Northwest Marine Technology, Inc.

Planning and Conducting Projects Using Coded Wire Tags

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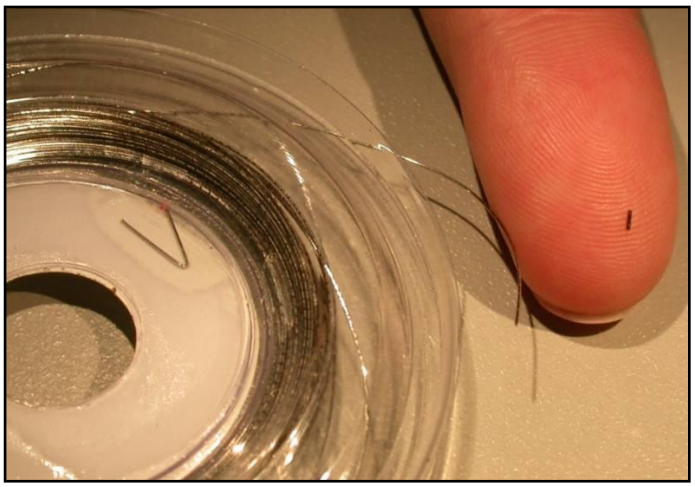

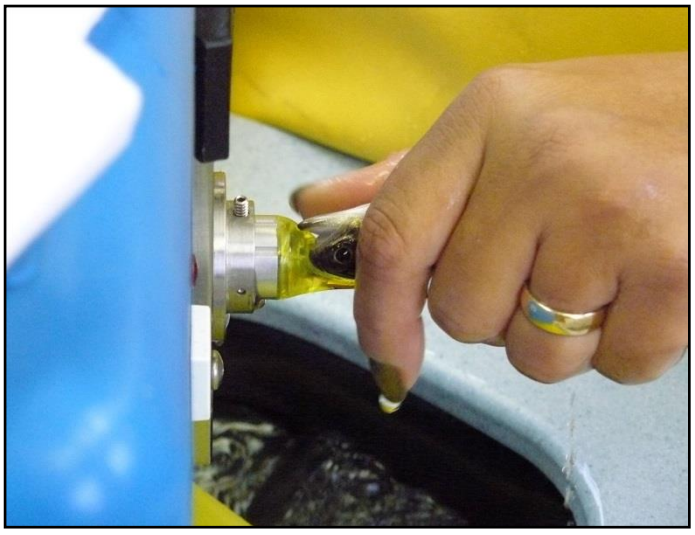

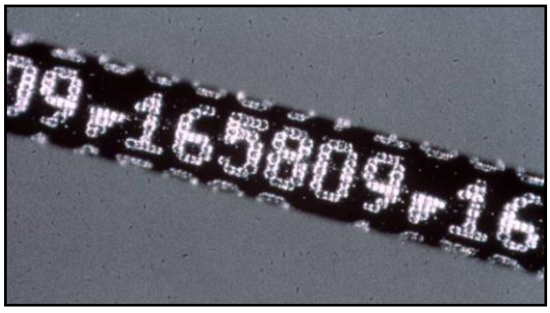

1 Contents

2 Introduction

This document describes all aspects of the deployment of the Coded Wire Tag (CWT) and ancillary equipment. It is primarily aimed at new and potential users, but experienced users may also find it a useful reference if they are considering tagging new species or tagging under different conditions. This manual is not intended to replace the user manuals for each piece of equipment, but rather to complement them to help in the design and execution of overall tagging programs and the selection of the most appropriate equipment. It contains many hints and suggestions for tagging, tag recovery, tag reading, and data handling. Additional resources are available on our website (www.nmt.us).

2.1 Background

The CWT was developed about 50 years ago (Jefferts *et al.*, 1963) for large-scale studies on migratory salmonids and this is still their dominant application. Annually, over 50 million Pacific salmon are tagged with CWT and about 250,000 CWT are recovered throughout the region (Nandor *et al.*, 2010). However, the CWT system is also suitable for smaller-scale projects with wild salmonids and a huge range of other fish and shellfish species. Projects of all types and sizes are described in later sections.

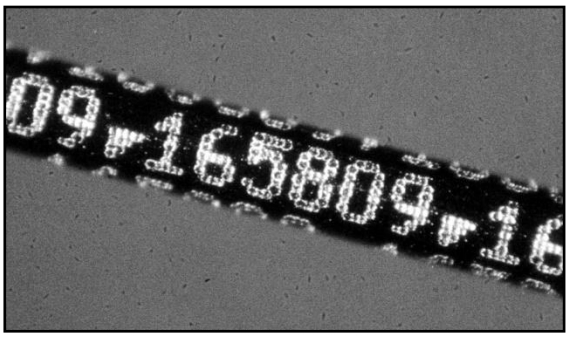

Figure 1 Magnified view of uncut coded wire. This tag shows standard formatting, and would be cut to 1.1 mm for injection. The code is repeated in four staggered rows along the wire to ensure that the code is readable, no

2.2 Overview of the CWT System

The following is a brief outline of the CWT system and its components. Each aspect is detailed in a later section.

The CWT is a small length of stainless steel wire (Figure 1) 0.25 mm in diameter and typically about 1.1 mm in length, though half, 1.5, and double length tags are also used in some circumstances. The tag is coded with a series of factory-etched numbers, which allow identification of the spool of wire from which it was cut (standard format), or particular batch, or even individual fish (sequential format). A spool usually holds 10,000 tags.

Coded Wire Tags are cut, magnetized, and implanted with an injector; two types are widely used. The Mark IV Tag Injector (Mark IV) is an electrically operated machine suitable for marking large numbers of animals, while the

Handheld Multishot Tag Injector is used where fewer animals are involved. The usual target in small salmonids is an area of muscle, connective tissue and cartilage in the snout, but other sites are also used, particularly in non-salmonids.

Coded Wire Tags do not transmit their codes. The presence of a CWT is verified using magnetic detectors. Handheld Wand Detectors are highly portable, while Tunnel Detectors are suitable for detecting tagged fish amongst large catches which can be passed through the detector. The tag must be recovered from the fish for code identification. This is usually done by dissecting the tag from a dead fish after capture by an angler or commercial fisher. The code is then read under a microscope. There are also possibilities for data recovery from live fish.

2.3 A Little History

In the 1960s, the CWT was developed in response to the need for a better way of tagging juvenile salmon for evaluation of hatchery performance. It was the result of collaboration between a Washington State salmon biologist, Peter Bergman, and a physicist from the Bell Telephone Laboratories, Keith Jefferts. The method of coding the tags has changed over the years, but the material used (stainless steel) and the dimensions of the standard size tag have remained unchanged. Although there have also been tremendous advances in the design and construction of the ancillary equipment (Vander Haegen and Blankenship, 2010), the principles of cutting, injection and magnetization of the tag, and of magnetic detection for recovery, are fundamentally the same as in the original concept. The first tags were coded with colored epoxy stripes which ran the whole length of a spool of wire. This provided a coding capacity of many thousands which was enough to see the CWT through its first years. It was superceded by binary coding in 1971, when Keith Jefferts set up Northwest Marine Technology to manufacture tags, injectors, and detectors. The decimal coding system was introduced in 2000.

Sequential Coded Wire Tags (sCWT) were introduced about 30 years ago, at that time in binary coding, but nowadays with decimal coding. These allow identification of small batches of tags cut from the same spool, and even individual identification. Although for many large-scale projects the standard coding system, where all tags cut from a spool are identical, is all that is required, the sCWT made a wide range of smaller scale projects viable.

2.4 Advantages and Limitations of CWT

Advantages of CWT

- can be used in very small fish
- can tag very large numbers of fish for large scale projects

- minimal impact on fish survival, growth and behavior
- very high retention rates are achievable, over considerable time periods and growth
- virtually unlimited coding capacity
- tags are inexpensive
- considerable scope for automatic scanning of large catches and samples
- tags recovered anywhere in the world will be correctly identified to their source

Limitations of CWT

- capital equipment is expensive (but can be leased from NMT or borrowed from other agencies)
- usually, the tag must be removed from fish for deciphering, though see section 4.5.5 for scope of benign data recovery
- returns can not be reported by anglers/fishermen unless the fish carry a secondary visible mark e.g. fin clip

The overwhelming advantages of the CWT over most other tagging methods with significant coding capacity are that they have virtually no adverse impact on the fish to which they are applied, and they can be applied to very large numbers of fish.

The CWT is tiny and biologically inert, and is injected beneath the skin or deeper within the tissues of the fish without a permanent wound or lesion. It has been demonstrated to have minimal impact upon subsequent survival, growth, and behavior of the fish.

The Pacific coast salmon program is an example of use of the coded wire tag for a very large scale project. This program involves many separate studies in the US and Canada. Over a billion hatchery produced salmon (mainly chinook and coho) have been marked with CWT's, and tens of thousands of tag recoveries are made each year. Tagging and recovery protocals are managed by the Pacific States Marine Fisheries Commission. They maintain a data base for all regional CWT releases and recoveries. Details of the organization and management of this program can be found in Nandor *et al* (2010).

The two main limitations of the CWT system are the capital cost of the injection and detection equipment, and the requirement to recover the tag to read the code. The injection and tag detection equipment is reliable and will operate with only routine maintenance for many years. The system is thus most suited to relatively large-scale projects, though there are some inexpensive options involving pre-cut tags and a Single Shot Tag Injector for trial or small-scale projects. Borrowing or renting of injection and detection equipment is another option especially for evaluation or start-up projects.

The Coded Wire Tag is a non-transmitting tag and must be recovered to read the code. This is inevitable with a tag as small and inexpensive as the CWT. The most widespread use of CWT is

in managing Pacific salmon where the tags are recovered from adult carcasses in the sport and commercial fisheries, on the spawning grounds and at hatcheries. In some circumstances tags can be retrieved without killing the animal (see Section 4.5.5). The presence or absence of a tag may also be enough so that the tag does not need to be removed.

3 Details of the CWT System

3.1 Tag Formats and Coding

All CWT are 0.25 mm in diameter and are etched with a series of decimal numbers 0.16 mm tall. Four lines of repeating decimal numbers are etched along the wire from which each tag is cut, at 90° intervals around the wire. Five formats are available: standard, half-length, one-and-a-half length, sequential, and agency only.

The machines that cut the tags are not indexed to the coding on the wire. Thus, the wire may be cut at any point in the code. This can make it difficult to read the data at the extremities of a cut tag, especially towards the end of the life of the cutter in the injector. Therefore, redundancy is built into the coding arrangement, with the code sequence repeated at a shorter interval than the tag length. Reliable reading of the code does not therefore depend upon being able to correctly decipher the data at the tag extremities, and the code can be read no matter where the cuts begin along the wire.

Warning: Any of the tags can be cut longer than the tag length designated on the spool label, but the code will not be readable if they are cut shorter than the intended length.

3.1.1 Standard CWT

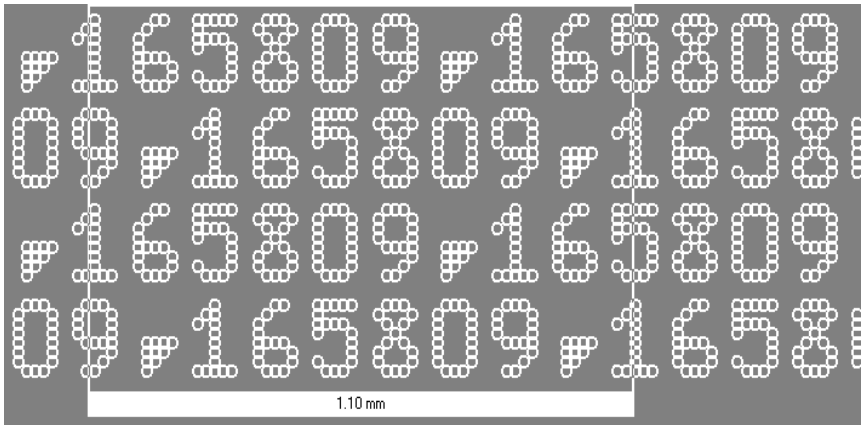

Figure 2: A sample of Standard tag wire that has been "unrolled". The triangular flag is pointing to the first digit in the code. The code (165809) is read from left to right. The white lines in the figure show the length of a Standard tag, and one possible cut.

For agencies using the traditional data conventions, the Standard tag has three words: Agency, Data 1 and Data 2. The tag in Figure 1 is Agency = 16, Data 1 = 58 and Data 2 = 09. Note the inclusion of the leading zero for Data 2 is to ensure that each data word has two digits.

This is the most commonly used tag. Standard Coded Wire Tags are 0.042 inches (1.1 mm) long and 0.010 inches (0.25 mm) in diameter. The code is 6 digits written on a single side of the tag and read from left to right. For reliability and ease of use, the code is replicated on four sides of the wire with the starting point offset by two character positions. This redundancy makes a tag readable no matter where the wire is cut. Standard Coded Wire Tags are not readable if cut shorter than 1.1 mm.

3.1.2 Half-length CWT

Half-length tags are 0.021 inches (0.5 mm) long and 0.010 inches (0.25 mm) in diameter. They are designed for use when fish size (less than approximately two grams) cannot accommodate a larger tag. The code is 6 digits long, and written on two longitudinal rows. The row with the flag character contains the first three digits of the code which is read from left to right. Aligned directly below are the last three digits of the code. The code is repeated once and offset to gain reliability.

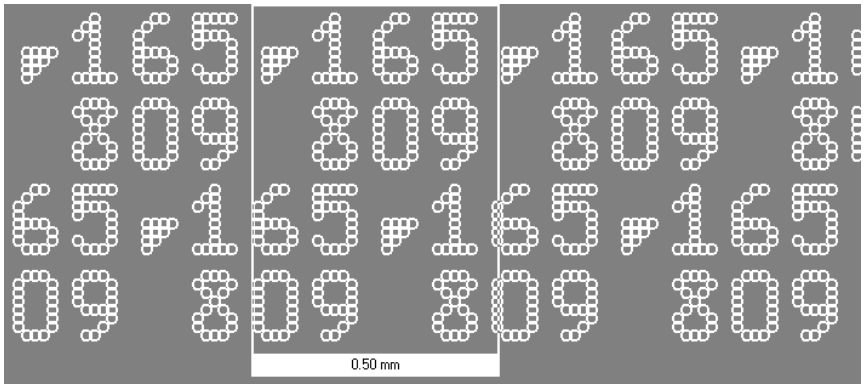

Figure 3: An example of the Half-length tag wire that has been "unrolled", with code 165809. The white lines in the figure show the size of the half-length tag, and one possible tag cut.

For agencies using the traditional data conventions, For agencies using this convention, the Half-length Tag has five words (Agency, Data 1, Data 2, Data 3, and Data 4). The Agency word is two digits and the four data words are two digits each. Figure 2 shows Agency = 16, Data 1 = 05, Data 2 = 08, Data 3 = 00 and Data 4 = 09. Half-length tags must be reported as 10 digits to RMPC.

3.1.3 One-and-a-half length CWT

1½-length tags are 0.062 inches (1.6 mm) long and 0.010 inches (0.25 mm) in diameter. This tag is designed for use in larger specimens or to enhance magnetic detection. The code is 6 digits and read from left to right. 1½–length tags are not readable if cut shorter than 1½-length.

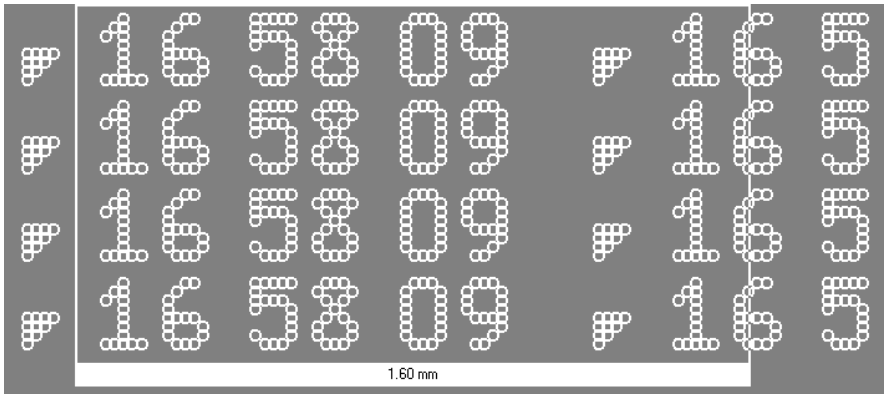

Figure 4: A sample of the 1½-length tag wire that has been "unrolled". The triangular flag points to the first digit of the 6 digit code (165809). The white lines in the figure show the size of the tag, and one possible cut.

For agencies using the traditional data conventions, the 1 ½-length tag has 3 words, Agency, Data 1 and Data 2. Figure 3 shows Agency = 16, Data 1 = 58 and Data 2 = 09.

3.1.4 Agency Only CWT

Agency Only tags are 0.042 inches (1.1 mm) long and 0.010 inches (0.25 mm) in diameter. They are batch coded with two digits. The Agency Only tag is designed for projects where the information required is related to the presence or absence of a tag in a fish.

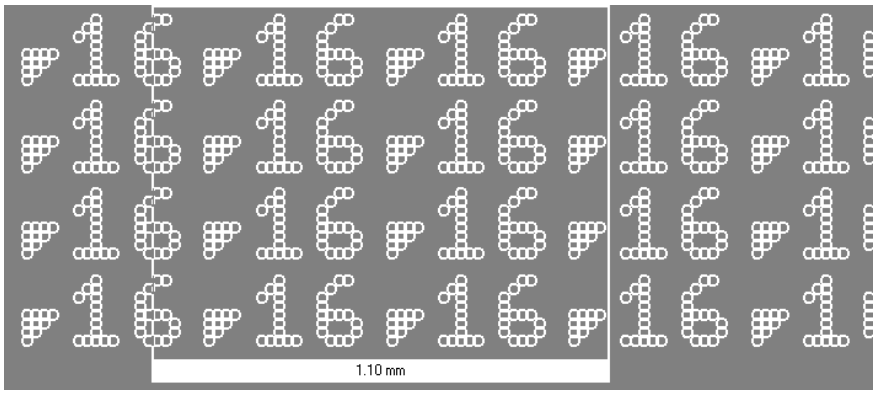

Figure 5: A sample of Agency Only tag wire that has been "unrolled". The triangular flag designates the start of the code and it is read from left to right (16). The white lines in the figure show the size of the tag and one possible cut.

For agencies using the traditional data conventions, the Agency Only Tag is only one word. Figure 5 shows Agency = 16. This code should be reported to RMPC as tag type 16

3.1.5 Sequential CWT

The sequential CWT (sCWT) is the same size as the standard CWT. Sequential Tags are 0.042 inches (1.1 mm) long and 0.010 inches (0.25 mm) in diameter. They have a batch code written along the axis of the tag in two rows and three columns, followed by a sequence number written around the circumference. The formatting of the Sequential Tag ensures that one entire sequential number is on each tag, no matter where the wire is cut. The sequential numbers are staggered by three digits around the circumference of the wire. This allows for greater reliability if a tag is scratched.

To resolve the ambiguity created when two complete sequential numbers are readable, the convention is to use the lesser number. Sequential Coded Wire Tags are not readable if cut shorter than 1.1 mm.

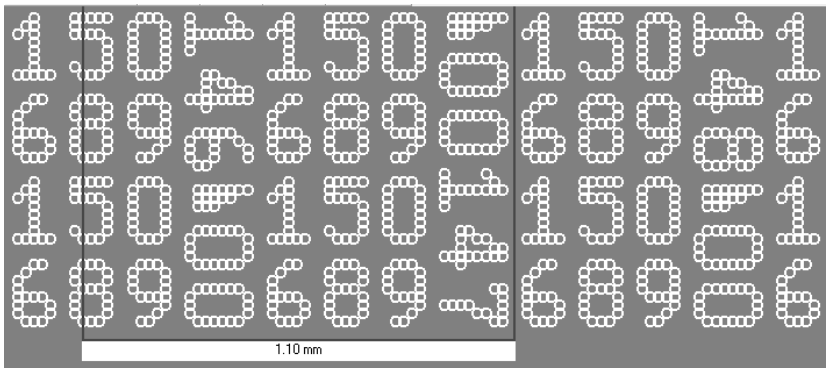

Figure 6: A sample of a Sequential Tag wire that has been "unrolled". The triangular flag points to the first digit in the batch code (165809) and in the sequential code (00146). The white lines in the figure show the size of the tag, and one possible cut.

For agencies using the traditional data conventions, the Sequential Tag has three static words (Agency, Data 1, and Data 2) and a sequential number. Figure 4 shows Agency = 16, Data 1 = 58, Data 2 = 09, and sequence = 00146 (the lesser of the 2 sequence numbers on the tag).

3.2 Injectors

There are two main types of CWT injectors in widespread use, the Mark IV Tag Injector and the Handheld Multishot Tag Injector. They are fully described in their instruction manuals so description here is limited to principles and essentials. A Single Shot Tag Injector is available for small-scale trials using precut tags (see Section 4.5.4).

3.2.1 Mark IV Tag Injector

The Mark IV Tag Injector (Mark IV; Figure 7) is designed for large-scale projects involving tens or hundreds of thousands, or even millions, of animals. It automatically cuts, magnetizes and injects the tag and can be used with head molds or with a needle support tube for tagging in other body locations. The Mark IV requires 12-24 V DC. This may be supplied by batteries or NMT's universal input-voltage power supply. Aspects of operation such as needle penetration depth and extent of needle movement are under software control and are adjusted by a waterproof keypad. Although often used in hatcheries or in research facilities, the Mark IV is suitable for field use in any situation where it and the required batteries can be carried.

Figure 7 The Mark IV Tag Injector is the workhorse of Coded Wire Tagging. It is designed for fast and accurate tagging where many thousands of fish are to be tagged.

For snout tagging, a head mold is used to position and hold the fish - see Section 0. When the "tag" button is pressed, the needle advances to penetrate the fish to the pre-set depth, the tag is injected, and the needle withdraws. For most other body locations, the needle is protected by a needle support tube. In this mode, the needle does not move, but the fish is impaled onto the needle (the support tube having been adjusted as a depth stop). Then, the tag button is pressed, the tag is injected, and the fish is withdrawn from the needle.

The Mark IV is usually used with a Quality Control Device (QCD; Section 3.2.1). This is a small tunnel detector that counts tagged and untagged fish and uses gates to separate them.

The Mark IV keypad and display are used to display tag counts and battery voltage, and to activate various operations. Other variables are controlled by physical adjustments including the needle type, head mold type (by species and size of fish), and needle penetration depth. This last variable is controlled by the position of the head mold or needle support tube in its holder.

Further aspects of Mark IV operation are discussed later. Full instructions for operation are given in the Mark IV Manual, which is available on our website (www.nmt.us) and is supplied with each injector.

3.2.2 Handheld Multishot Tag Injector

The Handheld Multishot Tag Injector (Multishot; Figure 8) is a portable device designed for mobile use or for projects where smaller numbers of fish are to be tagged. We would expect the Multishot to be used for projects involving hundreds or thousands of fish; for those involving many tens of thousands, the Mark IV is usually a better choice.

A tag is cut and advanced into the needle before the fish is picked up. The Multishot is held in one hand and the fish picked up and positioned with the other. The needle operates in a fixed position. When a head mold is used the fish is inserted into the mold and gentle pressure pushed in against light spring pressure. This allows the needle to penetrate to the pre-set required depth and the tag is injected. Operation using a needle support tube is similar to that with the Mark IV described above.

All adjustments are made physically with the Multishot. Tag magnetization is fixed, and tag

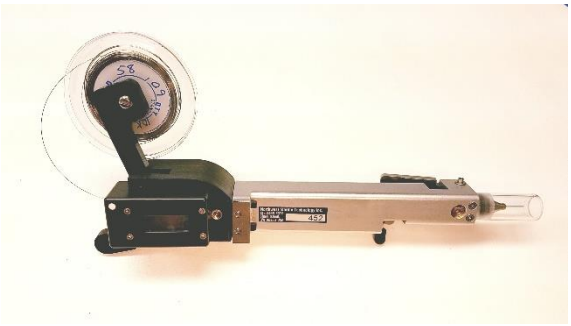

Figure 8 Handheld Multishot Tag Injector with a spool of tag wire loaded for injection.

length cut is set by adjusting the wire advance pawl or switching the drive roller assembly. The Multishot can cut tags that are 1.1 mm, 1.6 mm, or 2.2 mm long. Needle penetration depth is adjusted when using a head mold by moving the head mold in its carrier. When using a needle support tube, penetration depth is adjusted by use of different lengths of tube. The distance that the tag is injected beyond the tip of the needle is determined by adjusting the advancement of the push wire using a set screw. The tag cycle counter can be reset. The Multishot has no associated QCD; a Wand Detector or V-Detector (see Section 3.4) is usually used with a Multishot to verify that a tag was injected.

3.2.3 Single Shot Tag Injector

The Single Shot Tag Injector (Figure 9) is a simple syringe injector. It is used with precut sequential tags (see Section 4.5.4) which are

Figure 9 Single Shot Tag Injector for injecting precut sequential CWT.

supplied mounted on sheets. It is intended for laboratory trials and small-scale experiments involving perhaps up to a few hundred animals. Tags are loaded individually into the Single Shot Tag Injector by sliding the needle over the precut tag. The tagging process can be accelerted by having two injectors so that one person loads tags while another person injects the tags.

3.2.4 AutoFish System

NMT's AutoFish System is a self-contained mobile unit for handling very large numbers of

Figure 10 AutoFish System is contained in a mobile trailer (top) that can be moved between hatcheries. The interior (bottom) has a holding tank for fish waiting to be processed, a sorter that measures and sorts fish into size categories, and marking and tagging lines for injection of CWT and adipose fin clipping.

juvenile salmonids (Figure 10). This system can accomplish any combination of sorting, clipping and tagging with CWT. It incorporates Mark IV Tag Injectors and accomplishes adipose fin clipping and/or injecting CWT without the fish being anesthetized, dewatered, or touched by hand. It can process over 60,000 fish in 8 hours, and can handle fish from 57 mm to 142 mm. These systems are in use on the US Pacific Coast and in the Great Lakes region and are restricted to North America. Deployment of this system is viable where several million fish are to be marked and tagged annually. For more information about AutoFish contact NMT.

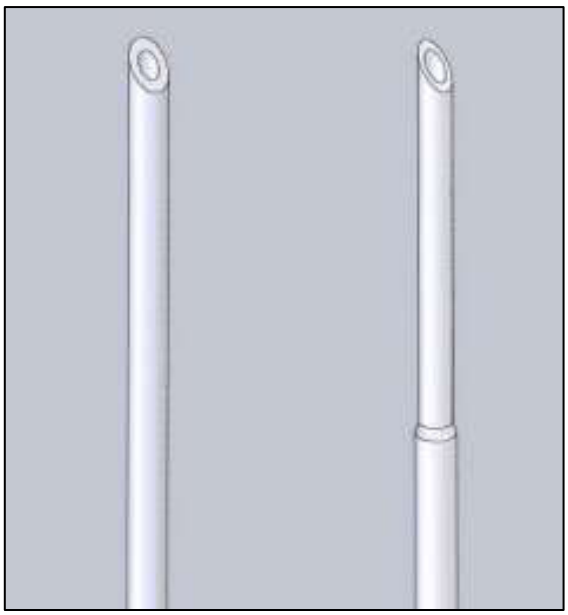

Figure 11 Needles for the Mark IV and Multishot are available as non-etched (left) or etched (right).

3.3 Needle types, head molds, and needle supports for Mark IV and Multishot injectors

3.3.1 Needles

A clean, sharp needle of the proper length and style is necessary for effective tagging. The type of injector, the species, the size of the fish, and the target location for the tag will determine the appropriate needle.

The Mark IV uses 2.5 inch (6.35 cm) and 3.5 inch (8.9 cm) needles. The shorter needle is designed for use with head molds. The longer needle is designed for use with the needle support tube which protect the needle from bending and reinforces the longer needle. Although either needle can be used without a needle support tube or head mold, experience has shown that a needle with the appropriate attachment is usually more effective.

The Multishot uses a 1.55 inch (3.94 cm) needle. This will accommodate head molds or a needle support tube. The needle support tube for the Multishot is not interchangeable with the tube for the Mark IV; however, head molds are interchangeable between the two.

Each of the three lengths of needle described above is available as either “etched” or “non-etched” (Figure 11). A non-etched needle has a constant outside diameter (0.0225 inch, 0.57

mm) from its base all the way to the beginning of the beveled tip. The etched needle is reduced to a smaller outside diameter (0.0185 inch, 0.47 mm) for about 0.3 inches (0.76 cm) from the beginning of the bevel. The etched needle is designed to make a smaller injection hole in the fish and has been very successful in conjunction with head molds for Pacific salmon. The etched needle will not work as well (i.e., it has a greater likelihood of bending) with fish with tougher tissue such as steelhead, nor will it work as well with most "body" tagging such as the cheeks of smallmouth bass, the scutes of sturgeon, and the rostrum of paddlefish. For these types of tagging, the non-etched needle in a needle support tube is often the better combination for

Figure 12 CWT being injected into the nape muscle of a herring using a needle support tube and non-etched needle.

penetration and tag placement (Figure 12).

The inside and the outside of the needles should be kept clean of dirt and fish slime. A dirty needle may cause tag jamming, improper tag placement, or infection. Clean the needle with detergent and water, disinfect with bleach, then rinse with bleach, water and alcohol. A sharp needle is required to repeatedly penetrate the fish and deliver the tag to the target site with minimal tissue damage. The tagging needles are multifaceted and can be very difficult to sharpen properly. We recommend replacing dull needles.

Figure 13 Two sizes of head mold, and a head mold base. The needle projects through the hole visible in the centre of the head mold base.

3.3.2 Head molds

Head molds (Figure 13) are designed to receive the head of a particular species and size of fish,to position it correctly for tag injection. A wide range of ready-made head molds are available from NMT (Table 1). The size stated is the middle of the range for which the individual mold is suitable; the size ranges of the molds listed overlap a little so that for example steelhead from a little smaller than 80 per pound (6 g) to a little larger than 2 per pound

(227 g) can be accommodated by the available range.

For head molds for other species or sizes of fish NMT can supply fabrication kits containing all supplies and instructions to make head molds. Head mold bases are also available for customers supplying their own materials.

For brown and sea trout (*Salmo trutta*), head molds for Atlantic salmon (but one size larger) are generally suitable.

Table 1 NMT offers many ready-made head molds. The size of the head mold corresponds to the size of the fish to be tagged.

Species	Weight (# of fish per pound)
Coho/ Chinook	5, 10, 15, 20, 30, 45, 65, 90, 120, 200, 300, 550, 1100
Steelhead	2, 3, 5, 7, 11, 20, 36, 80
Rainbow Trout	5, 8, 12, 18, 27, 50, 90, 200
Pink Salmon	2000
Atlantic Salmon	7, 9, 11, 15, 25, 30, 50, 100, 120
Lake Trout	5, 8, 12, 18, 27, 50, 90
Chum Salmon	700

Species	Head Mold Size (length of fish in mm)

3.4 CWT Detectors

Three types of electronic CWT detectors are available for deployment in different circumstances – wand detectors, the V-Detector and tunnel detectors. Details about each type are below. They all work by detecting the magnetic property of the injected tag, and require the tag or the detector to be moving relative to the other. The optimal speed of movement is about 3 ft/s (0.9 m/s). It is stressed that the detectors can detect and help locate the tag but they do not read the code; the tag has to be removed and viewed under a microscope to read the code.

Electronic CWT detectors are generally used during four stages of a project. First, because a CWT is usually invisible after injection, electronic detection is used right after tag injection to confirm that the tag is present. Second, they are used to sort tagged from untagged animals during a tag retention check, either as part of a laboratory study, or before the animals are released in field studies. Third, electronic detection is used in the field when tagged and untagged animals are captured together during tag recovery efforts (stream surveys, fishery sampling, or trapping, for example), and the tagged animals need to be sorted from the untagged animals. Finally, electronic detection is used during tag extraction in the laboratory to help identify the piece of tissue holding the tag. The most

suitable detector at each stage depends on the number and size of the animals being tagged, the way the detectors are designed, the location where the detector will be used, and the budget.

Stage 1: Tagging: The Quality Control Device (QCD, Section 3.2.1) and the V-Detector (Section 3.4.2) are most commonly used during juvenile fish tagging. The QCD is a small tunnel detector (63 mm internal diameter) which operates by connection with a Mark IV Tag Injector. It is detects, separates, and counts tagged and untagged fish. A V-Detector is also widely used during tagging, and is typically set next to the Mark IV, or conveniently close when using a Mulitshot Tag Injector or Single Shot Injector such that each tagged fish is passed through it to confirm a tag is present. The V-Detector allows for hands-free operation, and is suitable for any size of fish. While not as commonly used or quite as convenient as a V-Detector, a Wand (Section 3.4.1) can usually be used in its place, and has the advantage of being easily portable and rugged for field tagging applications.

Stage 2: Tag retention checks: We recommend that you measure tag loss by holding groups of tagged animals for at least 30 days before release if possible. These tagged animals should be left undisturbed, and then are all passed through some form of electronic detection to verify the presence or absence of a tag at the end of the holding period. The V-Detector is the usual choice for this operation because it is portable and allows for fast, hands-free operation of any species and most sizes. A Handheld Wand can also be used for this part of the project. As mentioned above, it is possible to use the QCD for small fish, but it must be connected to a Mark IV Automated Tag Injector.

Stage 3: Sorting tagged and untagged animals at recapture: For this stage, the most appropriate detector depends on the location and sampling environment as well as the size of the animal being sampled. Details about using each detector in the field are available below, but in our experience, handheld wands and tunnel detectors are the most widely used. The T-Wand is very portable, and is the most suited for situations where the sampler is walking streams, sampling at a variety of locations, is working from a boat, or is at a field station. This is the detector we recommend for most outdoor work. It is also very useful in hatchery settings. The V-Detector can be useful for sampling at fixed field stations, but it must be on a stable surface and is not as easy to carry as a T-Wand. Where very large volumes of fish must be sampled (like commercial catches), a tunnel detector may be a good choice

Stage 4: Removing the CWT for reading: In most projects, the CWT will be removed from the animal by dissection. The V-Detector is the best option for this as it can be set on a worktable and allows for fast, hands-free operation. A T-Wand can usually be substituted for the V-Detector, but it is not as convenient.

3.4.1 Wand Detectors

Wands are ideal field tools as they are easily carried, are powered by an internal battery, and can be used in moving boats, or in the presence of vibration. Their limited range is a distinct advantage in resolving body location of the tag when this is used to differentiate batches. Wands are often used as a QCD when tagging in the field with a Multishot. If you select a Wand Detector for your project, be sure to consider tag length and how it may affect detection rates.

The T-Wand (Figure 16) is a portable field detector and is replacing the Blue Wand Detector (Figure 14). The T-Wand has a larger tag detection range, is more rugged, and easier to use than the Blue Wand.

Both wands are operated by rubbing them over the suspected tag location with the specimen held still. The range of the T-Wand is about 5.25 cm while that of the Blue Wand is about 3.2 cm for a standard length CWT. Detection ranges are longer for length and a half and double length tags, and shorter for half length tags. To maximize tag detections, it is critical that both wands make physical contact with the animal.

Figure 16: T-Wand Detector

Figure 14 Blue Wand Detector being used to detect a tag in a Chinook Salmon.

Figure 15 V-Detector

3.4.2 V-Detector

The V-Detector (Figure 15) can detect all tag lengths within the V. The tagged specimen is moved relative to the detector and tag presence is indicated by a sound and light. The V-Detector is powered by a 9 V battery.

The V-Detector is sensitive to vibration, so it must be placed on a firm base and is not suitable for unstable locations – on a small boat, for example. Since the introduction of the Handheld Wand Detector, the use of the V-Detector is more concentrated in hatcheries and laboratories. It is the usual choice for tag recovery from fish or tissue in the laboratory (see Section 4.4) and is often used for verifying tag presence in programs where a QCD (see Section 3.2.1) is not used.

3.4.3 Tunnel Detectors

Tunnel Detectors are mainly designed for fish and detect tags passed at an appropriate speed through them. They may be powered by external batteries or by NMT's universal power supply. Four sizes are available; the R8000, R9500, T13, and QCD.

The two most commonly used tunnels are the R8000 (Figure 18) which has a tunnel cross-section of 4" x 7.875" (102 mm x 200 mm) and the R9500 which has a tunnel cross-section of 4.625" x 9.5" (119 mm x 241 mm). The R8000 and R9500 are most typically used for detecting tags in dead adult salmon. The larger R9500 can be used for salmon approaching 25 kg, and is also suitable for operating with small fish on a conveyor belt. Counters and diverter gates are available for both models.

Figure 18 R8000 Tunnel Detector with a diverter gate to separate tagged and untagged adult salmon returning to Marblemount Hatchery, WA. A tagged fish has just passed through and is going into the left bin.

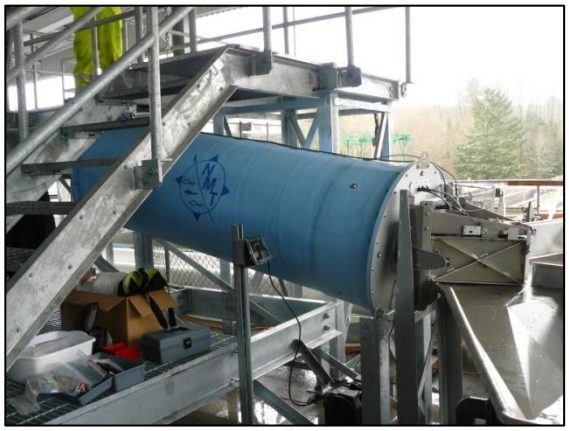

Figure 19 This T13 Detector is premanently installed at Cowlitz Hatchery in Washington and includes a gate for

The T13 (Figure 19), can accommodate very large, live or dead fish and will detect a tag anywhere in the tunnel as long as the fish is moving fast enough. The T13's tunnel is oval

with the maximum inside dimensions of 7.5" (190 mm) by 13" (330 mm). The T13 can be supplied with a diverter gate to sort tagged and untagged fish. It may be used with either the broad or the narrow dimension upright, but the gate only works when the larger dimension is horizontal. When using a gate, the detector and gate are powered with either the NMT 24V DC power supply or with 24V from two standard 12V deep cycle batteries. Because of their size and weight, most T13s will be in permanent installations, but the detector can be mounted on a small trailer so that it can be moved around on site.

The Quality Control Device (QCD, Figure 20) is a small tunnel detector (63 mm internal diameter) which operates by connection with a Mark IV. It is normally used to detect, separate and count tagged and untagged fish during a tagging operation, but it can also be used as a detector and sorter for suitably sized fish – for example, to check a group of juvenile salmon for tag retention some time after tagging. The main drawback in deployment in this way is the requirement for connection to a Mark IV.

Figure 20: Quality Control Device (QCD) in use connected to a Mark IV.

4 Using the CWT System

4.1 Tag Location and Retention Rates in Different Species

In general, CWT may be injected into any suitable tissue where they cause no harm and are likely to have a high retention rate. Adipose tissue, cartilage, muscle, connective tissue, beneath tough skin and in fins and fin bases are often good sites. Delicate organs and tissues (eg. brain, eye) should of course be avoided, as should sinuses and blood vessels where the tag might migrate. One factor to consider is the likely detectability of tags in animals that grow to a great extent between tagging and tag recovery; it may be difficult to detect tags deeply embedded in very large animals with some detectors. The suitability of particular sites varies between species, and available literature should be carefully reviewed when planning a new project. If no suitable experience can be found in the literature, then it may be

necessary to conduct small-scale trials before a field deployment is contemplated.

A wide range of experience of various tag locations is reviewed below, but this examination is not exhaustive. For a more extensive listing of published papers on CWT investigations and advice on tag location, please contact biology@nmt.us.

4.1.1 Fish

The snout is the usual location for CWT in salmonids. This is a well-established location for tagging, and over a billion fish have been tagged in this way. The target area is relatively large (Figure 21), it is some distance from sensitive organs and tissue, tag retention rates are very high, and a large range of head molds are available for different species and sizes (Section 0). Other potential advantages of using the snout location in salmonids are that it is well away from parts of the fish normally consumed by humans and it is an internationally recognized protocol that will maximize detection and reporting of captures in distant sampling programs. Numerous projects have indicated that properly-placed CWT have no effect upon the behavior, growth, and survival of the fish.

Retention rates over 95% between parr and adult are to be expected with properly injected tags along the mid-line of the snout. Isaksson and Bergman (1979) recorded a 98.3% retention between released smolts and returning adult Atlantic Salmon in Iceland, while Blankenship and Tipping (1993) recorded 100% retention on return to the river of Cutthroat Trout tagged as smolts. Another study reported an increase in retention rate from less than 80% to more than 98% in very small char (22-55 mm) marked with half-length tags, which was attributed to improved techniques of correct tag placement depth (Champigneulle *et al.*, 1987).

For most non-salmonid fish, the snout is not the optimal location for CWT. Further, in some situations tags may also be placed elsewhere than the snout in salmonids, for example where it is intended that tag location be used as a batch code or where recovery of the tag from live fish is desired (see Section 4.5.5).

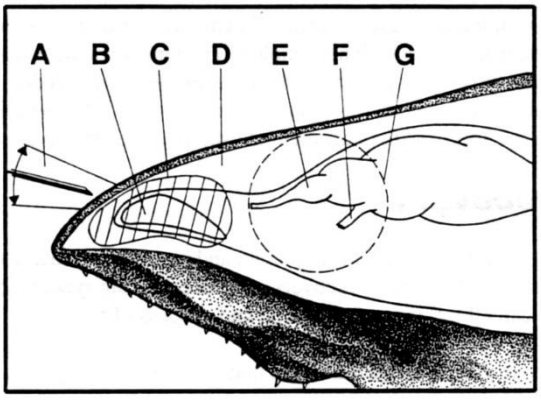

Figure 21 Target area for CWT in a salmonid snout. A – Usual range of tagging needle angles; B – Muscle, adipose and fibrous tissue; C – Tag target area (hatched); D – Cartilage; E – Olfactory lobe and nerve; F – Optic nerve; G – Position of eye.

Fletcher *et al.* (1987) obtained 100% tag retention in the cheek musculature of largemouth bass (*Micropterus salmoides*). Heidinger and Cook (1988) observed 92-100% tag retention in the nasal area, nape and cheek of Channel Catfish (*Ictalurus punctatus*), Golden Shiner (*Notemigonus crysoleucas*), Bluegill (*Lepomis macrochirus*) and Walleye (*Stizostedion vitreum*). Tipping and Heinricher (1993) experimented with different tag locations in Tiger Muskellunge (*Esox masquinongy x Esox lucius*). Tag retention rates were 88.3% between the rays of the dorsal fin, 99.4% in the cheek, and 99% in the anal fin.

Oven and Blankenship (1993) observed tag retention rates of 96% in post-ocular tissue, 99% in adipose fins, and 97% in dorsal fins in Rainbow Trout. Hale and Gray (1998) used CWT in several body locations in both Brown and Rainbow trout. Mean retention rates observed were; snout (98.5%); cheek (97%); base of pectoral fin (95.7%); muscle below the dorsal fin (98.4%); base of pelvic fin (97.3%); muscle below the adipose fin (99.5%); and musculature immediately anterior to the caudal fin (96.8%). The snout tags were injected using species-specific head molds and a moving needle; tags in all other locations were inserted using a fixed needle and needle support tube. Both Mark IV and Multishot injectors were used. The tagged fish ranged from 80 to 314 mm long.

Klar and Parker (1986) used four tag locations in Striped Bass (*Morone saxatilis*). Retention in the musculature below the dorsal fin and in the adductor mandibularis muscle was virtually 100%, whereas two locations in the snout gave retention rates ranging from 51-64% - a clear example where the nasal area is not the optimal target site! Bergstedt *et al.* (1993) examined two tagging sites in larval Sea Lamprey (*Petromyzon marinus*); retention was 99% in the dorsal musculature, and 82% for a subcutaneous location on the ventral surface.

Schram *et al.* (1999) double-tagged hatchery-produced Lake Sturgeon (*Acipenser fulvescens*) with two CWT, one in the snout and one beneath one of the dorsal scutes to indicate the year of release. They reported on the fate of the 1991 release, tagged beneath the sixth dorsal scute. Tag loss was estimated to be <1%.

4.1.2 Crustacea

A variety of crustacea have been tagged with CWT. Wickins *et al.* (1986) and Bannister and Edwards (1995) tagged over 91,000 juvenile European Lobster *(Homarus gammarus)*. Sharp *et al.* (2000) tagged individual Caribbean Spiny Lobster (*Panulirus argus*). Red Swamp Crayfish (*Procambarus clarkii*) Isely and Eversole (1998); Snow Crab (*Chinoecetes opilio*) Bailey and Dufour (1987); Blue Crab (*Callinectes sapidus*) Van Montfrans *et al.* (1986) and Fitz and

Wiegert (1991); Mud Crab (*Scylla paramamosain*) Le Vay *et al.* (1999); and Spot Shrimp (*Pandalus platyeros*) West and Chew (1968) and Prentice and Rensel (1977) have all been tagged with CWT with various retention and mortality rates depending on the size and age, molt stage of the animal and the location of the tag. If you need help locating other references, contact biology@nmt.us.

4.1.3 Other organisms

CWT have been successfully deployed in Molluscs, Amphibia, Reptilia and Annelids . Canner and Spence (2010) used CWT to tag seeds and track their dispersal by ants. It would appear that CWT can be effectively used in almost any animal or indeed plant of sufficient size, subject to investigation of suitable tag locations and assessment of retention rates. For questions about tagging other species contact biology@nmt.us.

4.2 Injecting the Tag

The procedure for injecting tags varies a little between the types of injector. The basic details have been described in Section 1.1. Detailed instructions are provided in the appropriate product manuals.

A paramount consideration in any tagging program is the well-being of the fish themselves. While the process of injecting the tag and the presence of the tag represents minimal trauma for the fish, careless handling and treatment of fish before, during, and after tagging can have significant adverse effects. Handling or tagging fish that are already sick or otherwise stressed can cause mortality. However, Sharpe *et al.* (1998) showed that tagging juvenile Chinook Salmon with CWT was no more stressful than other common hatchery practices such as pond-splits. Circulating levels of cortisol and glucose were used as indicators of stress and showed that carefully-conducted tagging operations did not compromise the well-being of the fish.

When hand tagging, it is usual to anesthetize the fish before tagging, to prevent the fish from struggling and protect them from potential injury while being held. It is also important to consider the layout of facilities and procedures of handling. Such issues will be specific to the site and situation, so it is not possible to offer explicit advice. In general, fish should not spend too much time in water containing anesthetic, a watching eye should be kept on water temperature in any holding tanks, and fish should be allowed to recover fully from the effects of anesthesia before they are returned to the wild.

4.2.1 Adjustments and procedures

Having decided on the optimal location for the tag (Section 1.1) the injector must be set up and adjusted appropriately. Where a head mold is being used this must be of the correct design and size for the species and size of fish and the appropriate needle should be used (Section 3.3). Procedures involved in setting-up and tagging salmonids in the snout, using a head mold and a Mark IV Tag Injector, are described in some detail below. This is partly because this represents a major usage of the system, and partly because the principles illustrated apply similarly to other tagging locations and species. We will use as an example tagging Coho Salmon or Atlantic Salmon from a hatchery pond.

The target tag location is an area of muscle and connective tissue in the snout of the fish. The exact anatomy will vary between species, but that of a typical salmonid is illustrated in Figure 21. At the start of any project with a new species, dissection to allow identification and familiarization with the optimal target area is an essential early step. Selection and use of the optimal implantation site is critical to tag retention, fish well-being, and tag recovery. This in turn involves selection of the correct head mold and correctly setting and adjusting the injector. When using standard length tags, the injector should initially be adjusted to SETUP (standard) and SHOW (96). See Section 3.2.1 and the Mark IV Coded Wire Tag Injector Instruction Manual for further explanation.

To select the correct head mold, weigh or measure a random sample of 30-40 fish from the group to be tagged. Pacific salmon head molds for 2 to 200 fish per pound are snout-only molds, whereas those for 300 to 1,000 per lb are whole-head molds. All Atlantic Salmon molds are snout-only. With snout-only molds, the snout should fit easily into the mold without the eyes entering the interior portion of the head mold; if the eyes enter inside the mold, the head mold is too big and if the nose will not reach the innermost section of the head mold, the head mold is too small. The largest and smallest specimens of the random sample should be checked for a proper fit. If the size variation is too great, it may be necessary to grade the fish and use two or more different sized head molds to get proper tag placement. If only a small proportion of the fish are too big or too small it may also be possible to reject these during tagging, without the need to re-grade the whole group. If the fish fall in-between head mold sizes, then select the larger size. Details of sizes of head molds available are in Section 0.

Once the head mold has been selected, the Mark IV should be set in the "SHOW" mode to move the needle to its deepest penetration position. Be sure to double check that the "SHOW" is adjusted correctly. Slide the head mold gently over the needle (it may be necessary to rotate the head mold back and

forth to slide it easily over the needle). Slide the head mold slowly into the head mold holder until the tip of the needle is slightly posterior to the short upper ledge of the head mold. Tighten the set screws on the Mark IV. This is generally a good place to begin checking tag placement. Exit the "SHOW" mode, tag a fish and kill it, then look on it's snout for the needle entry hole (this usually requires drying the snout with a paper towel). The needle hole should be centered between the nares. Using a scalpel, cut to the side of, and parallel to the needle hole back to the eyes. Twisting the scalpel blade slightly will reveal the inner section of the snout. A correctly placed tag should be in the center of the triangular shaped connective tissue within the snout (Figure 21).

If the tag is too deep in the target area, loosen the set screws and slide the headmold out slightly. To make it easier to determine how much you have moved the head mold you can draw a pencil line on the base where it enters the holder. If the tag is too shallow, loosen the holder set screws and slide the head mold in slightly. Re-check until the tag position is within the target area for the full range of fish sizes to be tagged; if it is not possible to find a single penetration depth that suits the full size range, the group must be regraded or the largest and/or smallest rejected during tagging.

Table 2 General guide of the needle penetration depths used for tagging Atlantic salmon.

Fish Length Range (mm)	Needle Penetration Depth (mm)
55-80	1.75
80-110	2.25
110-140	2.75
140+	3.25+

The tag must be placed entirely within the target area. Too shallow tag placement can cause high tag loss; too deep tag placement can cause nerve damage. It is important to check tag placement at least twice a day. The size distribution within a pond may change or the head mold may move slightly. The person tagging may also tire and develop a poor tagging technique.

The salmon group at the CEFAS Fisheries Laboratory in Lowestoft, England, have developed a slightly different approach to adjusting needle penetration depth for Atlantic salmon. Using a specially developed gauge they adjust the needle penetration depth directly, according to Table 2. It is stressed that this is only a guide for setting up; it is still essential to check tag placement by dissection.

When snout tagging salmonids, we recommend that the fish are held upside down (belly up) so that the tagger can properly see the fish doing into the head mold and ensure that the palate is vertical. In our experience, holdign the fish sideways in the head mold lead to poor tag placement and compromises retention rates.

4.2.2 Rates of tag application

With salmonid parr or smolts of 70 mm or above, tagging in the snout using an appropriate head mold on a Mark IV, marking rates of up to 1300 fish per hour have been achieved but usual rates range from 700 to 900. With smaller, more difficult to handle fish, rates are likely to be lower. Half-length tags were injected into emergent Pink Salmon fry (0.2 g, about 30 mm length) at rates of up to 800 per hour (Peltz and Miller, 1990). Champigneulle *et al.* (1987) report tagging rates for very small charr of 250-300 per hour for 20-30 mm fish, and 400-500 per hour for 40-50 mm fish. Snout tagging with a Multishot with a head mold could be expected to approach 500-600 fish marked per hour with easily-handled fish (e.g. 70+ mm).

Tagging rates in other body locations will vary considerably with species, size of fish, and arrangement of facilities. Buckmeier (2001) recorded tagging rates of 389 to 583 per hour tagging Black Bass (*Micropterus* sp), 32 –54 mm in length, in the nape muscle. Oven and Blankenship (1993) working with salmonids reported a rate of 200 fish per hour tagged in the post-ocular area, the adipose fin or between fin rays using a Mark IV. Thomassen *et al.* (2000) were able to tag about 400 eels per hour in the dorsal musculature. Slightly lower rates should be expected using a Multishot.

Attempting to tag at a very high rate can lead to carelessness and improper tag placement. Competition between tagging technicians should be avoided and tag placement efficiency should be checked regularly, especially in the early stages of a project.

4.2.3 Tagging very small fish

Successful application of CWT to very small fish is dependent upon finding a suitable site for the tag, careful handling of the fish, and accurate tag placement. The musculature is probably the most realistic site in the very smallest fish, and half-length tags are likely to be required to allow the lowest size limit to be approached. The smallest fish successfully coded-wire-tagged as far as we are aware, are 11 mm long damselfish (*Pomacentus moluccensis*) using half-length tags (Beukers *et al.*, 1995).

Other very small non-salmonids that have been successfully tagged with CWT include rockfish *Sebastes spp* (36 mm, single length tags in the nape musculature, Buckley *et al.* (1994)), Barramundi *Lates calcarifer* (30 mm, single length tags in cheek muscle, Russell and Hales (1992)) and largemouth bass *Micropterus salmoides* (28 mm, single length tags in cheek muscle, Copeland and Noble (1994)).

Some of the smaller salmonids marked with CWT are indicated in Table 3. Generally for tagging in the snout full length (1 mm) tags are recommended for fish 50 mm or larger, and half-length tags for smaller fish (Figure 22).

Table 3 Smallest salmonid fish that have been tagged with CWT.

Tag Length	Body location	Species	Size	Notes	Reference
0.5 mm	Snout	Salvelinus alpinus	22 mm	a	Champigneulle *et al.* (1987)
0.5 mm	Snout	Oncorhynchus gorbuscha	0.2 g	a	Kaill *et al.* (1990)

Although no results of use of the muscle in the nape have been published for salmonids, it is suggested that full-length tags could be used for fish over about 40 mm. Oven and Blankenship (1993) indicated that rainbow trout of 116 mm were close to the lower limit for post-ocular tagging with CWT, but did not indicate whether fish of 90 and 95 mm tagged in the adipose fin and between fin rays respectively represented a lower size limit.

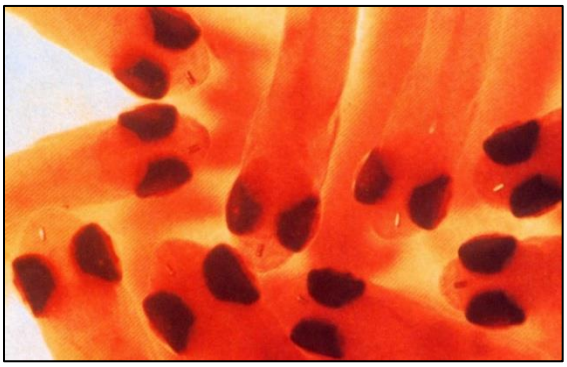

Figure 22 Half-length CWT in pink salmon fry (mean length 33 mm). Photo courtesy of Alaska Department of Fish and Game.

4.2.4 Tagging large numbers of fish

Large scale experiments where tens or hundreds of thousands, even millions, of fish are to be tagged will generally be accomplished using the Mark IV. Carefully designed facilities are important in such situations to ensure efficient operation and to minimize handling of fish. For example, as part of their program to tag about 40 million juvenile salmon per year, managing agencies in the Pacific Northwest of the USA use Mark IV Tag Injectors set up in specially designed manual marking trailers in addition to using AutoFish System (see Section 3.2.4). The interior of the manual trailers are laid out to optimize the use of space and typically have five tagging stations. Fish are delivered to each station via a pipe on the wall, and the QCDs are built into the benches and the tagged fish are returned directly to the pond via pipes constantly flushed with water. One of these five-injector trailers can be used to tag about 35,000 fish per 8 h shift. The design, construction and operation of one version of these trailer units is described by Schurman and Thompson (1990).

4.3 Detecting Tags

Tag recovery programs are specific to the particular situation but a number of common features will be apparent.

Where a significant proportion of the sample of fish to be scanned are likely to be tagged, a straightforward check of all fish in the sample is an ideal option. This can be done on individual fish using a Wand, V-Detector, or a tunnel detector. Some care will be needed to ensure that the tag detection process is efficient; we have observed the careless use of the Wand on occasions which would almost certainly cause some tagged fish to be overlooked. The instructions for each type of detector should be carefully studied and followed.

Where tagged specimens likely represent only a small part of the sample to be checked, some difficulties arise. Obtaining an adequate number of tagged fish will involve scanning large numbers of fish, which is not only time-consuming, but can lead to operator fatigue and careless use of the detectors. Missing the occasional tagged fish when they represent a large proportion of the catch may introduce only a minor bias in the results, but missing the one tagged fish in a sample of a thousand for example represents a serious matter. In some situations it may be appropriate to use a secondary visual mark – for example in salmonids it has been a widespread practice to clip the adipose fin of tagged fish. Even if other agencies were clipping fins for other purposes, only checking adipose clipped fish for the presence of tags reduces the effort required. For example, at the height of the program checking for CWT in the Greenland high-seas fishery for Atlantic Salmon, about one in a hundred fish was fin-clipped, and of these about one in four contained a CWT. Thus only 1% of the catch needed to be scanned for tags, and the tagged proportion of these (25%) was high enough to ensure thorough checking and reliable detection.

The adipose fin clip was also used to indicate the presence of a CWT in Pacific salmon for many years, but more recently the adipose fin clip has been re-assigned as a mark for all hatchery fish, whether or not a CWT is also applied. This created a requirement for more versatile tag detection equipment, and the Wand and the R-series tunnel detectors were the direct result. Tunnel detectors are useful for quickly and reliably scanning large numbers of fish. They are extensively used for adult Pacific salmon, the fish being passed through the detector by hand, one at a time. In this application the detectors are usually used with a diverter gate that automatically sorts tagged from untagged fish. A project in Denmark used an R8000 for scanning commercial catches and research catches of eels for presence of CWTs; handling live eels (the commercial catch is marketed live) individually would be very problematic (Bisgaard and Pedersen, 1991). A

study of herring stock assessment and migration in British Columbia used R9500 detectors with a conveyor belt to scan commercial landings of herring at a rate of about 40 tonnes per hour; any tagged fish detected are automatically diverted into a separate container for later tag recovery (Flostrand and Schweigert, 2005, Flostrand *et al.*, 2009 and Schweigert and Flostrand, 2001).

Where body location of the CWT is used to allow some level of benign data recovery (see Section 4.5.5), the high resolution of the Wand detector in fixing tag location is invaluable.

Figure 23 The Magniviewer is a portable, battery-operated device used to magnify and view CWT. Individual tags can be held with the brass pencil and viewed in the Magniviewer, or a strand of CWT wire can be inserted into it. The Magniviewer operates on AA batteries and can be used anywhere.

4.4 Recovering and Reading Tags

4.4.1 Recovering tags

Having identified a tagged animal, the tag must be retrieved to be read under a microscope. This usually requires the animal to be killed, but data can sometimes be recovered from live fish (Section 4.5.5).

Where the tagged fish is small it may be simplest to take the whole animal into the laboratory for tag recovery. However, where the animal is large, or represents a valuable commercial catch, the optimal approach may be to take a tissue sample that contains the tag.

Snout tagging salmonids generally results in the tag being in a predictable location for recovery. The most usual approach on the Pacific Coast is to use a detector to check whether a fish is tagged, and then, if the fish was tagged, to use a hack-saw or knife to remove the front part of the head just behind the eyes. With Atlantic salmon, the usual approach to recovery is to use a device like a cork borer to cut a “plug” of tissue about 25 mm in diameter from the top of the head through to the roof of the mouth in the area where the tag is believed to be. The core is then checked with the detector to ensure that it gives a tag signal, and the rest of the fish to ensure that it does not. If a steel borer is used the core must be removed from the borer before it can be checked, but if the borer is crafted from a copper tube it can be checked *in situ* as the copper does not trigger the detector

nor does it shield the tag from detection. The core is removed from the corer, labeled, placed in a small plastic bag (check bag for presence of tag to ensure that it was successfully transferred from the corer), and taken to the laboratory for extraction and reading of the tag.

Where such disfigurement of tagged fish is unacceptable, for example where large salmon are marketed whole, it is possible to recover the tag through the roof of the mouth without affecting the external appearance of the fish.

A laboratory tag extraction and reading area should be carefully laid out and maintained. A CWT is a tiny object and difficult to find if dropped or swept away with a sleeve. Care should also be taken to keep the area clear of extraneous tags so that there is no possibility of confusion arising. The tissue sample containing the tag should be placed onto a clean plastic tray, without joins and recesses, which has been checked for the absence of tag or tag-like signals at the start of the session.

The V-Detector is the device most often used to aid recovery of tags from a recaptured animal. The specimen (whole fish, or for example the head) is checked to confirm that a tag is present. The specimen is then split or a core sample is taken, by excising the section most likely to contain the tag. Each part is then checked with the detector, and the part containing the tag further divided. This process is continued until the tag is isolated in a small sample of tissue in which it may be seen and from which it can be recovered using a tag-reading "pencil". It may be helpful to use a headband magnifier. With practice this tag recovery procedure is likely to take less than a minute.

4.4.2 Reading tags

Tag reading is normally done under a suitable low-power microscope (magnification 20-40 X) or with the NMT Magniviewer (Figure 23). This is a portable device combining a 25X microscope, a high intensity light, and magnetic reading "pencil" to view individual tags or spools of tag wire. The MagniViewer is small,

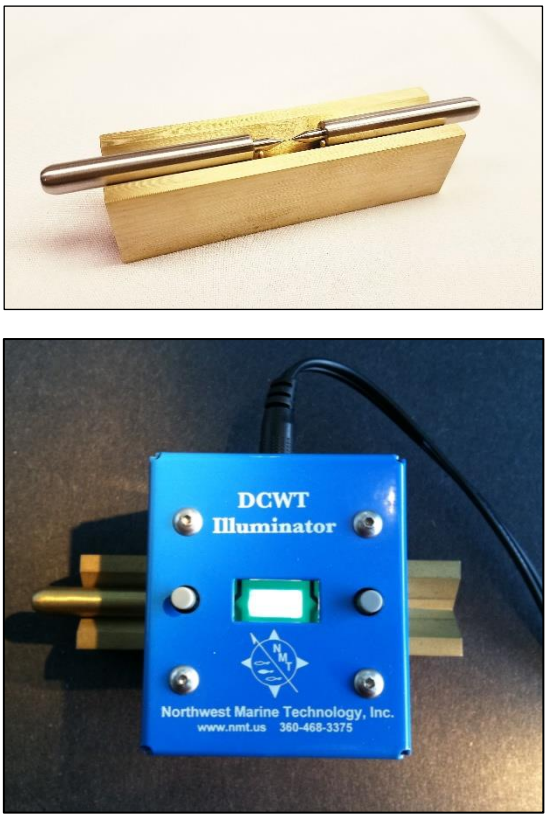

Figure 24 A tag reading jig (top) and Illuminator (bottom) facilitate reading CWT with a microscope. The brass pencils in the tag reading jig holds the tag so that the tag can be rotated during reading.

lightweight and easy to use – it actually fits in the palm of your hand. It is particularly suited for field use and in situations where limited numbers of tags must be read on site tag reading is assisted by the use of tag-reading pencils and a tag reading jig (Figure 24). The two pencils are brass rods with a small magnetic tips that holds the CWT end-on, allowing the tag to be rotated under the microscope to read all faces. The tag-reading jig is a specially-shaped brass block which holds the two pencils in an appropriate orientation for viewing the tag. Correct illumination is very important for reading tags under the microscope – this is discussed in detail below. A common protocol is to prepare a file card for each tag onto which all data is recorded. It is usual to affix the recovered tag to the card after reading, to allow for later quality control checks or confirmation of unexpected tag recoveries, and to prevent loose tags being confused with later recoveries.

4.4.3 Illuminating tags for reading

Tags should be illuminated properly for good contrast between the background and the code marks to ensure easiest reading. We recommend the use of the NMT Illuminator, which fits over the Tag Reading Jig (Figure 24), or the Magniviewer (described in Section 4.4.2) – these devices are specially designed for optimizing lighting for tag reading.

For users who prefer to set up their own lighting system we offer the notes below.

The most important idea to keep in mind when setting up a microscope and light for viewing CWT is that the unmarked surface of the wire acts like a smooth, curved mirror, while the marked dots act as small dimples or pits. The goal in setting up good lighting conditions is to make the smooth, mirror-like background look black, and the dimples or pits look white. The basic idea used to optimize lighting for viewing CWT; illuminate the laser-marked pits with light coming from the side, while orienting the mirror-like, smooth background of the tag so that you see the reflection of a black wall in it. The only complicating factor is that the "mirror" - the surface of the CWT - is curved. But one solution is simple: have the illuminating light coming from the side, with the light source positioned over the axis of the wire and directed so that it illuminates the wire at about 45 degrees, and have black or dark surfaces

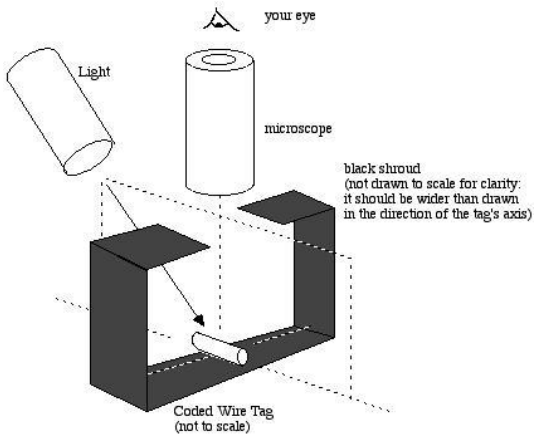

Figure 25 Sketch of recommended viewing and lighting geometry.

surrounding the CWT in all other directions (Figure 25).

With the axis of the CWT going from left to right and the CWT holder extending to the right, position the light source to the left of and above the CWT and direct the light so that it strikes the CWT at about 45 degrees. This will allow bright glints from the laser-marked dots, without significant lightening of the unmarked wire surface. Do not use uniform lighting, such as from a ring illuminator, since this will tend to light up parts of the tag other than the laser-marked pits.

- The CWT should be clean – adhereing material can significantly reduce readability.
- The room lights near the CWT should be dimmed
- The background to the view of the CWT should be black.

Various light sources can work well: a simple gooseneck desk lamp with a standard incandescent bulb (60W is fine, 100W may be better), a special-purpose microscope illuminator that puts out more directed or collimated light, and a white LED flashlight. An incandescent bulb works much better if a black shroud is used to prevent illumination of surfaces that should be dark.

If you need further assistance with setting up a CWT reading station, contact NMT at techsupport@nmt.us.

4.5 Use of Sequential CWT for Individual and Batch Identification

4.5.1 Identifying individual fish

As described in Section 3.1.5, each sCWT bears a unique code, although the sequential number (n) on any particular tag does not correspond to the nth fish tagged. So how can recaptures be identified? Let us first consider a situation where individual identification is required, for example where individual fish length has been recorded at the time of tagging in a growth study. There are two options:

1. Reading the tag before placing it in the fish and recording the sequence number. This is normally impractical, but may be feasible where precut tags are used in a Single Shot Injector – see Section 3.2.3.
2. The one-in-two option. Here, a reference tag is stored between each one used in a fish. This avoids all possible ambiguity, and recaptures are identified by reading the appropriate archived tags to locate the recovered tag in the sequence.

See Table 4 for a suggested layout for reference storage sheets for individual identification.

Table 4 Suggested tag storage sheet for individual identification, one in two option. We recommend laying down a strip of clear silicone in the shaded column. Once the caulking has cured, reference tags can be injected and stored in it.

Project:				Personnel:		Date:
Sheet #.....of.....		Tag Code:		Agency:	Data 1:	Data 2:
Line #	Fish #	Reference tag sequence #		Data (customized by project)		

4.5.2 Identification of batches

Use of sCWT for identifying small and variable sized batches of fish is simpler than that for individual identification, but similar care is required in archiving reference tags. In this case, however, it is only necessary to store one tag at the start of each batch, whatever the size of the batch. It is also necessary to store a reference tag at the end of the last batch, and it is prudent to do so at the end of any batch if there is the slightest doubt where and by whom the injector will next be used or if the wire is to be removed from the injector.

Examples of batches might be all fish of a particular length range or weight range in graded samples, or fish released at a particular place or at a particular time.

- The batch size can be variable, from single fish to many thousands.
- It is not necessary to decide the batch size in advance. A tag is stored before tagging the batch, and the group is bracketed by a reference tag stored at the end of each batch, or at the beginning of a subsequent batch.
- Record the number of tagged animals in each batch by reference to the counter on the injector.
- The batch to which a particular recovered tag belongs is established by locating the two reference tags between which its sequence number lies. It may not be necessary to read all reference tags unless they are adjacent to a batch from which a recovery is made. You may only need to read a few reference tags to locate the recovered tag.

See Table 5 for a suggested layout for reference storage sheets for batch identification.

Table 5 Suggested reference tag storage sheet for batch identification. We recommend laying down a strip of clear silicone in the shaded column. Once the caulking has cured, reference tags can be injected and stored in it.

Project:				Personnel:			Date:
Sheet #.....of.....		Tag Code:		Agency:		Data 1:	Data 2:
Line #	Batch #	Reference tag sequence #		Counter reading		# in batch	Batch data (customized by project)
				Start	End		

Combining batches in different ways can be very powerful. For example, suppose a hatchery was releasing groups of smolts at four times every day for 3 weeks. If each group comprised a tagged batch, we would have 84 batches (4 x 21). Each batch may in itself be too small to analyze for adult returns, but batches can be combined, for example by day of release (batches 1-4, 5-8, 9-12 etc) or by week of release (batches 1-28, 29-56, 57-84). The effect of time of day of release could also be examined by combining for example all fish released early in the morning (batches 1, 5, 9, 13 etc) or late evening (batches 4, 8, 12, 16 etc). An important point is that the way in which batches are combined can be decided when the return results are known, as long as the appropriate data concerning each batch are recorded.

4.5.3 Suggested designs of sheets for reference tag storage and data recording

On each sheet, we recommend that you lay down a vertical strip of silicone (indicated by a shaded bar), which can be clear silicon adhesive such as is used for aquarium tank construction or clear silicone caulking. Once cured, this is intended for holding reference tags injected into it; they are visible and readily removed for reading when required. It may be more convenient, especially where every other tag is being archived, to have these strips on a separate sheet from the other data, carefully cross referencing by a numbering system.

4.5.4 Precut Sequential Coded Wire Tags: Application and archiving

Large-scale CWT programs require injection and detection equipment that would be cost-prohibitive for a study where relatively few animals are tagged. To make these smaller programs feasible, NMT offers precut sequentiual tags (sCWT) that can be individually injected using a simple syringe type injector). All precut tags use the sCWT format (section 3.1.5). Detectors are not always required for tag recovery, and there is an option of equipment rental for short term projects.

Precut sCWT tags are supplied mounted in two columns on waterproof paper (**Error! Reference source not found.**). We cut the tags and lay them on the paper in order, starting with the initial reference tag, followed by a tag from the fish column, followed by a reference tag, then a fish tag, and so on down the columns. Because of the layout of the coding on the wire, the individual numbers on the tags do not increment by one for each following tag. For example, the third tag cut from the wire will not necessarily have the individual number 00003.

There are two ways to keep track of the tag code that is being injected:

1. Retain one tag at the beginning and end of each batch. If the batch is one animal (i.e. you want individual identification) then you will alternately retain a reference tag and inject a tag. Thus for individual identification, only one tag in two is deployed in an animal, and you will need to buy twice as many tags as animals you plan to identify. For larger batches, retain a tag, inject the tags sequentially from the reference tag column and fish column, then retain a tag at the end of the batch. When a tag is recovered, its position on the data sheet or its batch group can be determined by reading the reference tags.
2. Read all of the tags in the reference tag column before injecting any tag. The number on the tag in the “Fish” column can be deduced as it will be between the number above and below it in the reference column. Once you have read all of the reference tags, all of the tags can be deployed in animals. Inject tags alternately from the fish column and from the reference column so that they stay in order.

The precut tags are loaded, one at a time, into the syringe of a Single Shot Injector (Section 3.2.3) for injection into the animal. This process takes a little time and patience but is viable for experiments involving only hundreds of animals. Peterson and Key (1992) reported being able to tag juvenile walleye (*Stizostedion vitreum*) at a rate of up to one fish per 5-10 seconds using a Single Shot Tag Injector. Having two people and two Single Shot Injectors can increase the

rate of tagging; one person tags the fish while the other loads the second injector.

Correct tag placement is critical to obtaining high rates of tag retention. Once the depth of the tag has been determined, it can be helpful to wrap a piece of tape around the needle to use as a gauge.

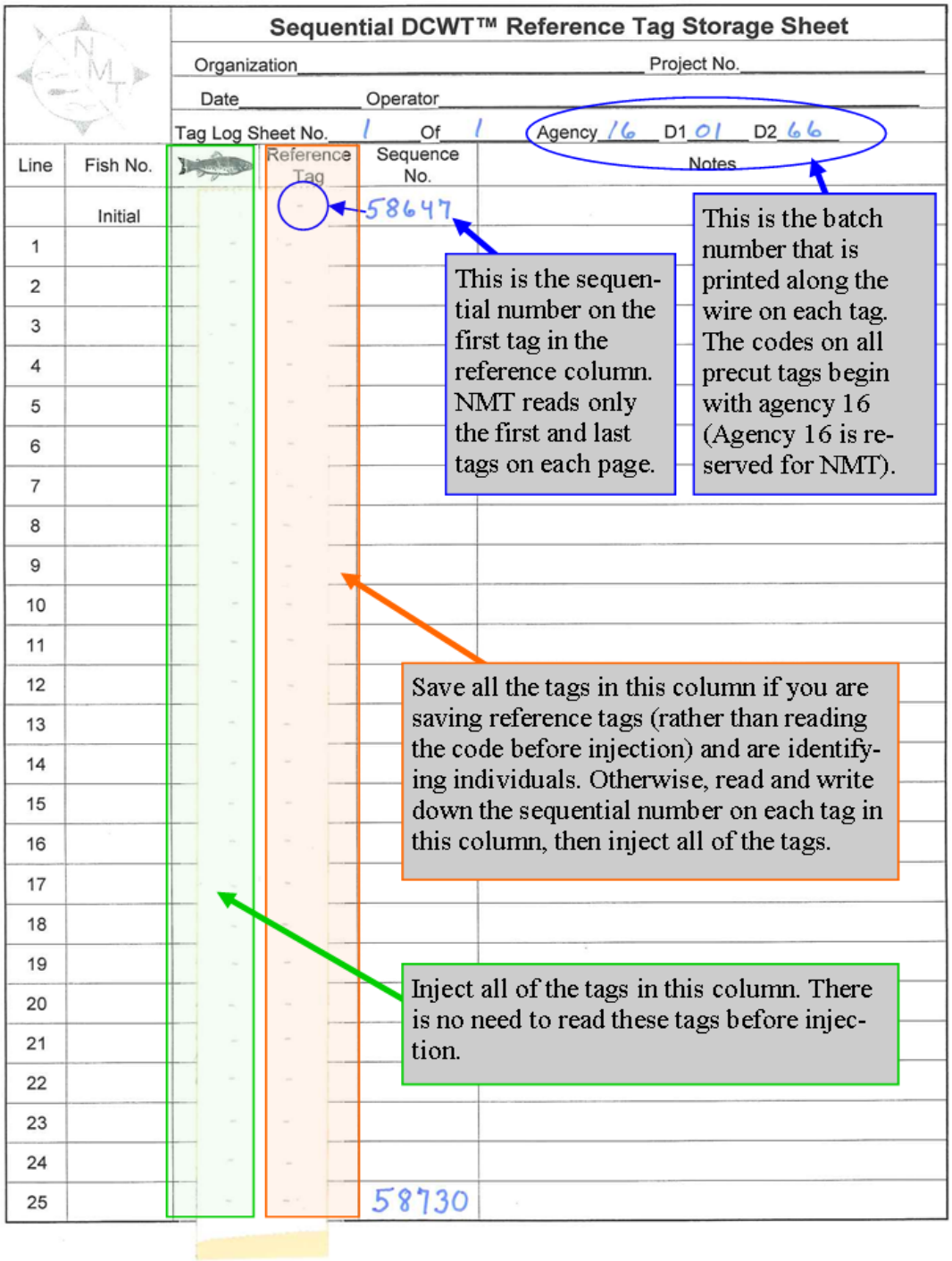

Figure 26: Precut tags are mounted on sheets of waterproof paper and loaded and injected individually with a Single Shot Injector

4.5.5 Benign Data Recovery

The CWT may sometimes be used in studies for data recovery from live fish. There are three approaches.

1. Tagged or untagged: Simply knowing whether or not the fish is tagged may be sufficient for some purposes.
2. Tag location, without tag recovery: In larger fish, detection of tags in different body locations may provide a simple batch coding system, exploiting the limited detection range of the wand detectors. This T-Wand can resolve tag location to about 5.25 cm, while the blue wand can resolve tag locations to about 3.2 cm. In large fish it is likely that several suitable, differentiable sites could be found (e.g. cheek muscles, bases of fins). In small fish, the tags can be put into locations which may not be differentiatable at the time, but will be when the fish grows. Tipping and Heinricher (1993) used three sites on tiger muskellunge (*Esox masquinongy x Esox lucius*, Esocidae) to differentiate between groups. A very useful study of the application of the approach to salmonids is reported by Hale and Gray (1998).
3. Recovering tags from live fish: By placing the tag in shallow tissue (e.g. post-ocular tissue or between fin rays), a detected tag may be excised and recovered without killing the fish. Oven and Blankenship (1993) described one approach; they used a magnetized scalpel to recover tags from between fin rays, and a modified syringe to extract tags from the adipose fin and from post-ocular tissue. They also found a 2 mm biopsy punch effective at removing tags from the adipose fin. An important feature of this method is that the tag should be visible once it has been magnetically detected; "blind" recovery may be more difficult or may involve more trauma to the fish. The rainbow trout used by Oven and Blankenship (above) more than doubled in size during the experiment and all tags remained visible, but the effectiveness of this approach for recovery of tags from adult salmon that were tagged as parr or smolts for example would need careful evaluation. If it is desired to have the fish identifiable on subsequent recapture after the tag had been recovered, it would be necessary to re-tag them.

4.6 Disinfecting Equipment

The possibility of spreading fish diseases between culture facilities and watersheds is of concern to both our customers and Northwest Marine Technology. Although we are unaware of a case of CWT equipment, moved between locations, as having served as a "vector' in spreading a disease, the potential consequences of such occurrences call for stringent

preventative measures. Disinfection procedures should also be implemented between groups of fish, within a facility, when signs of disease exist. Tagging should not be conducted during a disease outbreak.

Details of recommended procedures for disinfection are available on our website.

5 Potential Impacts of CWT on Humans

Coded Wire Tags (CWT) are lengths of stainless steel wire 0.25 mm in diameter and from 0.6 to 2.2 mm long. Whenever possible, we recommend that the incidence of ingesting CWT be minimized by placing the tags in parts of the animal that are not usually consumed (in salmon snouts, for example). Because of their very small size, their rounded shape, and benign material composition, we contend that the risk from ingesting a CWT is negligible and lies below the threshold of reasonable concern. In the absence of direct studies confirming this conclusion we offer the following comments:

- Billions of fish have been tagged with CWT since the early 1970's and there has not been a single report of anyone having any effect of ingesting a CWT.
- Stainless steel wire and other objects much larger than CWT are swallowed accidentally during dental procedures. These objects are typically egested without incident through the digestive tract (Milton *et al.* 2001; Obinata *et al.* 2011) and it follows that a much smaller CWT would also be harmless.
- The US Food and Drug Administration (FDA) classifies fish tags as food additives, and their use would theoretically require FDA approval. We are not aware of any type of fish tag having been reviewed for approval as a food additive. In practice, the FDA is well informed of the use of CWT and has allowed their widespread use without formal approval.
- Exports of fish that may contain CWT are accepted worldwide, and meet the highest quality standards in all cases.

6 Further Reading

Hundreds of published papers describe specific tagging techniques for fish, crustaceans, molluscs, and other animals. If you need assistance obtaining or searching the publications, please contact biology@nmt.us.

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