



## **6.0 Marking and Tagging of Finfish**

### **6.1 Introduction:**

This template is intended for use by instructors to train the Department of Fisheries and Oceans (DFO) staff and students in techniques for marking and tagging of research fish. An experienced instructor must demonstrate these procedures, and trainees must be deemed qualified in carrying out the techniques before they can be permitted to mark or tag fish without direct supervision. These procedures are specifically designed for the tagging of salmonids; species differences will have to be taught by the instructors based on the requirements of the individual facility.

Introductory and advanced level exercises require knowledge of anatomy, anaesthesia and handling of finfish. The advanced procedures also require more intensive training in surgical methods such as suturing and aseptic technique.

Templates provide the minimum requirements to be met in a training event, but the instructor may add additional material. Hands-on training of staff is a requirement for facility approval by the Canadian Council on Animal Care, of which DFO is a member. This template is part of a comprehensive DFO Science Branch series on training for users of aquatic research animals.

### **6.2 Rationale:**

Fish are marked or tagged so that individuals or groups of fish can be readily identified or differentiated from other individuals or groups of fish. Fish identification can provide information on fish movement, stock identification, population estimates, growth and also aid in laboratory based research studies. Where groups of fish need to be cohabited, the use of different marks such as fin clips or freeze branding can allow easy distinction between the groups. In situations where individual fish need to be identified for tracking, growth and performance; implanted tags such as PIT tags or VI tags can be used. Field studies may involve the tagging of large numbers of fish with visible external marks or tags for mark and recapture studies; alternately radio or acoustic tags can be surgically implanted in the peritoneal space so that fish movements can be detected in the environment without recapturing the fish.

There are many different methods used for marking and tagging fish, each with their own advantages and disadvantages. The choice of method used will depend on the species, age, size of the fish, number of fish required for the study, cost, amount of loss allowable, effects of the mark or tag on the performance of the fish during the study, as well as the length of time the mark or tag is required. The choice of the appropriate mark or tag used is the responsibility of the researcher.

### **6.3 Authority:**

The staff/consultant veterinarian or Animal Care Committee is responsible for providing information about marking and tagging procedures used on the fish



species used for scientific study in their respective regions. The introductory procedures are to be taught by instructors who have demonstrated knowledge and experience in the areas of anatomy and anaesthesia and has demonstrated excellent survival of fish that have been marked or tagged using the techniques outlined in this training template. The advanced techniques are to be taught by a veterinarian with experience in fish surgery.

Surgical methods are considered very advanced and only those trainees that are deemed to have excellent fish handling abilities, thorough knowledge of the anatomy and physiology of the fish being tagged, demonstrated experience in the principles and techniques of anaesthesia and euthanasia plus knowledge of the signs of distress in fish, should be allowed to perform surgical procedures on fish.

#### **6.4 Goals of this training exercise:**

1. Learn methods to humanely mark or tag finfish.
2. Understand benefits and drawbacks of each marking method.
3. Demonstrate humane handling of fish and excellent survival rates when live fish are used for training exercises.

#### **6.5 Theoretical training – to be completed before hands on sessions**

1. Completed 'The Experimental Fish'
2. CCAC guidelines: H: Experimental Procedures: Section 5 (Tagging and Marking), for tagging exercises other than surgical implantation plus Section 3 for advanced surgery template.
3. Completed anatomy, anaesthesia and euthanasia templates from the DFO series or has experience or training in this area.
4. WHMIS Training.
5. Review Theory material provided with this training template (Appendix A).

#### **6.6 Details of the Procedures:**

**Introductory Procedures:** Trainees learn how to perform common fish marking and tagging procedures on inanimate objects, euthanized fish and live fish.

1. Fin clipping
2. Freeze branding
3. Carlin tagging
4. Visible Implant tagging

**Advanced Procedures:** Trainees learn about the equipment required for invasive tagging procedures, perform the procedures on inanimate objects, euthanized fish and live fish.

5. PIT tagging
6. Surgical implantation of radio or acoustic tags



### **6.6.1 Time estimate required for hands on sessions:**

Time estimate provided with each exercise:

### **6.6.2 Equipment Required:**

Equipment list provided with each exercise.

### **6.6.3 Procedure:**

#### **6.6.3.1. Fin clipping**

##### **Time Estimate:**

Set up: 1 hour

Instruction and training: 1 hour

##### **Equipment Required:**

- 1 pair of sharp micro dissecting or iris scissors.
- 70% ethanol
- Dead fish
- Gloves (Latex or Nitrile)
- Sponge or holder to place fish in during the marking procedure

If live fish are used equipment required for anaesthesia must also be available.

- Dip nets
- Aquacalm™, TMS™
- NaHCO<sub>3</sub> if fish anaesthetized in fresh water.
- Mucus protectant (Vidalife™, Stresscoat™).
- Anaesthesia and recovery baths.
- Air stones and compressed air or oxygen.
- Water quality monitoring equipment, dissolved oxygen meter, thermometer.

##### **6.6.3.1.1 Procedure:**

An instructor that has demonstrated knowledge and experience in the areas of anatomy and anaesthesia plus has demonstrated excellent survival of fish that have been marked using this method may teach this procedure.

- Anaesthetize a fish. (Note: Ensure the fish have been taken off feed for 18-72 hours prior to being marked). Instructors may also choose teach the procedures on euthanized fish.
- Place the fish on the sponge or holder.



- Spray, dip or swab the tips of the scissors with 70% ethanol.
- Clip the fin or portion of the fin as directed by the instructor.
- Return the fish to a recovery bath and monitor closely until the fish revives.

**6.6.3.1.2 After the hands on session:**

- Monitor the fish closely during recovery for signs of distress.
- Trainees must wash hands with disinfectant soap.
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

**6.6.3.2. Freeze branding**

**Time estimate:**

Set up: 1 hour

Instruction and training: 1 hour

**Equipment Required:**

- Metallic rod for branding (copper or silver)
- Liquid nitrogen
- Safety glasses
- Safety glove for contact with rod & liquid nitrogen
- Dead fish
- Sponge or holder to place fish in during the marking procedure
- Stopwatch

If live fish are used equipment required for anaesthesia must also be available.

- Dip nets
- Aquacalm™, TMS™
- NaHCO<sub>3</sub> if fish anaesthetized in fresh water.
- Mucus protectant (Vidalife™, Stresscoat™)



- Anaesthesia and recovery baths
- Air stones and compressed air or oxygen
- Water quality monitoring equipment, dissolved oxygen meter, thermometer.

**6.6.3.2.1 Procedure:**

An instructor that has demonstrated knowledge and experience in the areas of anatomy and anaesthesia plus has demonstrated excellent survival of fish that have been marked using this method may teach this procedure.

- Anaesthetize a fish if using live animals. (Note: Ensure the fish has been taken off feed for 18-72 hours prior to being marked).
- Put on safety glasses and safety gloves.
- Have an assistant place the fish on the sponge or holder.
- Remove the metallic rod from the liquid nitrogen.
- Touch the dorsal surface of the fish with the tip of the rod.
- Time with the stopwatch the number of seconds required as directed by the instructor (Number of seconds is dependant on the species of fish being marked. Usually 3 – 5 seconds is sufficient for most salmonids).
- Return the fish to a recovery bath and monitor closely until the fish revives.

**6.6.3.2.2 After the hands on session:**

- Monitor the fish closely during recovery for signs of distress.
- Trainees must wash hands with disinfectant soap.
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.



### 6.6.3.3. Carlin tagging

#### **Time Estimate:**

Set up: 1 hour

Instruction and training: 1 hour

#### **Equipment Required:**

- Carlin tags with wires attached or modified Carlin tags with monofilament line
- Pair of injection needles that have been adhered together
- 70% ethanol
- Pinchers and wire cutters if using Carlin tags
- Scissors and haemostat if using modified Carlin tags
- Sponge or holder to place fish in during the tagging procedure
- Bread crust to practice tagging procedure

If live fish are used equipment required for anaesthesia must also be available.

- Dip nets
- Aquacalm™, TMS™
- NaHCO<sub>3</sub> if fish anaesthetized in fresh water.
- Mucus protectant (Vidalife™, Stresscoat™)
- Anaesthesia and recovery baths
- Air stones and compressed air or oxygen
- Water quality monitoring equipment, dissolved oxygen meter, thermometer.

#### **6.6.3.3.1 Procedure:**

An instructor that has demonstrated knowledge and experience in the areas of anatomy and anaesthesia plus has demonstrated excellent survival of fish that have been tagged using this method may teach this procedure.

#### **6.6.3.3.2 Practice handling the injector**

- Trainees must become comfortable with the tagging equipment before using it on live specimens.
- A practical exercise is for trainees to insert a Carlin tag through a crust of bread allowing them to familiarize themselves with the tagging needles and tying or twisting depending on which type of Carlin tag is used.



#### **6.6.3.3.3 Practice Carlin tagging on dead fish:**

- Once trainee is comfortable handling the equipment they can attempt tagging a euthanized fish.
- Place the fish in the holder with the dorsal side up.
- Remove the tagging needles from the 70% ethanol.
- Insert and push the two needles through the flesh of the fish directly below the dorsal fin. It is important not to penetrate the flesh too deeply below the dorsal fin as this may lead to wounds around the tagging area. It is also equally important not to attach the tag too close to the dorsal fin as this may lead to tag loss.
- Feed the two ends of the steel wire or monofilament lines into the needles once penetration is complete.
- Drag the needles out of the fish pulling the wires/line through at the same time.
- Tie off the ends as follows (Wire – hold both end of the wires with the pinchers and fold them crosswise. Twist them 6 – 8 times tightly to the fish's body. Cut the surplus wire with the cutters). (Monofilament lines – hold the ends of the monofilament line with the haemostat and tie with 5-6 knots. Cut the excess line with the scissors).

#### **6.6.3.3.4 Practice Carlin tagging on live fish:**

- Ensure fish have been taken off feed 18-72 hours prior to tagging exercise.
- Anaesthetize a fish.
- Place the fish on the sponge or holder.
- Insert and push the two needles through the flesh of the fish directly below the dorsal fin.
- It is important not to penetrate the flesh too deeply below the dorsal fin as this may lead to wounds around the tagging area. It is also equally important not to attach the tag too close to the dorsal fin as this may lead to tag loss.
- Feed the two ends of the steel wire or monofilament lines into the needles once penetration is complete.



- Drag the needles out of the fish pulling the wires/line through at the same time.
- Tie off the ends as follows (Wire – hold both end of the wires with the pinchers and fold them crosswise. Twist them 6 – 8 times tightly to the fish’s body. Cut the surplus wire with the cutters). (Monofilament lines – hold the ends of the monofilament line with the haemostat and tie with 5-6 knots. Cut the excess line with the scissors).
- Return the fish to a recovery bath and monitor closely until the fish revives.

#### **6.6.3.3.5 After the hands on session:**

- Monitor fish closely during recovery for signs of distress.
- Trainees must wash hands with disinfectant soap.
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

#### **6.6.3.4. Visible Implant (VI) tagging**

##### **Time estimate:**

Set up: 1 hour

Instruction and Training: 1 hour

##### **Equipment Required:**

- VI tag injector (size will depend on the size of the fish)
- VI tags (size will depend on the size of the fish)
- 70% ethanol
- Forceps
- Dead fish
- Fruit to practice tagging. Thin-skinned fruit such as an apple works best.
- Gloves
- Sponge or holder to place fish in during the tagging procedure





If live fish are used equipment required for anaesthesia must also be available.

- Dip nets
- Aquacalm™, TMS™
- NaHCO<sub>3</sub> if fish anaesthetized in fresh water.
- Mucus protectant (Vidalife™, Stresscoat™)
- Anaesthesia and recovery baths
- Air stones and compressed air or oxygen
- Water quality monitoring equipment, dissolved oxygen meter, thermometer.

#### **6.6.3.4.1 Procedure:**

An instructor that has demonstrated knowledge and experience in the areas of anatomy and anaesthesia plus has demonstrated excellent survival of fish that have been tagged using this method may teach this procedure.

#### **6.6.3.4.2 Practice handling the injector:**

- The trainees should handle the injector and get used to the feel of the instrument.
- Loading the injector can be accomplished by two methods:
  - Method 1: Dip the injector tip in 70% ethanol. Peel the plastic cover back or remove it from the strip of tags. Hold the strip of tags in one hand and curve the forefinger over the tag to be loaded. The tag code should not be visible at this stage. Point the open side of the needle downward aligning the end with the long axis of the tag. Slide the needle over the tag until the tag is entirely inside the injector. Push with a pair of forceps if resistance is observed to ensure the tag is all the way in the injector.
  - Method 2: Hold the tags with the clear plastic side facing upwards with the tag code visible. Peel back the plastic cover to reveal the tag to be loaded. Point the open side of the needle up and align the tip with the long axis of the tag. Slide the needle under the tag until the tag is entirely inside the injector. Push with a pair of forceps if resistance is observed to ensure the tag is all the way in the injector.
- Push the plunger to determine how much pressure is required to push the tag out.



- Reload the injector.
- Use a piece of fruit to practice injecting the VI tags. Trainees can inject the same fruit multiple times with several tags to get used to how to handle the equipment.
- Insert the needle into the skin of the fruit so that the tip is slightly beyond where the far edge of the tag is desired.
- Slightly withdraw the tip of the needle.
- Push the rod slightly with the palm of the hand to advance the tag so it fills the opening at the front of the needle. This is the tricky part, if you push too much; the tag will roll up and become distorted.
- Withdraw the needle while the push rod is held stationary relative to the tissue using the palm of the hand.
- Leave the tag in place.

#### **6.6.3.4.3 Practice VI tagging into dead fish**

- Once trainee is comfortable handling the equipment they can attempt tagging a euthanized fish.
- Place the fish on a sponge or holder with the lateral side upwards.
- Load the injector with a tag using method 1 or 2 as described above.
- Insert the needle into the adipose eyelid tissue so that the tip is slightly beyond where the far edge of the tag is desired.
- Slightly withdraw the tip of the needle.
- Push the rod slightly with the palm of the hand to advance the tag so it fills the opening at the front of the needle. This is the tricky part, if you push too much; the tag will roll up and become distorted.
- Withdraw the needle while the push rod is held stationary relative to the tissue using the palm of the hand.



- Leave the tag in place.
- Rub the surface of the injected area with the tip of your finger to insure the tag is in place and not on the surface where it will easily fall out.

#### **6.6.3.4.4 Practice VI tagging live fish**

- Ensure fish have been taken off feed 18-72 hours prior to tagging exercise.
- Anaesthetize a fish.
- Place the fish on the sponge or holder.
- Load the injector with a tag using method 1 or 2 as described above.
- Insert the needle into the adipose eyelid tissue so that the tip is slightly beyond where the far edge of the tag is desired.
- Slightly withdraw the tip of the needle.
- Push the rod slightly with the palm of the hand to advance the tag so it fills the opening at the front of the needle. This is the tricky part, if you push too much; the tag will roll up and become distorted.
- Withdraw the needle while the push rod is held stationary relative to the tissue using the palm of the hand.
- Leave the tag in place.
- Rub the surface of the injected area gently with the tip of your finger to insure the tag is in place and not on the surface where it will easily fall out.
- Return the fish to a recovery bath and monitor closely until the fish revives.

#### **6.6.3.4.5 After the hands on session:**

- Monitor fish closely during recovery for signs of distress.
- Trainees must wash hands with disinfectant soap.



- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

#### 6.6.3.5. PIT tagging

**Time estimate:**

Set up: 1 hour

Instruction and Training: 1 hour

**Equipment required** (numbers of items depend on number of people being trained to perform the procedure).

- PIT tags
- PIT tag injectors
- PIT tag reader
- Fruit for practice tagging, bananas are ideal but any available fruit will do.
- Dead fish
- Scalpel and forceps for dissection after tagging
- If live fish are used for the training procedure then equipment for sterilization of tags and tag injector must be available.
- 70% ethanol and sterile water or saline.
- Gloves (Latex or Nitrile)

If live fish are used equipment required for anaesthesia must also be available.

- Dip nets
- Aquacalm™, TMS™
- NaHCO<sub>3</sub> if fish anaesthetized in fresh water.
- Mucus protectant (Vidalife™, Stresscoat™)
- Anaesthesia and recovery baths
- Air stones and compressed air or oxygen
- Water quality monitoring equipment, dissolved oxygen meter, thermometer.

##### 6.6.3.5.1 Procedure:

An instructor that has demonstrated knowledge and experience in the areas of anatomy and anaesthesia plus has demonstrated



excellent survival of fish that have been tagged using this method may teach this procedure.

#### **6.6.3.5.2 Learning to use the scanner:**

- Have trainees pass the scanner over the tag on the desktop or in hand. This will enable them to become familiar with the speed of motion and the distance from the tag required for the scanner to activate and record the tag number.
- If the tag reader has a data storage function teach the trainees to use it. PIT tag numbers are very long and errors can be made if the number is transcribed.
- Comfort using the scanner will result in decreased time required during fish handling in the future.

#### **6.6.3.5.3 Practice handling the injector**

- The trainees should handle the injector and get used to the feel of the instrument.
- Place a PIT tag in the injector and push the plunger to determine how much pressure is required to push the tag out.
- Use a piece of fruit to practice injecting the PIT tags. Trainees can inject the same fruit multiple times with one tag to get used to how to handle the equipment.

#### **6.6.3.5.4 Practice IP injection of PIT tags into dead fish**

- Once the trainee is comfortable handling the equipment they can attempt tagging a euthanized fish.
- The fish is held in the hand with the belly facing upward.
- The needle should be placed on the ventral body wall midway between the caudal tip of the pectoral fin and the most cranial aspect of the pectoral girdle.
- The tag is injected 1 – 2 mm off the midline.
- Great care is to be taken not to push the needle too far into the body cavity; no more than 1 – 2 mm depth are required to get through the body wall of salmon smolts.



- The trainees should inject several fish and then perform a dissection to determine if the tag has been successfully placed at the level of the pyloric caecae.
- Other fish species have much deeper body walls and may require injection into the musculature rather than the body cavity. Instructors will need to determine which technique is ideal for the species and size of fish being studied.

#### **6.6.3.5.5 IP injection of PIT tags into live fish**

- Once the instructor is confident in the trainees' ability to perform the tag injection the trainees should be permitted to tag live fish.
- Ensure that the fish are taken off feed for 18 – 72 hours prior to being tagged.
- Given the cost of PIT tags and fish instructors may decide to perform this portion of the training session on fish that are destined to be PIT tagged for experimental purposes.
- When live fish are tagged, attention to disinfection of both tag and injector is required to prevent transmission of pathogens between individuals.
- Multiple injectors must be available, as they need to be soaked in 70% ethanol for 10 minutes between tagging of fish.
- After soaking in ethanol the injector should be rinsed in sterile water or saline to prevent tissue irritation from exposure to the alcohol.
- The instructor should demonstrate the injection of the PIT tag into an anaesthetized fish and then allow the trainee to tag the remainder of the experimental fish.
- The exercise may need to be altered for different species of fish. It may be more appropriate to make a small incision with a scalpel and slip the PIT tag through it rather than use an injector.

#### **6.6.3.5.6 After the hands on session:**

- Monitor the fish closely during recovery for signs of distress.



- Trainees must wash hands with disinfectant soap.
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

#### **6.6.3.6. Surgical Techniques for implanting Radio or Acoustic Tags**

A veterinarian with experience in fish surgery must teach surgical techniques. Once a trainee completes this training exercise they are not considered qualified to teach the techniques to others.

These procedures are advanced and complex. Individuals who perform these procedures must have an excellent understanding of and experience with anaesthesia, anatomy and the recognition of pain and distress in fish. Furthermore the trainees must have theoretical training in:

- Wound healing in fish.
- Behavioural and physiological consequences of tagging.
- Aseptic technique.
- Techniques for suturing.
- Choice of tag type and size for use on different fish sizes.
- How to create a recirculating anaesthesia table.

**Session 1:** Equipment and techniques: Instruments, sutures, knot tying, tags, activation and monitoring.

**Session 2:** Implanting tags into dead fish.

**Session 3:** Implanting tags into live fish.

Set up: 1 hour per session

Instruction and Training: 8 hours split between different sessions, plus trainees are expected to practice suturing in their own time until they become proficient at the techniques.

##### **6.6.3.6.1**

**Session 1: Equipment and Techniques:** Instruments, sutures, knot tying, tags, activation and monitoring.

##### **Goals of this training session:**

1. Learn the names of surgical instruments.
2. Learn the correct way to hold surgical instruments.



3. Learn to safely place a scalpel blade onto a scalpel handle and safely remove it.
4. Learn the basic types of surgical suture.
5. Understand the knots used for tying suture.
6. Correctly tie square knots and surgeons knots.

#### **6.6.3.6.1.1 Surgical instruments: terminology and uses**

- Introduce trainees to the basic surgical instruments they are likely to encounter. Trainees must be familiar with the correct terminology for the instruments and their use.
- Lay out the following instruments with labels attached:
  - Scalpel handles: Bard Parker #3, 4, 7 and 9 are the most common handles used.
  - Scalpel blades: #10, 11, 12 or 15 (to fit scalpel handles 3, 7 and 9) Blades #20, 21, 22 and 23 (fit handle #4).
  - Forceps: Present commonly used tissue forceps such as Adson, Alligator, Allis, Babcock, or Knowles.
  - Needle Driver (Needle Holder):
    - Mayo-Hegar needle holder and Olsen-Hegar needle holder (the latter is a combination of needle holder and scissors).
  - Hemostat: Present a variety of sizes.
- Demonstrate how to hold each of the instruments and have trainees handle them.
- Demonstrate how to place a scalpel blade onto the handle. This is done by grasping the dull edge of the blade with a haemostat (**emphasize not with fingers**) and snapping it into place on the scalpel handle.
- Demonstrate how to remove the scalpel from the handle using a haemostat (**emphasize not with fingers**).





#### 6.6.3.6.1.2 Types of suture

- Present a few different types of suture to the trainees.
- Include absorbable and non-absorbable, monofilament and braided types.
- Outline the characteristics of the suture presented.
- Absorbable suture:
  - Does not need to be removed.
  - Rapid loss of tensile strength usually within 60 days.
  - Can be natural (e.g. catgut) or synthetic (e.g. Polydioxanone).
  - Available as monofilament or braided.
- Non Absorbable suture:
  - Needs to be removed.
  - Tensile strength can remain for many months.
  - Can be natural (e.g. silk) or synthetic (e.g. nylon).
  - Available as monofilament or braided.
- Monofilament suture:
  - Suture made of a single filament.
  - Causes less irritation than braided suture.
  - Less likely to ‘wick’ bacteria into the skin.
- Braided suture:
  - Thin filaments braided into a single suture.
  - Provides strength, flexibility, ease of handling and good knot security
  - Braided suture is more likely to ‘wick’ bacteria into the skin.
- It is recommended that a strong, inert, non-hygroscopic monofilament suture material be used for suturing fish skin. Nylon (non absorbable) or Polydioxanone (PDS) (absorbable) are recommended.
- Suture Size: The finest gauge suture material that will perform adequately should be chosen.
- Needle types:



- Cutting: Have sharp cutting edges that will cut through tough tissue such as skin.
- Taper: have no sharp edges, used for suturing easily penetrated tissues such as peritoneum or intestine.

#### **6.6.3.6.1.3 Tying surgical knots:**

- Many surgical textbooks are available which provide good diagrams of knot tying techniques. It is advisable to provide references for the trainees to review prior to attending the hands on session.
- Demonstrate tying square knots and surgeons knots.
- Instructor may choose to teach single-handed tying, two-handed tying or instrument tying.
- Monitor students as they practice tying knots.

#### **Points to emphasize:**

- Failure to maintain even tension on both strands or pulling one strand upwards during knot tying will result in the production of a half hitch.
- Failure to change direction on successive throws results in the production of a granny knot instead of a square knot.
- Granny knots and half hitches are not recommended because they are subject to slippage.
- Prior to moving on to implanting tags into dead fish the trainees should practice knot tying until they are comfortable with the techniques. Provide suture and needle drivers so that the trainees can practice at home. Bananas are a good choice for practicing suture techniques.

#### **6.6.3.6.1.4 Become familiar with tags and receivers.**

- Present an array of the types of tags available so trainees become familiar with tag sizes, shapes and weights. If tags or dummy tags aren't available for display then pictures are acceptable. Vemco provides a pictorial guide to acoustic tag sizes and Lotek provides photographs of their tags on their respective web pages.  
[http://www.vemco.com/size\\_trans.htm](http://www.vemco.com/size_trans.htm)  
<http://www.lotek.com/products.htm#trans>



- Tags will have different methods of activation, for many tags a magnet will be attached to the tag; removing the magnet will activate the tag.
- Other methods of tag activation should be taught where necessary.

#### **6.6.3.6.2**

##### **Session 2: Tag implantation in dead fish**

Goals of this training session:

1. Learn to make incisions in fresh tissue.
2. Understand how to place the tag in the peritoneal space.
3. Learn to suture in fresh tissue.
4. Reinforce sterile technique.

- It is critical that trainees perform tag implantation on dead fish prior to attempting the procedure on live fish.

##### **6.6.3.6.2.1 Prior to practice tagging:**

- If the fish has been dead for more than a few hours or has been frozen the tissue will not handle like that of a live fish. Tissue handling on freshly euthanized fish is the best option for trainees to understand how the tissues will feel during the tagging of a live fish.
- Trainees should practice the same methods for instrument disinfection that they would use for a live fish.
- Instruments must be disinfected between uses on different fish. Set up a metal instrument tray containing a disinfectant (povidine iodine, benzalkonium chloride or chlorhexidine) and two trays containing sterile water or saline for rinsing the instruments.
- If multiple instrument sets are available they can be soaked in disinfectant between fish.
- Tags must also be disinfected and rinsed prior to implantation.
- Set up a surgical table or trough similar to that which will be used during surgery on a live fish.
- Remind trainees that during actual surgery the fish's eyes must be protected from light so they must be covered.



#### **6.6.3.6.2.2 Practice Tagging:**

- The instructor must demonstrate tagging a dead fish prior to the trainees conducting the procedure.
- While demonstrating the procedure the instructor must emphasize:
  - Safe loading of the scalpel blade onto the handle. The size and shape of the scalpel chosen will vary with the fish species being tagged.
  - Important anatomical features of the species being tagged.
  - Tissue preparation prior to surgery; generally consists of the gentle removal of dirt or debris. Do not perform extensive tissue preparation.
  - How to place a drape (if one is used for the procedure).
  - Location of the incision and reasons for its location.
    - Incision should not cross the lateral line.
    - Incisions should be placed on the longitudinal axis of the fish.
    - Ventral midline incisions may intrude on blood vessels so a paramedian incision may be preferable.
  - Removal of scales prior to making the incision may be necessary, only those scales directly over the incision are removed. Remove scales individually by pulling them in a posterior direction; thumb forceps can be used for this purpose.
- Demonstrate the size of the incision required for tag placement.
- Demonstrate how to place the tag in the peritoneal cavity:
  - Acoustic tags are often first pushed vertically through the incision and then turned and pushed posteriorly until the tag disappears in the body cavity.
  - On larger fish the tag may be pushed through the incision and disappear with no redirection necessary.



- Appearance of the incision after the tag has been placed in the peritoneal cavity.
- Tissue handling during suturing. Tissue can easily be crushed if handled roughly with forceps; gentle handling must be emphasized.
- Instrument management during surgery. If multiple fish are to be tagged then **either** sufficient sterilized instruments are present so that each fish has its own set of instruments **or** there are sufficient instruments to allow a 10-minute soak in disinfectant between procedures.
- The number of sutures to place and distance from the cut edge to start the suture.
- Once the procedure has been demonstrated the trainees should be monitored as they practice making incisions, implanting tags and suturing the incisions closed.

#### **6.6.3.6.2.3 After the hands on session:**

- Trainees must wash hands with disinfectant soap.
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

#### **Instrument care after use:**

Surgical instruments are expensive, (needle drivers can cost several hundred dollars per instrument) and proper care is critical to normal functioning.

- Remove all organic matter from the instruments; use a brush on grooved surfaces to remove blood or mucus. The use of surgical soap is helpful to remove blood.
- If an ultrasonic cleaner is available it should be used to ensure all organic matter is removed.
- Lubricate the instruments in surgical milk to prevent corrosion.



- Lay the instruments out and allow them to air dry.
- Wrap and autoclave instruments so they are ready for the next surgery day.

#### **6.6.3.6.3**

##### **Session 3: Tagging Live Fish**

Goals of this training session:

1. Learn to tag live fish.
2. Reinforce gentle handling and anaesthetic requirements.
3. Learn monitoring parameters for fish after tagging.

##### **6.6.3.6.3.1 Procedure:**

- Once the instructor is comfortable with the trainees' surgical skills as demonstrated on dead fish, the procedure should be carried out on live fish.
- A recirculating anaesthesia unit should be set up so that anaesthesia water is pumped over the fish's gills for the duration of the procedure.
- The fish are sedated with Aquacalm™ in their home tank prior to any handling.
- The fish are induced with TMS™ until they are at a surgical plane of anaesthesia.
- Once the fish is induced it is transferred to the surgical table or trough and a maintenance dosage of TMS™ is used to maintain the fish in a surgical plane of anaesthesia.
- Acceptable dosages for salmonid smolts are 0.5 ppm of metomidate for sedation, 70 ppm of TMS™ for induction, and 40 ppm of TMS™ for maintenance.
- Demonstrate the procedure on a fish and talk the trainees through each step as was described for the procedure using dead fish.
- Closely monitor the trainees as they perform the tag implantation.



- If the procedure performed by the trainee is traumatic to the fish, the instructor will have to make a decision whether to euthanize the fish rather than recovering it.
- Once the tag has been implanted the fish must be closely monitored during recovery for signs of distress.

**Evaluating tag placement on live fish:**

- The following are indications that the tag is too large for the fish:
  - The tag does not slide easily into the peritoneal space when pushed through the incision.
  - The edges of the incision are not apposed immediately after tag insertion.
  - The incision does not close easily.
  - The tag form is visible through the skin.
- Upon recovery the fish has difficulty maintaining neutral buoyancy or the swimming behaviour is otherwise abnormal. Salmonids are seen swimming on their sides and frequently surfacing to gulp air.

**6.6.3.6.3.2 After the hands on session:**

- Trainees should have clear instructions for carcass disposal if fish were euthanized.
- Increase monitoring for 2 – 3 weeks after the handling event if fish are recovered from anaesthesia.
- Anaesthesia baths must be disposed of in accordance with local waste management regulations.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Trainees must wash hands with disinfectant soap.
- Update inventory records to reflect the number of fish euthanized for this session (if any).



- Update drug use records to include anaesthetic use.

**Instrument care after use:**

Surgical instruments are expensive, (needle drivers can cost several hundred dollars per instrument) and proper care is critical to normal functioning.

- Remove all organic matter from the instruments; use a brush on grooved surfaces to remove blood or mucus. The use of surgical soap is helpful to remove blood.
- If an ultrasonic cleaner is available it should be used to ensure all organic matter is removed.
- Lubricate the instruments in surgical milk to prevent corrosion.
- Lay the instruments out and allow them to air dry.
- Wrap and autoclave instruments so they are ready for the next surgery day.

**6.7 ACC Notes**

- Locally significant differences required in training (e.g. species).
  
- Authorization required to teach/list of possible instructors for your region.
  
- Any other requirements for your region.





## **APPENDIX A: Review theory for fish tagging and marking.**

### **References:**

Thorsteinsson, V. 2002. Tagging Methods for Stock Assessment and Research in Fisheries. Report of the Concerted Action FAIR CT.96.1394 (CATAG). Reykjavik. Marine Research Institute Technical Report (79), pp 179.

### **Fin Clip Review:**

#### **Definitions:**

**Anal fin:** The mid-ventral fin located posterior to the anus.

**Pectoral fin:** The anterior and ventrally located fins whose principle function is locomotor manoeuvrability.

**Pelvic fin:** Paired fins corresponding to the posterior limbs of the higher vertebrates (sometimes called ventral fins), located below or behind the pectoral fins.

- Fin clipping is one of the more common methods used in fish marking.
- Fin clipping involves the removal of one or more of the fins.
- All fins have been used for marking at one time or the other. Currently, the clipping of the adipose, pelvic or pectoral fin is preferred.
- Fin clipping is often done in conjunction with other forms of marking.
- Fin clipping is inexpensive and easy and a large number of fish can be marked at one time.
- There have been no apparent detrimental effects on the growth, health or behaviour of fish that have been fin clipped.
- Fin clipping is suitable for all sizes and ages of fish.
- Fin clipping can be adapted for short or long term studies.
- Fin clipping requires minimal equipment and can be conducted on both field and laboratory research animals with ease.
- Fins not cut close to the body surface may regenerate thus there is a risk of losing the mark over time.



- The potential of secondary infections increases if the fin clip is not conducted properly.
- Fin deterioration may occur under poor husbandry conditions following fin clipping.
- Individual marks are not possible with this marking method and there are a limited number of group markings possible.
- Inexperienced personnel may misinterpret the identification of fish with clippings and as a result data collection can be disrupted.
- Fin clipping also poses the risk of potential negative public opinion especially in studies involving wild populations.

### **Freeze Brand Review:**

#### **Definitions:**

**Freeze branding:** Also referred to as cold branding, the use of supercooled instruments to destroy skin cells and result in a visible external mark on the skin.

- Branding destroys the outer cells of the skin and results in skin loss, scar tissue formation and eventual regeneration of scales.
- The brand shape is visible either as a scar or a group of disorganized scales and is recognizable within a few days following the brand.
- Fine detail is not generally visible in a brand, so the shape of the brand should be distinct.
- Branding can be accomplished by using hot or cold brands, laser, or silver nitrate pencils.
- Freeze branding utilizes a metallic brand (generally copper or silver), which has been cooled by liquid nitrogen (-196°C) or pressurized carbon dioxide.
- Once cooled, the brand is applied to the skin of the fish.
- The length of time the brand is visible seems to vary according to the species of the fish.
- This method of marking is best suited for fine-scaled species of fish to maximize the retention of the mark.



- Freeze branding is easy to do and a large number of fish can be branded at one time.
- There have been no apparent detrimental effects on the growth, health or behaviour of fish that have been freeze branded.
- This method of marking is suitable for all ages and sizes of fish.
- Freeze branding apparatus is very portable enabling marking to be carried out in remote locations with ease.
- Individual marks are limited to the number of brand shapes used.
- Freeze branding is best suited to short-term studies.
- Depending on the mark design, aged brands can often be difficult to see and interpret.
- Individuals using freeze branding must be trained to prevent the possibility of injury to the individual applying the brand.

#### **Carlin Tag Review:**

##### **Definitions:**

**Dorsal:** Pertaining to the back region, opposite of ventral.

**Dorsal Fin:** The fin on the back or dorsal side of the fish.

- Carlin tags are pieces of coloured plastic with a small hole at one end.
- They are shaped like a round-cornered rectangle and have printed identification information on one or both sides.
- Carlin tags are attached with a wire or a monofilament thread (modified Carlin tag).
- The Carlin tag is attached to the fish with a length of wire or monofilament that is knotted through the muscle immediately below the dorsal fin away from the internal organs.
- The size of the tag depends on the size of the fish.
- Carlin tags, when applied properly, have long retention rates and times.



- Retention of the Carlin tag is often a problem if not attached correctly necessitating training and experience of the tagging personnel.
- Carlin tags are easy to detect and the tags can display a lot of printed material enabling individual identification.
- Carlin tags yield little if any drag on the fishes swimming, which is especially important for pelagic fishes.
- Carlin tags may inhibit the growth and affect the health and survival of fish due penetration through the skin when applying the tag incorrectly providing a portal for secondary infections.
- Attaching Carlin tags is often a difficult and labour intensive procedure and skill is required to attach the tags.
- Carlin tags used in the field may become entangled in netting and other natural habitat structures.
- Carlin tags used in natural holding systems may accumulate algae or fungus.

**Visible Implant Tag Review:  
Definitions:**

**VI Tag:** Visible Implant Tag

**Adipose tissue:** Fatty connective tissue made up of specific cells capable of storing large amounts of neutral fats.

- VI tags are alphanumeric coded wire tags.
- VI tags are imbedded using specialized injection equipment in the subcutaneous tissue of the fish (usually in the clear adipose tissue caudal to the eye).
- VI tags remain readable for long periods of time after insertion without sacrificing the fish.
- VI tags have a large information capacity and a high retention time.
- VI tags lack any significant biological effects.
- Ideal sites of injection have not been worked out for some species of fish and the techniques for applying the tags in some species is still in the experimental phase.



- VI tags allow for individual tagging of fish for identification.
- VI tags can be applied to a variety of sizes and species of fish.
- Skill is required to apply VI tags and loss of tags during the tagging procedure is not uncommon thus making this method costly and labour intensive.
- Adipose tissue may thicken over time causing the tags to become less visible and more difficult to identify.

### **PIT Tag Review:**

#### **Definitions:**

**PIT Tag:** Passive Integrated Transponder

**IP:** Intraperitoneal, within the peritoneal cavity

**IM:** Intramuscular, within the musculature

- PIT tags are shaped like a grain of rice and are approximately 12 mm long, 2 mm in diameter and weigh 70 mg.
- PIT tags contain an electromagnetic coil and microchip that transmit a unique code when powered by a magnetic field. The PIT tag reader provides the magnetic field that allows the tag to transmit its code; without the reader the tag will not transmit its code.
- Portable hand held readers can read PIT tags at distances up to 10–15 cm.
- The tags are encapsulated in glass and have few apparent effects on fish tissues. The retention of PIT tags is very high, usually 90 – 100% in salmonids.
- It is recommended that fish are at least 65 mm in fork length before a PIT tag is placed in the peritoneal cavity. The ideal is that fish have reached 80 to 150 mm fork length before being PIT tagged.
- For larger fish, such as salmonid broodstock, the PIT tag can be placed in the dorsal musculature with good retention expected.
- In salmonids, PIT tags are generally placed using a tag injector that consists of a modified 10 cc syringe with a 12-gauge needle attached.



In other species it is often more effective to make a tiny incision with a scalpel rather than using the injector.

- PIT tags are so small the tag itself is unlikely to cause any interference with the fishes form or function. Any effects seen on the fish are more likely due to handling than effects of the tag.

### **Radio and Acoustic Tagging Review**

#### **Definitions:**

**Acoustic tag:** A tag that emits a sound at a frequency of 30 – 350 kHz.

**Radio tag:** A tag that emits a radio signal at high frequency, 20 – 250 MHz.

**Simple knot:** Simple knots form the basis for the three types of knots. Two simple knots are combined to form a square knot, a granny knot or a half hitch.

**Square knot:** A knot produced by throwing two simple knots with reversal of direction on each successive simple knot.

**Forceps:** a two bladed instrument with a handle for compressing or grasping tissues in surgical operations.

**Granny knot:** A knot produced by throwing two simple knots without reversal of direction on each successive simple knot.

**Hemostat:** A surgical clamp for constricting blood flow. These clamps come in a variety of sizes and shapes with different types of grooves on their gripping surfaces.

**Needle Driver (Needle Holder):** A strong scissor-type instrument used to hold a suture needle while pushing it through tissue. The handles are ratcheted and have to be squeezed to release the needle. The face of each blade is grooved so that the needle will not twist or swivel while being driven.

**Surgeon's knot:** Similar to a square knot but the first throw is wrapped through the loop twice.

**Scalpel Blade:** Small surgical knives that come in a variety of shapes and sizes.

**Scalpel Handle:** Handles used to hold scalpel blades.



- Radio and acoustic tags are generally bullet shaped tags that are described based on their diameter, length and weight in water.
- Radio tags can be placed into the stomach of the fish with an antennae protruding from the mouth or the tags can be implanted in the peritoneal space using surgical techniques.
- Acoustic tags are generally placed in the peritoneal space using surgical techniques.
- The lifespan of the tags depends on the battery present within the tags.
- There is a broad array of radio and acoustic tags available. Researchers must make themselves aware of the options in order to choose the best tag for their studies.
- Complications of surgical implantation of tags can include but are not limited to:
  - Wound infection.
  - Gut blockage.
  - Damage to internal organs.
  - Tag expulsion through the incision before completion of healing.
- Examples of the behavioural and physiological consequences of tagging can include but are not limited to:
  - Abnormal swimming behaviour.
  - Difficulty compensating for the weight of the tag resulting in postural disequilibrium.
  - Increased osmotic stress when the skin is breached.
  - Metabolic demands are increased due to the increased tag weight.
- To minimize the negative consequences the tag:fish weight ratio should be kept low. Moser et al, 1990 found no consequences on feeding and growth rate of juvenile Coho salmon where tag weight in water was up to 4.5%; higher proportions of body weight caused decline in feeding and growth.
- The behavioural and physiological consequences of surgically implanting tags must be seriously considered. Instructors and trainees should refer to the CCAC guidelines and the review provided in Thorsteinsson, 2002 for further information about the consequences of tagging.



- Trainees must be educated in the following areas:
  - Wound healing.
  - Choice of tag type and size for use on different fish sizes.
  - Behavioural and physiological consequences of tagging.
  - Use of recirculating anaesthetic tables.
  - Sterile technique.
  - Recognition of pain and distress in fish.