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[Article]

Long-Term Retention and Visibility of Visible Implant Elastomer Tags in Brook Trout

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Abstract.—Understanding how juvenile brook trout *Salvelinus fontinalis* from different sources contribute to fishable populations would be facilitated by a batch mark that could be applied to early life stages and be retained and visible throughout a fish's life. We evaluated visible implant elastomer (VIE) as a long-term batch mark for juvenile brook trout in hatchery and lake environments. Visible implant elastomer material was injected into the postocular eye tissue of 2,350 age-0 brook trout. Marked fish were stocked into three lakes, and an additional group was held in the hatchery. Tissue dissections revealed that VIE tag retention was 100% in both environments after 970 d. Tag visibility in hatchery fish examined under indoor fluorescent light was greater than 95% through 585 d, then dropped to 55–70% between 700 and 900 d. Tag visibility in lake fish examined under outdoor sunlight was 50–72% at 400 d and 0% at 959 d. When these same fish were observed in dark conditions with blue-filtered light and amber glasses, tag visibility was 75% for hatchery fish at 970 d and 100% for lake fish at 959 d. The high retention and increased visibility when viewed in dark conditions with blue-filtered light demonstrates that VIE tags are a suitable long-term batch mark for juvenile brook trout.

Historic reductions in the range and abundance of salmonid species have occurred throughout North America. Specifically, declines of brook trout *Salvelinus fontinalis* in the eastern United States have prompted initiatives aimed at conserving the remaining populations and restoring them to fishable levels (Hudy et al. 2005). Knowledge of juvenile recruitment to adult stocks is crucial to achieving these conservation and restoration goals for brook trout. Brook trout populations may originate or be sustained to various degrees from natural spawning or stocking programs, and identifying the sources of the juveniles that ultimately contribute to the adult stocks can be difficult. To understand how juvenile brook trout from naturally produced or stocked sources contribute to fishable populations requires a means by which the sources of brook trout can be determined. Identification of a batch mark that can be applied to early life stages and be retained and visible throughout a fish's life would provide such a tool.

The characteristics of an ideal batch mark include ease of application, affordability, high retention and visibility, a variety of unique marks, and negligible effects on growth and survival (Guy et al. 1996). Visible implant elastomer (VIE; Northwest Marine Technology, Inc., Shaw Island, Washington) is a liquid elastomer that when injected into fish tissue cures as a flexible solid. Short-term evaluations (<180 d) of VIE tags as a batch mark for juvenile salmonids have revealed high retention, high visibility, and minimal effects on growth and survival in rainbow trout *Oncorhynchus mykiss* (Hale and Gray 1998; Walsh and Winkelman 2004), brown trout *Salmo trutta* (Hale and Gray 1998; Olsen and Vollestad 2001), bull trout *Salvelinus confluentus*, and cutthroat trout *Oncorhynchus clarkii* (Bonneau et al. 1995). Longer-term evaluations in Atlantic salmon *Salmo salar* (28 months; Fitzgerald et al. 2004) and rainbow trout (35 months; Close and Jones 2002) found that VIE tag visibility was greatly reduced by the ends of these periods and therefore that VIE tags were not a suitable long-term batch mark. No short- or long-term evaluations of VIE tag retention and visibility have been conducted for brook trout.

We evaluated VIE tags as a batch mark for juvenile brook trout over a 3-year period, assessing tag retention in hatchery and lake environments. The visibility of VIE tags was evaluated under several light conditions,

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including indoor fluorescent light, outdoor sunlight, and darkness with only blue-filtered light.

Methods

Temiscamie-strain brook trout were used during this study; they were reared and held at the Little Moose Field Station hatchery in Old Forge, New York. Red VIE was injected into to the postocular eye tissue (Hale and Gray 1998; Close 2000; Fitzgerald et al. 2004) of 2,350 age-0 juvenile brook trout (mean length = 81 ± 18 mm) on September 16 and 17, 2004. Tagging was conducted with equipment and instructions provided by Northwest Marine Technology, Inc. Before they were tagged, the fish were anesthetized with tricaine methanesulfonate (MS-222). Adipose fins were removed to provide a secondary mark to use in evaluating VIE tag retention and visibility in the field. Adipose fin clips have negligible effects on brook trout survival and growth (Mears and Hatch 1976; Zerrenner et al. 1997). Anesthetized fish were fin-clipped and immediately tagged to reduce handling stress. The entire clipping and tagging process was completed in 17 h by two persons.

After being tagged, fish were held in four 1.2-m-diameter hatchery tanks for 10 d to monitor tag retention, tag visibility, and posttagging mortality. After 10 d, VIE tag visibility was checked and length was measured for 100 individual fish. Two thousand twenty-five VIE-tagged brook trout were stocked into three nearby lakes lacking natural reproduction (N = 80, 300, and 1,645, respectively) at a density of 25 fish per hectare on September 27, 2004. An additional group of fish (N = 75) was held in a 3-m-diameter circular cement tank at the Little Moose Field Station hatchery.

Tag visibility in the hatchery fish was checked 17 times from September 26, 2004, to May 14, 2007. To determine tag visibility in lake-stocked brook trout, we examined fish captured during spring gill-net surveys (2005, 2006, and 2007) and fall trap-net surveys (2005 and 2006) throughout the study. Fluorescent lights and sunlight provided background illumination for evaluating tag visibility in the hatchery and field, respectively. A blue-filtered light source and amber glasses provided by Northwest Marine Technologies, Inc., were used for all observations. To assess tag visibility, we again anesthetized the fish with MS-222, recorded tag visibility, and measured total length. A tag was considered visible if any portion of the red elastomer was observed.

To determine whether VIE tags had been lost from individual fish, the postocular adipose tissue was dissected from all dead fish observed in the hatchery or collected in the field that did not have visible tags. At the conclusion of the experiment, all hatchery fish without visible tags were euthanized with MS-222 and their postocular adipose tissue was dissected to determine whether red elastomer material was present.

During the experiment, we observed that the amount and type of ambient light influenced the visibility of the tags. To explore this further, we viewed tags in hatchery fish under indoor fluorescent light, outdoor sunlight, and inside a dark room with only blue-filtered light and amber glasses. Tagged fish from the hatchery were checked in this manner at 706, 770, 911, and 970 d posttagging. The brook trout stocked in the study lakes were observed under outdoor sunlight and under a dark blanket with only blue-filtered light and amber glasses at 755 and 959 d posttagging. The viewing of fish in the dark with only blue-filtered light and amber glasses is henceforth referred to as the no-light condition.

Tag visibility results are reported as the percentages of tags visible for each sampling event. Unpaired t-tests ($\alpha = 0.05$) were used to test for significant differences in the lengths of the hatchery and lake groups of brook trout sampled at similar time periods (N = 5) from spring 2005 to spring 2007.

Results

The 10-d VIE tag retention was 100% (N = 100 fish), and the mortality of all tagged fish was low (0.6%). Tagged fish showed no signs of stress or infection from the tag insertion wounds after 10 d. Long-term (970-d) VIE tag retention was 100%, based on direct observation of tags and the presence of tags in all dissected hatchery (N = 4) and lake (N = 54) fish. All visible and dissected tags were bright red with no signs of fading when illuminated with the blue-filtered light.

Tag visibility was always greater in hatchery brook trout than in brook trout captured in lake surveys when the fish were observed under indoor fluorescent light and outdoor sunlight, respectively. The percent visibility of tags in hatchery fish examined under indoor fluorescent light was greater than 95% through 585 d, then dropped to 55–70% between 700 and 970 d (Figure 1). The percent visibility of tags in lake fish examined under outdoor sunlight was 50–72% at approximately 400 d, declining to 0% at 959 d (Figure 1).

Examination under no-light conditions increased the percent visibility of tags in both the hatchery and lake groups of brook trout. The percent of visible tags in hatchery fish ranged from 25% to 70% when examined under indoor fluorescent and outdoor sunlight from 706 to 970 d, respectively, but was 100% at 706 and 770 d and 75–80% at 911 and 970 d when examined

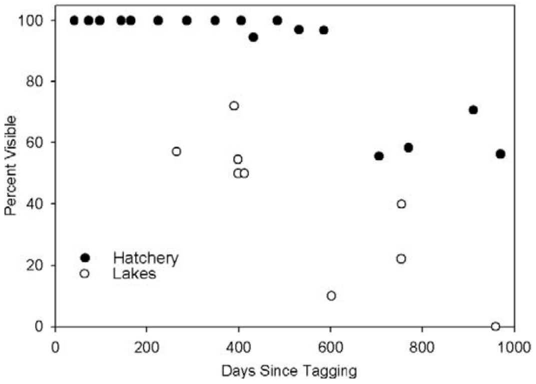

FIGURE 1.—Percent visibility of VIE tags in hatchery and lake groups of brook trout. Hatchery fish were examined indoors under fluorescent light and lake fish outdoors under sunlight. Blue-filtered light and amber glasses were used for all VIE tag observations.

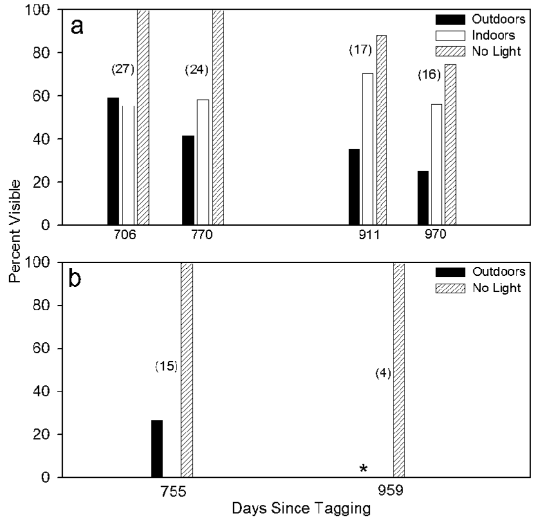

FIGURE 2.—Effect of different ambient light conditions on the visibility of VIE tags in brook trout raised in **(a)** hatchery and **(b)** lake environments. Hatchery fish were viewed under fluorescent light (indoors), under sunlight (outdoors), and in a dark room (no light). Lake fish were viewed under sunlight (outdoors) and under a dark blanket (no light). The number of brook trout examined in each period is noted in parentheses. The asterisk denotes 0% visibility of tags. Data from all three lakes were combined for the percent visibility estimates. Blue-filtered light and amber glasses were used to make all VIE tag observations.

under no-light conditions (Figure 2). The percent of visible tags in lake fish examined under outdoor sunlight was 25% at 755 d and 0% at 959 d but 100% under no-light conditions for 15 fish examined at 755 d and 4 fish at 959 d (Figure 2).

The measurements of length at age were not significantly different (unpaired *t*-tests; $P = 0.05$) for hatchery and lake groups of brook trout, except for one interval (spring 2006) in which lake fish were significantly longer than hatchery fish.

Discussion

In our study, retention of VIE tags was 100% and visibility ranged from 100% (lake group; $N = 4$) to 75% (hatchery group; $N = 16$) in brook trout when examined under no-light conditions after 959 and 970 d, respectively. The high tag retention in postocular tissue confirms this as an ideal location for salmonid VIE application (Hale and Gray 1998; Close 2000; Fitzgerald et al. 2004). Long-term studies (>180 d) on rainbow trout and Atlantic salmon attributed declines in tag visibility to tissue growth over the tags or tag loss (Close 2000; Close and Jones 2002; Fitzgerald et al. 2004). However, neither explanation was confirmed in those studies because the tagged fish were not dissected to determine whether elastomer tag material was present and the tags were not examined under no-light conditions. Our results demonstrate that VIE tag retention is high (100%) and that ambient light conditions have a substantial effect on tag visibility relative to other potential factors such as growth rate, diet, and rearing environment.

Hatchery and lake fish were similar in length at age throughout the experiment, suggesting that growth was not a causative factor in the variations in tag visibility. The diets of hatchery fish consisted of unpigmented fish pellets, whereas brook trout diets in lakes primarily consist of invertebrates (Gloss et al. 1989; Lacasse and Magnan 1992) that can have high pigment content. We observed that lake fish were darker colored and more pigmented than hatchery fish, which probably accounted for some of the lower tag visibility in lake fish. Similarly, Olsen et al. (2004) reported that VIE tags were less visible in more highly pigmented wild brown trout than in less pigmented hatchery fish.

Ambient light sources (fluorescence and sunlight) differentially reduced the visibility of VIE tags, which increased to up to 100% when examined in no-light conditions. The more rapid decline of tag visibility in lake fish suggests that outdoor sunlight had a greater influence on reducing tag detection than did indoor fluorescent light. The intensity of sunlight is greater than indoor light sources and reduces the ability to detect VIE tag material by using a blue-filtered light source and amber glasses (Terje Vold, Northwest Marine Technologies, Inc., personal communication). Previous studies have indicated that VIE tags were more visible when examined under shaded conditions.

These studies have included rainbow trout when examined under a blanket or shroud (Close and Jones 2002), Atlantic cod *Gadus morhua* when viewed under indoor light versus sunlight (Olsen et al. 2004), and snapper *Lutjanus campechanus* observed in deep versus shallow water under reduced sunlight levels (Willis and Babcock 1998). Willis and Babcock (1998) also noted that dark conditions in deeper water increased the attenuation of red light wavelengths, thereby increasing the visibility of the red VIE tags under ultraviolet light illumination.

Our study revealed that eliminating ambient light and examining fish under dark conditions with only blue-filtered light and amber glasses provided the best conditions for detecting VIE tags. Given the high retention and visibility in no-light conditions, we conclude that VIE tags are a suitable batch mark for long-term investigations of brook trout in hatchery and lake environments. Adipose fin clips could be used as a secondary mark to facilitate identification of VIE-tagged salmonids in field studies.

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