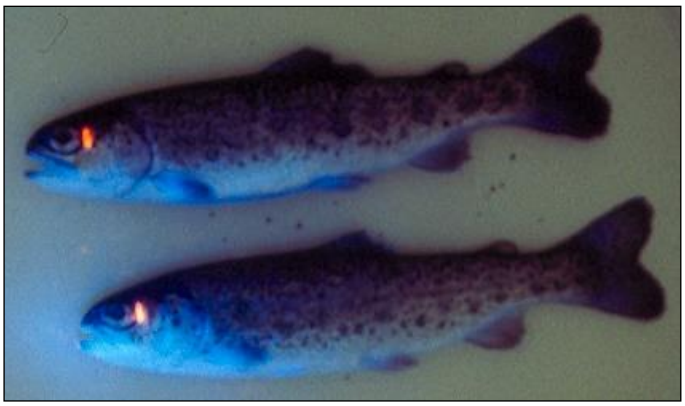
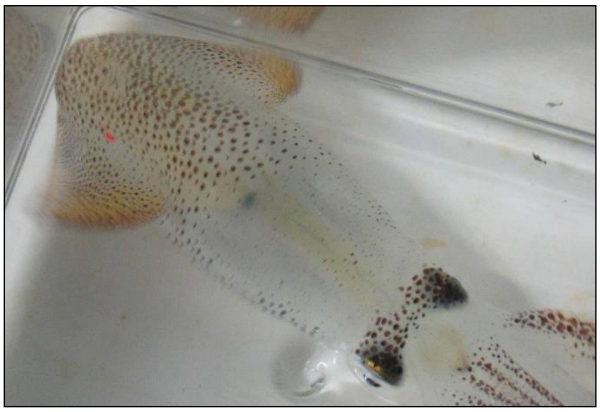
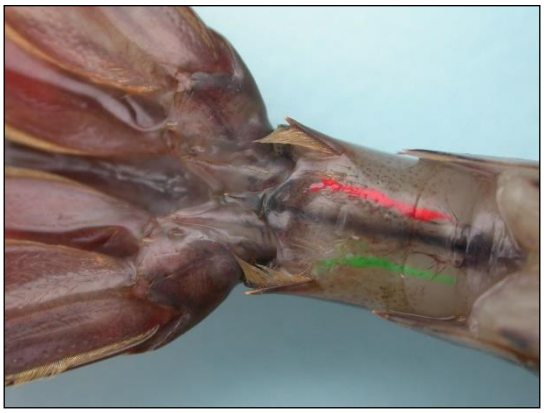
Northwest Marine Technology, Inc.

Visible Implant Elastomer Tag Project Manual

Guidelines on planning and conducting projects using VIE

Contents

1 INTRODUCTION

This document provides information on the uses and deployment of Northwest Marine Technology's (NMT), Inc. 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.

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

1.1 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 batch 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. The curing rate of the VIE tag is temperature dependent. At warm temperatures it can cure within hours and at very cold temperatures it may take days. The VIE cures into a pliable solid. The VIE tag holds the pigment in a well-defined tag, without damaging surrounding tissue. Using different tag locations, and perhaps two or more tags 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. In contrast, conventional external tags, attached via

penetration of the skin, cause a wound that is very slow to heal or may never heal.

1.2 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
- Fast to apply
- May be applied to very small fish and other animals
- Minimal impact on survival, growth and behavior
- Low capital and material costs make it viable for small-scale projects
- May be used in large scale projects quickly tagging many animals using the Air Driven Elastomer Injection System
- Tags detected visually in ambient light
- Fluorescing the tags with the VI Light significantly enhances detection
- Tags may be seen in the dark or underwater by fluorescing with the VI Light
- 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
- Tags may become difficult to detect in ambient light if growth is considerable and pigmented tissue is laid down over the tag
- 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°C this is of the order of 40 minutes; at 0°C it is many hours.

The material is biocompatible and carries no known human health hazards. A Material Data Safety Sheet is in Appendix 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.

Figure 1: Samples of the ten VIE colors under ambient light (left) and illuminated by the VI light (right). Note that only the six fluorescent colors show up with the VI light, and that the colors, especially yellow, appear differently.

2.2 The VIE Color Standard

Figure 2: VIE Color Standard

The color standard is a small transparent card with a sample of all ten colors of VIE that are available. It is supplied with all kits and extras are available free of charge. 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 tags 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.

2.3 Mixing supplies

Figure 3: Mixing and injection supplies for Manual VIE Kits include mixing cups, stirring sticks, transfer syringes, and injection needles.

VIE is supplied with enough mixing supplies for the material involved under most circumstances. Mixing supplies include green transfer syringes (without needles) for transferring the VIE to mixing cups, wooden stirring sticks, and plastic cups. All mixing supplies are intended for single use.

2.4 Injection syringes

The mixed VIE is loaded into a BD 0.3 cc insulin syringes with a 29 g needle for injecting into the animal. If you have a Trial Pack, the VIE Tag is injected using the 0.3 cc syringe. All other VIE Kits (except the 6 ml Refill) include a Manual Elastomer Injector for holding the 0.3 cc syringe for injecting. All VIE kits contain enough syringes for all the material supplied but if additional ones are required NMT can supply them.

A syringe with a removable needle is used with the Air Driven Elastomer Injection System (ADEIS). All orders for use with an ADEIS include enough syringes for the quantity of material supplied; additional supplies are available from NMT.

All syringes are single use and should be carefully handled and disposed.

2.5 Manual Elastomer Injector

Figure 4: Manual Elastomer Injector, ready for tagging.

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. 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.

2.6 Air Driven Elastomer Injection System

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

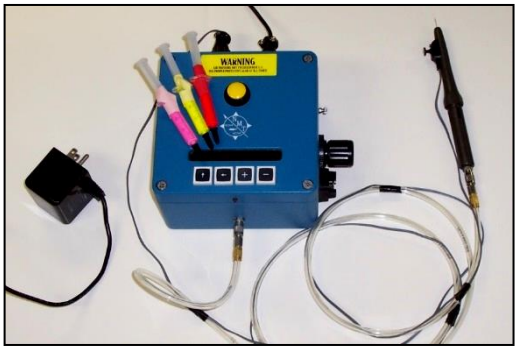


Figure 5: The ADEIS includes a blue control box and handpiece.

The ADEIS 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. If variable-sized tags are required, the machine can be set to maintain the supply of compressed air to the syringe for 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.

Compressed air is supplied to the machine from a suitable compressor. NMT does not supply compressors. 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 liters) per minute. It is recommended that it has an air tank of at least 5 liters to avoid constant cycling.

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, a token, and appropriate supplies of materials, syringes and mixing supplies for the number of tags purchased.

For more details about the ADEIS please refer to Air Driven Elastomer Injection System User's Manual available on our website www.nmt.us or contact NMT.

2.7 The VI Light

The VI Light is used to fluoresce the VIE and VI Alpha Tags. It has a nearly invisible, regulated, deep-violet beam. Deep violet (405 nm) is the optimum wavelength for fluorescing our tags. This causes the fluorescent VI colors (red, orange, pink, yellow, green and blue) to fluoresce, considerably increasing detection and readability.

Figure 6: The VI Light.

The VI Light is different from an ordinary flashlight. To hold detection efficiency constant, the VI Light maintains constant brightness and color right up until its battery fails completely. It provides a beam of uniform intensity, then begins flashing when the batteries near exhaustion.

The VI Light is waterproof to a depth of 150 m so is suitable for underwater studies.

2.8 Manual Elastomer Injection Kits

The VIE in all Manual Elastomer Injection Kits (except the Trial Pack) is packaged in 3 ml syringes.

The 60 ml Kit includes: 60 ml elastomer with curing agent - up to ten colors, mixing supplies, 200 injection syringes, VI Light, 2 Manual Elastomer Injectors, instructions and field carrying case.

The 24 ml Kit includes: 24 ml elastomer with curing agent - up to eight colors, mixing supplies, 60 injection syringes, VI Light, 1 Manual Elastomer Injector, instructions, and field carrying case.

6 ml Kit includes: 6 ml elastomer with curing agent - up to two colors, mixing supplies, 20 injection syringes, VI Light, 1 Manual Elastomer Injector, instructions, and field carrying case.

6 ml Refill includes: 6 ml elastomer with curing agent - up to two colors mixing supplies, 20 injection syringes, and instructions.

Trial Pack of Elastomer includes: 1 ml of elastomer with curing agent - one color, mixing supplies, 3 injection syringes, and instructions. This kit is intended to allow mixing of two or three batches of material for tagging small numbers of individuals and for evaluation of the system.

3 USING THE SYSTEM

Before using the system, we recommend a review of the available publications on experience with the same or related species. NMT maintains a list of up to date references. If you have questions, please contact us at biology@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. If no relevant experience exists elsewhere, experimentation to determine suitable tag locations, retention rates and visibility should be carried out before full-scale application. While the system works well with the overwhelming majority of applications, retention rates can be variable.

3.1 Color Selection

Proper color selection is a vital part of good experimental design. Your choice depends on how much contrast you need with the background pigmentation and how many different colors you require. Certain color combinations can be difficult to distinguish. We do not recommend that green and yellow be combined in a study because they are difficult to distinguish when fluoresced or when placed under pigmented tissue. The fluorescent colors are highly visible under ambient light and provide the option of greatly enhanced tag detection when fluoresced with the VI Light.

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.

Please contact biology@nmt.us if you would like assistance.

3.2 Mixing the material

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

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.

In transparent tissue such as the adipose eyelid of salmonids the VIE tag can be injected fairly deeply (Figure 7). 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.

Figure 7: Injecting a VIE tag into the clear tissue behind the eye of a brown trout

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 (Figure 8). 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:

No. 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.

Figure 8: Multiple VIE marks in a 55 mm turbot.

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 www.nmt.us.

One important consideration when using multiple tags is the scope for confusion if one or more tags are lost. For this reason, we recommend that all individuals in an experiment receive the same number of tags; 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 started with fewer tags.

If even a low rate of tag loss is likely 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 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 needles is about 0.2 mm, suggesting that the tags were of the order of 15,000 per ml of VIE. 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 are required to do this reliably.

Figure 9: A VIE tag being injected into a very small cyprinid (nase).

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 several 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 VIE 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.

3.8 How quickly can fish be tagged with manual VIE kits?

The rate at which fish can be tagged will depend upon several factors including species and size of fish, tag location, facilities available, and the experience of the tagger. Tag retention and animal survival is enhanced with practice and careful tag placement. 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 VIE 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/h.

3.9 Marking small numbers of fish

In some projects, marking small numbers of fish over an extended time 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 VIE material has only a limited useful life there is potential for wastage. We suggest three possible approaches to addressing this issue.

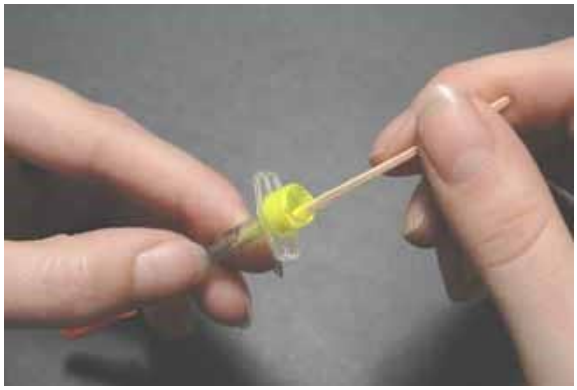


Figure 10: As little as 0.1 ml of VIE can be mixed in the barrel of the syringe.

First, mix the smallest volumes of VIE 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 colored elastomer. Wastage can be minimized 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 at www.nmt.us. 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.

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°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.

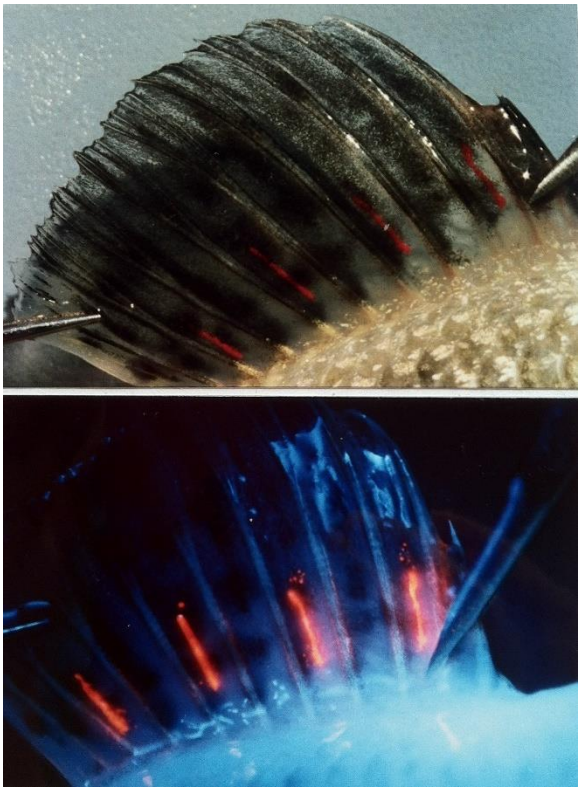


Figure 11: VIE is visible in the fin rays in ambient light (top) but is much easier to see when fluoresced (bottom).

3.10 Fluorescing and Detecting VIE

Six of the VIE colors (red, orange, green, yellow, pink and blue) are fluorescent. Black, white, purple and brown do not fluoresce.

NMT's VI Light has a nearly invisible, regulated, deep-violet beam. Deep violet (405 nm) is the optimum wavelength for fluorescing our tags. Shine the light on the area where the tag is, or is thought to be. Don't try to fluoresce tags in direct sunlight. Rather, you should work in a little shade – even the shade of your body is probably enough for most tags. Very faint tags are best seen when fluoresced in darkness.

To maximize tag identification:

- Proper color selection is a vital part of good experimental design.
- Place tags in clear tissue whenever possible.
- Train your samplers – let them practice with the tag colors they will encounter before they start collecting data.
- Fluoresce poor or obscured tags with the VI Light, working out of direct sunlight.
- Use the VIE Color Standard with the VI Light to correctly identify colors. The Color Standard presents the ten colors on a clear card. The sampler can place the color sample beside a tag for comparison, either under or over the tagged tissue.

3.11 Working underwater

The simple equipment required for tagging fish with VIE and for identifying and observing tagged fish means that VIE tags and VI Light are well suited for underwater use by divers or by observation from above the surface.

Frederick (1997) describes tagging small reef fish underwater and observing tagged fish on reefs at depths of up to 10 m. Tags were clearly visible from 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, fluorescing the tags enhanced detection and discrimination of tag colors. Willis and Babcock (1998) made underwater observations on VIE tagged snapper on a reef. Visibility was generally good, with some marks being detectable from up to 10 m. Bonneau *et al.* (1995) also took advantage of the tags' fluorescence 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 influence 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 around 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

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. NMT maintains a list of up to date references. If you have questions, please contact us at biology@nmt.us

4.1 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 VIE on cutthroat trout (*Oncorhynchus clarki*) and bull trout (*O. confluentus*) for behavioral investigations. Counts of tagged 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 tag retention; after 2 months retention was 100%, and one fish had lost its tag after 4 months.

Figure 12: VIE tags in juvenile Chinook Salmon.

Adams *et al*. (2000) undertook a similar study involving brook trout (*Salvelinus fontinalis*) using snorkeling to observe tagged fish. They were able to tag 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 tags from samples of fish retained after tagging 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 tagging and 96% of fish had a tag detectable in ambient light after six months.

Tagging of very small brown trout (*Salmo trutta*) by Olsen and Vollestad has already been described.

There have been a number of long-term studies tagging 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 (CWT) and red VIE which was applied using the Air Driven Elastomer Injection System. The VIE tag was placed in the post-ocular adipose eyelid. The first batch were tagged 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 VIE tags, 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 VIE tags in ambient light. A year later, 126 out of 138 (91.3%) returning CWT fish were found to have a VIE tag. In this case the tags were fluoresced to enhance detection. A second group was released in 1994, again as 80-100 mm juveniles. On release, VIE tag retention was estimated at 94.6%. Of 1752 CWT fish returning in 1995 (fish of about 2 kg), 1632 (93.2%) had VIE tags. These results indicate high retention and tag detectability between juvenile and adult salmon, and low rates of loss beyond a few weeks after tagging.

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 fluorescence. From the double marking it was calculated that about 73% of the fish that had received a VIE tag had a detectable tag on return. The use of a control group of fish (CWT and Adipose clip only) showed that VIE tagging had no impact on the survival, growth or return behavior of the fish. The authors suggested that the relatively high loss rates of tags 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) tagged smolts of Atlantic salmon (*Salmo salar*) and then reared them to maturity in net pens. This allowed regular observations on the level of detectable tags as the fish grew. About 9,000 smolts (mean length 213 mm, weight 99.7g) were tagged in the adipose eyelid, lower jaw or both. The tags were 3-5 mm in length. Tags 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 tags detectable in ambient light fell away to 52.2% for adipose eyelid tags at 28 months, and 14.4% for those in the jaw at 28 months. Better detection was possible with fluorescence (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 tagging of salmonids has been very successful with good retention and tag detectability over considerable periods and through many-fold increases in weight. Tags can become more difficult to detect due to growth and development of pigmented tissue but use of the VI Light to fluoresce the mark helps considerably.
In no case has a detectable impact upon survival, growth, or behavior been reported.

4.2 Cyprinids

Haines and Modde (1996) used VIE to mark small Colorado pikeminnow (Ptychocheilus lucius). Fish averaged 49.8 mm at time of

marking. VIE was "injected subcutaneously on the dorsal surface left of the dorsal fin". Mortality was less than 1%, and retention was 85% after 142 days.

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 tagged with an anal fin clip and two elastomer tags, one in the pre-ocular area and one between the first and second dorsal fin rays. The detectability rates of the two elastomer tags after various times are shown in the table below. Both locations gave good results but the preocular location would appear to be the best.

		Days since tagging			
Tag Location	n	0	19	42	292
Dorsal fin	39	100	100	100	83
Pre-ocular	39	100	100	100	93

Morgan and Farooqi (1996) used VIE to tag 79-232 mm barbel (*Barbus barbus*). Four tagging 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.

4.3 Percidae

Goldsmith *et al*. (2003) combined 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 tagged with three VIE tags 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) tagged 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 middorsal. 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 tag site, using a 5mm long tag. Tag detection at the time of release, 14 days after tagging, 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.

4.4 Amphibians

Visible Implant Elastomer 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 subcutaneous 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 tag 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.

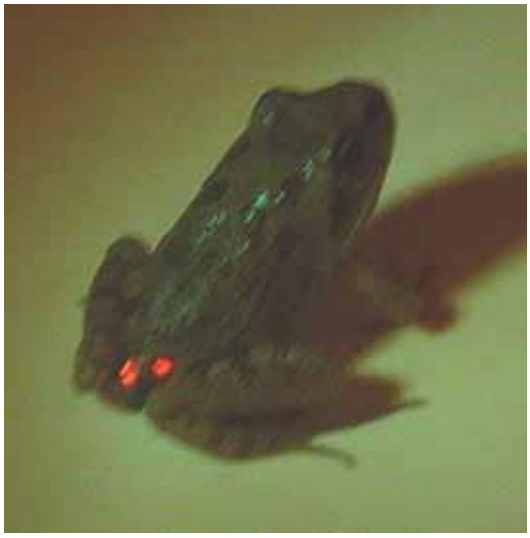


Figure 13: This frog, tagged as a tadpole, retained its VIE marks through metamorphosis. Photo courtesy of S. Hopkins.

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 Ovaska (2001). 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 tags 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 tag; this represents 96.8% retention overall.

Binckley *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. 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.

4.5 Crustaceans

Figure 14: VIE Tags in shrimp

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 VIE was injected into the musculature of the sixth abdominal segment. After 10 –14 weeks, tag retention was 99.9% in juveniles and 100% in adults, though UV light was required to identify tags 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 fluorescent light; 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.

No effect on growth and survival over six months in VIE marked spiny lobsters (*Jasus edwardsii*) was noted by Woods 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 tags lay across

the muscle fibers, and recommended aligning the long axis of the tag with the fibers.

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 tagging, and the VIE was injected into the upper (basal) segment of the swimming leg (5th periopod). Tag 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 tag 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 tagging method on growth.

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

4.6 Other fish and animal groups

Representatives of the following families have been successfully tagged with VIE. Please contact NMT biology@nmt.us for help if you can't find references. NMT maintains an up to date reference list.

Fish

Acipenseridae - sturgeons
Acanthuridae - surgeonfishes
Adrianichthyidae – ricefishes
Anarhichadidae – wolffishes
Anguillidae – freshwater eels
Aplocheilidae
Apogonidae – cardinalfishes
Balitoridae – river loaches
Blenniidae – combtooth blennies
Carangidae – jacks
Catastomidae - suckers
Centropomidae – snooks
Centrarchidae – sunfishes
Chaenopsidae pike-, tube- and flagblennies
Chaetodontidae – butterflyfishes
Chanidae – milkfishes
Characidae
Cichlidae - cichlids
Clupeidae – herrings
Cobitidae - loaches
Cottidae – sculpins
Cyclopteridae - lumpsuckers
Cyprinidae - carps and minnows
Cyprinodontidae - pupfishes
Esocidae - pikes
Eleotridae - sleepers
Engraulidae – anchovies
Fundulidae - topminnows
Gadidae – cod
Galaxiidae - galaxiids
Gasterosteidae - sticklebacks
Girellidae – nibblers
Gobiidae - gobies
Haemulidae – grunts
Hexagrammidae – greenlings
Ictaluridae - North American catfishes
Kuhliidae – flagtails
Labridae – wrasses
Lotidae - lings
Lutjanidae – snappers
Melanotaeniidae – rainbow fishes
Moronidae – temperate basses
Mugilidae – mullets
Nemacheilidae – stone loaches
Nothobranchiidae – African rivulines
Osmeridae – smelts
Osphronemidae
Paralichthyidae – sand flounders
Pempheridae
Percichthyidae – temperate basses
Percidae - perches
Petromyzontidae – lampreys
Platycephalidae
Pleuronectidae – righteye flounders
Poecilidae – livebearers
Polynemidae – threadfins
Pomacentridae – damselfishes
Salmonidae – salmon, trout, char
Sciaenidae – drums and croakers
Scophthalmidae – turbots

Scorpaenidae – scorpionfishes, rockfishes
Serranidae - sea basses and groupers
Siluridae - sheatfishes
Sparidae – sea breams and porgies
Syngnathidae – sea horses and pipefishes
Terapontidae – grunters or tigerperches
Tripterygiidae - triplefins

Reptiles

Agamidae
Chelydridae
Colubridae
Emydidae – pond turtles
Gekkonidae - geckos
Polychrotidae
Sincidae
Trionychidae

Amphibians

Alytidae
Ambystomatidae – mole salamanders
Ascaphidae
Bufonidae
Caeciliidae – caecelians
Cryptobranchidae – giant salamanders
Dicamptodontidae
Dicroglossidae – fork-tongued frogs
Hylidae – tree frogs
Hyperoliidae
Leptodactylidae
Pelobatidae – spadefoot toads
Plethodontidae – terrestrial salamanders
Proteidae
Ranidae – true frogs
Salamandridae
Scaphiopodidae – true salamanders, newts
Scincidae

Crustaceans

Astacidae
Atyidae
Xiphocarididae
Callianassidae – ghost shrimps
Cambaridae – crayfishes
Cancridae
Galatheidae – squat lobsters
Grapsidae – shore, marsh and talon crabs
Homaridae
Nephropidae – clawed lobsters
Palaemonidae
Palinuridae – spiny lobsters
Parastacidae
Penaeidae – penaeid shrimps
Portunidae – swimming crabs

Other

Slugs: Arionidae
Echinoderms: Asterinidae, Stichopodidae
Cephalopods: Octopodidae, Loliginidae, Sepiidae
Annelids: Hormogastridae, Lumbricidae
Elasmobranchs: Scyliorhinidae
Insects: Buthidae; Calliphoridae
Mammals: Soricidae – shrews

5 REFERENCES

Adams, S.B., C.A. Frissell and B.E. Rieman. 2000. Movements of non-native brook trout in relation to stream channel slope. Transactions of the American Fisheries Society 129: 623-638

Anholt, B. R., and Negovetic, S. 1998. Methods for anaesthetizing and marking larval anurans. Herpetological Review 29(3):153-154.

Astorga, N., Afonso, J.M., Zamorano, M.J., Montero, D., Oliva, V., Fernandez, H. and Izquierdo, M.S. 2005 Evaluation of Visible Implant Elastomer tags for tagging juvenile gilthead bream (*Sparus auratus* L.); effects on growth, mortality, handling time and tag loss. Aquaculture Research 36: 733-738

Bailey, L. L. 2004. Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias. Herpetological Review: 35:3841

Bailey, R. E., Irvine, J. R., Dalziel, F. C., and Nelson, T. C. 1998. Evaluations of visible implant fluorescent tags for marking coho salmon smolts. North American Journal of Fisheries Management 18:191-196

Bergman, P. K., F. Haw, H. L. Blankenship, and R. M. Buckley. 1992. Perspectives on design, use, and misuse of fish tags. Fisheries, 11(4):20-25

Binckley, C. A., et al. "Using the Visible Implant Fluorescent Elastomer (VIE) tagging system to mark amphibians.". Retrieved 7/12/2010, from http://www.pwrc.usgs.gov/marking/vie.html

Bonneau, J. L., R. F. Thurow, and D. L. Scarnecchia. 1995. Capture, marking, and enumeration of juvenile bull trout and cutthroat trout in small, low-conductivity streams. North American Journal of Fisheries Management 15: 563-568

Brennan, N.P., Leber, K.M., Blankenship, H.L., Ransier, J.M and DeBruler, R. 2005 An evaluation of coded wire and elastomer tag performance in juvenile common snook under field and laboratory conditions. North American Journal of Fisheries Management 25: 437-445

Bryant, M. J. and D. Reznick (2004). Comparative studies of senescence in natural populations of guppies. American Naturalist 163(1): 55-68.

Clough, S. 1998. Migration and habitat use of dace (*Leuciscus leuciscus*(L.)) in an English chalk stream. PhD thesis, University of St Andrews

Davis, T.M. and Ovaska, K. 2001. Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. Journal of Herpetology 35: 217-225

Davis, J. L. D., A. C. Young-Williams, and A. H. Hines & O. Zmora. 2004 Comparing two types

of internal tags in juvenile blue crabs. Fisheries Research 67:265-274

Dewey, M. R., and S. J. Zigler 1996. An evaluation of fluorescent elastomer for marking bluegill sunfish in experimental studies. The Progressive Fish Culturist 58: 219-220

Farooqi M.A. & C. E. Morgan. 1996. Elastomer visible implant (EVI) tag retention and effect of tagging on the growth and survival of Barbel, *Barbus barbus* (L.). Fisheries Management and Ecology 3:181-183

FitzGerald, J. L., T. F. Sheehan and J. F. Kocik. 2004: Visibility of visual implant elastomer tags in Atlantic salmon reared for two years in marine net-pens. North American Journal of Fisheries Management 24: 222–227

Frederick, J. L. 1997. Post-settlement movement of coral reef fishes and bias in survival estimates. Marine Ecology Press Series 150: 65-74

Godin, D. M., W. H. Carr, G. Hagino, F. Segura, J. N. Sweeney, and L. Blankenship. 1996. Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. Aquaculture 139: 243-248

Goldsmith, R. J., Closs, G. P. and Steen, H. 2003. Evaluation of visible implant elastomer as a method for individual marking of small perch and common bully. Journal of Fish Biology 63 (3), 631-636

Green, A. W., G. C. Matlock, and J. E. Weaver. 1983. A method of directly estimating the tag-reporting rate of anglers. Transcations of the American Fisheries Society. 112: 412-415

Haines, B. G. and T. Modde. 1996. Evaluation of marking techniques to estimate population size and first year survival of Colorado squawfish. North American Journal of Fisheries Management 16: 905-912

Hale, R. S. and J. H. Gray. 1998. Retention and detection of coded wire tags and elastomer tags in trout. North American Journal of Fisheries Management 18: 197-201

Jerry, D. R., T. Stewart, I. W. Purvis, L. R. Piper. 2001. Evaluation of visible implant elastomer and alphanumeric internal tags as a method to identify juveniles of the freshwater crayfish, *Cherax destructor*. Aquaculture 193:149-154

Jung, R. E. S. Droege, J. R. Sauer, and R. B. Landy. 2000. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. Environmental Monitoring and Assessment 63: 65-79

Nauwelaerts, S., J. Coeck, and P. Aerts. 2000. Visible implant elastomers as a method for marking adult anurans. Histological Review 31(3): 154-155

Olsen, E. M., L. A. Vollestad. 2001. An Evaluation of Visible Implant Elastomer for Marking Age-0 Brown Trout. North American Journal of Fisheries Management. 21: 967-970

Regester, K.J.and Woosley, L.B. 2005. Marking salamander egg masses with Visible Fluorescent Elastomer: retention time and effect on embryonic development. The American Midland Naturalist 153: 52-60

Roberts, J. H. and Angermeier, P. L. 2004. A comparison of injectable fluorescent marks in two genera of darters: effects on survival and retention rates. North American Journal of Fisheries Management 24: 1017-1024

Thompson, J. M., Hirethota, P. S. and Eggold, B. T. 2005. A comparison of elastomer marks and fin clips as marking techniques for walleye. North American Journal of fisheries Management, 25: 308-315

Uglem, I., Noess, H., Farestveit, E. and K. E. Jorstad. 1996. Tagging of juvenile lobsters (*Hommarus gammarus* (L.)) with Visible Implant Fluorescent Elastomer tags. Aquacultural Engineering 15: 499-501.

Walsh, M. G. and Winkelman, D. L. 2004. Anchor and Visible Implant Elastomer tag retention by hatchery rainbow trout stocked into an Ozark stream. North American Journal of Fisheries Management 24: 1435-1439

Willis, T. J. and R. C. Babcock. 1998. Retention and *in situ* detectability of visible implant fluorescent elastomer (VIFE) tags in *Pagrus auratus* (Sparidae). New Zealand Journal of Marine and Freshwater Research 32: 247-254

Woods, C. M. C. and P. J. James. 2003. Evaluation of visible implant fluorescent elastomer (VIE) as a tagging technique for spiny lobsters (*Jasus edwardsii*). Marine and Freshwater Research 54: 853-858

Woods, C. M. C and K. M. Martin-Smith. 2004. Visible implant fluorescent elastomer tagging of the big-bellied seahorse, *Hippocampus abdominalis*. Fisheries Research 66: 63-371

6 APPENDIX A

Material Safety Data Sheet	Visible Implant Elastomer Tags, 10:1 Formulation
Revised 2009/08/15	

1. PRODUCT AND COMPANY IDENTIFICATION	
Northwest Marine Technology, Inc. P.O. Box 427 Shaw Island, Washington 98286	Emergency Telephone: (360) 468-3375 Customer Service: (360) 468-3375

Trade Name: Visible Implant Elastomer Tag

Chemical Family: Silicone

Other Product Information: The base (Part A) is not a hazardous material as defined in the OSHA Hazard Communication Standard. The base contains a very small amount (less than 0.1%) of a potentially hazardous compound, formaldehyde. The maximum possible level of formaldehyde that could be released into the environment is far below the level allowed by OSHA. The information below applies to the curing agent (Part B) of the two-part kit. Handle freshly mixed elastomer material as recommended for the curing agent. After curing, the product is not hazardous. Visible Implant Elastomer Tags are available in various colors. All colors are equally non-hazardous.

National Fire Protection Association Profile: Health 0 Flammability 1 Instability/Reactivity 1

2. HAZARDS IDENTIFICATION

POTENTIAL HEALTH EFFECTS

Acute Effects

Eye: Direct contact may cause temporary redness and discomfort.

Skin: No significant irritation expected from a single short-term exposure.

Inhalation: No significant effects expected from a single short-term exposure.

Oral: Low ingestion hazard in normal use.

Prolonged/Repeated Exposure Effects

Skin, inhalation, oral: No known applicable information.

Signs and Symptoms of Overexposure

No known applicable information.

Medical Conditions Aggravated by Exposure

No known applicable information.

3. COMPOSITION/INFORMATION ON INGREDIENTS

CAS Number	Wt %	Component Name
68037-59-2	10.0 - 30.0	Dimethyl, methylhydrogen siloxane

The above component is hazardous as defined in 29 CFR 1910.1200.

4. FIRST AID MEASURES

Eye: Immediately flush with water.

Skin, inhalation, oral: No first aid should be needed.

Notes to physician: Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flash point: > 214 °F / > 101.1 °C (Closed Cup)

Autoignition temperature: Not determined.

Flammability limits in air: Not determined.

Extinguishing media: On large fires use AFFF alcohol compatible foam or water spray (fog). On small fires use AFFF alcohol compatible foam, CO2 or water sprays (fog). Water can be used to cool fire exposed containers. Do not allow extinguishing medium to contact container contents. Most fire extinguishing media will cause hydrogen evolution. When the fire is put out, hydrogen may accumulate in poorly ventilated or confined areas and result in flash fire or explosion if ignited. Foam blankets may also trap hydrogen or flammable vapors, with the possibility of subsurface explosion.

Unsuitable Extinguishing Media: Dry chemical.

Fire Fighting Measures: Self-contained breathing apparatus and protective clothing should be worn in fighting large fires involving chemicals. Use water spray to keep fire exposed containers cool. Determine the need to evacuate or isolate the area according to your local emergency plan.

Unusual Fire Hazards: None.

6. **ACCIDENTAL RELEASE MEASURES**

Use absorbent material to collect and contain for salvage or disposal.

Waste disposal method: All local, state and federal regulations concerning health and pollution should be reviewed to determine approved disposal procedures.

7. HANDLING AND STORAGE

Use with adequate ventilation. Avoid eye contact.

Product evolves minute quantities of flammable hydrogen gas which can accumulate. Adequately ventilate to maintain vapors well below flammability limits and exposure guidelines. Do not repackage. Do not store in glass containers which may shatter due to pressure build up. Clogged container vents may increase pressure build up. Keep container closed and store away from water or moisture.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Component Exposure Limits: There are no components with workplace exposure limits.

Engineering Controls: Local and general ventilation are recommended.

Personal Protective Equipment for Routine Handling and Spills

Eyes: Use proper protection - safety glasses as a minimum.

Skin: Washing at mealtime and end of shift is adequate.

Suitable Gloves: No special protection needed.

Inhalation: No respiratory protection should be needed.

Precautionary Measures: Avoid eye contact. Use reasonable care.

Comments: When heated above 150°C (300°F) in the presence of air, product can form formaldehyde vapors. Formaldehyde is a potential cancer hazard and a known skin and respiratory sensitizer. Vapors irritate eyes, nose, and throat. Safe handling conditions may be maintained by keeping vapor conditions within the OSHA permissible exposure limit for formaldehyde.

9. PHYSICAL AND CHEMICAL PROPERTIES

Odor, appearance, color:	little odor, liquid, some color
Specific gravity (at 77 °F):	0.972
Vapor pressure:	less than 5 mm

Percent volatile by weight (%):	less than 5
Solubility in water (%):	less than 0.1

10. STABILITY AND REACTIVITY

Chemical Stability: Stable.

Hazardous Polymerization: Hazardous polymerization will not occur.

Conditions to Avoid: None.

Materials to Avoid: Oxidizing material can cause a reaction. Water, alcohols, acidic or basic materials, and many metals or metallic compounds, when in contact with product, liberate flammable hydrogen gas, which can form explosive mixtures in air.

Hazardous Decomposition Products: Thermal breakdown of this product during fire or very high heat conditions may evolve the following decomposition products: Carbon oxides and traces of incompletely burned carbon compounds. Silicon dioxide. Formaldehyde. Hydrogen.

11. TOXICOLOGICAL INFORMATION/ ECOLOGICAL INFORMATION

No known applicable information.

12. TRANSPORT INFORMATION

DOT Road Shipment Information (49 CFR 172.101): Not subject to DOT.

Ocean Shipment (IMDG): Not subject to IMDG code.

Air Shipment (IATA): Not subject to IATA regulations.

13. REGULATORY INFORMATION

Contents of this MSDS comply with the OSHA Hazard Communication Standard 29 CFR 1910.1200.

TSCA Status: All chemical substances in this material are included on or exempted from listing on the TSCA Inventory of Chemical Substances.

EPA SARA Title III Chemical Listings

Section 302 Extremely Hazardous Substances (40 CFR 355): None.

Section 304 CERCLA Hazardous Substances (40 CFR 302): None.

Section 311/312 Hazard Class (40 CFR 370): Acute - No; Chronic - No; Fire - No; Pressure - No; Reactive - Yes

Section 313 Toxic Chemicals (40 CFR 372):

None present or none present in regulated quantities.

14. OTHER INFORMATION

These data are offered in good faith as typical values and not as a product specification. No warranty, expressed or implied, is hereby made. The recommended industrial hygiene and safe handling procedures are believed to be generally applicable in the context of the intended use.