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ARTICLE

Retention Rates of Passive Integrated Transponder Tags, Visible Implant Elastomer Tags, and Maxillary Marks in Wild Trout

Elizabeth R. J. M. Mamer* and Kevin A. Meyer
Idaho Department of Fish and Game, Nampa Fisheries Research, 1414 East Locust Lane, Nampa, Idaho 83686, USA

Abstract

Tagging or marking is a common method to identify individuals or groups of fish, but these tools are compromised if tags or marks are shed or deteriorate over time. We evaluated retention rates of passive integrated transponder (PIT) tags, visible implant elastomer (VIE) tags, and maxillary clips in stream-dwelling Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* and Rainbow Trout *O. mykiss* of spawning size. We tagged 2,891 fish ≥150 mm TL with PIT tags in the body cavity, muscle tissue posterior to the cleithrum, or muscle tissue ventral to the dorsal fin. Retention of PIT tags in the body cavity of fish at large for 1 year (72.1%) was lower than those implanted in the dorsal musculature (94.1%) or cleithrum (83.5%) locations. For PIT tags implanted in the body cavity, retention was lower for females (59.4%) than males (89.7%), suggesting that PIT tags implanted in the body cavity were expelled with eggs during spawning events. Retention of PIT tags implanted in the cleithrum and dorsal musculature were unaffected by sex, but retention rates decreased as body size increased for the body cavity tagging location. Annual growth rates were not affected by any PIT tag location. We observed a PIT tag failure rate of 1.7%. The likelihood of a PIT tag being retained in muscle tissue after filleting the fish was highest for the dorsal musculature location (65.1%), followed by the cleithrum (64.9%) and body cavity (4.0%) locations. One-year retention rates were 95.4% for VIE tags and 93.0% for maxillary clips. While our results demonstrate higher PIT tag retention in the musculature than the body cavity, human consumption concerns may prohibit the use of musculature implantation where angler harvest is possible.

Tags and marks are commonly used to assess the population dynamics of fish and wildlife populations. To be effective, tag loss needs to be minimal, and the tagging process should not affect animal survival or behavior (Guy et al. 1996).

In the world of freshwater fisheries science, fish tagging technology has advanced greatly in recent decades, especially with the development of passive integrated transponder (PIT) tags (Prentice et al 1990; reviewed in Pine et al. 2013). The use of PIT tags has greatly expanded biologists' ability to estimate important population metrics, such as fish movement, survival, growth, and vulnerability to predation. Because PIT tags are commonly made from glass and other materials not intended for human consumption, they are typically injected into the peritoneal (body) cavity of fish, so that if the fish is harvested and consumed by anglers, the tag theoretically is discarded with the carcass. Unfortunately, studies have shown retention rates in body cavity-placed PIT tags diminish as fish size increases, possibly due to spawning activity by females. For example, Bateman et al. (2009) found that smaller (<140 mm FL) Cutthroat Trout *Oncorhynchus clarkii* occupying headwaters were 1.4 times as likely to retain PIT tags as larger (>174 mm), presumably sexually mature fish, though they did not identify fish to sex. For Rainbow Trout *O. mykiss*, there was a marked difference in 1-year retention rates between females (67%) and males (90%) for larger fish (>150 mm TL) but not for smaller fish (95% females, 94% males; Meyer et al. 2011); the larger fish in their study were probably mature (Meyer et al. 2014), suggesting that the tags may have been forcefully ejected with the eggs. In a hatchery environment, Prentice et al. (1990) artificially spawned adult

*Corresponding author: liz.mamer@idfg.idaho.gov
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Atlantic Salmon *Salmo salar* that had previously been PIT-tagged in the body cavity with 12-mm tags and found distinct tag retention differences between females (83%) and males (100%). Alternate tagging locations (such as the dorsal musculature) may result in higher PIT tag retention rates (Dieterman and Hoxmeier 2009), but few comparison studies have been conducted, and such alternative tagging locations may increase the risk of human consumption of these tags.

In cases where groups of fish (rather than individuals) must be differentiated, batch marking can be a cost-effective alternative to unique individual tags. For example, visible implant elastomer (VIE) tags come in several different colors and can be injected into a number of different locations on fish, providing ample opportunity to batch mark numerous groups of fish within the same fish population or study. While the VIE technique has been used in various salmonid species and environments (Bonneau et al. 1995; Bailey et al. 1998; Mitro and Zale 2002), the duration of tag retention in stream-dwelling trout has seldom been evaluated.

To help fill these information gaps in PIT and VIE tag retention, we tagged trout in three streams across 2 years with PIT tags in one of three body locations: (1) body cavity, (2) muscle tissue posterior to the cleithrum, or (3) muscle tissue ventral to the dorsal fin, and with VIE tags in the lower jaw. Our objectives were to compare 1-year PIT tag retention rates in sexually mature fish (≥150 mm TL), evaluate the associated PIT tag consumption risk to anglers, and compare PIT tag retention rates to a simpler, inexpensive, but less informative batch mark (i.e., VIE tags). Because our study protocol required that we use an additional external mark to distinguish PIT tag injection locations, we used maxillary clips for this purpose and were also able to evaluate annual retention rates of this mark.

METHODS

Yellowstone Cutthroat Trout *O. clarkii bouvieri*, Rainbow Trout, and Yellowstone Cutthroat Trout × Rainbow Trout hybrids were collected annually (2012 to 2014) from three streams (Fall, Rainey, and Badger creeks) in eastern Idaho. These streams and species were selected because study fish would not be vulnerable to angler harvest due to state fishing regulations or restricted access to the public. Having unexploited populations was necessary to avoid anglers potentially consuming a PIT tag, such as that described by Phillips (2014). We pooled data from all streams because they had similar geomorphology, which was not likely to differentially influence tag and mark retention. We also pooled data by species for two reasons: (1) we assumed that their similarity in behavior, ecology, and populations dynamics (Griffith 1988; Behnke 2002) would probably not result in differential tag or mark retention; and (2) our study design did not provide adequate sample size to split analyses between these species and still maintain the ability to evaluate differences between PIT tag injection locations and sex, which were our primary interests.

In July and September of 2012, fish were collected using backpack electrofishing units (single upstream pass) and held in 19-L buckets while electrofishing. We retained only fish ≥150 mm TL because previous research has shown that retention of PIT tags injected into the body cavity of stream-dwelling trout is high for fish <150 mm TL (Meyer et al. 2011), and most fish ≥150 mm TL were or would become sexually mature during the year of study (Meyer et al. 2003, 2014). At periodic intervals, fish were sedated in an immersion bath of peppermint oil (1:10 stock solution ratio with ethanol, using 0.3–0.5 mL of stock solution per 1 L of water) and identified with a PIT tag, a VIE tag (Northwest Marine Technology, Shaw Island, Washington), and a maxillary clip. Specimens were held in freshwater until recovered and released near the area from which they were collected.

To evaluate retention rates of different PIT tag injection locations, a full-duplex PIT tag (Biomark, 12 mm long, 2 mm diameter, uncoated glass) was injected into the fish using a 12-gauge stainless steel veterinary hypodermic needle and modified syringe (Prentice et al. 1990). Each fish received a single tag in one of three locations: (1) body cavity (injected into the peritoneal cavity, anterior to the pelvic girdle, offset from the dorsoventral axis), (2) cleithrum (injected into muscle tissue, dorsoventrally, directly posterior and parallel to the cleithrum bone), and (3) dorsal musculature (injected into muscle tissue parallel to and directly ventral of the dorsal fin ray process). After tagging, the fish was scanned using a portable PIT tag reader (Biomark IS0-601 handheld) to confirm PIT tag placement and function and then released. Tagging wounds were not sealed by any surgical glue or closure.

Additional secondary physical batch marks were used to be able to assign fish to a particular treatment group. To differentiate between tagging years, a corresponding year-specific color of VIE was used and injected subcutaneously into the minimally pigmented tissue against and parallel to the bony structure of the lower mandible. Using a 28-gauge hypodermic needle on a 0.33-ml syringe in a manual injector, the needle tip was inserted into the tissue and elastomer was injected as the needle was withdrawn. Excess elastomer was removed to decrease the likelihood of shedding. Maxillary clip combinations (either right, left, or both sides) were used to indicate which of the three PIT tag locations a fish received, as needed for identification in the event the PIT tag was shed (Siepker et al. 2012). For this mark, only the tip (i.e., about 2–3 mm) of the maxillary was removed, using nail clippers.

In September and October of 2013, using the same sampling methods described above, fish were recaptured and interrogated for all marking types and locations. Fish were scanned for PIT tags using a portable PIT tag reader (Biomark IS0-601 handheld) and visually examined in ambient light for VIE tags and maxillary marks. Fish identified as recaptures had marks recorded and were released. New specimens encountered were PIT-tagged, tagged with the second-year VIE color, and

maxillary clipped. Recaptured fish that had shed a PIT or VIE tag were not retagged with a new PIT tag or VIE color.

Study sites were revisited again in August and October of 2014, and all fish found to bear a tag or mark of any kind were sacrificed and frozen until examined. In the laboratory, fish were thawed, scanned for a PIT tag using a PIT tag reader (Destron Fearing PTS FS2001F ISO ring-type), and X-rayed along the lateral plane using a portable digital X-ray machine (Sound-Eklin, tru/DRLX System). Presence and identity of a PIT tag were noted where possible. Samples were then moved to an adjacent laboratory where they were rescreened for PIT tags on a second PIT tag reader (Biomark IS0-601 handheld) by an individual who had no knowledge of the X-ray results. Fish length and weight were measured, and sex was assessed via dissection. For these samples collected in 2014, the presence of all external marks, including maxillary clip regrowth and VIE tag integrity (fragmentation) was noted, using a handheld UV light if no VIE tag was initially seen by the unaided eye.

As if to prepare the fish for human consumption, fish examined in the laboratory were then filleted parallel to the spine (severing rib cage bones) to produce two fillets and a carcass (consisting of head, spine, internal organs, tail, and residual meat). The individual performing the filleting had no awareness of marks that might indicate a possible PIT tag location. Each fillet and carcass was then scanned for a PIT tag with a third reader (Biomark IS0-601 handheld), after which determination of the terminal location (fillet or carcass) of the PIT tag, if found by the reader, was noted.

One-year tag or mark retention rates (i.e., the number of fish recaptured that retained their tag or mark in a given location divided by the number recaptured that had been tagged or marked in that location) were calculated for each tag or mark type. For dissected fish for which sex was obtained, we also estimated PIT tag retention for females compared with males. We calculated 95% confidence intervals (CIs) around retention estimates using the formulas in Fleiss (1981). A z-test at $\alpha = 0.05$ was used to evaluate statistical significance for PIT tag location retention rates. To evaluate the effect of fish size on PIT tag retention, we binned recaptures into 20-mm size groups and plotted tag retention against fish length bins. Relationships between fish size and PIT tag retention were tested using linear regression, but before statistical analyses were performed, retention rates were arcsine-square-root-transformed (Sokal and Rohlf 1995). We used analysis of covariance to evaluate whether growth was affected by PIT tagging location, with the growth (mm) of each fish from year x to year $x + 1$ as the response variable and initial fish length (at tagging) as a covariate. Too few fish at large >1 year were recaptured during the second sampling event to evaluate 1–2-year retention rates for each of the three different PIT tagging locations; however, the sample size was adequate to evaluate VIE tag and maxillary clip retentions in year 1 and year 2.

RESULTS

Sampling took place in three consecutive autumns, with days at large between marking and recapture ranging between 328 and 769 d, encompassing one or two opportunities for the trout to spawn after having been tagged. Collectively, 874 Cutthroat Trout and 596 Rainbow Trout were marked in 2012 and 816 Cutthroat Trout and 605 Rainbow Trout in 2013, for a total of 2,891 trout tagged over the 2 years. The total length of fish at tagging ranged from 150 to 415 mm (mean = 210 mm). A total of 589 tagged fish were recaptured, identified as such by the presence of either a PIT tag, a VIE tag visible by ambient light, or a maxillary clip. The majority (523) of recaptured fish had been at large for 1 year, 59 were at large for 2 years, and 37 fish were recaptured in both years. Seven of the 589 fish were not capable of being assigned to a specific PIT tag evaluation due to incomplete secondary mark retention. Since not every recaptured fish retained all tags or marks, the reported numbers of fish for each evaluation differed.

The PIT tags injected into the dorsal musculature had a significantly higher 1-year retention rate (94.1%, CI = 3.5) than the cleithrum (83.5%, CI = 5.6; $z = 3.10$, $P = 0.002$) and body cavity locations (72.1%, CI = 6.5; $z = –5.46$, $P < 0.001$). Similarly, PIT tags in the cleithrum location were retained at a significantly higher rate than the body cavity location ($z = 2.57$, $P = 0.010$). For the 231 fish from the third sampling event for which sex was determined, body cavity tags were retained at a significantly higher rate in males (89.7%, CI = 9.5) than in females (59.4%, CI = 17.0; $z = –2.98$, $P = 0.003$). There was no sex difference in PIT tag retention for the cleithrum (76.3% males, 74.4% females; $z = 0.20$, $P = 0.84$) or dorsal musculature (91.9% males, 91.9% females; $z = 0.0$, $P = 1.00$). Retention of PIT tags declined with increasing fish size for the body cavity tagging location but not the cleithrum or dorsal musculature locations (Figure 1). There was no significant difference in growth between fish receiving a PIT tag in any one of the three locations ($F = 1.66$, $P = 0.19$).

The use of X-rays accurately identified the terminal location of PIT tags when present in all 293 fish examined in the laboratory. In the fish from which X-ray images indicated a PIT tag was present, five tags were not detected by any of the three PIT tag reading devices used, suggesting a 1.7% PIT tag failure rate; all other tags were detected by all three PIT tag readers. Retention estimates reported above were not corrected for tag failure. During the filleting process, PIT tags were predominantly found in the fillets for the cleithrum (64.9%, CI = 10.7) and dorsal musculature (65.1%, CI = 10.3) sites, but PIT tags were also occasionally found in the fillet for the body cavity site (4.0%, CI = 44; Figure 2).

Secondary batch marks were retained at equal if not better rates than PIT tags, and retention was independent of size and sex of the fish. For VIE tags, 1-year retention was 95.4% (CI = 1.8, $n = 525$), whereas retention from year 1 to year 2 (i.e., fish

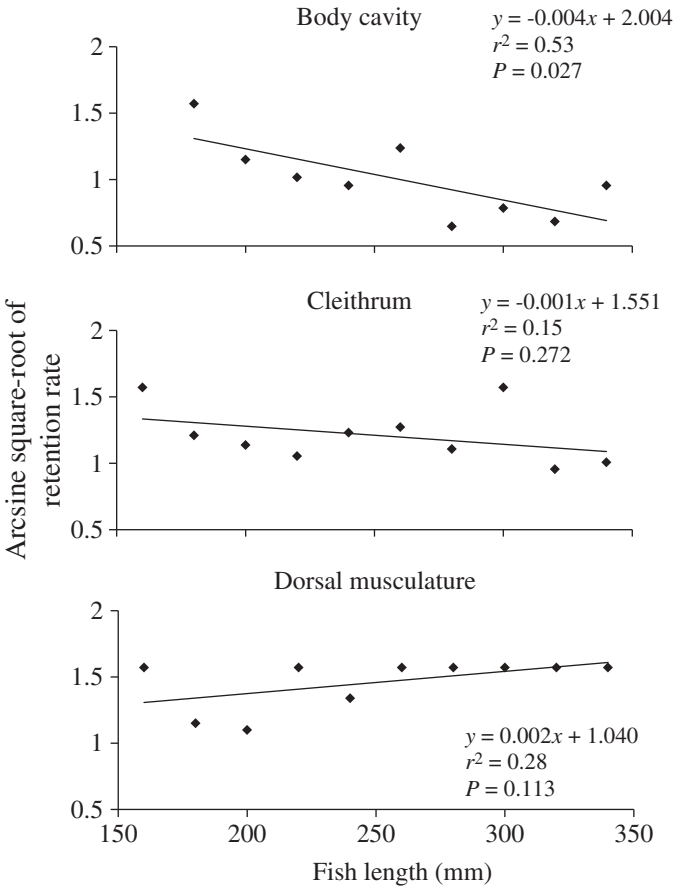

FIGURE 1. Relationship between annual PIT tag retention and total length of tagged Cutthroat Trout and Rainbow Trout by tagging location. Data for all fish were pooled into 20-mm length-groups and then averaged. Lines, equations, and statistics were taken from fitting least squares regressions to the data.

were recaptured two times) was 97.3% (n = 36). We examined 264 samples from the second recapture effort for VIE

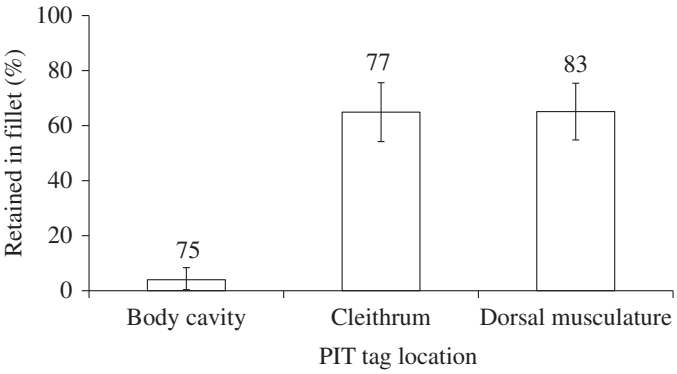

FIGURE 2. Percent of passive integrated transponder (PIT) tags located either in the carcass or the fillet (±95% confidence intervals) of Cutthroat Trout and Rainbow Trout when filleting as if for human consumption. Fish were at large for 1 year. Numbers at the top of each bar are sample sizes.

fragmentation and found that fragmentation occurred in 8.6% (CI = 3.8, n = 209) of those VIE tags at large for 1 year and 11.2% (n = 36) between year 1 and year 2. In 98.4% (CI, 2.0%) of the fish, VIE tags were easily visible in ambient light. Maxillary clip retention was also high for fish at large for 1 year (93.0%, CI = 3.3, n = 229) and between year 1 and year 2 (96.2%, n = 37).

DISCUSSION

We found that the use of alternative injection locations for PIT tags had a measurable effect on retention rate when used in stream-dwelling trout of spawning size. One-year retention of 12-mm PIT tags inserted into the body cavity of females was significantly lower than for males, probably because tags were expelled with the eggs during spawning, as has been suggested by previous salmonid studies (Prentice et al 1990; Bateman et al. 2009; Dieterman and Hoxmeier 2009; Meyer et al. 2011). This difference, supported by the negative relationship we observed between body cavity tag retention and fish length, compares favorably to a similar study examining 12-mm PIT tag retention rates in mature Rainbow Trout (>150 mm TL) where the 1-year retention rate of body cavity injected PIT tags was 67% for females and 90% for males (Meyer et al. 2011). Decreasing PIT tag retention in the body cavity with increasing fish size may occur because as female salmonids grow, egg size increases (Hutchings 1991; Klemetsen et al 2003), presumably requiring a larger vent size and providing more opportunity for tags to be shed with the eggs.

The nontraditional injection locations used in our study provided improved retention. However, those locations created additional concerns for use in fish that may be consumed because PIT tags are not intended for human consumption. Hence, they are commonly used in the body cavity injection location to avoid ingestion by anglers eating their catch. Our study confirms that cleithrum and dorsal musculature PIT tag locations should not be used on fish that are vulnerable to harvest and human consumption. When considering using the cleithrum injection location, our hope was that these 12-mm PIT tags could be inserted close enough to the cleithrum bone (without damaging vital organs) that normal filleting by anglers might leave the tag with the carcass and not in the fillet. However, inspection of X-ray images suggested that this location was more vulnerable to possible consumption than we expected because >50% of the cleithrum tags appeared to have migrated from the initial injection location (close to the cleithrum bone) further into the fillet tissue. Tag migration in tissue is a phenomenon noted in many species of animals (Gibbons and Andrews 2004; Hopko et al. 2010). The non-body cavity injection locations (cleithrum and dorsal musculature) might be usable on fish protected from harvest, but illegal or inadvertent harvest might still result in some anglers consuming tags. Our results demonstrated that, possibly due to

tag migration, even tags implanted in the body cavity carry a chance of ending up in fillet meat, creating the opportunity for possible human consumption of tags.

Retention of PIT tags in the body cavity may be improved in spawning-sized fish by use of a larger tag, making shedding with spawn less likely; similar differences in retention between male and female fish were reported for 23-mm PIT tags (Bateman et al. 2009). The 12-mm tag size we used was chosen for application in both internal and intramuscular injection sites to minimize impact on the fish. Smaller PIT tags (e.g., 8 mm) have been implanted in non-salmonids in non-body cavity locations that are not as vulnerable to angler consumption, such as the cheek muscle or isthmus (Younk et al. 2010; Kaemingk et al. 2011), but additional research is needed to more fully characterize tag retention rates in feasible implantation locations for salmonids and other freshwater fish species.

The rate of PIT tag failure we observed (1.7%) was similar to previous studies on Black Rockfish *Sebastes melanops* (0.9%; Parker and Rankin 2003) and Flathead Catfish *Pylodictis olivaris* (0.8%; Daugherty and Buckmeier 2009). The presence of failed PIT tags had a minor impact on observed retention rates because these tags were not actually shed. The tags that failed had been implanted in all three injection locations evaluated, suggesting that no specific injection location was more prone to causing tag failure. The cause of failure remains unknown because we did not recover these nonfunctional tags (i.e., all scanning was done independently and tags were not recollected from fish carcasses after filleting).

A VIE tag is inexpensive, easy to apply, easily observed with the naked eye, and requires little special equipment. However, the longevity of VIE tags can be affected by numerous factors, such as color selection, injection location, size at tagging as related to subsequent development of pigmentation or tissue overgrowth, and mark fragmentation (Close and Jones 2002; Curtis 2006; Younk et al. 2010; Bangs et al. 2013). Retention in our study (about 93% after 2 years) was higher than has been previously reported. For example, VIE retention for Muskellunge *Esox masquinongy* marked as fingerlings was 100% after 176 d but declined to nearly 0% 2 to 6 years later (Younk et al. 2010). Similarly, for Brook Trout *Salvelinus fontinalis* in both hatchery and lake environments, VIE tag retention rate (observed in ambient light) was 50–72% after 400 d and declined to 0% after 959 d (Josephson and Robinson 2008). Better retention of VIE in our study may be attributable to differences between species in lower jaw pigmentation and tissue overgrowth. Based on our results, using red and blue colors in the lower jaw for Cutthroat Trout and Rainbow Trout should produce excellent VIE retention for at least 2 years; use of a handheld UV light would provide added detection ability. Even if one were to limit VIE tagging to the lower jaw, multiple tagging locations and multiple colors would allow many batches of fish to be differentially marked.

A maxillary clip is a simple form of physical mark that has generally been shown to be benign (Stauffer and Hansen 1969; Weber and Wahle 1969) and provides a very effective, easily identified, and durable mark. Nevertheless, removal of bone can occasionally result in deformities that make later mark identification difficult. Bonham (1968) noted that along with deformations, exposure to fishing can complicate maxillary clip identification, though he reported 87% retention in Chinook Salmon *O. tshawytscha* marked as fingerlings and at large for 2 years. It is possible that maxillary clip retention in our study streams was high because the fish were marked at a larger size. However, the presence of study fish without a visible maxillary clip suggests that other processes have an impact on this type of mark, whether from partial regrowth or incomplete clipping. Angling pressure did occur in two of the three study streams, which could also alter the appearance of maxillary tissue. Given the high 1-year retention rate (93%), this mark is still very effective in trout >150 mm. Maxillary clips, when used in conjunction with VIE technology, would allow numerous subgroup delineations to be made with confidence for at least 2 years.

Alternative unique identifying tag technology exists that provides a perceived level of safety not possible with glass PIT tags. Plastic encapsulated transponder (PIP) tags are available and are used in muscle tissue in exploitable fish populations in Canada where they are considered food safe. These tags seem less prone to breakage, are easier to apply, have a wider read range, and are easier for anglers to identify in the muscle tissue (D. Ford, Golder Associates, personal communication). Retention of PIP tags appears to be as high or higher than glass-encapsulated tags (Siepker et al. 2012) and have been found to be 99% reliable for up to 2 years in Australasian Snapper *Pagrus auratus* (McKenzie et al. 2006). A caveat remains that while not encased in glass, PIP tags are rigid and may still present health issues if consumed by anglers (such as tooth fracture).

Our study demonstrates that in stream-dwelling, spawning-sized trout, PIT tag retention rates can be improved by implanting tags in two alternative (i.e., body cavity) locations, with the most effective being the dorsal musculature, though such PIT tag locations should not be used in fish populations available to angler harvest. Nonglass encapsulated tags circumvent the issue of glass consumption by anglers, though consumption of these tags may not be entirely benign. Biologists must weigh their options regarding tag type and what implant location to use. When remote sensing or tracking of individual fish is not required, VIE and other physical marks are effective methods of batch marking and carry none of the associated angler consumption risks.

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