PIT Tag Marking Procedures Manual

Prepared By:
Columbia Basin Fish and Wildlife Authority
PIT Tag Steering Committee

Version 2.0
1999

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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. The network has grown since those early days and now includes the PIT Tag Operations Center (PTOC), managed by the Pacific States Marine Fisheries Commission and funded by Bonneville Power Administration (BPA). PTOC houses, manages and maintains the PIT Tag Information System (PTAGIS) data repository where all anadromous PIT tag data in the Columbia River Basin are stored. PTOC collects and provides data obtained from the automated interrogation equipment at the mainstem dams. PTOC is also responsible for developing and maintaining the PIT tag data collection and validation software, providing training on PTAGIS and the data collection software, and maintaining the PIT tag interrogation equipment at the mainstem dams. PTOC 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 Columbia Basin Fish and Wildlife Authority's Fish Passage Advisory Committee (FPAC). PTSC is responsible for providing technical guidance for the field tagging, interrogation facilities and data management of PTOC. PTSC provides tribal, state, and federal agency representation with members from Idaho, Oregon, Washington, US Fish and Wildlife Service, National Marine Fisheries Service, and the Fish Passage Center, which is the representative for the Tribes. PTSC is responsible for coordinating the training of tagging personnel and facilitating the implementation of standardized tagging techniques and handling protocol to ensure consistent high quality marking.

This PIT Tag Marking Procedures Manual provides protocols and standards on the PIT tag marking station, fish handling techniques, anesthesia, tag injection, and information on data collection, verification, and transfer to PTAGIS. In addition to this manual, PTSC and PTOC plan to produce a PIT tag training video that will be available sometime in 2000. If you have specific questions that are not answered by this document, or if you would like to receive training, please contact your PTSC representative for assistance.

** Special Notice **

The Columbia Basin Fish and Wildlife Authority's Fish Passage Advisory Committee, at the request of the PIT Tag Steering Committee, decreed, in July 1992, that all PIT tag marking in the Columbia River Basin will be conducted using disinfected injectors and tags for all hatchery and wild anadromous fish. This requires that hand-held injectors be used, as there is currently no timely way to disinfect a semiautomatic injector between each fish. Disinfection is accomplished by immersing the needle and syringe in 70-80% ethyl alcohol or 60-80% isopropyl alcohol for a period of not less than 10 minutes. It is important that the push rod on the end of the plunger is also submersed in the disinfectant solution.

A. PIT Tag Marking Station

PIT tags are used to mark juvenile salmonids in a wide variety of situations in the Columbia River Basin, ranging from the collection and tagging of individuals in their natal streams to the marking of thousands of fish 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 small batches, anesthetized, and tagged;
3. Information about each animal is collected, recorded, and correlated with its injected tag; and
4. The fish are allowed to recover from the affects of the tagging, handling, and anesthetic before they are returned to their cohorts in the general population.

In most situations, a PIT tag marking station, developed around a 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.

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 streamside or backcountry marking. Marking station configurations and personnel/equipment requirements differ depending on the type of marking operation.

a. Mass Marking

Mass marking operations mark thousands of fish 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. To keep this type of tagging operation running efficiently you will need at least 500-750 tag injectors 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 if you have experienced personnel.

In the mass marking operation, such as tagging at a hatchery, 30 to 100 fish are anesthetized at one time. As each fish is PIT-tagged, it is routed to a person that scans the PIT code, records various information about the fish, and places it in a recovery tank. To minimize stress and the affect 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 marking population, **it is very important to scan all fish before tagging to ensure they are not already PIT-tagged**.

b. Traps and Weirs

PIT-tagging at traps and weirs normally is a much smaller operation than mass marking. Marking operations at traps and weirs generally mark run-of-river fish and mark several species and/or groups of hatchery and wild fish. Often in these situations 110 VAC power is not available and the PIT tag marking station is operated from battery power. The data collection equipment can be powered by any 12 VDC power supply, from small batteries with low amp-hour ratings to larger car type batteries that have up to a 100 amp-hour rating. Another way to supply power is with solar panels that recharge a battery system. With solar power you will have additional power to operate a water pump and/or aerator.

Aeration of work tubs and recovery tanks is highly recommended. This can be accomplished by using O_2 cylinders with a regulator to control pressure and a control valve system that will allow you to supply oxygen to all work tubs and recovery tanks. Other aeration methods are to run fresh water through the recovery tank using a water pump or to use an electric aerator. If aeration is not available the number of fish in a tub or recovery tank, and the time the animal spends in the tank, should be limited. Water in the tanks and tubs should be changed after every batch of fish processed.

At traps and weirs, a covered work area is very 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 multiport. The digitizer is generally housed in a Plexiglas cover to keep water off the device.

The person tagging selects a fish from the work tub and scans the fish by running it through the 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. Then length and/or weight can be recorded and the fish examined for other marks, clips, injuries, or descaling. The fish is then placed in the recovery tank.

PTSC recommends that fish should not be exposed to the anesthetic for more than five minutes. Only 20 to 50 fish should be anesthetized at one time, to ensure you can tag all the fish in the tub within five minutes. PTSC recommends that fish should be placed in a recovery tank and allowed to recover for a minimum of 30 minutes before being released back to the stream.

After fish have recovered, they can be placed in a net pen in the stream to protect them from predators and allow them additional recovery time. The net pen 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. The mesh in the upstream end of the net pen should be large enough so that fish can easily swim through it. With this type of net pen, fish can volitionally leave the sanctuary when they have recovered sufficiently.

c. Streamside

Streamside PIT-tagging operations are generally mobile situations where fish are collected by variously seining, electrofishing, or angling a segment of stream. The PIT-tagging equipment is operated using a 12VDC power supply and is either set up on the ground, on a portable table (roll-a-tables work well and are available in most sporting goods catalogs), or in a self-contained station.

Procedures for handling fish and PIT tagging are similar to that described for traps and weirs. Often O_2 is not available, so fish need to be worked quickly and returned to the stream for recovery. In this type of operation, the recovery tank can be a 30 gal. trash can or plastic box with a lid, with holes drilled in the sides to allow water exchange. 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.

Another option for streamside work is to pre-read the PIT tags and place each one, individually, in a scale envelope before going into the field. You can 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 this procedure you avoid having to transcribe the PIT tag code**. The PTSC strongly recommends that you <u>do</u> <u>not</u> transcribe PIT tag codes. The error rate when transcribing a PIT code is very high and unacceptable.**

Because streamside marking is generally performed during the summer, water temperature is very important. Water temperatures in excess of 15 degrees Celsius cause stress and additional care must be taken when handling and tagging fish. Temperatures above 17 degrees Celsius cause severe stress and high mortality. **The PTSC strongly recommends that all PIT tagging operations cease when water temperature exceeds 17 degree Celsius.**

2. Fish Handling Techniques

a. Minimizing Fish Stress

i. Fish Health. The health of the fish being tagged is very important in determining stress levels. Fish that are being attacked by a disease such as BKD or IHN will be difficult to handle, and mortality due to increased stress associated with the tagging operation will be elevated. The PTSC recommends that PIT-tagging operations be postponed until the outbreak is controlled.

ii. Water Temperature. Elevated water temperatures decrease a fish's ability to handle stress. Optimum tagging temperatures are between five and 10 degrees Celsius. Tagging fish below five degrees Celsius will not bother the fish but does cause problems for the people working with their hands in the water. As the temperature increases above 15 degrees, fish become stressed very easily. Great care must be taken when tagging fish in water temperatures greater than 15 degrees. Monitor fish in holding tanks and in anesthetic baths more closely and, if fish begin to show signs of stress, then cease operation immediately. Tagging fish in water temperatures greater then 17 degrees Celsius is not recommended and handling fish in water temperatures greater than 20 degrees must not occur. To avoid working with high water temperatures, you need to collect fish and PIT tag in the early morning hours when stream temperatures are at their lowest, or wait for cooler weather.

iii. Oxygen. Fish stress increases at low oxygen levels. It is important to supply recovery tanks and anesthetic baths with oxygen. As water temperature increases, oxygen becomes more important because warm water will hold less oxygen. There are two ways to supply oxygen. One is by bubbling air or oxygen through the water. Air can be supplied with an air pump and air stones. Oxygen can be supplied using oxygen cylinders. You will need to regulate the pressure from the oxygen bottle and some type of valve system so you can attach several air hoses and stones for the recovery tanks and anesthetic bath. The other way to supply oxygen is by running fresh water through your recovery tank. 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 depending on your situation. 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 your working area must be only a few feet above the water source. You can partially overcome the height differential between water source and working area by increasing the size of the bilge pump. The downside of a large 12 VDC pump is it will require a larger power supply.

iv. Fish Handling. How you handle fish can affect fish stress levels. Try and avoid chasing fish around when dip-netting them. Avoid putting too many fish in the dipnet and then having to release some. Set things up so you can net fish and immediately place them in an anesthetic bath. Use a sanctuary net if you have to carry fish short distances. Handling stress becomes more of a concern as water temperature increases. 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.

v. Crowding. Crowding can also increase stress levels, especially if you have both chinook and steelhead in the same sample container. Reduce fish density if you have chinook and steelhead together. When crowding fish in raceways, avoid overcrowding. 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. It is very important to frequently monitor fish that have been crowded.

b. Marking and Recovery Containers

Use plastic containers that do not have any toxic chemicals on them. It's recommended that you not use metal containers that have lead or zinc coatings. Aluminum or stainless steel works well for livewell 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. You can drill holes at about the 50-gallon level. You can run a water hose into the utility truck and have a recovery tank with water circulation. When you want to release fish you can simply roll the utility truck to the side of the trap or weir and dump the water and fish into the stream.

All fish containers should be dark in color. Avoid using white plastic buckets and tubs. Fish become very skittish when put in a white or light colored container. If you have aluminum or stainless steel containers, you can paint the inside of the container with a dark-colored non-toxic paint.

c. Anesthesia

Tricaine Methanesulfonate (MS-222), better known as MS, is approved by the FDA as an anesthetic for fishes and other cold-blooded animals. Neutralized MS (pH 7) is the most effective chemical 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 to have a copy on-site, as required by OSHA.

i. Concentration. The recommended concentration of MS to anesthetize salmonids is about 40 mg/l (Schoettger, R.A. and A. M. Julin. 1967). The required concentration will vary depending on water temperature, species of fish, and stress level of the fish. 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. Drug the tank so you can work the chinook first and then add more MS, as needed, to work the steelhead. Stress also plays a role in how fish react to the anesthetic. Fish that are suffering from stress will require less MS to be anesthetized.

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 50 mg/l. It takes about one milliliter of the stock solution per liter of water in the anesthetic bath to obtain the recommended concentration of about 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 (see Fisher Scientific Catalog or others)♣. 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. You can take a clear plastic container and wrap it with black electrician's tape to eliminate light penetration or you can purchase black or brown plastic containers.

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 at 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 one

♣ Reference to a specific manufacturer or product name does not constitute an endorsement by the PTSC.

to three minutes. You should still be able to see significant operculum movement and slight fin movement. 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 eminent. 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. In simple terms, fish should not be in the anesthetic bath for more than five minutes. **Under absolutely no circumstances should fish be allowed to stay in an anesthetic bath for more than 10 minutes.**

d. Fish Recovery and Release

Fish should be allowed to recover in a cool dark tank for at least a half-hour 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. 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 over night, tag them the next morning, and then hold them until that evening to release them. This allows the animal to partially recover from the stress of capture or being PIT-tagged before being subjected to the next phase. According to one researcher, the rate of interrogation at the dams the next spring is significantly higher for fish handled in this manner (R. Keifer, Idaho Dept. Fish and Game, personal communication).

e. Post-Tagging Mortality and Tag Retention

The PTSC recommends that 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 injector disinfection is a requirement for all PIT tagging in the basin, in order to reduce the chance of spreading disease. Because the injectors can be disinfected between fish, the hand-held injector is the only PIT tag injection device that has been approved by FPAC for use in the Columbia Basin. The recommended disinfection method is to put the injectors in an alcohol bath for at least ten minutes.

a. Injector Disinfection

PIT tag injectors 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. 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 to 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.

Alcohols are 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 re-hydrate 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. Alcohols have 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 PIT tag injectors and PIT tags be disinfected between fish in a 70-80% ethyl- or 60-80% isopropyl-alcohol solution for a minimum of 10 minutes.** 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 injector is accomplished by immersing the needle and pushrod in the alcohol solution for at least ten minutes. An easy method to disinfect injectors is to use a plastic case for carrying 20-gauge shotgun shells. There are two shelves in each case. Each shelf has impressions the individual shells set in. These impressions can be drilled out so the PIT tag injector will set in the impression with the needle pointing down. Shelf supports are riveted in the plastic case to hold the shelves. The alcohol solution is poured in the plastic case so it will cover the needle on the injector. The tags should be immersed in alcohol solution for at least 10 minutes prior to loading into the injectors. 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.

b. Injector Construction

Hand-held PIT tag injectors can be simple devices consisting of a 5-10 ml syringe with a 12-gauge veterinary-grade needle attached (see Figure 1). 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 1. A simple PIT tag injector.

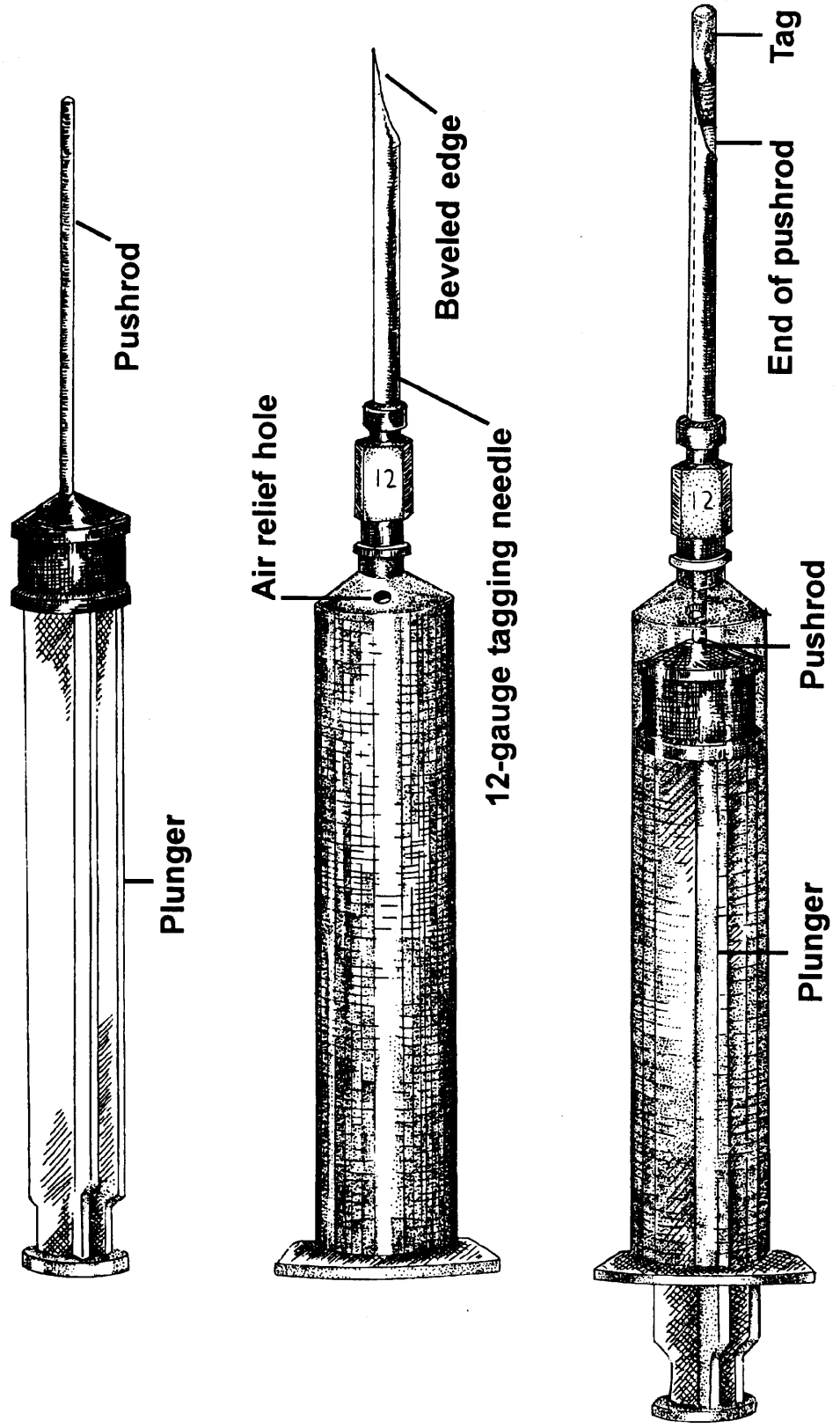
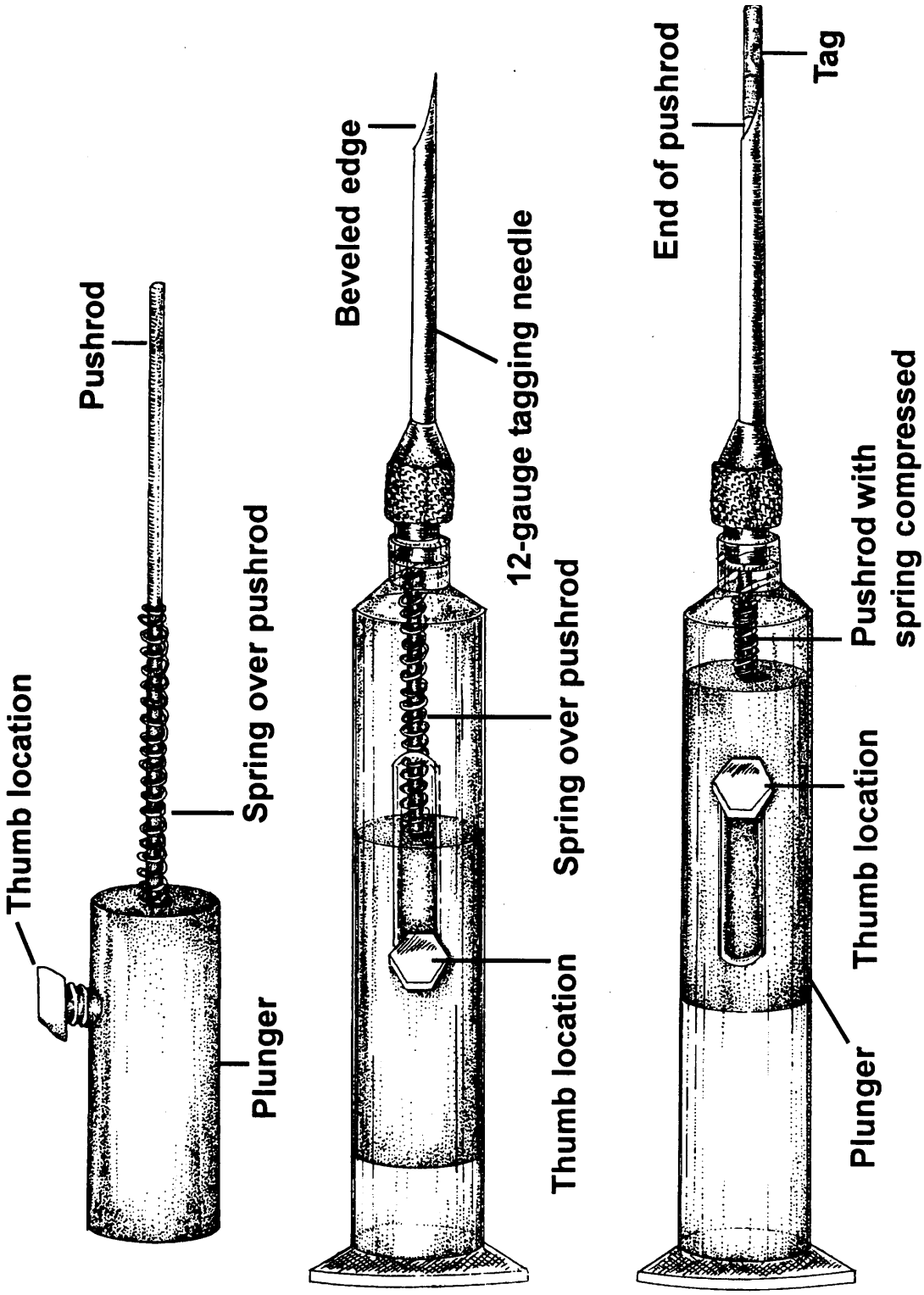


Figure 2. A spring-return PIT tag injector.



There are many possible variations to the hand held injector with the most elaborate incorporating thumb-operated plungers and springs in the barrel of the syringe to automatically retract the push rod (see Figure 2). 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 similar to that found in an ink pen,
- a piece of stainless steel welding rod about 1/16 inch in diameter and about 3 ¼ 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 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 vet 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.

List of components and suppliers:♣

Component	Specification	Common Suppliers
Vet Needle	1-1/2 inch long JorVet 12-gauge	Jorgensen Labs, Loveland, CO; Popper (516) 248-0300; VITA (781) 444-1780.
Springs	Part No. 353-B. 1.38 inches long and 0.188 inches wide.	Century Spring Co. Inc., Los Angeles, CA (213) 749-1466
Plastic Rod	1/2 inch diameter UHMW	Local industrial technologies supply store
Nylon Bolt	3/16 x 1/2 inch	Local hardware store
Stainless Steel Wire	1/16 inch diameter	Local welding supply store
Syringe	5-10 ml	Laboratory supply catalog (Fisher Scientific, Van Waters & Rogers, etc.)

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4. Tag Injection

a. Fish Position In Hand

The fish is held in the hand with the belly of the fish up, the tail toward your thumb and the head toward your little finger (see Figure 3). Position the fish in your hand so the point of injection is even with your middle finger. With an 80-150 mm fish, 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.

b. Injector Position in Hand

Both the thumb and plunger type PIT tag injectors 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 (see Figure 4). 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. 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. 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 maintain contact at all times between the barrel of the needle and the heel of the hand holding the fish.

c. PIT Tag Injection

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 (see Figure 5). If you necropsy a fish you will see that the pyloric ceca 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. The bevel of the needle is open toward the belly of the fish so the point of the needle is away from the internal organs. The puncture is made with a short, quick, jabbing 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 3. Fish held in hand in preparation to PIT-tag.

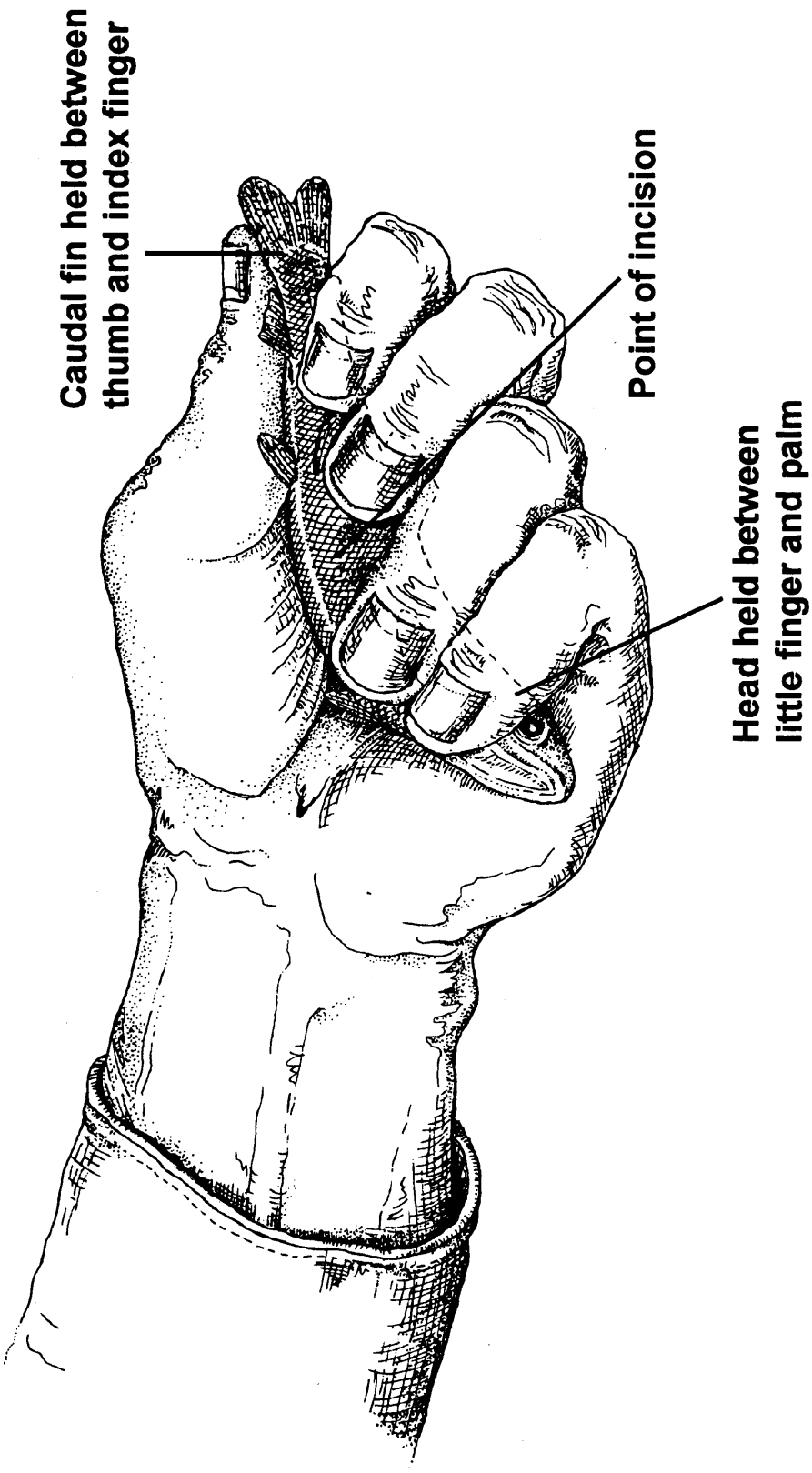


Figure 4. PIT tag injector placement in hand.

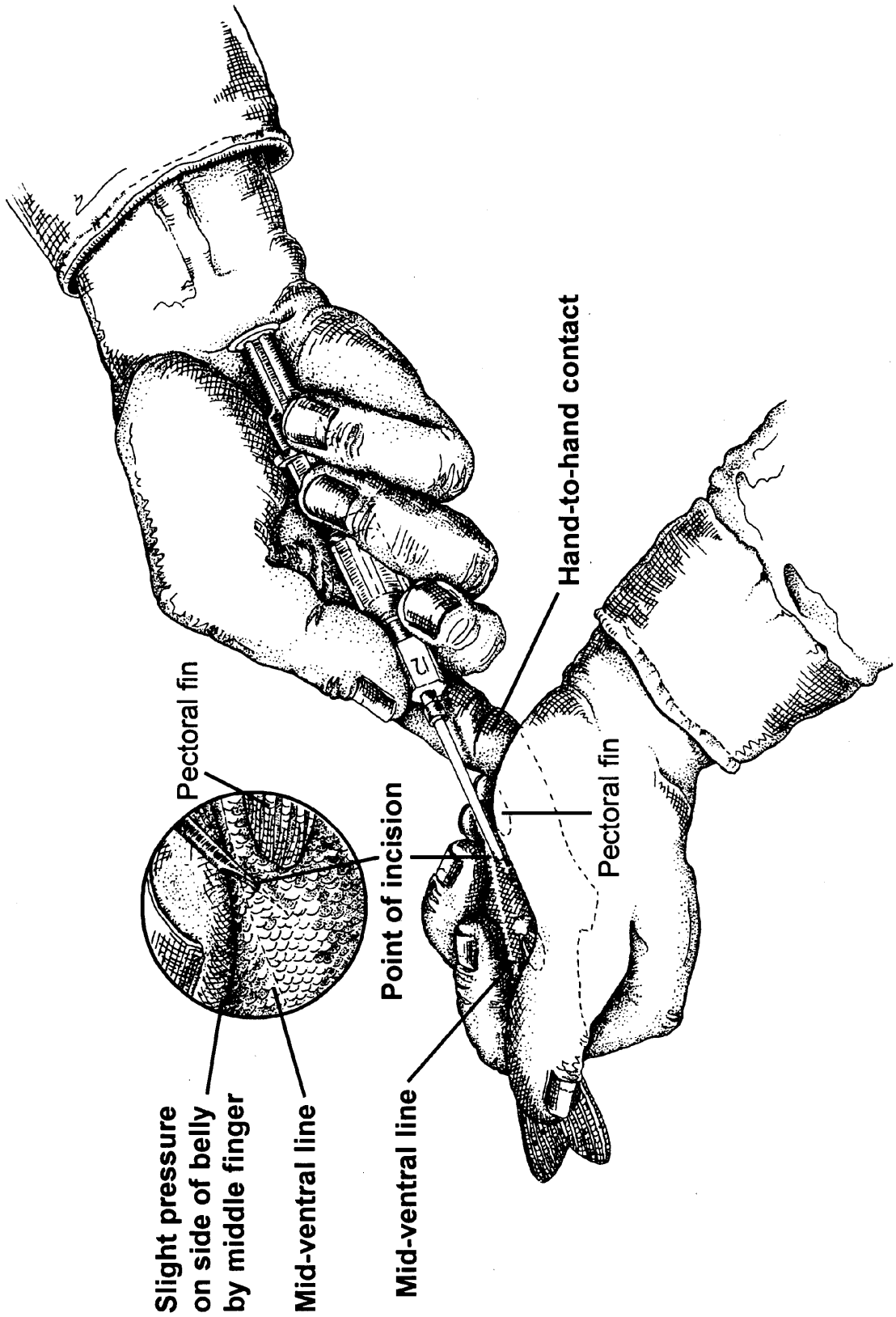


Figure 5. Inserting the PIT tag.

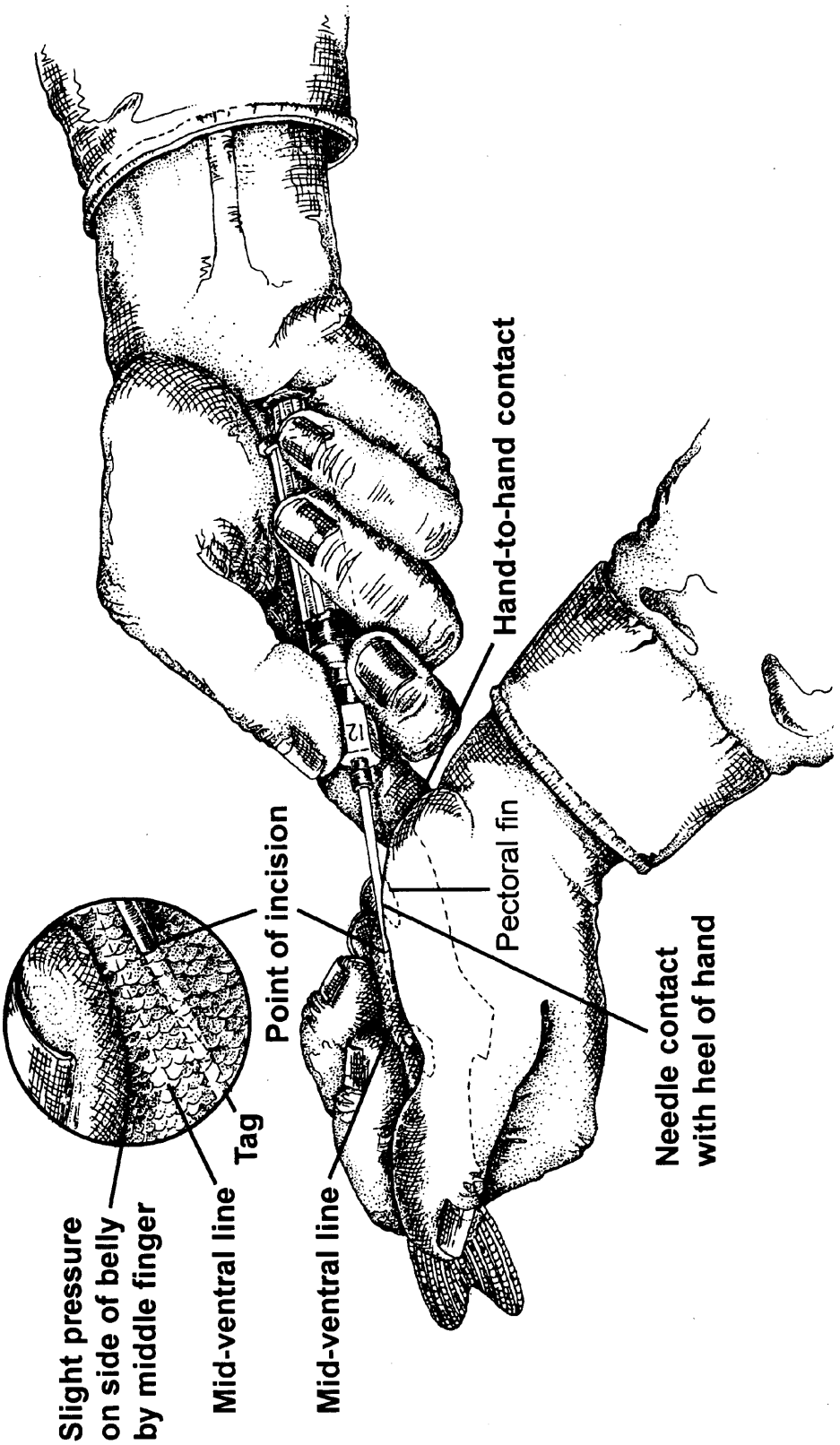
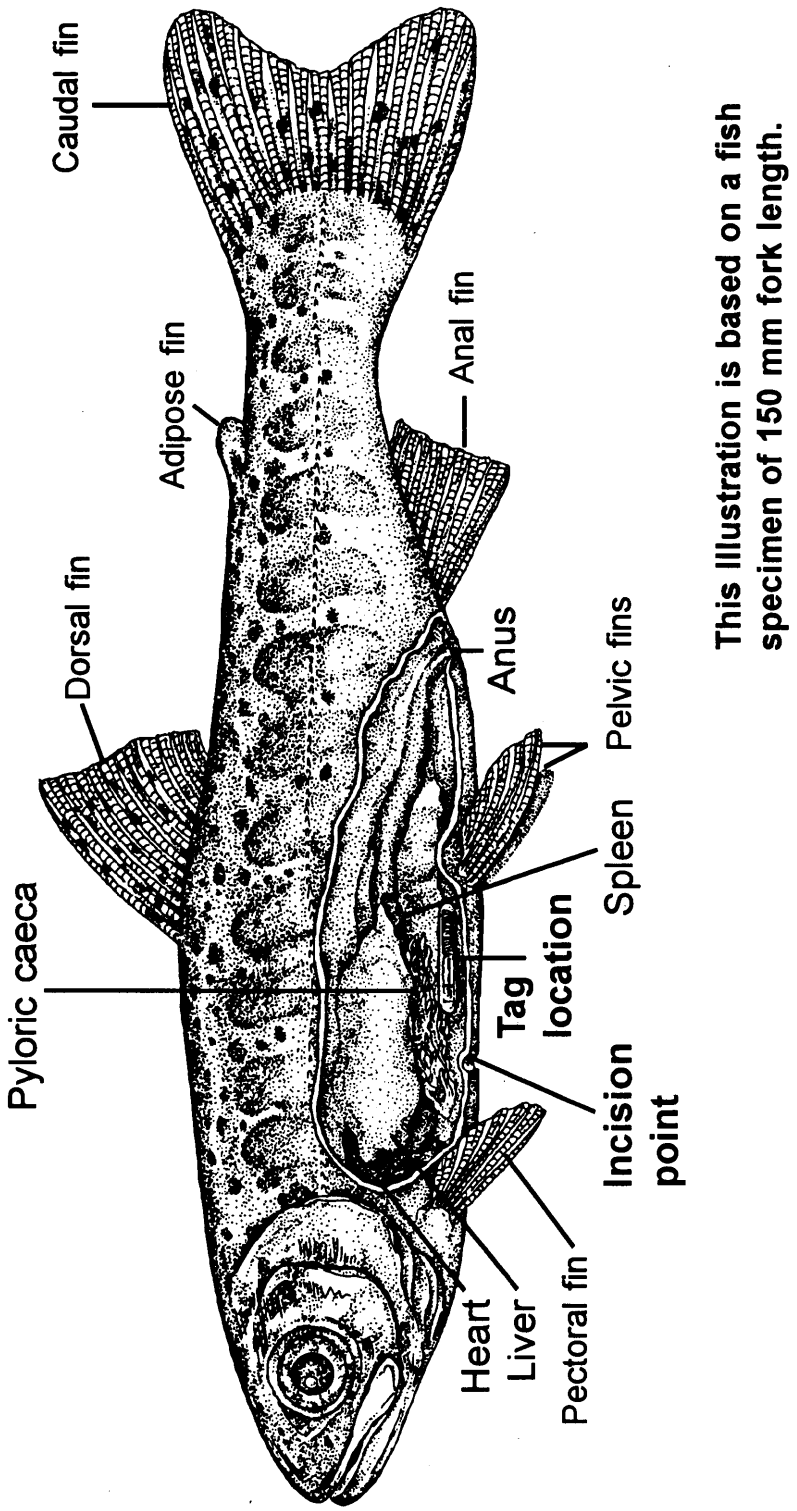


Figure 6. PIT tag location within the body cavity.



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.

d. Tag Location

The tag should be located in the ventral area of the abdominal cavity somewhere between the pyloric ceca and the pelvic girdle, generally in the fatty tissue just posterior to the pyloric ceca (see Figure 6). It is all right if the tag lies under the pelvic girdle.

e. Fish Size

Depending on species and rearing strategy, there can be a considerable size difference in fish. PTSC recommends not tagging fish less than 65 mm. Very small fish are difficult to handle and tagging can increase vulnerability to predation and decrease swimming stamina (McCann *et al*. 1993). Also, several researchers have found that the interrogation rate of small fish (<65 mm at tagging) at the dams is lower than fish of larger sizes (Achord *et al.* 1993).

The optimum size of fish to tag is about 80-150 mm. This size fish is easy to handle and the needle will penetrate the body wall smoothly. Small fish are very 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. Large fish (>200 mm) are difficult to hold, especially for people with small hands. Large fish are often hard to penetrate with the needle. The point of the needle will often hit a scale and the scale will adhere to the needle, preventing penetration of the body wall. In this situation, pull the needle away from the fish, remove the scale from the tip of the needle, and then penetrate the body wall where the scale was removed.

C. Computerized Data Entry

Each PIT tag code is unique. PTOC has developed and maintains specialized computer software to enable researchers to quickly record and store PIT tag data as it is collected, while simultaneously validating the format and values of those data. This software allows the user to connect PIT tag transceivers (readers) and other data devices directly to the computer, maximizing the ability to utilize direct electronic measurement and minimizing the potential for error due to keyed data entry. The software uses a system of shortcuts for program commands and data values that streamlines program operation and data entry, and permits consistent, standardized data input. The software also allows a user to import data previously stored to a transceiver, and the ability to append or modify corresponding information in the records associated with that imported data.

In general, there are two types of data collected during a "Tag Session." The first is the generic information global to the session. There are a number of standard session descriptors available to, and/or required from, the researcher. A partial list of these descriptors includes: the date and location of the Tag Session event, the date and location of the subsequent release of tagged fish, a verbose study description, various water temperature values, and the entity responsible for the research or monitoring. The second type of information collected is specific to each PIT tag code and provides detailed information on the tagged animal, such as fork length, weight, physical and/or physiological condition, as well as the observed species, run (if applicable), and rearing type of the fish. In addition to recording data at the time of tag insertion, the user can enter data on "recaptured" fish marked previous to the current tag session, and can document the mortality of a previously marked and released animal.

All collected information is verified against various domains of allowable values at the time of data entry. Besides helping to standardize data input, this also helps to ensure that data values are valid, complete, and properly correlated with the correct tagged fish. These variables are listed in the **PTAGIS PIT Tag Specification Document**, revised and published annually, and available from PTAGIS on the Web at http://www.ptagis.org.

PIT tag data sets should be inspected subsequent to data collection to double-check the data contents and context integrity. Some types of problems you may encounter are hatchery fish for which the adipose (AD) fin clip was not recorded or a wild fish that accidentally had an AD designation recorded next to it. These types of context errors or omissions generally occur when tagging run of the river fish. You can check the file visually or you can build an Excel program that will do the job. Scott Putnam (*sputnam@idfg.state.id.us*) has created a program in Excel (PITSUM) to look for this type of context error. You can create your own program or you can contact Mr. Putnam who will provide you with a copy of his PITSUM program. You will probably need to modify the program as it is very site specific. PITSUM type programs are very useful for finding errors in PIT Tag file that the validation program will not find and in summing different groups of fish. You can write the program to sum groups of fish also. This is important for such programs as the Smolt Monitoring Program where you may want to know the number of right or left ventral clips, the number of hatchery and wild fish, the number of elastomer tagged fish, etc.

The final product of the entry and validation software is a data file that can be uploaded to, and incorporated in, the regional PTAGIS mark/recapture database, operated and maintained by the PTOC. The individual detail records for the tag codes in that file can then be directly correlated with other tagging, recapture, or mortality observations, or automated interrogation records. The cooperative collection and exchange of these data allows any interested party to monitor the movement of individual marked fish across geographic, political, and jurisdictional boundaries.

For more information about acquiring and using the data collection software, instructions for transferring data files to PTOC, or help with the PTAGIS database, please contact the PTOC Program Manager at (503) 650-5400, or visit the PTAGIS website at http://www.ptagis.org.

Literature Cited

Adams, H.R., editor. Veterinary Pharmacology and Therapeutics. 7th edition. Iowa State University Press, Ames. 1995.

Achord, S., D.J. Kamikawa, B.P. Sandford, and G.M. Mathews. 1993. Monitoring the Migration of Wild Snake River Spring/Summer Chinook Salmon Smolts, 1993. Report to BPA, Project 91-28, Contract DE-A179-91BP18800. 88p.

McCann, J.A., H.L.Burge, and W.P.Connor. 1993. Evaluation of PIT Tagging of Subyearling Chinook Salmon. Pages 63-85 in D.W. Rondorf and W.H Miller, editors. Identification of the Spawning, Rearing, and Migratory Requirements of Fall Chinook Salmon in the Columbia River Basin. Annual Report 1991 to Bonneville Power Administration, Portland, Oregon.

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.

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.