

Evaluation of Injectable Fluorescent Tags for Marking Centrarchid Fishes: Retention Rate and Effects on Vulnerability to Predation

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Abstract.—We evaluated the performance of injectable fluorescent tags for use in mass marking of centrarchid fishes. Using age-0 largemouth bass *Micropterus salmoides* and bluegills *Lepomis macrochirus* (50–100 mm total length), we compared (1) the retention rate of injectable photonic dyes (IPDs) applied to the fin rays of largemouth bass, (2) retention rates of IPDs versus visible implant elastomers (VIEs), and (3) the effect of colorless and brightly colored marks on prey selectivity by adult largemouth bass. Retention rate of IPDs declined appreciably in age-0 largemouth bass (50 mm) after 120 d with only 19% of fish retaining visible marks by that time. By the end of the experiment (day 310), no marks were visible in anal fin rays of largemouth bass. For larger age-0 largemouth bass (100 mm), retention rates of IPDs after 48 d were greatest for fish marked in the anal fin (40%), followed by caudal (20%) and dorsal fin (5%) markings. Visible implant elastomers were retained at a higher rate (84.4%) than IPDs (68.4%) and were easier to observe after 210 d when placed in subcutaneous, opercular tissue. In general, bluegills marked with brightly colored tags were selected at a greater rate by largemouth bass than were fish marked with cryptic colors. Mean prey selectivities (Manly's alpha) were higher for brightly colored marks (blue = 0.36 and pink = 0.35) than for cryptic marks (colorless = 0.28). Only VIE marks had retention rates long enough for use in small centrarchids (<150 mm), but application of brightly colored marks to conspicuous areas should be avoided because that may increase susceptibility of the tagged fish to predation.

Considerable research has been invested in developing fish-marking techniques that (1) are easy to apply and identify, (2) can be used to characterize individual fish or cohorts, (3) are retained by fish through time, and (4) do not markedly increase fish mortality (Kelly 1967; Wydoski and Emery 1983; McFarlane et al. 1990). Techniques commonly used to mark fish externally include fin clips (McNeil and Crossman 1979), freeze-branding (Boxrucker 1982), anchor tags (Eames and Hino 1983; McAllister et al. 1992; Mourning et al. 1994), and visible implant tags (Kincaid and Calkins 1992; Mourning et al. 1994). In recent years, technologies that utilize fluorescent polymers and dyes have received increased attention because they can be relatively easy to apply and produce highly visible external marks.

Visible implant elastomers (VIEs; Northwest Marine Technology, Shaw Island, Washington) and injectable photonic dyes (IPDs; New West Technology, Arcata, California) are external marking techniques that use highly visible, fluorescent pigments of various colors injected beneath transparent tissue in fishes and invertebrates. Photonic dyes are composed of rigid microspheres filled with a colored dye and suspended in a biocompatible fluid, whereas VIEs consist of a liquid polymer that solidifies shortly after injection. Both of these techniques have potential applications for mass marking of fishes, and because they come in a variety of colors, they may be useful for identifying different cohorts.

Mark retention rate, ease of application, cost, and effects on behavior of marked fishes (i.e., differential predation, foraging, injury, etc.) are important considerations that need to be evaluated before incorporating marking techniques into research and management programs. Although VIE tags (Bailey et al. 1998; Hale and Grey 1998), and to a lesser extent IPD tags, have been evaluated in salmonid fishes, information on their usefulness and application to centrarchid fishes is limited (but

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see Dewey and Zigler 1996). Moreover, the effect of brightly colored tags on vulnerability to predation has not been experimentally evaluated for any fluorescent marks. Hence, information on mark retention rate and effects of fluorescent marks on predator selectivity is needed to determine the effectiveness of these techniques for use in mass marking of small, centrarchid fishes.

We evaluated the performance of fluorescent marking techniques for potential use in marking centrarchid fishes. Our objectives were to (1) evaluate effects of mark location on retention rate of IPDs, (2) compare retention rates of two fluorescent marks in age-0 largemouth bass *Micropterus salmoides*, and (3) determine the effect of mark color on vulnerability to predation.

Methods

Retention rate of IPD marks.—The retention rate of IPDs was evaluated by using 200 age-0 largemouth bass (mean size = 50.2 mm). In August 1998, each fish was marked at the base of the anal fin with 0.2 mL of pink photonic dye, the maximum amount of dye that could be injected into the anal fin rays (Pen-Ject System, New West Technologies). To reduce stress during marking, fish were anesthetized with clove oil at a concentration of 40 mg/L water (Peake 1998). After recovery, fish were placed in two recirculating tanks (1.1 m³) and were fed Biodiet formulated fish food two or three times a day for the duration of the study. Tanks were checked daily for mortality, and all dead fish were counted and examined for marks. We evaluated the retention rate of IPD marks by making a total census at 120, 210, and 310 d after marking.

To assess effect of mark location on retention rate of IPDs, we marked 90 age-0 largemouth bass (mean size = 104.1 mm) in June 1999. Sixty fish were marked in the anal fin and 30 were marked in both the caudal and soft dorsal fins. For these larger fish, we injected fins with 0.4 mL of pink photonic dye. The fish were fed a daily ration of Biodiet formulated fish feed and were examined for marks at 30 and 48 d after marking. Because the quality of retained marks varied among locations (e.g., fin placement), we categorized marks as good (mark clearly visible), poor (some pigment remaining in the tissue but not clearly visible), or absent (mark not visible). Our criteria for describing a “good” mark was that a fisheries professional could easily recognize the marked fish if given information on the location and color of the mark. If time had to be spent looking for faint

pigmentation under different angles of light (e.g., turning or moving fish around), we categorized the mark as poor. We used chi-square analysis to test the hypothesis that retention rate was equal among mark locations (PROC FREQ, SAS 1989).

IPD versus VIE marks in age-0 largemouth bass.—The relative performance of VIE and IPD marks was evaluated in 100-mm-long, age-0 largemouth bass. We marked 200 fish with either pink VIE tags ($n = 100$) or pink IPD tags ($n = 100$). Visible implant elastomers were placed into subcutaneous tissue of the right operculum, according to the manufacturer's suggestion (Northwest Marine Technology). Similarly, we injected 0.4 mL of pink IPD into the subcutaneous tissue of the left operculum. To verify lost fluorescent marks, VIE-marked largemouth bass were marked by removal of the right pelvic fin, whereas fish marked with IPDs were marked by removal of the left pelvic fin. Fish were then stocked into a 0.41-ha, predator-free pond on September 5, 1998. The pond was drained after 210 d and all fish were examined for marks. As in the tank experiments with 100-mm largemouth bass, mark quality was rated as good, poor, or absent for each fish. We compared retention rates of IPD and VIE in marked largemouth bass, using chi-square analysis.

Selectivity experiments.—To assess effects of colored marks on susceptibility to predation, we evaluated selectivity of largemouth bass feeding on age-0 bluegills *Lepomis macrochirus* marked with pink, blue, and cryptic IPDs. All bluegills were marked with 0.2 mL of dye in the anal fin ray. Fluorescent marks (e.g., pink and blue) appeared as 2–3-mm-diameter, brightly colored dots at the base of the anal fin. The cryptic dye resulted in a visible, opaque mark that could be identified at the base of the anal fin by trained observers. Preliminary marking evaluations indicated that 100% of marks were retained after 5 d. After being marked, bluegills were exposed to largemouth bass predators in a series of outdoor feeding trials involving six circular tanks (1.31 m³). Natural vegetation *Potamogeton* sp. was added to cover about 12% of the surface area of each tank. The inside of the tanks was painted a pale, yellow-green color with mottled dark brown areas, and the bottom was covered with sand and cobble substrates. To provide cover for adult largemouth bass in each tank, we added two cinder blocks that were covered by a 5 × 25 × 61-cm piece of wood. Each tank was allowed to acclimate, with aeration, for 1 week before the bluegills and largemouth bass were add-

ed. Water temperature in the tanks ranged from 22.5°C to 26°C.

Largemouth bass (266–355 mm) used in the experiment were angled from a local pond and a single bass was added to each tank. Largemouth bass were acclimated to the tanks for 7–9 d and fed age-0 bluegills until appreciable numbers had been consumed. Any remaining bluegills were removed from tanks 1 d before the start of the predation experiments.

We obtained age-0 bluegills from a local pond by seining. Age-0 bluegills used in feeding trials ranged from 36 to 64 mm long, representing prey/predator length ratios of 13.5–18.0% (mean = 15.2%). For each feeding trial, bluegills ($n = 300$) were anesthetized with clove oil and marked with either a pink ($n = 100$), blue ($n = 100$), or cryptic ($n = 100$) IPD. Mean length of age-0 bluegills was obtained by measuring 30–50 marked fish of each color. Marked bluegills were held in aquaria (0.19 m³) for approximately 24 h to recover from any handling stress associated with the marking procedure. To acclimate bluegills to the experimental tanks, groups of 12–15 fish of each color were placed into a small, porous metal container (20 L) and submerged in the tank 1–2 h before being released into the vegetated area of each tank. During the release of IPD-marked bluegills, largemouth bass predators remained hidden in the cinder block cover.

Tanks were checked daily to visually estimate the number of bluegills remaining in each tank and to monitor bluegill mortality. Dead bluegills were counted, removed from tanks, and checked for mark color. Experiments were ended when only 20–30% of the original number of marked bluegills remained in most tanks, which was usually after 3–7 d. At the conclusion of each trial, all remaining bluegills were removed, counted, and identified for mark color.

We conducted three trials, using the six circular tanks for a total of 13 different largemouth bass. Trials in which the largemouth bass died during the experiment or in which few prey were consumed (Manly 1974) were excluded from the analysis. To determine prey selectivity for IPD-marked bluegills, we calculated Manly's alpha (α_i) for variable prey populations using the equation

$$\alpha_i = \frac{\log_e p_i}{\sum_{j=1}^m p_j}$$

where α_i = Manly's alpha (preference index) for

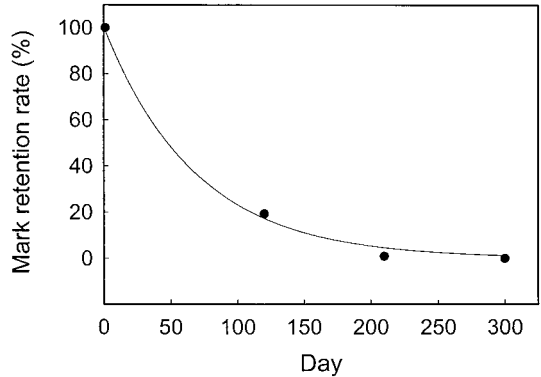


FIGURE 1.—Retention rate of fluorescent IPD tags injected into the anal fin rays of 50-mm largemouth bass. The line was fitted ($r^2 = 0.99$) using the regression equation $y = 100.2 \cdot e^{-0.0146d}$ and can be used to approximate mark retention rate (y) as a function of days after marking (d).

prey type i , p_i and p_j = proportion of prey i or j remaining at the end of the experiment, and m = number of prey types (i.e., three; Chesson 1978). Manly's selectivity index ranges from 0 to 1 with the high values indicating a preference. To assess effects of fluorescent marks on vulnerability to predation, we compared mean selectivity values for fish marked with brightly colored (e.g., pink and blue) and colorless (e.g., cryptic) marks, using a Student's t -test.

Results

Retention Rate of IPD Marks

Retention rate of IPD marks for 50-mm-long largemouth bass declined rapidly through 120 d (19%) and was 0% by the end of the experiment (day 310; Figure 1). Retention rate of IPD marks in 100-mm-long largemouth bass varied significantly among marking locations over the 48-d period ($\chi^2 = 28.5$, $df = 2$, $P < 0.001$). Retention rate was low for all locations but was highest for the fish marked in the anal fin (40%), followed by those marked in the caudal (20%) and dorsal fins (5%; Figure 2). Although retention rate was greater for fish marked in the anal fin, 22% of those fish retained marks that were poor quality. Initial mark retention immediately after application was not evaluated for 50- or 100-mm-long largemouth bass. Hence, although IPD marks appeared to degrade over time, marks that were lost gradually could not be distinguished from those that were lost due to the failure of mark application.

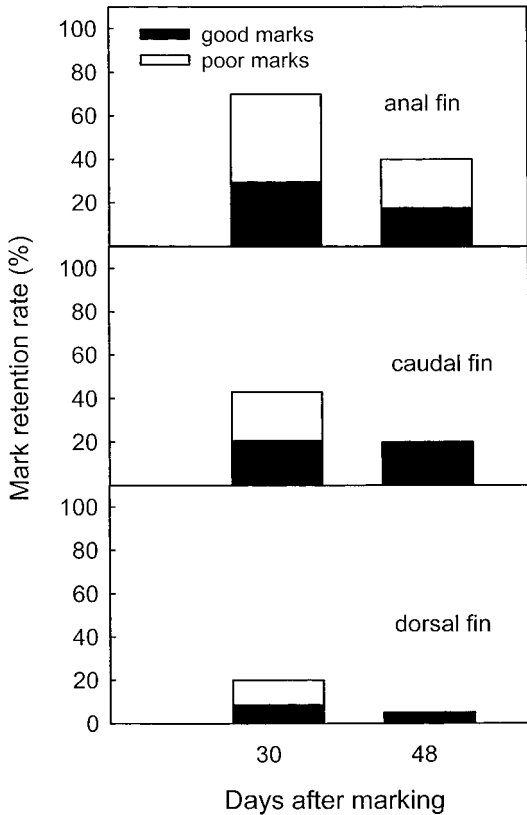


FIGURE 2.—Retention rates of fluorescent IPD tags at 30 and 48 d after injection into the anal (top panel), caudal (center panel), and dorsal (bottom panel) fin rays of 100-mm largemouth bass. Good marks were characterized as those that were easily identified without having to search for faint pigmentation under different angles of light.

IPD Versus VIE Marks in Age-0 Largemouth Bass

Survival of IPD- and VIE-marked largemouth bass at the end of the experiment (210 d) was similar, 78% and 77%, respectively ($\chi^2 = 0.006$, $df = 1$, $P > 0.1$). Retention rate of good marks was significantly greater for VIE than IPD tags ($\chi^2 = 34.8$, $df = 1$, $P < 0.001$; Figure 3). Moreover, poor quality marks were observed in fewer VIE-marked largemouth bass than IPD-marked fish. However, total retention rate (good plus poor quality) for IPD marks (66.6%) was similar to that of VIE marks (84.4%; $\chi^2 = 1.44$, $df = 1$, $P > 0.1$). Injectable photonic dye marks placed in opercular tissue of largemouth bass (Figure 3) exhibited greater retention rates than marks applied to the anal, caudal, or dorsal fins (Figure 2). Again, because initial mark retention was not evaluated for

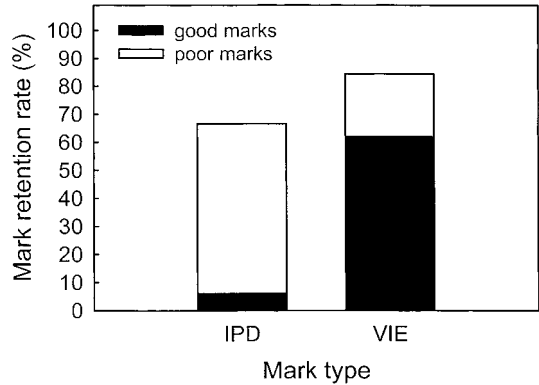


FIGURE 3.—Mark retention rates for IPD and VIE tags injected into opercular tissue of 100-mm largemouth bass. Largemouth bass ($n = 100$ for each mark) were reared in a 0.41-ha pond for 210 d.

VIE- or IPD-marked fish, we do not know whether the majority of VIE and IPD marks placed in the opercular tissue of 100-mm-long largemouth bass were lost initially after marking or gradually over time.

Selectivity Experiments

Mean initial size of bluegills used in the feeding trials was similar for each IPD mark (47.1 mm, pink; 47.7 mm, blue; 47.7 mm, cryptic; analysis of variance [ANOVA]: $F = 0.38$, $df = 2$, $P = 0.68$). Mean selectivity values were 0.36, 0.35, and 0.28 for fish marked with blue, pink, and cryptic IPD, respectively (Table 1). Because selectivities were similar for blue and pink IPD-marked fish (t -test: $F = 0.04$, $df = 1$, $P = 0.835$), we pooled these data and tested for differences between “brightly colored” and “colorless” marks. Mean selectivity for brightly colored marks (0.36) was significantly greater than for colorless marks (0.28; t -test: $F = 5.7$, $df = 1$, $P = 0.02$). On average, largemouth bass consumed over 13% more bluegills with brightly colored marks (mean number consumed = 9.75) than fish marked with cryptic dye (8.6).

Discussion

Long-term retention rate of IPD marks was poor in age-0 largemouth bass. Retention rate for 50-mm-long largemouth bass gradually declined with time to 0% after 310 d. Retention rate of IPD marks also varied significantly with mark location. Although low for all locations, retention rate of IPD marks was generally better when fish were marked in the operculum. When placed near the base of

TABLE 1.—Mean number of age-0 bluegills marked with pink, blue, or cryptic photonic dyes that were consumed by largemouth bass during each of three different feeding trials. Prey selectivity is measured by Manly's α (see Methods). Values in parentheses are SEs.

Date	Trial	<i>n</i>	Mark color	Prey per tank	Mean initial size (mm)	Mean number eaten	Mean prey selectivity
Jun 24–30	1	4	Pink	15	46.9 (0.7)	12.7 (0.6)	0.42 (0.04)
		4	Blue	15	47.3 (1.5)	11.7 (1.1)	0.34 (0.007)
		4	Cryptic	15	47.3 (0.8)	9.7 (1.8)	0.23 (0.04)
Aug 7–14	2	4	Pink	12	48.4 (0.8)	8.2 (0.6)	0.30 (0.04)
		4	Blue	12	48.7 (0.9)	9.2 (0.7)	0.39 (0.08)
		4	Cryptic	12	50.5 (0.9)	8.2 (1.3)	0.31 (0.06)
Sep 30–Oct 5	3	5	Pink	15	46.4 (0.8)	8.6 (1.4)	0.34 (0.03)
		5	Blue	15	47.2 (1.1)	8.6 (1.2)	0.35 (0.04)
		5	Cryptic	15	45.4 (0.5)	8.0 (1.3)	0.30 (0.02)
Total		13	Pink		47.1 (0.8)	9.7 (0.8)	0.35 (0.024)
		13	Blue		47.7 (1.1)	9.8 (0.7)	0.36 (0.026)
		13	Cryptic		47.7 (0.7)	8.6 (0.8)	0.28 (0.023)

fin rays, retention rate of IPD marks was greater in the anal fin than in the caudal or dorsal fins. Differences in fin morphologies may affect retention rate of IPD marks when applied within and across fish taxa.

Visible implant fluorescent elastomers were retained at a greater rate than IPDs in opercular tissue of age-0 largemouth bass. The greater retention rate of VIE marks is probably a result of the hardening process that occurs with elastomer tags shortly after injection. Unlike IPD tags, which remain fluid after injection, the solidification of VIE tags may help prevent loss or dissipation of the mark. However, we observed some evidence of tag degradation, and some of the VIE tags were of poor quality. Many of the low-quality tags were fragmented and smaller than the original tag. Similar findings were reported for barbel *Barbus barbatus*, in which VIE tags were degraded, fragmented, and subsequently lost (Farooqi and Morgan 1996). Improper curing of the liquid elastomer and handling may also reduce retention rates of VIE tags (Bailey et al. 1998), as may failure of some tags to fully solidify after injection. In our experiments, marked largemouth bass were stocked into holding ponds shortly after injection, so we were unable to estimate short-term tag loss resulting from uncured elastomer material and fish handling.

In our study, retention rate of VIE tags (84.4%) was within the range reported for other external tags. Visible implant tags (VIT) and Floy anchor tags, for example, exhibited retention rates of 82% and 89%, respectively, in rainbow trout *Oncorhynchus mykiss* after 120 d (Mourning et al. 1994). In a study of sea-run cutthroat trout *O. clarki*, retention rate of VIT tags was about 90% after 7–9 months (Blankenship and Tipping 1993). In cen-

trarchids, retention rate of VIE tags was less than that reported for other techniques. In an evaluation of retention of coded wire tags implanted into the cheek musculature of largemouth bass, Fletcher et al. (1987) reported retention was 100% after 9 weeks. Similarly, initial retention (15 d) of coded microwire tags in black crappies was high (96%; Myers et al. 2000). Retention rates of fin clips, freeze brands, and oxytetracycline marks in juvenile black crappies after 31 weeks were 100%, 96%, and 88%, respectively (Conover and Sheehan 1999).

Retention rates for VIE tags have been generally high (>90%) in short-term (<6 months) experiments (Dewey and Zigler 1996; Farooqi and Morgan 1996; Hale and Grey 1998; Willis and Babcock 1998) but can decrease over time (Bailey et al. 1998; Hale and Grey 1998). In a 2-year study of coho salmon *O. kisutch* smolts, 27% of VIE tags were lost (73% retention; Bailey et al. 1998). Despite some tag loss, the relatively high retention rates of VIE marks indicate that this technique can be useful across a variety of fish taxa.

Although retention rates of VIE and IPD tags varied, both marks shared a similar attribute with regard to visibility of fluorescent marks. Both techniques produce brightly colored external marks that aid in identification of individual cohorts. Similarly, both products are available as cryptic (e.g., colorless) marks, which are less conspicuous and can be easily observed with an ultraviolet light. In general, application of external marks often results in greater mortality of small fish than large fish (Kincaid and Calkins 1992; Blankenship and Tipping 1993). For many types of external tags, higher mortality in small fish has been attributed to increased severity of injury related to ex-

ternal marking procedures (Blankenship and Tipping 1993). However, color of external tags has also been suggested to increase susceptibility to predation in small fish. In a field study of yellow perch *Perca flavescens* and white suckers *Catostomus commersoni*, return rates of five different external tags were greater for colorless markers than for opaque yellow tags (Lawler and Smith 1963). These greater return rates of colorless tags were believed to result from lower predation by northern pike *Esox lucius*, although prey consumption was not directly quantified (Lawler and Smith 1963).

The presence of conspicuous marks or coloration patterns can affect selectivity by fishes. In a study of two color morphs of Midas cichlids *Cichlasoma citrinellum*, largemouth bass consumed significantly more natural-colored morphs than gold-colored morphs (Annett 1989). Preference for the natural-colored morph was attributed to the presence of dark lateral stripes and distinct lateral spots that were absent in the gold-colored morph. Moreover, studies with rudd *Scardinius erythrophthalmus* (Popham 1943), rainbow trout *O. mykiss* (Ginnetz and Larkin 1973), and three-spined sticklebacks *Gasterosteus aculeatus* (Ibrahim and Huntingford 1989) have demonstrated selectivities for different color prey. Similarly, we observed greater selectivities for fish marked with brightly colored tags (e.g., blue and pink) than for fish marked with a colorless tag (e.g., cryptic).

Poor retention rates of IPD marks make this a poor choice as a long-term mark in small, centrarchid fishes. In general, retention rate of non-hardening, injectable dyes is often lower for smaller fish (Kelly 1967; Dewey and Zigler 1996) and as a result may be more applicable as a marking technique for use with larger, adult fishes. Injection of VIEs is a useful technique for marking centrarchid fishes, given the marker's relatively high retention rates, ease of application, and economical feasibility (Dewey and Zigler 1996). Use and application of injectable fluorescent marks, however, should consider the size of fish and the conspicuousness of the tag because this may affect survival of marked cohorts. In particular, cryptic marks should be considered for use in fish that are vulnerable to predators.

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