Experimental Marking Techniques for Young-of-Year, Hatchery-Reared Striped Bass

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Abstract: Several experiments were tried with varying results using 5 different marking techniques in an attempt to permanently mark juvenile striped bass (*Morone saxatilis*). Techniques included: immersion staining, dye injection, tetracycline ingestion, streamer tagging, and fin-clipping. From a practical and economical standpoint, 2 methods were satisfactory, at least for short term marking: fin-clipping and tetracycline ingestion. The latter method showed some promise of permanency in mark retention. Other methods were either too ephemeral, too expensive, or caused considerable mortality.

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Since 1873, when attempts were made to tag Atlantic salmon in the Penobscot River, Maine, fisheries scientists have been looking for an ideal marking technique (Everhart et al. 1975). This ideal method still appears just beyond our grasp. Despite recent advances with examination of bone ultrastructure, electrophoresis, and the renewal of chemical injection or ingestion techniques, a method of delivering and detecting a suitable long term mark has yet to be established. Primary problems associated with present techniques include: (1) the mark is not permanent or, (2) high morality is directly or indirectly related to the marking technique.

Mortality associated with marking fish, especially juveniles, is usually so high that costs and effort of marking is frequently more than the benefits received. This is especially true if population enhancement is the ultimate goal and population estimates are secondary.

This study was the direct result of a project designed to enhance young-of-year striped bass (*Morone saxatilis*) population in the Santee River, South Carolina, by addition of hatchery stock. Concurrently, population estimates of this population at various points in time were to be obtained. Therefore, it was necessary to find a suitable method to "permanently" identify hatchery-reared striped bass.

To assist in the objective, an extensive literature review and compilation

of a bibliography of previous publications associated with marking or tagging fish were made and are included in a final report by Harrell (1982). For additional reviews of various marking techniques the following references are suggested: Arnold (1966), Stott (1971), Everhart et al. (1975), and Dolloff (1979). I would like to thank Jack D. Bayless, chief of the Rembert C. Dennis Wildlife Center, for his help and guidance and the Dennis Wildlife Center staff for their rearing and marking the fish involved in this study. I also thank Lou Villanova of the U.S. Fish and Wildlife Service and Sumter Moore of the S.C. Wildlife and Marine Resources Department, for administration of the funding for this study. Funding was made possible by the U.S. Fish and Wildlife Service through the Anadromous Fish Conservation Act (PL-89-304). Special thanks go to Kathie Dennis for typing and reviewing this paper.

Methods

Earthen ponds at the Dennis Wildlife Center in Bonneau, South Carolina, were stocked with striped bass larvae in April and harvested 30 to 45 days later. At harvest, fingerling striped bass were between 25 and 45 mm in total length. Fingerlings to be used for marking studies were brought into a fish holding house, placed in concrete raceways, and treated with salt (1%) and furacin (22 ppm active ingredient) before and after marking attempts were performed. Tests were divided into 5 categories: dye injection, dye immersion, the feeding of tetracycline, tagging, and fin-clipping. Each experimental design will be discussed individually.

Immersion Dyeing

Nine biological and histological dyes (Table 1) were tested by exposing experimental fish to an osmotic differential with the expectation that the dye would be absorbed into the tissue when the fish responded to ionic differences. Theoretically, dye particles would be transported across cell membranes while the organism is achieving isotonic equilibrium.

A total of 15 fish were used for each experiment. All test animals were placed in oxygenated 37-liter aquaria and salinity was gradually raised from 0 to 35 ppt. Dyes were diluted and aerated in ambient freshwater.

Prior to testing of the dyes, the effects of direct transfer from 35 ppt salinity to freshwater were determined. Then experimental fish were transferred from the high salinity concentration (Rila Marine Mix Synthetic Sea Salt) directly into freshwater to which the various dye concentrations had been added. Animals were placed into containers in 3 groups of 5 fish each. Behavior was recorded during the exposure time of 1 hour. After exposure, test fish were placed into clean freshwater containers. Dye retention and behavior were noted hourly for the first 10 hours following test initiation and

Table 1. Immersion dyeing experiments with fingerling striped bass ($N = 15$ for each test). Results are identified as positive dye uptake (+) or negative dye uptake or mortality (−). Exposure time = 1 hour.

Dye	Concentrations[a]	Results	Duration
Fluroscein	1 : 1,000	+	48 hours
Fuschsin	1 : 10,000	−[b]	
Aniline blue	1 : 1,000	+	48 hours
Aniline blue-black	1 : 1,000	+	48 hours
Red food coloring	1 : 100	−	
Green food coloring	1 : 100	−	
Red india ink	3 : 100	−[b]	
Black india ink	1 : 100	−[b]	
Animal tattoo ink	1 : 1,000	−[b]	

[a] Concentrations derived from pilot studies.
[b] Mortality within 10 hours after immersion.

twice daily thereafter. Results were recorded as positive (dye uptake) or negative (mortality with or without dye uptake). If tests were positive, duration of dye retention was determined.

Injection

Various techniques for injecting a biological stain or dye into fish tissue were tested as a means to permanently mark hatchery-reared striped bass. Two types of hypodermic syringes and 1 pneumatic injector (Schuco, American Caduceus Industries Inc.) were used to deliver a dye intramuscularly into juvenile striped bass.

One hypodermic was a standard, disposable 3 cc syringe with a 21-gauge needle. The second was a dental syringe with a 26-gauge intraosseus needle, 7.7 mm long. The pneumatic injector was a portable, hand-held, spring-loaded mechanical plunger device that when triggered delivered a relatively constant volume of liquid from a 4.0 ml reservoir.

Three dyes were tested with each device: aniline blue, black India ink, and a veterinarian grade tattoo ink. All were injected in either a dorsal or anal fin muscular region (Table 2). Total length (TL) of experimental fish ranged from 40 to 95 mm ($x = 75$ mm).

Two sets of experiments were designed to determine effects of injection and time mark retention. In 1 experiment, 2 groups of 20 fish were injected intramuscularly around the dorsal fin with the pneumatic injector as described by McIlwain and Christmas (1975). One group received aniline blue and the second received black India ink (Table 2). All injected fish were placed in aquaria and observed for 2 months. At the end of this period, surviving fish were removed, mark retention was noted, and the fish were stocked into an earthen pond (0.4 ha).

The second experiment utilized 6 groups of 50 fish each including con-

Table 2. Pneumatic and hypodermic dye injections of fingerling striped bass (total length $\bar{x} = 75$ mm).

Dye	*N*	Mark location	Injection method[a]	Survival w/mark		Duration[b] (months)
				N	%	
Aniline blue	20	Dorsal	Pneumatic	10	50	2
Black india ink	20	Dorsal	Pneumatic	10	50	2
Controls	50			20	40	3
Black india ink	50	Dorsal	Hypodermic	22	44	3
Black india ink	50	Anal	Hypodermic	25	50	3
Animal tattoo ink	50	Dorsal	Pneumatic	22	44	3
Animal tattoo ink	50	Anal	Hypodermic	42	84	3
Black india ink	50	Anal	Pneumatic	36	72	3

[a] Pneumatic injection with Schuco injection; Hypodermic with dental intraosseous syringe.
[b] Experiment terminated after 3 months.

trols, and varied location, type of dye, and method of injection (Table 2). All fish receiving marks with the hypodermic were injected with the small dental syringe, as the needle of the "standard" disposable syringe delivered too great a volume. After injection, fish were placed in earthen ponds (0.4 ha) where they remained for 3 months until harvest. They were then examined for mark retention.

Feeding

In 2 experiments, oxytetracycline was mixed in fish feed and fed to several thousand fish. In the first experiment 4 grams of tetracycline were hand-mixed in 3.8 liters of cod liver oil and combined with 45 kg of fish feed. Fish were then fed intensively in concrete raceways for a period of 1 week. After this feeding period, 50 fish were examined for tetracycline deposition in bone material. A hand-held ultraviolet light and a fluorescent light microscope were used for detection. Approximately 1,000 fish were placed in an earthen pond for 2 months to determine mark retention. Surviving fish were harvested and examined as previously described.

In the second experiment, tetracycline was premixed as oxtetracycline-quantenary salt (4,000 g/liter/ton) at the feed mill during pelleting process and then shipped as medicated feed (Murray Elevator Co., Murray, Utah). The second group of fish were fed this premixed tetracycline feed and treated in the same manner as the first group.

Tagging

The fourth method of marking was to use an external tag. Because of the trauma associated with needle insertion, this marking method was restricted to use with advanced fingerlings (>100 mm).

A shrimp streamer tag (FTSL-73, Floy Manufacturing Co., Seattle,

Washington) was inserted completely through the dorsal muscular region of tested fish. Care was taken to insure the middle portion of the tag was situated between 2 dorsal pterygiphores. This insertion allowed the tag to lie back along the body and not impede swimming motion. Two sizes of tags were used that varied in the width of the center portion of the tag (2 mm and 1 mm). A total of 15 fish were tagged (Table 3) and released into a 0.4 ha pond. After 2 months, the pond was drained and fish were examined for tag retention.

A second tagging experiment utilized the same procedure as the shrimp streamer tags but used braided multifilament nylon instead of the commercially produced vinyl tags. A total of 200 fingerlings (>100 mm TL) were tagged. Fish were divided into 2 lots of 100 each. The first group had sections of multifilament (50 mm long) simply inserted through dorsal muscle tissue and left dangling. The second group received the same treatment except both ends of multifilament had been heated and flattened to prevent the tag from slipping out of the site of insertion. These fish were held and fed in concrete raceways for 1 month at which time survivors were checked for tag retention.

Fin-Clipping

The most frequent method of tagging or marking utilized, fish finclipping, was also tested. Young-of-year striped bass were harvested and brought into a fish holding house. Once inside, they were placed in concrete raceways and prophylactic treatments were used as necessary to minimize losses from harvest and handling stress.

Fish were allowed to acclimate to oxygenated well water (20° C) for at least 24 hours before further handling. After the fish acclimated, small plywood fiberglassed clipping troughs (0.6 m × 0.6 m × 2.4 m) were set up beside each raceway. These troughs were filled with well water containing 2.0 ppm quinaldine sulfate to slow movement and facilitate handling. Small nets, 0.3-cm-ace mesh, suspended in the troughs, allowed workers to have an ample supply of anesthetized fish available for clipping.

Throughout the study, 4 different fins were clipped on different groups of fish to differentiate fish from different stocking sites and to monitor move-

Table 3. Results of tagging experiments using Floy shrimp tags and braided multifilament tags.

Tag type	Size or type	*N* tagged	*N* Survived
Shrimp streamer	Large	9	4
	Small	6	3
Braided multifilament[a]	Flattened	100	0
	Unflattened	100	8

[a] 96% loss due to heavy fungal infections.

ment following release. After all fish were clipped, prophylactic treatments were once again used to mitigate effects of handling. The fish were re-acclimated to ambient river temperature and stocked.

Results

Immersion Dyeing

None of the immersion dyes tested yielded satisfactory results (Table 1). Only 3 (fluorscein, aniline blue, and aniline blue-black), had positive results. Although the dye was readily taken into tissue, all traces of color had vanished within 48 hours.

Varying concentrations of dyes used were tested in pilot studies. Those concentrations shown in Table 1 were the most effective found. All other concentrations on the order of 1 or more magnitudes higher or lower resulted in rapid mortality (within 30 min) or no dye uptake. There was no evidence of osmotic shock found in the controls.

Injection Techniques

As a general rule, fish less than 70 mm could not be marked with the pneumatic injector because the force of the spray caused mortality. However, hypodermic injections from a small dental syringe were effective on fish >25 mm (Table 2). In all cases, an identifiable mark was still evident in surviving fish at the time the experiment was terminated.

Overall, survival rates were higher in fish injected by a needle than those injected by the pneumatic innoculator. There was also a higher survival rate in fish marked in the anal region versus those marked in the dorsal. This was true for fish marked with hypodermic or pneumatic methods.

Several thousand fish were marked with aniline blue and released into the Santee River. Seven of these were re-captured while sampling the river 1 year later. The mark, although still evident, was very faint and tissue had to be dissected to confirm the presence of the mark.

Feeding

Of the 2 experiments testing tetracycline feeding, the experiment in which tetracycline was mill-mixed had more successful marking results. Approximately 50% of the fish were definitely marked in the first experiment where tetracycline had been mixed by hand. In the second experiment with feed mill-mixed tetracycline, 99% were obviously marked.

After four mouths, only 1% of the fish from the first experiment survived, resulting in too low of a percentage to express valid results. However, after four mouths, surviving fish (6% of original stocking) from the second test were examined and 88% (53 out of 60) still had identifiable marks.

Tagging

In experiments on tagging methods, it was necessary to have larger fingerlings (>70 mm) than were used for other studies. Shrimp streamer tags similar to those used by Texas Instruments for marking Hudson River fingerling striped bass (Don Strout, Texas Instruments Inc., pers. commun.) were used for this study. As shown in Table 3, 15 shrimp streamer tags were used and at the end of 2 months, 7 of the 15 fish survived. Although all surviving fish had tags present, the legend stamped on the vinyl was not discernible.

The second experiment, using needles from the streamer tags and inserting braided multifilament, was considered a complete failure. Heavy fungal infections developed and only 8 out of the 200 fish survived (Table 3).

Fin-Clipping

Techniques described in the previous section allowed large numbers of fingerling striped bass to be marked and stocked in a relatively short period of time. Over the 5-year period of this study, more than 800,000 fish were marked by fin removal. A worker could remove the anal fin (most commonly used) at an average rate of 400/hour.

Mortality was relatively high (25%-30%) the first year due to heavy fungal infections; however, improved prophylactic treatments reduced this mortality to an average of less than 17% estimated 2 days post stocking from samples which were retained in wooden troughs. Mark retention and recognition lasted for 6 weeks in summer and 3 months in winter before regeneration occurred.

Discussion

As is usually true in most marking and tagging technique studies, unsuccessful results are more common than successful results. Such was the case with this study. It is obvious upon examination of the plethora of papers represented in the report by Harrell (1982) that a satisfactory solution has yet to be found. Each method tested and retested in its own right has merit, yet almost all leave something to be desired.

Each experiment had certain desirable aspects that would prove of value if the technique was successful. For example, if a suitable immersion dye could be developed, mass marking could be accomplished with limited handling and holding stress and the mark could be delivered quickly, economically, and efficiently. Yet in the case of the 2 dyes that were successfully absorbed, the mark was only present for 48 hrs. This is too brief a time if population estimates are to be determined. The other dyes were unsuccessful in that either no mark was evident or mortality was the end result. The failure of the dye to be absorbed into the skin may have been a function of mole-

cular structure of the dye being too large to cross cell membranes without some form of active transport.

Problems associated with dye injection techniques stem directly from the fact that it is necessary to individually handle each fish. Handling of small striped bass, especially those which have not been anesthetized, is traumatic and physically harmful for the fish. Mucous and scales which are primary defenses against bacterial and fungal infections are removed by handling. In addition, the technique is extremely tedious and time consuming. Use of the pneumatic injector speeded up the process of tagging but it could only be used on fish greater than 70 mm. Smaller fish were in most cases "blown apart" by the force of the spray or massive tissue damage resulted in impaired swimming performance .

Of the methods tested, feeding oxytetracycline directly to fish was the most suitable means to mark young-of-year striped bass. It now appears that all criteria necessary for an ideal mark were obtained with use of the premixed tetracycline diet. This marking technique is relatively inexpensive, has a simple means of delivery, eliminates excessive handling, and the mark does not appear to be as ephemeral in retention as other marks.

It was necessary to rely upon the mill-mixed formulation before suitable numbers of fingerlings could be marked because in the experiments attempted with on-site mixing of oxytetracycline with feed, it appeared that there was a leaching out of oxytetracycline and oil as it came in contact with water. Premixed medicated feed appears to have solved this problem in that tetracycline is bound in the feed during the pelleting process.

To date, it appears various agencies have decided to use this method of marking for population studies. Jim Maxwell, manager of Welaka National Fish Hatchery, Florida, has found mark retention of tetracycline-fed striped bass to be in excess of 1 year (pers. commun.). The Striped Bass Committee of the Southern Division of the American Fisheries Society decided on a tetracycline-fed mark as a means to identify young-of-year striped bass in an ongoing stocking evaluation program presently being undertaken on Lake Greeson, Arkansas. Future plans here at the Dennis Wildlife Center include possible elimination of fin-clipping as a marking technique and dependence entirely on tetracycline marking.

Detection is a major problem in marking small fish with tetracycline. Once the fish has grown to a yearling stage the mark will be covered by subsequent calcium deposits. It may be necessary to section bones of the fish and examine them under a fluorescent microscope before the presence of a mark can be determined. Therefore, an easier means of detecting tetracycline should be found that can be readily adapted to field use.

Tagging with Floy streamer tags and braided multifilament exhibited very similar problems as the dye injection experiment because each fish had to be individually handled. In addition, cost of streamer tags makes tagging of large numbers of fish impractical. Due to construction of the needle's eye

of the attachment, where the vinyl tag is connected, severe tears in muscle tissue often resulted with tag insertion. Irritation around the tagged area was evident in almost all 15 fish tagged. This was due to constant rubbing of the tag against the wound during swimming motion. The wound areas were also ideal locations for bacterial and fungal infections as evidenced by the massive die-off from the second test (Table 3).

Fin-clipping has long been a quick and easy method for marking fish of all sizes. Until recently, it was the only marking method adequate for population estimates at the Dennis facility. As with other studies, each fish had to be individually handled, but since they were anesthetized before clipping, stress was minimized. Careful prophylactic treatments before and after clipping greatly enhanced success.

Although fin-clipping was suitable for quick population estimates and movement patterns, long term studies will require a more reliable mark.

Recent work in fin-clipping warmwater fish and then freezing the wound (Boxrucker 1982) shows promise but this entails extra handling and added stress to the individual fish, not to mention increased time in marking the individual fish.

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