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[Article]

An Evaluation of Coded Wire and Elastomer Tag Performance in Juvenile Common Snook under Field and Laboratory Conditions

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Abstract.—From 1997 to 2002, retention of coded wire tags (CWTs) and visible implant elastomer (VIE) tags was evaluated in a series of stock enhancement studies with common snook *Centropomus undecimalis* (60–230 mm fork length [FL]). These experiments were conducted in both field and laboratory settings in Sarasota, Florida. Retention rates of CWTs were stable after 30 d and remained greater than 97% even 1 year after tagging. Retention of VIE tags was compared among different body implant locations, implant techniques, and fish sizes. Body location had the strongest influence on VIE retention, and tags implanted in the fins (anal and caudal) had significantly higher retention rates (mean ± SE = 76 ± 9%) than those implanted in the head (adipose eyelid, jaw, and preorbital nose tissue; 5.6 ± 1.8%, $P = 0.038$) 1 year after tagging. After 1 year, however, most VIE tags—regardless of body location—were nearly indistinguishable or lost. Fish implanted with two VIE marks had consistently higher mean tag retention rates and visibility than those with single marks (86% versus 64% mean retention in the caudal fins 7–12 months after tagging). Fish size at tagging was not a significant contributor to tag loss. Tagging rates were slowest with VIEs: 250–400 fish per hour and 200–300 fish per hour when one and two VIE marks, respectively, were injected per fish. Tagging mortality was less than 1.6%. No significant differences in tag retention or mortality were found between field and laboratory trials. Overall, we recommend CWTs implanted in cheek muscle and at least two VIE marks implanted in the caudal fin as tagging methods and locations for juvenile common snook because of the ability to tag large numbers of fish, high tag retention, and low tagging mortality.

Interactions among fish and their environments have been widely studied in the field by marking individual fish with various tag types to provide insight into the factors affecting growth, survival, habitat use, and ultimately recruitment to adulthood. Specific research conducted in experimental settings has estimated abundance and survival (Harris 1989; Skalski 1998), dispersal patterns (Martell et al. 2000; Appeldoorn et al. 2003; Brunton and Booth 2003), essential fish habitat (Martin-Smith et al. 1999), and habitat selection and connectivity (Adams and Ebersole 2002; Appeldoorn et al. 2003). Enhancement of depleted fish stocks through releases of hatchery-reared fishes has received increased attention as a potentially important component of stock restoration and management (Leber 1995; Kitada 1999; Svasand 1998). These enhancement projects are often evaluated by tagging many (thousands to millions) juvenile fishes over short periods of time and using tag

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returns to evaluate fates of released animals. Key issues associated with these tagging programs include benignity of tag type, implant location, high tag retention, low cost, and rapid application.

The common snook (''snook'') *Centropomus undecimalis*, a coastal, warm-water species, inhabits subtropical regions of North and South America within estuaries and river systems (Marshall 1958; Fore and Schmidt 1973). These habitats are often characterized by strong daily and seasonal environmental fluctuations, high productivity, diverse floral and faunal communities, and extensive anthropogenic alterations. Despite increasingly restrictive size, bag, and season regulations on common snook within Florida, the populations are considered overfished (Muller and Taylor 2002). Because of concerns over the status of this ecologically and recreationally important species, Mote Marine Laboratory in cooperation with the Florida Fish and Wildlife Conservation Commission launched an experimental snook stock enhancement program in 1996. Since then, over 42,000 hatchery-reared juvenile snook have been tagged and released into local estuarine habitats around Sarasota, Florida, of which over 38,000 were released with both coded wire tags (CWTs) and visible implant elastomer (VIE) tags. Evaluations of the interactions between hatchery and wild snook stocks, and of the integration of the two stocks, have been dependent on tag performance to identify hatchery-reared individuals. The objectives of this study were to investigate the performance of CWTs (Northwest Marine Technology Inc. [NMT], Shaw Island, Washington) and VIE tags (NMT Inc.) as tools to assess snook stock enhancement efforts by quantitatively evaluating optimal tag type, location, and tagging methods in long-term field and laboratory trials.

Methods

CWT tagging.—A stainless steel, magnetized, single-length (1.1-mm) CWT was injected ''freehand'' (manually impaling the tip of a stationary needle into the target area of the fish) with a Mark IV injector (NMT Inc.) in a ventral direction into the left adductor mandibularis muscle (''cheek''), parallel to the muscle fibers (Fletcher et al. 1987; Wallin et al. 1997). No other CWT implant locations or methods were tested in this study. From 1997 to 2000 and during 2002 all released common snook were batch-tagged according to specific release groups (release year, week, site, size-class, and VIE tag location; discussed below]). Reference tags for each of these groups were archived in silicone sheets. In 2001, sequential CWTs were archived for each tagged fish to link tag codes to specific records of fish lengths and weights at the time of release.

VIE tagging.—We used 0.3–0.5-mL hypodermic syringes with 29-gauge needles to inject the VIE material. For the field-release tagging operations, and some laboratory-based trials, VIE marks were applied with automatic pressure-regulated machines (supplied by the vendor); otherwise, we manually injected VIE marks with hand-held hypodermic syringes. To maximize tagging speed during tagging operations, twice as many technicians were assigned to VIE tagging as to CWT tagging. We injected VIEs into transparent and semitransparent tissue in juvenile common snook. Specifically, six locations were evaluated: (1) the adipose eyelid tissue posterior to the eye (Figure 1a), (2) under the preorbital nose tissue (Figure 1a), (3) in the lower jaw (Figure 1b), (4) into the ventral muscle of the caudal peduncle, (5) between the fin rays of the dorsal and ventral lobes of the caudal fin (Figure 1c), and (6) between the fin rays of the anal fin (Figure 1d). In 1999 in the laboratory, we tested placement in the adipose eyelid tissue and nose and injected a VIE mark into the right and left side of each fish to determine differences in ease of tagging, length of tag-retention, and how to maximize our use of each fish. In two other laboratory studies, ''full'' and ''half'' amounts of VIE were injected into the adipose eyelid and lower jaw tissue. We define a ''full'' VIE tag as the amount of elastomer required to fill a typical injection hole (approximately 0.001 g) and a ''half'' VIE tag as the amount of elastomer required to fill only the inner half of the injection hole (approximately 0.0005 g). In the 2001 and 2002 release experiments, we injected two VIE marks into each of the upper and lower lobes of the caudal fins to maximize retention and visibility. In a laboratory study in 2001, we also injected two VIE marks into the upper and lower lobes of the caudal fin, and three marks in the anal fin of each fish. Only one mark was injected between any given fin ray pair in all cases. We changed VIE tag implant locations with each release year as we sought to find optimal target location sites for the VIE tag.

Laboratory tagging procedures.—Age-0 common snook (60–275 mm fork length [FL], at least 90 d old) spawned from local wild stock were reared at Mote Marine Laboratory, Sarasota, Florida (S. Serfling, unpublished data). All snook reared were anesthetized with methane tricane sul-

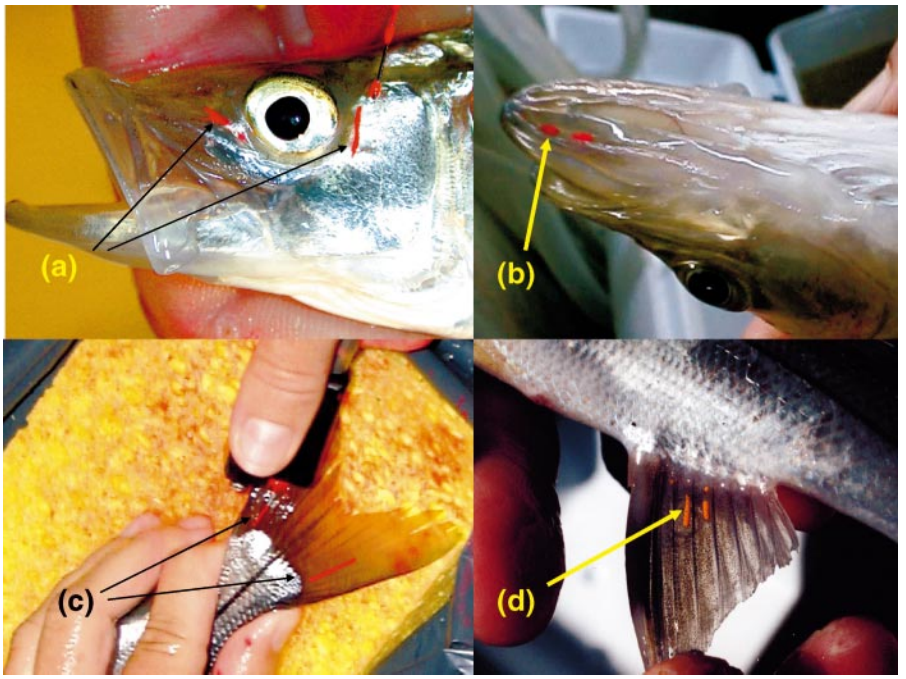

FIGURE 1.—Subcutaneous implantation of visible implant elastomer (VIE) tags in **(a)** the adipose eyelid tissue posterior to the eye and under the preorbital nose tissue, **(b)** the lower jaw, **(c)** between the fin rays of the dorsal and ventral lobes of the caudal fin, and **(d)** between the fin rays of the anal fin of common snook. The fish in (a) and (c) were recently tagged with VIE, whereas those in (b) and (d) were tagged 2 months before being photographed. All of the fish shown are between 120 and 200 mm FL.

fonate (MS-222, 70–80 mg/L), tagged with CWTs, and placed in a second anesthetic holding tub (MS-222 concentration, 35–40 mg/L) where VIE marks were then implanted by a second technician. To minimize harm from net contact, we used plastic-coated nets with plastic sides whenever possible, allowing the fish to be ''wet packed.'' Fork length and weight (g) subsamples were taken during the tagging process. All tagged snook in this study were separated by length and tagged according to three classes: small, 60–125 mm FL; medium, 125–165 mm FL; and large, 165–230 mm FL.

Field-release tagging procedures.—Tagging procedures for fish to be released into the wild (''field studies'' throughout) were produced and handled as above. After tagging, common snook were held temporarily in recirculating laboratory tanks for 3–18 d after tagging (to allow recovery from tagging stress and for VIE material to solidify; see below) and then released as part of our larger stock enhancement research efforts (N. Brennan, unpublished data).

Tag retention.—All released fish were double-tagged, that is, every fish received a CWT and an externally visible VIE tag. We obtained all our field tag retention estimates by using the presence of one tag to verify the presence or absence of another; we assumed that the presence or absence of one tag was independent of the other and that at least one tag type would be retained. We used all recaptured common snook containing a CWT to estimate retention of VIE tags placed in different body locations. Conversely, we used any recaptured fish with a VIE to estimate CWT retention. Unique combinations of VIE body location and fish size allowed us to differentiate between different release groups tagged with CWTs. Tag retention in the laboratory was determined by tagging all fish in each tank with a specific tag or a combination of tag implant body locations and then periodically examining all fish within each tank.

Field methods.—Each release of tagged common snook was followed by a series of recapture efforts to obtain results from each study. Before release, snook were harvested from their tanks (between 3 and 17 d after tagging), checked for the presence of CWTs and VIE tags, and loaded into transport tanks.

Postrelease sampling occurred roughly monthly

for the first 3 months, then at month 6, and intermittently thereafter. Standardized monthly sampling regimes and an annual fishing tournament were used to obtain recaptures of the tagged snook for analysis of tag performance and as part of other stock enhancement studies. Collections were made with a 21.3-m × 1.8-m (1-cm nylon mesh) bag seine, a 45.7-m × 3-m bag seine (1-cm nylon mesh), a 67-m × 3-m bag seine, a 2.4-m-diameter cast net (1-cm monofilament mesh), and hook and line. All snook captured were checked for the presence of CWTs (with magnetic tag detectors) and VIE tags (visually). Recaptured snook were preserved on ice and returned to the laboratory. Coded wire tags were extracted from the tissue and decoded. If no VIE tags were seen, we used an ultraviolet light to verify their absence. Data from each fish were uniquely recorded and tag presence was entered in a binary format.

Data analysis.—Common snook were grouped by variables at tagging (e.g., field versus laboratory source, year, lot, and fish size) and grouped further into time intervals according to days after tagging (DAT), namely, 1–30, 31–60, 61–90, 91–150, 151–365, and longer than 365 d, based on collection date. Time intervals that contained fewer than 5 fish within a particular tag group were removed from the analysis. Retention estimates (R) within a time interval (i) were calculated as

$$R_i = 100 \times t_i/T_i, \quad (1)$$

where t_i = the number of tagged snook at recapture time i and T_i = the number of snook in the sample at time i that originally had tags. In the field, retention estimates for a tag type in question were based on the presence or absence of the second tag. Mean retention estimates (R) were calculated from different release groups (e.g., study, lot, and fish size). For CWTs, we performed a regression analysis with mean percent retention and DAT to determine if retention rates differed; to do so, we examined whether the slope of the regression differed from zero. To simplify analysis, VIE body implant locations were grouped according to those implanted in the head region and those implanted in the fins. For VIE tag treatments (body location, fish size, and field versus laboratory results), we compared mean retention estimates with an analysis of variance (ANOVA). A main effects factorial ANOVA model was performed for data collected within each time interval to determine the dependent variables (body location, fish size, field or laboratory, amount of VIE, and number of marks) that most strongly influenced retention of all tag types. All analyses were performed with SAS software (SAS Institute 1990); $\alpha = 0.05$ was considered significant.

Results

Field

From 1997 to 2002, a total of 38,773 juvenile common snook were tagged and released with CWTs and VIE tags in the study area. These releases represented 270 general release groups based on release study, release lot, release site, and release size combinations. Field sampling over the same period recaptured 1,088 snook (2.8% of total released), of which 335 contained only CWTs, 47 contained only VIE tags, and 706 had both tags. Over 20% (267) of the released snook were captured at least 1 year after tagging; the longest time at liberty was 2,135 d (5.85 years). During release operations, tagging mortality was 0.14–1.55%. No tagging mortality occurred during the laboratory tagging trials.

Tagging rates for CWTs implanted in the cheek muscle were between 400 and 800 fish per hour. Rates of VIE tag application were 250–400 fish per hour per machine when one elastomer mark was implanted, 200–300 fish per hour when two elastomer marks were implanted, and 150–250 fish per hour when four elastomer marks (two VIEs in the upper and two in the lower lobes of the caudal fin) were implanted in each fish. Tagging rates were influenced by the experience of the tagger.

Laboratory

Because retention rates in laboratory studies for both tag types were not different from field results (all $P > 0.1$), we combined the retention results for both groups. These combined results are discussed below.

CWT Retention

Coded wire tags implanted in the cheek muscle had excellent retention for 31–90 DAT in both field and laboratory trials (mean = 97.4% and 99.6%, respectively). Furthermore, mean CWT retention estimates did not significantly change from initial estimates, even after 1 year (linear regression, $P = 0.53$; Figure 2). In 2001 CWT retention was extremely low (68%) because of operator error. To adjust for this, we retagged common snook that had lost their CWTs before release.

Fish size at tagging did not significantly affect mean CWT retention (ANOVA: df = 42, $P = 0.25$). When mean retention data were combined

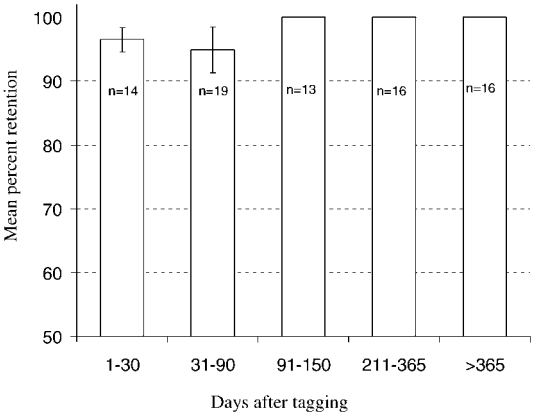

FIGURE 2.—Mean tag retention (100 × number of fish with tags/total fish originally tagged; ±SE) within time groupings (days after tagging) for coded wire tags implanted in the left adductor mandibularis muscle of common snook. Data are from field and laboratory settings. The numbers of experimental replicates (*n*) are also given; at least five fish were used to calculate each replicate estimate.

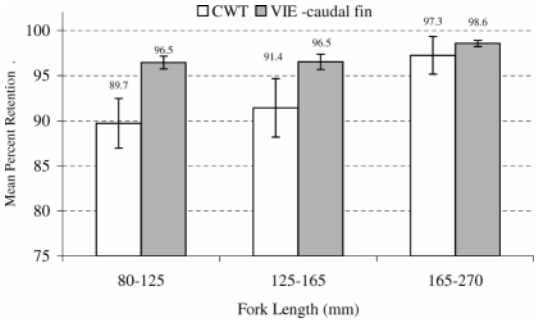

FIGURE 3.—Effects of size at tagging on mean retention rates (±SE) of coded wire tags (CWTs) and visible implant elastomer (VIE) tags implanted in the left cheek muscles and caudal fins, respectively, of juvenile common snook in different size-classes. Retention rates are from fish tagged for release and then collected 1–3 weeks after tagging. Mean retention rates were not significantly different between tag types, although retention tended to increase with fish size.

for all periods, the means for small, medium, and large common snook were 99.9% (n = 15 groups), 96.6% (n = 11 groups), and 97.6% (n = 17 groups), respectively. No differences in CWT retention estimates were found among all size-classes tagged for snook sampled from 1 to 30 DAT (Figure 3) nor for snook sampled after 30 DAT (ANOVA: P = 0.53 and P = 0.60, respectively).

VIE Tag Retention

Short-term retention of VIE tags (1–30 DAT) for all body locations and fish sizes tested averaged 88.8 ± 3.3% SE. The independent variables (body location, size-class, amount of VIE material, and number of marks) and the interactions among these variables did not strongly influence tag loss (ANOVA: df = 18, P = 0.79). Common snook sampled at 31–90 DAT averaged 63.6 ± 5.4% retention of VIE marks among all treatments. Among the independent variables related to retention, only body location was significant (ANOVA: df = 5, P = 0.01). When data were grouped into head and fin implant locations, the initial (1–30 DAT) estimates of retention, again, were not significantly different (ANOVA: df = 11, P = 0.84), but by 31–90 DAT, differences were significant: mean ± SE = 85 ± 6% for fin regions, compared with 55 ± 6% for head regions (ANOVA: df = 21, P = 0.04; Figure 4). By 1 year after tagging, these differences were even more pronounced: mean ± SE = 84 ± 3% for fin regions, compared with 5 ± 2% for head regions (ANOVA: df = 23, P = 0.0001; Figure 3). After 1 year, however, retention of all VIE tags was poor, regardless of independent variable (for cases where only one VIE mark was implanted, mean ± SE VIE retention estimates were 24.5 ± 6.6%).

Retention of VIE tags declined with fish size for tags implanted in both the head regions and in the fins, but the differences were not significant (ANOVA: $P < 0.05$) for all periods tested (Figure 3 shows short-term retention estimates). Visible implant elastomers were easier to implant in larger fish and tagging rates were faster, but retention was

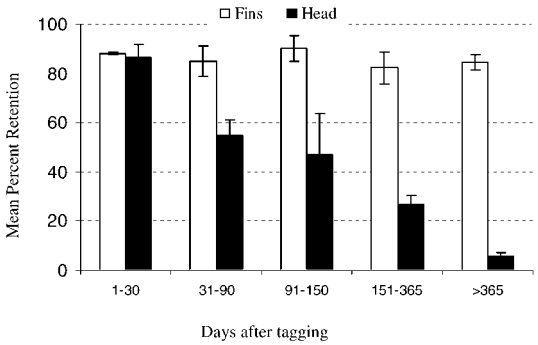

FIGURE 4.—Mean retention rates over time of visible implant elastomer (VIE) tags implanted in the heads and fins of juvenile common snook. Head tags were implanted in the adipose eyelid tissue, subcutaneous nose tissue, and lower jaw tissue. Fin tags were implanted in the caudal and anal fins between fin rays toward the base of the fin.

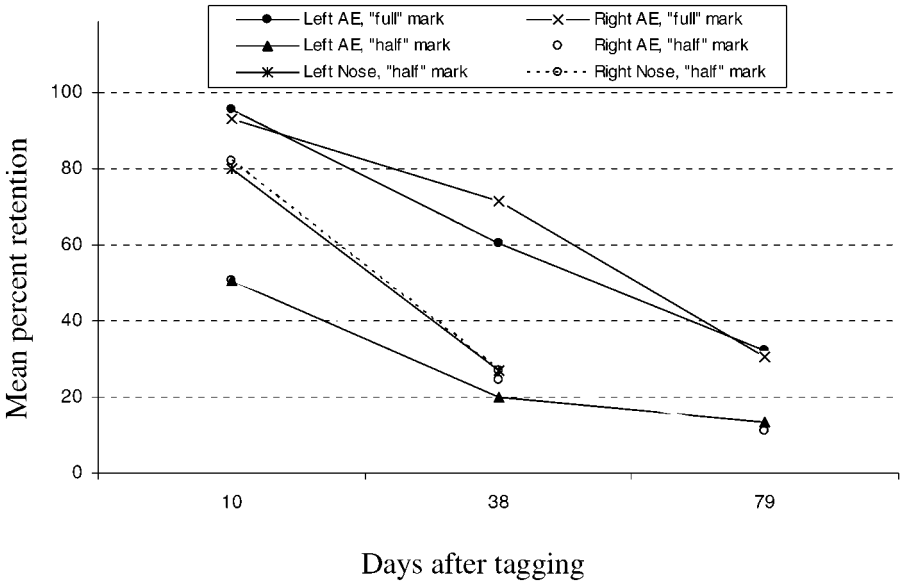

FIGURE 5.—Retention rates of visible implant elastomer tags implanted in the adipose eyelid (AE) and nose tissues on opposite sides of common snook. The terms ''full'' and ''half'' represent different amounts of elastomer (i.e., whether the injection hole was filled completely or only halfway).

poor for time periods longer than 1 year. Differences in retention of VIE material injected into opposite body sides for the adipose eyelid or preorbital nose tissue were very slight (Figure 5). Multiple VIE marks improved tag presence, being more detectable than single marks. These effects were tested only with VIE tags in fin rays (caudal fin and anal fin), but results showed mean tag retention estimates were always higher for fish tagged with two VIE marks. However, these differences were only significant at 1 year after tagging (mean retention = 84% versus 38% for common snook tagged with two and one marks, respectively; ANOVA: df = 5, $P = 0.02$; Figure 6). Two VIE marks also improved the overall visibility of the tag, making the tags more readily detected by observers. Inconsistent results were obtained when full and half amounts of VIE were injected in different body locations: In the jaw, half the amount of elastomer resulted in approximately double the retention estimates obtained by injecting full amounts; in the adipose eyelid, however, the opposite effect was observed (Figure 7).

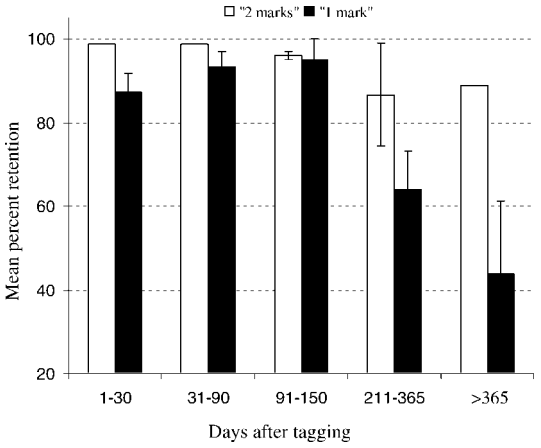

FIGURE 6.—Mean retention rates of visible implant elastomer tags over time when one or two marks were implanted in the lower lobe of the caudal fin. Error bars indicate the standard errors of the mean.

Discussion

Coded wire tags were selected for this study because of their widespread application in large-scale stock enhancement studies, particularly for Pacific salmon *Oncorhynchus* spp. (Johnson 1990). Coded wire tags used in juvenile fishes are benign and show little to no effect on growth and survival when tags are implanted in appropriate body locations (Jefferts et al. 1963; Heidinger and Cook 1988; Dunning et al. 1990; Russell and Hales 1992; Buckley et al. 1994). In a short-term study with juvenile common snook, Wallin et al. (1997) found CWTs had no significant effect on growth and survival, and retention rates were high in the

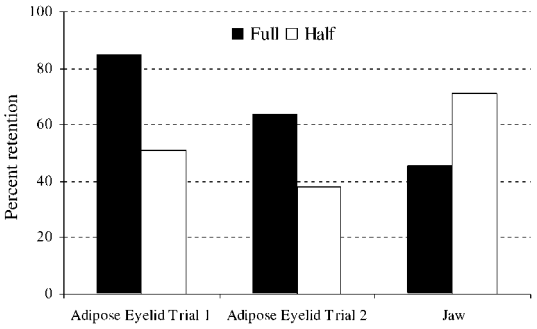

FIGURE 7.—Thirty-day retention rates of full and half amounts (see Figure 5) of visible implant elastomer injected into the adipose eyelid or lower jaw tissue of juvenile common snook.

laboratory for 60 d. Our release experiments have shown that CWTs implanted in muscle tissue in juvenile snook had high retention rates (mean rates over 95%), and no apparent detrimental effects of the tag on behavior, growth, or survival were observed.

Although our method for documenting tag retention in the field relied on the presence of at least one tag type (thus potentially positively biasing retention rates because individuals that lost both tag types were considered to have never been tagged), we found that the resulting recovery rate of CWTs was so high, and thus the expected rate of losing both tags so low, for simplicity we ignored the latter group in the analyses. Furthermore, because all fish in the laboratory were originally tagged, tag retention rates in the laboratory were direct measures of retention for each tag type, and because retention was not significantly different between field and laboratory settings, we consider any possible bias in the field retention rates to be insignificant.

Large-scale tagging applications require tags that can be rapidly applied, are cost-effective, and cause minimal mortality. In our studies, where over 38,000 juvenile common snook were tagged for release, tagging mortality was minimal (0.14–1.55%). Most mortality in the tagging operations occurred from overdose of MS-222 and handling stress. Overall, fish in good condition before tagging procedures tended to have excellent survival rates. Because of animal welfare concerns and the precision needed to apply these tags, it was necessary to use anesthesia for tag application. However, prolonged exposure to anesthetics (~70–90 mg/L, >10 min) sometimes resulted in mortality. A more lenient anesthetic, such as clove oil or Metomidate (5 mg/L) (Mattson and Riple 1989), may minimize tagging mortality; nonetheless, MS-222 is the only drug authorized for use in U.S. waters.

Tagging free hand into the cheek muscle was slower than typical head-mold tagging, which is capable of achieving such rates as 800–1,400 fish per hour (Leber et al. 1998; N. Brennan, unpublished data). Although the rates of CWT application in this study are acceptable, faster tagging could be achieved if needle-directed head molds were implemented for common snook. The morphology of the nose cartilage area is porous in snook, but careful selection of a target site and an oblique needle approach may prove successful. Injection of CWTs into the nape muscles also shows promise, but if head molds are used, a skewed needle approach would be necessary because of the elongated head shape of the snook. In any case, because of the high harvest rates from the snook fishery, CWTs should be implanted in ''disposable'' regions of the body, such as the head, and not in regions that are more likely to be consumed by anglers.

Visible implant elastomers in marine fishes can have good retention for relatively long periods, say, 1–6 months (Buckley et al. 1994; Frederick 1997) and are benign (Bergman et al. 1992). Furthermore, multiple colors and body locations of VIE tags are useful to externally identify experimental treatments with minimal harm to the fish (Dewey and Zigler 1996; Frederick 1997; Willis and Babcock 1998).

In our study, we achieved good retention rates of VIE tags in the fin rays for up to 1 year after tagging. At other tag implant locations, however, tag loss was significant after 2–3 months, which demonstrates the importance of experimentally identifying optimal body implant locations before large-scale projects are underway. For short-term studies, the adipose eyelid, jaw, nose, and fin ray tissue were all sufficient implant sites. No noticeable differences were found in retention of VIE tags when implantation occurred in opposite body sides, and we recommend using whichever side is convenient for the researcher or study design. Although VIE tags are visible in caudal peduncle muscles in very young common snook (less than 8 months old), it is not a suitable target site for long-term studies because skin pigmentation obscured VIE tags in older snook. Pigmentation also reduced visibility of VIE material in the lower jaw and to some extent in the caudal fin.

Size at tagging had no significant effect on re-

tention, but generally the smallest fish (60–125 mm FL) were the most difficult to tag (Figure 3). Small fish had less target tissue available for tag implantation, but a careful, focused effort could produce good tag retention no different from that achieved in larger common snook. The difficulty in tagging small snook was more obvious with VIE tags than with CWTs and resulted from the scarcity of transparent tissue necessary for VIE identification—compared with the relatively abundant muscle tissue used for implanting CWTs. Obviously as fish size decreases, tag and tagging effects will become apparent, but these must be weighed against the benefits of marking small fish. Although pink salmon *O. gorbuscha* fry tagged with half-length CWTs had significantly greater initial mortality than unmarked fry, the tags successfully identified specific release groups in adult returns years later (Wertheimer et al. 2002). Small snook (30–50 mm FL) potentially have sufficient muscle tissue (in the nape or dorsal muscle) to retain CWTs and such tissue may serve as a suitable tag implant location. Although cheek muscle is a useful target site for CWTs in the sizes we tested, it would probably be insufficient for retaining CWTs in smaller snook. Overall, however, we have shown that, for applications under laboratory conditions, these tags can be applied to fingerlings with high retention and low mortality rates and are suitable for stock enhancement studies in complex release designs on a fairly large scale.

The results from our studies on full and half amounts of elastomer in different body locations may be explained by the degree of porosity in the target tissue. In the adipose eyelid tissue, jaw, and nose, elastomer fluid often spread out beyond the needle hole and filled nearby pores. In other areas such as the caudal and anal fins, we detected no migration of the elastomer outside of the injection hole, even when elastomer was injected under high pressure. This suggests that target sites absent of large body pores may have the highest retention rates for VIE material. The lack of significantly improved retention with increasing body size (and thus more tissue available for tag injection) for VIE material injected in the head locations further exemplifies this, tag loss probably occurring through the porous tissue. Besides tag loss, unintentional spreading of VIE material into body pores also may detrimentally affect specific body functions. We recommend always wiping excess elastomer from the injection wound so that the tags are lodged and can cure subcutaneously.

Stock enhancement studies have unique research requirements that must be followed stringently (Blankenship and Leber 1995). The tags evaluated here provide a means to identify experimental treatment groups for moderate numbers of small fish in a cost-effective manner while minimizing harm and mortality to the fish. In our pilot studies, where relatively small numbers of fish were released, a tag system that minimized harmful health effects allowed us to capitalize on returns from the fishery and obtain necessary feedback. By identifying a reliable tagging system, we were able to design complex experiments related to our release programs for use in each of our common snook stock enhancement studies

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