RECLAMATION

Managing Water in the West

High Pressure Injection of a Photonic Marking Agent: Mark Retention and Effects on Survival of Juvenile Striped Bass, *Morone saxatilis*, and Adult Delta Smelt, *Hypomesus transpacificus*

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14. ABSTRACT
Employing appropriate marking techniques and marking agents, that ensure adequate mark retention and fish survival, are critical when developing mark and recapture experimental designs. Fluorescent pigments (BMX-1000, New West Technology, Arcata, California) injected into the dorsal, caudal, and anal fins of adult delta smelt *Hypomesus transpacificus* (53 – 86 millimetres [mm] fork length [FL]) using a pressurized CO_2 gun, exhibited adequate retention rates (> 80 percent) through 77 – 105 days (in two experiments) and resulted in survival rates > 82.5 percent through 28 days. Employing the same techniques and injectable fluorescent pigments using juvenile striped bass *Morone saxatilis* (74 – 170 mm FL) resulted in mark retention rates through 28 – 35 days between 30 and 100 percent, as well as 100 percent survival of test fish. In general all test fish displayed highly visible marks, appeared healthy, and exhibited no immediate adverse effects after mark injection. This study demonstrates the applicability of injectionable fluorescent pigment marks for short-term mark and recapture study designs using adult delta smelt and to a lesser degree juvenile striped bass.

15. SUBJECT TERMS
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by

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EXECUTIVE SUMMARY

The performance of a fluorescent pigment (BIOMETRIX SYSTEM-1000, NewWest Technology, Arcata, California) injected into the dorsal, caudal, and anal fins using a pressurized CO_2 (carbon dioxide) gun was evaluated using adult delta smelt, *Hypomesus transpacificus*, (53 – 86 millimeter [mm] fork length [FL]) and juvenile striped bass, *Morone saxatilis*, (74 – 170 mm FL). Adult delta smelt and juvenile striped bass exhibited the lowest amount of fin damage when the marking agent was injected using CO_2 pressures of 150 pounds per square inch (psi) and 200 psi, respectively. Mark retention and survival were evaluated at days 77 and 105 in two experiments using delta smelt, and at 28 and 35 days (d) in two experiments using striped bass. Delta smelt 28-d survival was not affected by mark color or location, and ranged from 82.5 – 100 percent in both experiments. Delta smelt survival rates at days 77 and 105 did not appear to be effected by mark location or color, and ranged from 85 – 95 percent, and 80 – 92.5 percent, respectively. Striped bass incurred no mortalities through the duration of the 28 or 35-d experiments. Initial mark loss was first observed at 56-d when evaluating 77-d delta smelt mark retention, and mark retention rates through 77-d ranged from 82.5 – 100 percent. Initial mark loss was first observed at 91-d when evaluating 105-d delta smelt mark retention, and retention rates through 105-d ranged from 86 – 100 percent. Initial mark loss was first observed 21-d after initial injection in juvenile striped bass. Striped bass mark retention rates through 35-d ranged from 30 – 100 percent, with lowest retention rates exhibited in the dorsal fin and using yellow pigments. All fish survived initial marking and displayed highly visible marks. This study demonstrates the applicability of injectable fluorescent pigment marks for short term mark and recapture study designs using adult delta smelt and to a lesser degree juvenile striped bass.

INTRODUCTION

Marking fish has been in practice since the early 1600s and is a valuable research technique employed during mark and recapture study designs to distinguish between groups, and acquire data for population dynamics, age and growth, observational studies, mortality, and movement and migration studies (Hilborn *et al*., 1990; McFarlane *et al*., 1990; Guy *et al*., 1996). The following are a few key characteristics that fisheries biologists have identified as important when selecting a marking agent and technique (Wydoski and Emery, 1983; Stott, 1968):

- Efficient: marks per unit time and unit effort
- Low handling stress
- Low effect on fish health and condition (i.e. swimming performance)
- Longevity
- High number of distinguishable marks
- Low cost
- Low mortality

Many methods and styles of external marks currently employed do not meet the needs of mark recapture studies. For example, branding is time consuming and provides inconsistent marks, and mark loss, and negative biological and physiological effects can result from fin-clipping, anchor tags, spaghetti loops, and floy-tags (McNeil and Crossman, 1979; Boxrucker, 1982; McAllister *et al*., 1992).

BIOMETRIX SYSTEM-1000 (BMX-1000) injectable photonic marking dyes (PMD), developed by NewWest Technology (NWT), Arcata, California, are fluorescent pigments that have been used successfully as a marking agent for fisheries mark and recapture study designs. However, current literature on injectable pigments is limited to a few families: Salmonids (Bonneau *et al*., 1995; Hayes *et al*., 2000), Centrarchids (Dewey and Zigler, 1996; Catalano *et al*., 2001) and coral reef fishes (Frederick, 1997), and indicates that mark retention of injectable pigments is dependent upon taxon and tag location (Hill and Grossman, 1987; Goforth and Foltz, 1998; Albanese, 2001).

Little information is available detailing the retention time and ease of application of PMD, or its affects on health and survivability, when applied to adult delta smelt and juvenile striped bass. Delta smelt and striped bass are important members of the aquatic fauna of the San Francisco Bay Estuary (SFE) and their distribution and abundance are closely monitored by Federal and State agencies. Delta smelt are small (60 – 110 millimeter [mm] fork length [FL]), fragile, planktivorous, lean-bodied members of the *Osmeridae* family (Moyle, 2002; Swanson and Cech, 1995). They are endemic to California and were formally listed as threatened under the Federal Endangered Species Act in 1993 (*Federal Register,* 1993; Sweetnam and Stevens, 1993). Striped bass are large (> 1000 mm FL) predators, first introduced into the SFE in 1879 (Dill and Cordone, 1997). Due to their status as a sporting fish, they are possibly the most economically important fish in the delta.

Populations of pelagic fish species of the SFE and Sacramento-San Joaquin Delta (SSJD) have steadily declined over the last decade, and popular culprits leading to this problem are the two major south delta pumping facilities, the U.S. Department of the Interior, Bureau of Reclamation's (Reclamation) Bill Jones Pumping Plant and California State's Harvey O. Banks Pumping Facilities (Bennett and Moyle, 1996; Moyle, 2002). In combination, these pumping facilities divert nearly one quarter of the SSJD average annual inflow, creating flows attractive to migratory fish and too strong for larval fish to resist (Mitchell, 1996). Adjacent to each of these pumping facilities are fish collection facilities, designed to divert and collect fish prior to them encountering the pumping facilities.

In response to the concern over the loss of fish at SSJD pumping facilities, both Federal and State agencies continue to develop and coordinate mark and recapture study designs to make improvements on, and to better understand the effectiveness of SSJD fish collection facilities. Mark and recapture study designs are an important part of fish facility research and are devised in a manner that require identification of individual test fish, and multiple fish injections per experiment, with multiple repetitions per day. Therefore, marking methods and agents that provide a diversity of marks are necessary to meet the demands of the experimental methods employed for SSJD fish facility research.

The primary objective of this research was to determine if the subcutaneous injection of PMD, using a high pressure photonic tagging gun, is a suitable marking technique for short-term delta smelt and striped bass mark and recapture studies at Reclamation's Tracy Fish Collection Facility (TFCF) and California's Skinner Fish Protection Facility (SFPF), the two fish salvage facilities in the southern region of the SSJD. The primary goals of this project were to assess PMD retention rates and effects on fish survival. The secondary goals were to evaluate optimal CO_2 (carbon dioxide) injection pressures, color reliability, variations in fin retention, and whether a post injection prophylactic medicinal treatment reduced fungal development and increased fish survival.

METHODOLOGY

PMD Solution and Tagging System

A high-pressure, CO_2 powered tagging gun (POW'R-JECT BMX-1000, NWT; figure 1) was used to subcutaneously inject BMX-1000 PMD (polymethylacylate fluorescent pigment, New West Technologies) into the fins of fish. Affixed to a high-pressure CO_2 tank, the gun is capable of injecting PMD at pressures between 50 and 430 pounds per square inch (psi). Gauge pins allow PMD to be injected, from 0.05 – 2.0 milliliter (mL) per injection, in 0.05 mL increments. The PMD is comprised of aqueous paint containing latex microspheres. A marking bioadhesive is added (1 mL per 45 mL of PMD) to the PMD prior to injection to maximize retention. The marking solution is available in nine colors and fluoresces when exposed to ultraviolet light.

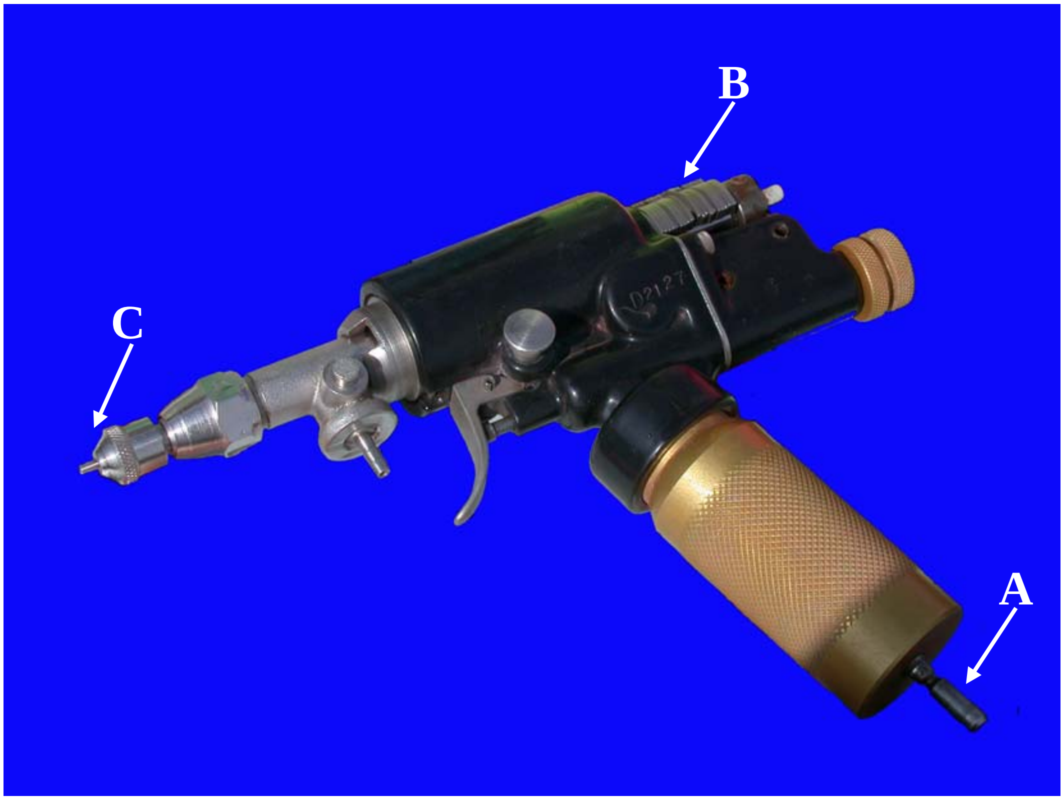

FIGURE 1.—Photonic marking gun equipped with (A) high pressure hose attachment, (B) pins to adjust injection quantity and (C) injection needle and PMD intake.

Fish Source and Care

Delta smelt

Cultured adult delta smelt (age-1, 53 – 86 mm FL) were held at the University of California Davis' Fish Conservation and Culture Laboratory (FCCL) in Byron, California. Delta smelt tests were completed using indoor, black, 1,000 liter (L) (264 gallon [gal]) cylindrical fiberglass tanks (153 centimeter [cm] diameter × 61 cm height) maintained on a 11,356 L (3,000 gal) recirculation system. Make-up water from the Clifton Court Forebay was ozone purified before use. Tanks were maintained at 10.1 ± 0.1 (mean ± S.E.) degree Celsius (°C) in experiment No. 3, and 13.3 ± 0.2 °C in experiment No. 4. Salinity levels were low throughout testing (0.2 – 1.0 parts per thousand [‰]). Total ammonia, nitrite, pH, dissolved oxygen, and ozone levels were monitored and maintained near optimal levels (as recommended by Meade, 1989). Fish were observed actively feeding (2:1 mix of 600 – 800 micrometer [µm]) Lancy feed, and 370 µm Hikari plankton feed (Kyorin Co Ltd., Himeji City, Hyogo Prefecture; approximately 1 percent body weight per day) throughout testing.

Striped bass

Wild striped bass (age-1, 74 – 170 mm FL), were collected from the TFCF, and maintained and tested at the Tracy Aquaculture Facility (TAF) located in Tracy, California. Striped bass photonic marking experiments were conducted in shade cloth-covered, outdoor, black, 757 L (200 gal) cylindrical polyethylene tanks (120 cm diameter × 76 cm height). A flow through water system provided 18.0 °C, air saturated, brackish (5.0 – 5.5 ‰) well water to all tanks. Fish were observed actively feeding (Silver Cup 1 mm Salmon Feed, Nelson and Sons, Inc., Murray, Utah; approximately 1 percent body weight per day) throughout testing. Total ammonia, pH, temperature, and dissolved oxygen levels were monitored and maintained near optimal levels (as recommended by Meade, 1989).

Fish Marking

Prior to being marked, fish were anaesthetized by 1 – 2 minutes (min) of exposure to a solution (40 milligram per liter [mg/L]) of tricaine methanesulfonate (MS-222, Sigma-Aldrich). Marking took place in a small, shallow, tray (40 × 35 × 3.8 cm), in 4 L of water. During marking, fish were placed ventral side up and the targeted fin was placed directly against a sloping tile (15.5 × 5.0 cm) to facilitate tagging. The tagging gun tip was gently placed at the base of the fin at an approximate 75 degree angle, so that the PMD hit the fin perpendicular to the fin rays (figure 2). For all experiments, two shots of PMD, each approximately 0.05 mL, were injected into a pre-determined fin.

Mark Quality and Fin Damage: Assessment

Mark quality and fin damage were assessed 24-hour (h) post-injection, and every 7-day (d) throughout the duration of each experiment. To assess mark quality, fish were lightly sedated (MS-222, 10 mg/L) to minimize handling stress, removed from their respective holding tanks using fine mesh dip nets, assessed for mark quality and fin damage, and transferred to a tank void of fish. During the assessment of mark quality, fish exposure to the atmosphere was minimized (< 3 seconds). Upon visual inspection, marks were given the following ratings based on the following criteria:

A. **Good:** No apparent fin damage. PMD mark clearly visible between at least one set of fin rays (figure 3).

B. **Bad:** No visible damage and PMD mark not clearly visible. Typically observed as a small ring of pigment surrounding the injection area (figure 4).

C. **Not Visible:** No PMD color visible.

D. **Damaged Fin:** Visible fin damage and PMD mark clearly visible (figure 4).

FIGURE 2.—Photonic marking equipment being used to mark adult delta smelt.

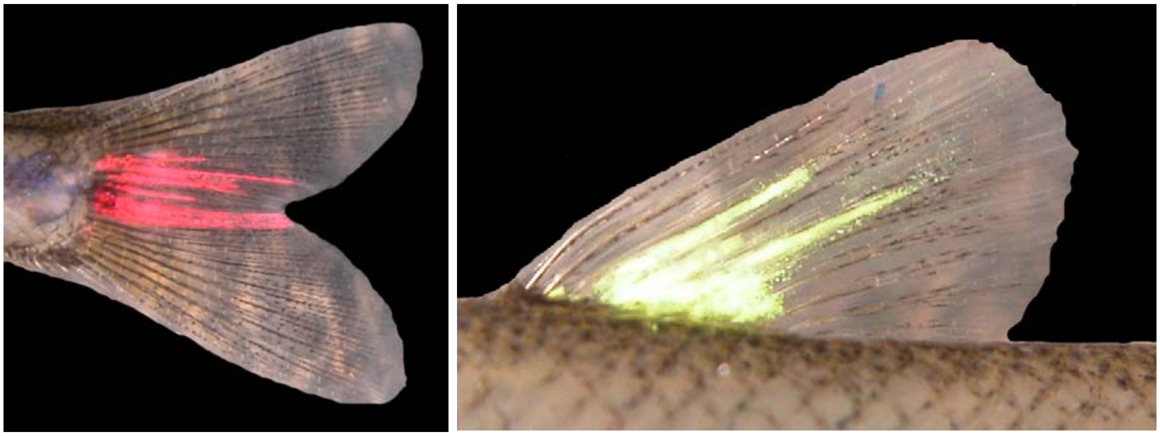

FIGURE 3.—“A” quality PMD marks displayed on the caudal and dorsal fins of adult delta smelt.

FIGURE 4.—“B” and “D” quality PMD marks displayed on the anal fin of an adult delta smelt.

Tanks were checked daily, throughout the duration of each experiment for sick fish, fungal development, and mortalities. All dead fish were removed, measured (FL in mm), and examined for mark quality, and damage or fungal development near the PMD injection site.

Fish Marking Experiments

Four laboratory experiments (No. 1 – 4) were conducted between September 2004 and May 2005 to assess the subcutaneous injection of PMD using a high-pressure tagging gun as an effective agent and method for marking wild juvenile striped bass and lab-reared adult delta smelt.

Experiment No. 1

Past marking efforts using the described materials and methods have often resulted in holes and rips in fish fins, as well as body damage near the base of the fin that has lead to a noticeable increase in post-injection fungal development. This has been attributed to excessively high CO_2 pressure in the tagging gun, resulting in elevated PMD and biobead velocities during injection. The objective of experiment No. 1 was to determine the optimum CO_2 pressure range necessary to obtain a high quality mark. Tests were performed on September 14, 2004, using adult delta smelt, and on September 16, 2004, using juvenile striped bass. Red PMD solution was the standard for all replicates in Experiment No. 1. For both species, CO_2 pressure was initially regulated to 50 psi, as this was the minimum pressure needed to efficiently propel PMD from the tagging gun. Throughout each replicate, pressure was increased in 25 psi intervals until fin damage was clearly noticeable. At each pressure three delta smelt and five striped bass were marked with two injections on the dorsal, caudal, and anal fin. Following injection fish were given 10 minutes to recover in a black 18.9-L (5-gal) bucket, and were then removed and given a rating for tag quality and fin damage using the criteria previously described (see page 4).

Experiment No. 2

Results from Experiment No. 1 indicated a broad range of suitable marking pressures (150 – 250 psi) when PMD was applied to all striped bass fins (see Results, page 10). In Experiment No. 2 (initiated on October 3, 2004) a narrower range of pressures, and a larger sample size of fish were tested to determine a narrower range of appropriate marking pressures for juvenile striped bass. During Experiment No. 2, red PMD (two 0.05 mL injections per fin) was used in all replicates, and for each treatment, only one fin per fish was marked. Three CO_2 pressures (50, 150, and 250 psi) were tested on three fins (dorsal, caudal, and anal) with four replicates and 20 fish per treatment to assess 28-d mark retention. After marking, test fish were maintained in four tanks with 180 fish per tank. To verify loss of mark and location of loss, each treatment group was given a unique fin clip corresponding to each unique application pressure and fin tag location.

Experiment No. 3

Experiment No. 3 was initiated on January 13, 2005, using delta smelt and on March 9, 2006, using striped bass to evaluate survival and mark retention as a function of PMD color and fin tag location. For both species, three PMD colors (blue, red, and yellow) were injected into each of three fins (dorsal, caudal, and anal) at one CO_2 injection pressure (delta smelt = 175 psi, striped bass = 200 psi) with four replicates and 10 fish per treatment. Optimal PMD injection pressures were determined from Experiments No. 1 and No. 2 for delta smelt and striped bass, respectively. For each treatment, one fin was marked for each individual fish. For each replicate, ten fish were not injected with a PMD, yet subjected to identical handling techniques, to act as a control group. To verify loss of mark and location of loss, each treatment group was given a unique fin clip corresponding to each unique PMD color and fin tag location. After 28-d mark retention and survival assessment, delta smelt were grouped into a common tank, and mark retention and survival were assessed through 105-d. Striped bass mark retention assessment was stopped after 35-d because for all colors and locations, except yellow caudal, mark retention was ≤ 90 percent, which was below the a priori decision to stop the experiment.

Experiment No. 4

Adult delta smelt were marked on March 2, 2005, to assess the effect of a post-injection prophylactic treatment on PMD retention and fish survival. The same methods were used as employed in Experiment No. 3, except fish were subjected, post tagging and clipping, to an anti-fungal/parasitic treatment. On day 0 through day 3, post tagging fish were provided a 1-h prophylactic bath containing salt at 7 ‰ and 0.52 mg/L of Pond Rid Ich (Novalek Inc. Haywardm California; 11.52 percent formalin 4.26 percent U.S.P. grade, and 0.038 percent zinc-free chloride salt of malachite green). Pond Rid Ich has proven to be an efficient treatment used by delta smelt culturists to treat against external protozoans and fungal infections (Bridges personal communication, 2007). After the 28-d mark retention and survival assessment, all fish were grouped into a common tank and mark retention and survival were assessed through 77-d.

For experiments No. 2, No. 3, and No. 4, 28-d and 35-d post-treatment assessments were chosen, as this length of time was deemed suitable by TFCF fisheries biologists conducting mark and recapture experiments at SSJD fish collection facilities. Twenty-eight days provides adequate time for post-tagging prophylactic treatment (3-d), recovery (11-d), acclimation to test environment (7-d), and experimentation (7-d). Therefore, if application of a marking agent coincides with low survival rates and or low retention between 28 and 35-d, it will be difficult to use for mark and recapture experiments conducted at SSJD fish collection facilities. Mark retention rates were assessed until the majority of treatments in each experiment showed ≤ 90 percent retention or until it became logistically impossible to continue evaluations.

Fin Meristics

In December of 2004, fin measurements were obtained from juvenile striped bass and adult delta smelt. Measurements were taken to better ascertain whether fin thickness influenced PMD retention. Five delta smelt and nine striped bass were euthanized using a high dosage of MS-222 (200 mg/L, 1 – 2 min exposure). All fish sampled were in the same size ranges as indicated in Experiments No. 3 and No. 4. A 1-mm cross-section of the dorsal, caudal, and anal fins at points approximately 1 – 2 mm from the body were attained, as this was the targeted area for PMD injections. Each sample was placed into a 5 percent formalin solution under a Leica MZ75 stereomicroscope and oriented in a fashion so that five measurements, and associated average fin thickness, fin ray thickness, and space between fin rays could be obtained from each fin (figure 5). Measurements were made using a micrometer and digital images were recorded and saved at 1.0 X magnification.

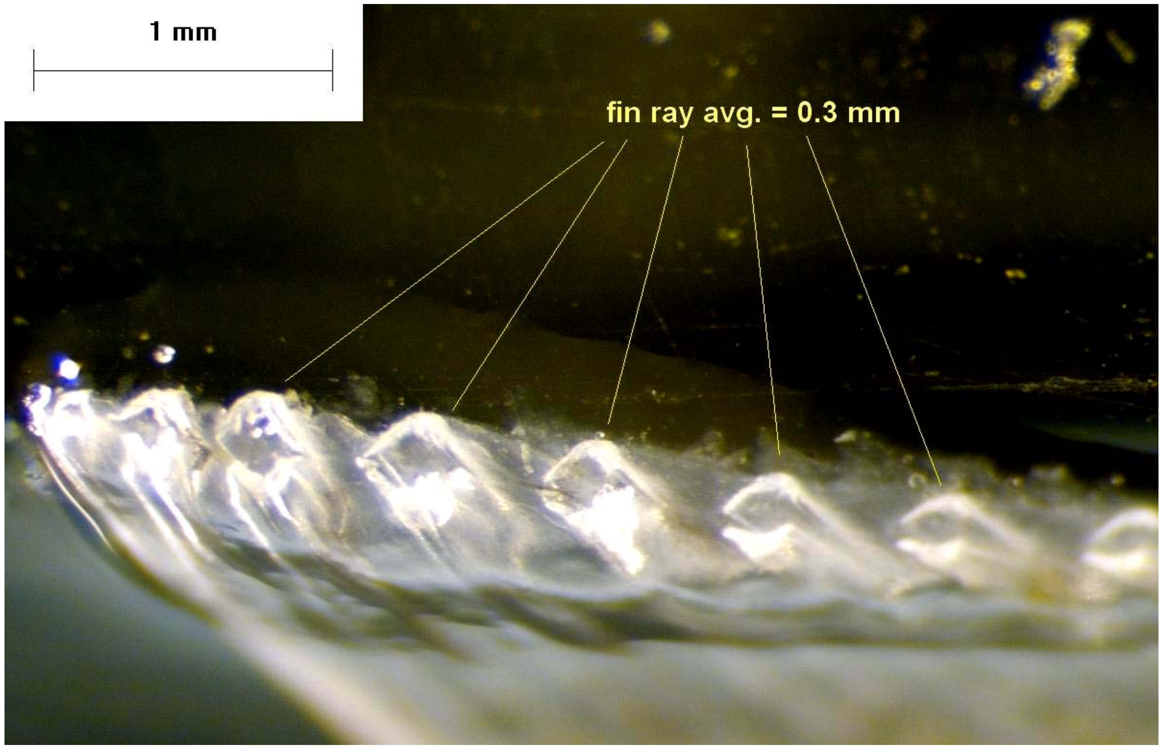

FIGURE 5.—Cross section taken from a delta smelt dorsal fin and used to determine fin thickness.

Data Analysis

A one-way ANOVA (Tukey's) test, comparing means, was used in all experiments to determine if there was a difference between fish lengths amongst treatments, and again to detect differences between dorsal, caudal, and anal fin thickness. No statistical analysis was performed for Experiment No. 1, because all data collected were qualitative. Due to low sample sizes (n = 4), and the large proportion of survival and retention data that resulted in 100 percent totals in Experiments No. 2, No. 3, and No. 4, assumptions necessary to model using parametric or regression methods could not be met. Therefore, a non-parametric alternative, the Kruskal-Wallis test, was used to independently test the hypothesis that the distribution of both alive *vs*. dead fish, and lost *vs*. retained marks, were not significantly different for each combination of mark location and color on day 28 of Experiments No. 2, No. 3, and No. 4. In cases where the null hypothesis was rejected using the Kruskal-Wallis test, individual group medians were compared using the Nemenyi test, a non-parametric multi-comparison test (Zar, 1999). Because no mortalities were recorded for striped bass in Experiments No. 2 or No. 3, and survival was 100 percent for delta smelt through day 28 in Experiments No. 3 and No. 4, no statistical analyses were performed to measure the effects of mark pressure, location, or color on fish survival or mark retention. Delta smelt were grouped into common holding tanks and separated by treatment after 28 days in Experiments No. 3 and No. 4. The small sample size (n = 1) did not allow for a statistical analysis measuring the affects of the described marking methods on long-term (≥ 77-d) mark retention and fish survival to be performed. All statistical analyses were conducted using SAS 9.1 (SAS, 2005), and the alpha level for all analyses was set at 0.05 (Waters and Erman, 1990).

RESULTS

Effects of Fin Location and Marking Pressure: Experiments No. 1 and No. 2

Experiment No. 1

Delta smelt (77.5 ± 1.1 mm FL, mean ± standard error; *n* = 30) and striped bass (83.0 ± 2.7 mm FL; *n* = 50) lengths were the same across treatments (injection pressures 50 – 300 psi, in 25 psi intervals) during Experiment No. 1 (P > 0.05). Of the pressures tested, 150 and 175 psi resulted in "A" quality marks on all delta smelt fins. Fin damage (puncture wounds on all fins) as a result of tagging was initially noticeable at 225 psi. For use in delta smelt Experiments No. 3 and No. 4 (see below), 175 psi was preferred over 150 psi, as pressures lower than 150 psi tended to leave PMD obstructing the tag gun, resulting in clogging and additional time loss due to gun maintenance.

A broad range of pressures (50 – 250 psi) were found to produce high quality marks in juvenile striped bass, and therefore an additional experiment (Experiment No. 2) was developed to determine an appropriate injection pressure for marking juvenile striped bass. Striped bass fin damage (puncture wounds on the dorsal fin) as a result of tagging was initially noticeable at 300 psi in experiment No. 1.

Experiment No. 2

Juvenile striped bass lengths (86.21 ± 0.94 mm FL, *n* = 80) were not different when compared across treatments (P > 0.05). Striped bass incurred no mortalities and showed no evidence of fungal development through 28-d during the experiment. Mean 28-d mark retention ranged from 12.75 percent (dorsal tag, 150 psi application pressure) to 98.6 percent (caudal tag, 200 psi application pressure). The 28-d retention of marks applied to the dorsal fin, using a pressure of 150 psi, were lower than those applied to the caudal fin using pressures of 200 and 250 psi (χ^2 = 26.7, DF = 8, P < 0.05; figure 6). There were no other differences comparing all other combinations of application pressures and fin types (P > 0.05). Because no differences were found comparing mark application pressures of 200 and 250 psi on any fin type, 200 psi was chosen for use when marking striped bass in Experiment No. 3.

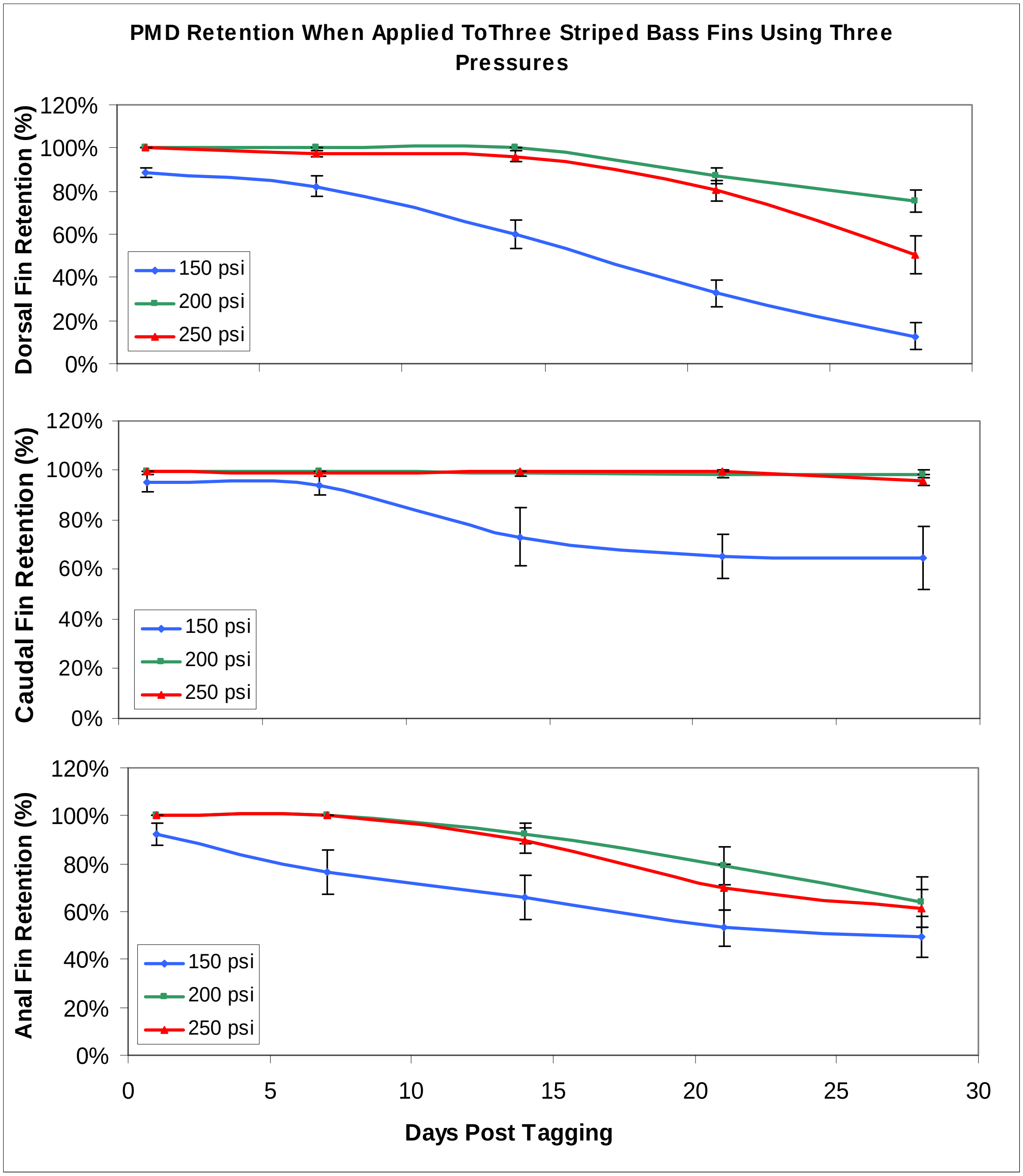

FIGURE 6.—Experiment No. 2: 28-d red PMD mark retention applied to the dorsal, caudal, and anal fins of striped bass using three different application pressures. Individual dots are representative of treatment means, and error bars are displayed as ± the standard deviation of the mean.

Effects of Mark Location and Color: Experiments No. 3 and No. 4

Mark Longevity

Striped Bass Experiment No. 3

There was no significant difference in striped bass lengths when compared amongst treatments in Experiment No. 3, both pre- (119.63 ± 1.14 mm, p = 0.82, *n* = 200, P > 0.05) and post (136.53 ± 1.21 mm, *n* = 200, P > 0.05) experimentation, respectively. Mean 35-d mark retention ranged from 30 percent (yellow dorsal tag) to 100 percent (yellow caudal tag). The retention of yellow PMD marks applied to the dorsal fin were lower than yellow PMD marks applied to the caudal fin on day 35 of testing ($\chi^2 = 24.4$, DF = 8, $P < 0.05$; figure 7). There were no other differences comparing all other combinations of application colors and fin types ($P > 0.05$; figure 8).

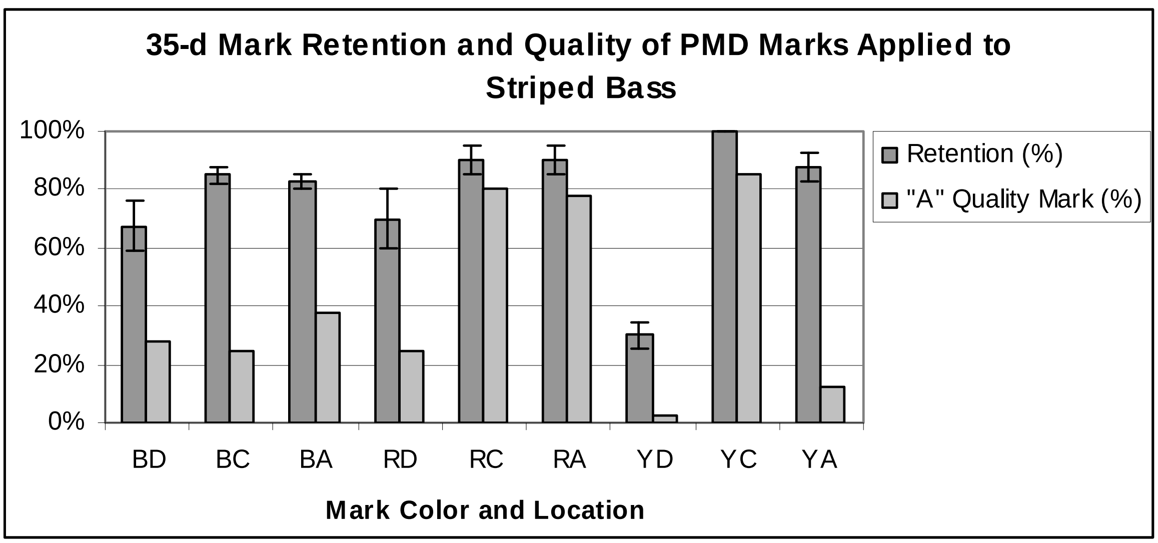

FIGURE 7.—Juvenile striped bass 35-d mark quality and retention rates when applied in blue (B), red (R), and yellow (Y) PMD to the dorsal (D), caudal (C), and anal (A) fins (Experiment No. 3). Thirty-five day mark retention of yellow dorsal marks was significantly different compared to yellow caudal marks, no other differences were significant. Bars represent means ± one standard deviation.

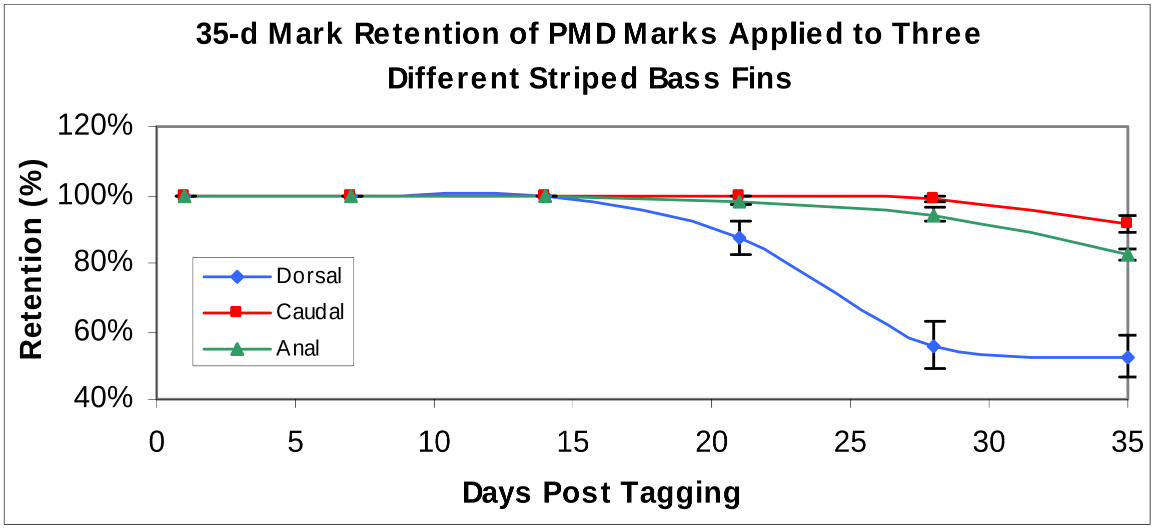

FIGURE 8.—Thirty-five day PMD mark retention when applied to the dorsal, caudal, and anal fins of juvenile striped bass, averaged over PMD color. Individual dots are representative of treatment means, and error bars are displayed as ± the standard deviation of the mean.

Delta Smelt Experiment No. 3

There were no differences in the size of delta smelt, across treatments, in Experiment No. 3 (68.4 ± 0.42 mm FL, $n = 200$, $P > 0.05$). Adult delta smelt mark retention was 100 percent for all colors and locations on day 28, and initial mark (red anal fin mark) loss was first noticed 91 days after the initial injection. Mark retention on day 105 of the experiment was high for all colors and locations, but highest for blue dorsal fin (100 percent), pink dorsal fin (100 percent), and pink caudal fin marks (100 percent), and lowest for blue anal fin marks (86 percent; figure 9). Although mark retention rates on day 105 were high, all combinations of mark color and fin location produced a portion of low quality (“B”) marks. The percentage of B quality marks through 105 days in Experiment No. 3 ranged from 17.9 percent (blue dorsal fin) to 60 percent (yellow anal fin; figure 9).

Delta Smelt Experiment No. 4

There were no differences in delta smelt fork lengths, across treatments, in Experiment No. 4 (76.0 ± 0.60 mm FL, $n = 200$, $P > 0.05$). Adult delta smelt mark retention was 100 percent for all colors and locations on day 28 of testing, and initial tag loss was noticed on the 56th day after initial marking (yellow dorsal fin mark). Mark retention on day 77 in Experiment No. 4 was highest for yellow caudal fin marks (100 percent) and lowest for pink caudal fin marks (85.50 percent; figure 10). All combinations of PMD mark color and fin location produced a portion of low quality (“B”) marks by the 77th day in Experiment No. 4, and ranged from 9.7 percent (yellow caudal fin) to 68.8 percent (red anal fin; figure 10).

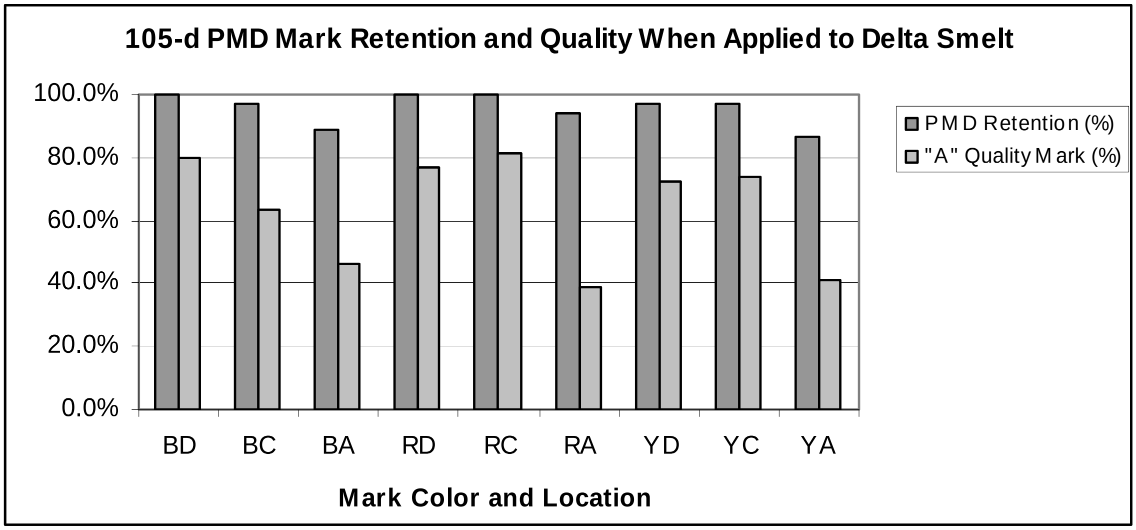

FIGURE 9.—Experiment No. 3: Mean delta smelt 105-d mark quality and retention rates when applied in blue (B), red (R), and yellow (Y) PMD on the dorsal (D), caudal (C), and anal (A) fins.

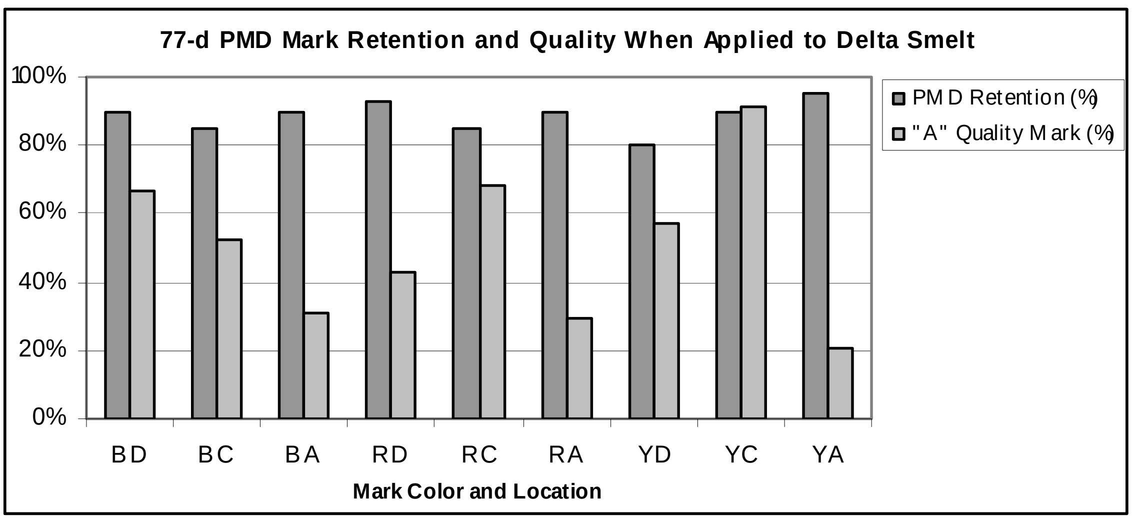

FIGURE 10.—Experiment No. 4: Mean delta smelt 77-d mark quality and retention rates when applied in blue (B), red (R), and yellow (Y) PMD on the dorsal (D), caudal (C), and anal (A) fins.

Survival and Fungal Development

Striped Bass Experiment No. 3

Striped bass (119.6 ± 1.13 mm FL) incurred no mortalities and showed no evidence of fungal development through the 35th day during Experiment No. 3.

Delta Smelt Experiment No. 3

There were no differences between 28-d survival of delta smelt, across treatments, in Experiment No. 3 (χ^2 = 14.5, DF = 9, P > 0.05), and mean 28-d survival was 92.8 ± 0.01 percent (*n* = 40, figure 11), and ranged between 60 and 100 percent. Mean survival, and ranges of survival on the 77th and 105th days of the experiment were 89 and 87 percent, respectively, and between 80 and 92.5 percent, and 77.5 and 92.5 percent, respectively (figure 11). Fungal growth did not appear within the first 28 days of experimentation, but by the 105th day appeared on 5.0 percent of test fish, and was present on 73 percent of observed mortalities.

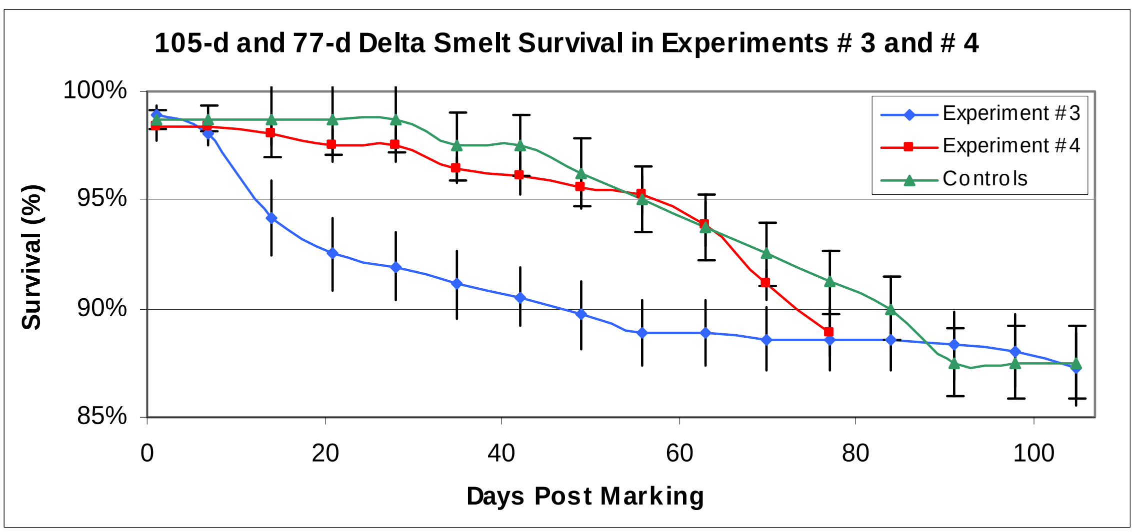

FIGURE 11.—Effects of the high-pressure injection of PMD on delta smelt survival in Experiments No. 3 and No. 4. Fish in Experiment No. 4 were provided a 3-d anti-fungal/parasitic treatment. Survival of control fish in both experiments was very similar, and for ease of comparison were averaged over both experiments. Individual dots are representative of treatment means, and error bars are displayed as ± the standard deviation of the mean.

Delta Smelt Experiment No. 4

Twenty-eight-day survival of delta smelt was the same across all treatments in Experiment No. 4 (χ^2 = 7.8, DF = 9, P > 0.05). The mean 28-d survival was 97.5 ± 0.01 percent (*n* = 40), and ranged between 90 and 100 percent. The mean 77-d survival was 84.8 ± 0.02 percent (*n* = 10), and ranged between 85.5 and 95 percent (figure 11). Fungal growth did not appear within the first 28 days of experimentation, but by the 77th day appeared on <1.0 percent of test fish and was present on 10 percent of observed mortalities.

Fin Meristics

There were no differences between fin thickness (mm) across samples taken from the dorsal, caudal, and anal fins of either delta smelt (76.4 ± 1.4 mm FL; P > 0.05), or striped bass (107.8 ± 2.8 mm FL; P > 0.05). Mean fin thicknesses for delta smelt dorsal, caudal, and anal fins were 0.19, 0.23 and 0.20 mm, respectively (table 1). Mean fin thicknesses for striped bass dorsal, caudal, and anal fins were 0.24, 0.38, and 0.28, respectively (table 1). Comparisons between species were not attempted because striped bass were significantly longer (P < 0.05), and therefore were expected to have longer and thicker fins.

TABLE 1.—Mean dorsal, caudal and anal fin thickness for adult delta smelt and juvenile striped bass (**n* = 4)

		Mean FL		Fin Membrane (mm)				Fin Ray (mm)		
	n	**(mm)**	**S.E.**	**Dorsal**	**Caudal**	**Anal**	**p-value**	**Dorsal**	**Caudal**	**Anal**
Delta Smelt	5	76.4	1.4	0.19	0.23	0.2	0.57	0.19	0.198	0.2
Striped Bass	9	107.8	2.8	0.24	0.38	0.28	0.31	*0.415	*0.4675	*0.18

DISCUSSION

High pressure injection of PMD proved an effective method and agent for marking adult delta smelt, particularly for short-term needs, but had some characteristics that rendered less favorable results for use with juvenile striped bass. The retention of PMD marks, as well as the survival of delta smelt, remained high throughout testing, and was unaffected by location or color. This was particularly evident through the 28th day, when retention and survival were 100 percent. Though retention and survival decreased with time through 105 and 77 days in Experiments No. 3 and No. 4, respectively, survival rates were similar to control fish in both experiments, suggesting the injection of PMD had no effect on fish survival. Most research has shown that the subcutaneous injection of dyes or elastomers into freshwater fish has no effect on survival (Pauley and Trout, 1988; Thedinga and Johnson, 1995; Dewey and Zigler, 1996). However, Dussault and

Rodriguez (1997) reported increased mortality associated with the jet injection of Alcian blue into the pelvic and pectoral fins of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*).

The current study reported > 80 percent retention through 15 weeks, for all combinations of injection location and color, when applied to adult delta smelt. Using similar injection methods, and the same marking agent, Catalano *et al*., (2001) reported lower retention rates when applied to smallmouth (19 percent retention after 17 weeks) and adult largemouth bass (5 – 40 percent retention after < 7 weeks). However, the subcutaneous injection of liquid elastomers, as well as other injectable dyes, tends to have similar retention times as the current study (Dewey and Zigler, 1996). Pauley and Trout (1988) reported > 90 percent retention of Alcian blue dye after 6 weeks, injected into different fins of brook trout and Atlantic salmon. Thedinga and Johnson (1995) observed > 76 percent retention of Alcian blue after 6 months, jet injected into the caudal fin of coho (*Oncorhynchus kisutch*) and sockey salmon (*Oncorhynchus nerka*), and Dussault and Rodriguez (1997) jet injected dye into the fins of brook trout and Atlantic salmon, and reported > 88 percent mark retention after 8 weeks.

A significant portion of delta smelt developed fungus on the body cavity near the mark injection site in Experiment No. 3, and the development was pronounced on test fish that experienced mortality. An evaluation of post-test delta smelt identified fluorescent microbeads, using a UV (ultraviolet) light source, in the body near the base of targeted fins. This lead us to believe that errant microbeads punctured external tissues near the base of the targeted fin, resulting in an open wound and increased opportunity for infection. The appearance of fungal development near injection wounds appeared to decrease significantly when fish were provided a 3-d prophylactic anti-fungal/parasitic treatment after handling and tagging. This was expected, as handling and confinement are stressful to fish, which may lead to reduced performance of the immunity system, resulting in increased rates of infection (Pironet and Jones, 2000). Anti-fungal/parasitic treatments can reduce the advancement of fungal development, parasites, and disease in fish (Leteux and Meyer, 1974; Pickering and Pottinger, 1989).

Survival of striped bass was also high and unaffected by mark location and color. However, using the described methods, most combinations of PMD color and fin location rendered less than adequate short-term (35-d) retention time, and high-pressure photonic marking is likely an inadequate marking method for juvenile striped bass mark-recapture studies. It appeared that the reduction in mark retention was due to the sensitivity of striped bass fins to chronic deterioration near mark application sites. Injection sites, for both species, appeared void of damage immediately after marking in Experiment No. 3. However, the area of the puncture wound in striped bass, and in particular puncture wounds on the dorsal fin, appeared to worsen as time post-marking increased, significantly deteriorating mark quality. This observation, however, was not supported by our laboratory studies comparing the meristic characteristics of the dorsal, caudal, and anal fins of both striped bass and delta smelt, as the mean thicknesses of all striped bass fins were greater than those sampled from delta smelt.

The application of PMD initially produced highly visible marks, all test fish appeared to recover equilibrium and began swimming efficiently within 1 min after handling and injection, and no mortalities were observed within 2-h post tagging. Employing the described methods, and two employees, approximately 300 fish could be tagged per hour, with the estimated cost of application to be approximately $0.10 per fish (or $0.05 per injection). This is similar to the efficiency and cost of injecting elastomer products and other types of dyes, but can be applied more rapidly and at a cheaper cost compared to visible implant coded tags, coded wire tags, and other external tags (Kelly, 1967; Blankenship and Tipping, 1994; Dewey and Zigler, 1996).

High retention rates, lasting at least 2 – 3 months, and high survival rates make this method of applying PMD a good choice for short-term adult delta smelt research applications. All marks were highly visible, distinguishable and versatile (high number of possible marks). Though a large portion of marks had reduced in quality, from A marks to B marks over the time frame of each experiment, they were still easily recognizable. Colors provided by the manufacturer (red, yellow, green, orange, pink, black, blue, brown, purple, and white) can be mixed to create numerous marks. A standard CO_2 high pressure cylinder, regulator, and high pressure hose are necessary for applying the PMD as indicated by the manufacturer's methods. However, similar products have been administered successfully using techniques applicable for field research (Roberts and Angermeier, 2004).

The procedures and injection pressures outlined in this report are good starting points for marking similar species. Given the extreme sensitivity of delta smelt to handling stress, it is likely that the described methods and marking agent will allow for even higher survival in other freshwater fish (Swanson and Cech, 1995). However, fish condition and survival rates post-tagging are prone to be highly variable as they rely on the experience of the person marking and the quality of post-marking care. Mark retention rates can be variable between fin type, likely due to differences in fin sensitivity and thickness. This research has shown that a standard injection pressure cannot be used for all species and life stages. Fish taxon, size, fin type, and fin thickness should ultimately dictate application pressure. This makes the adjustable nature of a CO_2-regulated photonic marking gun advantageous when a study design calls for the application of marking agents to different fins, and sizes and species of fish.

RECOMMENDATIONS

Continued research is necessary in developing marking techniques and possible areas of injection for PMD for all species of fish studied at the TFCF. It is reasonable to consider that other injectable solutions or injections in other areas on the body would result in higher retention rates in fish. Hayes *et al*. (2000) had increased success when applying an injectible solution into the pectoral girdle of adult fish, while Roberts and Angermeier (2004) recommend the use of visible implant elastomer tags over PMD injections for two genera of darters. The effects any marking agent has on fish performance should also be evaluated, especially if it is to be considered for mark and recapture research at a fish collection facility where fish collection and survival is dependent upon swimming performance. I would recommend evaluating the impact visual implant elastomer (VIE) and PMD marks applied to the caudal fin of south delta fish species has on the maximum sustained swimming velocity (U_{crit}).

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