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ARTICLE

## Evaluation of Internal Tag Performance in Hatchery-Reared Juvenile Spotted Seatrout

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### Abstract

Stock enhancement programs rely on the ability to recapture and identify stocked fish to evaluate stocking effectiveness. Since 2006, the Seatrout Population Enhancement Cooperative (SPEC) has released almost 600,000 Spotted Seatrout *Cynoscion nebulosus*, about 100 mm TL, tagged with opercular coded wire tags (CWTs) into coastal Mississippi waters. However, only about 50 fish have been recaptured and initial retention of the opercular CWT has rarely exceeded 75%. This study first evaluated the suitability of visible implant alpha (VIA) and visible implant elastomer (VIE) tags for use in juvenile Spotted Seatrout. The VIA tags performed poorly. Based on those results, the study evaluated the effects of tagging site and fish size on survival, growth, and retention of CWTs and VIE tags and VIE tag fragmentation in juvenile Spotted Seatrout. Three separate growth experiments with juvenile Spotted Seatrout that had mean initial TLs of 93, 138, and 152 mm, respectively, were conducted to assess the effects of tagging. Each growth experiment had nine treatments consisting of a control, fish with either an opercular CWT, a dorsal muscle CWT, a ventral caudal fin VIE tag, or a jaw VIE tag, and four false-tagged treatments corresponding to each tagged treatment. Dorsal CWTs were retained better than opercular CWTs; VIE tags were equally retained regardless of body location. However, VIE tag quality was affected over the long term by pigmentation overlap and fragmentation. Growth rates and survival were not different within any size-class experiment or among treatments. This study has shown that CWTs and VIE tags are effective marking methods for juvenile Spotted Seatrout.

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Almost 40% of known fisheries stocks are considered overfished, depleted, or recovering from depletion (FAO 2010). Even though capture-fisheries production has been stable since the late 1980s (Diana 2009; FAO 2010), a burgeoning human population, which is predicted to grow from 7 to 9 billion by 2050 (Cohen 2003), and shifts in social and economic factors have substantially increased the demand for fisheries products for both food and recreation. Management responses to declining and overfished stocks as required by the Magnuson–Stevens Act have routinely included two approaches: the regulation of fishing effort through restrictions on catch, season, or gear and habitat restoration. Although potentially effective, these techniques often do not produce quick results.

Stock enhancement, or the release of cultured fish into the wild to supplement wild populations, constitutes a third approach. Stock enhancement is a well-developed and accepted

practice in inland freshwater systems dating back to the 17th century (Stickney 2000). Marine stock enhancement (MSE) historically suffered from the difficulties in culturing marine larvae and monitoring releases in an open system (Leber 2004). After 80 years of stocking billions of unmarked fry of a variety of species into marine waters, the expected improvements in fishery yields never materialized and the practice fell out of favor. Despite management efforts, the decline of wild fisheries continued throughout the latter part of the 20th century and renewed interest in alternative management strategies. Blankenship and Leber (1995) argued that technology had advanced such that a comprehensive quantitative assessment of MSE could be accomplished to explain the success or failure of the practice and, as such, this approach should be considered a viable tool in a comprehensive fisheries management strategy.

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Along with improvements in feeding and filtration technology, the success of MSE depends on the ability to identify and track hatchery-reared fish as they recruit to and supplement wild populations. Tagging has permitted the assessment of important ecological questions such as dispersal (Hendon et al. 2002; Miller and Able 2002; Able et al. 2012), straying (Candy and Beacham 2000), and the use of essential habitat (Able et al. 2006, 2012). While oxytetracycline and genetic marking play an important role, physical tags are the most widely used method of identifying individuals or batches of fish. Different tag designs, however, elicit different host responses and have different retention and recovery rates. The demand for a tag that is well retained and inflicts little physical damage has led to the development of visible implant elastomer (VIE) and visible internal alpha (VIA) tags, which are embedded just beneath the epidermis (Guy et al. 1996) and are externally visible, and the coded wire tag (CWT), which is injected below the epidermis and not externally visible.

The CWT is a 1-mm-long piece of magnetized stainless steel imprinted with an identifying code. This small size makes it ideal for use in small fish (Leblanc and Noakes 2012). The imprinted codes are typically in a sequential format that allows for hundreds of thousands of coding possibilities that facilitates the efficient, long-term marking of large numbers of fish in a relatively short period of time. However, CWTs require postmortem recovery.

The Spotted Seatrout *Cynoscion nebulosus* is an obligate estuarine species with high natal fidelity and limited migration (Hendon et al. 2002; Comyns et al. 2008; Johnson et al. 2011) and is the most popular recreational catch in the Gulf of Mexico (Perret et al. 1980; Hettler 1989; Johnson et al. 2011). As with many heavily fished populations, the spawning potential ratios (SPRs) often have been less than what is generally considered ideal. In Mississippi, Fulford and Hendon (2010) noted that the annual fishing mortality is close to that for maximum sustainable yield and the population is highly dependent on annual recruitment. The combination of the Spotted Seatrout's biological characteristics and its popularity, therefore, make it a potentially suitable candidate for MSE. As a result, an evaluation of the feasibility of MSE as part of a comprehensive management strategy for Spotted Seatrout began in 2005, and since 2006 almost 600,000 juvenile seatrout with opercular CWTs have been released in Mississippi waters. Few tagged fish, however, have been recovered, and 30-d laboratory tag retention evaluations have rarely exceeded 75% retention (authors' personal observations), which limits the ability to assess the potential effectiveness of the program.

To improve the ability to assess the success of a potential MSE program, we examined the performance of internal tags in juvenile hatchery-reared Spotted Seatrout. We first assessed the efficacy of VIA and VIE tags for use in Spotted Seatrout in an attempt to narrow the choices for potential tagging sites on the fish. Based on those results, we then used three separate size-classes (mean TL of 93, 138, and 152 mm) of fish to eval-

uate the effects of the tagging site (including the currently used CWT location) and fish size on survival, growth, and retention of CWTs and VIE tags in juvenile Spotted Seatrout. We also evaluated VIE tag fragmentation.

## METHODS

*Source of fish.*—Spotted Seatrout for this study were maintained at the University of Southern Mississippi (under Institutional Animal Care and Use Protocol 08050801). Larvae were spawned from captive adults held in closed recirculating systems under controlled environmental conditions in a manner similar to Arnold et al. (1978). To standardize age and growth, all experimental fish, except for those used in the preliminary evaluations, were derived from a single spawn. After about 50 d of rearing in a series of recirculating tanks in which the larvae were weaned from rotifers (*Brachionus* sp.) to brine shrimp (*Artemia* sp.) and finally onto dry pelleted food, the fish were maintained on commercial pelleted food (Skretting, Stavanger, Norway) at about 3% of their body weight per day until they were needed for the growth experiments. Dissolved oxygen was maintained above 4.5 mg/L, water temperature was maintained at about 27°C, and salinity was maintained at 25‰. Ammonia (as ammonia nitrogen, NH<sub>3</sub>-N) and nitrite (as nitrite-nitrogen, NH<sub>2</sub>-N), maintained at <0.25 mg/L through the use of some combination of Polygeyser bead filters, sand filters, foam fractionators, activated carbon, and ozone, were measured daily using Hach test strips (Hach, Loveland, Colorado).

*Preliminary research.*—Pilot research was conducted with only VIA and VIE tags (both Northwest Marine Technology) to narrow the choices for suitable tagging locations in juvenile Spotted Seatrout (105–146 mm TL). Twenty-four fish were injected with red VIE tags either anteroposteriorly into the ventral surface of the caudal peduncle ( $n = 8$ ), the ventral jaw tissue ( $n = 8$ ), or the ventral surface just anterior to the pelvic girdle ( $n = 8$ ). These tags were all retained with no mortalities at 18 d post tagging (DPT); however, pelvic tag retention had declined to 63%. Mortality associated with VIE tags was low, but cannibalism was greater than 50%, which thus compromised long-term retention data on all tags. Actual retention is unknown, but at 51 DPT six of the initial 16 ventral jaw and caudal peduncle tags were still present and of good quality. Thus, the caudal peduncle and ventral jaw localities were selected as the experimental tagging sites.

Twenty fish were implanted with VIA tags anteroposteriorly in either the caudal peduncle ( $n = 10$ ) or ventral jaw tissue ( $n = 10$ ) and by 4 d after tagging, all VIA tags had been lost or the fish had died. Because all fish had been lost, a second trial was conducted with 20 additional fish. In this trial tag retention at 51 DPT was 30% (ventral jaw) and 0% (caudal peduncle). The tags that were retained were unreadable due to pigmentation overlap. Due to their poor performance, VIA tags were omitted from the remainder of the study.

*Experimental design.*—The experimental system for the main portion of the study consisted of thirty 60-L black polyethylene tanks, each supplied with its own aeration, but connected to a common water supply and filtration system. Three separate 30-d growth experiments using progressively larger fish were conducted in succession. Experiment 1 used 135 fish (mean length, 93 mm TL at the beginning of the experiment), experiment 2 used 135 fish (mean length, 138 mm TL), and experiment 3 used 120 fish (mean length, 152 mm TL). At the beginning of each of the three experiments, fish were moved individually from the holding tank, anesthetized with tricaine methanesulfonate (MS-222) until they lost equilibrium while held in water treated with StressCoat (Aquarium Pharmaceuticals, Chalfont, Pennsylvania), weighed (wet weight [WW, g], measured [TL, mm]), tagged before being allowed to recover, and moved into the experimental system. Fifteen fish (or 12 fish in the case of experiment 3) were tagged in each of the following ways before five (or four) of each were randomly assigned to each of three replicate tanks for a total of 27 tanks: (1) a standard sequential CWT (Northwest Marine Technology) injected in the opercular musculature in a dorsoventral direction using a Mark-IV automatic injector (Northwest Marine Technology); (2) a standard sequential CWT injected in the epaxial dorsal musculature in a posteroanterior direction using a Mark-IV automatic injector; (3) a red VIE tag manually injected into the ventral caudal fin tissue in a posteroanterior direction using a 29-gauge, hand-pressurized, hypodermic syringe; or (4) a red VIE tag injected into the ventral jaw tissue in an anteroposterior direction using a 29-gauge, hand-pressurized, hypodermic syringe. Each of the four tag treatments had a corresponding “handling” control in which an equal number of fish in an equal number of tanks were false-tagged, meaning the tag-specific protocol for each of the four groups including the insertion of the needle was performed on the appropriate body part without tag injection. An additional overall experimental control group consisted of fish that were moved from the grow-out tank, anesthetized, weighed, measured, and handled only when tagged fish were assessed for growth at days 15 and 30. The three remaining tanks were stocked with five unmarked fish each to serve as replacement fish for treatments that experienced mortality in order to maintain similar growth conditions and densities between treatments. Fish were fed 3.0- or 4.0-mm dry feed (Skretting) at 3% of total body weight per day based on initial measurements.

Tag retention was evaluated on days 3, 6, 9, 12, and 15 during the first 15 d to assess immediate retention (Wagner 2009). On retention assessment days, only fish belonging to the false-tagged and tagged treatments were netted, removed from their experimental tank, anesthetized, checked for tag retention, elastomer quality (if applicable), and signs of infection before being returned to their respective tank. Elastomer quality was judged as being a full tag (continuous elastomer) or a fragmented tag (no continuous elastomer). On days 15 and 30, the same retention assessment was conducted; however, fish from all treatments, including the handling control group, were removed and each fish was anesthetized, weighed, and measured. Based on these

measurements, feed rate and pellet size were adjusted to accommodate fish growth and maintain a 3% body weight per day feeding rate. Upon completion of each 30-d growth experiment, all tagged fish were grouped together and moved into a 600-L tank within a closed recirculating raceway for estimation of long-term tag retention. Retention and tag quality were monitored once a month for a total of 4 months after tagging. These fish were also weighed and measured at 60, 90, and 120 d after transfer.

*Statistical analyses.*—A two-way ANOVA was used to compare 30-d growth rate (g/d) between each tagging treatment ( $n = 9$ ) and size-class ( $n = 3$ ). Sidak's post hoc tests were used to separate mean growth rates among the main factors.

Analysis of percent tag fragmentation (expressed as the number of fish with fragmented tags over the total number of VIE-tagged fish per location) of jaw ( $n = 3$ ) and caudal VIE ( $n = 3$ ) tag treatments only (between-subjects factors) across four time periods (30, 60, 90, and 120 d; within-subject factors) was conducted with a split-plot ANOVA for each growth experiment. All tag fragmentation percentage data were arcsine-transformed prior to analysis. Mauchly's test was also conducted to assess the sphericity assumption of the repeated measures analyses. When significant  $F$ -values were found for the between-subjects factors, mean values were separated using Sidak's post hoc test. If sphericity was violated, the Greenhouse–Geisser correction was used to adjust the degrees of freedom in order to determine significance for the within-subjects tests.

All analyses were performed with SPSS (version 15 or 20); all values were considered significant when  $P \leq 0.05$ . All data used in ANOVA procedures were tested for normality (one-sample Kolmogorov–Smirnov [K–S] test) and heterogeneity (Levene's test) assumptions prior to analysis (Field 2005).

## RESULTS

### Survival

At 30 DPT, Spotted Seatrout survival was high in all treatments across all growth experiments and with both tag types. There was only a single tagging-associated mortality throughout the duration of the 30-d study that occurred at 6 DPT in a fish with a VIE tag inserted in the jaw during experiment 2.

Fish in all treatments within experiments were present at the 120 DPT assessment except for (1) the loss of a replicate of a fish with a VIE tag inserted in the caudal fin in experiment 2 due to an air line malfunction at the day-30 assessment and just prior to combining the fish for continued assessment, and (2) the loss of all fish with dorsal CWTs and fish with VIE tags in the jaw at 93 DPT in experiment 2 as a result of a filter malfunction.

### Tag Retention

*Coded wire tags.*—Tag retention after 30 d varied slightly between experiments for fish tagged with a CWT in the opercular musculature (Table 1). The retention of opercular CWTs for growth experiments 1, 2, and 3 was 87, 80, and 92%, respectively, indicating high retention across size-classes. These

TABLE 1. Tag retention after 30 d for coded wire tags (CWTs) and visible implant elastomer (VIE) tags for three successive growth experiments in juvenile Spotted Seatrout. A bacterial infection prior to growth experiment 3 reduced replicate totals to  $n = 4$  instead of 5 fish per replicate and subsequently to  $n = 12$  fish per treatment instead of 15 as in experiments 1 and 2. Initial TL is included for each experiment.

Tag and location	Experiment 1 (fish TL, 93 mm)			Experiment 2 (fish TL, 138 mm)			Experiment 3 (fish TL, 152 mm)		
	$n$	Tags lost	% retention	$n$	Tags lost	% retention	$n$	Tags lost	% retention
Dorsal CWT	15	0	100	15	0	100	12	0	100
Opercular CWT	15	2	86.7	15	3	80	12	1	91.7
Caudal VIE tag	15	0	100	15	0	100	12	0	100
Jaw VIE tag	15	0	100	15	0	100	12	0	100

percentages remained constant to 120 DPT. No tag loss in fish with the opercular CWT was observed after 9 DPT. Dorsal CWT retention was 100% at 120 DPT for all experiments.

*Visible implant elastomer tags.*—All VIE tags were 100% retained at 120 DPT regardless of growth experiment (size-class) or tag location. The use of ultraviolet light and amber-shaded sunglasses was often required to increase the visibility of both tags, especially in the caudal location. However, tag fragmentation was present in fish in all growth experiments for both tag locations. Fragmentation did not change significantly by time (split-plot ANOVA:  $df = 3$ ,  $P = 0.075$ ) nor tag location (split-plot ANOVA:  $df = 1$ ,  $P = 0.613$ ; Figure 1) for either growth experiment. However, there was a possible trend over time for decreased fragmentation with increased fish size in the jaw-tagged fish (Figure 1A) and final 120-d fragmentation percentages were 66.7, 50.0, and 41.7% for growth experiments 1, 2, and 3, respectively. In contrast, final caudal tag fragmentation was 20, 90, and 25% for experiments 1, 2, and 3, respectively (Figure 1B). Caudal VIE tags experienced the highest fragmentation rates in experiment 2 (fish TL, 138 mm) with percentages ranging from 26.7% at 30 DPT to 90% at 120 DPT. Additionally, darker pigments in the tail region of juvenile Spotted Seatrout resulted in pigmentation overlap, which affected tag visibility in the caudal VIE treatment.

### Growth

Mean 30-d growth rates (g/d) were not significantly different (Figure 2; Table 2) among treatments within an experiment (ANOVA:  $F_{1,8} = 0.514$ ,  $P = 0.844$ ), but there was a significant size-class effect (ANOVA:  $F_{1,2} = 21.914$ ,  $P < 0.001$ ). Sidak tests indicated that growth rate was significantly greater in size-class 1 (experiment 1) compared with those in experiments 2 and 3 (all  $P < 0.001$ ) and that growth rate in size-class 2 (experiment 2) equaled that in size-class 3 (experiment 3) ( $P = 0.619$ ). However, while the data were normally distributed (K-S test: all  $P > 0.428$ ) there was a slight deviation in the homogeneity of variances (Levene's test:  $P = 0.036$ ). We consider this difference negligible, however, based on Underwood's (1997) discussion of the robustness of ANOVA to these minor violations.

### DISCUSSION

Survival was excellent for Spotted Seatrout of all sizes tagged with either a VIE tag or a CWT. Except for a single fish with a VIE tag inserted in the jaw that died at 6 DPT, no large-scale, unexplained mortalities occurred during the 120-d duration of

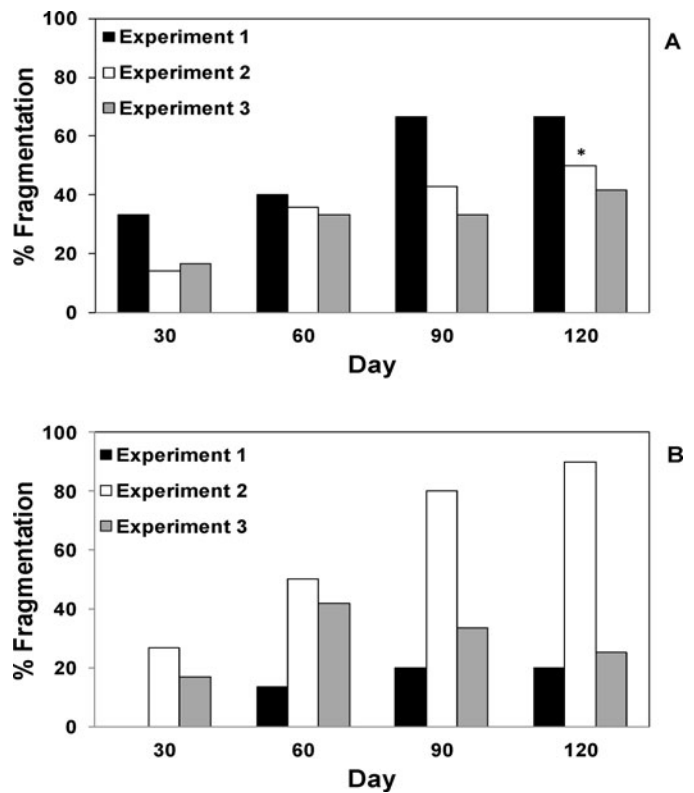


FIGURE 1. Percent VIE tag fragmentation over 120 d for (A) jaw and (B) caudal body locations in three successive experiments using progressively larger juvenile Spotted Seatrout. Mean starting lengths were 93 mm TL (experiment 1), 138 mm (experiment 2), and 152 mm TL (experiment 3). A tag was considered fragmented when no continuous piece of elastomer was present in the tissue. The percent fragmentation is expressed as the number of fish with fragmented tags over the total number of VIE-tagged fish for that body location. In experiment 1, the 30-d caudal tags (shown in panel B) were unfragmented, thus there is no bar for that entry. The asterisk indicates the final fragmentation assessment for jaw-tagged fish in experiment 2 was at 93 d instead of 120 d.

TABLE 2. Summary of mean 30-d growth rate (g/d) of juvenile Spotted Seatrout for three growth experiments. Each experiment used control fish and fish with dorsal CWTs (DCWT), opercular CWTs (OPCWT), caudal VIE tags (CFVIE), jaw VIE tags (JVIE), as well as four corresponding false-tag (F plus tag abbreviation, e.g., FDCWT) treatments. Initial TL is included for each experiment.

Tag treatment	Growth rate (g/d)		
	Experiment 1 (fish TL, 93 mm)	Experiment 2 (fish TL, 138 mm)	Experiment 3 (fish TL, 153 mm)
Control	0.41	0.23	0.07
DCWT	0.32	0.23	0.11
OPCWT	0.28	0.17	0.25
CFVIE	0.27	0.20	0.18
JVIE	0.28	0.23	0.24
FDCWT	0.32	0.16	0.12
FOPCWT	0.26	0.23	0.15
FCVIE	0.30	0.23	0.21
FJVIE	0.31	0.18	0.28

the studies. We therefore concluded that neither CWTs or VIE tags themselves nor the process of using them caused juvenile Spotted Seatrout increased mortality. Reeves and Buckmeier (2009) found that VIE tag-induced mortality was species- and size-specific. We are unaware of any previous studies of CWTs in juvenile Spotted Seatrout. Although this study did not address the issue, it is possible that an externally visible tag like a VIE tag could contribute more to postrelease mortality than an invisible CWT due to increased visibility to predators. Reeves and Buckmeier (2009) found no evidence for such an effect associated with VIE tags, but noted that the literature is somewhat equivocal on the issue. Additionally, retention for CWTs implanted into epaxial dorsal and opercle muscle averaged 93%

for both body locations through 120 DPT, and VIE tags in both the lower jaw and ventral caudal fin were 100% retained at 120 DPT. Thus, the CWT and VIE tag are both well suited for use in juvenile Spotted Seatrout.

Although CWT retention rates across both body locations were high, the only tag that was lost during the study was the opercular CWT, with experiment 2 showing the greatest degree of tag loss (20%). Additionally, there was a potential negative pattern between fish size and tag loss, and the highest retention rate for the opercular CWT occurred in the largest size-group of fish. Brennan et al. (2005) observed a similar pattern in juvenile Common Snook *Centropomus undecimalis*. Perhaps the size of the area targeted for tagging plays a key role in initial retention. The opercle muscle of juvenile Spotted Seatrout is relatively thin, thus considerable precision is required when tags are inserted. Therefore, opercular tags easily could be injected too deeply or at an inappropriate angle in small fish, which may result in tags being pushed through to the buccal cavity where they could be initially detected in standard quality control checks but ejected soon thereafter (Bumgardner et al. 1992).

One of the goals of this study was to evaluate a possible alternative site for CWTs because of the lower than expected retention of opercular CWTs in the mass tagging program (~75%, authors' personal observations). The retention rates observed in this study for opercular CWTs (mean = 86.1%) were, however, higher than those observed during mass tagging events. Although we cannot explain the difference, one possible explanation is that the speed required in a mass tagging event reduces accuracy and retention. Another possible explanation is that we did not use the quality-control device (QCD) that is used in mass tagging operations to verify the presence of a tag before release. The QCD acts in coordination with the Mark IV automatic tag injectors to sort successfully tagged fish from those tagged unsuccessfully. This is accomplished by passing the fish through a tunnel where a detector separates tagged and untagged fish. In

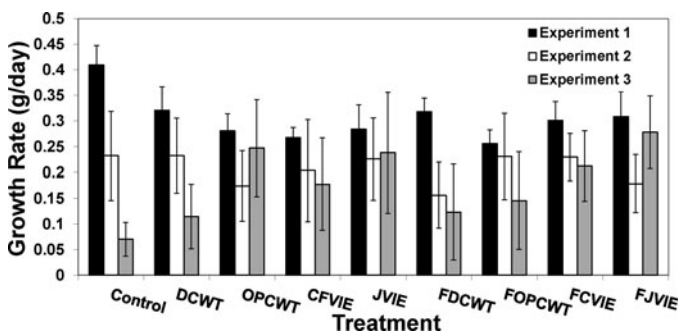


FIGURE 2. Effects of tagging and false-tagging with CWTs and VIE tags on 30-d growth rate (g/d; mean  $\pm$  SE) of juvenile Spotted Seatrout across three successive 30-d growth experiments. Mean starting lengths were 93 mm TL (growth experiment 1), 138 mm TL (growth experiment 2), and 152 mm TL (growth experiment 3). Treatment codes are as follows: DCWT, CWT placed in epaxial dorsal muscle; OPCWT, CWT placed in opercular cheek muscle; CFVIE, VIE tag placed in ventral caudal fin tissue; JVIE, VIE tag placed in lower jaw tissue; FDCWT, false-tagged (CWT) in epaxial dorsal muscle; FOPCWT, false-tagged (CWT) in opercular cheek muscle; FCVIE, false-tagged (VIE tag) in ventral caudal fin tissue; and FJVIE, false-tagged (VIE tag) in lower jaw tissue. False-tagged treatments were those where the appropriate needle for the tag was inserted but no tag was injected.

the situation where high precision is required for proper insertion, as the case may be for Spotted Seatrout, water current in the QCD or the process of dropping the fish into water as they exit the QCD could facilitate loss of tags that were not ideally placed. Therefore, this study suggests that, with some modification of procedure, opercular CWT retention in the mass tagging program can be increased.

This study also demonstrated that the epaxial dorsal musculature was an excellent body location for the use of CWTs because no tags were lost at 120 DPT and there was no effect on survival or growth. In a short-term study with Red Snapper *Lutjanus campechanus* Brennan et al. (2007) found dorsal CWTs to be highly successful with a retention rate of 90.4% at 6 weeks after tagging with no mortality. Dorsal CWTs also have been used successfully in Rainbow Trout *Oncorhynchus mykiss* with retention percentages ranging from 92% to 100% (Hale and Gray 1998). Despite its success in this study, the dorsal tag brings with it the criticism of being in a potentially edible area of the fish, which could influence the success of fisheries-independent monitoring due to the potential unwillingness of anglers to part with an edible portion of the fish. Opercular tags are not in a portion of the body that is typically consumed and, therefore, are conducive to angler participation. Consumption of the tag, albeit unlikely due to the small size (length, 1.1 mm; diameter, 0.25 mm) is also a possibility, but the risk to the consumer would be low.

All VIE tags were visible at 120 DPT, but ultraviolet light and amber-shaded glasses were required throughout the duration of the study for effective tag detection. At the base of the caudal fin there was a proliferation of dark, white, and silvery pigments that increased with age and resulted in overlap with the VIE tag. In marine fishes, VIE tags can be retained well over the short term (Olsen et al. 2004; Bushon et al. 2007), but tag fragmentation is common with VIE tags as muscle tissue spreads with fish growth, which can lead to increased fragmentation over time. Brennan et al. (2005) observed pigmentation overlap of VIE tags in the caudal peduncle of older Common Snook and did not recommend this body location for long-term use. The jaw tag was not affected by pigmentation and was more visible than the caudal tag.

For both anteroposteriorly tagged VIE tag locations there was a general increase in fragmentation over time. During experiment 2, however, there was a much higher degree of caudal tag fragmentation (90%) compared with experiments 1 and 3 and the jaw tag location (50%) in experiment 2. This could not, however, be explained by differential growth over the 30-d time period; fish in experiment 2 had intermediate growth rates. Astorga et al. (2005) demonstrated that anteroposteriorly injected tags in juvenile Gilthead Seabream *Sparus auratus* fragmented less than dorsoventrally injected tags because fish tend to grow in length more than depth. Thus, although we did not specifically examine it in this study, anteroposterior tag orientation may be a better option than a dorsoventral orientation. Size at tagging did have an effect on the amount of fragmentation in

the jaw tag. Fish tagged at larger sizes experienced less fragmentation (41.7%) than those tagged at smaller sizes (61.7%). This finding may be attributable to size of the target area, which is proportional to the size of the fish and, therefore, inversely proportional to the difficulty in applying the tag. Transparent tissue is limited in juvenile Spotted Seatrout; thus, more precision is required when injecting a VIE tag into smaller individuals. Therefore, we conclude that VIE tags, particularly those in the jaw, work well for juvenile Spotted Seatrout. However, because of fragmentation they may be most ideal for short-term studies in larger juveniles in which visual, nonlethal identification of the fish is required. The combination of the lack of automation in their application and the limited number of coding possibilities compared with CWTs limits the use of VIE tags in mass tagging operations.

As has been determined in previous studies (Heidinger and Cook 1988; Peterson and Key 1992; Astorga et al. 2005; Hoey and McCormick 2006) there was no effect of tag type, location, or handling on 30-d growth rate of juvenile Spotted Seatrout in any of the experiments. In our study, small fish had a greater growth rate than large fish, which is not unusual during fish ontogeny (Weatherley and Gill 1987; Wootton 1992).

The data from this study provide new information regarding the applicability of current and potential tagging methods for juvenile Spotted Seatrout. Overall, except for VIA tags, which clearly require further research, internal tags appear to have little negative effect on the well-being of juvenile Spotted Seatrout and fish can be tagged effectively at small sizes without affecting growth. Target size and the selection of the tagging site should be considered carefully when choosing tagging locations as both may play a role in the overall performance of the tag.

The study suggests that for the critical task of evaluating large-scale enhancement programs (Blankenship and Leber 1995), properly applied CWTs are ideal. When combined with the tag's cost effectiveness and its inherent capacity for complex coding patterns, the excellent retention provides the capability for robust experimental designs and long-term reliable identification of hatchery-reared fish. The study also establishes that VIE tags are practical and reliable, but it also confirms the conclusions of Leblanc and Noakes (2012) that their use for long-term identification is inadvisable pending further research. Future research also should evaluate how to effectively increase the retention of CWTs in mass tagging operations for Spotted Seatrout as well as improved methods for the use of the CWT in other body locations that are not vulnerable to human consumption.

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