Visible Implant Elastomer Tag
Project Manual

Guidelines on planning and conducting projects using VIE and associated equipment

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1 INTRODUCTION

1.1 Aim of this document

This document provides information on the uses and deployment of the Visible Implant Elastomer (VIE) system and its associated equipment. It is primarily aimed at new and potential users, but existing users may also find it useful, particularly if they are considering marking new species or working under different conditions. It is not intended to replace the instructions issued with each kit or piece of equipment – such information is generally not repeated in detail here.

1.2 Overview

The VIE system provides internal colored tags that are visible externally, for fish and other animals that are too small for the NMT VI Alpha tag, or when group codes are sufficient. The system uses a bio-compatible, two-part, elastomer material. After mixing, the elastomer is a liquid that is injected into tissue with a hypodermic syringe; most species of fish, and many other animals, have suitable areas of transparent or translucent tissue. Within hours or days this material cures into a pliable solid. The elastomer holds the pigment in a well defined mark, without damaging surrounding tissue. By the use of different marking sites, and perhaps two or more marks on each individual, development of numerous group or individual codes is possible. Some of the colored pigments used are fluorescent, and use of appropriate lighting can significantly enhance detection of tags.

The VIE tag lies beneath the skin or deeper within the tissues, without a permanent wound or lesion. It has been demonstrated to have minimal impact upon subsequent growth and behavior of fish and other animals. In contrast, conventional external tags, attached via penetration of the skin, cause a wound that is very slow to heal or may never heal (Roberts *et al.* 1973 a-c). A study in Iceland indicated that salmon smolts tagged with Carlin tags experienced a 38% mortality and a depressed growth rate (typically 100-300 g lighter as adults) (Isaksson and Bergman 1978). An investigation on juvenile char indicated that Carlin tags reduced the survival to return by at least 28.8% (Berg and Berg, 1990).

1.3 Some history

The VIE System was developed by NMT biologists in the early 1990's while they were seeking better fish marking methods than traditional external tags and fin clips, which may have adverse impacts on the growth, survival and behavior of fish.

The system was initially used with salmonids, exploiting an area of transparent tissue behind the eye. Since then, however, it has been used on hundreds of species of fish, amphibians, crustaceans and other animals, with many body locations being used. Again, further details are provided in later sections of this manual.

1.4 Advantages and limitations of the VIE system

The advantages and limitations of the system are summarized in the table below. Each of these factors is discussed in detail later in the report.

Advantages of VIE tags

- High retention rates
- May be applied to very small fish and other animals
- Minimal impact on fish survival, growth and behavior
- Low capital and material costs make it viable for small-scale projects
- Fast to apply
- Tags detected visually in ambient light
- Detection can be further enhanced with appropriate illumination
- Well-established technique with extensive literature on successful applications in hundreds of species of fish, amphibians, crustaceans and other animals

Limitations of VIE tags

- Limited coding capacity (but use of several colors, several body locations, and possibly more than one tag allows a greater coding capacity to be developed)
- Tags may become difficult to detect in ambient light if growth is considerable and pigmented tissue is laid down over the tag, though it can usually be detected using the VI light
- Tags may not be noticed and reported by casual observers

2 DETAILS OF THE SYSTEM

2.1 The material

VIE tags are formed from a two-part mixture. When first mixed, the material is a viscous liquid. This hardens to a pliable solid mark that generally retains its structural integrity as the animal grows; this avoids the gradual dissipation of pigment that tends to occur with injections of particulate material in liquid suspension such as Alcian Blue.

The rate at which the material hardens, and thus the length of time that it remains usable, depends on temperature. At 20^{o}C this is of the order of 40 minutes; at 0^{o}C it is many hours.

The material is biocompatible and carries no known human health hazards. A Material Data Safety Sheet is attached as Annex A.

Ten VIE colors are available. Six (red, pink, orange, yellow, green, blue) are fluorescent; the other four (black, white, purple and brown) are not.

Samples of the ten VIE colors under ambient light (left) and illuminated in a darkened room by the VI light. Note that only the six fluorescent colors show up in the VI light, and that the colors, especially the yellow, appear somewhat different when fluoresced.

2.2 The VIE Color Standard

The color standard is a small transparent card with a sample of all ten colors of material that are available. It is supplied with all kits other than the Trial Pack, and is available free of charge on request. It allows consideration and selection of the most appropriate color for any particular application. However, perhaps its greatest value is a color standard when identifying tag recoveries, especially when using the VI Light to fluoresce the material. Customized color standards can easily be made by the user by loading small volumes of material of each color being used onto a transparent sheet and covering them, when cured, with transparent tape. This has the advantage that the volumes loaded onto the customized standard can be similar in shape and size to the marks being used in the particular project. Labeling the samples is advisable to avoid any risk of confusion over colors that may appear similar under certain lighting conditions.

VIE color standard on a white background

2.3 Mixing supplies

All hand kits and supplies of VIE material include enough mixing supplies for the material involved. Mixing supplies include syringes (without needles) for dosing the material and transferring it to mixing cups, where it is mixed with small wooden spatulas (supplied). All mixing supplies are intended for single use and should be discarded.

Mixing supplies and injection syringes from a VIE hand kit

2.4 Injecting syringes

The mixed material is loaded into a hypodermic syringe for injecting into the animal. Two types are used. For use on their own (for example with the Trial Pack) or with a Manual Elastomer Injector (supplied with all other manual injection kits) we supply BD 0.3 cc insulin syringes with a 29 g needle. All kits and refill packs contain enough syringes for all the material supplied but if additional ones are required NMT can supply them. However, freight costs may exceed the price of the syringes, so if you need extra syringes we suggest seeking a local source; this brand is widely available internationally from medical suppliers and retailers. Other similar brands are likely to be suitable if they will fit into the manual injector.

A somewhat different syringe is used with the air-driven injector. All orders for use with an air-driven injector include enough syringes for the quantity of material supplied; additional supplies are available from NMT.

All syringes are single use only and should be handled and disposed of with appropriate care.

2.5 Manual Elastomer Injector

The Manual Injector is a machined plastic device into which a loaded 0.3 cc injection syringe is placed. It is designed to make holding and deployment of the syringe comfortable, and allows extended use without fatigue. In particular, it allows carefully controlled pressure to be applied to extrude the desired volume of material. While it is

perfectly possible to use the bare syringe for small numbers of tags (for example while using a Trial Pack for evaluation or very small projects) we advise the use of the Manual Elastomer Injector for anything involving a hundred or more tags.

VIE Manual Elastomer Injector

2.6 Air Driven Elastomer Injection System

The Air Driven Elastomer Injection System is intended for large-scale use, for marking many thousands of individuals. Some experiments in N. America have involved over 750,000 fish per year. Marking rates well in excess of 500 per machine per hour have been achieved.

The Air Driven Elastomer Injection System comprises a machine that delivers a pulse of compressed air to a specially designed handpiece containing a VIE syringe to inject a VIE tag. The air pressure and the length of the pulse are under software control, so that the optimal combination can be found and then reliably reproduced for injecting large numbers of tags. The pulse of air is triggered by a button on the handpiece. Alternatively, if variable-sized tags are required, the machine can be set to maintain the supply of compressed air to the syringe as long as the button on the handpiece is depressed.

The injector works using a credit token system. When you purchase supplies to use with this system you are also provided with a token which allows the machine to be operated for the number of tags purchased (plus an allowance for setting up and testing). Enough VIE material is supplied for the number of tags purchased. The customer therefore pays by the tag rather than by the quantity of material supplied.

Air Injection System complete with air hose and handpiece

Compressed air is supplied to the machine from a suitable compressor. NMT does not normally supply such compressors as they are usually readily and cheaply available locally, and are expensive to ship internationally. The required specification is given in the next section.

The injector has a number of controls and an LED display which shows a range of information about the machine and its settings. When first switched on it displays the version and serial numbers of the unit. Other information than can be scrolled through includes the following:-

Batch – the number of tags injected since the batch counter was last re-set.

Credit – the number of tags in credit within the injector, and in brackets the credit contained in any token that is inserted into the machine. This machine credit reading will decrement by one as each tag is injected. If a charged token is installed a credit of 100 tags will automatically be downloaded into the machine whenever the machine credit falls below 100. Larger volumes of credit can be downloaded from a token, and credit can also be uploaded from the machine to the token for transfer to another machine.

Pressure – the pressure setting of the internal regulator is displayed and can be re-set. For most applications a pressure of 45-65 psi (3-4.3 Bar) is recommended.

Timer - here the option of a timed pulse of air (as opposed to compressed air being supplied to the handpiece and syringe while the button is pressed) can be selected and the length of the pulse is set. Typical pulse lengths are well under a second, but it is possible to set the pulse up to 9.99 seconds.

Total – this displays the total number of tags the unit has injected and cannot be reset.

Token – this displays the token identification number.

Error messages – in the event of equipment problems an error message may appear in the display.

Other features of the machines include:-

Debit button – this reduces the batch reading by one tag for each press. This is used to remove test or accidental pulses from the register to maintain an accurate tally of the number of animals actually tagged.

Front panel LED – this lights for the duration of the pulse of compressed air for each tag.

Drip control knob – this can be adjusted to provide a small extent of negative pressure to the handpiece between injection pulses. This prevents dripping of material from the syringe needle.

Blow-out plugs – these are two white rubber plugs in the base of the machine. They allow the machine to breathe and prevent pressure build-up in the machine should an internal pressure component fail.

Supplied with the machine are a detailed instruction manual, an appropriate power supply for the region where the equipment is to be used (input 120-250 V AC, output 12 V DC), handpiece and associated air hose and control cables, a VI Light (see below), a token and appropriate supplies of materials, syringes and mixing supplies for the number of tags purchased.

2.7 Compressor for the Air Driven Injection System

As already explained, NMT does not usually supply a compressor for this system as they are usually readily and cheaply available locally, and are expensive to ship.

The compressor must be able to supply air at a pressure of at least 60 psi (4 Bar), preferably 100 psi (6.5 Bar), at a rate of at least 1.5 cubic feet (42 litres) per minute. It is recommended that it has an air tank of at least 5 liters to avoid constant cycling. Such machines are commonly used with spray painting and other equipment. As an example, in the UK a suitable compressor is the Direct Power 1.5 hp 6 liter compressor (see picture) which is available from Screwfix (www.screwfix.com) at a price of £60 excluding VAT (June 2008).

Small compressor suitable for supplying the Air Injection System

NMT will be happy to advise prospective purchasers regarding appropriate fittings for connections to a compressor.

2.8 The VI Light

The new VI Light is designed for use with both VIE and VI Alpha tags. It radiates a deep purple light with the peak of its spectrum at the limit of visibility to the human eye at about 405 nm. This causes the fluorescent VI colors (red, orange, pink, yellow, green and blue) to fluoresce, increasing visibility considerably. In contrast to the earlier light system supplied by NMT there is no requirement for the use of amber sunglasses for optimal visibility. The working of the system is described in section 3.10.

VI Light

The VI light is fitted with a voltage regulator that maintains full intensity of the light until the batteries are virtually exhausted; the light then flashes on and off as a warning. Without this, the performance of the light would deteriorate, perhaps un-noticed, as the batteries aged.

The VI light is waterproof to a depth of 500 feet (150 m) so is suited to underwater use.

2.9 Manual Elastomer Injection Kits

All Manual Elastomer Injection Kits (with the exception of the Trial Pack) are based on multiples of VIE units. One VIE unit contains material to generate 6 cc of mixed VIE, mixing supplies and injection syringes. Each unit can be any of the available colors.

The Master Kit contains: 10 units of VIE, two Manual Elastomer Injectors, one VI Light, a VIE Color Standard, a field carrying case, an instructional CD and written instructions.

The Four Color Kit, contains: 4 units of VIE, one Manual Elastomer Injector , one VI Light, a VIE Color Standard, a field carrying case, an instructional CD, and written instructions.

The Single Color Kit, contains: 1 unit of VIE, one Manual Elastomer Injector, one VI Light, a VIE Color Standard, a field carrying case, an instructional CD, written instructions and.

The Trial Pack, with 1 cc of elastomer, mixing supplies and injection syringes. This kit is intended to allow mixing of two or three batches of material for tagging small numbers of individuals, for evaluation of the system, or for very small projects.

When the supplies in the kit have been exhausted, NMT offers Refill Kits based on units of VIE.

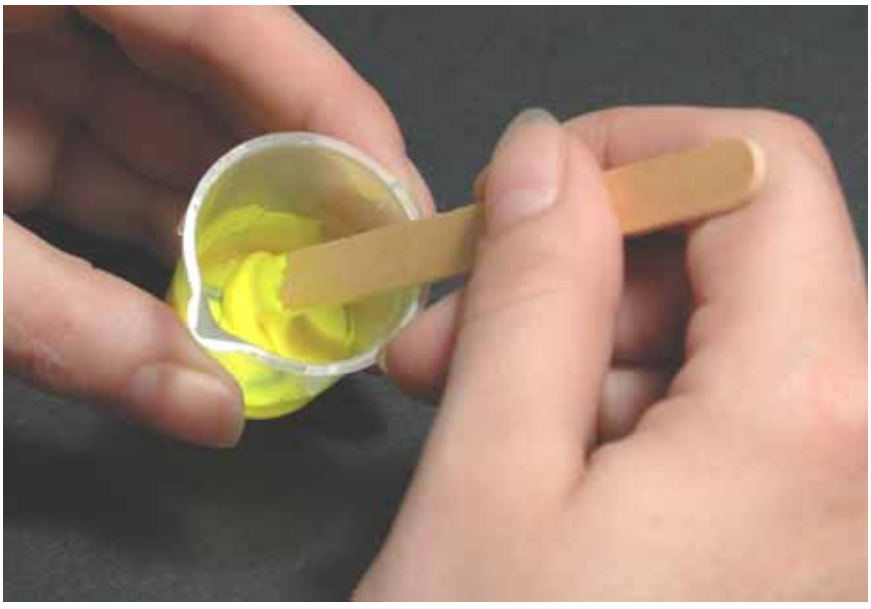
3 USING THE SYSTEM

3.1 Introduction

In this section we discuss in general terms a range of aspects of deployment on the VIE system; in Section 4 we consider projects on a species by species basis. Before using the system, we recommend a review of the available reports on experience elsewhere with the same or similar species. A number of published papers are cited in this manual, and an up-to-date list of publications known to NMT is kept on our website (www.nmt.us). We may also be able to provide advice from our own experience or guide you to someone else who may be able to provide information, so please ask us about any proposed project; contact details are provided at the end of this manual. If no relevant experience exists elsewhere, experimentation to determine suitable tag locations, retention rates and visibility should be carried out before full-scale application is undertaken. While the system works well with the overwhelming majority of fish, amphibians and crustaceans, a few species, notably Tilapia, have shown poor results with elastomer (as they have with most other marking methods!).

3.2 Mixing the material

Full mixing instructions are provided with all VIE kits and refill packs, and are available for download from our website (www.nmt.us).



Mixing yellow VIE material

3.3 Injecting the tag

To inject a tag, the syringe needle is inserted into the marking location, and is slowly withdrawn as the material is injected, so that a long narrow mark is created. It is important that the tag created is fully contained within the target tissue; extrusion of the material from the needle must cease before the needle is withdrawn so that material does not project through the needle wound, as this is likely to cause rapid loss of the tag.

Injecting a VIE tag into the clear tissue behind the eye of a brown trout

In transparent tissue such as the adipose eyelid of salmonids the VIE tag can be injected fairly deeply. However, if the material is being injected into fully or partly pigmented tissue it is important to place it just beneath the skin. Frederick (1997), and Olsen and Vollestad (2001) describe achieving maximum detectability by making sure that the syringe needle was pushed back towards the surface of the skin after the initial penetration.

3.4 Tag location and retention rates.

Clear tissue, such as behind the eye in salmonids, is the ideal site. Similar tissue exists in many other fish families behind and above the eye. Clear tissue is not present in all species, but semi-transparent and translucent tissue may also be suitable for elastomer implants, especially in smaller animals. In trials with turbot, VIE tags were implanted just under the skin in less pigmented areas. Tagging shrimps in the last abdominal segment has been very successful. The base of fins and beneath the jaw are also good sites in many species.

Fin membrane tissue, in spaces between rays, is another potential target. Such a technique offers the potential to develop a variety of unique codes based upon tags in specific spaces.

A detailed description of some successful applications with different species is given in Section 4; this includes a consideration of tag locations and retention rates.

3.5 Coding capacity of single and multiple tags

Although the VIE system was developed as a batch mark, there is significant scope for use of different colors, different tag locations and multiple tags to generate a significant number of batch marks or even individual identification. For example, use of a single tag but using four colors in five different body locations immediately gives 20 unique marks.

Using more than one tag greatly increases the coding capacity, according to the formula:-

Number of unique codes = $(L!/[L-N]!N!)C^N$

Where C = number of colors used, L = number of body locations available, and N is the number of tags used on each fish.

For example, use of three tags in three body locations with four colors would give 64 combinations; three tags, four locations and five colors would give 500. This approach has been used on sea horses to identify up to 500 individuals (Dr Keith Martin Smith, Pers. Comm.), over 1,000 in guppies *Poecilia reticulate* (Bryant and Reznick, 2004) and Jung *et al*. (2000) used three colors and four body locations to create 255 individual codes in their study of salamanders. A computer program for calculating the number of combinations (NMT VIE color code generator) can be downloaded from our website.

One important consideration when using multiple tags is the scope for confusion if one or more tags are lost. For this reason we strongly recommend that all individuals in an experiment receive the same number of tags (eg three); then, if a tag is lost, the fish is correctly recognized as one that has lost a tag rather than being mistaken for a fish that has a two-tag code.

Multiple VIE marks in a 55 mm turbot.

If even a low rate of tag loss is likley to be critical to a project it is worth considering double marking, placing two tags in different locations, with a protocol such that the retention of both or either tag will allow correct batch identification.

3.6 How big is a VIE tag?

In contrast to conventional tags or coded wire tags, the size of a VIE tag is controlled by the user. Very small fish are likely to require a very small tag, while it may be desired to put a larger one in larger fish to aid visibility. Some general guidance is useful for new users.

The biologists who have used VIE on some of some of the smallest fish, 26 mm brown trout (Olsen and Vollestad, 2001) and 8 mm damselfish (Frederick,1997) both stated that the amounts used were "minute", but the former reported that the tags made were 2-3 mm long made with a 29 g needles. The inside diameter of such a needles is about 0.2 mm, suggesting that the tags were of the order of 15,000 per ml. Dewey and Zigler (1996) reported tagging around 1000 fish per ml. Willis and Babcock (1998) used large tags on *Pagrus auratus* of the order of 10 mm x 1 mm x 1 mm (127 per ml). We normally advise assuming 300 to 500 tags per ml of material for planning purposes, where efficient use of mixed material can be achieved.

3.7 Tagging very small fish

Some remarkably small fish have been tagged with VIE, although care and experience is required to do this reliably.

The smallest fish that we have record of being tagged are 8 mm long Pomacentrids *Chromis ovalis* and *Dascyllus albisella* (Frederick, 1997). Several other species of reef fish between 9 and 20 mm in length were tagged in the same study. The fish were tagged at one of a number of body locations on their flanks. Injections were done using an insulin syringe as supplied with the manual VIE kits. The most visible marks were those made close to the surface of transparent tissue, but effective tags in pigmented tissue were possible by bringing the needle tip close to the skin from the inside, without breaking the skin. Surgical gloves were worn to reduce abrasion of the very delicate fish. In the field, fish were tagged under water while being held in a hand net; "trauma to the fish marked in this manner appeared to be considerably less than when they were brought to the surface and anaesthetized for marking". Some mortality was observed with the smallest fish, mostly within 2 hours of tagging. It was higher for fish of less than 20 mm than for larger fish. However, mortality fell steadily during the project even though the average size of fish being tagged was falling; this was ascribed to the operator gaining experience. "In fact, after accounting for this learning curve, there was no significant difference between initial mortality of individuals marked and that of the control group". Tag retention was virtually 100% for most species over periods varying from 24 days to 76 days.

The smallest salmonids reported tagged are brown trout (*Salmo trutta*) down to 26 mm (Olsen and Vollestad 2001). Again, the scientists involved stated how experience improved the tagging performance. An insulin syringe was used, with a 29 g needle. A tag 1-3 mm long was injected alongside the anal fin, as close to the skin as possible. No

mortality or mark loss occurred in fish held for 77 days in the laboratory. At the end of the experiment all tags were detectable, but two out of 50 required blue light to enhance visibility. Growth was unaffected. The technique was then used successfully on a project in small streams.

A VIE tag being injected into a very small cyprinid (nase).

3.8 How quickly can fish be tagged with manual kits?

The rate at which fish can be tagged will depend upon a number of factors including species and size of fish, tag location, facilities available, and the experience of the tagger. Further, we do not recommend working to any particular target rate as this might encourage careless working. Nevertheless, some idea of tagging rates that have been achieved is useful for project planning.

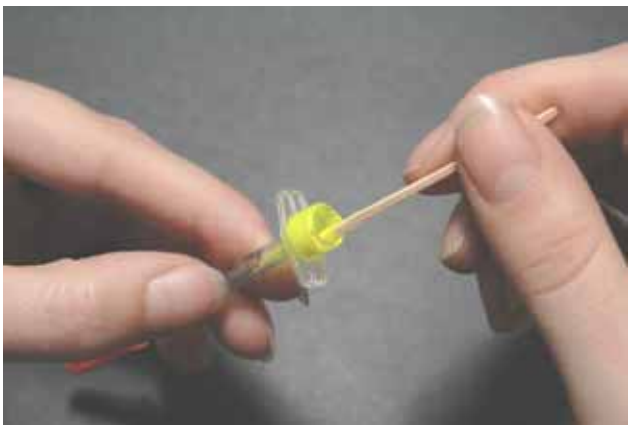
Where fish size and species are not limiting it appears that a rate of about 250-300 tags per hour is a good rate; actual examples from the literature include Bailey *et al* (1998) (300-400 per hour for juvenile coho salmon); Astorga *et al* (2005) (230 per hour for *Sparus* of 7-18 g); and Dewey and Zigler (1996) (288 per hour for *Lepomis* of 33-133 mm).

Using more than one mark slows the operation down somewhat; Brennan et al (2005) reported handling rates for snook of 250-400 per hour for a single tag, and 200-300 per hour for two tags. Similarly, tagging very small fish takes much longer; Olsen and Vollestad (2001) were only able to process 40 mm trout fry at a rate of about 60 per hour.

3.9 Marking small numbers of fish

In some projects, marking small numbers of fish over an extended time period may be required – for example in a field study where just a few fish per site are likely to be captured and marked. As mixed material has only a limited useful life there is potential for wastage. We suggest three possible approaches to addressing this issue.

First, mix the smallest volumes that can be achieved. This is generally limited by the ability to measure the volume of the hardener which is mixed with ten times the volume of coloured component. Wastage can be minimised by placing the two components directly into the barrel of the injection syringe and mixing them there with a toothpick. Instructions and hints on doing this are included in the mixing instructions which come with each kit, and which can be found on our website. We have found that as little as 0.1 ml can be mixed, with care and practice. Allowing for wastage and dead space in the syringe needle, this could allow creation of the order of 20 to 50 tags, depending on their size.



Mixing a minimal volume of VIE material inside the injection syringe

Second, the life of mixed material can be extended for many hours or even a few days by placing it in an ice chest or freezer; Goldsmith *et al* (2003) were able to store mixed material on ice for at least 48 hours. If possible mixed material to be stored in this way should not be loaded into the injection syringe until shortly before it is to be used as, with prolonged contact, the rubber part of the plunger in those syringes may react with the material and prevent curing. As mixed material stored at minus 20^{o}C remains liquid and can be handled and manipulated with syringes, storage in the mixing cup or transfer syringe are viable options.

Third, the problem of wastage may be minimized by arranging for any coding required to be achieved using one color at a time. For example, if several field sites are involved in a single day it may be feasible to code them by different body locations of a single color, using another color for the same body locations on other days.

3.10 Fluorescing VIE tags

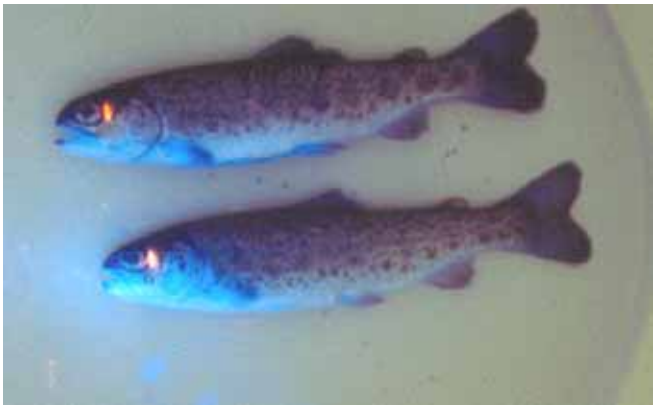
Six of the VIE colors (red, orange, green, yellow, pink and blue) are fluorescent under short-wavelength (far blue and UV) light and visibility can be enhanced by the use of suitable illumination. Black, white, purple and brown do not fluoresce. We usually recommend that all options with the fluorescent colors be exhausted before using the non-fluorescent colors. The non-fluorescent colors are most useful where a high coding requirement demands the use of the maximum number of colors, or there is some other specific advantage.

NMT supplies an illumination system based upon a custom-made deep purple flashlight, which we term the VI Light (it is equally effective for use with VI Alpha tags). The peak of the radiated spectrum is at the very limit of human visual detection (around 405 nm) so that there is only very limited illumination with visible wavelengths, so that the

fluorescing tag stands out. The VI Light includes a voltage regulator that maintains optimal performance until the batteries start to weaken; the light then flashes, warning that the batteries require replacement. While flashing in this mode, the light intensity during the "on" phase is still adequate for effective flouresence.

The VI light system operates best where ambient light is reduced; even simple shade from direct sunlight will help considerably. For the most difficult marks to detect (for example those overlain with pigmented tissue in fish which have grown substantially since marking) operation in a darkened room will maximize detection.

Although the wavelengths and thus the perceived colors of the fluoresced light from the different colors of VIE are different from each other, they are somewhat different from the colors as perceived in ambient light. In particular, green and yellow appear rather similar, as do the pink and red to a lesser extent (see picture in Section 2.1 of this manual). When using fluorescence for mark detection we recommend the use of the VIE Color Standard to check color discrimination, and where possible avoidance of the simultaneous use of the colors that can appear similar.



VIE tags in small rainbow trout being fluoresced

A number of users have deployed UV light sources to fluoresce VIE marks. This works well but we believe that the VI Light provides better performance than commercially available UV light sources.

3.11 Working underwater

The simple equipment required for tagging fish with VIE and for identifying and observing tagged fish means that the system is well suited for underwater use by divers or by observation from above the surface. Frederick (1997) describes tagging small reef fish underwater (see section 3.7), and observing tagged fish on reefs at depths of up to 10 m. Tags were clearly visible from a distance of 1 m, in clear water (visibility 15 m), without the need for additional illumination. However, when visibility was reduced due to low light, and at night, use of a UV light source enhanced detection and discrimination of tag colors. Willis and Babcock (1998) made underwater observations on VIE tagged snapper on a reef. Visibility was gernerally good, with some marks being detectable from a distance of up to 10 m. They experienced some problems differentiating between colours using blue light, but these issues would probably have been resolved if the current deep-purple illumination system had been available at that time. Bonneau *et al.* (1995) used

UV light to allow night observations on small tagged bull trout in streams by divers or by observers on the bank.

3.12 The approach to tag detection.

A critical factor in the design of tagging experiments is the manner of recovery and identification of tagged fish. Workers often state that returns from fishermen are critical to the conduct of the project, and that tags must therefore be large and colorful. However, depending upon such returns introduces an unquantifiable and potentially serious bias to the experiment. First, large and conspicuous external tags are likely to have an effect on the survival, growth and behavior of the fish itself, as discussed above. Second, fishermen may not notice even large tags, they may forget or not get round to making a report of their recapture, or they may choose not to report such captures out of apathy, or a perception that their interests will not be advanced by doing so. In one study anglers catches were secretly tagged after capture. In spite of rewards being offered for return of tags, only 29% of the tags were reported (Green *et al*. 1983). Even if all recaptures are reported, it is difficult to establish the true size of the "sample" of which the tagged recaptures formed a part.

A more robust approach from the statistical viewpoint is for the scientist to scan samples of fish catches for tagged individuals. Such sampling can be planned and appropriately stratified to address specific questions and to obtain reliable and unbiased answers. Although this approach involves an additional phase in the project it can be highly cost-effective; a reasonable volume of robust data may be much more valuable than a large volume of possibly biased data of doubtful validity. The VIE system is particularly suited to this latter approach, although they may be noted and reported by anglers. If dependence upon angler returns is essential for the project this can be greatly enhanced by training a team of interested fishermen to look for tags and also to maintain a log book of all fish caught (so that the sample size is recorded).

A useful discussion of these and other aspects of fish tagging programs is provided by Bergman *et al.*(1992).

4 SOME SUCCESSFUL APPLICATIONS WITH DIFFERENT SPECIES

4.1 Introduction

Successful applications of the VIE system are too numerous to describe in full detail. Instead, applications with some important groups of fish, plus amphibians and crustaceans are discussed in some detail, together with an extensive list of other animal families that have been tagged with VIE. A regularly updated list of publications by family and species is available on our website (www.nmt.us), together with abstracts of many of the papers cited. We also provide links to other websites which feature successful tagging applications, and in some cases, detailed tagging instructions.

4.2 Salmonids

There have been many published papers reporting on the use of VIE in salmonids. This review just deals with a selection of them, chosen to represent a range of species and situations.
Bonneau *et al* (1995) used the system on cutthroat trout (*Oncorhynchus clarki*) and bull trout (*O. confluentus*) for behavioral investigations. Counts of marked fish were undertaken both day and night in different stream reaches using a snorkel diver. The night-time observations involved the use of an underwater light. The following body locations were used as batch marks; top of the head, post ocular tissue, adipose fin, dorsal fin, pectoral fin and caudal fin. A group of 85 fish were retained in captivity to evaluate mark retention; after 2 months retention was 100%, and one fish had lost its mark after 4 months.

Adams *et al*. (2000) undertook a similar study involving brook trout (*Salvelinus fontinalis*) using snorkeling to observe marked fish. They were able to mark fish as small as 50 mm in the adipose eyelid and lower mandible, and from 75 mm upwards between the rays of the dorsal and caudal fins. Overnight losses of marks from samples of fish retained after marking were 2-13% from the adipose eyelid, and 0-27 % from the fins. These relatively high loss rates may have been partly due to the small size of the fish involved.

Walsh and Winkelman (2004) used VIE in hatchery-reared rainbow trout (*Oncorhynchus mykiss*) stocked into streams. The fish averaged about 250 mm in length at the time of marking and 96% of fish had a mark detectable in ambient light after six months.

Marking of very small brown trout (*Salmo trutta*) by Olsen and Vollestad has already been described in Section 3.7.

VIE tags in juveile Chinook salmon

There have been a number of long-term studies marking juvenile migratory salmonids which are then sampled as adults. One unpublished study was undertaken with juvenile Chinook salmon (*Oncorhynchus tshawytscha*) by the Washington Department of Fisheries. Batches of fish were double marked with coded wire tags and red elastomer which was applied using a compressed air injector. The elastomer mark was placed in the post-ocular adipose eyelid. The first batch were marked at a length of about 80-100 mm in late 1992 a few weeks before release to the wild. On release, a sample was checked for elastomer marks, which indicated 92.1% retention. Of 124 cwt fish returning to the hatchery in 1994 (as 5-6 kg fish), 107 (86.3%) had observable elastomer marks; no blue or UV light was used to enhance visibility. A year later, 126 out of 138 (91.3%) returning

cwt fish were found to have an elastomer mark. In this case a blue light was used. A second group was released in 1994, again as 80-100 mm juveniles. On release, elastomer retention was estimated at 94.6%. Of 1752 cwt fish returning in 1995 (as fish of about 2 kg), 1632 (93.2%) had elastomer marks. These results indicate high retention and mark detectability between juvenile and adult salmon, and low rates of loss beyond a few weeks after marking.

Hatchery pre-smolts of coho salmon (*Oncorhynchus kisutch*) were marked and released by Bailey *et al* (1998). About 10,000 fish averaging 108 mm in length were VIE marked in the adipose eyelid; they were also tagged with a coded wire tag (CWT) and adipose clipped to facilitate recovery. Two groups of 100 fish were retained for 24 hours, indicating VIE mark loss rates of about 5%. Returning adults were sampled in the stocked river, and heads from ocean catches were obtained from the CWT sampling program. Most VIE marks were visible in ambient light but detection was improved by the use of UV. From the double marking it was calculated that about 73% of the fish that had received a VIE mark had a detectable mark on return. The use of a control group of fish (CWT and Adipose clip only) showed that VIE marking had no impact on the survival, growth or return behavior of the fish. The authors suggested that the relatively high loss rates of marks may have been affected by operator inexperience and a failure of the material to set properly; small droplets of uncured VIE were noted when the fish were released some months after marking. The formulation of the material has been improved so that curing problems are now very unusual.

FitzGerald *et al* (2004) marked smolts of Atlantic salmon (*Salmo salar*) and then reared them to maturity in net pens. This allowed regular observations on the level of detectable marks as the fish grew. About 9,000 smolts (mean length 213 mm, weight 99.7g) were marked in the adipose eyelid, lower jaw or both. The marks were 3-5 mm in length. Marks in the adipose eyelid were detectable in ambient light in more than 92% of fish after 17 months (mean length 547 mm, weight 1.7 kg), and those in the lower jaw at more than 92% at 16 months. From then onwards the level of marks detectable in ambient light fell away to 52.2% for adipose eyelid marks at 28 months, and 14.4% for those in the jaw at 28 months. Better detection was possible with UV illumination (87.8% for adipose eyelid, 72.2% for jaw) suggesting that the deterioration was due to the marks being obscured by growth and pigmented tissue rather than loss of marks.

In conclusion, VIE marking of salmonids has been very successful with good retention and mark detectability over considerable periods and through many-fold increases in weight. Marks can become more difficult to detect due to growth and development of pigmented tissue but use of appropriate lighting to fluoresce the mark helps considerably. In no case has a detectable impact upon survival, growth, or behavior been reported

4.3 Cyprinids

Haines and Modde (1996) used Elastomer to mark small Colorado squawfish (*Ptychocheilus lucius*). Fish averaged 49.8 mm at time of marking. VIE was "injected sub-cutaneously on the dorsal surface left of the dorsal fin". Mortality was less than 1%, and retention was 85% after 142 days.

VIE tags in small barbel

Clough(1998) undertook retention trials on dace (*Leuciscus leuciscus*) prior to deploying the system in the field. Thirty nine fish of 154-169 mm were marked with an anal fin clip and two elastomer marks, one in the pre-ocular area and one between the first and second dorsal fin rays. The detectability rates (= retention rates?) of the two elastomer marks after various times are shown in Table 1. Both locations gave good results but the pre-ocular location would appear to be the best.

		Date (days elapsed since marking)			
Location	n	Marking day	19 days	42 days	292 days
Dorsal fin	39	100	100	100	83
Pre-ocular	39	100	100	100	93

Morgan and Farooqi (1996) used VIE to mark 79-232 mm barbel (*Barbus barbus*). Four marking sites used:- scalp, post-orbital, base of anal fin and base of caudal fin. Retention rates after 57 days 82.6%, 44.8%, 82.6% and 91.3% respectively. There was no impact on growth rate.

4.4 Percidae

Goldsmith *et al*. (2003) used a combination of colors and body locations to individually identify small perch *Perca fluviatilis* (mean length 88 mm, mean weight 5 g). In a tank trial, 25 fish were marked with three VIE marks each, along a horizontal line between the lateral line and the base of the dorsal fin; using four colors this allowed a coding capacity of 64, though not all combinations were used. Retention was 100% after 125 days, and there was no effect on growth or survival.

Roberts and Angermeir (2004) marked 40 mm or larger Roanoake darter (*Percina roanoka*) and riverweed darter (*Etheostoma podostemone*) in a laboratory study. Mark locations used were mid-ventral, lower caudal peduncle, upper caudal, peduncle and mid-dorsal. There was no impact on survival, and retention rates after 240 days were 90 for *Percina* and 79% for *Etheostoma*.

Thompson *et al*.(2005) compared VIE with fin clipping as marking methods for evaluation of stocking with walleye (*Sander vitreum*). After tank trials they selected the ventral surface of the lower jaw as the mark site, using a 5mm long mark. Mark detection at the time of release, 14 days after marking, was 97%. Recaptures were made over the five years following release. Overall, VIE detection rate was calculated at 82.5%, and no effects on growth rate were observed.

4.5 Amphibians

Visible Implant Elatomer (VIE) is the most widely used alternative to toe clipping for identifying amphibians. It also allows the marking of tadpoles with the mark generally being retained through metamorphosis.

Anholt and Negovetic (1998) tagged 1000 tadpoles of *Rana lessonae* and *R. esculenta*. The tadpoles were anaesthetized and tagged under a stereo microscope with 6.5 x magnification. Placing a single tag took about 10 seconds per individual, and two sub-cutaneous tag locations were used; above the musculature of the tail and on the back. The smallest individuals marked were 8 mm snout to vent length. Overall mark retention was 85% after 8 days, but the authors suggest that losses would have been less if only the tag location on the back had been used. Survival was close to 100% after five weeks, during which time some of the tadpoles had metamorphosed. Although the tags were obscured by pigment in the metamorphosed individuals they were retained and could be recovered by dissection. The authors concluded that the consistency and biocompatibility of the VIE tags allows tagging of small animals, including larvae, that could not be tagged using other methods.

Nauwelaerts *et al*. (2000) tagged 40 adult *Rana esculenta* in the transparent tissue between the toes. Retention was 100% over eight months.

Three tags per individual were applied to 20 salamanders (*Plethodon vehiculum*, 36-60 mm snout to vent length) and 12 tree frogs (*Hyla regilla*, 16-34 mm SVL) by Davis and Osaka (1999). Tag locations were all on the ventral surface; anterior to the front leg, posterior to the hind leg at the anterior end of the vent, and at the posterior end of the vent in the salamanders; and anterior end of the thigh, posterior end of thigh, and mid-calf in the tree frogs. No tagging-related mortality was noted after 10-11 months. Retention was high; 10.5% of the salamanders and 22.2% of the tree frogs had lost one of their marks at the end of the experiment, representing 96.5% and 92.6% retention overall for the two species. Subsequent field trials involved using three tags per individual on 115

salamanders. Forty two were recaptured up to five times, and 9.5% had lost one mark; this represents 96.8% retention overall.

Binkley *et al*.(undated) reported on a project undertaken by Karl Mallory which involved tagging 421 Pacific giant salamanders (*Dicamptodon tenebrosus*) with VIE in the wild. A total of 55 recaptures were recorded in the following year, and 63 after two years, indicating high retention and detectability. Tadpoles were also tagged; 127 out of a total of 471 individuals were recaptured at least once.

In a novel approach to monitoring the development of salamander egg masses, Regester and Woosley (2005) used VIE to identify and track the egg masses.

Other amphibian species which have been successfully tagged with VIE include *Ambystoma maculatum, Anolis sagrei, Ascaphus truei, Plethodon cinereus , Rana sylvatica, Xenopus tropicalis* and many others.

Many researchers do not anesthetize amphibians for tagging , while others prefer the ease of handling that anesthetic provides.

This frog, tagged as a tadpole, retained it's VIE marks through metamorphosis. Photo courtesy of S. Hopkins.

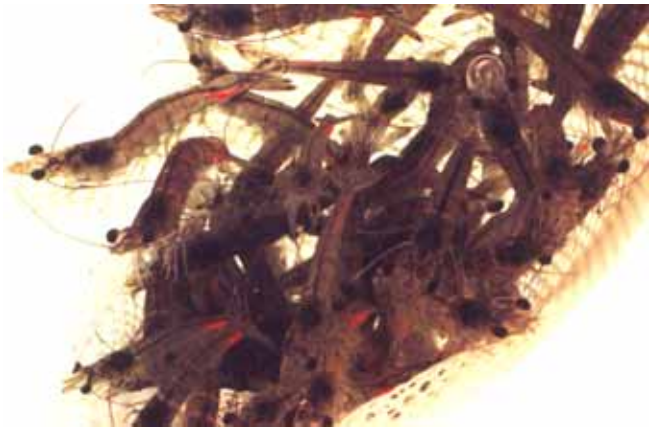
A technique for non-anaesthetized amphibians has been developed in which the animal is placed in a plastic bag with some water and is tagged through the bag.

Some amphibians lack septa between the skin and underlying tissue.VIE tags injected in these animals can therefore migrate from the original tagging location, making it impossible to use those tagging locations to create individual codes. In such cases where individual identification is needed, we recommend the use of VI Alpha tags.

4.6 Crustaceans

The first publication on the use of VIE in crustaceans was Godin *et al* (1996) who marked juvenile (mean weight 1.63 g) and adult (mean weight 38.22 g) shrimps (*Penaeus vannamei*). The material was injected into the musculature of the sixth abdominal segment. After 10 –14 weeks, mark retention was 99.9% in juveniles and 100% in adults, though UV light was required to identify marks in about 9% of the juveniles. The juveniles had increased in weight to 15-20 g, and had molted 17-23 times by the end of the experiment. All marks were readily identified in shrimps tagged as adults without the use of UV; the adults had molted 5-7 times during the experiment.

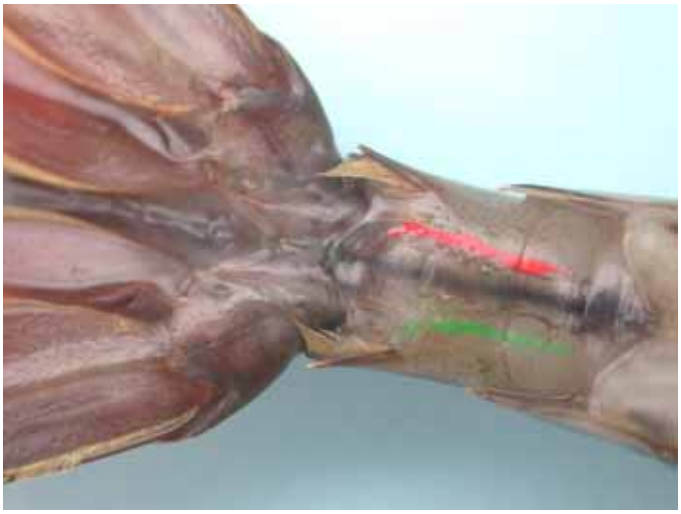
Uglem *et al*. (1996) used VIE placed beneath the epidermal layer in the abdomen of juvenile lobsters (*Homarus gammarus*). After three molts retention was 100%, and overall survival 92%.



Jerry *et al.* (2001) used VIE in the freshwater crayfish or yabby (*Cherax destructor*). They used three sites in animals averaging 0.9 g in weight; after ten weeks retention was 94% in the coxa of the last pair of walking legs, 92% in the 3rd schlerite of the abdomen, and 82% in the uropod. The animals had averaged three molts during the experiment.

VIE tags in shrimp

No effect on growth and survival over six months in VIE marked spiny lobsters (*Jasus edwardsii*) was noted by Wood and James (2003). The marks were injected into the muscle block of the second abdominal segment of juveniles with a mean weight of 9.6 g. Retention was 100% through the mean of 1.78 molts per animal, but marks injected transversely tended to break up somewhat; those placed longitudinally did not. The authors suggest that breaking up was due to the 3mm long marks lay across the muscle fibers, and recommended aligning the long axis of the mark with the fibers.



Double VIE tag in *Penaeus vannamei*

Davis *et al*.(2004) compared the performance of coded wire tags (CWT) and VIE in small blue crabs (*Callinectes sapidus*), and concluded that each had advantages and limitations which depended upon animal size and the duration of the project. The crabs were 6-25 mm carapace width at the time of marking, and the VIE was injected into the upper (basal) segment of the swimming leg (5th periopod). Mark loss was 9.2% over 8 days, mainly due to the shedding of the marked limb. In the longer term, there was also some loss due to the mark migrating into the carapace. This was avoided in slightly larger crabs (30 mm CW) by placing the material into the distal segment of the leg. There was no impact of either marking method on growth.

Overall, VIE appears to be a very successful marking system for crustaceans, with very high retention rates through multiple molts.

4.7 Other fish and animal groups

Representatives of all the following families have been successfully tagged with VIE; details of published papers and other information will be found on our website, along with links to other relevant websites.

Fish

Acanthuridae - surgeonfishes
Adrianichthyidae – ricefishes
Anarhichadidae – wolffishes
Apogonidae – cardinalfishes
Carangidae – jacks
Centropomidae – snooks
Centrarchidae – sunfishes
Chaetodontidae – butterflyfishes
Chanidae – milkfishes
Clupeidae – herrings
Cottidae – sculpins
Cyprinidae - carps and minnows
Cyprinodontidae - pupfishes
Eleotridae - sleepers
Engraulidae – anchovies
Gadidae – cod
Galaxiidae - galaxiids
Girellidae – nibblers
Gobiidae - gobies
Ictaluridae - North American catfishes
Kuhliidae – flagtails
Labridae – wrasses
Lutjanidae – snappers
Moronidae – temperate basses
Mugilidae – mullets
Percichthyidae – temperate basses
Percidae - perches
Petromyzontidae – lampreys
Poecilidae – livebearers
Polynemidae – threadfins
Pomacentridae – damselfishes
Salmonidae – salmon, trout, char
Scophthalmidae – turbots
Scorpaenidae – scorpionfishes and rockfishes
Serranidae - sea basses
Sparidae – sea breams and porgies
Syngnathidae – sea horses and pipefishes
Terapontidae – grunters or tigerperches

Reptiles
Gekkonidae - geckos
Polychridae
Sincidae

Amphibians
Ambystomatidae
Ascaphidae
Caeciliidae – caecelians
Hylidae – tree frogs
Pelobatidae – spadefoot toads
Plethodontidae – terrestrials salamanders
Ranidae – true frogs
Salamandridae

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