
PIT Tag Marking Procedures Manual

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PIT Tag Information System
Columbia Basin | ptagis.org

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Introduction

The passive integrated transponder (PIT) tag has been used since 1987 as a research and management tool by the Columbia River Basin's fisheries agencies and tribes to mark and track anadromous fish. An information network has been developed to collect and share mark and recovery information. In 1991, the PIT Tag Information System (PTAGIS) was developed and managed by the Pacific States Marine Fisheries Commission with funding from the Bonneville Power Administration (BPA). PTAGIS houses, manages and maintains the data repository where all PIT tag data in the Columbia River Basin are stored. PTAGIS collects and provides data obtained from the automated interrogation equipment at the mainstem dams, as well as from other sites throughout the basin. PTAGIS is also responsible for developing and maintaining the PIT tag data collection and validation software (for both tagging and interrogation events), managing the separation by code system at hydropower facilities, providing training on the database and associated data collection software, as well as maintaining the PIT tag interrogation equipment at the mainstem dams. PTAGIS is the clearinghouse for procurement and distribution of all PIT tags for BPA-funded projects.

The PIT Tag Steering Committee (PTSC) is a subcommittee of the Fish Passage Advisory Committee (FPAC). The PTSC members are from Idaho, Oregon, Washington, US Fish and Wildlife Service, NOAA Fisheries, the Fish Passage Center, the Columbia River Inter-Tribal Fish Commission, and Bonneville Power Administration. The PTSC is responsible for

1. Providing technical guidance to the region for best tagging practices throughout the basin to facilitate standardized tagging techniques, handling protocols, and tag data reporting.
2. Helping to guide and coordinate training of tagging personnel to facilitate the implementation of standardized tagging techniques and handling protocols to ensure consistent high-quality marking.
3. Providing regional technical input and guidance to PTAGIS for the interrogation sites and facilities, and for the management of the database to benefit the region.

This PIT Tag Marking Procedures Manual provides protocols and standards on the PIT tag marking station(s), fish handling techniques, anesthesia, tag injection, and information on data collection, verification, and transfer to the regional database. In addition to this manual, the PTSC and PTAGIS produced a [PIT tag training video](#). If you have specific questions that are not answered by this document or the video, or if you would like to receive training, please

contact your [PTSC representative](#) for assistance.

The reader should be aware that this manual contains guidelines based on nearly four decades of researching and using PIT tag technology. It is understood that specific studies may have to deviate from the guidelines due to the life history characteristics of the species being studied. If a researcher must deviate from the guidelines, it is hoped this manual can aid the researcher in reducing the biological consequences of such deviations.

The PTSC recommends only PIT tags that have been tested against the equipment employed within the Columbia River Basin be used within the basin. While we appreciate the necessity of looking for ways to reduce costs, untested PIT tags may not function as well as tested tags, affecting not only the researcher's results, but possibly affecting other researchers' studies, especially if the untested tags overwhelm detection sites causing other tags to not be detected.

Separate guidelines for marking some non-salmonids (e.g., lamprey) and the use of PIT tags smaller than 12 mm are now included in this manual due to their increasing involvement in PIT tag marking. Smaller PIT tags allow the tagging of smaller, and sometimes more representative sizes of fish. The tradeoff is that smaller tags can have poorer remote detection efficiencies. We will add guidelines to this document as procedures for tagging other non-salmonids and the use of non-traditional tags becomes more established.

A. PIT Tag Marking Station

PIT tags are used to mark fish, often juvenile salmonids, in a wide variety of situations in the Columbia River Basin. These range from the collection and tagging of individuals in their natal streams to the marking of thousands of juvenile salmonids within a hatchery. Regardless of the magnitude of the marking programs, certain elements of these programs are held in common:

1. Fish are collected prior to tagging;
2. Fish are taken from the collection in batches, anesthetized, and tagged;
3. Information about each animal is collected, recorded, and linked to its injected tag; and
4. The fish are allowed to recover from the effects of tagging, handling, and anesthetic before they are returned to the general population.

In many situations where large numbers of juvenile salmonids are tagged at a fixed PIT tag marking station, a sophisticated semi-automated computerized

data-entry system is used to maximize the tagging rate and simultaneously minimize the time a fish is deprived of fresh water. At a minimum, a marking station consists of a PIT tag transceiver (reader) connected to a computer running specialized data collection and validation software. More complex stations include peripheral hardware such as a digitizer (to record fork lengths or speed entry of predefined data codes) and an electronic balance (to record weights). The complexity of the marking station used is generally dependent on the type of marking procedure, as described below.

In these situations, scanned tag codes are populated into a local database along with other biological data during tagging, minimizing the chance of data transcription errors. When use of these data entry systems is not feasible due to equipment costs, availability of electrical power, or challenges associated with remote site access, there are strategies to minimize the error-prone transcribing of PIT tags codes. One option is to pre-read the PIT tags and place each one, individually, in a referenced scale envelope before going into the field. Another option is to make a printout, on write-in-the-rain paper, of the PIT tag codes with blanks for the appropriate data (length, weight, flag codes, etc.) next to each PIT code. You can use the printout as a field data sheet to collect the appropriate data and then create a tagging file when you return to the office with the data. Using these procedures minimizes having to transcribe the PIT tag code. *The PTSC strongly recommends that you do not transcribe PIT tag codes while tagging fish. The high error rates associated with transcribing PIT codes by hand are unacceptable.*

There are currently two types of tag injectors used within the Columbia River Basin, the typical syringe/needle assembly that is used multiple times (multiple-use injectors, MUI), with disinfection between uses, and the single-use injector system (SUI) utilizing a pre-loaded needle, containing the tag and a push rod, that is used just once with an injection device designed to be used thousands of times without the need for disinfection.

B. Tagging Operations

1. Major PIT-tagging Scenarios

There are three major tagging scenarios: mass marking operations, small scale tagging operations at fixed locations such as traps or weirs, and remote marking. Marking station configurations and personnel/equipment requirements differ depending on the type of marking operation. The guidelines presented in the following sections assume the use of MUI's. The use of SUI's eliminates the need to disinfect injectors and eliminates the need to

disinfect and load tags into the injectors.

a. Mass Marking

Mass marking operations mark thousands of juvenile salmonids in a short period of time. These types of operations generally utilize some type of fish marking trailers specifically designed to handle large quantities of fish and minimize fish stress. Fish marking trailers usually are equipped with recirculating anesthetic systems and flow-through fish holding tanks. It is important to monitor water chemistry and temperature in these systems. Normally, 110 VAC electricity is available in the tagging trailer. One or more PIT tag marking stations can be set up in a trailer. It will take at least one person tagging and one person entering data to operate a station. In many situations, each station can handle two people tagging.

Mass marking can be done with Single-use Injectors (SUIs) or Multiuse Injectors (MUIs). Determining which type of injector is suitable for any given mass marking operation is dependent on several factors, including (but not limited to) budget (MUIs are typically lower in cost), space considerations (MUIs typically require less space), marking logistics and safety (SUIs are typically more efficient and require less preparation between marking), fish health (SUI needles do not require sharpening to maintain clean insertion), waste/disposal (MUI needles can be reused after proper disinfection), etc. See [Section 3](#) of this manual for more detail on each of these types of injectors.

In the mass marking operation, such as tagging at a hatchery, 30 to 100 fish may be anesthetized at one time. As each fish is PIT-tagged, it is routed to a person that scans the PIT code, records various types of information about the fish, and places it in a recovery tank. To minimize stress and the effect of the anesthetic on fish, the PTSC recommends that total anesthetic exposure time should not exceed five minutes. If there is a possibility of encountering previously PIT-tagged fish in the population being marked, *it is very important to scan all fish before tagging to ensure they are not already PIT-tagged.*

To maintain efficiency in mass marking with MUIs, you will need at least 500-750 MUIs per station and at least three or four people disinfecting, loading tag injectors, and supporting the operation for each tagging station. This type of operation can PIT tag up to 5,000 fish per day per station with experienced personnel.

b. Traps and Weirs

PIT-tagging at traps and weirs normally is a much smaller operation than mass marking. Marking operations at stationary traps and weirs generally mark run-of-river juvenile salmonids, including several species of hatchery and wild fish. Often in these situations 110 VAC power is not available, and the PIT tag marking station is operated from auxiliary battery power. The data collection equipment can be powered by any 12-24 VDC power source, with a range of battery options including: lead acid, AGM, or newer lithium phosphate batteries that have up to a 100-amp-hour rating. Another way to supply power is with solar panels that recharge the battery system. Solar power can provide additional power for operation of other accessories (i.e. water pumps, aerators, etc.).

At traps and weirs, a covered work area is beneficial to keep tagging equipment and personnel out of the weather. Some of the larger traps have covered work areas built on the back of the trap. Smaller traps and weirs often will use a wall tent for cover with tables set up inside. Tagging equipment is set up on cabinets or tables with one area that is protected from water for the computer and any peripherals. The digitizer is generally housed in a Plexiglas cover to keep water off the device.

At traps and weirs, all captured fish should be run through a PIT tag reader antenna to determine if it was previously PIT-tagged. *It is very important to scan all run-of-river fish before tagging to ensure they are not already PIT-tagged.* If the fish was not tagged previously, a tag is injected. The fish is then scanned for the PIT tag code.

c. Remote

Remote PIT-tagging operations are generally mobile situations where juvenile salmonids are collected by seining, electrofishing, or angling a segment of stream. The PIT-tagging equipment can be operated off auxiliary battery power (similar to what is used at traps and weirs), or electronics can be simplified to include only a small handheld PIT tag reader and aeration pumps operated on integrated batteries. These operations can take place on the ground, on a portable table (roll-a-table styles are especially effective), or within a self-contained tagging station—a portable aluminum box equipped with fold-out legs that contains everything required for tagging and collecting data on fish.

Similar to trap and weir tagging operations, all remotely captured fish should be run through a PIT tag reader antenna to determine if it was previously PIT-tagged. *It is very important to scan all run-of-river fish before tagging to ensure they are not already PIT-tagged.*

Since these tagging operations are often mobile, it is best to take actions that minimize the chance that invasive or non-native species are moved between streams or watersheds by tagging activities. This could include using a set of tagging equipment exclusively for a certain watershed or stream, or by cleaning, draining, drying, and possibly treating tagging equipment between changes in location. Commercially available disinfectants like Virkon Aquatic are often used and proven effective when sprayed on sampling equipment (e.g. waders, boots, nets) to sanitize surfaces. Other disinfectants or control methods could include bleach, hot water/steam, or freezing potentially exposed equipment. Permitting agencies may recommend or dictate what options are available for proper disinfection when sampling. Since control methods are often species-specific, practitioners who understand which invasive species may be present at tagging sites are best equipped to take the appropriate preventive measures.

Because remote marking is generally performed during the summer, monitoring water temperature can be particularly important during these operations (see the following section). Water temperatures more than 15°C cause stress and additional care must be taken when handling and tagging fish. Temperatures above 17°C cause severe stress and high mortality. *The PTSC strongly recommends that all juvenile salmonid PIT-tagging operations cease when water temperature exceeds 17°C.*

2. Fish Handling Techniques

a. Minimizing Fish Stress

i. Fish Health. The health of the fish being tagged is very important. Diseased fish typically experience much higher stress levels in the handling and tagging process, resulting in elevated mortality rates among the tagged fish. In a situation where diseases are prevalent, the PTSC recommends that PIT-tagging be postponed until the disease outbreak diminishes and/or, where possible, is controlled by treatments. Injured fish may also experience higher levels of stress and mortality when handled and tagged. In most tagging scenarios, fish deemed unlikely to survive the tagging process are typically rejected.

ii. Water Temperature. Elevated water temperatures decrease a juvenile salmonid's ability to handle stress. Optimum tagging temperatures are between 5°C and 10°C. Tagging fish below 5°C will not bother the fish but does cause problems for the people working with their hands in the water. As water temperature increases above 15°C, fish become stressed very easily. Great care must be taken when tagging fish in water temperatures greater than 15°C. Monitor fish in holding tanks and in anesthetic baths more closely. If fish begin

to show signs of stress, cease the operation immediately. Tagging juvenile salmonids in water temperatures greater than 17°C is not recommended and handling juvenile salmonids in water temperatures greater than 20°C must not occur. Avoid working when water temperatures are high by collecting salmonids and PIT-tagging in the early morning hours when stream temperatures are at their lowest or waiting for cooler weather. Lamprey are more tolerant of warmer temperatures. For lamprey tagging, we recommend a maximum of 26°C for larval lamprey, and 21°C for juvenile lamprey. Continuously monitor lamprey within 3°C of these maximum temperatures for excessive stress.

iii. Oxygen. Fish stress increases at low oxygen levels. It is important to ensure adequate oxygen levels for the supply tanks, holding and recovery containers, and anesthetic baths. As water temperatures increase, oxygen becomes more important as warm water holds less oxygen. A common solution is to bubble air or oxygen through the water. Oxygen can be supplied using oxygen cylinders at fixed tagging sites like traps, weirs, and where mass marking occurs. You will need to regulate the pressure from the oxygen bottle and use a valve system to route air hoses and stones to each recovery tank and anesthetic bath. At remote sites air can be supplied with a small air pump and air stones. Another way to maintain oxygen levels is to run fresh stream water through holding and recovery containers. This will also help keep the water temperature in the recovery tank the same as the stream.

A small water pump, either 12 VDC or 110 VAC, will work to circulate water through tanks. A bilge pump works well if you have a 12 VDC power supply. It's important to remember that bilge pumps are not designed to lift water very high, so the working area must be only a few feet above the water source. Using a bigger bilge pump can help offset the difference in height between the water source and the working area. However, a large 12 VDC pump needs a stronger power supply.

If aeration is not available, the number of fish in a holding or recovery container, and the time the animal spends in the containers, should be limited. Water in any containers or tanks should be changed with fresh water after every batch of fish is processed.

iv. Fish Handling. How you handle fish affects fish stress levels. Avoid chasing fish around when dip-netting them. Avoid putting too many fish in the dip-net and then having to release some. Set up the tagging station so netted fish can be immediately placed in an anesthetic bath. Use a sanctuary net if carrying fish short distances. Larval and juvenile lamprey are sensitive to crushing and pinching injuries, so make sure lamprey do not become pinned between a rigid net and their holding container during movement or capture. Handling stress becomes more of a concern as water temperature increases. Fish held in

temporary enclosures or buckets should have a constant flow of fresh water or protection from ambient heat. Handling fish can also disturb the protective mucous layer on a fish's skin. To avoid this, handle fish with wet hands and ensure any surface the fish touches is wet. Avoid double-handling fish. Stress is cumulative and if you handle the same fish several times during the same day the fish's level of stress will increase dramatically.

When PIT-tagging run-of-the-river juvenile salmonids where there are multiple sizes, species, and rearing types, process the fish based on the following order to reduce stress: small fish before large fish, less stress tolerant before more stress tolerant (i.e. Chinook before steelhead), and wild reared before hatchery reared.

When tagging in hatchery situations, it is best to cease feeding fish 24-48 hours prior to tagging and to wait 24-48 hours post-tagging to resume feeding. This allows for better tag retention. Tagging fish that have just fed not only increases the chances for tag shedding but also increases the chances of hitting vital organs when tagging due to less empty space within the peritoneal cavity. Feeding fish just after tagging can cause the tag to be shed (through the insertion hole) due to pressure from the expanding gastrointestinal tract.

v. Crowding. Crowding can also increase stress levels, especially when both Chinook and steelhead are in the same sample container. Reduce fish density if you have Chinook and steelhead together. When crowding fish in raceways, avoid overcrowding. It is very important to monitor crowded fish frequently for signs of excess stress. Be aware that when barometric pressure drops, such as when a weather front approaches, overcrowded steelhead will pack onto the floor and into the corners of raceways or holding tanks. There have been several instances, in hatchery situations, where large numbers of steelhead have suffocated one another in such a situation. When this phenomenon occurs several thousand steelhead can die in a matter of minutes. This phenomenon has not been observed with Chinook salmon.

b. Marking and Recovery Containers

Use plastic containers that do not have any toxic chemicals on them. Do not use metal containers that have lead or zinc coatings. Aluminum or stainless-steel works well for live-wells or holding containers. Square or rectangular plastic three-gallon tubs work well for anesthetic tanks in small-scale tagging operations. Thirty-gallon trashcans filled with about 20 gallons of water work well for recovery tanks. If you are working on a flat surface, for instance the deck on a trap or weir, you can use a half-cubic yard molded plastic utility truck. For containers with salmonids, drill holes at about the 50-gallon level to drain water without overflowing the container. Run a water hose into the utility truck to have a recovery tank with water circulation. Attach a mesh bag over the hose outlet to prevent fish from swimming into it. Roll the utility truck to the side of the trap or weir and dump the water and fish into the stream

when the fish are ready for release. Monitor the sedated fish when using this method so they do not get impinged on the drain holes.

All fish containers should be dark in color. Avoid using white plastic buckets and tubs. Fish become skittish and will undergo chromatosis when put in a white or light-colored container, putting them at higher risk for predation when released. If you have aluminum or stainless-steel containers, you can paint the inside of the container with dark-colored non-toxic paint.

Streamside tagging operations with limited aeration or large numbers of tagged fish to recover can use large plastic storage containers that have been perforated. The container is set in the stream in a configuration that allows sufficient water exchange and minimizes the risk of impingement. Rocks can be used to hold the recovery tank in place.

Recovery containers for larval and juvenile lamprey should not have large, uncovered drill holes since they may escape through them. Any holes can be covered with 1/8" (3.2 mm) sized mesh to ensure (eyed) juvenile lamprey remain in the recovery container. If holding smaller larval lamprey ~40-90 mm, a 2-mm sized mesh is needed. When holding lamprey for long periods of time, a thin layer of sand or fine sediment (larvae) or pebbles/cobbles (juveniles), should be placed into the bottom of the recovery container to allow for burrowing or hiding. This will reduce lamprey stress and prevent them from becoming exhausted by swimming. Long-term holding of juvenile lamprey at water temperatures $\geq 18^{\circ}\text{C}$ has been associated with fungal outbreaks.

c. Anesthesia

The two most common compounds used to anesthetize juvenile fish are Tricaine Methanesulfonate and Eugenol, the active compound found in clove oil. Tricaine Methanesulfonate (MS-222), better known as MS, is approved by the FDA as an anesthetic for fishes and other cold-blooded animals in areas where there is no active retention of the fish or potential for consumption during a 21-day withdrawal period. Neutralized MS (pH 7) is one of the most effective chemicals for anesthetizing salmonids but can cause permanent damage or death if the concentration is too strong and/or if the fish is left in the anesthetic bath too long (Wedemeyer, G. 1970). MS can slow movement of the opercula enough to affect the flow of water over the gills, thereby affecting the oxygen exchange rate between water and blood, causing a state of asphyxia. If fish are left in the anesthetic bath too long, they can suffer from lack of oxygen, which may cause permanent brain damage or death. Be sure to read and follow all recommendations of the Material Safety Data Sheet (MSDS) provided by the manufacturer and have a copy on-site, as required by OSHA.

i. Concentration. The recommended concentration of MS to anesthetize salmonids is 40 mg/l (Schoettger, R.A. and A. M. Julin. 1967). The required concentration will vary depending on water temperature, species of fish, life stage, and stress level of the fish. Especially with ESA listed stocks, it is best to be conservative, starting with a low concentration of anesthetic and working up to a concentration that is effective with the fish and environmental conditions with which one is working. As water temperature increases, so does fish metabolism, which means the drug is absorbed at a faster rate. Therefore, at warmer water temperatures fish require less MS. Salmon are more susceptible to MS than steelhead and require less MS to anesthetize. If you are PIT-tagging run-of-river fish you may have both salmon and steelhead in the anesthetic tank. Add anesthetic to the tank so you can work the Chinook first and then add more MS, as needed, to work the steelhead. Lamprey are more tolerant of MS than salmonids. The recommended MS concentration for larval and juvenile lamprey is 75-150 mg/l (Christiansen et al. 2013; Jolley et al. 2017, Moser et al. 2017). Sodium bicarbonate (baking soda) is often added in equal parts to buffer the acidity of MS. Eugenol (AQUI-S 20E) has also been used to safely anesthetize juvenile lamprey at 10-100 mg/l. Stress and fish condition (e.g., descaling) also play a role in how fish react to the anesthetic. Fish suffering from stress will require less MS to be anesthetized, as will fish that have lost their mucus layer because of recent descaling.

ii. Stock Solution. It is recommended that you make a stock solution of MS and use this stock solution to drug the anesthetic bath. There is a much lower chance of over-drugging the anesthetic bath using this method. The concentration of the stock solution can vary slightly, depending on your preference, but should be between 40 and 60 gm/l. With a stock solution of 40 gm/l, it takes one milliliter of the stock solution per liter of water in the anesthetic bath to obtain the recommended concentration of 40 mg/l. There are many delivery methods for the stock MS solution. Plastic automatic burettes are commonly used, but many models can be very expensive. There are some less expensive alternatives. Several suggestions are a *Repipet Jr. Dispenser* or a 500-ml *Nalgene Variable Volume Dispenser* (Fisher Scientific Catalog or others)¹. One thing to remember: MS is photo-sensitive and will deteriorate when exposed to light, so you need to store it in a container that will not allow light penetration. Clear plastic containers can be wrapped with black electrician tape to eliminate light penetration if black or brown plastic containers cannot be purchased.

iii. Anesthetization. The PTSC recommends that you begin a marking operation with a canary test. Use a light dose of anesthetic and anesthetize only a few fish first. Monitor their reaction to the anesthetic to determine if the concentration is correct. You can always add more anesthetic, if required. The induction time (the time it takes the fish to lose equilibrium and roll on their sides) should be

¹ A reference to a specific manufacturer or product name does not constitute endorsement by the PTSC.

one to three minutes². You should still be able to see significant operculum movement and slight fin movement in salmonids. At this time fish can be tagged, the length and/or weight can be recorded, and the fish can be examined for other marks, clips, injuries, or descaling. *It is very important that all run-of-river fish are scanned before tagging to ensure they are not already PIT-tagged.* Monitor the fish constantly as you work them. If movement of the operculum becomes weak or stops, add fresh water to the anesthetic bath immediately or place the fish in fresh water. When movement of the operculum stops, death is imminent. Without water movement across the gills, the fish will suffocate in a matter of minutes. Once the fish from the canary test are placed in a recovery tank, they should begin to regain equilibrium and maintain an upright swimming position within about five minutes. If recovery time is greater than five minutes, reduce the concentration of MS in the anesthetic bath.

The PTSC recommends that an appropriate number of fish be placed in the anesthetic bath at one time such that PIT-tagging and data collection are completed within five minutes of the fish first entering the anesthetic i.e., fish should not be in the anesthetic bath for more than five minutes. When tagging with limited personnel at traps, weirs, or at remote sites, only 20 to 50 fish should be anesthetized at one time to ensure all the fish in the tub can be tagged within five minutes. *Circumstances where fish are left in an anesthetic bath for more than 10 minutes should be avoided.*

d. Fish Recovery and Release

Fish should be allowed to recover in a cool, dark tank. The PTSC recommends that fish should be allowed to recover for a minimum of 30 minutes before release back into the stream. The fish must have recovered sufficiently from the anesthesia to be able to avoid predation once they are released. Fish can also be placed in a net pen or perforated tub in the stream to protect them from predators and allow them additional recovery time. The net pen or container needs to be placed in the stream where the velocity is relatively slow, so that fish are not impinged on the downstream side of the pen. If fish are released volitionally, the mesh or holes in the pen or container should be large enough so that fish can easily swim through it when they have recovered sufficiently. In a hatchery situation, if fish are released back into a raceway or pond before they have recovered from the anesthetic, they may fall victim to predation by their cohorts. During summer and fall parr marking, some researchers will collect fish in the afternoon, hold them overnight, tag them the next morning, and then hold them until they have had adequate time to recover prior to release. Stream releases of larval lamprey should be conducted over fine sediment to allow for burrowing of released fish. Juvenile lamprey hide in the interstitial spaces of coarse substrate, so they should be released over some kind of cover. Larval and juvenile lamprey become largely stationary after

² Some chemical manufacturers recommend higher than 40 mg/l dosage for 1–3-minute induction. The PTSC recommendations are conservative and based on experience and the possible presence of ESA-listed species.

hiding in the substrate, so avoid releasing lamprey directly on or adjacent to tag detection equipment that could become overwhelmed by detections.

Prior to release, shed tags and mortalities should be collected and PIT tag records for these fish dotted out of the tagging file. A [training video is available on the PTAGIS website](#) to excise (dot-out) these records via tagging software. *Only reuse a PIT tag if it was recovered before release and its record has been removed (dotted-out) from all files submitted to PTAGIS. Tags that are recovered after release (e.g., recovered from a mortality in the field) should never be reused. If in doubt, do not reuse tags.*

e. Post-Tagging Mortality and Tag Retention

The PTSC recommends that, when applicable, a sub-sample of the marked population should be held and observed for up to 24 hours to obtain information on post-tagging mortality and tag loss.

3. PIT Tag Injectors

The Columbia River PIT tag community has agreed that needle disinfection is a requirement for all PIT-tagging in the basin when using MUIs, to reduce the chance of spreading disease. SUIs are exempt from this requirement if the needle assembly is not reused. Because MUIs can be disinfected between fish and SUIs don't need to be disinfected, they are the only PIT tag injection devices approved by FPAC for use in the Columbia Basin. The recommended disinfection method for MUIs is to put the needles in an alcohol disinfectant bath for at least ten minutes.

a. Single-use Injector (SUI)

SUIs have become the industry standard for most researchers within the Columbia Basin. These devices not only eliminate the need for disinfection but create a more efficient and safe process for each tagging operation. Single-use laser sharpened needles mean more depth control during the tagging process and a cleaner incision, which promotes faster healing of the wound. The ease and efficiency of use mean less time spent prepping before tagging operations. Also, SUIs make it easy and efficient to alternate tag sizes (e.g. 9mm v 12mm) when tagging different species or sizes of fish. In rare circumstances, improper functioning push rods can cause "deep insertions" from needles when attempting to tag fish. SUIs and needles should be checked for proper function prior to tagging live animals.



Figure 1. Common single-use injector (SUI) and associated needles.

b. Multiple-use Injector (MUI) Disinfection

MUIs remain a viable option for PIT-tagging but require some level of maintenance to maintain fish health and ensure clean insertion for faster wound healing. MUIs need to be disinfected between fish. The disinfection is intended to reduce or eliminate the possibility of lateral transfer of Bacterial Kidney Disease (BKD) and other diseases. In a lab or hatchery setting it may be possible to sterilize using an autoclave; however, the standard process involves an alcohol disinfectant. Two different alcohols are recommended as disinfectants. Pure ethyl alcohol (ethanol) is nontoxic “in moderation”. Denatured or “reagent” grade ethanol will work for disinfection purposes, but it contains methyl and/or isopropyl alcohols, which make it toxic for human consumption and is slightly more toxic on the skin.

Denatured ethanol should be diluted as if it were 100% ethanol. Isopropyl alcohol can also be used but is toxic for human consumption and slightly toxic by inhalation and absorption through the skin. *Never use methyl alcohol*. It is very toxic to humans if consumed or absorbed through the skin. Other alcohols are not effective disinfectants.

Alcohol is most effective when diluted with water to a final concentration of 70-80% ethanol or 60-80% isopropyl solution (by weight). Water in the alcohol is very important. Water acts to rehydrate a dried cell so the alcohol can penetrate the cell membranes. Alcohols act by dissolving the membrane lipids, denaturing soluble proteins, and by depressing surface tension. Alcohol has excellent antibacterial activity against most vegetative gram-positive bacteria, gram-negative bacteria, and tubercle bacillus organisms, but do not inactivate bacterial spores (Adams H.R. 1995). *FPAC requires that all MUIs and PIT tags used with MUIs be disinfected between fish in a 70-80% ethyl- or 60-80% isopropyl-alcohol solution for a minimum of 10 minutes.* Equipment and PIT tags

should be completely air-dried following the disinfecting process and prior to use on fish. Be sure to read and follow all recommendations of the Material Safety Data Sheet (MSDS) provided by the manufacturer and have a copy on-site, as required by OSHA.

Disinfection of the MUI is accomplished by immersing the needle and pushrod in the alcohol solution for at least ten minutes. The PTSC recommends that the needles be cleaned between each use to remove slime and scales that adhere to the needles. Slime and scales can be a path of infection between fish even if the needles are otherwise disinfected properly. The bore of needles should be checked periodically and cleaned, if necessary, using a pipe cleaner. Dried accumulation of scales on needles may require soaking and scrubbing. *To maintain clean insertion (promoting faster wound healing), MUI needles may need to be sharpened or replaced after several uses.*

c. MUI Construction

MUIs can be simple devices consisting of a 5-10 ml syringe with the appropriately sized veterinary-grade needle attached. A 12-gauge needle is used for standard 12mm tags shown in Figure 2. A push rod is attached to the plunger of the syringe. When the plunger is depressed, the push rod should protrude no more than two millimeters past the tip of the needle. The end of the push rod must be smoothed and rounded so it won't damage internal organs. If the rubber seal has not been removed from the plunger, a small hole must be made at the bottom of the syringe to allow air to escape. If this isn't done, you will inject air into the fish along with the PIT tag.

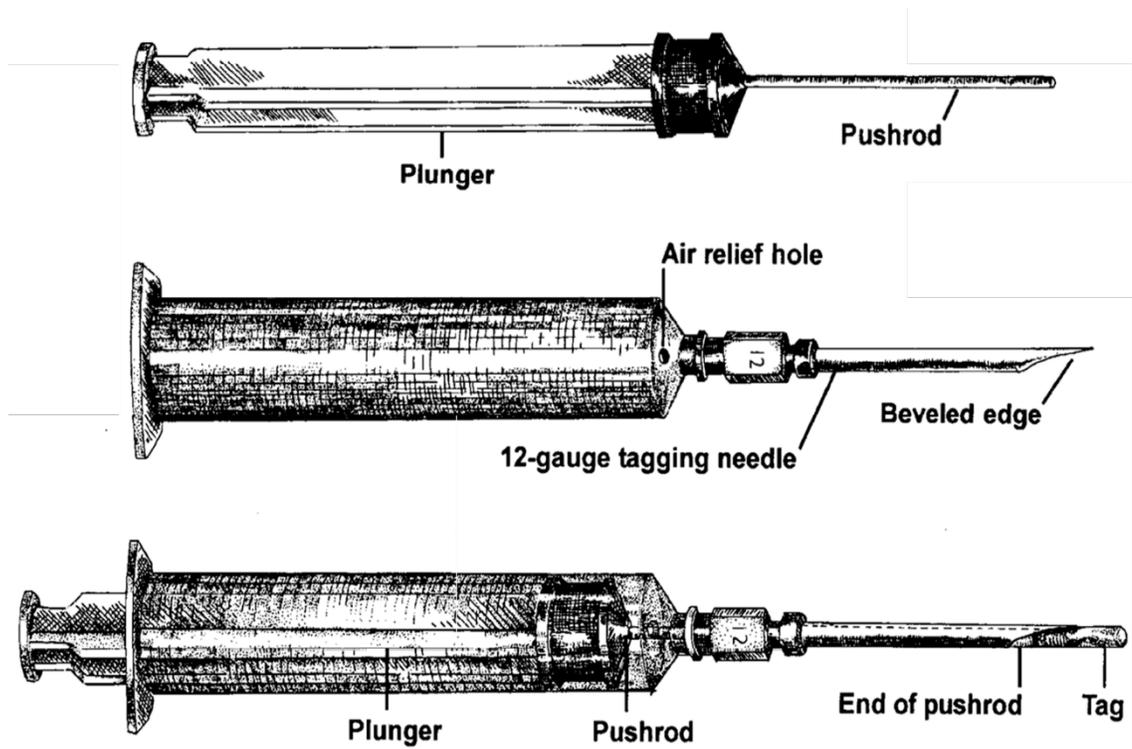


Figure 2. A simple PIT tag multiple-use injector (MUI).

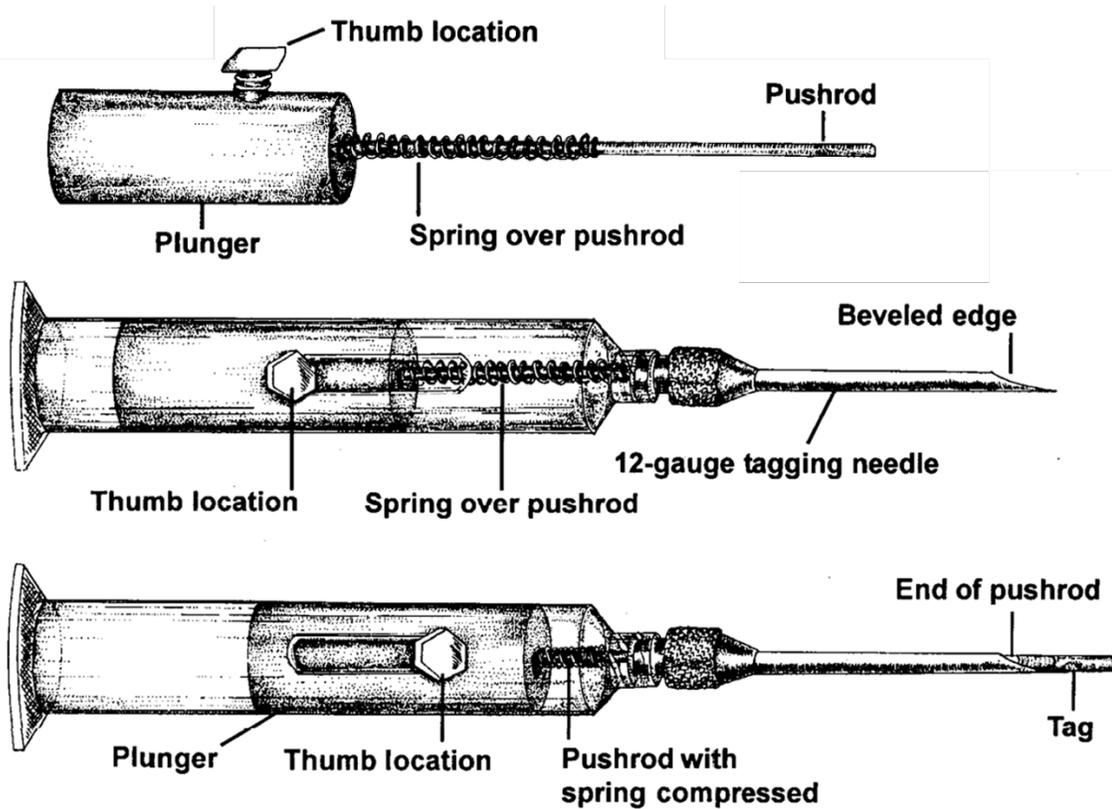


Figure 3. A spring-return PIT tag multiple-use injector (MUI)

There are many possible variations to the MUI with the most elaborate incorporating thumb-operated plungers and springs in the barrel of the syringe to automatically retract the push rod (Figure 3). The purpose of the spring is to keep the plunger retracted, so it will not accidentally push the PIT tag out of the needle. To make this type of injector you will need:

- a 10 ml syringe,
- a spring like that found in an ink pen,
- a piece of stainless-steel welding rod about 1/16 inch in diameter and about 3¼ inches long,
- a piece of round solid plastic about 1/2 inch in diameter and one inch long, and
- a 1/4 x 1/2-inch nylon screw or bolt with a large head.

Remove the plunger and cut a slot about 1/4 inch wide and about 1-1/8 inch long in the side of the syringe. The slot should start about 3/4 inch from the bottom of the barrel. Take the 1/2-inch piece of round plastic and drill a 5/32" hole in the side to a depth of 5/16". The hole should be drilled about one third of the distance from one end. Heat one end of the stainless-steel wire until it becomes red hot and then push it about 3/8-1/2 inch into the center of the end farthest away from the hole you drilled. Slip the spring over the wire and then slip the wire and round plastic into the barrel of the syringe. Put the nylon bolt through the slot in the syringe and screw it into the hole in the round piece of plastic. Put a 1-1/2 inch 12-gauge veterinary-grade needle on the syringe, push in the plunger all the way, and cut off the stainless-steel push rod not more than 1/16- inch past the tip of the needle. Smooth and round the end of the wire.

Finally, round off the side of the hex head nut where your thumb will be placed to push the plunger in. This will prevent a sore spot from developing on the tagger's thumb when thousands of fish are tagged per day. If properly cared for, this type of injector will last for three to five years.

4. Tag Insertion in Juvenile Fish

The PTSC and FPAC members do not endorse or recommend any tagging location in anadromous salmonids or lamprey other than what is described in this manual.

a. Tag Location

In juvenile salmonids the tag should be inserted into the ventral area of the abdominal cavity somewhere between the pyloric caeca and the pelvic girdle,

generally in the fatty tissue just posterior to the pyloric caeca (see Figure 9). It is all right if the tag lies under the pelvic girdle.

The abdominal cavity of larval and juvenile lamprey is small compared to juvenile salmonids. Tag insertion should occur at the largest part of the abdominal cavity, just posterior to the last gill pore.

b. Fish Size

Depending on species, life stage, and rearing strategy, there can be a considerable size difference in fish. The optimum size of juvenile salmonids to tag with 12 mm tags is about 80-150 mm in fork length. This sized fish is easy to handle, and the needle will penetrate the body wall smoothly. Small fish are difficult to hold in your hand and the point of insertion may need to be moved slightly forward of the posterior tip of the pectoral fin. This will provide a little more room for the tag in the abdominal cavity. Great care must be taken with small fish not to damage internal organs with the needle or when injecting the tag. The needle should not be inserted beyond the bevel and ideally only 2-3mm into the cavity of the fish. Large juvenile salmonids (>200 mm) can be difficult to hold and can be hard to penetrate with the needle. The point of the needle will often hit a scale, which adheres to the needle point and prevents penetration of the body wall. In this situation, pull the needle away from the fish, remove the scale from the point of the needle, and then penetrate the body wall where the scale was removed.

Specialty PIT tags can be obtained as small as 8 mm. For Chinook salmon, a study by Tiffan et al. (2015) showed survival and growth were not affected by 8 mm and 9 mm tags implanted at fork lengths of 40-49 mm, and by 12 mm tags implanted at fork lengths of ≥ 50 -69 mm. However, different taggers and projects have reported varied experiences tagging relatively small salmonids. This could be due to a variety of factors like tagger experience, tagger dexterity, or morphological variability in small fish. For this reason, projects tagging fish at these small sizes may want to evaluate their growth and survival (see Section 2.e. post-tagging mortality and tag retention). As a guideline, PTSC recommends that Chinook Salmon implanted with 8 mm PIT tags should have a fork length of at least 40 mm, and 9 mm PIT tags should be implanted in fish with fork lengths of at least 50 to 55 mm. Taggers often begin implanting 12 mm PIT tags in Chinook salmon at fork lengths of 60 or 65 mm (range 55 to 70 mm). Tagging recommendations are often required to be species specific to these small body sizes due to their morphological differences (see Section 2e. Post-tagging Mortality and Tag Retention). Species that have relatively large body depth like Chinook salmon may more easily tolerate larger tags at these smaller body sizes, compared to steelhead that have less body depth. Steelhead can be difficult to tag below a 60 mm fork length with a 9-mm PIT tag and below a 70 mm fork length with a 12-mm PIT tag.

The relatively small abdominal cavity of larval and juvenile lamprey constrains what sized PIT tags can be inserted. Larval lamprey ≥ 70 mm total length can be tagged with a narrow-width (1.4 mm) 8-mm tag. Larval and juvenile lamprey ≥ 80 mm total length can be tagged with a narrow-width (1.4 mm) 10-mm tag. Reserve standard 12-mm PIT tags for larval and juvenile lamprey at sizes ≥ 120 mm (Mueller et al. 2006, Mosier et al. 2017).

c. Body Position for Tagging Juvenile Salmonids

Juvenile salmonids are held in the hand with the belly of the fish up, the tail toward your thumb and the head toward your little finger (Figure 4). Position the fish in your hand so the point of injection is even with your middle finger. The head is held between the little finger and the heel of the hand. The tail is held between the thumb and index finger. Slight pressure is applied with the middle finger by pressing the side of the fish's belly. This will tighten the belly tissue so the needle will penetrate more easily.

Relatively small salmonids can be difficult for the tagger to hold in their hand. Some taggers use a foam board to help hold small fish. The board is prepared by cutting a foam wedge out of the corner of the board in a shape that allows for the fish's head to be inserted and supported (Figure 8). If using this technique, make sure the foam is wet or treated with synthetic mucus (e.g. a fish aloe product) to avoid damaging the fish's skin, operculum, or eyes.

d. Injector Position for Tagging Juvenile Salmonids

For injection of tags into juvenile salmon, both the thumb and plunger type MUI are held in the hand in a similar fashion. The injector is laid in the hand, so the tip of the index finger rests on the barrel of the needle (Figure 5). The barrel of the syringe is held by the fingers, and the little finger rests on the flange at the top of the syringe. The top of the syringe rests against the palm near the base of the little finger. The index finger rests on the barrel of the needle. If you have a thumb type injector, the thumb will rest on the bolt attached to the plunger. If you have a plunger type injector, the end of the syringe plunger will rest against the palm of your hand. (People with small hands may have difficulty holding plunger type injectors). The barrel of the needle rests against the heel of the palm of the hand holding the fish. This provides maximum support for the needle. It is recommended that you have as many contact points between you and the injector as possible. This will allow precise movement of the point of the needle. Without maximum control of the injector, the needle can penetrate too deeply and damage the internal organs of the fish. If you are using a plunger-type injector, use the heel of the hand, rather than your thumb to push the plunger. If you use your thumb, you can lose control of the needle and cause internal damage to the fish. Be sure to always maintain contact between the barrel of the needle and the heel of the hand holding the fish.

Positioning of the SUI in the tagger's hand should be based on the manufacturer's recommendation. Ask the manufacturer for a demo video, if available, to aid in training you and your staff. As with the MUI, the barrel of the needle should rest on the index or middle finger, and you want as many contact points between you and the injector as possible. This will allow precise movement of the point of the needle. Without maximum control of the injector, the needle can penetrate too deeply and damage the internal organs of the fish.

In some scenarios, a fixed-depth micro scalpel (1.5 mm) can be used for small juvenile salmonids tagged with 8 or 9 mm tags. This can give the tagger greater control, ensuring a needle or blade does not go too deep into the body of a fish.

e. PIT Tag Injection of Juvenile Salmonids

The needle should penetrate the fish's belly between the posterior tip of the pectoral fin and the anterior point of the pelvic girdle. Avoid placing the point of the needle too far posterior. There is a loop of the intestine under the pelvic girdle so you should avoid inserting the needle in that area. The puncture should be made one to two millimeters off the mid-ventral line (Figure 4). If you necropsy a fish, you will see that the pyloric caeca and the spleen lie below the puncture wound. The tip of the needle can nick either organ without injury to the fish (Prentice et. al. 1986).

Using the middle finger of the hand holding the fish to add pressure, place the tip of the needle on the belly of the fish 1-2 mm from the mid-ventral line. It is recommended that the bevel of the needle opens toward the belly of the fish, so the point of the needle is away from the internal organs. This keeps the needle tip away from the internal organs and prevents the tag from catching the skin and sliding along the outside of the fish or into the muscle. However, it can prevent the tagger from being able to see that a tag has entered the fish. This has led some taggers to angle the bevel to some degree. The puncture is made with a short, quick, steady motion.

Maximum control is needed because the tip of the needle should move forward only about 1-2 mm. The angle of the needle should be at about 45° above the belly of the fish and the motion of the needle should be directed through the fish and at your middle finger. Once the needle has penetrated the abdominal wall, and with about 2- 3 mm of the needle inside the fish (for fish up to about 150 mm), the tag can be injected. Using this technique, if you slip and the tip of the needle travels more than 1-2 mm the needle will either follow the inside wall of the abdominal cavity or will pass back out the abdominal wall without passing through any internal organs.

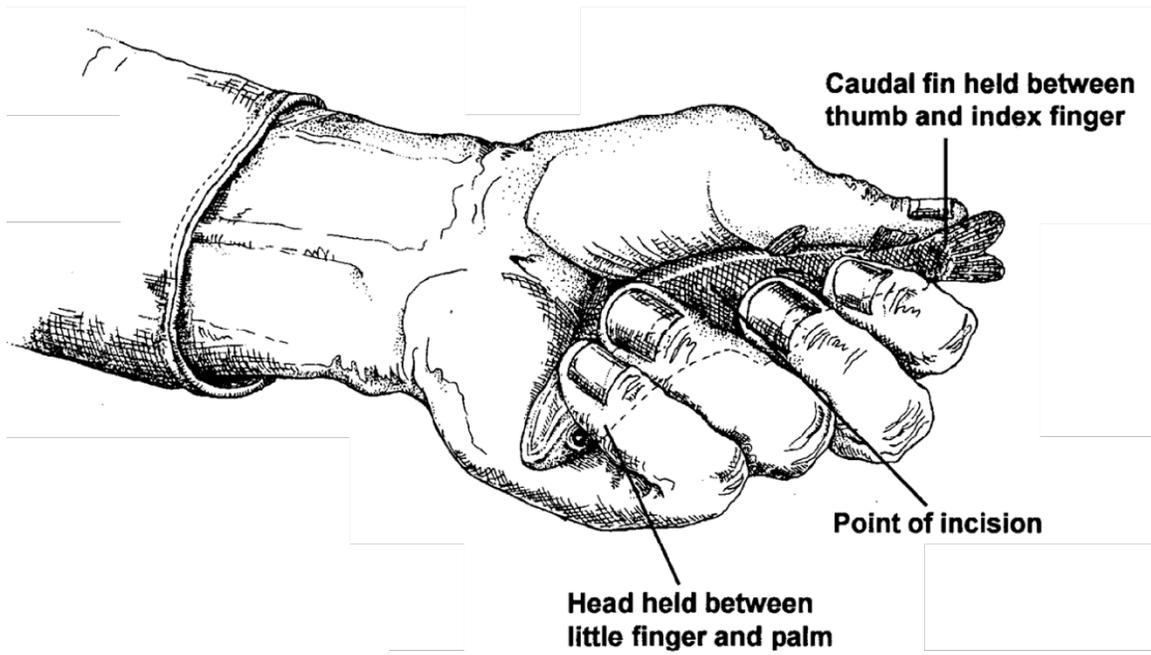


Figure 4 Fish held in hand in preparation for PIT-tagging.

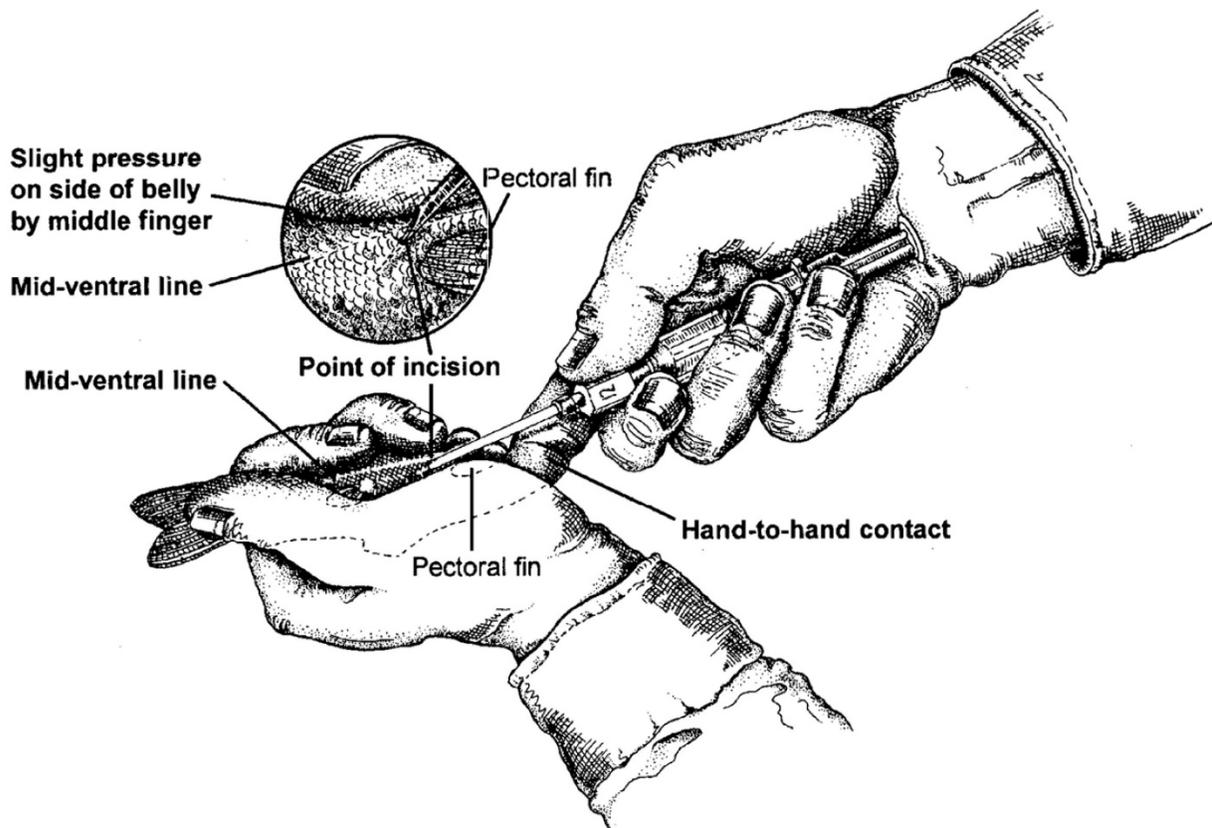


Figure 5. PIT tag MUI injector placement in hand

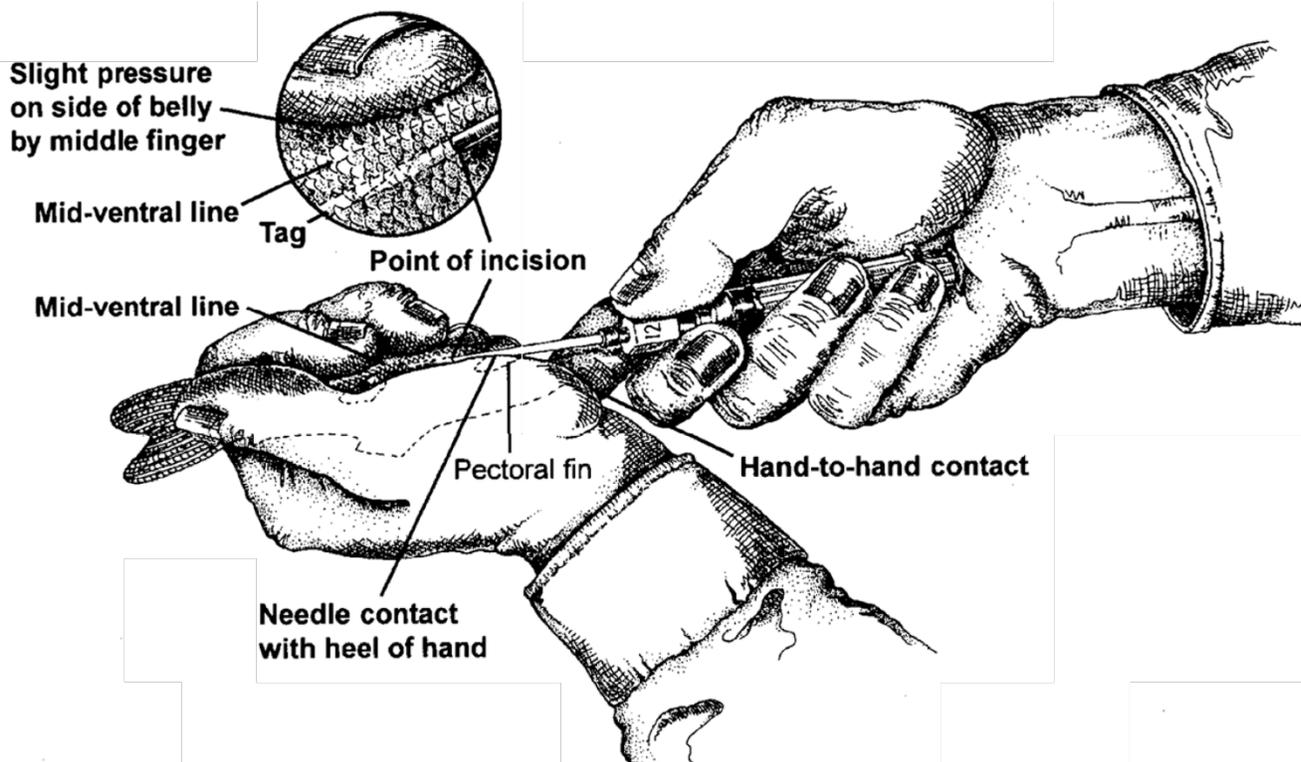


Figure 6. Inserting a PIT tag using a MUI

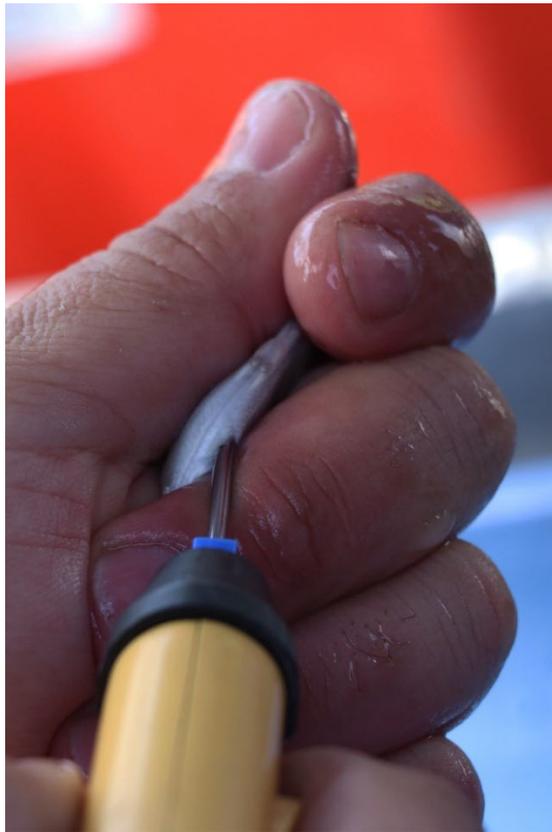


Figure 7. Inserting a PIT tag using a SUI (photo credit Benjamin Sandford, NOAA Fisheries).



Figure 8. Holding and injector placement for small salmonids (photo credit Ken Tiffan, US Geological Survey).

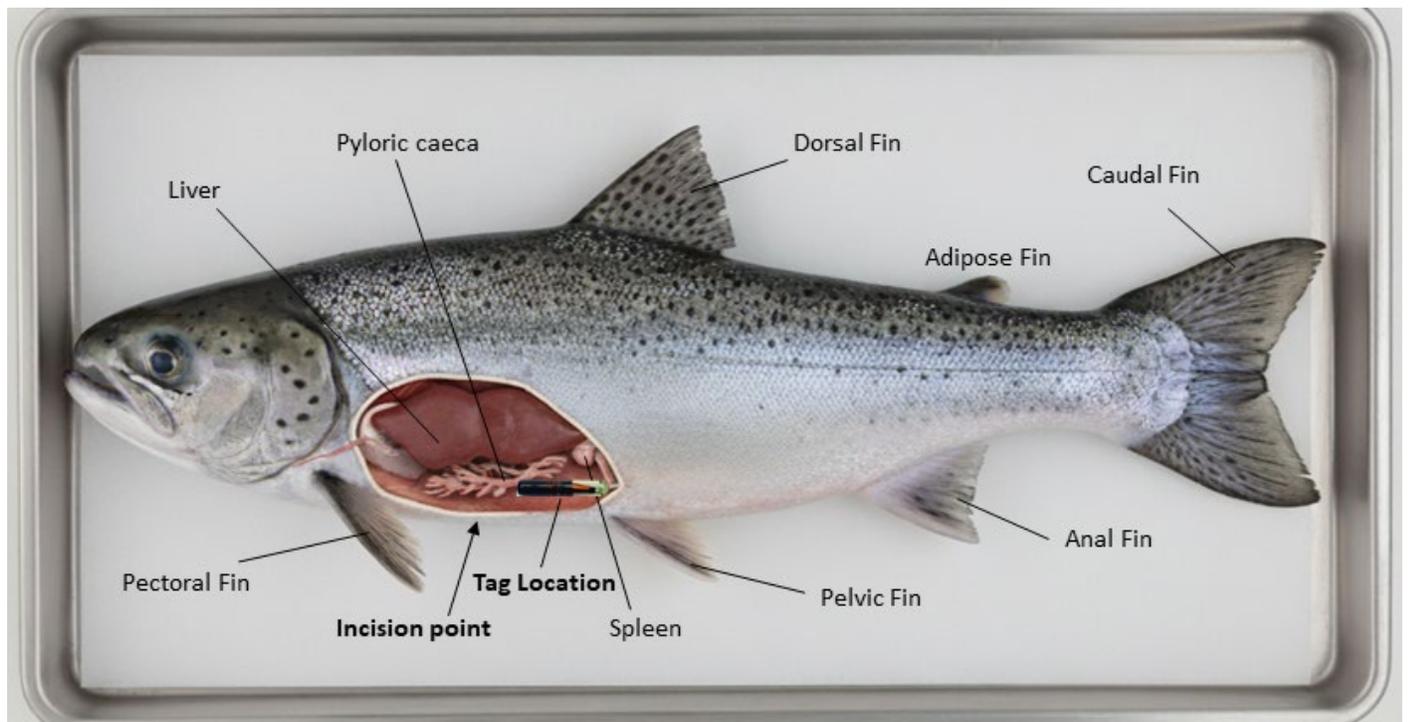


Figure 9. PIT tag location within the body cavity.

If you are using sharp needles, the puncture wound should be a straight incision about 1-2 mm in length. This type of wound may be difficult to see immediately after tagging and will heal quickly. If the needle is dull, the puncture wound will probably be a ragged hole, which will heal more slowly. There will also be some discoloration around the ragged wound that is caused from bruising of the tissue. Needles should be handled very carefully. Even slight contact between the needle and any hard surface will dull the needle and may cause a burr at the tip of the needle. The tagging supervisor should inspect all MUI's frequently to ensure all needles are sharp. SUI type needles should be checked for proper push rod operation prior to tagging operations. If there is any question about the quality of the needle, replace it with a new one. With SUIs, each block (100 count) of needles can have slightly different operation but should be laser sharp from the manufacturer.

f. PIT Tag Insertion for Larval and Juvenile Lamprey

Prior to PIT tagging larval or juvenile lamprey, ensure the fish is identified to species or genus when possible. Differences between juvenile (eyed) Pacific Lamprey and adult (eyed) Brook Lamprey (*Occidentis* spp.) are based on dentition and the appearance and coloration of the eye, body, and tail. Species identification of larvae can be challenging and is based largely on tail pigmentation. Wearable glasses with magnification can be useful for identification.

After the lamprey is anesthetized, place the fish on a grippy surface, like a firm closed-cell foam board. Some taggers cut a groove in the board to hold the lamprey during tag insertion. Place the lamprey on its side with the gill pores facing up and next to a 150-200 mm ruler. A 3-mm 15° fixed blade micro scalpel will be used to make an incision for tag insertion, since a longer blade or injector may increase the chance that an internal organ is injured. The scalpel should be sanitized in 0.8% Chlorohexidine when possible. The horizontal body location of the incision site is 10-15% of the fish's body length posterior to the last gill pore. The vertical body location of the incision is halfway (45°) on the body radius, between the lateral side of the fish (gill pore) and the ventral side of the fish (Figure 10). The small incision should run parallel to the fish's length. For smaller fish (approx. < 105 mm) the entire depth of the blade may not be necessary for an incision in the body wall long enough to fit a PIT tag. For larger lamprey insertion of the entire blade and a small sawing motion may be necessary to make an incision in the body wall large enough to insert a PIT tag. Insert the PIT tag perpendicularly through the incision until the body wall is breached, then angle the PIT tag posteriorly (parallel to the body) and push the tag into the body cavity. For the most current information on tagging lamprey consult materials produced by the Tagging Subgroup of the Lamprey Technical Workgroup at <https://www.pacificlamprey.org/ltwg>.

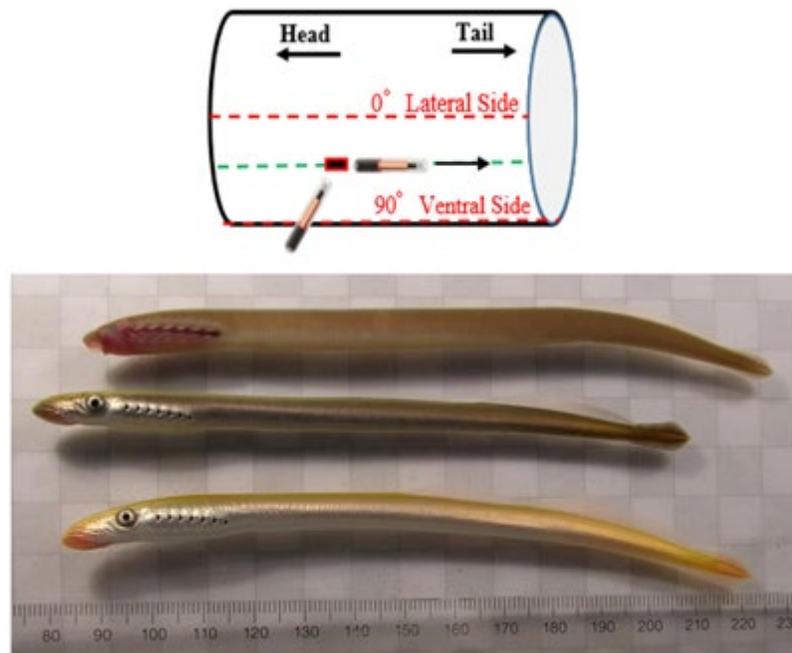


Figure 10. Incision location on the body radius of lamprey for PIT tag insertion. Gill pores on the lateral side should be facing up, and the mouth on the ventral side should be facing the tagger (diagram credit Tyler Beals and Ralph Lampman, Yakama Nation Fisheries; photo credit Jesse Lamb, NOAA Fisheries).

C. Computerized Data Entry

Each PIT tag code is unique, making accurate recording essential for subsequent identification. PTAGIS offers free software (P5) that enables researchers to efficiently record, validate, and manage PIT tag data. Users can connect readers and other peripheral devices directly to their computers, minimizing manual entry errors. Tag codes previously stored on a reader may be imported into this software.

This software captures events such as tagging, recapture following tagging, or recovery after death as [MRR \(mark/recapture/recovery\) data](#). These records are typically obtained while the fish is in hand and include both biological and condition details, as well as information regarding the timing, location, and personnel involved in data collection. The software offers validation features to catch common data entry errors and check if a scanned fish was previously tagged using vendor clip files. A clip file, provided by the tag manufacturer, is matched to the unique vial number and lists all tag codes expected inside. When imported, the software uses them to instantly verify scanned tag codes and flag any unexpected or misread tags. The *PTSC does not advise relying solely on clip files as a source of data; each fish should be scanned and recorded in the software when tagged.*

When data are entered, all collected information is checked against defined domains of acceptable values known as validation codes. This process not only promotes regional standardization in data entry but also ensures that the data are accurate, complete, and correctly associated with the tagged fish. Validation codes and other data conventions are described in the [PTAGIS Data Specification](#) which is governed by the PTSC and exclusive to the region. Researchers outside the region can use this software with custom validation codes, but PTAGIS cannot provide extended support in these situations.

PIT tag data should be inspected after collection to ensure integrity using the robust quality assurance features of the software. This is especially important when multiple species and rearing types are PIT-tagged.

The final product of the software is a uniquely identified data file that can be uploaded to, and incorporated in, the regional database operated and maintained by PTAGIS. The individual detail records for the tag codes in that file can then be directly correlated with other tagging, recapture, or mortality observations, as well as automated interrogation records. The cooperative collection and exchange of these data allow any interested party to monitor the movement of individual marked fish across the region.

For further information regarding the acquisition and utilization of data

collection software, guidance on transferring data files, or assistance with the PTAGIS database, please contact PTAGIS at (503) 595-3100, or visit their website at <http://www.ptagis.org>.

Literature Cited

- Adams, H.R., editor. Veterinary Pharmacology and Therapeutics. 7th edition. Iowa State University Press, Ames. 1995.
- Christiansen, H. E., L. P. Gee, M. G. Mesa. Anesthesia of juvenile Pacific Lampreys with MS-222, BENZOAK, AQUI-S 20E, and Aquacalm. North American Journal of Fisheries Management 33:269–276.
- Jolley, J. C., C. T. Uh, G. S. Silver, T. A. Whitesel. 2017. Low mortality of larval lampreys from electrofishing, suction-pumping, anesthesia, and handling. Journal of Fish and Wildlife Management 8:640–647.
- Moser, M. L., A. D. Jackson, R. P. Mueller, A. N. Maine, M. Davisson. 2017. Effects of passive integrated transponder (PIT) implantation on Pacific Lamprey ammocoetes. Animal Biotelemetry 5:1.
- Mueller, R. P., R. A. Moursund, M. D. Bleich. 2006. Tagging juvenile Pacific Lamprey with Passive Integrated Transponders: methodology, short-term mortality, and influence on swimming performance. North American Journal of Fisheries Management 26:361-366.
- Prentice, E.P., D.L. Park, T. A. Flagg, and C.S. McCutcheon. 1986. A Study to Determine the Biological Feasibility of a New Fish Tagging System. Report to Bonneville Power Administration, Project 83-319, Contract DE-AI79-84BP11982. 89p.
- Schoettger, R.A. and A.M. Julin. 1967. Efficacy of MS-222 as an Anesthetic on Four Salmonids. United States Bureau of Sports Fisheries and Wildlife Investigations in Fish Control. No 13. Washington, District of Columbia. USA.
- Tiffan, K. F., R. W. Perry, W. P. Conner, F. L. Mullins, C. D. Rabe, D. D. Nelson. 2015. Survival, Growth, and tag retention in age-0 Chinook Salmon implanted with 8-, 9-, and 12-mm PIT tags. North American Journal of Fisheries Management 35:845-852.
- Wedemeyer, G. 1970. Stress of Anesthesia with M.S. 222 and Benzocaine in Rainbow Trout (*Salmo gairdneri*). J. Fish Res. Bd. Canada. 27: 909-914.