Quality Assurance Project Plan

Evaluation of Migration Behavior and Survival of Juvenile Steelhead in Satus Creek, Washington

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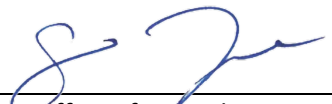
Tobias J. Kock
Supervisory Research Fish Biologist
U.S. Geological Survey
Western Fisheries Research Center
5501A Cook-Underwood Road
Cook, Washington 98605
tkock@usgs.gov

Approved by:

Signature: 	Date: 10/28/2024
Tim Resseguie, Yakama Nation Fisheries Research Scientist	
Signature: 	Date: 10/29/2024
Tobias Kock, USGS Supervisory Research Fish Biologist	
Signature: 	Date: 10/29/2024
Scott Tarbutton, Office of Columbia River Quality Assurance Coordinator	
Signature: 	Date: 10/29/2024
Kevin Haydon, Office of Columbia River Project Manager	

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1.0 Background and Project Description

The Yakima River, in central Washington state, supports four distinct populations within the Yakima Major Population Group (part of the Middle Columbia River Steelhead Distinct Population Segment). These include Satus Creek, Toppenish Creek, Naches River, and Upper Yakima River populations. The Satus Creek population is noteworthy because Satus Creek and its tributaries spatially comprise approximately 9% of the Yakima River Basin but support the largest percentage of returning adult steelhead spawners during recent years. Estimates from PIT tag detections during the 2019–2021 spawn years indicated that 64% of Yakima River adult steelhead were detected in Satus Creek. Overall, the number of returning adult steelhead to the Yakima River basin has been very low since 2017, which is consistent with other steelhead populations throughout the Columbia River basin. Previous studies have provided numerous insights into life history characteristics of Satus Creek steelhead. Adults primarily return to Satus Creek during December–April, peak spawning occurs during February and March, and emergence occurs during May and June. Juvenile steelhead spend 1–3 years in Satus Creek (age-1 is the predominant age class at migration) before outmigrating to the Pacific Ocean. Recent data (2019–2021) suggests that Satus Creek juvenile steelhead begin outmigrating in January, earlier than their counterparts from Toppenish Creek and the Naches River, and continue to do so through May each year. Preliminary data suggests that outmigration survival within Satus Creek is very low based on survival estimates of approximately 20% from tagging locations in the upstream reaches to the PIT interrogation site located near the creek mouth. Factors that could be resulting in high mortality include degraded habitat resulting from overgrazing and predation from piscivorous fish (e.g., smallmouth bass, Northern pikeminnow) and birds (e.g., American white pelicans, Great blue herons). As a result of these factors, there is a need to evaluate and describe factors influencing survival of juvenile steelhead in Satus Creek and in the lower Yakima River. A collaborative study design involving the U.S. Geological Survey, Yakama Nation Fisheries, and Washington Department of Ecology was developed to begin collecting data useful for assessing behavior and survival of juvenile steelhead in these stream reaches. The proposed study is a two-year effort that will primarily rely on radiotelemetry to monitor movements and survival of juvenile steelhead. A total of 100 fish will be tagged during the study with 50 fish planned for 2024 and the remaining 50 fish planned for 2025. The study will focus on evaluating behavior in the lower 25 miles of Satus Creek and in the lower Yakima River (Figure 1).

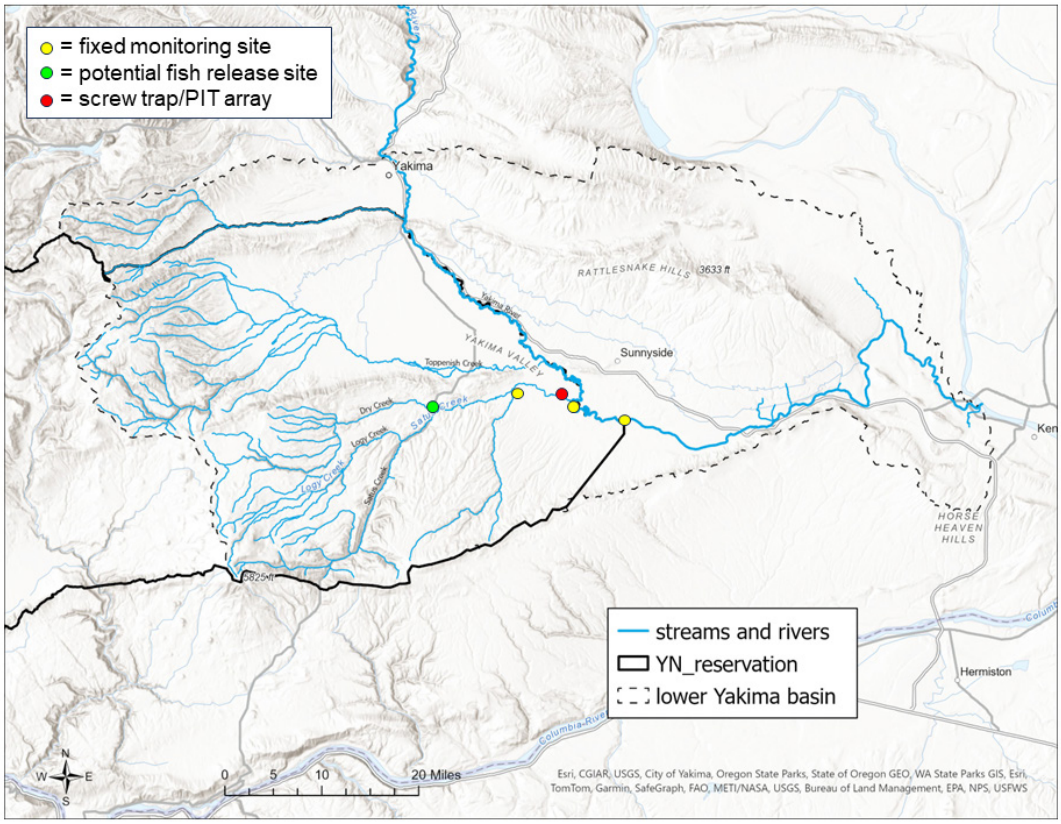

Figure 1. Study area map.

2.0 Organization and Schedule

Principal Investigator

Toby Kock, Supervisory Research Fish Biologist, United States Geological Survey: 23 years of experience leading telemetry studies throughout the western United States. Toby has been leading telemetry studies in the Yakima Basin since 2012 evaluating juvenile and adult salmon behavior and survival. https://www.usgs.gov/staff-profiles/tobias-j-kock

Field Lead/Telemetry Expert

Brian Ekstrom, Fish Biologist, United States Geological Survey: 21 years of experience with telemetry studies throughout the western United States. https://www.usgs.gov/staff-profiles/brian-ekstrom

Fish Surgeon

Jamie Sprando, Fisheries Technician. United States Geological Survey: 24 years of experience leading fish tagging crews throughout the western United States.

Data Analyst

Amy Hansen, Fish Biologist, United States Geological Survey: 24 years of experience analyzing telemetry datasets from studies conducted throughout the western United States. https://www.usgs.gov/staff-profiles/amy-c-hansen

Yakama Nation Fisheries Lead
Tim Resseguie, Fisheries Research Scientist IV, Yakama Nation Fisheries. 21 years of experience in the Satus Creek watershed working with rotary screw traps and PIT-tagging studies.

Ecology OCR QAPP Coordinator
Scott Tarbutton, Hydrogeologist, Washington Department of Ecology - Office of Columbia River. Provides initial review and feedback of QAPP, approves QAPP.

Ecology OCR Project Manager
Kevin Haydon, Environmental Planner, Washington Department of Ecology - Office of Columbia River. Coordination of QAPP development and finalization. Coordinate with the project completion of deliverables, timelines, and budget.

Anticipated Schedule
October 2024: mobilize telemetry stations and tagging equipment.

October-December 2024: tag and release 50 juvenile steelhead

October 2024-February 2025: maintain telemetry stations, conduct mobile tracking, download data.

March 2025-September 2025: process Year 1 data, provide preliminary results to stakeholders, consider study design modifications to improve Year 2 study.

October 2025: mobilize telemetry stations and tagging equipment.

October-December 2025: tag and release 50 juvenile steelhead

October 2025-February 2026: maintain telemetry stations, conduct mobile tracking, download data.

March-December 2026: process Year 2 data, conduct multi-year analysis, provide preliminary results to stakeholders, prepare and complete final multi-year report.

3.0 Quality Objectives

The overall goal of the telemetry data collection is to describe migration behavior and estimate survival of juvenile steelhead in Satus Creek and in the Lower Yakima River. A primary assumption of survival modeling is that the fate (alive/dead) of tagged fish is correctly determined at each monitoring gate. Therefore, it is critical to maintain receiver functionality throughout the entire study period. The processes that will be implemented to ensure receiver functionality are described in Section 6.2 below. Additional information on survival modeling is available in pages 453-475 of Adams et al. (2014). False-positive detections can occur using

telemetry applications and can lead to biased survival estimates if they remain in the final dataset. We use an automated proofing program to remove false-positive detections from the final dataset described in pages 505-518 of Adams et al. (2014).

4.0 Study Design

Juvenile steelhead will be radio-tagged and released in Satus Creek to describe migration behavior and estimate survival during fall/winter months. Data will be analyzed using a mark-recapture framework with radio-tagging serving as the "mark" and detections on fixed telemetry stations and via mobile tracking serving as "recaptures". A similar study design was used to evaluate migration and survival of juvenile coho salmon on the Cowlitz River during 2007–2010 (Attachment A; Kock et al. 2012). Detections on fixed telemetry stations in Satus Creek and in the lower Yakima River (Figure 1) will provide general information on movement patterns of tagged fish. Bi-monthly mobile tracking will be used to determine fine-scale (<10 m) instantaneous locations of tagged fish to provide additional information regarding habitat use and fish fate (alive/dead).

5.0 Field Procedures

Fish Collection

Fish will be collected for tagging by Yakama Nation staff using a screw trap located in the lower portion of Satus Creek (Figure 1). Yakama Nation's protocol for screw trapping is available at: https://www.monitoringresources.org/Document/Protocol/Details/111. We anticipate that collection will occur for 2-3 days in each study year.

Fish Holding, Tagging, and Release

For this study, USGS staff will adhere to procedures and protocols for fish holding, fish tagging, and fish release outlined in Liedtke et al. (2012; Attachment B). This document is a peer-reviewed, publicly available (https://pubs.usgs.gov/of/2012/1267/pdf/ofr20121267.pdf) document that was developed by USGS scientists with the Western Fisheries Research Center. Fish holding and release procedures are in *pages 10–13* in Liedtke et al. (2012). Fish tagging procedures are in *pages 14–26* in Liedtke et al. (2012). All fish collection, holding, tagging, and release procedures will be conducted in accordance with animal welfare considerations outlined in Attachment C (CRRL–KF1 Institutional Animal Care and Use Committee).

Monitoring of Tagged Fish

USGS will deploy three fixed telemetry stations in Satus Creek and in the Lower Yakima River (Figure 1) to determine general movement patterns of tagged fish. Fixed monitoring sites will include: a steel storage box that houses a 12-volt battery and telemetry receiver (Model SRX 400; Lotek Wireless, Inc., Newmarket, Ontario, Canada), a solar panel, and a three-element Yagi antenna. Each site will operate continuously throughout the study period to detect tagged fish. USGS staff will visit each fixed monitoring every two weeks to check on receiver operating status and to download data. Detections on fixed telemetry stations will be used to inform mobile tracking excursions that will be used to determine fine-scale (<10 m) instantaneous

locations of tagged fish. Mobile tracking will likely occur using a combination of approaches including walking-, vehicle-, and boat-based options. When tagged fish are located their position will be documented using a GPS along with the date and time. Fish that are repeatedly detected at the same location will be assumed to be dead. Note: there may be an option to use snorkeling to confirm fish fate, but this will be location- and condition-dependent.

Additional Data

Juvenile salmon behavior and movement is often affected by environmental factors such as streamflow and water temperature. River flow and water temperature data will be acquired from the following sources:

- Satus Creek data will be obtained from the Lower Satus Creek Gage operated the Yakama Nation Water Resources Program.
- Yakima River data will be obtained from USGS's Kiona, Washington gage (Gage #12510500) at the following site: https://waterdata.usgs.gov/nwis/uv?site_no=12510500&legacy=1

6.0 Quality Control

Quality control measures are implemented during laboratory preparation and field sampling phases of telemetry studies to ensure that equipment is fully functional during the study period. These steps are described in sections 6.1 and 6.2 below.

6.1 Steps in preparation of field work

Fish holding, tagging and transport containers/equipment are always sanitized and inspected prior to transporting to field sites. Telemetry receivers, 12-volt batteries, and solar panels are tested for functionality prior to transporting to field sites. Otherwise, not applicable for this study.

6.2 Steps taken in field

Fish holding, tagging, and transport containers/equipment are always sanitized and inspected after each use in the field. 12-volt battery voltages are always checked during downloading visits to minimize risk of data loss due to battery failure. Telemetry receiver functionality is tested with a 'test tag' that operates on the same frequency as study fish. Otherwise, no applicable for this study.

7.0 Data Management Procedures

At the conclusion of each study year, telemetry data records will be merged with tagging and release data and river flow water temperature data. This final merged dataset will be used to determine the number of fish detected at each site, travel times between sites, and to assess for behavior patterns influenced by water temperature and river flow. Survival analyses will be conducted using a Cormack-Jolly-Seber mark-recapture model following methods described in Skalski et al. (1998; Attachment D).

At the conclusion of the two-year study the datasets will be merged to create a final dataset from the study. This dataset will undergo USGS's peer review process and will be publicly available at https://sciencebase.gov/

USGS's fundamental science practices are outlined at: https://www.usgs.gov/office-of-science-quality-and-integrity/fundamental-science-practices

8.0 Reporting and Field Activity Assessments

A final report will be produced as either (1) a peer-reviewed fishery journal article; or (2) a peer-reviewed USGS Open-File Report that will be publicly available at: https://pubs.usgs.gov/

9.0 References

The following documents are referenced in this Quality Assurance Project Plan and provided as attachments.

Attachment A

Kock, T.J., Liedtke, T.L., Rondorf, D.W., Serl, J.D., Kohn, M. and Bumbaco, K.A., 2012. Elevated stream flows increase dam passage by juvenile coho salmon during winter: Implications of climate change in the Pacific Northwest. *North American Journal of Fisheries Management*, *32*(6), pp.1070-1079.

Attachment B

Liedtke, T.L., Beeman, J.W. and Gee, L.P., 2012. *A standard operating procedure for the surgical implantation of transmitters in juvenile salmonids* (No. 2012-1267). US Geological Survey.

Attachment C

USGS Western Fisheries Institutional Animal Care and Use Committee Fish Handling and Tagging Approval

Attachment D

Skalski, J.R., Smith, S.G., Iwamoto, R.N., Williams, J.G. and Hoffmann, A., 1998. Use of passive integrated transponder tags to estimate survival of migrant juvenile salmonids in the Snake and Columbia rivers. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(6), pp.1484-1493.

Scientists with USGS's Western Fisheries Research Center are widely recognized as global experts in aquatic telemetry. In 2012, we published a book entitled "Telemetry Techniques: A User Guide for Fisheries Research" that is currently available for purchase at the American Fisheries Society webpage: https://fisheries.org/bookstore/all-titles/professional-and-trade/55068c/

The citation for this book is:

Adams, N.S., Beeman, J.W., Eiler, J.H. 2014. Telemetry Techniques: A User Guide for Fisheries Research, American Fisheries Society, Bethesda, Maryland.

Several sections within this book are particularly useful in relation to this Quality Assurance Project Plan, including the following:

> *Techniques for Telemetry Transmitter Attachment and Evaluation of Transmitter Effects on Fish Performance: Section 4*

Radio Telemetry–Principles and Practice: Sections 5.1, 5.2, 5.3

Using Mark-Recapture Models to Estimate Survival from Telemetry Data: Section 9.2

Additional references are included below as examples highlighting USGS's extensive experience publishing peer-reviewed fisheries studies using telemetry-based techniques.

Courter, I.I., Garrison, T.M., Kock, T.J., Perry, R.W., Child, D.B. and Hubble, J.D., 2016. Benefits of prescribed flows for salmon smolt survival enhancement vary longitudinally in a highly managed river system. *River research and applications*, *32*(10), pp.1999-2008.

Kock, T.J., Tiffan, K.F. and Connor, W.P., 2007. *Investigating passage of ESA-listed juvenile fall Chinook salmon at Lower Granite Dam during winter when the fish bypass system is not operated*. Bonneville Power Administration.

Kock, T.J., Henning, J.A., Liedtke, T.L., Royer, I.M., Ekstrom, B.K. and Rondorf, D.W., 2011. Behavior and movement of formerly landlocked juvenile coho salmon after release into the free-flowing Cowlitz River, Washington. *Northwestern Naturalist*, *92*(3), pp.167-174.

Kock, T.J., Liedtke, T.L., Ekstrom, B.K., Tomka, R.G. and Rondorf, D.W., 2012b. *Behavior and passage of juvenile salmonids during the evaluation of a behavioral guidance structure at Cowlitz Falls Dam, Washington, 2011* (No. 2012-1030). US Geological Survey.

Kock, T.J., Liedtke, T.L., Ekstrom, B.K. and Hurst, W., 2015. *Evaluation of two juvenile salmon collection devices at Cowlitz Falls Dam, Washington, 2014* (No. 2015-1054). US Geological Survey.

Kock, T.J., Perry, R.W. and Hansen, A.C., 2016a. *Survival of juvenile chinook salmon and coho salmon in the Roza Dam fish bypass and in downstream reaches of the Yakima River, Washington, 2016* (No. 2016-1210). US Geological Survey.

Kock, T.J., Perry, R.W., Gleizes, C., Dammers, W. and Liedtke, T.L., 2016b. Angler harvest, hatchery return, and tributary stray rates of recycled adult summer steelhead Oncorhynchus mykiss in the Cowlitz River, Washington. *River Research and Applications*, *32*(8), pp.1790-1799.

Kock, T.J., Perry, R.W., Pope, A.C., Serl, J.D., Kohn, M. and Liedtke, T.L., 2018. Responses of hatchery-and natural-origin adult spring Chinook Salmon to a trap-and-haul reintroduction program. *North American Journal of Fisheries Management*, *38*(5), pp.1004-1016.

Kock, T.J., Evans, S.D., Ekstrom, B.K. and Hansen, A.C., 2019a. *Adult sockeye salmon (Oncorhynchus nerka) behavior and movement from Roza Dam to Cle Elum Dam, Washington, 2018* (No. 2019-1053). US Geological Survey.

Kock, T.J., Ekstrom, B.K. and Liedtke, T.L., 2019b. *Distribution of adult Chinook salmon (Oncorhynchus tshawytscha) in relation to water temperatures, Lake Scanewa, Cowlitz River, Washington, 2012* (No. 2019-1055). US Geological Survey.

Kock, T.J., Hansen, A.C., Evans, S.D., Visser, R., Saluskin, B., Matala, A. and Hoffarth, P., 2021. *Evaluation of factors affecting migration success of adult sockeye salmon (Oncorhynchus nerka) in the Yakima River, Washington, 2020* (No. 2021-1075). US Geological Survey.

Kock, T.J., Evans, S.D., Perry, R.W., Monk, P.A., Porter, M.S., Hansen, A.C. and Pope, A.C., 2024. Survival implications of diversion entrainment for out-migrating juvenile Chinook Salmon and steelhead. *Transactions of the American Fisheries Society*, *153*(2), pp.200-215.

Perry, R.W., Kock, T.J., Courter, I.I., Garrison, T.M., Hubble, J.D. and Child, D.B., 2016. Dam Operations Affect Route-specific Passage and Survival of Juvenile Chinook Salmon at a Main-stem Diversion dam. *River research and applications*, *32*(10), pp.2009-2019.

Tiffan, K.F., Kock, T.J., Connor, W.P., Steinhorst, R.K. and Rondorf, D.W., 2009. Behavioural thermoregulation by subyearling fall (autumn) Chinook salmon Oncorhynchus tshawytscha in a reservoir. *Journal of Fish Biology*, *74*(7), pp.1562-1579.

Tiffan, K.F., Kock, T.J., Connor, W.P., Mullins, F. and Steinhorst, R.K., 2012. Downstream movement of fall Chinook salmon juveniles in the lower Snake River reservoirs during winter and early spring. *Transactions of the American Fisheries Society*, *141*(2), pp.285-293.

Tiffan, K.F., Kock, T.J., Connor, W.P., Richmond, M.C. and Perkins, W.A., 2018. Migratory behavior and physiological development as potential determinants of life history diversity in fall Chinook salmon in the Clearwater River. *Transactions of the American Fisheries Society*, *147*(2), pp.400-413.

10.0 Appendices

See Attachments A, B, C and D.

North American Journal of Fisheries Management 32:1070–1079, 2012

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ARTICLE

Elevated Streamflows Increase Dam Passage by Juvenile Coho Salmon during Winter: Implications of Climate Change in the Pacific Northwest

Tobias J. Kock,* Theresa L. Liedtke, and Dennis W. Rondorf
U.S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory, 5501A Cook-Underwood Road, Cook, Washington 98605-9717, USA

John D. Serl
Washington Department of Fish and Wildlife, Cowlitz Falls Fish Facility, 1379B Falls Road, Randle, Washington 98377, USA

Mike Kohn
Public Utility District Number 1 of Lewis County, Cowlitz Falls Project, Post Office Box 1387, Morton, Washington 98356, USA

Karin A. Bumbaco
Office of the Washington State Climatologist, Joint Institute for the Study of Atmosphere and Ocean, University of Washington, Box 355672, Seattle, Washington 98195-5672, USA

Abstract

A 4-year evaluation was conducted to determine the proportion of juvenile coho salmon *Oncorhynchus kisutch* passing Cowlitz Falls Dam, on the Cowlitz River, Washington, during winter. River and reservoir populations of coho salmon parr were monitored using radiotelemetry to determine if streamflow increases resulted in increased downstream movement and dam passage. This was of interest because fish that pass downstream of Cowlitz Falls Dam become landlocked in Riffe Lake and are lost to the anadromous population. Higher proportions of reservoir-released fish (0.391–0.480) passed Cowlitz Falls Dam than did river-released fish (0.037–0.119). Event-time analyses demonstrated that streamflow increases were important predictors of dam passage rates during the study. The estimated effect of increasing streamflows on the risk of dam passage varied annually and ranged from 9% to 75% for every 28.3 m^3/s increase in streamflow. These results have current management implications because they demonstrate the significance of dam passage by juvenile coho salmon during winter months when juvenile fish collection facilities are typically not operating. The results also have future management implications because climate change predictions suggest that peak streamflow timing for many watersheds in the Pacific Northwest will shift from late spring and early summer to winter. Increased occurrence of intense winter flood events is also expected. Our results demonstrate that juvenile coho salmon respond readily to streamflow increases and initiate downstream movements during winter months, which could result in increased passage at dams during these periods if climate change predictions are realized in the coming decades.

Juvenile coho salmon *Oncorhynchus kisutch* typically reside in freshwater streams and rivers during their first winter of life. Because significant mortality can occur during this period, their ecology has been well studied (Bustard and Narver 1975; Tschaplinski and Hartman 1983; Giannico and Hinch 2003). Coho salmon juveniles are commonly found in areas containing

*Corresponding author: tkock@usgs.gov
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large woody debris during winter, and riverine ponds, alcoves, and side channels typically contain the highest densities of overwintering fish (Tschaplinski and Hartman 1983; McMahon and Hartman 1989; Shirvell 1990). These habitats provide cover and velocity refugia during high flow periods, which is important because juvenile coho salmon respond readily to streamflow increases by moving downstream in areas lacking woody debris and side-channel habitat (Bustard and Narver 1975; Hartman et al. 1982; Taylor 1988; Shirvell 1990). Downstream movements by juvenile coho salmon that occur prior to the spring migration period are associated with reduced survival, so minimizing this behavior during winter is important in areas where these populations can be managed (Thedinga and Koski 1984; Holtby 1988).

Although previous studies have been insightful for understanding the general behavior patterns of juvenile salmonids during winter months, additional research is required. Factors that affect the survival, behavior, and habitat use of juvenile salmonids in the winter are complex, and many of the previous studies have been limited due to the small size of fish and difficulties associated with sampling during this period (Huusko et al. 2007; Brown et al. 2011; Tiffan et al. 2012). Recent reviews by Huusko et al. (2007) and Brown et al. (2011) provide thorough summaries of the contemporary understanding of stream-rearing juvenile salmonids during winter months. Both papers call for additional research to better understand juvenile salmonid behavior during the winter and habitat use patterns and the effects of environmental (temperature, discharge) and physical (dams, reservoirs) factors on these relationships. Evolving technologies will undoubtedly aid in the development of studies to address these factors. For example, radio transmitters have recently been developed that are relatively small (<0.4 g) and operate for long periods of time (>100 d). Tiffan et al. (2012) used these transmitters to monitor downstream movements of juvenile fall-run Chinook salmon *O. tshawytscha* through reservoirs in the lower Snake River and were able to describe annual, seasonal, and spatial variations in downstream movement rates while also documenting dam passage during winter months. The availability of these radio transmitters and monitoring systems allowed the researchers to monitor individual fish throughout a relatively large study area (>150 river kilometers, hereafter rkm), whereas previous studies had to draw inferences about fish behavior using fine-scale observations and fish counts at various points during their study periods (Holtby 1988; Nickelson et al. 1992; Giannico and Hinch 2003).

Winter passage of juvenile coho salmon at Cowlitz Falls Dam, located on the Cowlitz River in southwestern Washington State (Figure 1), may result in significant losses to the coho salmon population, which is listed as threatened under the U.S. Endangered Species Act (NMFS 2005). Cowlitz Falls Dam provides flood control in the upper Cowlitz River during high-flow periods, which can result in large volumes of water passing

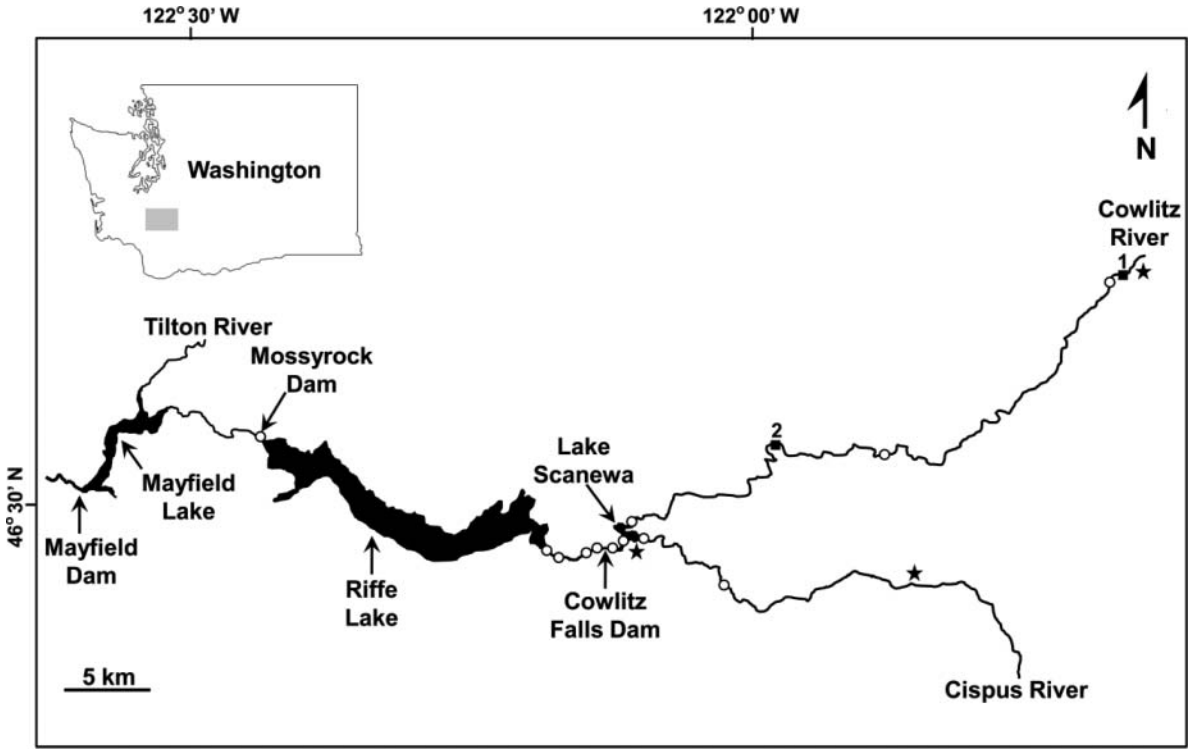

FIGURE 1. Map of the upper Cowlitz River basin showing the location of fixed sites (open circles), collection and release locations (stars), and USGS flow gauging stations located near Packwood, Washington (Gauge #14226500; square #1) and Randle, Washington (Gauge #14231000; square #2).

through the dam when streamflow increases occur. Flood control operations are handled in various ways depending on the size of the flow increase. River flows $\leq$227 m^3/s (measured at U.S. Geological Survey [USGS] Gauge #14231000) do not require flood control measures because the dam's two turbines can accommodate flows at or below this level. Streamflows that range from 227 to 425 m^3/s are usually controlled by maximizing turbine output and passing additional water through spillbays or low-level sluiceways at the dam. However, once river flows exceed 425 m^3/s the dam is required to pass enough water (through turbines, spillbays, etc.) to lower the surface elevation of Lake Scanewa (the reservoir created by Cowlitz Falls Dam; Figure 1) by as much as 6 m (hereafter reservoir drawdown). On average, reservoir drawdowns have occurred 2.3 times (range = 0–6) during November–March each year between 1994 and 2010. Winter streamflow increases are believed to cause significant dam passage by juvenile coho salmon for the following reasons: (1) juvenile coho salmon are commonly observed rearing in Lake Scanewa during the fall and would be susceptible to dam passage during drawdown events; (2) the largest number of coho salmon smolts ever collected (334,718 fish compared with the 10-year average of 136,519 fish) at the Cowlitz Falls Fish Facility (CFFF) occurred during 2001, the only year in which a reservoir drawdown did not occur during the winter prior to a fish collection season; and (3) the CFFF is only operated during April–August each year, which means that fish moving downstream during high winter flow events are not collected and pass downstream of the dam.

The fish that are collected at Cowlitz Falls Dam are transported downstream and released into the lower Cowlitz River, where they can migrate to the ocean, whereas fish that pass Cowlitz Falls Dam enter Riffe Lake, where they become landlocked and are lost to the anadromous fish population. Several anadromous fish species reside within the study area including coho salmon, steelhead *O. mykiss*, Chinook salmon, and cutthroat trout *O. clarkii*. The construction of Mossyrock Dam in 1968 threatened anadromous fish species upstream of rkm 105 because the dam blocked upstream volitional fish passage and created Riffe Lake (Figure 1). These species have persisted upstream of Mossyrock Dam because of a trap-and-haul program, which transports fish around the Cowlitz River hydropower system. Returning adults are captured downstream of Mayfield Dam (rkm 80), loaded onto trucks, and transported upstream of Cowlitz Falls Dam (rkm 143), where they are released (Figure 1). High quality spawning habitat is located throughout the basin upstream of Cowlitz Falls Dam, and progeny from the transported adults are distributed throughout river and reservoir habitats, where they rear before moving downstream toward the ocean. Juvenile out-migrants are collected at Cowlitz Falls Dam and transported downstream of Mayfield Dam, where they are released (Figure 1). Juvenile out-migrants that are not collected at Cowlitz Falls Dam pass downstream and enter Riffe Lake, where they become landlocked due to the large size of the reservoir and lack of fish collection facilities at Mossyrock Dam. Mark–recapture studies conducted by the Washington Department of Fish and Wildlife at Cowlitz Falls Dam from 1997 to 2009 show that about 32% of the coho salmon smolts are collected at Cowlitz Falls Dam each year, which means that approximately 2.3 million coho salmon smolts were passed into Riffe Lake during that period (Serl and Morrill 2011). This estimate demonstrated the losses of anadromous production that occur in the upper Cowlitz River basin when juvenile coho salmon smolts pass Cowlitz Falls Dam and become entrapped in Riffe Lake.

Climate change predictions for western Washington suggest that flow regimes will be significantly altered in the coming decades, which could increase the winter passage of juvenile coho salmon at Cowlitz Falls Dam. The Cowlitz River is classified as a transient watershed, which means that river flows are influenced by both rainfall and snowmelt events (Elsner et al. 2010; Mantua et al. 2010). Peak streamflow events typically occur twice annually in transient watersheds: once during the winter when seasonal precipitation peaks and once during the late spring or early summer when snowmelt occurs (Elsner et al. 2010). Climate change predictions suggest that annual air temperatures in the Pacific Northwest will increase by 3.0 °C (on average) by the 2080s, which is expected to have significant effects on hydrological regimes in western Washington (Elsner et al. 2010; Mote and Salathe 2010). Transient watersheds, like the Cowlitz River, are expected to experience a significant shift in streamflow timing that will include increased winter runoff and decreased summer runoff due to the shift towards more rain-dominated winter precipitation that does not contribute to the snowpack (Mote et al. 2003; Elsner et al. 2010; Mantua et al. 2010; Figure 2). Studies have shown that high streamflow events are significant factors in overwinter mortality of juvenile coho salmon, so predictions of increased peak flows during the winter may increase early life stage mortality of coho salmon (Reeves et al. 1989; Beechie et al. 1994; Mantua et al. 2010).

Frequent winter streamflow increases resulting in reservoir drawdowns prompted an evaluation of coho salmon passage at Cowlitz Falls Dam during winter. We conducted an evaluation of fish passage using radiotelemetry to monitor downstream movements and estimate dam passage during October–February from 2007 to 2011. Our objectives were to (1) quantify dam passage of juvenile coho salmon at Cowlitz Falls Dam during winter, (2) determine if river and reservoir populations of juvenile coho salmon experienced similar passage rates, and (3) identify potential long-term effects of climate change predictions to inform future management actions aimed at protecting anadromous fish populations in the upper Cowlitz River basin.

METHODS

Study site.—This study was conducted in the upper Cowlitz River, which is a tributary to the Columbia River, in southwestern Washington State. Tagged fish were monitored in two riverine reaches (Cowlitz and Cispus rivers) upstream of Lake

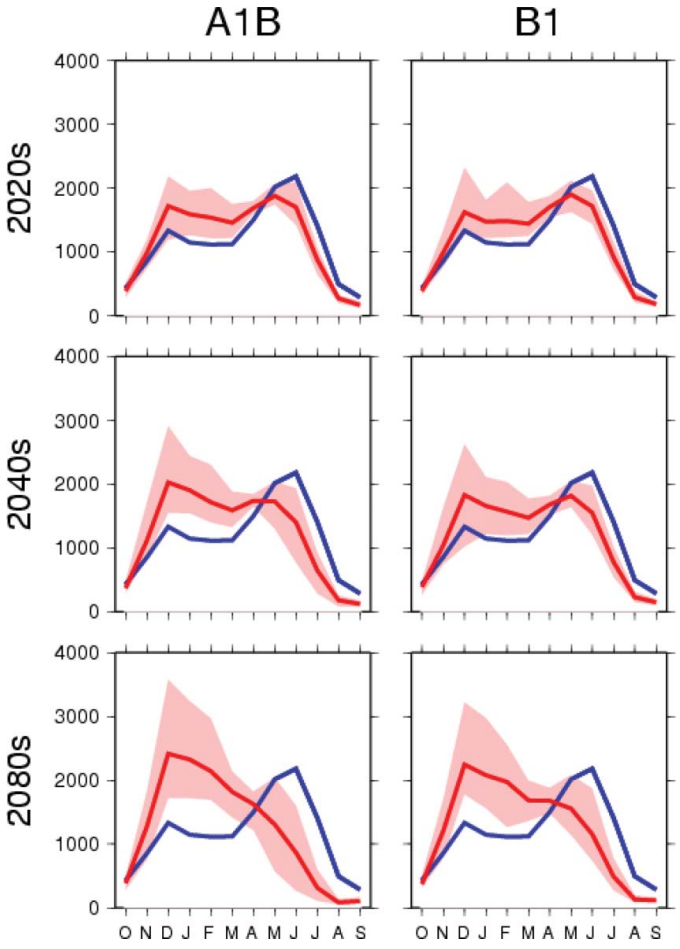

FIGURE 2. Relationship between historical streamflows (blue line) and predicted streamflows (red line; ensemble averaged estimates) for the Cowlitz River at Packwood, Washington during the 2020s, 2040s, and 2080s. The light red envelope represents the range of predicted streamflows, and A1B and B1 refer to two emission scenarios used for estimating future climate change scenarios. Values on the *y*-axis represent streamflows (ft^3/s) and values on the *x*-axis represent individual months beginning with October and ending with September. This figure was downloaded from the Columbia Basin Climate Change Scenarios Project website and was produced by the Climate Impacts Group at the University of Washington in collaboration with the Washington State Department of Ecology, Bonneville Power Administration, Northwest Power and Conservation Council, Oregon Water Resources Department, and the British Columbia Ministry of the Environment. [Figure available in color online.]

Scanewa, in Lake Scanewa, and downstream of Cowlitz Falls Dam (Figure 1). Lake Scanewa is a 284-ha reservoir that was created by the construction of Cowlitz Falls Dam in 1994. The confluence of the Cowlitz and Cispus rivers is located near the center of the reservoir (Figure 1).

The Cowlitz and Cispus rivers drain large areas on the western slope of the Cascade Mountains, and rainfall and snowmelt events control water temperatures and streamflows throughout the year. The Cowlitz River, upstream of Lake Scanewa, is larger and warmer than the Cispus River during most months of the year. Streamflows in the Cowlitz River averaged 82.0 m^3/s (range = 7.5–736.2 m^3/s; 264.9–25,991.6 ft^3/s) from January 2007 to March 2011 compared with 31.2 m^3/s (range = 7.4–257.4 m^3/s; 261.3–9,075.9 ft^3/s) in the Cispus River. River temperatures in the Cowlitz River averaged 4.8°C (range = 0–7.8°C) from December 2010 to March 2011 compared with 4.2°C (range = 0–7.2°C) in the Cispus River.

Fish collection and tagging.—Juvenile coho salmon were collected by angling or electrofishing, radio transmitters were surgically implanted, and tagged individuals were monitored to determine dam passage proportions and dam passage rates during our study. A total of 17 tag-and-release efforts were conducted during four field seasons, which included October to February of 2007–2011. Coho salmon juveniles were collected for tagging in Lake Scanewa during 2007, 2009, and 2010, and in the Cowlitz and Cispus rivers during 2008–2010 (Table 1). Reservoir collection efforts occurred primarily near rkm 1.0 on the Cispus River (Figure 1). River collection on the Cowlitz and Cispus rivers occurred near rkm 195.5 and 27.0, respectively. Following collection, juvenile coho salmon were transported to the CFFF, where they were held for 24–72 h in floating 208-L, perforated containers that received a continuous supply of flow-through river water. On each tagging date, fish were anesthetized using buffered tricaine methanesulfonate (70 mg/L) and radio transmitters (Model NTC-3-1; Lotek Wireless, Canada) were surgically implanted using techniques described by Adams et al. (1998). Radio transmitters were 13.5 mm long, 5.3 mm wide, and weighed 0.37 g in air. Transmitters included an antenna that was 16 cm long and emitted a signal every 20 s. A subsample of 25 transmitters was monitored in a laboratory setting during 2009 and we found that mean tag life was 104 d. Fish were not tagged if the radio transmitter weighed more than 5% of their body weight (in air) at the time of tagging. We were able to tag fish that were 7.4 g or larger. Following tagging, fish were held for 24 h and were then transported by truck to one of three release sites. Fish were segregated throughout the holding, tagging, and release process to ensure that tagged fish were released near the location where they were originally collected.

Monitoring system.—Fixed monitoring sites (hereafter fixed sites) were established and maintained to collect information about movement patterns of tagged fish during the study (Figure 1). Fixed sites were located on the Cowlitz River (rkm 173.2 and 193.9), Cispus River (rkm 3.2 and 9.6), in Lake Scanewa (rkm 136.7, 139.1 on the Cowlitz River; rkm 1.8 on the Cispus River), and on Cowlitz Falls Dam (rkm 135.5) to monitor fish movements prior to passage at the dam (Figure 1). Fixed sites in the tailrace of Cowlitz Falls Dam (rkm 135.2 and 135.3) monitored for tagged fish passing the dam. Two sites located downstream of the dam's tailrace (rkm 130.3 and 131.6) provided a secondary array for confirming dam passage by tagged fish. Additionally, fixed sites were operated on Mossyrock Dam (rkm 105.8) during 2007–2009 to determine if tagged fish moved through Riffe Lake after passing Cowlitz Falls Dam.

TABLE 1. Release dates and the number of radio-tagged juvenile coho salmon that were released in the upper Cowlitz River basin during a 2007–2010 evaluation.

Release dates and totals	Release location			Total number of fish released
	Reservoir	Cispus River	Cowlitz River	
October 23, 2007	16	0	0	
October 24, 2007	24	0	0	
October 25, 2007	10	0	0	
November 30, 2007	29	0	0	
2007 totals	79	0	0	79
November 6, 2008	0	0	28	
November 7, 2008	0	28	0	
January 23, 2009	0	0	6	
January 24, 2009	0	20	0	
2008 totals	0	48	34	82
October 22, 2009	0	33	0	
October 23, 2009	4	0	0	
October 24, 2009	0	0	21	
October 30, 2009	28	0	6	
November 6, 2009	23	0	0	
2009 totals	55	33	27	115
October 5, 2010	26	0	0	
October 8, 2010	63	0	0	
October 9, 2010	39	0	0	
October 14, 2010	0	12	12	
2010 totals	128	12	12	152
2007–2010 totals	262	93	73	428

Estimating passage proportions.—We used a Cormack–Jolly–Seber (CJS) mark–recapture model (Cormack 1964; Jolly 1965; Seber 1965) to estimate detection probabilities at fixed sites and the proportion of juvenile coho salmon that passed Cowlitz Falls Dam during our study. These models are commonly used to estimate recapture and survival probabilities from mark–recapture studies. We anticipated that some tagged fish that passed Cowlitz Falls Dam would not be detected by fixed sites in the tailrace of the dam because fish tended to move downstream quickly during high flow periods, the transmitters emitted a signal every 20 s, and the signal strength was relatively weak due to the small size of the transmitters. The use of the CJS model allowed us to obtain unbiased estimates of the proportion of tagged fish that passed Cowlitz Falls Dam during our study because these estimates were corrected for missed detections at fixed sites.

Release and detection records from river- and reservoir-released fish were summarized to create detection histories that were analyzed using Program MARK (White and Burnham 1999). A three-occasion detection history was created for each fish that was released during the study. Each detection history summarized the release and subsequent detection or nondetection of tagged fish at sites downstream of the dam. All fish received a "1" in the first occasion of the detection history, which represented tagged fish being released upstream of the dam. The last two occasions in the detection history represented detection or nondetection (0 = not detected; 1 = detected) in the tailrace of the dam and at fixed monitoring sites located approximately 5 rkm downstream of the dam. The CJS model produced three types of estimates: P_{dam}, which was the joint probability of tagged fish being released, surviving and moving downstream from the release sites, and passing Cowlitz Falls Dam; d, which was the detection probability of fixed sites located in the tailrace of the dam; and λ, which was the joint probability of tagged fish surviving, moving downstream from the tailrace, and being detected at fixed sites located 5 km downstream of the dam. Dam passage data for river-released fish during 2010 were sparse, so we pooled river-released groups of tagged fish from 2009 and 2010 for the CJS analysis. The fully parameterized model that we used contained 15 parameters, which included P_{dam}, d, and λ estimates for reservoir-released fish in 2007, 2009, and 2010 and river-released fish in 2008 and 2009–2010.

Estimating passage rates.—Cox proportional hazards regression was used to examine effects of covariates on rates of dam passage by tagged fish during the study period (Allison 1995; Castro-Santos and Haro 2003). Dam passage, as indicated by detection of individual tagged fish downstream of the dam, was the event of interest, and the hazard rate was the proportion of

tagged fish that passed the dam during each day of the study period. Four predictor variables were included in the analysis: release year (2007, 2008, 2009, 2010), fish size (<100 mm fork length; ≥100 mm fork length), release location (river, reservoir), and river flow. We used a 100-mm fork length criterion because this has been identified as a threshold for smoltification of coho salmon (Sandercock 1991) and is applicable to coho salmon in the Cowlitz River. River flow was included as a continuous predictor variable. Mean daily flow data were obtained from the USGS gauging station (#14231000) located near Randle, Washington. All fish not detected downstream of the dam were considered at risk of passing the dam until 105 d after release (the maximum observed time from release to passage), at which point they were censored.

The event-time analysis consisted of three general steps, which included identifying significant covariates and two-way interactions between covariates, assessing whether assumptions of the Cox proportional hazards model were satisfied for the data, and determining hazard ratios for significant predictor variables. Hazard ratios were used with river flow data to estimate the effects of river flow on risk of dam passage. Flow data were grouped by 28.3 m^3/s bins, and a risk statistic, described by Allison (1995), was used to understand the relationship between river flow and dam passage. The risk statistic is informative because it described how risk of passing the dam changed relative to each 28.3 m^3/s increase in river flow. The risk statistic was calculated as follows:

$$\text{risk statistic} = 100 \times (\text{hazard ratio} - 1)$$

The results from these analyses were used to understand the effects of covariates on the rates of dam passage during our study and to infer how coho salmon passage at Cowlitz Falls Dam could be affected in the future if climate change predictions are realized.

River flows and climate change.—As previously discussed, climate change predictions suggest that the timing of peak streamflow events will shift in future decades in the Pacific Northwest as a result of climate warming. Cowlitz River streamflow data were used to verify that historical and current streamflow responses support predicted shifts in streamflow timing. Cowlitz River monthly streamflow data were downloaded for the USGS flow gauging station #14226500 near Packwood, Washington (http://waterdata.usgs.gov/wa/nwis/) for the years 1930–2009. Area-averaged monthly temperature data were downloaded from the National Oceanic and Atmospheric Administration's Climate Division dataset (http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp) for Washington's climate division 4 (East Olympic Cascade Foothills) for the same time period. Climate Division 4 includes the west slopes of the Cascade foothills, parts of southwestern Washington, and the east Olympic foothills and includes the upper Cowlitz River basin, where our study was conducted. Climate data from this region are represented as an area average using cooperative observer weather stations within the boundaries of the area.

We used Cowlitz River streamflow data to calculate a ratio that compared melting-season streamflows (May–July) to late-winter streamflows (January–March). The ratio was obtained by dividing the average melting-season streamflow by the average late-winter streamflow, representing the proportions of annual streamflow that occurred during the two periods. Higher flows during the melting season than during the late-winter period result in a relatively large ratio (>1). Conversely, the ratio would be near 1 or <1 when streamflow timing was composed of higher flows during the late-winter period than during the melting season. This ratio was then plotted against the average late-winter air temperature (January–March) to assess the relationship between streamflow timing and temperature.

RESULTS

A total of 856 juvenile coho salmon were collected during the study period and 428 of these fish were tagged and released. Of these, 262 fish were released in the reservoir and 166 fish were released in the Cowlitz and Cispus rivers (Table 1). Cispus River releases comprised 56% of the river releases (93 total fish; 48 in 2008, 33 in 2009, 12 in 2010) compared with 44% for the Cowlitz River (73 total fish; 34 in 2008, 27 in 2009, 12 in 2010). During 2007 tagged fish were only released in the reservoir, and during 2008 tagged fish were only released in the rivers (Table 1). Mean weight and fork length of river-released fish was 10.4 g and 98.3 mm, respectively, compared with 10.5 g and 97.2 mm for reservoir-released fish. Mean fork length of fish that were not tagged during the study period was 82.1 mm and mean weight was 6.3 g.

More than one-third of the fish released in the reservoir passed Cowlitz Falls Dam during our study, whereas few of the river-released fish moved downstream of the dam. Tagged fish exhibited one of two behaviors during the study period, either remaining near the release location or moving downstream and passing Cowlitz Falls Dam. We did not observe downstream movements by tagged fish that did not pass the dam. A total of 102 (38.9%) juvenile coho salmon from the reservoir were detected downstream of Cowlitz Falls Dam during the study period compared with only 13 (7.8%) of the river-released fish. Of the river-released fish that passed downstream of Cowlitz Falls Dam, six fish were from the Cowlitz River release group and seven fish were from the Cispus River release group. The average elapsed time from release to dam passage by reservoir-released fish was 25.0 d (range = 1–85 d) compared with 54.8 d (range = 6–105 d) for river-released fish (Figure 3).

Some of the fish that passed Cowlitz Falls Dam were eventually detected at Mossyrock Dam. Eight of the fish known to have passed Cowlitz Falls Dam were detected at Mossyrock Dam during the study (3 in 2007, 2 in 2008, 3 in 2009). Mean elapsed time from passing Cowlitz Falls Dam to detection at Mossyrock Dam was 24.4 d (range = 3.4–69.7 d) and mean travel rate in Riffe Lake was 1.1 km/d (range = 0.1–2.4 km/d).

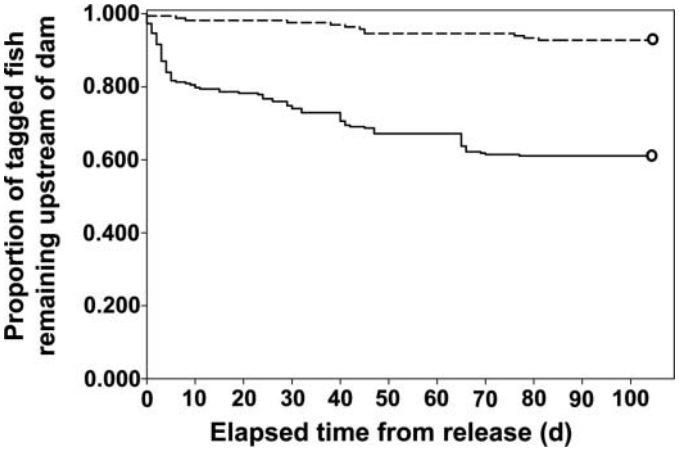

FIGURE 3. Kaplan–Meier survivorship function for tagged fish from river (dashed line) and reservoir (solid line) release locations. Open circles represent the time when data were censored due to expected failure of all radio transmitters in the study.

Dam Passage Proportions

We found that d varied throughout the study period and that P_{dam} was higher for reservoir-released fish than for river-released fish. Estimates of d ranged from 0.100 to 0.682 across release groups and study years (Table 2). Estimates of P_{dam} were relatively high for reservoir-released fish (0.391 in 2007, 0.480 in 2009, 0.445 in 2010) compared with river-released fish (0.037 in 2008, 0.119 in 2009 and 2010; Table 2). Estimates of λ ranged from 0.666 to 1.000 (Table 2).

Dam Passage Rates

Event-time analyses demonstrated that release location ($P = 0.0051$), river flow ($P < 0.0001$), and year ($P < 0.0001$) were significant predictors of dam passage rates during the study period, whereas fish size was not ($P = 0.2110$). We also observed that the two-way interaction between river flow and year was significant ($P < 0.0001$) so the final model that was used for testing assumptions and determining hazard ratios included the following variables: release location, river flow, year, and the river flow × year interaction. Multiple tests were conducted using techniques described by Patetta (2006) to confirm that assumptions of the Cox proportional hazards model were satisfied. Release location did not interact with other covariates during our study, so the hazard ratio of 6.493 demonstrates that reservoir-released fish had a dam passage rate that was nearly 6.5 times greater than that of river-released fish throughout the study period. Because we observed an interaction between river flow and year, hazard ratios were estimated for river flow during each year of the study. Hazard ratio estimates were 1.491 during 2007, 1.187 during 2008, 1.748 during 2009, and 1.086 during 2010, which means that the risk of dam passage increased by 49.1%, 18.7%, 74.8%, and 8.6% for every 28.3 m^3/s (1000 ft^3/s) increase in river flow during 2007, 2008, 2009, and 2010, respectively. Individual passage events were plotted with daily streamflow data to illustrate the relationship between dam passage and streamflow increases (Figure 4). This showed that most dam passage occurred during periods of increasing streamflow.

TABLE 2. Estimates of dam passage proportions (P_{dam}), detection probability (d), and the joint probability of surviving and being detected downstream of Cowlitz Falls Dam (λ) for radio-tagged juvenile coho salmon in the Cowlitz River during 2007–2010. Estimates were obtained using a Cormack–Jolly–Seber mark–recapture model. The number of fish passing downstream of Cowlitz Falls Dam from river-release groups was low so λ was fixed to 1.000 to facilitate estimating the remaining parameters.

Year	P_{dam} (SE)	d (SE)	λ (SE)
Reservoir-released fish			
2007	0.391 (0.071)	0.259 (0.084)	0.875 (0.117)
2009	0.480 (0.073)	0.682 (0.099)	0.833 (0.088)
2010	0.445 (0.057)	0.579 (0.080)	0.666 (0.082)
River-released fish			
2008	0.037 (0.021)	0.667 (0.272)	1.000
2009–2010	0.119 (0.035)	0.100 (0.095)	1.000

River Flows and Climate Change

The 80-year analysis of Cowlitz River streamflow patterns demonstrated that warm winters were characterized by increased river flows during late winter (January–March) and decreased flows during the melting season (May–July), and this relationship was reversed during cool winters (Figure 5). The melting-season flow to late-winter flow ratio is smaller during warm winters, which demonstrates that increased temperatures during winter months leads to higher winter flows and lower melting-season flows. The five warmest winters (1934, 1941, 1981, 1983, 1992) had an average late-winter flow of 48.2 m^3/s (1700 ft^3/s) and an average melting-season flow of 42.8 m^3/s (1510 ft^3/s), whereas the five coldest winters (1936, 1937, 1949, 1950, 1956) had an average late-winter flow of 29.2 m^3/s (1030 ft^3/s) and an average melting-season flow of 106.8 m^3/s (3770 ft^3/s). The 80-year historical record of streamflow on the Cowlitz River exhibits behaviors consistent with regional climate change predictions; the warming temperatures are expected to result in higher late-winter flow and lower melting-season flow as the basin becomes more rain dominant. These findings are consistent with predictions illustrated in Figure 2 from two climate change models. Expected warming patterns will increase December–February flows by about 30% by the 2020s, and flows are expected to double during this same period by the 2080s (Figure 2).

DISCUSSION

We used recent developments in radio transmitter technology to further advance the existing information about winter behavior of juvenile coho salmon. The ability to effectively evaluate parr-sized fish with telemetry technologies has traditionally been limited by the availability of small transmitters. However, recent efforts to reduce transmitter size and increase operating life resulted in the development of the transmitter that

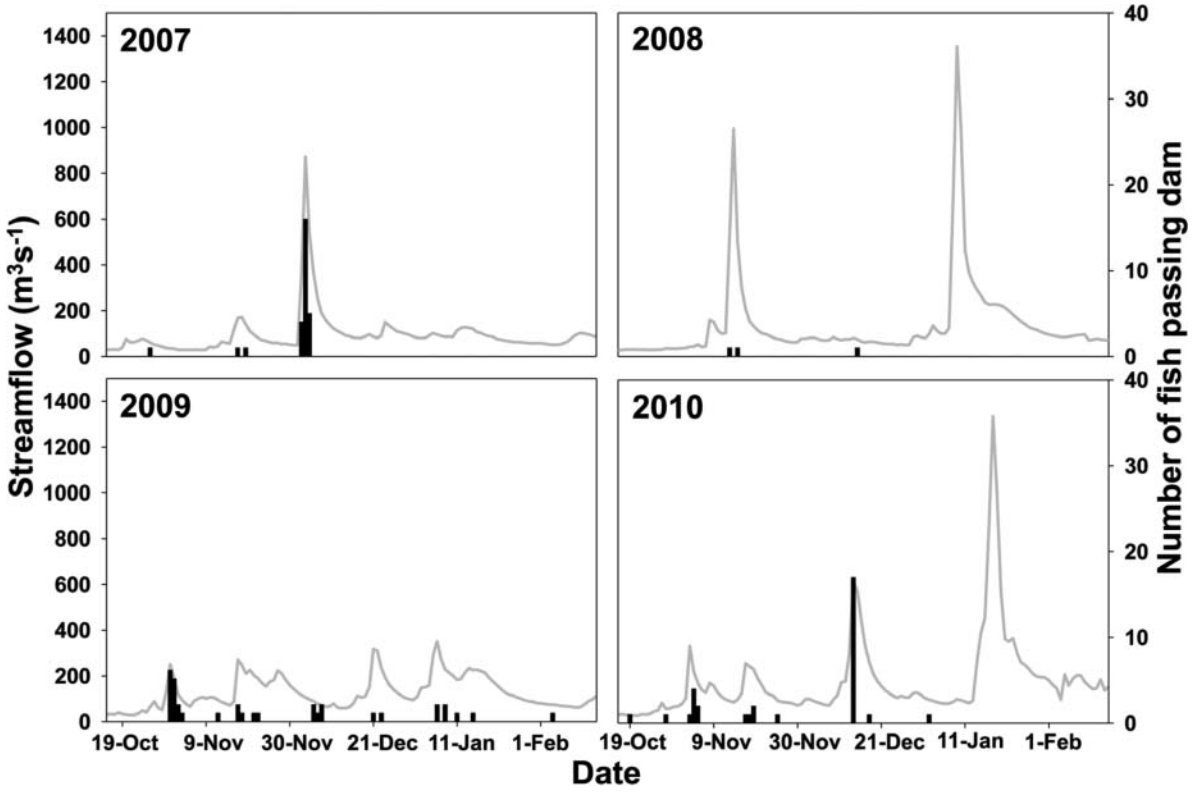

FIGURE 4. Mean daily streamflow data (grey line) and number of radio-tagged juvenile coho salmon that passed Cowlitz Falls Dam each day during evaluations conducted during 2007–2010.

we used, which weighed 0.37 g (in air) and operated for about 3 months. This transmitter allowed us to tag juvenile coho salmon as small as 7.4 g (approximately 85 mm fork length) and to monitor the movements of individual fish for 3 months throughout a study area that encompassed approximately 100 rkm. Previous studies did not have the advantage of using this technology, so most studies either relied on enumerating fish in certain habitats or monitoring fine-scale movements of individuals to draw inferences about juvenile coho salmon responses to streamflow increases (Shirvell 1990; Shirvell 1994; Giannico and Healey 1998). Our data support findings from these studies while also providing additional information (i.e., dam passage proportions, dam passage rates).

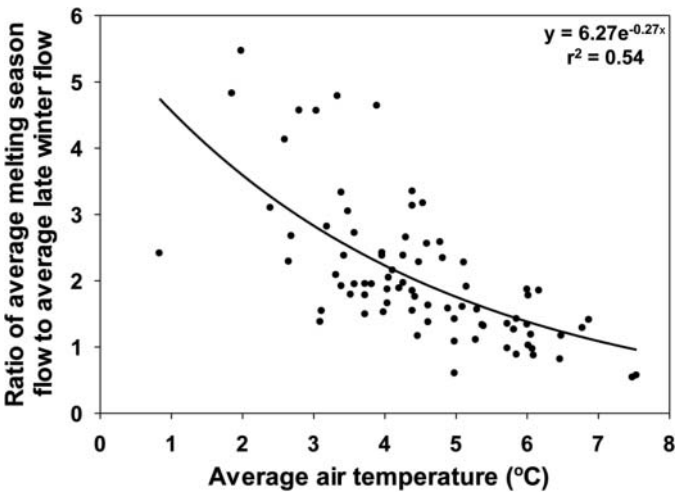

FIGURE 5. Ratio of melting-season flow (May–July) to late-winter flow (January–March) for 80 individual water years (1939–2009) on the Cowlitz River compared with the average late-winter temperature (January–March) in the region.

Our data showed that winter streamflow increases in the upper Cowlitz River resulted in increased dam passage by juvenile coho salmon at Cowlitz Falls Dam. Mean estimates of dam passage proportions during our study were 44% for reservoir-released fish and 8% for river-released fish. These findings are important because they identify a significant source of lost production to the anadromous coho salmon population of the upper Cowlitz River. Research has shown that large numbers of coho salmon smolts enter Riffe Lake during the fish passage season (April–August) each year (Serl and Morrill 2011), and fishery managers have suspected that winter dam passage could be substantial at Cowlitz Falls Dam. Our study provided the first empirical estimates of dam passage by juvenile coho salmon during winter months in the system.

Reservoir populations of juvenile coho salmon appear to be more susceptible to dam passage than river populations in the

upper Cowlitz River. Our analyses estimated that nearly half of the reservoir-released fish (39–48%) passed Cowlitz Falls Dam during the study compared to 4–12% of the river-released fish. Both CJS modeling analyses and event-time analyses found these differences to be significant. The difference in dam passage between the two release groups can likely be attributed to habitat differences between the river and reservoir environments. Riverine habitat located between the river release sites and Lake Scanewa is complex and diverse, containing large accumulations of woody debris, numerous side channels, and deep pools, all of which have been shown to maintain high numbers of juvenile coho salmon during winter freshets (Tschaplinski and Hartman 1983; McMahon and Hartman 1989; Nickelson et al. 1992; Giannico and Hinch 2003). Conversely, habitat in Lake Scanewa is largely homogenous with steep-sided shorelines that contain little woody debris, and the areas that do contain woody debris are typically dewatered when the reservoir is drawn down. Drawdowns return much of the reservoir to riverine-like conditions, and the lack of suitable habitat for juvenile coho salmon to hold in may result in the increased dam passage that was observed during our study. McMahon and Hartman (1989) found that most coho salmon juveniles in their study emigrated from test channels during simulated freshets unless complex habitat conditions (i.e., low velocity, shade, woody debris) were present. Similarly, Tschaplinski and Hartman (1983) found that stream sections containing log jams, undercut banks, and debris retained high numbers of juvenile coho salmon during winter freshets compared with stream sections where these habitat characteristics were absent. Results from these studies suggest that dam passage by reservoir-released juvenile coho salmon in the upper Cowlitz River would exceed those of river-released fish, and our study confirmed this. It is possible that reservoir-released fish passed Cowlitz Falls Dam at a higher rate than river-released fish because of their proximity to the dam (river release sites >30 rkm from Cowlitz Falls Dam; reservoir release site $= 3.1$ rkm from Cowlitz Falls Dam). Although we cannot rule out the effects of this factor on our results, it seems apparent that coho salmon juveniles in Lake Scanewa are susceptible to winter dam passage. Based on these findings it is also clear that future evaluations will be required to understand how the juvenile coho salmon population is distributed between river and reservoir environments in the upper Cowlitz River basin. This information will be essential for understanding population loss during winter months.

We found that substantial numbers of tagged juvenile coho salmon responded to winter streamflow increases by moving downstream and passing Cowlitz Falls Dam, and this behavior suggests that winter dam passage will be exacerbated during coming decades if climate change predictions are realized. At the beginning of the study we assumed that large numbers of fish were passing Cowlitz Falls Dam during drawdowns, when river flows were >425 m^3/s. We were surprised to find that the largest proportion of tagged fish passed during 2009, the only winter during our study when a drawdown did not occur. However, river flows peaked multiple times during 2009 and cumulative passage during that winter resulted in an estimated passage of 48% of the reservoir-released fish (Figure 4; Table 2). This observation supports our findings that flow increases lead to increased risk of dam passage, and large streamflow increases that occur occasionally may result in lower dam passage than moderate streamflow increases that occur frequently. The effects of flow-related winter passage by juvenile coho salmon are evident if we consider a simplistic example in which a population of 100 juvenile coho salmon are residing upstream of Cowlitz Falls Dam during winter months under the following two scenarios: (1) daily streamflows average 28.3 m^3/s, representing current, cool winter conditions; and (2) daily streamflows average 84.9 m^3/s, representing predicted future, warm winter conditions. We assumed that the risk of passing Cowlitz Falls Dam would increase by 38% for every 28.3 m^3/s increase in streamflow, which is the 4-year average of risk estimates that we observed during our study. If 25 of the juvenile coho salmon pass Cowlitz Falls Dam under the first scenario, we would expect to see 44 juvenile coho salmon pass under the second climate change scenario. This finding indicates that substantial dam passage could occur during winter months in coming decades, which could result in additional losses to the anadromous coho salmon population of the upper Cowlitz River.

Although these data were collected in the Cowlitz River basin, the findings from this study have implications throughout the entire range of Pacific salmon *Oncorhynchus* spp. Juvenile salmonid collection facilities are being developed, are under construction, or are in operation at hydroelectric facilities on midsized rivers (Baker River, Cowlitz River, Lewis River, Willamette River, etc.) located along the western slope of the Cascade Mountains, in Washington and Oregon, and are being considered in California. These facilities comprise a common strategy throughout the region for restoring anadromous salmon populations in watersheds where volitional access is no longer present due to dam construction, but important habitat is available upstream of dams. Our data suggest that out-migration timing at these locations will change in the coming decades. If this occurs, contemporary collection efforts that focus on smolt-sized fish that out-migrate during May–August will likely have to adapt to target parr-sized fish that move downstream during January–April. Given these concerns, future habitat restoration efforts upstream of Cowlitz Falls Dam, and at other locations, may be useful for increasing winter habitat for juvenile salmonids to reduce winter flow-related passage.

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A Standard Operating Procedure for the Surgical Implantation of Transmitters in Juvenile Salmonids

Open-File Report 2012–1267

U.S. Department of the Interior
U.S. Geological Survey

Cover:

Upper row, Left: A variety of electronic transmitters that are commonly implanted into juvenile salmon.
Center: Juvenile salmon.
Right: Releasing tagged juvenile salmon near The Dalles Dam.

Lower row, Left: Inserting an acoustic transmitter into the body cavity of a juvenile salmon.
Center: Two simple interrupted sutures used to close an incision.
Right: A radio transmitter being implanted into a juvenile salmon.

All images are from Columbia River Research Laboratory.

A Standard Operating Procedure for the Surgical Implantation of Transmitters in Juvenile Salmonids

By T.L. Liedtke, J.W. Beeman, and L.P. Gee

Open-File Report 2012–1267

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
°F=(1.8×°C)+32.

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

A Standard Operating Procedure for the Surgical Implantation of Transmitters in Juvenile Salmonids

By T.L. Liedtke, J.W. Beeman, and L.P. Gee

Introduction

Biotelemetry is a useful tool to monitor the movements of animals and is widely applied in fisheries research. Radio or acoustic technology can be used, depending on the study design and the environmental conditions in the study area. A broad definition of telemetry also includes the use of Passive Integrated Transponder (PIT) tags, either separately or with a radio or acoustic transmitter. To use telemetry, fish must be equipped with a transmitter. Although there are several attachment procedures available, surgical implantation of transmitters in the abdominal cavity is recognized as the best technique for long-term telemetry studies in general (Stasko and Pincock, 1977; Winter, 1996; Jepsen, 2003), and specifically for juvenile salmonids, *Oncorhynchus spp.* (Adams and others, 1998a, 1998b; Martinelli and others, 1998; Hall and others, 2009). Studies that use telemetry assume that the processes by which the animals are captured, handled, and tagged, as well as the act of carrying the transmitter, will have minimal effect on their behavior and performance. This assumption, commonly stated as a lack of transmitter effects, must be valid if telemetry studies are to describe accurately the movements and behavior of an entire population of interest, rather than the subset of that population that carries transmitters.

This document describes a standard operating procedure (SOP) for surgical implantation of radio or acoustic transmitters in juvenile salmonids. The procedures were developed from a broad base of published information, laboratory experiments, and practical experience in tagging thousands of fish for numerous studies of juvenile salmon movements near Columbia River and Snake River hydroelectric dams. Staff from the Western Fisheries Research Center's Columbia River Research Laboratory (CRRL) frequently have used telemetry studies to evaluate new structures or operations at hydroelectric dams in the Columbia River Basin, and these evaluations typically require large numbers of tagged fish. For example, a study conducted at the dams on the Columbia River and funded by the U.S. Army Corps of Engineers required tagging and monitoring of 40,000 juvenile salmon during a 3-month migration period (Counihan and others, 2006a, 2006b; Perry and others, 2006). To meet the demands of such a large study, the authors and CRRL staff refined the SOP to increase efficiency in the tagging process while maintaining high standards of fish care. The SOP has been used in laboratory and field settings for more than 15 years, and consistently has produced low mortality rates (<1 percent) and transmitter loss rates (<0.01 percent) in the 24–36 hours after tagging.

In addition to describing the detailed surgical procedures required for transmitter implantation, this document provides guidance on fish collection, handling and holding, and the release of tagged fish. Although often overlooked, or at least underemphasized, these processes can have a large impact on the outcome of the tagging procedure. Stress associated with the individual steps in handling and tagging can be cumulative and lethal (Maule and others, 1988; Wedemeyer and others, 1990; Portz and others, 2006), so the goal is to provide the best possible fish care at every step in order to manage the overall effect on study fish.

Purpose and Applicability

The purpose of this document is to provide guidelines and procedures for the surgical implantation of radio or acoustic transmitters into juvenile salmonids. Guidelines for fish collection, handling, holding, and release are included to reduce stressors to fish and to increase the likelihood of a positive surgical outcome. The clear, specific guidance in the SOP, when monitored and enforced, keeps the application of procedures consistent across studies, personnel, and time, and increases opportunity for comparisons across telemetry studies using similar procedures.

The SOP can be applied in studies that surgically implant radio or acoustic transmitters in the body cavity of juvenile salmonids of both natural and hatchery origin. Radio and acoustic transmitters require slightly different implantation procedures because radio transmitters commonly have an external antenna, whereas acoustic transmitters do not have an external antenna. The SOP also can be used for the surgical implantation of PIT tags, either separately or with a radio or acoustic transmitter.

Although the procedures were developed and refined in the Columbia River Basin for large-scale telemetry studies, the guiding principles behind the procedures can be applied readily to other studies and locations. The SOP has been adapted for work in the Sacramento and San Joaquin Rivers in California (San Joaquin River Group Authority, 2008, 2009; California Department of Water Resources, 2012) and in the marine waters of Puget Sound, Washington. Although the SOP is intended to guide the surgical implantation of transmitters in juvenile salmon, it can be modified and expanded to provide guidance for other species or life stages. Many elements of the procedures generally are applicable to the handling and tagging of all fish.

Guiding Principles

Several guiding principles should be applied to studies using telemetry to minimize transmitter and handling effects. Entities that use telemetry invest considerable amounts of money into monitoring equipment, transmitters, surgical equipment, and staff time in order to address their research questions. Each individual tagged fish is a significant investment; therefore, efforts should be made to ensure that the potential for handling and transmitter effects are controlled, and that tagged fish can reliably represent the untagged population.

Develop Surgical Proficiency

Personnel performing any transmitter attachment procedure (hereafter referred to as taggers) must be proficient in order to minimize stress to fish (Cooke and others, 2003; Wagner and Cooke, 2005). Surgical implantation generally is more challenging than other attachment methods (that is, external or gastric), and requires a level of manual dexterity that may not be possessed by all taggers. Practice sessions are essential to positive tagging outcomes (Smith and others, 2009), and ideally, taggers will practice on fish of the same species, size, and life stage that will be used for the study. The use of surrogates may be required when the study fish are threatened, endangered, or otherwise difficult to obtain. Surrogates should provide the best possible match to the target species and size. In cases where proficient taggers have been inactive for a period, or when a new species or size class is being studied, taggers should participate in a refresher session to reinforce proper technique.

A tagger training program should include a knowledgeable mentor, the opportunity to practice on model systems, and a continuous evaluation and feedback loop. New taggers must have access to a proficient mentor with broad knowledge of fish handling and tagging procedures. Many veterinarians have specialized training in fish medicine, making consultation and collaboration powerful approaches to developing or refining transmitter implantation techniques (Wagner and Cooke, 2005; Harms and

Lewbart, 2011). The use of model systems, rather than live fish, can be helpful during the initial tagging practice sessions. For example, when learning suturing techniques, taggers can practice on bananas, orange peels, or artificial skin until surgical knots are tied correctly and consistently. Once proficient with the model system, taggers can advance to a more realistic surrogate, such as dead fish, where they can refine their skills without attention to fish handling. Once they have mastered basic tagging skills, trainees can advance to practice on live fish and the corresponding fish-handling requirements.

Mentors should evaluate proficiency and provide feedback to taggers throughout the training program. Tagging proficiency can be evaluated by holding practice fish in tanks and monitoring short-term (days to weeks) survival, transmitter loss, and the external and internal condition of the fish. Necropsies should be conducted post-tagging, with both the tagger and the mentor present to visualize transmitter position within the body cavity, incision apposition, and any potential effects on internal organs. Photographs of practice fish can help document proficiency, and video recordings can be useful in documenting tagging methods and as a learning tool for future trainees. Another measure of tagging proficiency that is easily quantifiable and linked with the stress response of fish is the length of time needed to complete a surgical procedure. Taggers should strive to become competent and efficient in order to limit fish exposure to anesthesia and handling. Speed, however, should not compromise proper execution of the procedure. The time required to complete a surgery should be monitored, and the time required by a trainee can be compared to the time required by an experienced tagger as one indicator of proficiency.

Many resources are available to researchers who want to develop surgical proficiency. Surgery text books (for example, Slatter, 2003) should be reviewed for the principles underlying suture material and surgical knots. We recommend that researchers consult an experienced veterinarian or medical professional for specific instruction in surgical knot-tying techniques. Additionally, numerous medical videos are available on the Internet that illustrate basic surgical knots.

Anticipate and Manage Tagger Effects

When multiple taggers are used, the experimental design should anticipate some level of "tagger effects," with potentially different outcomes for individual taggers. Even with experienced and proficient taggers, there are likely to be some minor, short-term differences, although these are often difficult to assess. The probability that fish tagged by different taggers will have different short-term mortality rates (the easiest response to measure) is low, but mortality is only a crude indicator of tagging success (Mulcahy, 2003; Jepsen and others, 2008). Differences in stress response or wound healing among fish tagged by different taggers are more likely to occur, but are more difficult to detect. For example, response differences may arise from fish being held out of water for varying lengths of time because of differences in surgical procedure time.

Anticipating tagger effects is the best approach to managing them. The most simplistic approach is to use a single, experienced tagger throughout the study. If a single tagger is not sufficient, minimize the number of taggers and train them all using the same SOP and training program. When multiple taggers are used, the schedule should be configured so each tagger contributes equally to all study treatment groups. Researchers should be prepared to quantitatively assess tagger effects by formally evaluating response metrics by tagger (for example, see Beeman and others, 2011).

Reduce and Refine Fish Handling

Researchers should use the utmost care when handling fish before, during, and after the surgical procedure to minimize the stress to fish and to control the risk of infection. Stress due to handling may reduce survival and the capacity to handle additional stressors such as tagging (Kelsh and Shields, 1996). Fish will be more vulnerable to infection as a result of any handling procedure that interrupts the outer mucus layer (Harms, 2005), including contact with nets, measuring, weighing, and transmitter implantation. The tagging operation should be designed to limit fish handling, and especially fish transfers, to control these risks. Study fish should be collected using the least destructive and least stressful method that is effective. Holding containers should have dark interiors and covers to minimize disturbance and fish loss due to jumping (Portz and others, 2006), and should be monitored to maintain appropriate water-quality. Dissolved oxygen (DO) concentrations should be maintained near saturation, and water temperature should be maintained within a few degrees of the water where the fish were collected and will be released (Kelsh and Shields, 1996). Differences in water temperature larger than several degrees Celsius (°C) can be managed by mixing the source and destination water to produce a gradual temperature change (Stickney and Kohler, 1990; Kelsh and Shields, 1996). Researchers should use extreme caution when handling and tagging juvenile salmonids at high water temperatures (about 18–20°C).

Tagging operations should be designed to reduce the number of fish transfers in an effort to control the stress to study fish. Typically several transfers are involved as fish are moved from the pre-tagging location, through the anesthesia process, into a post-tagging holding location, and finally to the release location. Working within the logistical constraints for the study and the tagging location, researchers should establish the fish movement path with the fewest transfers. For example, the source of fish to be tagged should be near the tagging operation so that fish can be netted directly into anesthesia. As fish are tagged, they can be placed in a portable holding container, such as a bucket, that can then be transported to the release location without an additional transfer.

Standard handling techniques should be refined to reduce stress to fish and to avoid damage to the mucus layer. Where possible, use a crowding device to aggregate fish gently in a container prior to removal rather than pursuing them aggressively with a net. Such devices can be quickly fabricated from plastic pipe and mesh fabric or more rigid netting. Reducing the water level in the container is another approach to crowding. We recommend use of a sanctuary net, which is designed to transfer fish in water, rather than removing them from water in a standard net. The use of water-to-water transfers has been shown to reduce handling stress (Matthews and others, 1986; Flagg and Harrell, 1990). Although sanctuary nets can be difficult to purchase in appropriate sizes for juvenile salmonids, they are relatively easy to fabricate using standard nets and water-resistant fabric.

Practice Aseptic Techniques

When surgeries are performed on animals in a veterinary hospital, the goal of aseptic technique is to create a surgical environment that is completely sterile. This level of asepsis is difficult to achieve in field settings, and the aquatic environment poses additional challenges to keeping the equipment, incision site, and transmitter free of contamination. Even in controlled laboratory settings, strictly sterile procedures cannot be accomplished on fish (American Fisheries Society, 2004; Harms, 2005). Despite these challenges, steps should be taken to reduce the risk of infection when invasive procedures such as surgery are performed on fish.

Medical-grade exam gloves should be worn by the tagger and any personnel handling fish, instruments, or transmitters. The primary function of gloves is to reduce the transmission of potential pathogens from fish to fish, but they also protect the tagger from anesthetics, disinfectants, and potential waterborne pathogens. Ideally, sterile gloves would be used and changed between fish; however, it is virtually impossible to avoid contamination from either the fish or the water source during a procedure. At a minimum, clean gloves should be worn and gloves should be changed regularly.

Equipment, such as tanks, containers, nets, air stones, and the tagging platform, should be disinfected between successive tagging sessions. A thorough drying period is a useful addition to chemical disinfection for equipment (Harms, 2005). Because the water source is the route of pathogen exchange, the environment should be kept as clean and dry as possible during a given tagging session.

Irrigation of the gills during surgery presents a risk of contamination of the peritoneal cavity. The water used for irrigation likely is pathogen-rich, so steps must be taken to prevent this water from contaminating the incision area or entering the body cavity (Harms, 2005). This need can be met using a tagging platform that holds fish in a reclined position so that the incision is higher than the head. Taggers must monitor the irrigation system to ensure that water does not overflow the gills and enter the body cavity through the incision.

Surgical instruments should be sterilized prior to each individual procedure to reduce infection and tissue reaction (Marty and Summerfelt, 1986; Moore and others, 1990; Mulcahy, 2003; Harms, 2005). Small autoclaves or pressure cookers can be used for this purpose. When tagging large numbers of fish in a field setting, there may not be enough instruments available to allow for prior sterilization and packaging of instruments for each procedure. Under these circumstances, instruments should initially be sterile and then should be disinfected and rinsed between procedures (Lucas, 1989; Lacroix and others, 2004). This SOP requires that chlorhexidine diacetate (hereafter referred to as chlorhexidine) be used to disinfect instruments between procedures, and several sets of instruments are used in rotation to ensure sufficient contact time (10 minutes) for full efficacy. If instruments are not rotated, sequential procedures must be separated by enough time to allow for the full contact time in the disinfectant. Following disinfection, instruments must be rinsed well with distilled or deionized water to remove potentially toxic chemical residue. Instrument sterilization or disinfection procedures must be strictly enforced because fish-to-fish transmission of pathogens has been documented (Elliott and Pascho, 2001).

The transmitter deserves special care in regard to aseptic technique because it will remain in contact with the tissues of the fish for extended periods. Ideally, transmitters would be sterilized prior to implantation, but this expectation is difficult to meet because the most common sterilization technique uses heat, which can damage transmitter components. Alternative approaches, such as gas and chemical sterilization, are available, but typically are limited to controlled laboratory settings because of their potentially hazardous nature. A veterinary or medical office may be able to pre-sterilize transmitters and package them for transport into the field (Mulcahy, 2003). A common approach is to disinfect transmitters using the procedures established for disinfecting instruments. Extra care should be taken to ensure adequate contact time with the disinfectant and thorough rinsing of residues from the transmitter. Researchers also should ensure that the disinfectant will not damage the transmitter coating. After transmitters have been disinfected or sterilized, they should be handled only with clean instruments or gloved hands, avoiding contact with any source water or potentially contaminated surfaces until they are implanted.

Manage Anesthesia

Tricaine methanesulfonate (MS-222®), also known as Finquel®, is the only chemical anesthetic approved by the U.S. Food and Drug Administration for use in fish at the time of this writing (2012). Ideally, anesthetics should have a rapid induction time (about 3 minutes), a short recovery time (<5 minutes), and no persistent effects on fish physiology, allowing for immediate release of fish (Marking and Meyer, 1985; Summerfelt and Smith, 1990). MS-222® does not meet the definition of an ideal anesthetic because it requires a 21-day withdrawal period for fish that may be captured and used as food (Schnick, 2006). Fisheries researchers clearly need additional options in the choice of anesthetics, and efforts are underway to evaluate several candidate chemicals. MS-222® was selected for use in this SOP because of its proven efficacy, and the 21-day withdrawal period is not limiting since humans are not likely to consume juvenile salmon.

Because of the hazardous nature of MS-222®, the stock solution should be prepared in a laboratory setting, following the guidance on the package label and the material safety data sheet. Stock solutions should be kept in amber bottles because the material will degrade in sunlight. To ensure full efficacy, avoid exposing the solution to high temperatures and regularly replace the stock solution.

The effectiveness of MS-222® as an anesthetic varies with working concentration, water temperature, species, and individual fish response. Adjustments to the anesthesia concentration should be made based on fish response and water conditions at the tagging location. The depth of anesthesia can be recognized by monitoring a series of physiological changes, beginning with a loss of reactivity to stimuli (stage 1), and progressing to a total loss of equilibrium (stage 4), loss of all reflex activity (stage 5), and eventual medullary collapse (stage 6) (Summerfelt and Smith, 1990). Surgical implantation procedures require stages 4–5 anesthesia and regular monitoring of the ventilation rate to prevent respiratory failure (stage 6). The concentration of the anesthesia should not be any higher than needed to achieve an induction time of about 3 minutes because the risk of mortality is inversely related to induction time (Summerfelt and Smith, 1990). Total exposure time also is an important consideration because prolonged exposure can lead to mortality (Mulford, 1984; Summerfelt and Smith, 1990).

A stock solution of 100 mg of MS-222®/mL water has a pH of about 2 in deionized water (Summerfelt and Smith, 1990), but may be less acidic in other water sources, depending on their buffering capacity. To counteract the low pH and to minimize the corresponding physiological effects on fish, the addition of sodium bicarbonate is recommended to buffer the anesthetic solution to a pH of about 7 (Wedemeyer, 1970; Soivio and others, 1977; Harms, 2005). Separate stock solutions should be prepared for MS-222® and sodium bicarbonate because they form a white, oily precipitate when they are mixed at high concentrations.

Exposure to MS-222® induces immediate physiological changes (Wedemeyer, 1970; Houston and others, 1971; Strange and Schreck, 1978), some of which can be long-lasting (Soivio and others, 1977). A state of asphyxia develops because of reductions in the heart rate and gill ventilation rate, and is compounded by swelling of erythrocytes, which further restricts circulation (Soivio and others, 1977). The oxygen debt that is incurred during exposure to anesthesia must be overcome during the recovery period. To facilitate recovery, fish should be placed in water with a high DO concentration immediately following the surgical procedure.

To maintain effective and consistent anesthesia throughout a tagging session, the anesthesia bath must be replaced regularly. In a typical field setting, a single anesthesia container is used to anesthetize a number of fish. Each fish added to the container removes part of the oxygen and anesthetic and adds mucus, carbon dioxide, and ammonia to the solution. The transfer of fish to the container typically also involves the addition of at least nominal amounts of water, diluting the working concentration of the anesthetic. Water temperature in the isolated anesthesia container typically will increase with time, influencing fish response. Regularly changing the anesthesia bath will minimize these variables and ensure consistent application.

Construct an Effective Tagging Station

A tagging station should include a tagging platform to hold fish with the ventral surface exposed and some space to allow easy access to equipment and supplies (for example, transmitters and instruments). Plastic, closed cell foam, or other materials that do not absorb significant amounts of water should be used in the construction of the tagging station so that it can be effectively disinfected. Any surfaces that will directly contact fish should be smooth and kept moist to prevent damage to the skin, scales, or mucus layer of the fish. Similarly, surfaces designed to hold equipment and supplies should be kept dry to prevent exposure to any waterborne pathogens in the water source. Designing the station to hold the fish in a reclined position, with the head at the lowest point, allows for good irrigation while controlling the risk of irrigation water entering the body cavity through the incision. Good lighting is another consideration. Adjusting the height of the tagging surface so that the tagger is positioned comfortably will improve the efficiency of the tagging process. Example tagging platforms include an acrylic glass (or PLEXIGLAS®) frame (fig. 1) or a block of closed-cell foam modified to hold a fish (fig. 2).

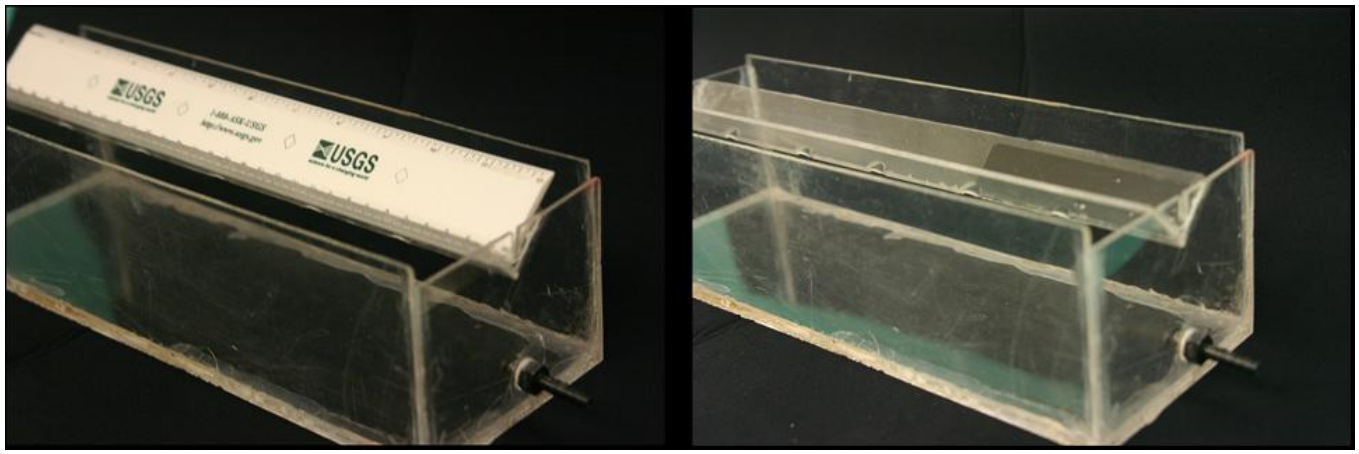

Figure 1. An example surgical platform constructed of acrylic glass.

Figure 2. An example surgical platform consisting of closed-cell foam with sections carved to support the fish in proper surgical orientation.

Fish must have their gills irrigated during the surgery, so the tagging station must accommodate either gravity-feed irrigation or a pump system. This SOP recommends the use of a gravity feed irrigation system due to its ease of operation (no power is needed) and high reliability (fig. 3). The irrigation system includes two water containers (buckets or carboys): one labeled "sedation" that delivers a light dose of anesthetic, and one that delivers fresh water. The containers are positioned side-by-side on an elevated platform or shelf over the tagging platform. The containers should be about 45–60 cm above the tagging platform to provide adequate flow. Each container has tubing that links the container to a junction connector, which joins the two tubing lines (fig. 3). Following the junction, there is a single line of tubing that enters the fish's mouth to provide irrigation. Each container has a valve that allows the tagger to control the rate of flow through the tubing. This system allows the tagger to provide fish with sedation water, fresh water, or a combination of sedation and fresh water. An example tagging station, including the gravity feed irrigation system, is shown in figure 4.

Figure 3. An example gravity feed irrigation system. One container holds fresh water and one container holds a light dose (sedation) of MS-222®. Both containers have valves to control flow. The tubing from each container is joined, and a single line of tubing continues to the surgery platform.

Figure 4. An example tagging station with gravity feed irrigation system, instrument disinfection and rinse trays, and surgical platform.

Procedures

Because of the tight linkages between fish handling and surgical outcome, the SOP outlines procedures prior to and following the implantation of the transmitter. The procedures begin with fish collection and continue until tagged fish are released for monitoring. Although procedures may vary somewhat between individual studies, based on study design and logistics, the general workflow should be similar. Workflow steps generally include: (1) fish collection or acquisition, (2) possible transport to the tagging location, (3) a holding period before tagging (pre-tag holding), (4) an anesthesia process, (5) morphometric data collection and review of fish condition, (6) implantation of the transmitter, (7) short-term recovery, (8) a holding period following tagging (post-tag holding), (9) possible transport to a release location, and (10) release of tagged fish for monitoring. For the purposes of this SOP, 19-L buckets are used to hold fish, beginning with the anesthesia process and continuing at least through short-term recovery, and most commonly through the release of tagged fish.

To aid in study planning, a detailed list of suggested materials is provided in appendix A. Abbreviated, step-by-step procedures are provided in appendix B. The narrative procedures below provide rationale and background for the critical SOP steps to allow the researcher to understand the principles behind the procedures.

Fish Collection, Holding, and Transport

Collection: Study fish should be collected using the least destructive and least stressful method that is effective. Seining, trapping, or other collection techniques may be used or fish can be obtained directly from a rearing facility, such as a hatchery or research laboratory. Dams or other barriers often have fish collection facilities that can provide access to fish for studies. When selecting a collection method, consider any biases inherent in the technique. For example, a given trapping method might be biased toward catching primarily small fish, which will have the effect of restricting the size range of the fish that will be tagged and monitored. Fish that obviously are injured, diseased, or excessively burdened by parasites should not be retained for tagging. If fish are collected by a group other than the group performing the surgeries, coordination of the fish-handling details will be critical. Groups should agree on the most appropriate handling processes and perhaps generate an SOP to ensure consistent procedures throughout the study.

Holding periods: Following collection or acquisition, fish should be held for 12–36 hours (ideally 24 hours) prior to tagging. The pre-tag holding period for a group of fish begins once fish are in the care of the researcher and ends when surgical procedures begin. If fish must be transported from the collection location to the tagging location, the pre-tag holding time begins when fish arrive at the tagging location. Following tagging, fish should be held 18–36 hours (optimally about 24 hours). The post-tag holding period begins when the last fish for a given tagging session is placed in the post-tag holding container. The holding period ends when fish are removed from the post-tag holding container in preparation for release.

The rationale for pre-tag and post-tag holding is to allow fish to recover from acute stressors, such as collection, transport, and tagging (Stickney and Kohler, 1990; Kelsh and Shields, 1996). A combination of stressors (for example, collection and tagging) can be lethal even if fish can tolerate them independently (Portz and others, 2006), so the stressors should be separated in time (Stickney and Kohler, 1990). Although the duration and severity of the stressor can produce significant variability in stress responses, such as plasma cortisol (Pickering and others, 1982), levels frequently return to baseline conditions within about 24 hours following an acute stressor, such as handling (Strange and Schreck, 1978; Jepsen and others, 2001). An additional rationale for the pre-tag holding period is to restrict access to food so that fish enter a post-absorptive state prior to the invasive procedure (Summerfelt and Smith, 1990).

Although there are compelling reasons to hold fish both before and after tagging, these reasons must be balanced against the risk of additional stress to fish, particularly fish in migratory life stages, such as juvenile salmonids. Actively migrating juvenile salmonids may incur high mortality when held for extended periods, even without the stress of a surgical procedure. The pre-tag and post-tag holding periods must be considered in light of the entire duration of holding, including the time needed to complete the tagging. The total amount of time that juvenile salmon are held captive should be limited to about 48 hours. The recommended holding periods are defined as ranges so that adjustments can be made based on individual study objectives or logistical constraints, while accommodating an overall limited holding period of about 48 hours.

Holding conditions: Fish should be held at low stocking densities in dark containers with lids. Container lids reduce visual disturbances, such as bright sunlight or passing shadows, and reduce the risk of fish loss from jumping. If multiple species of fish are collected, hold the species in separate but comparable containers. This separation is especially important when there are large size differences between the species that could induce a stress response in the smaller fish. Pre-tag holding densities should not exceed 20 g of fish per L of water, and post-tag holding densities should not exceed 10 g of fish per L of water (see appendix B for formula to calculate holding density). The recommended pre-tag and post-tag holding densities are conservative relative to standard hatchery transportation practices for salmonids, which vary but range from 60 to 240 g/L (Piper and others, 1982). The post-tag density is more restrictive than the pre-tag density for several reasons. First, there is commonly a need to transport untagged fish, and the recommended pre-tag density allows some flexibility in transport options while controlling crowding stress. Second, the tagged fish reflect a significant financial and time investment, and will be the basis of the planned study, so a conservative approach is warranted to minimize potential crowding stress post-tagging.

Pre-tag and post-tag holding conditions should be configured to minimize fish transfers and to facilitate the release of tagged fish. An approach we commonly use to meet this challenge is to hold fish in small, portable containers so that the container can be moved as needed without direct handling of the fish. The small containers can be immersed in a larger container to maintain water-quality if water exchange is established between the containers and the tank (fig. 5). At the start of the tagging procedure, a transfer can be avoided if the holding of untagged fish is well designed. Specifically, untagged fish should be held close to the tagging location (or in a small tank or cooler that can be carried or moved on a dolly system to the tagging location), rather than holding fish in a distant, stationary container and then netting small groups of fish into a container closer to the tagging location for ease of processing. If large numbers of fish need to be held, add additional small containers rather than move to a larger container so that portability is maintained. Alternately, the anesthesia container can be carried from the distant holding tank to the tagging location.

Figure 5. Holding tank with immersed 19-liter perforated recovery containers. The perforations allow water exchange between the containers and the larger volume of the tank.

At the end of the tagging procedure, fish ideally would be held in small containers that can be used for recovery, post-tag holding, transport (if needed), and release. At a minimum, the post-tag holding should be configured so that tagged fish can be released without a net transfer. When there are limited options for releasing tagged fish, use water-to-water transfer techniques such as pouring fish out of a container or moving them through a pipe.

In addition to the general holding recommendations outlined above, both pre- and post-tag holding have specialized requirements: pre-tag fish should be monitored for behavior and condition, and post-tag fish must have access to air. Monitor untagged fish for significant scale loss, wounds, or atypical behavior, such as compromised swimming ability that may result from stress or injury from collection or transport. The discovery of moribund or dead fish during the pre-tag holding period is an ominous sign and likely will lead to increased post-tagging mortality. Following tagging, and throughout the post-tag holding period and any needed transport, salmonids (physostomes) need access to the air-water interface in order to regain neutral buoyancy. Attachment of the transmitter to fish induces negative buoyancy, which could cause altered depth distribution (resting on the bottom) or increased metabolic requirements due to increased fin use or faster swimming (Gallepp and Magnuson, 1972). Juvenile salmon can counteract the weight of the transmitter relatively quickly by gulping air and increasing the volume of the swim bladder, provided they have access to air. Fried and others (1976) reported that tagged Atlantic salmon smolts (*Salmo salar*) denied access to air after tagging were unable to regain neutral buoyancy, even after 24 hours. Alternately, tagged fish provided access to the air-water interface were able to adjust their buoyancy within 6 hours (Fried and others, 1976). Handling can cause fish to expel air, which changes their buoyancy (Harvey and others, 1968), so gentle handling in all aspects of fish collection and tagging should be emphasized.

Water-quality during holding: Water-quality must be monitored and maintained in all containers used to hold fish. Water temperature, DO, and total dissolved gas (TDG) are the water-quality parameters that need to be maintained within the limits defined by the SOP. Ideally, fish would be held using the water where they were collected or into which they will be released. When the water source does not offer appropriate water-quality, the holding system must have the capacity to adjust to

meet the minimum SOP standards. If, during monitoring, a water-quality parameter is outside of the defined limits, action must be taken to bring the parameter into compliance with the SOP. A proactive monitoring approach generally will detect water-quality deviations before they become severe, and then they are more quickly remedied.

Water temperature is a critical consideration for tagging operations because of the risk of thermal stress. Fish should not be transferred between water sources until the difference in water temperature between the water sources is less than or equal to 2°C. Changes in water temperature exceeding 2°C require tempering to prevent thermal stress (Stickney and Kohler, 1990; Kelsch and Shields, 1996). Tempering is the process of mixing water sources to reach an intermediate temperature. Therefore, prior to exposing fish to a new water source, the temperature of the current water source and the new water source must be measured. If the temperature difference is less than or equal to 2°C, the transfer can be made without tempering. If the temperature difference is greater than 2°C, then water in the container holding fish should be tempered at a rate of 0.5°C per 15 minutes until the temperature difference between the two water sources is less than or equal to 2°C. New source water should be added in small amounts multiple times over 15 minutes to gradually change the temperature. Once the temperature difference between the two water sources is less than or equal to 2°C, fish can be transferred to the new water source. The same instrument should be used to measure both water sources to ensure accurate measurement of the temperature differential.

The DO concentration in all holding containers must be 80–130 percent saturation. Supplemental oxygen or aeration can be used, in combination with air diffusers, to supplement the DO in source water, as needed. We caution that supplemental systems are prone to supersaturating the water, so they must be carefully regulated. There is one exception to these DO saturation standards, and it will be addressed in the post-tagging recovery procedures.

Total dissolved gas should be monitored when it has the potential to be an issue, and should not exceed 110 percent saturation in water sources that contain fish. Water passed over high-head spillways can entrain gas and create dissolved gas supersaturation in the water. Exposure to TDG can be an acute or chronic source of stress, and can lead to gas bubble trauma (Mesa and others, 2000). Control of TDG levels typically is done with a degassing column where source water cascades over a collection of high-surface-area objects (for example, plastic rings) so that gas is released. Following passage through the degassing column, water has reduced TDG levels and can be supplied to holding containers.

Water-quality in small containers that lack exchange with a larger volume can quickly become compromised. Many such small containers are used during tagging procedures, including anesthesia and recovery containers. Taggers and support staff must be vigilant in monitoring water-quality and refreshing containers regularly to maintain SOP standards.

Fish transport: Study fish may need to be transported before tagging, after tagging, or both, depending on the study design and site logistics. Untagged fish might need to be moved from the collection point to the tagging location, and tagged fish might require transport from the tagging location to the point of release. Transport operations should be designed to minimize stress to fish and to maintain the water-quality parameters outlined in the SOP. Select a route of travel for the shortest and smoothest ride to minimize jarring. If water temperature rises significantly during transport, cooling actions, such as the addition of ice, may be required. Researchers should be aware that most commercially produced ice contains chlorine, which can be harmful to fish. We recommend freezing source water in small containers or double-bagging commercial ice to prevent the exchange of melted ice water with the source water.

Fish Size Criteria

The size of fish suitable for tagging depends on the size of the transmitter used. The smallest transmitter that will meet study objectives should be used because larger transmitters (in similar size fish) are more likely to induce transmitter effects. An estimate that can be used to evaluate the risk of transmitter effects is the transmitter-to-body weight ratio (or tag burden). The tag burden should be calculated using the combined mass of all transmitters or tags implanted. For example, in some cases, a PIT tag is implanted in combination with a radio or acoustic transmitter, and the tag burden should include the weight of both tags. The most commonly accepted limit for tag burden is from Winter (1996), who recommends that transmitter weight in air does not exceed 2 percent of fish body weight in air. Transmitter effects correlated with high tag burdens include increased mortality and transmitter expulsion (Moore and others, 1990; Lacroix and others, 2004; Jepsen and others, 2008; Hall and others, 2009), reduced growth (Jepsen and others, 2008), and reduced swimming performance (Adams and others, 1998b; Zale and others, 2005).

Transmitter sizes have been reduced through technological advances, but even small transmitters, in small fish, can exceed the 2-percent tag burden rule of thumb. In our experience, the performance of juvenile salmonids is not significantly compromised when tag burdens of up to 5 percent are used (Adams and others 1998a, 1998b; Martinelli and others, 1998; Perry and others, 2001). When the tag burden exceeds 5 percent, the transmitter effects are variable, and may occur more frequently (Hall and others 2009; Brown and others, 2010). Considering the consequences of an excessive tag burden, we have set a maximum acceptable tag burden limit of 5 percent for this SOP; smaller tag burdens are preferable.

In addition to tag burden, other considerations may influence the fish size criteria established, including transmitter volume, length, diameter, shape, density, and coating, any of which also may influence fish behavior and performance (Sakaris and others, 2005; Penne and others, 2007).

Tagging Preparations

Transmitters: Transmitters should be prepared for implantation by confirming their operation and disinfecting them. The transmitter characteristics (that is, frequency, pulse rate, and so on) should be confirmed to be as specified, as errors can occur when transmitters are labeled or packaged. A pre-tag water exposure trial can be useful in evaluating whether water intrusion into transmitters might cause failure. If PIT tags are used, their function also should be confirmed before tagging. Although PIT tags do not carry a battery, they can still fail to function when energized. If transmitters were not sterilized and pre-packaged for use during a tagging session, they must be disinfected before they are implanted. Transmitters should be immersed in chlorhexidine disinfectant solution for a minimum of 15 minutes. The disinfectant exposure time for transmitters is somewhat longer than instruments (15 minutes versus 10 minutes) to ensure efficacy because transmitters stay in close contact with tissue for extended time periods. Following disinfection, transmitters should be rinsed well with distilled or deionized water, and rinsing should continue until there are no signs of suds or residue. Once the transmitters have been disinfected, they must not be handled by anything other than clean instruments or clean, gloved hands.

Tag station and supplies: Prepare the tag station and tagging supplies. Set up the irrigation system (gravity feed or pump) by filling containers from the pre-tag holding or post-tag holding water source. Do not fill containers until the near the time of tagging to prevent the water temperature from fluctuating. A scale, weigh boat, and measuring board will be needed for tagging, and they should be clean at the start of the tagging session. Scales should be calibrated weekly to ensure accuracy. The

measuring board should be made of smooth material and be in good condition to avoid damage to the skin or scales of the fish. As further protection against skin damage, we placed 1–2 mL of a diluted mucous restorative solution on the weigh boat and the measuring board. We typically have used a mucous restorative product, Stress Coat® (manufactured by Aquarium Pharmaceuticals Inc.), but there are many similar products on the market and any of them will be effective in protecting fish (the SOP will continue to reference Stress Coat® for clarity). Working surfaces will need to be coated regularly with Stress Coat® throughout the tagging session to keep the working surfaces wet. Expendable tagging supplies (suture packets, gloves, and solutions) should be positioned near the tagging platform, on a dry surface, to prevent contamination.

Instruments: Surgical instruments should be organized into sets and arranged in individual disinfection trays. Each tagging session should begin with sterile surgical instruments. The standard procedure for instrument sterilization is to wrap instruments in a cloth and to secure them with autoclave tape, which is designed to indicate exposure to sterilization temperatures. At the start of a tagging session, the instrument packet should be examined to confirm that the autoclave tape has changed color (confirming exposure to the appropriate temperature), the packet should be unwrapped, and instruments should be sorted into sets. A complete set of instruments includes a needle driver, a microscalpel holder, and forceps (see appendix A for detailed descriptions of the instruments). Each instrument set should be placed into a separate disinfection container with chlorhexidine disinfectant solution. At least one rinse container, filled with distilled or deionized water also will be needed.

We recommend the use of micro scalpel blades rather than standard scalpel blades because of the thin body wall of juvenile salmonids and the risk of damage to organs when making the incision. Additionally, the micro scalpel blades can improve tagger confidence and, therefore, can improve tagging speed and efficiency. The blades can be purchased in a wide variety of blade angles and lengths, and the appropriate blade should be selected through experimentation and practice. Our preference is to use two lengths of micro scalpel with a 15-degree blade angle: a 3-mm blade for thin-bodied fish such as Chinook, sockeye, and coho, and a 5-mm blade for steelhead or other fish with a relatively thicker body wall. Micro scalpels are disposable and can be purchased as a complete unit (a full blade and handle) or as a stand-alone blade. The stand-alone blades are less expensive than the full scalpels, and are designed to be attached to a reusable stainless steel micro scalpel handle (fig. 6). Narratives in this SOP assume that micro scalpel holders will be used.

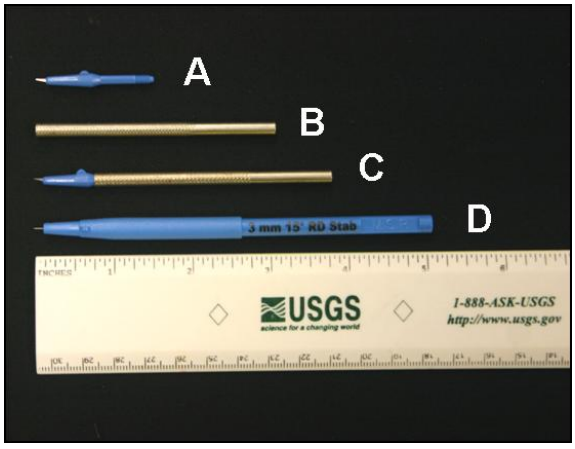

Figure 6. Example micro scalpel configurations. A disposable micro scalpel blade with a threaded end (A) can be combined with a stainless steel handle (B) to make a complete scalpel (C). A fully disposable micro scalpel (D) is an alternate configuration.

Surgical instrument sets will be rotated throughout the tagging session to ensure 10 minutes of exposure time in chlorhexidine. Depending on the speed of the implantation procedure, 3–6 sets of instruments may be needed for each tagging session. Alternately, successive surgeries can be separated by at least 10 minutes to allow for appropriate contact time with the disinfectant, or the appropriate number of instrument sets can be sterilized so that a single set is used for each fish. The later approach eliminates the need for instrument rotation and disinfection, but is more expensive.

Recovery containers: Recovery containers are used to hold fish immediately after surgery. Depending on the study design, these containers also may be used to hold fish during the post-tag holding period, and then be transported to the release location. This approach is ideal, and is the assumed approach in the SOP narratives because it limits the number of fish transfers. Our general procedure is to use commercially available 19-L buckets as recovery containers. We recommend selecting a color that is dark enough to restrict light penetration (that is, avoid white), but light enough to avoid absorbing significant amounts of solar radiation, leading to risk of elevated water temperatures (that is, avoid black); we use green. Lids that fit securely on these containers are readily available and are needed to minimize disturbance.

In this SOP, because the recovery containers are used for post-tag holding, we perforate the side of the containers (fig. 7) to allow water exchange when they are immersed in a tank. The containers are only perforated in their upper portion so that they retain a 7-L volume for fish when the containers are not immersed in a larger reservoir or tank.

Figure 7. A perforated 19-liter recovery container. The bottom of the container is not perforated so that the reservoir holds 7-liters of water.

Immediately prior to starting the tagging operation, fill several recovery containers with source water and position them near the tagging station. Avoid filling the containers too far in advance of the tagging operation in order to prevent loss of water-quality. We recommend a container labeling system that allows you to record the identity of each fish placed into each recovery container.

The DO concentration in recovery containers should be 120–150 percent saturation. This increased DO concentration is critical to assisting fish recovery from the oxygen debt incurred during anesthesia. The increased DO concentration can be established using an oxygen cylinder and a diffuser.

Careful monitoring is required to ensure that the high concentration of DO is maintained in all recovery containers until the containers are put in a tank for the post-tag holding period.

A recovery process must be established for source fish that are deemed unsuitable for tagging. Fish that may be rejected from tagging include fish that do not meet the size criteria, fish that respond poorly to anesthesia, or fish that experience excessive handling or stress during the tagging process (for example, fish may have been dropped or may have jumped out of containers). Rejected fish should be set aside during the tagging operation and then released or euthanized, as directed by study objectives and scientific permits. A 19-L container should be filled with approximately 10 L of source water, labeled "reject," covered with a lid, and positioned near the tagging operation. Because a single container will be used throughout the tagging operation, equip the container with a battery-operated aerator to ensure that DO concentrations remain near saturation.

Anesthetic: Anesthetic should be added to10-L of source water in a 19-L container. Effective working concentrations of MS-222® for juvenile salmonids are in the range of 50–90 mg/L. The exact anesthetic concentration for a tagging session should be based on the induction and recovery times of fish. Individual fish often have varied responses to MS-222®, so several fish must be monitored in order to evaluate the effectiveness of a given concentration at a given water temperature. Start with a low dose of anesthetic (for example, 60 mg/L), monitor fish response, and make adjustments as needed (see section, "Anesthetizing Fish"). Following the addition of anesthetic, add an equal amount of sodium bicarbonate to buffer the solution. Approximately 10 mL of diluted Stress Coat® should be added to the anesthesia container to protect and to restore the mucus layer of tagged fish. To provide guidance for future tagging sessions, record the anesthetic concentration used for each tagging session (or each group of fish within a session), along with the water temperature.

A maintenance dose of anesthetic is delivered to the fish's gills during surgery through a gravity feed or pump irrigation system. The fish initially is exposed to a relatively high dose of MS-222® to induce deep anesthesia. Following loss of equilibrium, the fish is moved to the surgery platform and the tagging procedure is started. During the procedure, the anesthesia is maintained by delivering a light dose of anesthetic through the irrigation system. Add 2 mL of MS-222® to the 10 L of source water in the gravity feed container, and add 2 mL of sodium bicarbonate solution as a buffer. Connect the tubing from the water source to the tagging station.

Communication among tagging personnel is critical during anesthesia preparation to ensure that anesthetic is not administered to the same container repeatedly or not at all. Our general procedure allows only one person to administer anesthetic in all needed containers to avoid confusion.

The solution in the anesthesia container should be changed periodically to minimize dilution of the working concentration of anesthesia (when fish are added) and to prevent water temperature changes of more than 2°C from the source water. Similarly, water in the gravity feed containers should be changed regularly to prevent water temperature differences and to ensure that irrigation can continue throughout a surgery without interruption. For general guidance, we recommend changing the anesthesia and gravity feed containers after 5–6 surgeries have been completed.

Anesthetizing Fish

Fish must be handled carefully and monitored closely while undergoing anesthesia. To ensure effective determination of the stage of anesthesia and the anesthesia exposure time, fish are processed individually. The process begins by carefully removing a fish from the pre-tag holding container and placing it directly in an anesthesia container. The container lid is immediately positioned and a timer is started to document exposure time. Induction time to stage 4–5 anesthesia will vary, but generally should be 2–4 minutes (average of about 3 minutes) if the appropriate concentration is used. Remove

the container lid after approximately 1 minute to monitor the stage of anesthesia. Fish that lose equilibrium within the first minute of exposure to anesthesia are assumed to be especially sensitive to the anesthetic and are not used for tagging. These fish should be removed from the anesthesia container and placed in the reject container for recovery and later handling. Typically, at 1 minute of exposure, fish are partially responsive to stimuli and oriented. Once the fish loses equilibrium (estimated to be about 3 minutes), its condition should be examined. Keep the fish submerged during the exam, and look for fungus, descaling, injury, signs of disease, or other factors that would eliminate the fish from consideration for tagging (for example, an existing mark or tag). Fish that are not acceptable for tagging should be transferred to the reject container. If fish are acceptable for tagging, they are removed from the anesthesia container using a gloved hand or a net, and transferred to the balance or measuring board. A timer is started when they are removed from the anesthesia container to record the amount of time they are held in air to complete the surgery. Fish that are exposed to MS-222® for 5 minutes or longer prior to surgery are not acceptable for tagging because of the risk of medullary collapse and mortality (Mulford, 1984; Summerfelt and Smith, 1990).

The need for a change in the anesthetic concentration should be determined based on the responses of the first few fish to undergo anesthesia. If after anesthetizing several fish, you find that they are losing equilibrium too quickly (<1 minute) or too slowly (>3.5–4 minutes), or if their recovery time is extensive (>10 minutes), the anesthesia concentration should be changed. We recommend small changes in the anesthesia concentration and continued monitoring. For example, if 60 mg/L MS-222® results in slow induction times, adjust the concentration to 65 mg/L and process another group of fish before changing to 70 mg/L. Recording the anesthesia concentration used during each tagging session can provide a reference for taggers when making a decision on the initial concentration to use.

More than one anesthesia container may be useful, depending on the scale and pace of the tagging operation. Add a container for each tagger used in the tagging session. In addition, taggers that perform the surgery rapidly may have greater efficiency with an additional anesthesia container, considering that each container holds a single fish. Adding anesthesia containers holds some risk in that any delay in removing the fish and starting the surgery (that is, the previous surgery is not yet complete) may result in the fish being rejected if the exposure time is more than 5 minutes.

Measuring Fish Size

Fish morphometrics are recorded after anesthesia and before tagging. First, fish are transferred from the anesthesia container to the measuring board. The fork length (FL) of the fish is the distance from the snout to the fork in the caudal fin, and it is measured to the nearest millimeter. Following the FL measurement, the fish is transferred to a weigh boat on a scale. Ensure that the scale is properly zeroed to account for the weight of the weigh boat before the fish is added. Measure and record fish weight to the nearest 0.1 g. Both the measuring board and the weigh boat should be kept moist to reduce damage to the fish's skin. This is accomplished through the regular addition of diluted Stress Coat® to these surfaces.

Fish transfers should be done by cradling fish in two gloved hands while moving quickly and carefully. Position the measuring board and scale close to the anesthesia container to facilitate the transfers. Although the fish are anesthetized, there is a risk that fish will be dropped. If a fish is dropped to the floor it must be rejected, but if a fish is dropped a shorter distance (for example, from the handler to the tagging platform), it should be tagged unless there is an obvious injury.

The tagger generally completes the morphometric data collection, but this will vary with the size of the tagging operation. We encourage taggers to weigh and measure the fish themselves, rather than use an assistant, because doing so gives taggers insights into the fish's condition and level of anesthesia.

The first full view of the fish comes on the measuring board, and this is a good time to complete a secondary inspection of fish condition before time is spent performing a surgery. If the measuring or weighing steps are difficult to complete because of the activity level of the fish, then the level of anesthesia is too shallow to proceed with surgery. To grant these benefits while maintaining efficiency in the tagging operation, we recommend using a dedicated data recorder. The tagger can then measure and weigh the fish and verbally relay the information to the data recorder. The recorder should then log the measurements on the datasheet and repeat the data back to the tagger to avoid any miscommunication and to allow for corrections.

Implantation of Transmitters

Irrigation: Immediately after the morphometric data have been collected, the fish should be placed on the surgery platform, ventral side up, and irrigation should be established. Place the irrigation tubing in the mouth of the fish and ensure consistent water flow over the gills. Inadequate flow through the irrigation system will cause the fish to become agitated, which can mimic a shallow depth of anesthesia. Excessive flow through the irrigation system can push overflow water toward the incision area and, therefore, should be avoided.

The irrigation system is configured to deliver sedation (a low concentration dose of anesthetic), freshwater, or a combination of both. Fish should receive sedation at the start of the surgery, but as the procedure continues, the sedation should be reduced to begin the recovery process. At approximately the mid-point of the surgery, the tagger should consider providing irrigation water that is an equal mix of sedation and freshwater. When the surgery is almost completed, we recommend switching the irrigation to fresh water. The tagger must monitor the level of anesthesia throughout the surgery and make adjustments to the irrigation flow to manage the level of anesthesia, as needed.

Incision: The incision is made near the pelvic girdle, using a micro scalpel. Locate the pelvic girdle of the fish by visual exam and palpation. In a ventral view, the pelvic girdle has a “V” or “U” shape, with the single point oriented anteriorly (toward the head) (fig. 8). The most anterior point of the pelvic girdle is the positioning guide for the location of the incision. Using a micro scalpel, make an incision about 3 mm anterior to the anterior point of the pelvic girdle, and about 3 mm away from and parallel to the mid-ventral line. Draw the blade toward the head of the fish as the incision is lengthened to avoid damaging the pelvic girdle.

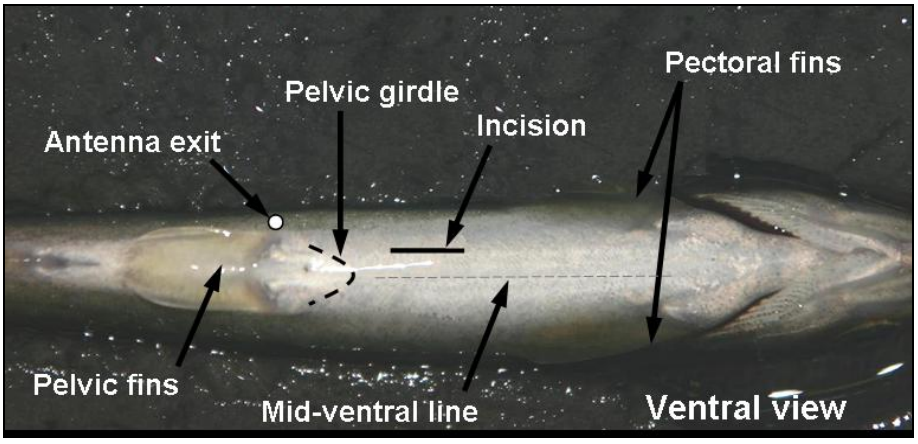

Figure 8. Ventral view of a juvenile salmon on a tagging platform. Locations of the pelvic girdle, incision, and antenna exit site are shown.

The incision should be only long enough to allow insertion of the transmitter without tearing the adjacent tissue. A good estimate of appropriate incision length is the diameter of the transmitter because the transmitter's length can be inserted through the incision after its head has entered the body. A short incision will minimize risk of organ damage, reduce the area vulnerable to infection, minimize tissue damage due to sutures, and reduce the time needed for closure. The incision should be deep enough to penetrate the peritoneum (the thin membrane separating the abdominal cavity from the musculature) without damaging internal organs. The spleen and pyloric caeca are often located near the incision, so the incision must be made carefully (see fig. 9).

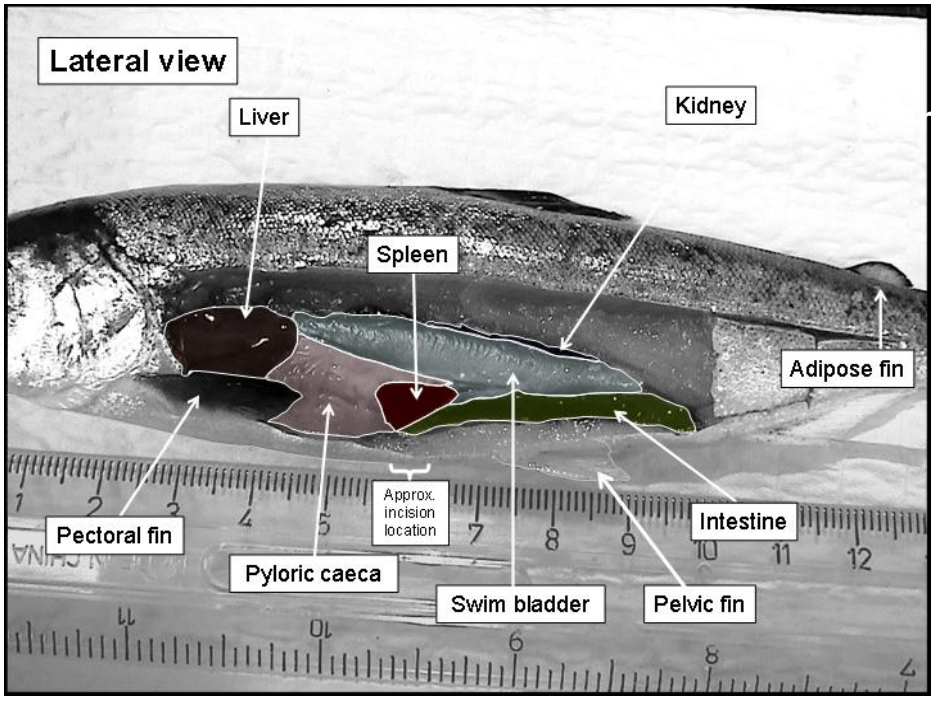

Figure 9. Lateral view of a juvenile salmon with location of structures and organs and approximate location of the surgical incision.

The optimal incision has clearly defined wound margins that will promote complete apposition and healing. Ideally, the incision will be completed with a single pass of the micro scalpel rather than the use of a "sawing" motion. If, after several fish, the tagger finds that multiple passes with the scalpel are needed to penetrate the peritoneum, a longer micro scalpel should be considered. Because a single micro scalpel will be used on several fish, it is important to monitor scalpel effectiveness to determine the timing for replacement. If the blade fails to move smoothly through the tissue or requires additional pressure to make the incision, it should be replaced.

After the incision has been made, use forceps to open the incision to quickly evaluate effectiveness and any potential organ damage. Insert the forceps into the incision to ensure that the peritoneum was penetrated along the full length of the incision and that there is clear access to the body cavity. Assess any potential organ damage by looking for bleeding. The spleen is located near the incision site and will cause significant bleeding if damaged. If the fish is bleeding excessively, it should not be implanted with a transmitter. The rejected fish may be sutured without a transmitter and recovered for release or sacrificed, depending on study design and scientific permit authority.

Transmitter antenna: If the transmitter has an antenna, an antenna exit site must be made in the lateral body wall so that the antenna can exit the body and trail behind the fish as it swims. We use a modified shielded needle technique (Ross and Kleiner, 1982) where a plastic catheter is positioned over the point of a needle so that it cannot damage organs as it is guided through the abdominal cavity. Following purchase, the catheter typically must be modified slightly to function as a shield. The non-tapered end of the catheter typically has a plastic tip with a connector, and this part of the catheter should be removed (fig. 10).

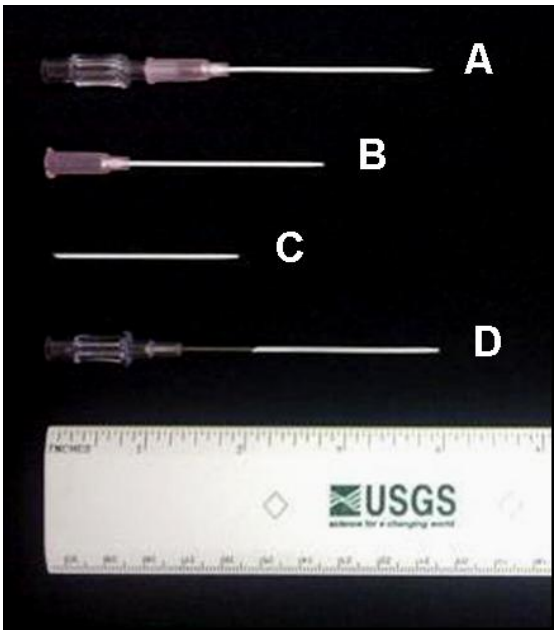

Figure 10. Example catheters used to perform a shielded needle technique to create an exit location for a trailing antenna on a radio transmitter. Image A shows the needle and catheter combination as it comes packaged from the manufacturer. The pink catheter tip (shown in B) must be removed (as in C) prior to use. The modified catheter is then positioned over the needle (D) as it will be used during surgical procedures.

Insert the shielded needle (catheter covering the tip of the needle) into the abdominal cavity through the incision and guide it to the antenna exit site. Hold the shielded needle between thumb and forefinger, keeping the needle tip covered by the catheter to protect the organs. The antenna exit site position should be even with the insertion of the pelvic fins on the longitudinal axis of the fish, and should be about 40 percent of the distance from the mid-ventral line to the lateral line on the vertical axis of the fish (fig. 11).

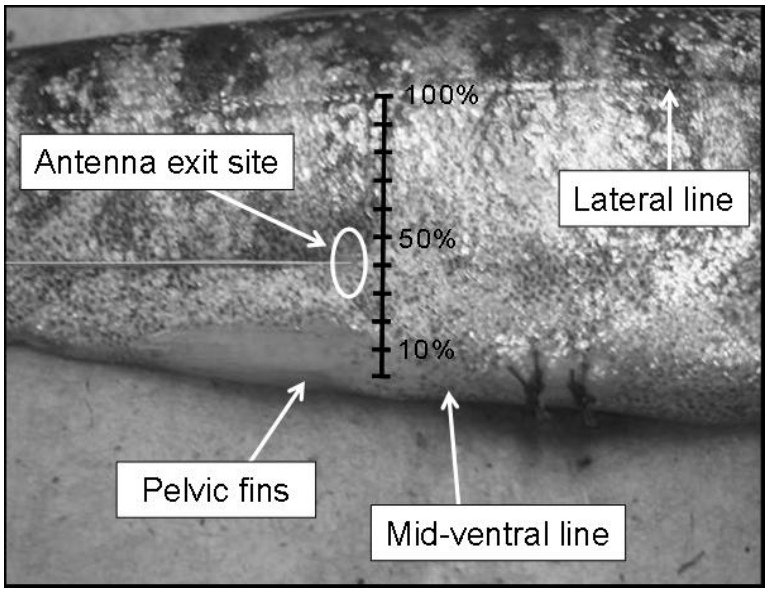

Figure 11. Lateral, external view of a juvenile salmon with the location for the antenna exit site.

After the catheter is positioned at the antenna exit site, draw back the catheter to expose the tip of the needle, and use the needle to puncture the body wall. The orientation of the tip of the needle is important for a clean puncture. The cutting edge of the needle should be facing away from the body wall (see fig. 12). If the cutting edge is against the body wall, the needle may scrape along the peritoneum rather than make a clean cut. Depending on the manufacturer, there is usually a tab or other mark on the shaft end of the needle that can be used as a landmark to indicate the orientation of the cutting edge. Prior to puncturing the body wall, be certain that the catheter is withdrawn far enough along the length of the needle to expose the tip. If the catheter is covering the tip of the needle during the puncture through the body wall, the puncture wound will be larger and more irregular and healing may be delayed. Following the puncture of the body wall, advance the catheter through the wound so that it is visible from the outside of the fish.

Hold the catheter in position, extending out the incision anteriorly and the exit wound posteriorly, and withdraw the needle. The catheter now forms a channel that allows the antenna to be threaded through the incision to the antenna exit site.

Route the transmitter antenna through the catheter, starting at the incision. Keep the body of the transmitter in your gloved hand to avoid it contacting the surface of the fish or other surfaces where it could become contaminated. After the transmitter antenna has exited the lateral body wall, pull the catheter out of the body wall and off the free (non-transmitter) end of the antenna.

There is no exact specification as to what diameter or length of catheter to use. The catheter with the smallest diameter that will accommodate the transmitter antenna is optimal because it will minimize the size of the exit wound. In our experience, a 20-gauge needle will accommodate most transmitter antennas. The length of the needle may be limiting, however, based on fish length. The needle and catheter need to be long enough to bridge the distance between the incision and the exit site. Needles and catheters can be used for several surgeries and should be disinfected between surgeries along with the other surgical instruments. The tapered end of the catheter will begin to fray or bend and the cutting edge of the needle becomes dull after repeated use, so they should be replaced at regular intervals.

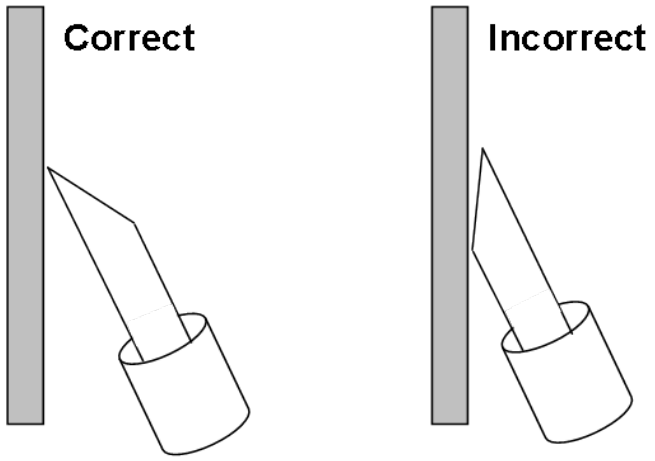

Figure 12. Schematic showing the correct and incorrect orientation of the tip of the needle against the body wall when the shielded needle technique is used to create an antenna exit site.

Transmitter insertion: Orient the transmitter vertically, and carefully insert it in the body cavity through the incision. If there is resistance during insertion, suggesting that the transmitter may tear the tissue at the edges of the incision, the incision should be enlarged.

Once inserted, position the transmitter directly beneath the incision. This positioning will protect organs during incision closure because the suture needle will contact the transmitter rather than any organs.

If additional tags will be used, insert them through the incision. For some study designs, PIT tags are used in conjunction with radio or acoustic transmitters. All tags should be tested for proper function and disinfected prior to implantation.

Incision closure: Two sutures, in a simple interrupted pattern, are used to close the incision. The simple interrupted pattern (fig. 13) is used because it involves independent closure efforts. This suture pattern ensures that the integrity of the entire incision (and corresponding risk of transmitter loss) will not be affected if one of the sutures becomes loose or untied. A modified surgeon's (or friction) knot is used to secure each suture. The knot consists of three double-wrap throws (that is, a single wrap of the suture material around the surgical instrument) and is commonly described as a 2 × 3 knot (2 wraps on each of 3 throws). The direction of the wraps should be alternated between throws to aid knot security. For example, on the first throw, the direction of the wrap is away from the tagger, on the second throw, the wrap is towards the tagger, and on the third throw, the wrap is again away from the tagger. The first throw should be lightly tensioned, so that the edges of the incision are drawn closely together (are well apposed) but do not overlap. The second and third throws have less impact on the apposition of the sides of the incision and can therefore be applied with increased tension to aid knot security. Ensure that the wraps from each throw lie flat and do not twist on themselves and form a ball.

After the knot is complete, cut the suture so that the tag ends of the knot are about 3 mm long. The length of suture tag ends should be long enough to ensure the integrity of the knot, but as short as possible to reduce the available surface area for bacterial or fungal growth.

The suture needle entry and exit sites should be about 2 mm from the edge of the incision. This distance is sufficient to anchor the suture material and to resist the tendency of the suture to pull through the entry or exit site, tearing the tissue in a line perpendicular to the incision. When entry and exit sites

are positioned farther from the incision the knot is less effective at creating and maintaining good apposition of the sides of the incision. Locking the suture needle in the jaws of the needle holder may allow the best control during needle entry and exit.

Ideally, the sutures should penetrate the full depth of the body wall and peritoneum. The incision will heal best when the full thickness of the body wall (including the peritoneum) on both sides of the incision is pulled into apposition. Examination of the internal aspect of sutures, looking to confirm penetration of the peritoneum, is an important part of developing surgical proficiency.

The two sutures should be positioned with equal spacing along the length of the incision. The goal is to have approximately equal distance between the two sutures as well as between each suture and the adjacent edge of the incision. For example, with a 6-mm incision, sutures should be positioned 2 mm from each other and 2 mm from the anterior and posterior margins of the incision (fig. 13). A third suture may be added if needed to adequately close the incision. The need for a third suture will depend on the length of the incision (which depends on the size of the transmitter) and the placement of the first two sutures. Although adding a third suture poses some additional risks (for example, infection and tearing) compared to a well-closed incision with two sutures, the addition is warranted if there is risk of transmitter loss due to a partially open incision.

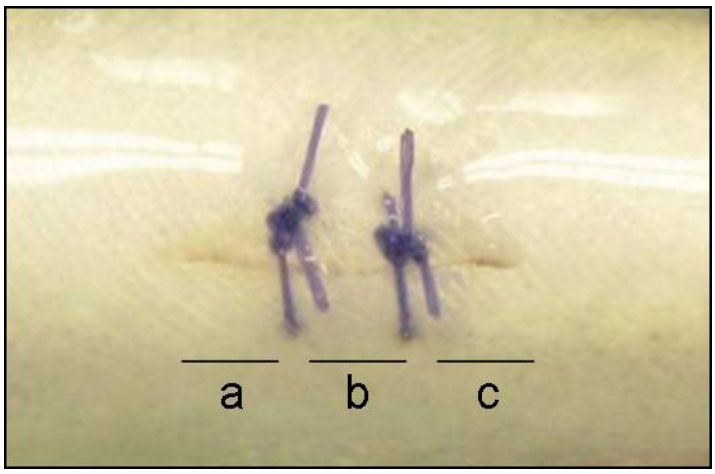

Figure 13. Two simple interrupted sutures. Note the suture positioning, where distances a, b, and c are approximately equal.

There is no exact specification for suture size. The general recommendation is to use the smallest diameter suture material that will secure the incision without tearing through the adjacent tissue. Typically a 4–0 suture is used for fish weighing more than about 50 g, and a 5–0 suture is used for fish weighing less than about 50 g.

Reduce sedation exposure: If the ventilation rate of the fish has been slow and regular throughout the surgical procedure, consider irrigating with fresh water to begin the recovery from anesthesia. A good time to switch to freshwater irrigation is after the completion of the first suture, as the second suture generally can be completed quickly and effectively, even while the fish is beginning to revive. An alternate approach is to continue to provide sedation through the gravity feed and to open the valve for the freshwater gravity feed container. This approach reduces the sedation concentration by approximately one-half. Full sedation can be continued for fish that have been active on the surgery platform during the initial phases of surgery.

Rotate instruments: A single set of surgical instruments should be used for a given surgical procedure and adequately disinfected prior to use in subsequent procedures. Prior to the surgery, the instruments should be removed from the disinfectant tray and placed into a rinse tray filled with distilled or deionized water. Following use, instruments should be returned to the disinfectant tray. A new tray is then selected for the next procedure to allow sufficient exposure time to the disinfectant. Heavy organic debris (for example, scales and blood) should be removed from instruments prior to disinfection. Small toothbrushes are useful for this purpose. Rotate unused portions of suture (along with the attached needle) with a given set of instruments so that they are effectively disinfected.

Disinfectant and rinse trays should be replaced regularly during a tagging session. Organic debris will accumulate in the disinfectant tray through multiple surgeries (reducing effectiveness of the disinfectant) and the solution will be diluted slowly as instruments are moved between the rinse tray and the disinfectant. Replace the water in the rinse trays as needed to be sure they are effectively removing all disinfectant residues.

Record special conditions: Although most surgeries will proceed as planned, there likely will be a few surgeries where atypical conditions arise. For example, the micro scalpel may begin to dull so the incision is somewhat irregular. Perhaps a third suture may be used or the fish may show some bleeding. These situations with individual fish should be recorded because they may be linked with wound healing, behavior, or survival. Additionally, analysis of the frequency of such events might guide modifications to tagging procedures in future studies. Consider having a field on the datasheet where generalized comments can be recorded.

Post-Tag Recovery

After the surgical procedure is complete, fish are transferred from the surgery platform to a recovery container. The transfer is performed by holding the fish in position on the surgery platform and carrying the platform to the recovery container. This approach eliminates the need to pick up the fish from the platform and reduces the risk that the fish will be dropped during the transfer. If a fish is dropped to the floor after it is tagged, it should be euthanized, and the transmitter should be removed, disinfected, and implanted into another fish.

As soon as a tagged fish is placed in a recovery container, the container cover is positioned and the timer recording air exposure time is stopped. The air exposure time for each fish is recorded and is a good indicator of the risk of stress to the fish because it measures the amount of time the fish was exposed to air, not just the time needed to complete the surgical procedure.

The density in recovery containers should not exceed 10 g of fish per L of water. Depending on the size of the fish, this generally translates to 2–3 fish per 19-L recovery container (the containers are not full of water due to the perforations). This is a highly conservative density level, designed to optimize holding conditions for tagged fish.

Recovery containers are maintained at increased DO concentrations to help fish recover from the oxygen debt incurred during anesthesia. Because the recovery containers are closed systems (no water exchange) the increased DO concentrations can readily be maintained for short time periods. Fish should remain in the high DO saturation environment for at least 10 minutes to allow them to regain equilibrium and to demonstrate at least nominal swimming activity. Use a timer to record recovery time for each container of tagged fish. The timer should record the minimum recovery time for the group of fish in the container; therefore, it should be started when the last fish is placed in the container. The first fish placed into the container will have a slightly longer recovery time.

Fish generally regain equilibrium in 3–5 minutes (depending on water temperature, anesthesia exposure, and individual sensitivity). Fish that take more than about 5 minutes to regain equilibrium require close monitoring. Fish that do not show regular ventilation rates, are not readily recovering from surgery, or both should be manually ram ventilated to increase the flow of oxygenated water over the gills. Ram ventilation is accomplished by grasping the fish gently and moving them forward and back within the recovery container. The increased water flow over the gills will increase and stabilize the heart rate, which leads to increased blood flow to the gills to help eliminate the anesthesia (Ross and Ross, 2008). Tagged fish that do not recover well, even with assistance, or for other reasons are determined to be inappropriate for release, should be euthanized. The transmitter should be removed, de-activated, and disinfected or sterilized for later use.

After fish regain equilibrium and show responsiveness to stimuli, the recovery container is moved to a post-tagging holding tank. The perforations in the recovery containers allow exchange with tank water to enable the maintenance of water-quality during the holding period.

Release of Tagged Fish

The last opportunity a researcher has to visually examine tagged fish is immediately prior to release. This is the time to assess whether fish are behaving as expected and whether they are appropriate subjects for the telemetry study. Examine all post-tag holding containers for dead fish, moribund fish, and shed transmitters. The exam should be conducted with as little disturbance as possible. The lid of the container should be partially removed, just enough for the fish to be visible. Try to avoid letting significant amounts of light into the container as fish will likely jump and may escape.

Observe fish condition by noting swimming activity and the vertical position of fish in the container. Carefully remove moribund, dead, or poorly performing fish so as not to disturb the other fish in the container. Stressors, such as chasing the fish immediately prior to release, may induce a stress response that could influence fish behavior or survival. Remove the transmitters from fish that will not be released and record the final condition of the individual tagged fish.

We recommend that transmitter function be validated immediately prior to the release of tagged fish. This step is valuable because transmitters may have failed after exposure to water, or there may have been errors during the activation procedure. Monitoring equipment (radio or acoustic telemetry receivers) will be needed, and several approaches can be used, but they are outside the scope of this SOP.

The final step in the tagging procedure is to release tagged fish from their holding containers so they can be monitored. If transportation is required to move fish from the post-tag holding location to the release location, be sure to maintain water-quality standards and access to the air-water interface during transport. It is useful and convenient to document water temperature during transport with a thermograph. These devices are readily available and inexpensive. Place one in the transport container to monitor temperature changes during the transport effort. Review the data and make any necessary modifications to the transport procedures to ensure compliance with water-quality standards. In addition to water-quality, it is important to maintain fish access to the air-water interface during transport because rough handling can cause fish to expel air and become negatively buoyant. Fish that cannot maintain neutral buoyancy likely will show altered behavior, such as variable depth profiles or delayed movements.

After tagged fish arrive at the release location, put the holding containers in or near the water and let tagged fish volitionally swim out of the container. Record the release time for individual tagged fish or for the groups of fish within each holding container.

Clean-Up and Disinfection

Surplus fish: Source fish that were not needed for tagging and fish that were deemed inappropriate for the tagging operation need to be released, returned, or euthanized. Reference the appropriate collection or handling permit or use local guidance to determine the best course of action for surplus fish. Record the numbers of surplus fish to help refine fish needs for future tagging operations.

Discard tagging solutions: The chlorhexidine and anesthetic solutions used during the tagging operation should be discarded based on local guidance and label instructions. The MS-222® solutions commonly are poured onto pavement or gravel surfaces for evaporation or filtration. The chlorhexidine solution may be handled like MS-222® or may be discarded through any water system that receives wastewater treatment. For settings where disposal may be challenging, chlorhexidine can be collected during the tagging operation and transported to a facility for disposal. Full solution capture generally is not an option for MS-222® because of the high volume of solution used during a tagging operation.

Clean and disinfect: Surgical instruments, working surfaces, and holding containers need to be cleaned and disinfected regularly. At the end of each tagging operation, surgical instruments should be cleaned and prepared for sterilization. Discard any open or partially used suture packets and micro scalpel blades in a sharps container. If catheters were used, discard the plastic catheter as it cannot be sterilized. The needle portion can be retained and packaged with the instruments. Use a small toothbrush to scrub organic debris off all surgical instruments. Pay close attention to the jaws of the needle drivers and the forceps where organic matter is most likely to accumulate. Rinse and dry all instruments. Consider an application of an instrument lubricant to enhance instrument performance and longevity. Wrap instruments in a cloth or place them in an autoclave bag to prepare them for sterilization. Apply autoclave tape or another marker system so that sterilization can be confirmed prior to the next use of the instruments. Consider wrapping each complete instrument set individually to speed the set-up of the next tagging operation.

All working surfaces and tagging equipment should be disinfected. Spray countertops, the tagging platform, the tagging station, nets, and any other working surfaces with Virkon® Aquatic solution. Alternately, a large container of disinfectant solution can be used so that equipment can be soaked. Allow 10 minutes of contact time and rinse with clean water. Position equipment to drain, and allow a thorough drying prior to the next use.

Clean and disinfect all water and holding containers regularly. The need for cleaning and disinfection will depend on the level of use and local water-quality. Tanks should be scrubbed and disinfected when the sediment load is sufficient to influence water-quality. Anesthesia, reject, recovery, and gravity feed containers should be disinfected weekly if the tagging operation occurs daily. Disinfect containers by applying Virkon® Aquatic, allowing 10 minutes of contact time, rinsing, and allowing containers to drain and dry.

Recommendations

Maximize Efficiency

The procedures for surgical implantation of transmitters in juvenile salmonids can be used in any tagging operation, regardless of scale. A small-scale tagging operation may involve a tagger, an assistant, and potentially a dedicated data recorder. A larger operation may involve multiple taggers and numerous support personnel. The scale of the tagging effort will depend on the number of fish that will be tagged during a single tagging session (that is, the tagging load). Regardless of the scale of the tagging operation, our recommendation is to maximize efficiency by adopting some simple procedures and providing appropriate support staff. When the tagging load is high or the timing is particularly

critical (for example, tagging groups of fish to be released hourly), the tagging operation must operate efficiently. There is no downside to introducing efficiency into smaller scale tagging operations; efficiency is just less critical in these circumstances. The largest source of inefficiency in a tagging operation is the delay in starting a surgery because a fish is not yet anesthetized. If the tagger is ready to perform the surgery and the fish is not ready to be removed from the anesthesia container, minutes of delay are incurred for each fish. Because a proficient tagger can weigh, measure, and tag a fish in about 3 minutes, waiting 2 minutes for anesthesia is a significant delay. This delay is readily managed by providing a dedicated assistant to the tagger who can move source fish into anesthesia and deliver them to the tagging area. Close coordination between the tagger and the assistant is required. The tagger should request that a fish be added to the anesthesia container before the previous fish has been removed from the surgery platform. The exact timing will depend on the proficiency and speed of the tagger, but a general rule is for taggers to request a new fish once the first suture has been tied on their current fish. Using this approach, the taggers have sufficient time between surgeries to rotate their instruments, to prepare the next set of instruments, to conduct a quick exam of the fish in the anesthesia container, and to begin the next procedure.

Providing appropriate support staff is another approach to improving efficiency. A dedicated data recorder keeps the tagging operation running smoothly because the person is constantly available to record tagger comments and to assist with monitoring anesthesia and preparing surgery materials. When multiple taggers are used simultaneously, we recommend that each tagger have a dedicated data recorder.

Monitor and Document SOP Compliance

We recommend that compliance with the SOP be monitored and documented. Staff can "drift" from the protocol through time, especially when more than a few people are involved in tagging operations. To control protocol drift across time or personnel, researchers should draft a checklist of measurable SOP elements, such as DO concentrations or water temperatures and use it to conduct compliance inspections. An example checklist for such inspections is provided in appendix C. Complete several inspections over the course of the study so that all staff involved in tagging operations are included in at least one inspection. Reporting of study findings should include full details of the SOP and a summary of the findings from the compliance inspections.

Evaluate Transmitter Effects

Researchers should consider evaluating transmitter effects in a controlled setting to support the collection of telemetry data on study animals. There are various approaches that can be used to evaluate the risk of transmitter effects, ranging from monitoring simple outcomes, such as mortality and transmitter loss, to more complex evaluations, such as swimming performance or predator avoidance ability. An overview of procedures that can be used to conduct evaluations of transmitter effects is available in Liedtke and Wargo-Rub (2012). The ideal evaluation would use the same personnel, fish source, and procedures as were used for the field telemetry study so that their influence on the fish can be documented. An easily performed approach is to surgically implant transmitters in fish and then hold them under controlled conditions for the duration of the expected transmitter battery life. Inactive or "dummy" transmitters can be used as long as they accurately reflect the weight and external material, size, and shape of active transmitters. Check for mortalities and shed transmitters regularly and monitor general fish condition. This type of evaluation, even with a few fish, provides compelling evidence about the likely fate of fish released for the telemetry study.

Report Methods in Detail

Researchers should report, in detail, the procedures used for transmitter implantation and the training and experience of the taggers. We encourage rigor in reporting of procedures such as pre-tag and post-tag holding, fish handling, and efforts toward aseptic technique. The skill of the tagger can have a significant effect on the outcome of the tagging procedure (Wagner and Cooke, 2005), so the number of taggers, their contribution to the study effort, and their experience and training should be reported in detail.

Recapture Tagged Fish

Finally, we recommend that researchers take advantage of any opportunities that arise to examine tagged fish after they have been released. The use of a secondary mark (for example, PIT tag, fin clip, or a non-electronic tag) will aid this effort, especially in the event of shed transmitters. Fish may be incidentally recaptured through recreational or commercial harvest or purposefully recaptured by first locating the tagged fish using the transmitter signal and then netting or electroshocking to recover it (Jepsen and others, 2000, 2002; Koed and Thorstad, 2001; Jepsen, 2003). In some cases, the fish may not be physically recaptured, but may be detected by researchers conducting video surveillance.

Regardless of the means, recapture provides an opportunity to evaluate, in a real life setting, the effectiveness of the surgical implantation procedure. Conduct both external and internal exams on recaptured fish, looking for tissue or organ damage, infection, healing, and location of the transmitter relative to the original placement. Document findings with photography, and provide detailed descriptions during study reporting. Negative outcomes of transmitter attachment procedures are rarely reported, but they provide valuable insight. For example, based on recaptured fish, Bauer and others (2005) found that the location of the exit site for a trailing antenna had migrated 3 cm, and Thorstad and others (2001) described a transmitter antenna that had accumulated significant biofouling.

Summary

Surgical implantation procedures have become a common tool for fisheries applications. Surgical techniques generally are reported with few details, however, limiting the learning opportunities for surgeons. Most fish surgeons learned their techniques and choose their materials based on the experience of colleagues who have performed surgeries or through their own trial-and-error process (Cooke and Wagner, 2004). There are diverse opinions on the most appropriate surgical techniques and materials (for example, suture material, knot, and suture pattern), and they generally are aggressively defended, despite the potential lack of experimental evidence supporting them. This Standard Operating Procedure (SOP) does not purport to describe the single best set of procedures for surgical implantation of transmitters in juvenile salmonids. The purpose of the SOP is to share the procedures that have allowed the Columbia River Research Laboratory to implant transmitters into large numbers of fish with very high survival rates and very low transmitter losses. We believe that this SOP is sound, as it has been rigorously tested in field settings with large numbers of fish, taggers with varied skill levels, variable fish sizes and species, and a range of environmental conditions (for example, water temperature, dissolved oxygen, and total dissolved gas). Additionally, the SOP has been used to evaluate fish performance and transmitter effects in laboratory studies. Because there is no single tagging SOP that will fit all situations, each researcher must adopt procedures and materials that are defensible based on their experimental evidence, experience, or the scientific literature.

This SOP can be applied as published, or modified to fit specific study objectives or site logistics. We commonly make minor adjustments to fit new applications, while maintaining the guiding principles and details of the procedures. Although the SOP details procedures specific to juvenile salmon, many of the principles apply generally to fish surgeries and can be used to develop SOPs for other species or life stages.

Good fish-handling practices are an overarching theme in the SOP because we believe they are integrally linked with good surgical outcome. Fish-handling procedures generally are underemphasized in descriptions of surgical techniques because of the focus on the details of the surgery itself. Good surgical performance certainly contributes to a good outcome, but we believe that the effects of poor fish handling or poorly managed anesthesia can quickly outweigh the benefits of excellent surgical technique. In our experience, good fish handling, combined with a well-executed tagger training program and sound aseptic technique, will produce consistently positive surgical outcomes.

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Appendix A: Materials Needed

A list of materials is provided for study planning. The study design and numbers of fish to be tagged will dictate the quantities of materials needed and will be determined on an individual study basis. Our materials list is divided into equipment (non-expendable items) and supplies (expendable items). In some cases, there are specific recommendations for materials or the number of items needed based on our experience. In most cases, however, materials are described in general terms and can be substituted with equivalent materials as needed.

We provide some guidance on the amount of supplies to purchase, based on the number of fish to be tagged (table A1). Using the number of fish to be tagged to estimate supply needs is simplistic, however, because needs will vary depending on the number of tagging sessions, the number of fish tagged each session, the environmental conditions, and the number and experience of the taggers. For example, experienced taggers will generally use less suture material per fish than novice taggers. Some supplies, like chlorhexidine and MS-222® are needed in similar quantities whether a single fish or 25 fish are being tagged. Table A1 can be used as a broad planning guide for materials and supplies, and planning can be refined using the specific details for a given study. We encourage researchers to order supplies to accommodate practice surgeries in addition to the supplies needed for the execution of the study.

Equipment

- Digital thermometer
- Dissolved oxygen meter
- Total dissolved gas meter (depending on study conditions)
- Dark-colored 19-L containers marked with 10-L volume line and labeled “Anesthesia”
- Dark-colored 19-L containers perforated to hold 7 L of water in reservoir
- Dark-colored 19-L containers labeled “Reject”
- Dark-colored lids to fit 19-L containers
- Bench, shelf, or platform to hold gravity feed containers 45–60 cm above work platform
- Two gravity feed buckets or carboys, each marked with 10-L volume. One will be labeled “Sedation” and one will be labeled “Fresh Water” (see fig. 3)
- Tubing to connect the two gravity feed containers to each other and to the work platform, with in-line valves to allow flow from each container individually, and from both containers simultaneously (see fig. 3)
- Scale that measures weight to the nearest 0.1 g, with calibration weight
- Smooth surface measuring board with a ruler in millimeters
- Surgical table or platform to hold fish with ventral surface exposed and allow gills to be perfused. A block of closed-cell foam with a groove cut out or an acrylic glass “V” are recommended options (see fig. 1 and fig. 2)
- Dip nets
- Sanctuary nets: modified dip nets with non-porous material in deepest part of the net to retain water and allow fish to be moved without exposure to air
- Trays (n=6–8) to hold transmitters and instruments during disinfection and rinse procedures (approximately 20 cm x 10–15 cm and about 5 cm deep) (see fig. 4)

- Surgical instruments: need 4–6 complete sets of instruments per tagger to allow for rotation
    - Needle holders/drivers: recommend 12 cm Olsen-Hegar combination with scissors
    - Forceps: recommend micro dissection with 0.5–0.8 mm serrated tip
    - Micro scalpel handle (if using disposable micro scalpel blade tips)
- Small toothbrush to scrub surgical instruments
- Autoclave or pressure cooker to sterilize instruments
- Timers (count-down or count-up): water resistant timers are recommended
- Transmitters and any required activation or validation equipment (for example, receivers, hydrophones, antennas)

Supplies

- Distilled or deionized water
- Chlorhexidine diacetate solution (diluted to 30 mL/L); (Brand name: Nolvasan® by Fort Dodge)
- Tricaine methanesulfonate (MS-222®) solution (100 g/L) in an amber bottle to prevent reduction of activity due to photosensitivity
- Sodium bicarbonate (baking soda) solution (100 g/L)
- Stress Coat® (Aquarium Pharmaceuticals Inc.) or similar product: undiluted solution and 25 percent solution in squirt bottles
- Virkon® Aquatic Solution (Western Chemical); 1 percent solution in spray bottle
- 10 ml plastic syringes or graduated cylinders for dispensing solutions
- Oxygen cylinder, regulator, tubing, and airstones
- Battery-powered aerators (equipped with tubing and airstones)
- Medical-grade exam gloves (non-latex): a variety of sizes to fit all personnel
- Large plastic weigh boats (~12 cm square)
- Surgical supplies:
    - Disposable 3 mm and/or 5 mm micro scalpels with 15 degree blade angle (disposable blades or full disposable scalpels)
    - 4–0 and/or 5–0 Ethicon Vicryl Plus® sutures with RB-1 tapered needle
    - Catheters (20 gauge, 8–10 cm long); for radio transmitter implantation only
- Sharps container for disposal of needles and scalpel blades (one per tagging station)
- Autoclave bags and/or tape and towels to wrap instruments

Table A1. Estimated materials needed to tag between 10 and 100 fish.

Material	Unit multiplier	10 fish	25 fish	50 fish	100 fish
Chlorhexidine (undiluted)	2.4 ml/fish	24 ml	60 ml	120 ml	240 ml
MS-222® (powder)	0.13 g/fish	1 g	3 g	7 g	13 g
Sodium bicarbonate (powder)	0.13 g/fish	1 g	3 g	7 g	13 g
Stress Coat® (undiluted)	0.53 ml/fish	5 ml	13 ml	27 ml	53 ml
Virkon® Aquatic (powder)	5 g/tag date	5 g	5 g	10 g	20 g
Exam gloves	0.13 gloves/fish	2 gloves	4 gloves	8 gloves	14 gloves
Microscalpels	0.22 blades/fish	2 blades	6 blades	11 blades	22 blades
Suture packets	0.33 pkts/fish	4 pkts	9 pkts	17 pkts	33 pkts
Catheters	0.25/fish	3 catheters	7 catheters	13 catheters	25 catheters

Appendix B: Abbreviated Procedures

Fish Collection, Holding and Transport

1. Collect study fish using the least destructive and least stressful method that is effective.
   A. Fish that obviously are injured, diseased, or excessively burdened by pathogens should not be retained for tagging.
   B. Document the number of fish collected, but not retained, as well as the rationale used for rejection (for example, injury or disease).
2. The pre-tag holding period for a group of fish begins once fish are in the care of the researcher and ends when surgical procedures begin.
   A. The pre-tag holding period should be between 12 and 36 hours and ideally 24 hours.
   B. Record the time the pre-tag holding period begins for each container of fish.
   C. Fish should not have access to commercial fish feed during the pre-tag holding period.
3. Hold fish in containers with lids to reduce disturbance and fish loss due to jumping.
   A. Pre-tag holding densities should not exceed 20 g of fish per L of water (Formula: [mean fish wt (g) x number of fish]/L of water is less than or equal to 20 g/L).
   B. If multiple species are collected they should be held in separate, but comparable containers.
4. Monitor and maintain water-quality during pre-tag holding (and any required transport process).
   A. Dissolved oxygen concentration (DO) in pre-tag holding containers should be between 80 – 130 percent saturation.
   B. Do not transfer fish between water sources until the difference in water temperature between the sources is less than or equal to 2°C.
   C. Total dissolved gas (TDG) in pre-tag holding containers should not exceed 110 percent saturation.
5. Monitor fish behavior and condition during pre-tag holding (or at a minimum, prior to tagging).
6. Transport of untagged or tagged fish should be designed to minimize stress to fish.
   A. Monitor and maintain water-quality parameters established for fish holding.
   B. If water temperature rises significantly during transport, the addition of ice may be required. Be aware that most commercially produced ice contains chlorine, which may be harmful to fish.
   C. Select a transport route of travel for the shortest and smoothest ride to minimize jarring.

Fish Size Criteria

1. The weight of the transmitter in air must not exceed 5 percent of the weight of the fish in air.

Tagging Preparations

1. Monitor and maintain water-quality in all fish containers before, during, and after the tagging session.
   A. DO should be between 80 – 130 percent saturation in all water sources that hold fish.

B. TDG should be less than 110 percent in all water sources that hold fish.

C. Do not transfer fish between water sources until the water temperature difference is less than or equal to 2°C.

2. Prepare tagging equipment and supplies.

A. Confirm specifications (that is, frequency, pulse rate, and so on) and operation of transmitters, and/or PIT tags.

B. Disinfect and rinse transmitters.

i. Immerse transmitters in chlorhexidine solution for 15 minutes.
ii. Thoroughly rinse transmitters in distilled or deionized water.
iii. Following disinfection, handle transmitters only with clean instruments or clean, gloved hands.

C. Prepare the tagging station.

i. Set up the irrigation system (gravity feed or pump).
ii. Pour chlorhexidine solution into disinfection trays.
iii. Pour distilled or deionized water into a rinse tray.
iv. Put tagging supplies (that is, sutures and blades) near the tagging station, and load each tray with a complete set of sterile surgical instruments.

D. Prepare the measuring board and scale.

i. Measuring board should be made of smooth material and in good condition.
ii. Ensure that the scale is functioning, and calibrated regularly.
iii. Place a plastic weigh boat on the pan of the scale.
iv. Wet the measuring board and weigh boat with diluted Stress Coat® solution.

3. Prepare recovery containers.

A. Fill recovery containers with source water just prior to the start of tagging to maintain optimal water-quality.

B. DO concentration in recovery containers should be between 120 – 150 percent saturation.

C. Fish density in recovery containers should not exceed 10 g of fish per L of water.

D. Position containers near the tagging station.

4. Prepare a reject container.

A. Fill reject container(s) with source water just prior to start of tagging to maintain optimal water-quality.

B. Equip the container with a battery-operated aerator.

C. Position the container near the tagging station.

5. Prepare anesthesia.

A. Prepare the anesthesia container.

i. Fill the19-L anesthesia container with 10 L of source water and add MS-222® stock solution.
ii. For each mL of MS-222® stock solution added to the container, add the same amount of sodium bicarbonate stock solution.
iii. Add approximately 10 mL of diluted Stress Coat® solution to the container.

iv. Cover the anesthesia bucket with a lid, place a timer on the lid, and position the container near the source of fish to be tagged.

B. Prepare gravity feed containers.

i. Fill both gravity feed containers with 10-L of source water. Add 2 mL of MS-222® stock solution and 2 mL of sodium bicarbonate stock solution to the sedation container.

ii. Place both containers on an elevated platform and connect tubing between the containers and the tagging platform.

Anesthetizing Fish

1. Net one fish from the pre-tag holding container and place directly into the anesthesia container.
   A. Immediately place a lid on the container.
   B. Start a timer to document the MS-222® exposure time.
2. Remove the lid after approximately 1 minute to monitor the stage of anesthesia.
   A. Induction time should be 2-4 minutes, with an average time of 3 minutes.
   B. If a fish loses equilibrium in less than 1 minute it should not be tagged.
   C. Net the fish from the anesthesia container into the Reject container.
3. Once the fish loses equilibrium examine the fish for condition.
   A. Keep the fish submerged during the examination.
   B. Look for marks, tags, clips, fungus, descaling, injury, parasites, and signs of disease.
   C. Fish that are not acceptable for tagging should be transferred to the Reject container.
4. Wait 30–60 seconds after the fish has lost equilibrium to remove it from anesthesia.
   A. Use a net or gloved hand to remove the fish.
   B. Start a timer to monitor the air exposure time.
   C. Note the anesthesia exposure time.
   D. Fish exposed to MS-222® for 5 minutes or longer should be rejected.
5. Monitor anesthesia for several fish and adjust concentration as needed.
   A. Adjust concentration (up or down) in 5 mg/L increments of working concentration (or 0.5 mL increments of stock solution).
   B. Note any change of anesthesia on the datasheet.
   C. Anesthetize another group of fish and monitor the new concentration for effectiveness.
   D. If more than one tagger is operating, coordinate the change of concentration so that it applies to all taggers.
6. Add anesthesia containers as needed to minimize delays, keeping a single fish in each container, with a separate timer.

Measuring Fish Size

1. Transfer fish from the anesthesia bucket to a measuring board.
   A. Ensure that a timer is started to measure air exposure time.
   B. Measure and record fork length (FL) to the nearest millimeter.

C. The FL is the distance from the snout to the fork in the caudal fin.

D. Regularly add diluted Stress Coat® to keep the surface of the board wet.

2. Transfer the fish to the weigh boat on the scale.
   A. Tare the scale so that it shows zero with the weigh boat and Stress Coat® added.
   B. Measure and record weight to the nearest 0.1 g.
   C. Regularly add diluted Stress Coat® to keep weigh boat wet.
3. Make all transfers by cradling fish in two hands.
   A. If a fish is dropped to the floor before it is tagged it must be rejected.
   B. If a fish is dropped on the tagging surface it may be repositioned and tagged at the tagger's discretion.
   C. If a fish is dropped to the floor after it is tagged it should be euthanized, and the transmitter should be removed, disinfected, and used in another fish.
4. Vocally relay fish size data to a recorder to speed fish handling and ensure data accuracy.

Implantation of Transmitters

1. Place the fish on the surgery platform ventral side up and establish irrigation.
   A. Place the gravity feed tubing into the mouth of the fish.
      i. Deliver sedation water from the sedation gravity feed container.
      ii. Ensure water flow over the gills; adjust flow using the valves.
      iii. Inadequate flow will cause the fish to become agitated.
      iv. Recline the head of the fish to avoid water entering the body cavity through the incision.
   B. Lighten the sedation dose as the surgery progresses to begin recovery.
      i. At the mid-point of the surgery consider providing a mix of freshwater and sedation.
      ii. Near the end of the surgery try to provide completely freshwater irrigation.
2. Conduct a second external exam to evaluate fish condition and determine suitability for tagging.
   A. Briefly examine fully anesthetized fish now that it is out of the water.
   B. Record fish condition notes.
3. Use a micro scalpel to make an incision.
   A. In general use a 5 mm blade for fish that weigh more than about 50 g and a 3 mm blade for smaller fish.
      i. The choice of micro scalpel blade will depend on the thickness of the body wall.
      ii. Blade selection should be done through experimentation.
   B. Locate the pelvic girdle of the fish by visual exam and palpation.
      i. In a ventral view the pelvic girdle has a "V" or "U" shape.
      ii. The most anterior point of the pelvic girdle is the positioning guide.
   C. Make an incision about 3 mm anterior to the anterior point of the pelvic girdle, and about 3 mm away from and parallel to the mid-ventral line .
      i. Draw the blade toward the head of the fish.

ii. The incision should be only long enough to allow insertion of the transmitter without tearing the adjacent tissue.
iii. The incision should be deep enough to penetrate the peritoneum without damaging internal organs.
iv. The incision would ideally be made with a single pass of the micro scalpel.
v. One micro scalpel blade can be used on several fish before it becomes dull.

4. Use forceps to open the incision and quickly evaluate any potential organ damage.
   A. Insert forceps to ensure that the peritoneum was penetrated along the full length of the incision.
   B. Assess any potential organ damage by looking for bleeding.
   C. If the fish is bleeding excessively it should not be implanted with a transmitter.
5. If the transmitter has an external antenna, make an antenna exit site in the lateral body wall using a modified shielded needle technique. If the transmitter has no external antenna, proceed to step 6.
   A. A plastic catheter, positioned over a needle, is termed a "shielded needle" (see fig. 10).
   B. Following purchase, the catheter typically must be modified slightly to function as a shield.
   C. Insert the shielded needle through the incision and guide it to the antenna exit site.
      i. Keep the needle tip covered by the catheter to protect the organs.
      ii. The antenna exit site should be even with the insertion of the pelvic fins and about 40 percent of the distance from the mid-ventral line to the lateral line (see fig. 11).
   D. Once positioned at the antenna exit site, use the needle to puncture the body wall.
      i. The cutting edge of the needle should be facing away from the body wall (see fig. 12).
      ii. Puncture the body wall with the needle, not the catheter.
      iii. Advance the catheter through the wound until it is visible from the outside of the fish.
   E. Hold the catheter in position, extending out the incision and the exit wound, and withdraw the needle.
      i. In this position the catheter forms a channel for the transmitter antenna.
      ii. Select the catheter with the smallest diameter that will accommodate the transmitter antenna and is of appropriate length.
      iii. A single needle can be used for several surgeries.
   F. Route the transmitter antenna through the catheter, starting at the incision.
      i. Keep the body of the transmitter in your gloved hand.
      ii. Pull the catheter out of the body wall and off the antenna.
6. Insert the transmitter into the abdominal cavity.
   A. Orient the transmitter so that the top or bottom is inserted first.
   B. Carefully insert the transmitter through the incision.
   C. Position the transmitter directly beneath the incision.
   D. If additional tags (for example, PIT tags) will be used insert them through the incision.

7. Close the incision with sutures.
   A. Two sutures, in a simple interrupted pattern, are used to close the incision.
      i. The simple interrupted pattern involves independent closure efforts.
      ii. Use a modified surgeon's (or friction) knot to secure each suture.
      iii. Suture entry and exit sites should be approximately 2 mm from the incision edge.
   B. A third suture may be added if needed to adequately close the incision.
      i. Position sutures so that there is an approximately equal distance between the sutures and between the sutures and the anterior and posterior edges of the incision (see fig. 13).
      ii. Adding a third suture poses some risks but is warranted if there is risk of transmitter loss due to a partially open incision.
      iii. Note on the datasheet the addition of a third suture.
   C. There is no exact specification on suture size.
      i. Use the smallest diameter suture material that will secure the incision without tearing through the adjacent tissue.
      ii. Typically a 4–0 suture is used for fish that weigh about 50 g and a 5–0 suture is used for smaller fish.
      iii. A single suture packet is used for several fish and must be disinfected and rinsed between fish surgeries, following the same procedures as for instruments.
8. Switch irrigation water from sedation to freshwater.
   A. The ventilation rate of the fish must be monitored throughout the procedure.
   B. Irrigation should be switched to freshwater to begin the recovery from anesthesia.
   C. Sedation can be continued for active fish.
9. Rotate surgical instruments and suture material to ensure adequate disinfection.
   A. Multiple sets of instruments must be available.
   B. Gently place micro scalpel blades into the disinfectant bath to avoid damaging delicate blades.
   C. Organic debris should be removed from instruments before disinfection.
   D. Replace the chlorhexidine in the disinfectant trays to maintain efficacy.
   E. Rotate unused portions of suture with a set of instruments.
   F. Replace the water in the rinse tray as needed to ensure adequate rinsing.
10. Record any special conditions that occurred during the procedure for an individual fish.

Post-Tag Recovery

1. Monitor the water-quality in recovery containers and make any needed adjustments.
   A. The temperature of the containers should be less than 2°C different from the water source.
   B. The DO concentration in the containers should be 120–150 percent saturation.
   C. TDG should not exceed 110 percent saturation.
2. Transfer fish from the surgery platform to a recovery container.
   A. Hold the fish in position on the surgery platform and carry the platform to the recovery container to reduce the risk of dropping fish.
   B. Cover the container immediately after the fish enters.

C. Stop the timer that records the air exposure time and note the time on the datasheet.

D. The density in recovery containers should not exceed 10 g of fish per L of water.

E. Start a timer to record the minimum recovery time for fish in a given recovery container.

   i. Monitor fish for short-term recovery before transferring them to a post-tag holding container.

   ii. Tagged fish should remain in recovery containers for at least 10 minutes to ensure access to high DO saturation.

   iii. Monitor fish for recovery of equilibrium.

F. Fish that take more than about 5 minutes to regain equilibrium require close monitoring.

   i. Manually ram ventilate fish with slow recovery. Move the fish forward and back within the recovery container.

   ii. Tagged fish that do not recover should be euthanized.

G. Transfer fish to the post-tag holding container.

Post-Tag Holding and Fish Release

1. Configure post-tag holding to reduce fish transfers and facilitate release.
   A. Hold fish using a system where the small, mobile containers used for recovery can be used for post-tag holding.
   B. Post-tag holding should be configured so that tagged fish can be released without a net transfer.
2. Allow tagged fish access to the air-water interface throughout the post-tag holding period.
3. The post-tag holding density should not exceed 10 g of fish per L of water.
4. The post-tag holding period should be 18–36 hours, and optimally about 24 hours.
   A. The holding period begins when the last fish is placed into the post-tag holding container.
   B. The holding period ends when fish are removed in preparation for transport or release.
5. Maintain water-quality in post-tag holding containers.
   A. Water temperatures should be maintained within approximately 2°C of the tagging or release water source.
   B. DO concentrations should be near 100 percent saturation.
   C. TDG should not exceed 110 percent saturation.
6. Examine fish to assess condition before release.
   A. Visually examine fish with as little disturbance as possible.
      i. Partially remove the lid of the container to visualize fish.
      ii. Observe fish condition (for example, swimming activity and/or vertical position).
   B. Carefully remove moribund, dead, or poorly performing fish, minimizing disturbance to the remaining fish.

7. Release the tagged fish.
   A. Ensure transmitter function following surgery and water exposure.
   B. Compare water temperatures in the post-tag holding container and at the release location.
      i. If the temperatures are more than 2°C different, mix the water sources until the difference is less than 2°C.
      ii. Tempering should occur at a rate of 0.5°C/15 minutes.
   C. Maintain water-quality and access to the air-water interface during any required transport.
      i. Maintain the water temperature in transport containers within 2°C of the release location.
      ii. Use insulated containers, a refrigerated transport truck, or non-chlorinated ice to prevent significant temperature increases.
      iii. Document water temperature during transport with a thermograph.
      iv. Maintain access to the air-water interface during transport.
   D. Let tagged fish volitionally swim out of the release container.
   E. Record release time for individual tagged fish or groups.

Clean-Up and Disinfection

1. Release, return, or euthanize rejected fish and source fish that were not needed for tagging.
2. Discard tagging solutions following local guidance.
3. Clean surgical instruments and prepare them for the next tagging operation.
   A. Remove all micro scalpel blades and dispose of them in a sharps container.
   B. Discard any partially used suture packets in a sharps container.
   C. If catheters were used, discard the plastic catheter as it cannot be sterilized.
   D. Use a small toothbrush to scrub organic debris off of all surgical instruments.
   E. Wrap instruments in a cloth or place them into an autoclave bag with autoclave tape.
   F. Sterilize instruments in an autoclave and confirm appropriate temperature exposure by visualizing a color change in the autoclave tape or bags.
4. Disinfect all working surfaces and equipment.
   A. Spray countertops, the tagging platform, and any other working surfaces with Virkon® Aquatic. Allow 10 minutes of contact time and rinse with clean water.
   B. Disinfect all water containers regularly. Spray the containers with Virkon® Aquatic, allow 10 minutes of contact time, and rinse with clean water.
5. Clean and disinfect pre-tag and post-tag holding containers regularly.
6. Allow a thorough drying period for all equipment.

Appendix C: SOP Compliance Form

Tagging Procedures SOP Compliance Inspection

Crew: _____ Time:

Inspector: _____

☐ ☐ No ☐ Did not observe

_____

---

2. Were fish held at appropriate densities?
for pre-tag holding: ((mean fish weight(g)) x (number of fish) / (L of water) less than or equal to 20g/L

☐ ☐ ☐

Comments:

Corrective action (if applicable):

---

3. Were transmitters checked to ensure that they were operating prior to implantation?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

Tagging Procedures SOP Compliance Inspection—Continued

4. Were water containers filled with water immediately prior to tagging to prevent loss of water quality?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

5. Were transmitters disinfected in chlorhexidine and rinsed prior to implantation?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

6. Were MS-222® and bicarbonate added to the anesthesia containers resulting in the proper concentration?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

7. Was Stress Coat® used appropriately on surfaces and in containers?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

Tagging Procedures SOP Compliance Inspection—Continued

8. Were sanctuary nets used for netting fish? Was care taken to minimize chasing?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

9. Were lids used on all containers holding fish?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

10. Did staff ensure that all fish in a recovery container had regained equilibrium before moving them to post-tag holding containers?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

11. For dissolved oxygen (DO) and temperature measurements using a YSI-55:

Was the meter on for 15 minutes prior to calibration? ☐ Yes ☐ No ☐ Did not observe

Was the meter calibrated? ☐ Yes ☐ No ☐ Did not observe

Were measurements taken correctly? ☐ Yes ☐ No ☐ Did not observe

(that is, Was the probe continuously moved? Did the values stabilize before being recorded?)

Comments:

Corrective action (if applicable):

Tagging Procedures SOP Compliance Inspection—Continued

12. Were the following water-quality measurements taken:

Temp and DO before tagging in the pre-tag holding area	☐ Yes	☐ No	☐ Did not observe	
Temp and DO after tagging in river near holding containers	☐ Yes	☐ No	☐ Did not observe	
Temp and DO in recovery buckets	☐ Yes	☐ No	☐ Did not observe	

Comments:

Corrective action (if applicable):

13. If water-quality measurements were outside the acceptable range, was corrective action taken?

☐ Yes ☐ No ☐ Did not observe ☐ Readings were within acceptable range

Comments:

Corrective action (if applicable):

14. Were all datasheets filled out correctly?

☐ Yes ☐ No ☐ Did not observe

Comments:

Corrective action (if applicable):

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U.S. Geological Survey
6505 NE 65th Street
Seattle, WA 98115
http://wfrc.usgs.gov/

CRRL-KF IACUC
PROTOCOL REVIEW FORM

Please check: ☒New project
☐3-year renewal of IACUC Protocol # Click or tap here to enter text.
(Please complete this form as well as Appendix B)

Project Title: Monitoring downstream migration behavior and survival of juvenile salmon and steelhead
Principal Investigator: Tobias Kock
Project Dates: March 2024-November 2027
Ideal project start date (target IACUC approval date): 2/21/24
Funding Source(s)**:** US Army Corps of Engineers (ACOE), Upper Columbia United Tribes (UCUT) Yakima Nation (YN), Bureau of Reclamation (BOR), Washington Department of Ecology
Project Partners:
Will any project partners ever handle animals without USGS staff present? ☐ Yes ☒ No

Classification of project: *check all that apply:*

☒ Field Research

☐ Laboratory Research

☐ Other (describe): Click or tap here to enter text.

Animal housing (for animals held > 12 hours, for example in net pens, tanks, etc.): *check all that apply:*

☐ NO HOUSING of animals

☒ Field study sites

☐ Laboratory housing

☐ Other (*describe*): Click or tap here to enter text.

(FOR IACUC USE ONLY, PLEASE DO NOT WRITE BELOW THIS LINE

Pre-review confirmation of completed training:

☐ All project personnel have completed required training.

Verified by: Dorothy Chase

Date: 12/11/2023

CRRL-KF Pain Category: **B** ☐ **C** ☐ **D** ☒ **E** ☐	Initial IACUC Approval: 2/16/2024
Comments: Veterinary evaluation completed bt Michelle Rub, DVM, on 2/14/2024	3-Year Expiration Date: 2/16/2027

Date sent out by e-mail for Committee Review: 12/11/2023
Designated Reviewer: Marty Liedtke approved 2/14/2024

Protocol ID:
2023-08

SECTION I

A. Brief Project Summary: In a few sentences, *and using language understandable to non-scientists, please. respond to the prompts below. Keep your responses brief, as detailed descriptions are requested in other form sections.*

1. *Briefly describe the proposed project and the species and life stages involved.*
   This protocol covers numerous studies that rely on applying the same research techniques to evaluate outmigration behavior and survival of juvenile salmon and steelhead in various river systems of the Pacific Northwest, United States. These studies are focused on parr/smolt life stages for the following species: Chinook salmon, steelhead, coho salmon, and sockeye salmon. All fish are surgically tagged with an active transmitter (radio or acoustic) and most fish also are tagged with a PIT tag. The active transmitter provides the opportunity to monitor fine-scale behavior and survival for weeks/months after tagging and the PIT tag provides the opportunity to monitor general fish movement and survival through the adult life stage.

2. *What are the goals of the project?*
   Salmon management is a high priority in the Pacific Northwest where costs for flow-management decision making and infrastructure improvements aimed at protecting salmon and steelhead populations are commonly in the $100,000s million range. Data collected during studies described in this protocol are imperative for resource managers. These projects are designed to provide accurate and precise measurements of the following:
   - Travel time, migration rate, and migration survival through focal river and reservoir reaches.
   - Approach and egress behavior at high head, run-of-river, and diversion dams
   - Route-specific passage probabilities and survival
   - Behavior at and near prototype fish passage devices

3. *Please BRIEFLY list procedures that may result in animal pain/distress, morbidity, or mortality. Optional: see CRRL-KF guidance on the classification of pain and distress categories (available on IACUC Sharepoint site).*

   - Netting and holding fish in 20 or 30-gallon containers for the pre-tag holding period
   - Manipulative procedures: weighing and measuring
   - Administration of anesthetic
   - Surgical implanting of acoustic transmitters and PIT tags
   - Recovering and post-tag holding inside perforated 5-gallon buckets
   - Releasing streamside from buckets
   - Euthanasia

B. Summary of Procedures: *Provide a description of the proposed animal studies to be conducted over the next 3 years. For each species on the protocol, describe, in simple language, what the animals will experience, step by step. Begin your narration with animal capture if applicable. For complicated experimental designs, a flow*

chart, diagram, or table is recommended. Do not describe the details of the procedures as that narration will be included in Section II (below).

For the studies under this protocol all juvenile salmon and steelhead will be netted and held in perforated 20 or 32-gallon containers that are irrigated with pass-through river water (at fish collection facilities) or that are floating in-river (at sites where fish are collected using screw traps) prior to the tagging procedure. Fish will be anesthetized prior to handling for weighing, measuring, and surgery . Most fish will be implanted with a PIT tag and acoustic or radio tag; fish smaller than ~110 mm total length will not receive a PIT tag. After the surgery, tagged fish are placed into a perforated 5-gallon recovery container (with a lid) and observed periodically during a 10-minute recovery period to confirm they regained equilibrium. Once they are upright, the recovery bucket lid will be tightly secured and the container will be transferred to float inside a tank or in-river holding location for a 18-36 hour recovery period. A small subsample of yearling Chinook will be euthanized to assess key assumptions of survival models that are used during data analysis. Euthanized fish will be tagged, transported and released along with live tagged fish. Following tagging and recovery, the perforated 5-gallon recovery buckets will be moved to transport tanks and transported (30 minutes to 2.5 hours) to release sites. Water quality parameters (water temperature, dissolved oxygen) are closely monitored throughout the entire process (from pre-tag holding to release) and these data are included in final tagging and release datasets that are analyzed for the study.

Table 1. Detailed summary of projects and procedures

Study	Species	Fish Source	Pre-tag holding	Tagging location	Tagging details	Post-tag holding	Transport		Release
							Holding	Duration	
UCUT	Yearling Chinook	Hatchery in tanks	Netted out of tank and placed into 20-30 gallon can on day of tagging with lid	Hatchery building	Anesthetized measured, weighed & surgically implanted with acoustic and PIT tags	Perforated 5 gallon buckets	5 gallon buckets held inside a tank on a truck	30 min-2.5 hrs.	By bucket at rivers edge
Tieton Dam	Yearling Chinook Coho Salmon Sockeye Salmon Subyearling Chinook	Hatchery in tanks or raceway	Netted out of tank and placed into 20-30 gallon can on day of tagging with lid	Hatchery building	Anesthetized, measured, weighed & surgically implanted with acoustic and PIT tags (no PIT tag for subyearling)	Perforated 5 gallon buckets	5 gallon buckets held inside a tank on a truck	2.5 hrs.	By bucket at rivers edge
Lower Yakima River (A)	Yearling Chinook Subyearling Chinook	Collected at in-river fish collection facility & held for study	32 gallon cans with flow through water inside fish facility with lid	Inside fish facility	Anesthetized, measured, weighed & surgically implanted with acoustic and PIT tags (no PIT tag for subyearling)	Perforated 5 gallon buckets	5 gallon buckets held inside a tank on a truck	1 hrs.	By bucket at rivers edge
Lower Yakima River (B)	Steelhead	Collected at in-river fish collection screw trap	20-32 gallon perforated can held in-river	At screw trap along river's edge	Anesthetized measured, weighed & surgically implanted with acoustic and PIT tags	Perforated 5 gallon buckets	5 gallon buckets held inside a tank on a truck	1 hrs.	By bucket at rivers edge

C. *Animal Housing:*

Complete this section if animals will be held more than 12 hours. Describe the holding location, duration, and holding procedures separately for each species in the protocol.

All the fish under this protocol are in the custody of hatchery employees or are collected for us by tribal personnel as fish pass through the in-river collection facility or pass into the screw trap.
For the UCUT and Tieton Dam studies fish will be fed, cared for and in the custody of hatchery employees until USGS arrival on-site for a tagging event. The day before tagging occurs, USGS staff will take custody of the fish and net fish into their pre-tag holding flow-through containers (for a minimum of 12 hours) so fish can evacuate their stomachs prior tagging.

For the Lower Yakima River study, tribal employees will sort and collect fish for us at the Chandler Fish Facility or from the in-river screw trap located on Satus Creek the day before tagging occurs. Tribal staff will place fish in 20 or 32-gallon containers that are irrigated with pass-through river water (at fish collection facility) or floating in-river (at sites where fish are collected using screw traps) where the fish will begin their pre-tag holding time to evacuate their stomachs. We take custody of these fish on the day of tagging after their pre-tag holding period is complete.

Pre-Tag Holding:
Table 1 is provided as an overview of study-specific details (species, pre-tag holding locations, etc.). All projects share the following elements in common: (1) from a non-USGS organization; (2) each species are held separately and placed into 20- or 30-gallon holding containers that float in-river or that are irrigated with flow-through river water; (3) holding densities of ≤20g of fish per L will be maintained throughout the entire process; (4) and fish will be held for 12-36 h prior to tagging to allow stomach evacuation to allow stress levels to normalize after collection. Collection and tagging are done at the same location.

Post-Tag Holding:
The recovery process is the same for all studies and species in this protocol and all fish are held separated by species. Recovery buckets are maintained at holding densities ≤10g of fish per Liter and the dissolved oxygen saturation is maintained between 120 and 150% in a flow-through system, for 10 min after the surgical procedure (SOP-5). After the surgical procedure is completed, fish are placed into perforated 5-gallon recovery buckets, monitored for 10 minutes then transferred to a recovery tank/raceway or to an in-river holding site. All fish will remain undisturbed (18-36 h) until they are loaded for transport to the release location.
The tank/raceway provides flowing water and dissolved oxygen is near 100% saturation. The UCUT hatchery has supplemental bubblers in all tanks that continuously provide air to ensure dissolved oxygen levels are maintained. Tieton and Lower Yakima River studies have large recovery tanks/raceway with continuous flow-through river water.

During the transportation and release stage fish remain in their 5-gallon containers and are transported in a holding tank on a truck. The number of fish we implant per tagging session almost always results in a full capacity transport tank, but if there is extra space, empty recovery buckets are put in to minimize sloshing and movement during the drive. The water temperature and dissolve oxygen are monitored and recorded during the 30 minutes to 2.5-hour transport. Before the release, the water will be tempered at 0.5℃ per 15 minutes if there is a 2℃ temperature difference between the transport tank and release site water.

D. Experience and Training of Project Personnel:

All personnel must fulfill the training requirements outlined in SOP A-1 "CRRL-KF IACUC training requirements". This applies to all USGS staff and all staff from project partners (i.e., non-USGS personnel) handling animals under the protocol. These requirements must be met prior to PRF submission for the primary project staff. Please complete ***APPENDIX A*** *of the PRF, which includes a PI certification and a roster of primary project personnel. The PI should be listed on the roster where personnel experience, duties and training are described.*

- *Note that additional personnel can be added to the project later using a personnel change form. This approach might be useful if not all personnel have been identified or some staff have not completed IACUC training at the time of submission. This form does not require committee review, so can be completed quickly.*
- *Abbreviated training is available for non-USGS animal handlers that conduct minimally invasive procedures under the direct supervision of USGS personnel for no more than 14 days per calendar year*

(See WFRC Policy on Abbreviated Animal Care Training for Shor-term or Intermittent non-USGS Animal Handlers).

Might non-USGS short-term or intermittent animal handlers work under this protocol?

Yes ☒ (complete list of animal handling tasks below)
No ☐

List tasks that short-term or intermittent non-USGS animal handlers might perform (examples might include netting, external exam, measuring, transporting).

- Carrying buckets containing tagged fish

E. *Summary of Animal Numbers:*

Indicate the planned number of animals for the next three years and provide the total numbers in table E.

A. New: Animals to be ordered/purchased/received:			
Species	Sex	Ages	Number
NA	NA	NA	NA

B. Breeding: anticipated number of animals to be born/hatched as part of this protocol:	
Species	Number
NA	NA

C. Transfers: animals to be acquired from another approved protocol:				
Protocol #	Species	Sex	Ages	Number
NA	NA	NA	NA	NA

D. Captures: Anticipated number of animals to be captured: 500-2000 fish tagged/study			
Species	Permitted/year	Estimated captures/year	Total permitted captures (3 years)
Chinook Salmon	5,500	5,000	16,500
Steelhead	500	250	1,500
Coho Salmon	500	250	1,500

*Collection requests typically include a 5-10% buffer to account for rejections based on size and overall health. (Ex. When tagging 100 fish we would request a minimum of 105 to account for fish who do not meet the study criteria)

E. TOTAL:	
Species	Total Number by Species
Chinook Salmon	16,500
Steelhead	1,500
Coho Salmon	1,500

F. Final Disposition of Animals:

Indicate the ultimate disposition of all animals in the study. If multiple dispositions are selected, please indicate the approximate percentage of animals for each disposition.

1. Release: *Describe timing and location of release relative to study activities.*
After their 18-36 hour post-tagging holding time, all fish will be visually inspected to confirm they are in good health and then transported to their designated release site. The release sites for all projects are between 30 minutes to 2.5-hour drive from the tagging site.

2. Retain: *If you plan to retain the animals for future research, when will you submit a protocol for that next research activity? Briefly describe the planned future research activity.*
NA

3. Euthanasia: *Describe the method to be used or cite the appropriate elements from an approved SOP.*

For the small subsample of yearling Chinook Salmon selected to be part of the dead fish release, they will be euthanized using 2-step method outlined in detail in the Euthanasia SOP F-2. First, the fish will be placed in a water bath with a high dose of buffered MS-222 at a dosage of 250-500 mg/L for 30 minutes or longer until operculum movement stops. Then the next step is to pith their brain and severe one side (all four) of the gill arches with scissors.

4. Death as Endpoint: *If the protocol involves observing or studying animals until death occurs provide justification as to why an earlier endpoint is not acceptable. If the animals are collected by lethal means, describe and justify the method.*
NA

5. Other:(*describe any planned disposition not previously addressed***)** NA

G. Replacement, Reduction, and Refinement:

Federal policies require documentation that "the three R's" (replacement, refinement, and reduction) have been addressed. Narration for "Reduction" is addressed in Section J. Please complete each section below.

REPLACEMENT: *discuss what non-animal methods were considered in lieu of using live animals in this study, or why this approach was not feasible.*

Currently there is not a non-animal product on the market that can exactly mimic fish behavior or run timing as fish navigate down a body of water or into a passage structure. Salmon and steelhead each exhibit behaviors that cannot be exactly duplicated with a lower order animal species or using a non-animal method. The data we acquire from each fish that migrates downstream aids researchers on how to enhance fish survival and conservation of the species.

REFINEMENT: *discuss how your procedures have been refined to reduce or eliminate unnecessary pain and distress to the animals in the study.*

- Before any fish handling or surgery, fish are anesthetized using the lowest effective dose of buffered MS-222

- Handling time will be minimized by efficiently collecting only the biometric needed for the study
- Diluted Stress Coat, a mucus restorative solution, is sprayed on all equipment where fish have physical contact and into all water buckets where fish are held
- Surgical tools are sterilized between tagging sessions and soaked in disinfectant solution between individual surgeries. After tagging is complete for the day, all surgical tools are sterilized using the autoclave
- All of the release items (buckets, lids and tanks) are disinfected as needed as long as there is no contact with a different water source than the fish originated from. If another water source makes contact, then the release items are sprayed with disinfectant, set for 15 minutes and then thoroughly rinsed
- To minimize the pathogen load and curb the possible spread of disease at the tagging location, all release and tagging equipment (buckets, nets, pre-tag holding containers, etc.) are regularly disinfected throughout the entirety of the study
- Gravity feed buckets with a lower dose of buffered MS-222 or the option of fresh water is readily accessible during surgery (through a tube in the fish's mouth) to keep fish calm and reduce stress during surgery
- Pre-and post-tag holding densities are monitored as well as dissolved oxygen and water temperature
- During the transportation and release process, transport tanks are double walled and foam insulated to maintain temperature and supplementary oxygen is turned on to compensate for oxygen consumed during transport. The most efficient route is taken to the release site to minimize duration of transport time
- Fish are gently released into the river by slightly submerging the bucket and allowing the fish to swim out
- When euthanizing the fish two methods are used to reduce stress and pain. First the chemical method, overdosing the fish in a buffered MS-222 water bath then followed by two physical methods after operculum movement has stopped

H. Avoiding Unnecessary Duplication of Research:

As part of the 3 R's and consideration of alternatives, the IACUC seeks to avoid unnecessary duplication of previous research. Describe how the proposed study meets this requirement, using a literature search or professional experience with the research objectives, water bodies, and/or species involved.

We work closely with other agencies to confirm there is no duplication of research projects. Most of the monitoring equipment we deploy has the capability of detecting other agency's tags, thus we share data for different telemetry projects, but we do not have duplication of studies. Each study has specific objectives to accomplish with critical questions to answer. Some studies also occur over multiple years as environmental conditions change from year-to-year which allows multi-year comparisons to find data trends.

The UCUT project is a collaboration between the USGS and UCUT Staff, working to reintroduce anadromous salmon and steelhead in the Columbia River basin upstream of Grand Coulee and Chief Joseph dams. This is a multi-year effort study, tagging Chinook salmon to evaluate downstream migration and survival in the Lake Roosevelt, the Spokane River, and the Columbia River in the reach between Grand Coulee and Wells dams. Dam passage will be monitored at three dams on the Spokane River and at two dams on the Columbia River. These studies will help to demonstrate the effect of variable environmental conditions on fish migration and survival as they move downstream.

The USGS is the only entity performing the high-level tagging and equipment deployment at Tieton Dam. Efforts are underway to develop upstream and downstream fish passage at Tieton Dam and this project will aid managers in making informed decisions related to downstream fish passage development. This is the first study to describe behavior and movement patterns in juvenile salmon in the Rimrock Reservoir.

The Lower Yakima River studies are multi-year studies where each year we gain more knowledge about environmental conditions and insight on how juvenile fish utilize the river, pass through dams or into irrigations canals during low and high-water events. Acquiring yearly data is essential to evaluate the overall river system and allow managers to make informed decisions to aid downstream passage on the Yakima River.

I. Alternatives to Procedures That Cause Pain or Distress:

A literature search (or other documentation) is required to determine that less painful/distressful alternatives to your proposed animal use and procedures are not available or feasible. Procedures that follow CRRL-KF approved SOPs are considered supported by other documentation. If ALL animal procedures described in the protocol reference approved SOPs, a literature search is not needed (but SOPs must be cited in text and in Section II on Animal Use Procedures). ANY animal procedures not described in an approved SOP must be supported by a literature search. The search should focus on the procedure and provide evidence that the procedures are the currently accepted methods to meet animal welfare considerations and meet study goals. Alternative procedures that reduce pain/distress are not available to meet study goals.

NOTE: Literature search must be within 30 days of the submission date for the protocol

☒ All animal procedures based on approved SOPs
☐ Literature search was conducted focused on procedures for: Click or tap here to enter text.

Please complete the table summarizing the literature search:

Databases/Sources	Date of Search	Years Searched	Key Words or Strategy
Click to enter text.	Date	Click to enter text.	Click to enter text.
Click to enter text.	Date	Click to enter text.	Click to enter text.
Click to enter text.	Date	Click to enter text.	Click to enter text.
Click to enter text.	Date	Click to enter text.	Click to enter text.

Summary of Literature Search Findings: NA

J. Justification for Animal Numbers Requested:

Provide in detail the method used to determine the number of animals needed over the next 3 years. Inclusion of a Power Analysis is recommended, if preliminary data are available for the necessary calculations. For studies where Power Analysis is not appropriate (e.g., pilot studies, tissue protocols, etc.), provide a narrative describing how requested animal numbers were determined. Be sure to explain how you determined group sizes, the planned groups necessary, etc. This section addresses the "Reduction" requirement. The IACUC is interested in whether meaningful results could be obtained with fewer animals than requested.

Note: The justification that the project has permit authority to handle a certain number of animals does not address the IACUC concerns of reducing the pain/distress experienced by individual animals.

Most rigorous scientific studies rely on power analyses and desired precision targets for specific parameters to identify sample sizes. However, telemetry studies designed to estimate survival are limited

by the extreme cost of transmitters (~$250-400 apiece) and therefore commonly use sample sizes below those that might be suggested by a power analysis. Power analyses are still conducted to determine "ideal" sample sizes, but ultimately, the actual number of tagged individuals is less than ideal and based on funding availability. We would need to tag several thousand fish per study to achieve the desired precision targets. Therefore, we tag as many individuals as they can afford and deal with larger-than-desired confidence intervals on survival estimates.

In addition to sample size, confidence interval size is affected by other factors such as detection probabilities of monitoring sites. Reduced detection probability increases the confidence intervals because it effectively reduces the sample size of detected fish. We are meticulous in deploying and maintaining each site during the studies to maximize detection probabilities, to generate the most data from each tagged individual. Detection probabilities at our sites commonly exceed 95% which is exceptionally high relative to other telemetry studies.

SECTION II

Procedures:

Mark the box ("yes" or "no") for each listed procedure. Complete each question for all procedures marked "yes". If a specific part of that procedure is not applicable to your project, indicate "NA".

See the list of approved Standard Operating Procedures (SOP's) on the CRRL-KF IACUC SharePoint site and cite them to document planned procedures. Adherence to the listed SOP's will facilitate review and approval of protocols.

If procedures vary by species, describe procedures specific to each species.

 # Yes No

1. ☐ ☒ ***Capture:***

Capture method (make selections):

☐ ***Angling***

a. *Describe methodology including platform (vessel, shore) and gear (hook type):*

b. *Approximate duration that fish will be engaged with the gear:*

c*. Total number of lines/hooks and anglers engaged in the collection effort:*

d. *How will non-target species be handled?*

☐***Electrofishing***

a. *Describe methodology.*

b. *How will non-target species be handled?*

☐***Active netting*** (net is attended at all times and fish interaction with net is generally brief, examples include seining such as beach, lampara or purse).

a. *Describe net material (mesh size), net size, and deployment methodology.*

b. *Approximate duration fish will be engaged with the gear:*

c. *Does the net have an area designed for fish collection (i.e., bag end or sanctuary box)?*

Yes, we use a sanctuary net which allows to be transferred in a small amount of water

d. *Describe catch processing procedures.*

e. *How will non-target species be handled?*

☐***Passive netting_trapping*** (net or trap is set and left unattended for a "soak" period, examples include gill netting, rotary screw trapping).

a. *Describe net material (mesh size), net size, and deployment methodology.*

b. *Planned net soak time and approximate duration fish will be engaged with the gear:*

c. *Does the net have an area designed for fish collection (i.e., bag end or sanctuary box)?*

d. *Describe catch processing procedures.*

e. *How will non-target species be handled?*

☐***Other (describe collection procedures)***

2. ☒ ☐ ***Anesthesia:***

a. *What parameters will be monitored to ensure adequate anesthesia (e.g., reparation rate, heart rate, loss of equilibrium, corneal reflex, etc.)?*

Loss of equilibrium (SOP F-1_Anethesia_17 Oct 2023.pdf)

b. *If fish will be released back into the wild, is selected drug approved for use in this species and life stage and are appropriate drug withdrawal times being observed?*

Yes, all of the juvenile fish in this protocol will be released back into the wild. We are using Finquel (MS-222), an approved drug for the life stage and species listed here. Yes, the appropriate drug withdrawal time is observed and the juvenile salmon life stage in this protocol is not a desired size for anglers

c. *Describe procedures and complete table:*

Animal Species	Anesthetic Agent or Method	Dose	Route	Procedure (e.g., handling, tagging)
Chinook Salmon	Buffered MS-222	50-85 mg/L depending on fish size and water temperature.	Anesthetic bath	All live fish handling, measuring, and sampling procedures (length, weight, tagging).
Steelhead	Buffered MS-222	50-85 mg/L depending on fish size and water temperature.	Anesthetic bath	All live fish handling, measuring, and sampling procedures (length, weight, tagging).
Sockeye Salmon	Buffered MS-222	50-85 mg/L depending on fish size and water temperature	Anesthetic bath	All live fish handling, measuring, and sampling procedures (length, weight, tagging).
Coho Salmon	Buffered MS-222	50-85 mg/L depending on fish size and water temperature	Anesthetic bath	All live fish handling, measuring, and sampling procedures (length, weight, tagging).

3. ☐ ☒ ***Non-lethal tissue sampling or removal (e.g., blood sample, fin clip, opercular punch, scales, fin rays):***

a. *Tissue to be collected:*
b. *Timepoint for collection*:
c. *Tissue size or blood volume to be removed*:
d. *Frequency of sampling*:

4. ☐ ☒ ***Imaging procedures (photographs, radiographs, ultrasound, etc.):***

a. *Type of procedure:*
b. *Frequency:*
c. *Potential effects on animals:*

5. ☒ ☐ ***Implanted tags (e.g., passive integrated transponder, acoustic, radio, etc.):***

a *Type of tag:*
- ATS SS300
- ATS SS400
- PIT Tag- 12mm

b. *Tag dimensions and weight:*
- SS300 = 430 mg, 11.9 x 6.3 x 3.7 mm
- SS400 = 210 mg, 15.0 x 3.3 mm
- PIT= 0.11 g, 12.5 x 2.03 mm

c. *Maximum tag burden (ratio of weight of tag to weight of fish, expressed as %):*
5%

d. *Implantation technique: (select one)*

☐ Insertion via syringe or scalpel puncture, with no wound closure

☒ Surgical incision, tag insertion and wound closure (sutures, etc.)

☐ Gastric insertion into the stomach

e. *Describe procedure(s) or cite approved SOP:*

Approved SOP F-5_ Surgical implantation of transmitters for juv salmon_17 Oct 2023.pdf

f. *Pre-operative protocol (e.g., food/water restriction, animal prep):*
All fish will have food withheld for 12-36 hours prior to tagging for stomach evacuation and limited disturbance to reduce stress.

g. *Aseptic precautions (if applicable, include method of instrument sterilization/disinfection prior to initial use and between animals):*
Following SOP F-5, all surgical tools will be sterilized via autoclave prior to starting each tagging session. In between each fish surgery, all surgical tools and suture will soak in a chlorhexidine solution for a minimum of 15 minutes and rinsed prior to use. Prior to each daily tagging session, the acoustic transmitters are soaked in a chlorhexidine solution for 15 minutes and the PIT tags are disinfected using 80% isopropyl alcohol, both tags are then rinsed using de-ionized/distilled water. During the entire surgical process, the tagger wears nitrile gloves.

h. *Supportive care during procedure (e.g., irrigation, resuscitation):*
Following SOP F-1 and SOP F-5, each individual fish will be directly observed during the entire tagging process. If a fish were to drift too deep under anesthesia, surgeons can provide freshwater, immediately, in a gravity fed system to aid the fish in supportive care. After the procedure is completed, the fish is placed in their perforated holding bucket with a lid, where they will recover and periodically be observed for 10 minutes to confirm they have regained equilibrium. They will remain in the same perforated recovery bucket until released to reduce stress.

i. Typical d*uration of procedure:*
The entire process from the time the fish is placed in the anesthetic bath to the time it's placed in the recovery bucket is somewhere between 4.5-8 minutes. The induction time is around 3:30 minutes (between 2-4 min.) with the average surgery length being between 1:30-2:45 minutes depending on how many sutures are being tied and the experience of the tagger.

j. *Type and size of suture material if used (if applicable):*
Ethicon coated Vicryl Plus antibacterial suture with a taper RB-1 needle.
We use the 5-0 size for smaller sized fish (usually the Chinook, sockeye and coho) & 4-0 for larger sized fish (steelhead).

k. *Post-procedure monitoring protocol (include the duration of the monitoring period):*

We will follow the holding procedure from SOP F-5 for all studies. Fish will be placed inside a perforated 5-gallon recovery bucket where they will be monitored as they regain equilibrium for 10 minutes or longer if needed. A lid will be placed on each bucket and placed in a large flow-through holding tank/raceway or in-river where the fish will recover 18-36 hours prior to transportation to the release site.
Each holding bucket will only contain the same species.

l. *Will the animal be released into the wild following tag implantation? Briefly describe.*
Yes, once the post-tag holding period is complete, fish will be released into the wild. Following the SOP F-5, after the post-tagging holding period is complete, USGS employees fill up a large transport tank that has been placed in the bed of a pickup truck (or on a flatbed trailer), with the same water source the fish are currently in. Each transport tank is dual-walled, foam insulated and is accompanied with a bottle of oxygen, tubing and a 3–4-foot oxygen sock to disperse oxygen throughout the tank of water during transport. On the day of release, the tank is filled up, oxygen is turned on and all water quality data is written down on the datasheet. Recovery buckets are removed from the holding tank(s), lids briefly removed to visually inspected for mortality and overall condition, and then loaded into the transport tank. Once the transport tank is full of floating recovery buckets, the lid is secured on with a rachet strap and we drive to the release location. Depending how long the drive is multiple water quality stops may be needed. For all projects, the release sites are between 30 minutes and 2.5-hour drive from the tagging location. Upon arrival at the release location, water quality is done to confirm water temperature is within 2° C between the two water sources (the transport tank compared to the river/stream). If the water temperature difference is greater than 2°C, staff would blend the 2 water sources at a rate of 0.5°C every 15 minutes until the temperature is within the 2°C, recording the data on the datasheet. When it is time for release, buckets are removed from the transport tank, carried into the river flow, the lid is removed, and the bucket is slightly submerged so the fish can volitionally swim out of the holding bucket. Some release locations require a boat to transport buckets of tagged fish due to inaccessibility or predetermined location. For all studies the time of release is recorded on the datasheet.

m. *Will the animal be physically re-captured after release? (if yes, for what purpose?)*
No

n. *Deficits that may occur as a result of the procedure:*

We strive to follow all the procedures and SOP's, but sometimes there are deficits that occur. Since all our tagged fish are released back into the wild there is always the risk of infection or fungus at the incision site. Each fish responds to the tagging procedure differently so the time each fish needs to adjust their buoyance after the surgical tag implantation could impact the initial fish behavior with the additional weight of the transmitters. There is also the possibility of increased predation during the release due to repeated use and disturbance at the release sites.

6. ☐ ☒ ***Marking other than implanted tags (e.g., floy tag, coded wire tag, visual elastomer tag):***

a. Type of tag:
b. Describe procedure(s)
c. Typical duration of procedure:

7. ☐ ☒ ***Special diet (e.g., high fat, etc.):***

a. *Composition of diet:*
b. *Amount/feeding rate:*
c. *Duration:*
d. *Anticipated side effects (e.g., anticipated % weight loss or gain, dehydration, etc.):*

8. ☐ ☒ ***Food restriction of 48 hours or more:***

a. *Duration of food restriction:*
b. *Anticipated side effects (e.g., anticipated % weight loss, dehydration, etc.):*
c. *What parameters will be monitored, and how often will animals be monitored for health and well-being:*
d. *Scientific justification for restriction:*

9. ☒ ☐ ***Non-physical euthanasia or physical euthanasia on anesthetized animals:***

a. *Agent (drug, dose, route) for non-physical method:*

- Drug: buffered MS-222
- Dosage 250-500 mg/L
- Route: anesthetic bath

b. *Physical method for anesthetized animals:*
Following the SOP F-2, fish slated to be euthanized will have their large branchial vessels cut (gill arches) and they will also be pithed with a sharp tool. We have a datasheet specifically used for documenting this process.

c. *Criteria used to decide upon euthanasia (e.g., illness, tissue harvest, end of study, etc.):*
One assumption of telemetry survival studies is that the status of the smolt (i.e., alive or dead) is correctly assessed. Dead fish drifting downstream could result in false-positive detections, positively biasing survival estimates. Therefore, releases of dead tagged fish are made to validate this assumption. This will only be done for a small sample of randomly selected Chinook salmon in the UCUT study. All collection, holding, tagging and release procedures for the euthanized fish match that of fish released live.

d. *Scientific justification for methods not approved by the AVMA Panel on Euthanasia (JAVMA 218:669-696, 2000):*
None

10. ☐ ☒ ***Physical Euthanasia without anesthesia:***

a. *Method:*

b. *As per AVMA Panel on Euthanasia recommendation, scientific justification is required:*

c. *Personnel performing procedures must be certified by Veterinary Officer:*

1. *Personnel:*
2. *Date of certification:*

d. *Criteria used to decide upon euthanasia (e.g., illness, tissue harvest, end of study, etc.):*

11. ☐ ☒ ***Other procedures not listed elsewhere (such as survival surgery for other than tag implantation). Please complete Appendix C.***

PRF SUBMISSION CHECKLIST:

☐ All project personnel listed on Appendix A have completed IACUC training prior to submission.

☐ **Appendix A** has been completed, with all required signatures. Appendix A is REQUIRED.

☐ **Appendix B** has been completed if this is a 3-year renewal of a previously approved protocol.

☐ **Appendix C** has been completed if procedures in Section II, Item 11 will be used.

CRRL-KF
Institutional Animal Care and Use Committee

Protocol Review Form APPENDIX A
PI Certification and Project Personnel Roster

IACUC Protocol PI: Tobias Kock

IACUC Protocol Title: General protocol for acoustic studies monitoring downstream migration behavio

Principal Investigator Certification Statement:

The Principal Investigator (PI) is the person with responsibility and authority for the research activities being conducted, and oversight of the staff, volunteers, and students participating in the work.

- The PI certifies that this Protocol Review Form accurately describes all aspects of the proposed animal use for this project.
- The PI affirms that this proposal does not unnecessarily duplicate previous research.
- The PI ensures that all work proposed herein is designed to avoid discomfort, distress, and pain to animals to the extent possible; and the alternatives (replacement, reduction, and refinement) have been considered.
- The PI understands that any unauthorized use of animals by personnel for this project may constitute grounds for scientific misconduct.
- The PI accepts responsibility that all personnel working on the project will adhere to this protocol, the regulations regarding the humane treatment of laboratory animals, and will receive proper training as required by the IACUC.
- The PI acknowledges that emergency care, including euthanasia, may be administered at the discretion of the veterinary staff.
- The PI agrees to obtain approval prior to instituting any significant changes to the project.
- The PI understands that the IACUC can require changes to the protocol and that approval to conduct this work is not final until I receive notification of such in writing.
- The PI understands that approval of projects is for a maximum of three years **from the date of IACUC approval** and that I will need to apply for a renewal after this time.

TOBIAS KOCK

Digitally signed by TOBIAS KOCK
Date: 2023.12.05 09:34:09 -08'00'

Project Personnel Roster: please complete the table.

Animal Use Personnel Certification Statement

I certify that I have **read the Protocol Review Form,** have **completed the required training** per SOP A-1, and that I will **only perform procedures that have been approved by the IACUC**. I understand that any Significant Changes in procedures must be approved by the IACUC prior to implementation.

Name	Duties	Years of Experience	Signature and Date
Gabriel Hansen	Fish collection, handling, anesthesia, euthanasia	22	GABRIEL HANSEN Digitally signed by GABRIEL HANSEN Date: 2023.12.05 10:37:25 -08'00'
Jamie Sprando	Fish collection, handling, anesthesia, euthanasia, surgeon	23	JAMIE SPRANDO Digitally signed by JAMIE SPRANDO Date: 2023.12.05 10:31:06 -08'00'

(CRRL-KF ANIMAL CARE COMMITTEE USE ONLY - DO NOTWRITE BELOW THIS LINE)

Training Requirements are up to date: ■

Initial Reviewer(s): D. Chase Review Date: 12/11/2023 Comments:

Use of passive integrated transponder tags to estimate survival of migrant juvenile salmonids in the Snake and Columbia rivers

John . Skalski, Steven G. Smith, Robert N. Iwamoto, John G. Williams, and Annette Hoffmann

Abstract: Single-release and modified single-release statistical models were evaluated as means to generate reliable survival estimates from release–recapture studies of migrant salmonid smolts in the Snake and Columbia rivers of the northwestern United States. Monte Carlo simulation studies were used to assess robustness of estimation methods to violations of model assumptions. To field test model assumptions, passive integrated transponder tagged chinook salmon (*Oncorhynchus tshawytscha*) smolts were released on seven consecutive days in 1993 above Lower Granite Dam on the Snake River. These releases were used to estimate sampling variability of survival estimates for comparison with model-based variance estimates and to assess mixing of detected and nondetected individuals. Field results satisfied model assumptions. The average survival estimate from point of release to the tailrace of Lower Granite Dam (31 km) was 0.902 ± 0.004 (mean ± SE). From the tailrace of Lower Granite Dam to the tailrace of Little Goose Dam (60 km) the average survival estimate was 0.859 ± 0.013.

Résumé : On a évalué l'efficacité de modèles statistiques de lâcher simple et de lâcher simple modifié pour produire des estimations fiables de la survie à partir d'études de lâcher–recapture de smolts en migration dans la rivière Snake et le fleuve Columbia, dans le nord-ouest des États-Unis. On s'est servi de la méthode de Monte Carlo pour évaluer la robustesse des méthodes d'estimation dans les cas où les hypothèses du modèle ne sont pas respectées. Afin de mettre ces hypothèses à l'essai sur le terrain, des saumons quinnats (*Oncorhynchus tshawytscha*) étiquetés à l'aide d'une marque transpondeur intégré passif ont été libérés pendant 7 jours consécutifs en 1993 en amont du barrage Lower Granite, sur la rivière Snake. On s'est servi de ces lâchers pour estimer la variabilité due à l'échantillonnage dans les estimations de la survie afin de les comparer aux estimations de la variance fondées sur le modèle et d'évaluer le mélange des individus détectés et non détectés. Les résultats obtenus sur le terrain correspondaient aux hypothèses du modèle. L'estimation de la survie moyenne entre le point de lâcher et le canal de fuite du barrage Lower Granite (31 km) était 0,902 ± 0,004 (moyenne ± ET). Entre le canal de fuite du barrage Lower Granite et celui du barrage Little Goose (60 km), l'estimation de la survie moyenne était 0,859 ± 0,013. [Traduit par la Rédaction]

Introduction

In the middle to late 1980s, the National Marine Fisheries Service (NMFS) developed a miniature passive integrated transponder (PIT) tag that, when implanted in the body cavity of juvenile fish, allows for unique identification of individuals (Prentice et al. 1990*a*, 1990*b*, 1990*c*). For migrant juvenile salmonids PIT-tagged in the Snake River Basin, detectors automatically decode the tags as fish pass through juvenile fish collection facilities at selected hydroelectric dams (Fig. 1).

In 1988, the University of Washington (UW) began to develop statistical theory and software to estimate survival probabilities and assess survival relationships from data generated by release and detection of PIT-tagged fish in the Snake and Columbia rivers. From this work was developed the statistical software SURPH.1 (Smith et al. 1994) used in the studies described herein. In 1993, these technologies came together in a joint NMFS–UW pilot study of smolt survival in the Snake River (Iwamoto et al. 1994). This collaboration of electronic technology, statistical theory, and fisheries biology to devise an effective system for information gathering and analysis is a first in the science of animal-tagging studies.

This paper describes the statistical models used to analyze survival studies with PIT-tagged fish, their assumptions, sampling precision, and robustness to model violations. Further, we describe the field methods used in 1993 to collect, PIT-tag, and release migrant juvenile salmonids in the Snake River to gather data for the models. Monte Carlo simulation results are presented, along with the results of seven replicate releases in 1993, to determine the validity of the estimation procedures. The PIT-tag studies in 1993 also provided some of the first survival estimates for juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Snake River since a summary of survival estimates in the 1970s was made by Sims and Ossiander (1981). We discuss extension of these technologies to the investigation, mitigation, and recovery of salmonid stocks in the Snake–Columbia River Basin.

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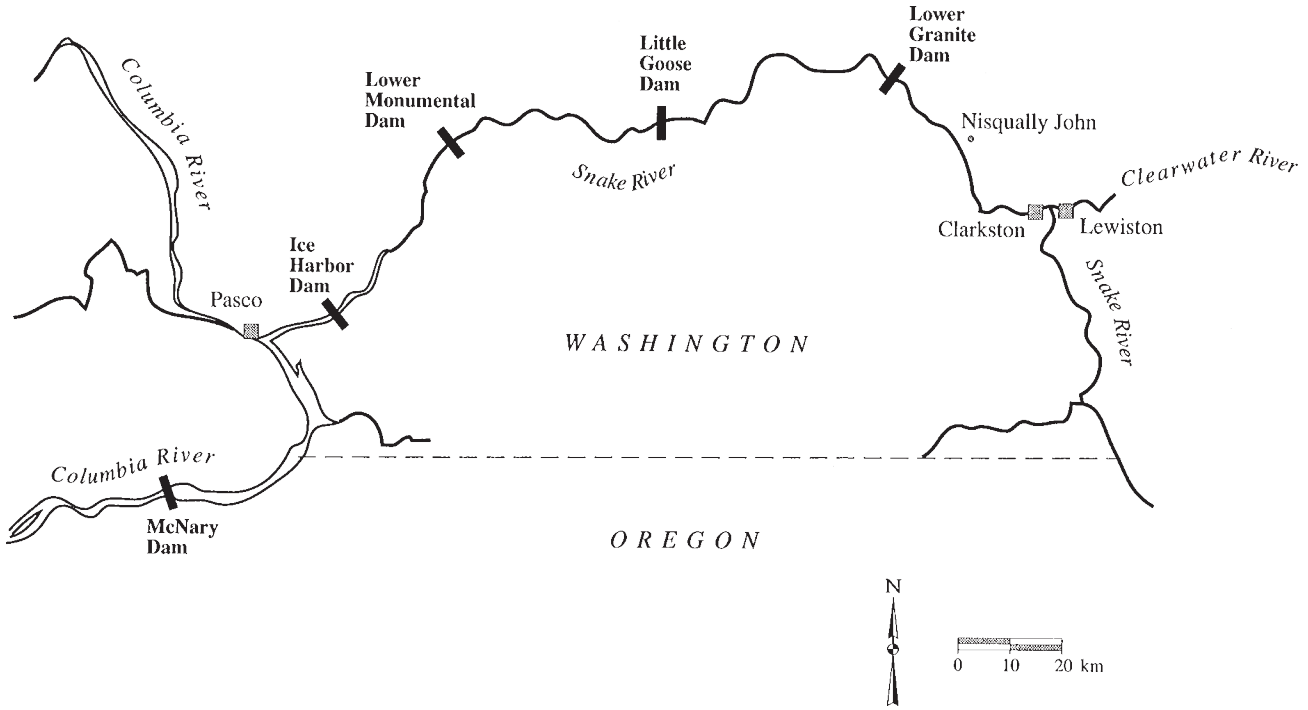
J.R. Skalski. School of Fisheries, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, WA 98101-2509, U.S.A.
S.G. Smith, R.N. Iwamoto, and J.G. Williams. Northwest Fisheries Science Center, National Marine Fisheries Service, 2725 Montlake Boulevard E, Seattle, WA 98112, U.S.A.
A. Hoffmann.[1] School of Fisheries, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, WA 98101-2509, U.S.A.

[1] Current address: Washington Department of Fish and Wildlife, 600 Capitol Way N, Olympia, WA 99504, U.S.A.

Fig. 1. Study area showing release and detection sites.



Materials and methods

Field methods

Study area

PIT-tagged fish were released in Lower Granite Reservoir near Nisqually John boat landing (river kilometer (Rkm) 726), at Lower Granite Dam (Rkm 695), and at Little Goose Dam (Rkm 635) on the Snake River in Washington State. PIT-tagged fish were detected at Lower Granite Dam, Little Goose Dam, and Lower Monumental Dam (Rkm 589) on the Snake River and at McNary Dam (Rkm 470) on the Columbia River (Fig. 1). Lower Granite and Little Goose dams also had facilities to return PIT-tagged fish detected in the bypass systems back into the river in the tailrace (Fig. 2).

Survey design

The primary objective of the field study was to estimate smolt survival from Nisqually John boat landing to the tailrace of Lower Granite Dam (S_1) and survival from the tailrace of Lower Granite Dam to the tailrace of Little Goose Dam (S_2). A secondary objective was to estimate survival from the point of detection in the bypass system to the point in the tailrace at each dam where detected fish remixed with nondetected fish ($\hat{\tau}_2$,$\hat{\tau}_3$) (Fig. 2). Primary release groups (R_1) consisted of hatchery-reared yearling chinook salmon captured by purse seine in Lower Granite Reservoir and PIT-tagged near the Nisqually John boat landing (Fig. 2). There was one primary release per day for seven consecutive days. Daily releases ranged from 797 to 1405 tagged fish, depending on availability. Capture histories from each group were the basis for estimating survival in the river sections above Little Goose Dam (Table 1).

Secondary, paired releases of PIT-tagged fish were made at Lower Granite (R_{B2}, C_{B2}) and Little Goose Dams (R_{B3}, C_{B3}) to investigate potential mortality of fish returned to the river after detection. If such mortality occurs in the bypass system, it could bias estimates of reach survival obtained from the primary releases. Test groups were released in the bypass system at the juvenile collection facilities, just downstream from the PIT-tag detector. Control groups were released in the river below the dam at a point where bypassed fish remixed with fish that passed via turbines or spillways. Each paired release was replicated three times at each dam (Table 2).

Release and detection data were transmitted to the PIT-tag information system (PTAGIS) for later retrieval and analysis. PTAGIS is a computer database developed for the Columbia River Basin and managed by the Pacific States Marine Fisheries Commission (PIT Tag Operations Center, 45 SE 82nd Drive, Suite 100, Gladstone, OR 97207, U.S.A.).

Fish collection and handling

For the primary release groups in Lower Granite Reservoir, fish were collected using two purse-seine vessels (Durkin and Park 1967). Purse seines were approximately 229 m long and 11 m deep with 1- to 2-cm webbing (stretch measure). Effective fishing depth was about 6 m. Seines were towed upstream in a U shape for 10–30 min prior to pursing. For the secondary releases, fish were obtained from the juvenile collection facilities at the respective dams.

Only hatchery-reared yearling chinook salmon, determined by the absence of either adipose or ventral fins (clipped at hatchery), were used in this study. Fish with extreme injuries, excessive descaling, or obvious bacterial kidney disease (BKD) symptoms were excluded, as were previously PIT-tagged fish (identified by scanning with a PIT-tag detector). During sorting and marking, fish were kept anesthetized with tricaine methanesulfonate (MS-222) in a recirculating anesthetic system at a dosage of approximately 50 ppm.

Fish were PIT-tagged using modified hypodermic syringes containing a push rod, terminal air hole, and 12-gauge needle (Prentice et al. 1990c; Nielsen 1992). The PIT-tag needle was inserted alongside the midventral line between the ventral and pelvic fins, and the tag was placed into the body cavity posterior to the pyloric caeca (Prentice et al. 1990*c*). Studies that have looked at the effects of PIT-tagging on salmon smolt growth or survival have found no significant effects (Prentice et al. 1987, 1990*a*; Prentice 1990). The small entry wound tends to heal quickly.

Smolts used in the primary releases (i.e., R_1) were kept in net-pens (1.8 × 0.9 × 0.7 m) for 32–54 h prior to release. Mortalities were

removed, scanned, and recorded prior to release. Smolts used to estimate post-detection bypass mortality were held in aluminum tanks with flow-through water at the dams. These smolts were generally held for at least 24 h prior to release with all mortalities recorded and counts adjusted accordingly.

Detection of PIT-tagged fish

At each dam, a variety of passage routes were available for migrating smolt. Fish could either pass over the spillway or enter the powerhouse (passage through navigation locks is negligible). The powerhouses of each dam were equipped with screening devices to guide fish away from turbine intakes and into "juvenile bypass systems," that have historically been used to collect fish to be transported downstream in barges or trucks. Not all fish were successfully guided and consequently some passed through turbines. PIT-tag detectors were installed only in the juvenile bypass systems. As a PIT-tagged fish passed through a detector, the tag code was recorded automatically (the fish was not handled). At Lower Granite and Little Goose Dams, the PIT-tag detector triggered a "slide gate" to divert the fish away from the transportation collection facilities and back into the river. However, not all detected fish were successfully diverted back to the river. Nondiverted fish were transported, and their capture histories were subsequently censored at that point.

Statistical methods

Single-release (SR) model

Survival probabilities through a section of a river or reservoir can be estimated using a single release of tagged fish upstream with multiple detection sites downstream. The minimal design configuration is an upstream release site and two downstream detection sites with the uppermost detection facility capable of returning detected fish to the river. In 1993, the Snake–Columbia River study used four detection sites. The two uppermost sites (Lower Granite and Little Goose dams) had both detection and rerelease capabilities and the two lower sites (Lower Monumental and McNary dams) had only detection and removal capabilities. For purposes of data analysis, detections at Lower Monumental and McNary Dams were not differentiated. The single release–recapture (SR) model was first presented by Cormack (1964), Jolly (1965), and Seber (1965). This model is also a special case of paired-release models using complete capture histories introduced, but not fully developed in its own right, by Burnham et al. (1987). The single release–recapture design is a special case of designs that can readily be analyzed using the interactive computer program SURPH.1 (Smith et al. 1994).

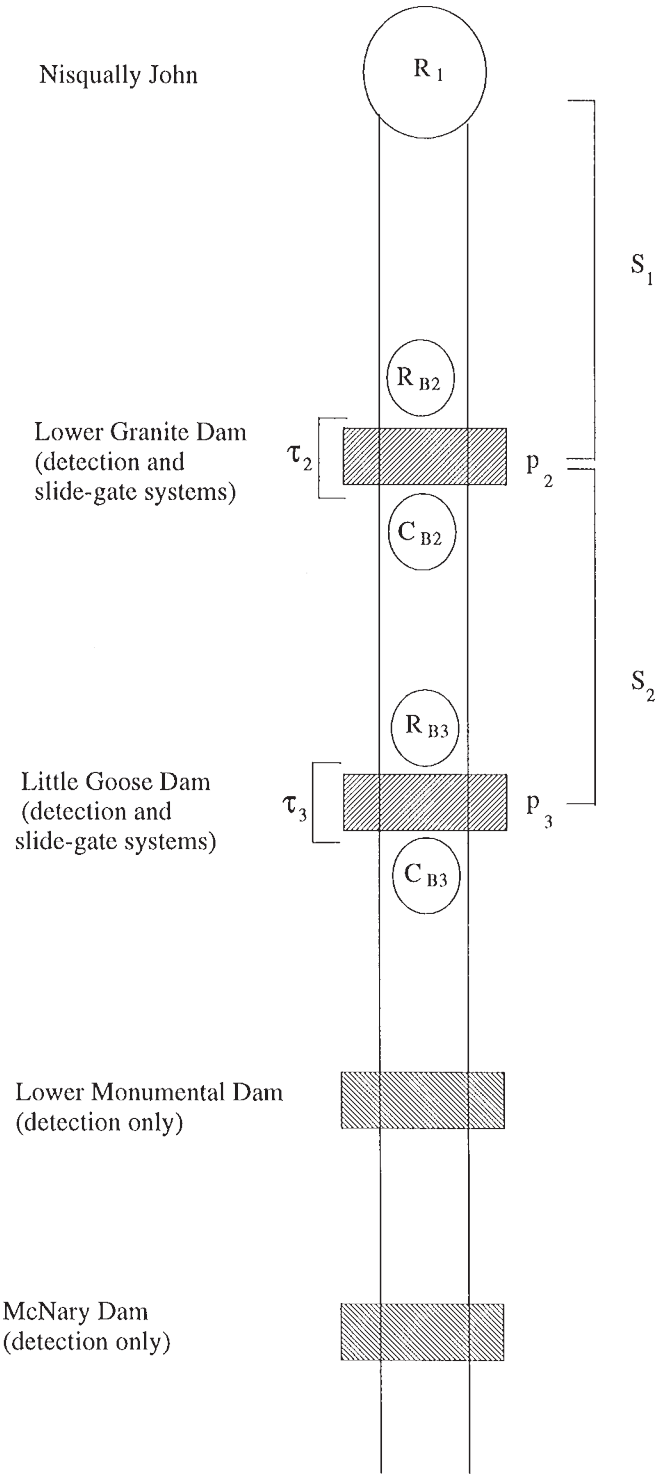
The likelihood model (SR model) for each single release of the 1993 Snake River survival study can be written as follows:

(1) $$L(S,p,\lambda|R,m_1,m_2,m_3)=$$

$$\binom{R_1}{m_{12},m_{13},m_{14}}(S_1p_2)^{m_{12}}(S_1(1-p_2)S_2p_3)^{m_{13}}$$
$$\times(S_1(1-p_2)S_2(1-p_3)\lambda)^{m_{14}}$$
$$\times(1-S_1(p_2+(1-p_2)\lambda+(1-p_2)S_2(1-p_3)\lambda))^{R_1-r_1}$$
$$\times\binom{R_2}{m_{23},m_{24}}(S_2p_3)^{m_{23}}(S_2(1-p_3)\lambda)^{m_{24}}$$
$$\times(1-S_2(p_3+(1-p_3)\lambda))^{R_2-r_2}\binom{R_3}{m_{34}}\lambda^{m_{34}}(1-\lambda)^{R_3-r_3}$$

where R_1 is the number of PIT-tagged fish released above Lower Granite Dam; R_2 is the number of PIT-tagged fish detected and rereleased at Lower Granite Dam; R_3 is the number of PIT-tagged fish detected and rereleased at Little Goose Dam; m_{ij} is the number of fish released at the ith site ($i = 1, 2, 3$) first detected at the jth detection site ($j = i+1,..., 4$); $r_i = \Sigma^4 \; m_{ij}$, for $i = 1, 2, 3$; S_1 is the survival probability

Fig. 2. Schematic of release locations, detection, and slide-gate rerelease facilities used to estimate survival (S), capture rates (p), and post-detection survival (τ).



from point of release to tailrace of Lower Granite Dam; S_2 is the survival probability from Lower Granite tailrace to tailrace of Little Goose Dam; p_1 is the detection probability at Lower Granite Dam; p_2 is the detection probability at Little Goose Dam; and λ is the joint probability of surviving from tailrace of Little Goose Dam and being detected at either Lower Monumental Dam or McNary Dam.

Table 1. Number of fish in each primary release group and their associated capture histories (0, not detected; 1, detected and released; 2, detected and removed).

Capture history	Release group and date						
	R_{11} 15 April	R_{12} 16 April	R_{13} 17 April	R_{14} 18 April	R_{15} 19 April	R_{16} 20 April	R_{17} 21 April
1 1 1	66	118	91	74	75	52	96
0 1 1	111	146	128	120	86	69	97
1 0 1	81	119	100	106	107	82	130
0 0 1	129	142	132	160	136	109	171
1 1 0	46	52	33	42	33	28	53
0 1 0	70	93	66	48	50	36	80
1 0 0	87	110	103	114	115	67	156
0 0 0	223	280	259	296	260	177	351
2 0 0	148	174	171	182	204	133	226
0 2 0	54	71	69	66	47	44	45
1 2 0	30	44	36	27	29	23	43
Total	1015	1305	1152	1208	1113	797	1405

Table 2. Number of fish released (i.e., R_{B2} and C_{B2} or R_{B3} and C_{B3}) and subsequent capture histories for each pair of replicate releases (0, not detected; 1, detected and released; 2, detected and removed).

(A) Lower Granite Dam.

Capture history	28 April		30 April		12 May	
	R_{B2}	C_{B2}	R_{B2}	C_{B2}	R_{B2}	C_{B2}
1 1	132	155	107	152	15	20
0 1	155	189	193	189	137	129
1 0	79	85	99	82	65	81
0 0	287	263	250	260	525	501
2 0	75	68	47	59	6	12
Total	728	760	696	742	748	743

(B) Little Goose Dam.

Capture history	7 May		8 May		13 May	
	R_{B3}	C_{B3}	R_{B3}	C_{B3}	R_{B3}	C_{B3}
1	273	413	452	423	117	98
0	186	279	285	307	634	637
Total	459	692	737	730	751	735

When all fish detected at a dam are rereleased (i.e., $R_2 = m_{12}$, $R_3 = m_{13} + m_{23}$), the statistical model can be simplified.

Formulae for the parameter estimates are simplified by defining the following variables:

$$m_j = \sum_{i=1}^{j-1} m_{ij}, \qquad \text{for } j = 2 \text{ and } 3$$

(3) $$\widehat{\mathrm{Var}}(\hat{S}_1 \mid S_1) = (S_1)^2\left[\frac{1}{r_1} - \frac{1}{R_1} + (1-p_2)^2\left(\frac{1}{r} - \frac{1}{R}\right)\right] + (S_1)^2(1-p_2)^2\left(1 - \frac{r_2}{R_2}\right)^2 \frac{m_2}{z_2 T_2}$$

(4) $$S_2 = \frac{r_2}{R_2}\left[\frac{(m_3 - z_3 R_3)}{T_2} + \overline{T r}\right]$$

with

(5) $$\widehat{\mathrm{Var}}(\hat{S}_2 \mid S_2) = (S_2)^2\left[\frac{1}{r_2} - \frac{1}{R_2} + (1-p_3)^2\left(\frac{1}{r_3} - \frac{1}{R_3}\right)\right] + (S_2)^2(1-p_3)^2\left(1 - \frac{r_3}{R_3}\right)^2 \frac{m_3}{z_3 T_3}$$

while

(6) $$\hat{p}_2 = \frac{m_2}{m_2 + z_2 R_2 / r_2}$$

(7) $$\hat{p}_3 = \frac{m_3}{m_3 + z_3 R_3 / r_3}$$

and

(8) $$\hat{\lambda} = \frac{r_3}{R_3}$$

$$z_j = \sum_{i=1}^{j-1} \sum_{k=j+1}^{4} m_{ij}, \qquad \text{for } j = 2 \text{ and } 3$$

$$T_j = m_j + z_j, \qquad \text{for } j = 2 \text{ and } 3$$

The parameter estimates and associated variances can be written as follows:

$$(2)\qquad S_1 = \frac{r_1}{R}\left(\frac{m_2}{T} + \frac{r_2 R_2}{r_1 r_2}\right)$$

with

These estimators were first derived by Cormack (1964) and later by Burnham et al. (1987, pp. 112–116). Approximate 95% confidence interval estimates for survival probabilities (S_k) can be calculated according to the formula:

$$(9)\qquad 95\%\ \mathrm{CI} = S_k \pm 1.96\,(\mathrm{var}(S_k))^{1/2}$$

Although we used the standard formula (eq. 9), normality assumptions could be avoided by using profile likelihood methods (Hudson 1971).

Assumptions associated with the SR model are as follows:

(A1) The test fish are representative of the population of inference.
(A2) Test conditions are representative of the conditions of interest.
(A3) The number of fish released is exactly known.
(A4) PIT-tag codes are accurately recorded at the time of tagging and at all detection sites.
(A5) For replicated studies, data from different releases are statistically independent.

(A6) The fate of each individual fish is independent of the fates of all other fish.
(A7) All fish in a release group have equal survival and detection probabilities.
(A8) Prior detection history has no effect on subsequent survival and detection probabilities.

Assumptions A1–A5 are pertinent for the validity of statistical inferences to the population of interest and to the proper conduct of the study. These assumptions (i.e., A1–A5) are largely satisfied by appropriate capture, handling, marking, and release procedures of the study protocol. Postrelease handling mortality could violate assumption A1 and tend to underestimate actual survival probabilities. Careful handling is therefore needed to avoid such bias and was the reason fish were held at least 24 h prior to release.

The key assumptions in constructing the multinomial likelihood (eq. 1) are A6–A8, which imply that the fates (i.e., capture histories) of all PIT-tagged fish in a release group are independent, identically distributed, multiple Bernoulli trials. Assumptions A6–A8 are mathematical constraints in the formulation of likelihood (eq. 1), and investigators have less direct control over them than assumptions A1–A5. For this reason, these assumptions warrant closer scrutiny. In the sections that follow, we first describe the effects of various violations of assumptions A6–A8 on survival estimates and associated variance estimates from the SR model. We then present a series of data analyses that were performed to test whether the assumptions were satisfied in the 1993 Snake River survival study.

Monte Carlo simulations

Heterogeneity in survival probabilities among the fish within a release group can violate assumption A7. Monte Carlo simulation studies were used to evaluate the robustness of the SR model to three forms of survival heterogeneity. The first potential violation of assumption A7 can be stated as follows:

(V7a) Survival probabilities for individual smolts are heterogeneous because of inherent differences in viability of fish.

To evaluate the robustness of the model in eq. 1 to violation V7a, Monte Carlo simulations were conducted, each consisting of 1000 fish released above Lower Granite Dam. A total of 1000 simulations were conducted for each scenario investigated. Detection probabilities were 0.50, 0.55, and 0.60 at Lower Granite, Little Goose, and McNary dams, respectively. The distribution of survival probabilities among individual smolts depended on the distribution of a condition index. Each fish was assigned a standardized condition index from a normal distribution with mean (μ) = 0 and standard deviation (σ) = 0.1 for convenience. Heterogeneous survival probabilities among individual smolts were created by assuming a proportional hazards relationship based on the condition index. In terms of survival probabilities, a smolt with condition index x_i was assigned survival probabilities $S_i^{1} = S^{e^{x_i}}$, $S_i^{10} = S^{e^{x_i}}$, $S_i^{2} = S^{e^{x_i}}$, $S_i^{20} = S^{e^{x_i}}$, $S_i^{3} = S^{e^{x_i}}$, and $S_i^{30} = S^{e^{x_i}}$ from release to Lower Granite, Lower Granite to Little Goose, and Little Goose to Lower Monumental, respectively. Two scenarios were investigated. In scenario V7a.1, the baseline survival (i.e., S_{k0}) probability was 0.88 for all three reaches. In scenario V7a.2, the baseline survival probabilities were $S_{10} = 0.6$, $S_{20} = 0.9$, and $S_{30} = 0.5$. In both scenarios, 20% of detected fish were removed for barging downstream at each dam. Program SURPH.1 (Smith et al. 1994) can relax assumption A7 by permitting the survival and detection probabilities of individual fish to be modeled as functions of individual-based covariates. Individual-based models will not be explored in this analysis (see Skalski et al. 1993; Smith et al. 1994).

The second potential violation of A7 can be stated as follows:

(V7b) Survival probabilities for individual smolts are heterogeneous because survival rates differ among the various routes through the hydroelectric projects (i.e., spill, bypass, turbines).

Risks of mortality are not the same in each of the passage routes through a dam. For the simulated scenarios, survival probabilities were calculated as the product of two independent probabilities. The first component was survival from the top of the reach to the forebay of the next downstream dam (i.e., the "pool"). This survival probability was set at 0.95. The second component of survival was the probability of surviving dam passage. Survival through the dam depended on whether passage occurred through the spillbays (S_{spill}), turbines ($S_{turbine}$), or bypass system (S_{bypass}). In this set of simulations, mortality in the bypass system occurred before detection. The proportion of fish going through the alternative routes was allowed to vary (Table 3). In all V7b scenarios, of those fish entering the powerhouse (not the spill), the proportion passing through the bypass system (i.e., fish guidance efficiency (FGE)) was 0.45, 0.50, and 0.55 at Lower Granite, Little Goose, and McNary dams, respectively. In all scenarios, 20% of detected fish at each dam were removed for barging downstream (i.e., right censored).

Table 3. Passage route-specific survival rates for scenarios simulated under violation V7b.

	Probability	Survival probabilities		
Scenario	through spill	S_{spill}	S_{bypass}	$S_{turbine}$
V7b.1	0.2	0.90	0.90	0.90
V7b.2	0.6	0.90	0.90	0.90
V7b.3	0.2	0.97	0.98	0.85
V7b.4	0.6	0.97	0.98	0.85
V7b.5	0.2	0.80	0.50	0.30
V7b.6	0.6	0.80	0.50	0.30

Note: Release sizes of 1000 PIT-tagged fish were simulated 1000 times under each scenario.

A potential violation of assumption A8 can be stated as follows:

(V8) Survival probabilities for individual smolts are heterogeneous because the route taken through a hydroelectric project affects downstream survival (or detection) probabilities.

Currently, PIT-tagged fish can be detected only in the juvenile bypass facilities of the dams. Consequently, detected fish traverse a different part of the tailrace than fish that pass via turbines or spillway. Assumption A8 would be violated if the subsequent level of mortality experienced by detected fish differs from that experienced by nondetected fish. For example, such a difference could be caused by differential predation mortality in the tailrace between fish passing through the bypass system and those using other passage routes, prior to remixing below the dam. The parameters for scenarios under violation V8 were identical to those under V7b (Table 3), except that mortality in the bypass system occurred after detection and before remixing with nondetected fish at Lower Granite and Little Goose Dams.

Tests of assumptions based on field trials

The validity of assumption A8 was evaluated using three distinct

approaches. If two or more groups of tagged fish are mixed as they travel down the river, they will experience the same river and dam passage conditions. Thus, mixing of distinct groups is a sufficient, but not necessary, condition for equal capture and survival probabilities. The hypothesis that groups are mixed can be tested by comparing distributions of daily detections at downstream dams, using Pearson χ^2 tests of homogeneity based on contingency tables (Snedecor and Co- chran 1989, pp. 210 and 211). The first test of assumption A8 was a comparison of the distributions of daily detections at downstream sites for subgroups of primary releases defined by their upstream capture history. Contingency table entries for Little Goose Dam dis- tributions were the number of fish detected each day from two sub- groups of each release: those detected at Lower Granite Dam, and those not detected at Lower Granite Dam. Similar tests of homogene- ity were based on daily tag detections at Lower Monumental Dam for four subgroups defined by capture histories at Lower Granite and Little Goose dams.

The second method for testing assumption A8 was presented by

Burnham et al. (1987) and called TEST 3. This test checked the internal consistency of survival and capture probabilities by dividing a single release group into subgroups based on their capture histories up to a specified location. The two detection and diversion sites followed by two detection-only sites provided sufficient data to construct one contingency table analysis under TEST 3.

Each series of contingency table tests (one test for each of seven primary releases) was considered to be a single experiment, and significance levels were selected to control the experimentwise type I error rate at $\alpha_{EX} = 0.05$. With seven tests in each experiment, the testwise significance level was $\alpha_T = 0.0073$.

A third approach to testing assumption A8 was based on data from the secondary paired releases and is presented in the next section.

If the fates of individual fish are not independent (i.e., assumption A6 violated), point estimates of survival and detection probabilities for a release group remain valid and unbiased. However, model-based variance estimates tend to underestimate true variability under nonindependence. To determine whether the likelihood model (eq. 1) provided an accurate estimate of the variance of the survival estimates (eqs. 3 and 5), the seven primary releases in Lower Granite Reservoir were clustered in time as closely as possible. As such, the variability in the respective point estimates of survival should be almost exclusively the result of sampling variability and not because of changing survival probabilities associated with varying river conditions. The empirical variance among the seven point estimates (i.e., $s^2_{\hat{S}}$) was compared with the average variance estimated from the model (i.e., $\widehat{\mathrm{var}}(\hat{S}_i|S_i)$). There was no formal test of significance. However, if the empirical variance was much greater than the average variance predicted by the model, this would imply that the model was not accounting for a substantial source of variability.

Modified single-release (MSR) model

Assumption A8 is violated if fish detected in the juvenile fish bypass facility experience differential mortality before remixing with fish that passed though turbines or spillways. Data from the secondary paired releases at Lower Granite and Little Goose Dams, in conjunction with a modification of the SR model (eq. 1), can be used to estimate post-detection bypass mortality and provide valid estimates of smolt survival in the river reaches. The MSR model provides a robust alternative to the traditional SR model (Dauble et al. 1993).

The modification to the design for the SR model consists of concurrent releases of PIT-tagged fish in the bypass system just downstream from the PIT-tag detector and control fish in the zone of the tailrace where detected and nondetected fish remix. The MSR model explicitly estimates mortality between the detector and the remixing zone at Lower Granite and Little Goose Dams, and adjusts the reach survival estimates (S_1 and S_2).

Additional terms for the MSR model (Fig. 2) are defined as follows:

R_{B2} is the number of fish released in the bypass system at Lower Granite Dam; C_{B2} is the number of fish released in the tailrace at Lower Granite Dam; R_{B3} is the number of fish released in the bypass system at Little Goose Dam; C_{B3} is the number of fish released in the tailrace at Little Goose Dam; n_{R2} is the number of fish recovered downstream from the treatment release (R_{B2}); n_{C2} is the number of fish recovered downstream from the control release (C_{B2}); n_{R3} is the number of fish recovered downstream from the treatment release (R_{B3}); n_{C3} is the number of fish recovered downstream from the control release (C_{B3}); τ_2 is the survival probability between detector and remixing zone at Lower Granite Dam; τ_3 is the survival probability between detector and remixing zone at Little Goose Dam; λ_2 is the joint probability of secondary release fish from Lower Granite Dam surviving and being detected downriver; and λ_3 is the joint probability of secondary release fish from Little Goose Dam surviving and being detected downriver.

(10) $$L(\underset{\sim}{S},\underset{\sim}{p},\underset{\sim}{\tau},\underset{\sim}{\lambda}|\underset{\sim}{R_1},\underset{\sim}{R_p},\underset{\sim}{R_B},\underset{\sim}{C},\underset{\sim}{m},\underset{\sim}{n}) = \binom{R_1}{m_{12},m_{13},m_{14}} (S_1p_2)^{m_{12}} (S_1(1-p_2)S_2p_3)^{m_{13}} \times (S_1(1-p_2)S_2(1-p_3)\lambda)^{m_{14}} \times (1-S_1(p_2+(1-p_2)S_2p_3+(1-p_2)S_2(1-p_3)\lambda))^{R_1-r_1} \binom{R_2}{m_{23},m_{24}} (\tau_2S_2p_3)^{m_{23}} (\tau_2S_2(1-p_3)\lambda)^{m_{24}} \times (1-\tau_2S_2(p_3+(1-p_3)\lambda))^{R_2-r_2} \binom{R_3}{m_{34}} (\tau_3\lambda)^{m_{34}} (1-\tau_3\lambda)^{R_3-m_{34}} \times \binom{R_{B2}}{n_{R2}} (\tau_2\lambda_2)^{n_{R1}} (1-\tau_2\lambda_2)^{R_{B2}-n_{R2}} \binom{C_{B2}}{n_{C2}} (\lambda_2)^{n_{C2}} (1-\lambda_2)^{C_{B2}-n_{C2}} \times \binom{R_{B3}}{n_{R3}} (\tau_3\lambda_3)^{n_{R3}} (1-\tau_3\lambda_3)^{R_{B3}-n_{R3}} \binom{C_{B3}}{n_{C3}} (\lambda_3)^{n_{C3}} (1-\lambda_3)^{C_{B3}-n_{C3}}$$

Iterative numerical methods were used to estimate reach survival and post-detection bypass survival simultaneously. However, the following formula (the "relative-recovery" estimate) can be given for estimates of post-detection bypass survival:

(11) $$\hat{\tau}_i = \frac{(n_{Ri}/R_{Bi})}{(n_{Ci}/C_{Bi})}, \text{ for } i = 2 \text{ and } 3.$$

The likelihood model for the MSR design can be written as the joint likelihood for the primary and secondary releases:

The null hypotheses H_0: $\tau_2 = 1$ and H_0: $\tau_3 = 1$ were tested using likelihood ratio tests (LRT). Fish from the secondary releases were pooled in performing the LRTs.

A critical assumption of the MSR likelihood model (eq. 10) model is that the treatment fish of the paired release have the same survival and capture probabilities as the control fish downstream from the remixing zone (i.e., the difference between the downstream detection rates for the two groups is entirely due to mortality in the bypass system). For paired groups that pass two or more detection and diver- sion sites, an alternative formulation of the MSR model is available, based on survival probabilities from the point of release to the next site downstream. Post-detection bypass survival probability is then based on relative survival for the two groups, rather than on relative recovery rates.

Results

Assessment of the validity of using PIT-tag data in single release–recapture analysis was first evaluated using Monte Carlo methods to identify nonrobust properties of the model and then by empirical results from replicated field trials.

Monte Carlo simulations

Effect of heterogeneity of individuals on estimates of survival probabilities

Under conditions of heterogeneity of survival probabilities among individuals (violation V7a), the estimates of survival were unbiased, while theoretical variance estimates were slightly inflated (Table 4). Consequently, nominal 95% confi- dence intervals (eq. 9) covered the true survival probability (S_1 and S_2) slightly more than 95% of the time. These results are consistent with properties of independent but nonidentical Bernoulli trials used to estimate binomial proportions (Feller 1968, pp. 230–231).

Effect of route-specific dam passage mortality on estimates of survival probabilities

When bypass mortality occurred before detection (violation

Table 4. Results from 1000 Monte Carlo simulations for scenarios with heterogeneous survival rates due to individual differences (violation V7a).

Scenario	Reach	True S_k	$\bar{S}_k$	$\overline{\widehat{\mathrm{var}}(\hat{S}_k \mid S_k)}$	$s^2_{\hat{S}_k}$	95% CI coverage
V7a.1	Rel–LGR	0.878	0.878	0.000 564	0.000 563	95.0
	LGR–LGO	0.878	0.880	0.001 651	0.001 565	95.4
V7a.2	Rel–LGR	0.598	0.598	0.000 679	0.000 663	94.9
	LGR–LGO	0.898	0.904	0.005 343	0.005 100	95.7

Note: True mean survival probability (S_k), average survival estimate ($\bar{S}_k$), average variance estimate ($\overline{\widehat{\mathrm{var}}(\hat{S}_k \mid S_k)}$), empirical variance among survival estimates ($s^2_{\hat{S}_k}$), and 95% confidence interval coverage are given. Survival probabilities were estimated for release to Lower Granite Dam (Rel–LGR) and Lower Granite to Little Goose Dam (LGR–LGO) reaches.

V7b) and the dead fish went undetected, there was no bias in overall release survival estimates (S) or associated variance estimates ($\widehat{\mathrm{var}}(\hat{S}_k \mid S_k)$) (Table 5). Confidence interval coverage for S was nominal.

When mortality occurred in the bypass system after detection but before remixing with nondetected survivors, the PIT-tagged fish are counted as survivors. This type of route-specific mortality (violation 8) resulted in biased point estimates and variance estimates of survival and poor confidence interval coverage (Table 5). These simulation results motivated the development of the MSR model and the paired post-detection bypass releases in 1993.

Field trials

Effect of upstream detection on the probability of downstream detection

Daily distributions of detections at Little Goose Dam for the two subgroups of each primary release that passed Lower Granite Dam (i.e., detected or not detected) differed significantly ($P = 0.007$) only for the fifth release. Among the detection distributions at Lower Monumental Dam for four subgroups of each primary release defined by capture histories at Lower Granite and Little Goose Dams, again only the fifth release had significant differences ($P = 0.004$).

Differences in detection distributions at Little Goose Dam may have been the result of a 1- or 2-day delay for fish detected at Lower Granite Dam. However, river conditions over the peak days of passage for both groups were sufficiently stable and unlikely to cause a significant difference in survival or detection probability at Little Goose Dam. Furthermore, the test of homogeneity (TEST 3) proposed by Burnham et al. (1987) did not indicate significantly different survival and capture probabilities for detected and nondetected fish at Lower Granite Dam. The distribution of capture histories at Lower Monumental and McNary Dams did not depend on the capture history at Lower Granite and Little Goose dams for any of the seven primary releases.

Effect of upstream detection on the probability of downstream survival

Estimates (eq. 11) of post-detection bypass survival using relative recovery numbers from paired releases at Lower Granite Dam (τ_2) ranged from 0.915 to 0.986 with a weighted average (weights inversely proportional to respective estimated variances) of 0.950 ± 0.022 (mean ± SE). Based on the weighted average, an approximate 95% confidence interval for post-detection bypass survival at Lower Granite Dam of 0.907–0.993 does not include 1.0, suggesting that significant mortality may have occurred. Likelihood ratio tests based on the model in eq. 10 also indicated significant mortality at Lower Granite Dam ($\chi^2_1 = 4.774$, $P = 0.029$). However, there is some indication that the assumption of equal detection probabilities for the paired releases was violated at Little Goose Dam for the second paired release. Applying the SR model independently to the two groups, the estimated detection probabilities were 0.405 ± 0.028 and 0.502 ± 0.026 for the second treatment and control groups, respectively. The weighted average of post-detection bypass survival at Lower Granite Dam based on the relative survival method was 1.001 ± 0.070. Consequently, conclusions regarding post-detection bypass mortality depend on whether the relative recovery or the relative survival method is used in the analysis of the paired releases. Differential detection probabilities within the paired releases suggest the better estimate of post-detection bypass mortality is provided by the relative survival method. Therefore, survival estimates for the primary releases were calculated using both the SR and MSR models.

Accuracy of model-based estimates of measurement error

For the seven survival estimates in the reach from release to Lower Granite Dam (Table 6), the average estimated sampling variance was 0.000 49 (SD = 0.022 1), while the empirical variance among the seven point estimates of survival was 0.000 12 (SD = 0.010 9). The two variance estimates are of the same order of magnitude, and the model-based variance is greater than the empirical variance. For survival estimates in the Lower Granite Dam to Little Goose Dam reach (Table 7), the average estimate of sampling variance was 0.000 13 (SD = 0.011 4), while the empirical variance among the seven point estimates was 0.000 12 (SD = 0.010 7), almost identical. There was no evidence that the model failed to adequately measure any significant source of variability.

Survival estimates for Snake River reaches

The primary releases, besides testing model assumptions, also provide an opportunity to examine survival of run-of-the-river hatchery-reared yearling chinook salmon during their outmigration to the ocean. However, when examining the resulting survival estimates, it must be remembered that these estimates represent only 1 week in the seasonal and interseasonal history of the river.

The following reach survival estimates are based on the SR model (i.e., assumption of 0% post-detection bypass

Table 5. Results from 1000 Monte Carlo simulations for scenarios with passage route-specific mortality.

Scenario	Reach	True S_k	$\bar{S}_k$	$\widehat{Var}(S_k\|S_k)$	$s^2_{S_k}$	95% CI coverage
V7b.1	Rel–LGR	0.855	0.858	0.001 71	0.001 62	95.9
	LGR–LGO	0.855	0.860	0.005 27	0.005 26	95.3
V8.1	Rel–LGR	0.889	0.954	0.002 20	0.002 16	75.3
	LGR–LGO	0.893	0.862	0.005 67	0.005 60	90.3
V7b.2	Rel–LGR	0.855	0.868	0.012 85	0.013 70	95.9
	LGR–LGO	0.855	0.888	0.043 06	0.043 17	94.2
V8.2	Rel–LGR	0.872	0.972	0.018 00	0.017 57	97.0
	LGR–LGO	0.874	0.883	0.048 58	0.052 37	91.4
V7b.3	Rel–LGR	0.875	0.878	0.001 59	0.001 69	94.9
	LGR–LGO	0.880	0.883	0.004 92	0.005 10	95.0
V8.3	Rel–LGR	0.882	0.897	0.001 67	0.001 68	95.2
	LGR–LGO	0.887	0.881	0.004 98	0.004 91	94.2
V7b.4	Rel–LGR	0.894	0.904	0.011 84	0.012 77	94.3
	LGR–LGO	0.898	0.922	0.037 79	0.034 58	94.3
V8.4	Rel–LGR	0.902	0.940	0.013 16	0.013 79	95.8
	LGR–LGO	0.904	0.913	0.037 58	0.035 57	93.2
V7b.5	Rel–LGR	0.448	0.456	0.003 71	0.003 32	95.7
	LGR–LGO	0.456	0.484	0.017 87	0.015 83	94.6
V8.5	Rel–LGR	0.619	1.044	0.022 92	0.022 91	2.3
	LGR–LGO	0.646	0.538	0.023 63	0.022 38	72.1
V7b.6	Rel–LGR	0.604	0.624	0.021 18	0.020 30	93.2
	LGR–LGO	0.608	0.696	0.132 30	0.100 98	92.5
V8.6	Rel–LGR	0.690	1.371	0.214 11	0.198 25	92.4
	LGR–LGO	0.703	0.774	0.472 80	0.287 62	87.3

Note: True survival probability (S_k), average survival estimate ($\bar{S}_k$) , average variance estimate ($\widehat{Var}(S_k|S_k)$), empirical variance among survival estimates ($s^2_{S_k}$), and 95% confidence interval coverage are given. Survival probabilities were estimated for release to Lower Granite Dam (Rel–LGR) and Lower Granite to Little Goose Dam (LGR–LGO) reaches. Mortality in the bypass system occurs either before detection (violation V7b) or after detection (violation V8) at Lower Granite and Little Goose dams.

mortality). For the river section between Nisqually John and the tailrace of Lower Granite Dam (Table 6), smolt survival estimates (S_1) ranged betwe_en 0.886 ± 0.020 and 0.920 ± 0.024 with a weighted average (S_2) of 0.902 ± 0.004. The S_2 values between the tailrace of Lower Granite Dam and the tailrace of Little Goose Dam (Tab_le 7) ranged from 0.818 ± 0.034 to 0.902 ± 0.044 with a S_2 of 0.859 ± 0.013. These estimates correspond well with other survival estimates of wild and hatchery-released yearling chinook over the wider range of the 1993 outmigration season reported by Iwamoto et al. (1994), and over the 1994 and 1995 seasons reported by Muir et al. (1995, 1996). Under the SR model, the estimated survival from Nisqually John to the tailrace of Little Goose Dam is 0.775 (= 0.902 × 0.859).

Using the relative recovery method, post-detection bypass mortalities at Lower Granite and Little Goose dams are estimated to be 6% and 0%, respectively. If these survival figures are correct, the pooled estimates of survival for the primary releases are 0.875 ± 0.014 from Nisqually John to Lower Granite Dam tailrace and 0.913 ± 0.021 from Lower Granite Dam tailrace to Little Goose Dam tailrace. The effect of post-detection mortality is to lower the estimated survival rate in the subsequent reach. Essentially, the MSR model redistributes this "extra" dam mortality to the proper reach. Under the MSR model, the estimated survival from Nisqually John to the tailrace of Little Goose Dam is 0.799 (= 0.875 × 0.913) compared with 0.775 under the SR model.

Discussion

By establishing the validity of the statistical and field method- ology, we have shown that reliable survival estimates can be obtained from release–recapture models of detection data from PIT-tagged migrant juvenile salmonids in the Snake–Colum- bia River Basin. The ability to obtain reliable survival esti- mates is a powerful tool for the investigation, mitigation, and recovery of threatened and endangered salmonid stocks.

Our Monte Carlo simulation studies showed that hetero- geneity of survival and detection probabilities among animals had no effects on estimates of mean survival probabilities but resulted in overestimation of the sampling variance and pro- duced confidence intervals that were too wide. Conversely, the fates of fish within a release group are not independent; model- based variance estimates will tend to underestimate the true variance. While it is intuitive that there is heterogeneity among animals, there is no empirical evidence that fates of fish within a group are not independent. The empirical variance estimates from seven replicate releases estimated very near the model- based error variances estimated from the likelihood model. Hence, not only do the point estimates generated by this pilot study appear to be accurate and robust, but the standard errors of the estimates appear to be reliable measures of the uncer- tainty of the survival estimates. These results indicate the use of PIT-tags and release–recapture models can provide valid estimates of survival probabilities. Since 1993, diversion

Table 6. Estimated survival probabilities, estimated sampling variances, and average estimated variance from release to Lower Granite Dam tailrace based on primary releases.

Release	Point estimate of survival (S_{1i})	Estimated variance ($\widehat{var}(S_{1_i}\|S_{1_i})$)
R_{11}	0.920	0.000 576
R_{12}	0.900	0.000 361
R_{13}	0.911	0.000 484
R_{14}	0.903	0.000 529
R_{15}	0.901	0.000 484
R_{16}	0.895	0.000 576
R_{17}	0.886	0.000 400
Overall estimated variance		0.000 487 (0.022 1)
Empirical variance ($s^2_{S_{1i}}$)		0.000 119 (0.010 9)

Note: Values for average estimated variance and empirical variance are variance and standard deviation.

Table 7. Estimated survival probabilities, estimated sampling variances, and average estimated variance from Lower Granite Dam tailrace to Little Goose Dam tailrace based on primary releases.

Release	Point estimate of survival (S_{2i})	Estimated variance ($\widehat{var}(S_{2i}\|S_{2i})$)
R_{11}	0.888	0.001 52
R_{12}	0.889	0.000 84
R_{13}	0.831	0.000 96
R_{14}	0.818	0.001 16
R_{15}	0.831	0.001 37
R_{16}	0.902	0.001 94
R_{17}	0.869	0.001 30
Overall estimated variance		0.000 130 (0.011 4)
Empirical variance ($s^2_{S_{2i}}$)		0.000 115 (0.010 7)

Note: Values for average estimated variance and empirical variance are variance and standard deviation.

systems have been completed at Lower Monumental and McNary dams extending the capability to perform survival studies much further downriver.

The assumption that survival and detection probabilities were not affected by upstream capture histories was evaluated by comparing temporal passage distributions for detected and nondetected smolts. Coincident downstream migrations were evident in six of seven cases. In one case, the timing was delayed 1 or 2 days for detected fish. Coincident passage is a sufficient condition for assuring that all fish are exposed to similar survival and detection conditions. However, coincident passage is not necessary for equal probabilities if river conditions vary little over a 1- or 2-day period. Indeed, for the group with apparent noncoincident passage, the Burnham et al. (1987) test of homogeneity (TEST 3) found no significant difference in downstream capture histories for groups of fish with differing upstream detection histories.

Our smallest release group of only 797 PIT-tagged fish on 20 April 1993 resulted in standard errors of 0.024 and 0.044 on survival estimates from the release to Lower Granite Dam and Lower Granite Dam to Little Goose Dam reaches, respectively. The largest release of 1405 fish resulted in standard errors of 0.020 and 0.036 for the two reaches, respectively. These levels of precision for survival estimates have not been attainable using more conventional marking techniques in the past (e.g., freeze-brand, fin-clipping), even with tens of thousands of marked fish. Hence, the PIT-tag has the capability of providing accurate and precise estimates of survival using a minimum number of fish at a time when the need for information is the greatest and the opportunity to handle large numbers of fish is low because of listings of salmonids stocks under the Endangered Species Act.

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