

ARTICLE

## Visible Implant Elastomer (VIE) Tags for Marking Small Rainbow Trout

C. A. Leblanc\*

Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA; Institute of Biology, University of Iceland, Sturlugata 7, 101 Reykjavík, Iceland; and Department of Aquaculture and Fish Biology, Hólar University College, Háeyri 1, 550, Saudarkrókur, Iceland

D. L. Noakes

Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis, Oregon 97331, USA; and Oregon Department of Fisheries and Wildlife, Oregon Hatchery Research Center, 2418 East Fall Creek Road, Alsea, Oregon 97324, USA

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### Abstract

Being able to individually follow fish <40 mm in length is a challenge for fish research. In the present study we examined whether the use of visible implant elastomer (VIE) tags is suitable for research on first-feeding rainbow trout *Oncorhynchus mykiss* raised under laboratory conditions. We assessed the effect of tagging and tagging location on the growth and survival of juveniles over a 34-d period. We also documented the retention rate and visibility of the tags under natural light. This study validates the use of VIE tags for 25–40-mm fish under laboratory conditions, using the base of the dorsal fin and upper or lower caudal fin as tagging locations.

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Several types of tags are available to tag fish, e.g., passive integrated transponder (PIT), Carlin, Floy, and coded wire tags (see Jensen et al. 2008 for a review). All of these methods are limited by the “minimum-sized” fish on which they can be used or require the death of the fish for visual identification. Alcian blue injection has been used to tag cohorts of small fish, but it does not allow for sufficient individual identification (Porto et al. 1999). Other individual marking methods, such as fin-clipping, are not an option in juvenile fish since fins regenerate quickly (McNicol and Noakes 1979). The use of visible implant elastomer (VIE; Northwest Marine Technology, Inc.) may be a solution to this problem. Several studies have evaluated the visibility of VIE; the results appear to vary greatly according to species (Reeves and Buckmeier 2009), tagging location (Bonneau et al. 1995), fish size (Close and Jones 2002), and study duration (Bailey et al. 1998). Those results show that there is a need to study (at different life stages of a species) tag retention, visibility (Zeller and Cairns 2010), and the effect of

the VIE tagging procedure on the growth, behavior (Kerwath 2006; Imbert et al. 2007), and survival of tagged fish. In the present study, we tested the use of VIE tags on juvenile rainbow trout *Oncorhynchus mykiss* immediately after the initiation of first feeding and examined the influence of tagging and tagging location on growth, survival, retention rate, and visibility over 1 month under laboratory conditions.

### METHODS

We randomly divided 60 first-feeding, hatchery-reared rainbow trout from the Siletz stock (Oregon Hatchery Research Center, Alsea) into two treatments: VIE-tagged fish and control fish. Each treatment had two replicates of 15 fish each. Fish were raised in four baskets (one basket per replicate) following standard hatchery operating procedures. Baskets were placed in a single tank with flow-through water (temperature,  $14.8 \pm 4.5^\circ\text{C}$  [mean  $\pm$  SE]; photoperiod, 10 h light : 14 h dark).

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\*Corresponding author: camille@mail.holar.is  
Received October 26, 2011; accepted March 30, 2012  
Published online July 20, 2012

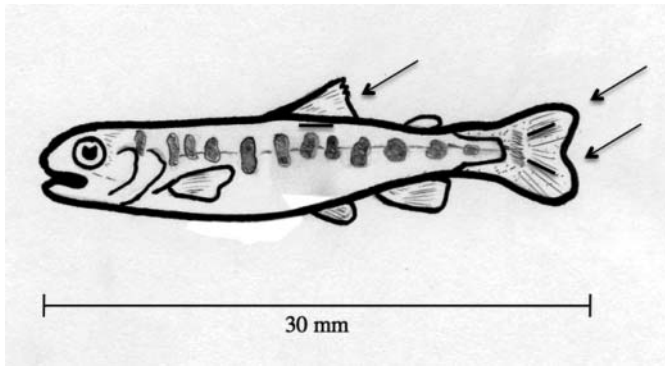


FIGURE 1. Locations of VIE tags implanted in juvenile rainbow trout. Each tag consisted of red or green elastomer, measured 2–3 mm, and was inserted below the skin. The red tags were implanted at the base of the dorsal fin and the green tags between the rays of the caudal fin. This drawing was modified from Pollard et al. (1997).

Prior to tagging, we anesthetized fish using tricaine methanesulfonate (MS-222; 50 mg/L MS-222 buffered to pH 7.0 with 125 mg/L NaHCO<sub>3</sub>). Then fish were placed on a plexiglass board and tagged with an insulin syringe mounted with a 29-gauge needle (Northwest Marine Technology, Inc.). The needle was inserted below the skin and parallel to the dorsal fin. Red elastomer was injected as the needle was withdrawn (following the description in Olsen and Vollestad 2001 and the manufacturer's instructions). We used the same procedure to tag the caudal fin rays of the fish with green elastomer. Extreme care was taken not to perforate the fin ray with the needle. We gently wiped a thumb on the other side of the caudal fin to ensure that no elastomer went through the fin. Each reliable marking corresponded to a 2–3-mm stripe of colored elastomer. Each fish received two tags: one at the base of the dorsal fin and one between the caudal fin rays (either in the upper or the lower caudal fin; Figure 1).

These two tagging locations were selected after preliminary observations. Based on previous work on salmonids (Bonneau et al. 1995; Close and Jones 2002; Josephson et al. 2008), we preliminarily tagged fish with VIE at several locations: postocular adipose eyelid tissue, lower jaw, base of the dorsal, pectoral, and pelvic fins, and caudal fin. We selected two preferred tagging locations, the base of the dorsal fin and the caudal fin (Figure 1), based on the size of the fish, the ease of tagging, and the amount of time required to tag each fish. In the preliminary tagging exercise, we measured (total length and wet weight) and tagged small fish (two tagging locations) in less than 1 min (mean  $\pm$  SE time per fish, 55  $\pm$  7 s).

In the primary study, the control fish were anesthetized, measured, and held out of water for the same amount of time as marked fish but not touched with the needle. This design allows testing for the effect of the overall tagging procedure. Fish were fed equal amounts of commercial pellets (Silver Cup Diet size 0) 4–5 times a day. No mortality occurred at tagging, and the wounds associated with needle insertion were not visible 12 d

TABLE 1. Mean  $\pm$  SE wet weight (mg) and total length (mm) of tagged and untagged rainbow trout juveniles at various times after tagging with VIE tags. The results from Mann–Whitney *U*-tests are also shown. Only two tagged fish and two untagged ones died during the experiment.

Time	Tagged fish	Control fish	<i>U</i>	<i>P</i>	<i>N</i>
<b>Wet weight</b>					
Outset	25.97 $\pm$ 0.53	26.6 $\pm$ 0.53	553.5	0.85	60
12 d	56.71 $\pm$ 1.59	57.00 $\pm$ 1.24	394.5	0.53	59
19 d	85.23 $\pm$ 1.78	82.00 $\pm$ 2.00	425.5	0.72	57
26 d	128.79 $\pm$ 2.85	127.48 $\pm$ 2.49	402	0.67	56
34 d	185.16 $\pm$ 3.51	174.22 $\pm$ 3.00	340.5	0.35	56
<b>Total length</b>					
Outset	32.71 $\pm$ 0.18	33.68 $\pm$ 0.19	608	0.97	60
12 d	37.90 $\pm$ 0.33	37.72 $\pm$ 0.33	373.5	0.40	59
19 d	43.06 $\pm$ 0.28	42.28 $\pm$ 0.36	408	0.62	57
26 d	47.32 $\pm$ 0.4	47.42 $\pm$ 0.34	395.5	0.63	56
34 d	54.10 $\pm$ 0.41	53.17 $\pm$ 0.35	345.5	0.38	56

after tagging. Fish were anesthetized, measured, and screened for tag visibility and loss rate at 12, 19, 26, and 34 d posttagging. Tag visibility was consistently assessed between 0900 and 1300 hours under natural light conditions and always by the same observer (C. Leblanc). Visibility was described as 1 when the tag was a 2–3-mm colored stripe or 0 when the tag was a colored dot or absent. Visibility was assessed out of water at an approximately 30 cm distance above the anesthetized fish.

Because the data were not normally distributed, we used a Friedman test with wet weight or total length as the dependent variable and treatment and time as the independent variables. Mann–Whitney *U*-tests were used to compare the weights and lengths of the fish in the two treatments at each age.

## RESULTS

At the outset of the experiment there were no significant differences in mean total length or wet weight between treatments (Table 1). Tagged fish were 33  $\pm$  2 mm long (mean  $\pm$  SE; range, 27–37 mm) and weighed 26  $\pm$  6 mg (range, 17–42 mg).

Over time, the mean total length and mean wet weight did not differ between tagged and untagged fish (total length:  $n = 288$ , Friedman  $\chi^2 = 1.8$ ,  $df = 1$ ,  $P = 0.18$ ; wet weight:  $n = 288$ , Friedman  $\chi^2 = 0.2$ ,  $df = 1$ ,  $P = 0.65$ ). Additionally, the mean total length and mean wet weight did not differ at any age (Mann–Whitney *U*-tests;  $P > 0.05$  at each age; Table 1). Over the course of the experiment mortality was equal (3.3%) in both treatments. There was no indication that mortality was associated with the marking procedure, since no fish died in the first few hours after tagging. Only one fish lost the dorsal fin tag (3 weeks into the experiment); all other fish retained their tags, such that tag retention was 97%. In all of the fish that retained their tags, tag visibility was 100% at each sampling time. The

visibility of the tags did not decrease with fish age, indicating that surrounding tissue did not cover the tags.

## DISCUSSION

Our experiment demonstrates that VIE tags are a suitable way to follow individual juvenile rainbow trout in the short to medium term under laboratory conditions. Both color marks were visible under natural light when fish were out of the water. Development of pigmentation and tissue growth did not cover the marks at the base of the dorsal fin and the caudal fin rays. We demonstrated that VIE tags did not negatively affect the growth and survival of rainbow trout (25–40 mm) and that visibility and retention rate were excellent. We recommend the use of caudal fin rays as an implant location for 25–40-mm fish (fin ray tags were 100% retained and visible after 34 d). The upper and lower fin rays could easily be marked and distinguished (out of the water), increasing individual tag code possibilities (see Figure 1).

Steingrímsson and Grant (2003) also reported two possible locations at the base of the dorsal fin of young salmonids: one anterior and one posterior on either side, which would increase the number of individual tag codes. They used VIE tags to identify individual fish during a snorkel survey, indicating that these tags are visible under water. However, they tagged each fish in two of eight possible tagging locations, and they mentioned that some fish were tagged a second time because some of the tags were fading. They did not report whether tag fading was associated with specific tag locations. Our work validates the visibility of dorsal and caudal VIE tags under natural light and out of the water but additional work is needed to specifically test whether such tags are visible under water.

Additionally, we recommend that VIE tagging be implemented by a skilled tagger to maximize survival and increase tagging reliability (see also Frederick 1997). From our preliminary tagging exercise, we established that a skilled tagger can insert one VIE tag in a single shallow injection. To achieve the ability to measure and tag a small fish in two locations in less than 1 min, our tagger (C. Leblanc) repeated the tagging technique with 15–20 fish before conducting the primary study. This study is the first to validate the use of such methodology for age-0 rainbow trout under laboratory conditions (see also Olsen and Vollestad 2001 for work on brown trout *Salmo trutta*). We especially recommend the use of VIE tags for individual-level analysis of early life history traits in young salmonids under laboratory conditions. However, more research needs to be conducted, especially on the effects of VIE tags and tag color on the behavior of first-feeding fish.

Mass marking of juvenile fish is important for evaluating releases of hatchery fish for management and research purposes (Elle et al. 2010). Calcein marking is a technique that has been used to externally tag a large number of small fish. That technique has been successful for the short-term identification of Chinook salmon *Oncorhynchus tshawytscha* and rainbow trout

juveniles raised indoors (Elle et al. 2010; Hill and Quesada 2010). However, in those studies the external calcein mark disappeared very rapidly in tanks exposed to direct sunlight. Visible implant elastomer tags are not sensitive to direct sunlight, and they have been successfully used for long-term (6–42-month) capture–recapture studies in the wild (Summers et al. 2006). Thus, VIE tags may also be an alternative for mass marking young and small fish.

This is the first study validating the use of VIE tags in two consistent tagging locations on first-feeding salmonids (25–40 mm) and indicating the possibility of identifying individual fish by using a wide set of tag colors. We identified two tagging locations that optimize fish handling time and tag detection while maintaining a low mortality rate. Tags implanted at the base of the dorsal fin and injected in caudal fin rays (dorsal and ventral positions) allowed shallow injections that guaranteed good tag visibility and retention for the 34-d duration of this study. Previous studies on slightly larger salmonids tested either a single tagging location per fish (the anal fin; Olsen and Vollestad 2001) or two tagging locations randomly chosen from eight possible ones (Steingrímsson and Grant 2003). In this last study, the authors did not specifically report on tag visibility, tag retention, and the effect of multiple tagging locations on juvenile growth. Based on our results and on the literature, it appears that VIE tags are the best option for marking individual small fish at a very low cost per tag (see Dewey and Zigler 1996) under both laboratory and field conditions (Bonneau et al. 1995; Frederick 1997; Bailey et al. 1998; Bushon et al. 2007; Olsen and Vollestad 2001; Steingrímsson and Grant 2003). In the context of fishery management and fish implementation programs, VIE tags are a promising technology for following fitness differences and possible adaptations to local environments among very small fish over a short time period. However, additional research is needed to provide definitive conclusions regarding their use in behavioral studies and over a longer time period (several months).

## ACKNOWLEDGMENTS

All care and handling of fish were carried out under the approval of the Institutional Animal Care and Use Committee of Oregon State University (ACUP 3951). We thank the technical personnel and assistants of the Oregon Hatchery Research Center, R. Couture, J. O’Neil, and J. Mahr, for their assistance with care and maintenance of the fish. We also thank M. Frank and his crew from the Alsea Hatchery and Oregon Department of Fisheries and Wildlife (Newport) for providing fish from the Siletz stock. Reviews by two anonymous reviewers as well as B. K. Kristjánsson, P. J. Woods, and A. Millet improved this manuscript. C. Leblanc was supported by scholarships from the Icelandic Center for Research (RANNÍS), the Eimskip University fund, and the Leifur Eiríksson Foundation. Operations of the Oregon Hatchery Research Center are supported by the Oregon

Department of Fish and Wildlife and the Fisheries and Wildlife  
Department of Oregon State University.

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