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Use of coded-wire and visible implant elastomer tags for marine stock enhancement with juvenile red snapper *Lutjanus campechanus*

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Abstract

As part of a stock enhancement research project in the Gulf of Mexico, we evaluated optimal tag placement sites, retention, and detection in hatchery-reared juvenile red snapper *Lutjanus campechanus* (46–110 mm standard length) with coded-wire tags (CWT) and visible implant elastomer tags. Coded-wire tags were implanted in nose cartilage, left cheek muscle, the nape, dorsal musculature, and the caudal peduncle. Three weeks after tagging, all implant locations tested showed tag retention over 97% except for those implanted in the dorsal musculature (90%). No additional tag loss was found between 3 and 6 weeks after tagging regardless of implant location or fish size. We selected the nape musculature for larger-scale tagging because of high tag retention and ease of tagging. Retention of CWTs in the nape after 6 months was 99% ($n = 285$). Visible implant elastomer (VIE) tags were implanted in muscular and fin tissue of juvenile snapper and retention rates were ≥95% (after 6 weeks) for all implant locations except for anal fin muscles (87% tag retention). In a separate study, 6 months after tagging, fish size-at-tagging and the number of VIE marks implanted in the caudal fin significantly influenced tag retention (two-way analysis of variance, $P = 0.026$). After experimental stock enhancement releases, divers 17–20 m deep, found that VIEs in caudal fins were highly visible, but those in the nose area were difficult to see due to pigmentation over the tags and fish orientation. Tag color influenced tag visibility and red and orange elastomer tags were the most visible among eight colors tested (red, orange, yellow, green, pink, black, blue, and purple). Divers commonly confused green elastomer tags for yellow, and sometimes confused red, pink and orange. No color confusion occurred when similar colors were observed side-by-side however.
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Keywords: Artificial reefs; Coded-wire tag; *Lutjanus campechanus*; Marine stock enhancement; Red snapper; Visible implant elastomer

1. Introduction

Ecological research with aquatic organisms often requires marking or tagging individuals to monitor growth, movement, and population characteristics. Since the late 1980's research on marine stock enhancement has expanded rapidly owing much success to reliable and applicable tagging systems and tagging methods for rigorous scientific experimentation (e.g. see reviews in Munroe and Bell, 1997; Leber, 2002; Hilborn, 2004). Typically, stock enhancement programs require tags to be applied to many juvenile fishes with minimal harm yet have high information content and low associated costs (Blankenship and Leber, 1995). A variety of tag types have been used to monitor stocked individuals including genetic tags (Perez-Enriquez and Taniguchi, 1999; Saillant et al., 2004; Taniguchi, 2004),

chemical and thermal marks (Smoker et al., 2000; Jenkins et al., 2004), internal tags (Davis et al., 2004; Brennan et al., 2005), electronic archival tags (e.g. Prentice et al., 1990), and external tags (Stoettrup et al., 2002).

In this study we investigated the use of coded-wire tags (CWT) and visible implant elastomer (VIE) tags (Northwest Marine Technology, Shaw Island, WA) for stock enhancement purposes with juvenile red snapper *Lutjanus campechanus*. Red snapper populations in the Gulf of Mexico are subjected to high fishery mortality due to over-exploitation and high rates of juvenile loss through commercial shrimp bycatch (Gulf of Mexico Fishery Management Council, 2004). Among other things, in 1999 investigative research on marine stock enhancement with red snapper (Pruder et al., 1999) was initiated as an additional management tool to aid in population recovery.

While CWTs and VIE tags are internal, comparatively benign, and can be used with small fish, CWTs typically have excellent long-term retention and can be quickly applied, but tagged animals must be harvested to obtain internal tag codes.

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We chose CWTs to identify our experimental treatments over the long-term from fishery dependent and fishery independent recaptures. Visible implant elastomer tags can be used for external identification with various color and body implant locations (Buckley et al., 1994; Frederick, 1997; Willis and Babcock, 1998; Curtis, 2006). Short-term (6 months or less) retention of VIE tags is typically good (Godin et al., 1996; Hale and Gray, 1998; Olsen et al., 2004; Woods and Martin-Smith, 2004), but after this, retention and visibility can become unreliable (FitzGerald et al., 2004; Brennan et al., 2005). We used VIE tags as short-term external identifiers of our experimental treatments. A series of laboratory-based and field experiments were conducted to accomplish the following objectives: (1) identify optimal tag implant locations for CWTs and VIEs in juvenile snapper with the highest long-term retention rates, and best underwater visibility of the VIE tags and (2) develop a tagging system with CWT and VIE tags that minimized mortality and streamlined the tagging process.

2. Materials and methods

All red snapper used in this study were hatchery-reared from wild parental stock captured in the northern Gulf of Mexico. Fish were reared in salt water (salinity, 28–32 ppt) closed circulation tanks at the Gulf Coast Research Laboratory, Ocean Springs, Mississippi, USA (Blaylock et al., 2000).

2.1. General tagging procedure

Juveniles (45–125 mm standard length [SL]) were anaesthetized for 1–4 min in 70 ppm methane tricane sulphonate (MS-222), tagged, and placed in recovery water. Weights and lengths of anesthetized fish were recorded for each source tank, and tagged as follows: CWTs were injected free-hand with Mark IV tagging machines (Northwest Marine Technology, Shaw Island, WA, USA). We used a needle guard to regulate the depth of needle penetration and tag presence was verified with a magnetic field detector (Northwest Marine Technology products). Visible implant elastomer marks were injected with 27 gauge needles with both hand-pressurized syringes and pneumatic tag machines (Northwest Marine Technology).

2.2. Initial tag placement studies

Coded-wire tags were implanted in five body locations of juvenile snapper (48–107 mm SL) (Fig. 1, Table 1): (1) in a posterior direction into the nose cartilage ($n = 35$), (2) in a ventral direction into the left cheek muscle parallel to muscle fibers ($n = 35$), (3) in a posterior direction into the anterior epaxial musculature ("nape", near skull) ($n = 40$), (4) in an anterior and ventral direction also into the epaxial musculature, but near the dorsal fin (the needle was wedged beneath the scales before injection) ($n = 35$), and (5) in an anterior direction into the hypaxial musculature of the caudal peduncle (the needle was wedged beneath the scales before injection) ($n = 75$). Tagged groups were then placed in separate 400 l smooth fiberglass
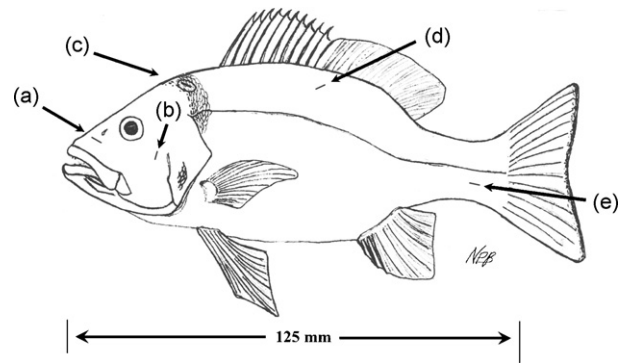

Fig. 1. Schematic of coded-wire tag implant locations and injection angles (indicated by arrows): (a) nose cartilage (b) left cheek, (c) nape, (d) dorsal muscle, and (e) caudal peduncle. Coded-wire tags (dashes) not to scale.

tanks with a common supply of recirculating sea water (~30 ppt, 29–30 °C). Tag retention was checked at 3 and 6 weeks after tagging.

Fluorescent red VIE was also injected in various body locations of juvenile snapper (48–107 mm SL) (Fig. 2): (1) anteriorly and diagonally across the nose bridge ($n = 40$ snapper) (specifically, two VIE injections were performed in an anterior direction, posterior and anterior to the eyes in an "X" like pattern), (2) under the skin at the base of the anal fin ($n = 35$), and (3) under the skin of the ventral caudal peduncle muscle ($n = 35$). Excess elastomer was removed from injection points to insure that marks were completely internal. Tagged fish were then placed in separate 400 l smooth fiberglass tanks with a common supply of recirculating sea water (about 30 ppt, 29–30 °C). Tag retention was checked at 3 and 6 weeks after tagging.

In another experiment, fluorescent red, orange, and yellow VIE marks were injected subcutaneously between the fin rays in the upper and lower lobes (Fig. 2) of the caudal fin of three size classes of hatchery-reared red snapper: "small" 46–65 mm SL (mean = 57 mm, $N = 100$), "medium" 55–83 mm SL (mean = 70 mm, $N = 100$), and "large" 75–111 mm SL (mean = 94 mm, $N = 100$). Again, excess elastomer was removed from injection points to insure that marks were completely internal. Tagged

Table 1
Results of initial tag retention trials for coded-wire tags (CWT) and visible implant elastomer tags (VIE)

Tag type	Implant location	Number tagged	Tag retention (weeks after tagging)	
			3	6
CWT	Nape	20	100	100
	Cheek	31	100	100
	Nose cartilage	35	97.1	97.4
	Dorsal muscle	35	91.4	90.4
	Caudal peduncle	51	98	98
VIE	Nose bridge	40	100	100
	Caudal peduncle	35	100[a]	95
	Anal fin muscles	35	87.1[a]	87

Tag retention is stated as percent tagged of total population sampled.
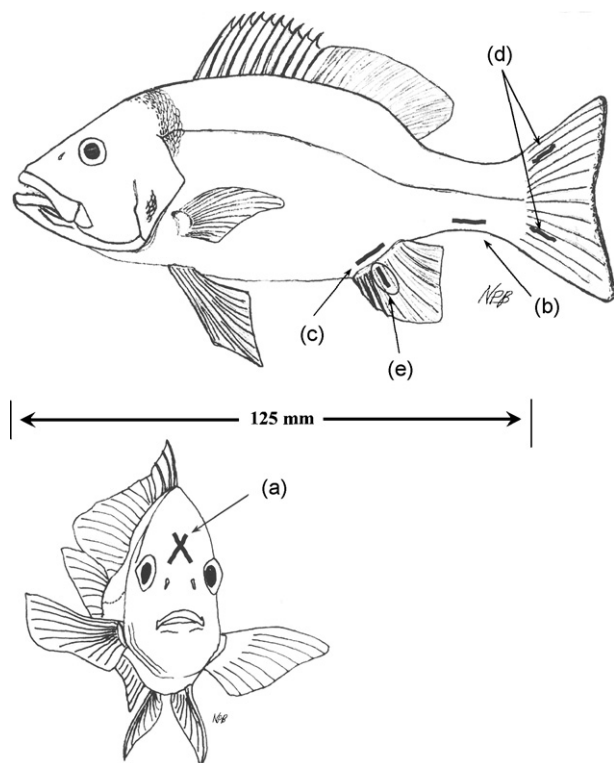[a] Difficult to see under direct observation.

Fig. 2. Schematic body implant locations of visible implant elastomer tags: (a) nose bridge, (b) caudal peduncle, (c) base of the anal fin, (d) dorsal and ventral caudal fin lobes, and (e) anal fin.

fish, separated according to the above size classes, were placed in indoor smooth fiberglass 400 l tanks supplied with common recirculating sea water (30 ppt, 29–30 °C at time of tagging). Tag retention was checked at 1, 2, 5, and 6 months after tagging. We performed a two-way analysis of variance (ANOVA) on arcsine transformed retention data (Sokal and Rohlf, 1995) to test the effects of size class-at-tagging and number of marks implanted in the caudal fin (upper lobe [1 mark], lower lobe [1 mark], any lobe [2 marks]) on tag retention after 6 months.

2.3. Tag and release studies

Stock enhancement release experiments were performed with red snapper in 1999–2001. Tagging activities were as follows: fish were harvested from source tanks in small batches (about 20–40 juveniles per batch) and transported to anesthetized water. Individuals were then measured and weighed and transferred to a second holding tub with anesthetized water. Coded-wire tags were implanted, then scanned for tag presence with magnetic tag detectors, and transferred to a third tub of anesthetized water before VIE tags were injected. After VIE injection, individuals were placed in non-anesthetized water to recover for about 10–30 min then transported in buckets with about 10 l of water to 1.5 m square nylon net pens (19 mm square mesh) until release.

Each year, juvenile snapper were tagged according to experimental treatments. Coded-wire tags were injected with Mark IV automatic injectors, and VIE tags were injected with pneumatic

tag machines at 70 psi (Northwest Marine Technology) and with hand-held syringes.

In October 1999, CWTs were implanted into the "nape" muscles just posterior to the skull of 1201 juvenile snapper (Fig. 1). Each fish was checked with a magnetic field detector for the presence of a magnetized CWT. Following this, two fluorescent red VIE marks were applied in the nose bridge as described above (Fig. 2). Tagged fish were held according to release groups in net pens for 14–16 days until release. On the day of release, fish were packed into plastic bags with 10 l of seawater at a density of about 50 g/l. Bags were packed into Styrofoam boxes and transported to the release site by truck and boat. Boxes were individually unpacked and about 10 l of seawater was added to the bags to allow fish to acclimate prior to release. After 5–10 min, fish in opened plastic bags were released into submerged bags (0.5 m × 0.25 m rigid rectangular, 5 mm mesh) and transported by divers to artificial reefs where they were released in the Gulf of Mexico 20 km off the coast of Mississippi. Water depth was about 20 m.

In 2000, we tagged 1201 juvenile snapper each with a CWT in the nape and a VIE mark subcutaneously between the fin rays of the dorsal and ventral lobes of the caudal fin (Fig. 2). Our experimental treatments were three artificial reef types (rubble reef, oyster reef, and stacked cinder block reef, Leber et al., unpublished data). Fluorescent red, orange, and yellow VIE were used to externally identify the fish stocked on different reef types. Tagged snapper were held in 1.5 m square net pens and tag checks were performed 7–8 days after tagging on the day of release. A sub-sample of 270 snapper (74 large, 97 medium, and 99 small) were held back from release to monitor tag retention. Each size class was placed in an indoor 1000 l smooth fiberglass cylindrical tanks all supplied with closed-circulation seawater. A tag retention check was performed 5 months after tagging. The remaining snapper were released in the Gulf of Mexico, approximately 24 km due west off the coast of Sarasota, FL, USA (Fig. 3) in approximately 18 m deep water. Divers performed point counts (e.g. Stone et al., 1979) on days 1, 8, 13, 15, 30, 45, 56, 68, 90, 118, 146, 168, and 200. For this study we only compared color confusion by divers.

In 2001, 353 juvenile snapper were each tagged with CWTs in the nape, and VIE tags subcutaneously between the caudal fin rays (Fig. 2). After tagging, all tagged fish were held in net pens according to experimental treatment for 35 days then transported from Mississippi to Florida in 1000 l tanks by truck, and stocked into three 3800 l fiberglass cylindrical tanks all supplied with closed-circulation seawater. Tag retention was checked at 35 days and 147 days after tagging. At this point, VIE material had fragmented (see Astorga et al., 2005), so all fish were retagged with fresh VIE marks 1 week before release. Release methods were similar to releases described above. However, in 2001 some groups were acclimated in situ. Fish were stocked into a 2.4 m × 2.4 m × 2.4 m PVC frame covered with 1 cm nylon mesh that enclosed one of the reef's subunits. These fish were acclimated for 3 days then released from the pens. Non-acclimated groups were stocked directly on to the remaining reef subunits.
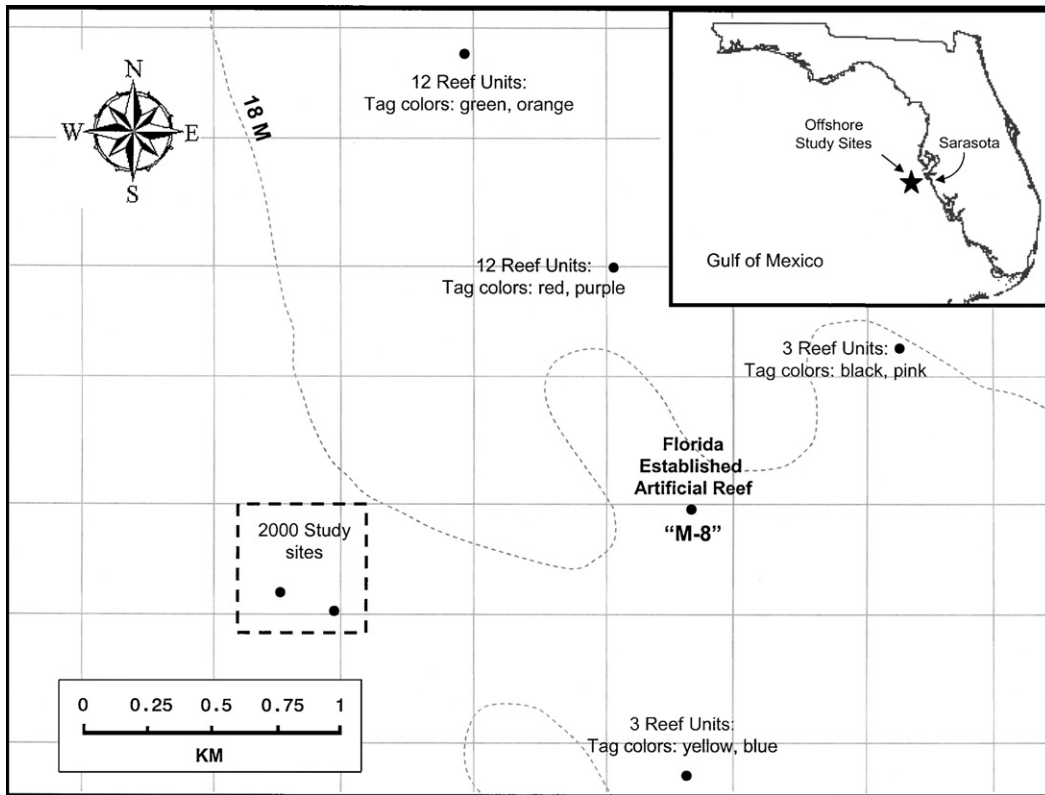
Fig. 3. Site map of artificial reefs where red snapper juveniles were released off of Sarasota, Florida. In the 2001 study, tag colors of snapper released are indicated. Depth contour is indicated by dashed curvy line. Map is not to scale.

In the 2001 release study there were eight experimental treatments as follows: number of artificial reef subunits (3 units versus 12 units; each unit was a $1\,m \times 1.12\,m \times 1.01\,m$ high concrete-block artificial reef), acclimation treatment or not. We used eight VIE colors in the caudal fins to individually identify treatments (2 reef types × 2 acclimation treatments × 2 replicates = 8 colors). Four artificial reef systems (about 1 km apart, Fig. 3) were each stocked with snapper tagged with one of two VIE colors (because within each reef system half were acclimated and half were not). Each reef was stocked with one of the following color combinations: green-orange, red-purple, black-pink, and yellow-blue (Fig. 3).

Post-release data were used to evaluate in situ tag visibility and tag color confusion by divers. Divers assessed stocked fish at 1, 4, 7, 10, 17, 24, 31, 38, 60, 79, 92, and 156 days after release. For every assessment day, a minimum of two experienced divers visited each of the release sites and counted the number of snapper tagged with each VIE color. Again, divers used point counts to assess fish on each reef. All dives were performed during daylight hours and tags were identified with the naked eye. One assessment (day 60), however, was performed at night and tags were identified with the assistance of standard dive lights. We used data from days 1–38 to evaluate color confusion by divers. Because each release site was stocked with fish tagged with one of two VIE colors, we expected those released at the site to represent the most abundant colors. Occasional migrants were represented by additional colors at the site.

3. Results

3.1. CWT retention

Initial tag placement studies showed that at 3 and 6 weeks after tagging CWT retention was good for all body locations tested (all greater than 90% tags retained), with little change in retention results between the two time periods (Table 1). At 6 weeks, no CWT loss was found from the nape and cheek muscle, and slight loss from the caudal peduncle muscle (98%), the nose cartilage (97%) and the posterior dorsal muscle (90%) (Table 1). Growth rates averaged 0.81 mm/day and one mortality occurred. In 2000, CWTs placed in the nape muscle of 270 juvenile snapper had high overall retention of 97.6% after 6 months. Size-specific retention of CWTs in the nape at 6 months was 98.9% for "small" snapper ($n = 92$), 99.2% for "medium" snapper ($n = 94$), and 99.6% for "large" snapper ($n = 88$). Growth rates for this period were 0.69, 0.66 and 0.52 mm/day for small medium and large size classes, respectively. No mortality was observed in these groups.

3.2. VIE retention

Elastomer mark retention 3–6 weeks after tagging was also high (Table 1); 95% or greater VIE mark retention was found in all of the body locations tested except those implanted in the anal fin muscles (87%). Although VIE material was initially quite visible in all locations, 3 weeks later VIE marks in the caudal
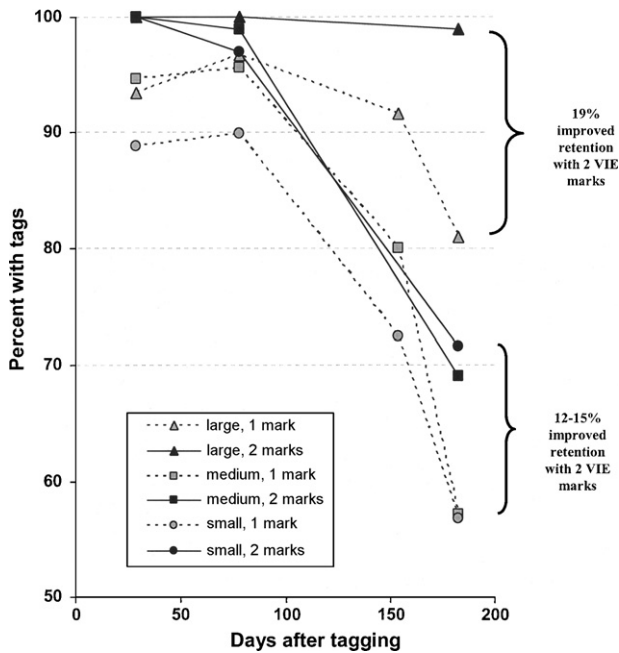
Fig. 4. Effects of one vs. two visible implant elastomer marks in the caudal fin on tag presence over time for different fish sizes. "Small" fish at the time of tagging were 46–65 mm SL (*N* = 100), "medium" fish were 55–83 mm SL (*N* = 100), and "large" 75–111 mm SL (*N* = 100).

peduncle and anal fin muscle were difficult to see under natural lighting conditions due to pigmentation over the VIE. Although the nose bridge showed high retention, visibility was poor and best visibility and retention of all sites tested was achieved in the caudal fin rays. Among the VIE colors tested in the caudal fin of laboratory-held snapper, fluorescent red and fluorescent orange VIE were more visible to the naked eye than fluorescent yellow VIE.

Both size-at-tagging and number of marks implanted in the caudal fin significantly influenced VIE retention after 6 months (*P* = 0.035, d.f. = 2 for size class; *P* = 0.018, d.f. = 1, for number of marks used) in the caudal fin rays and the small and medium size classes tagged had the highest tag loss rates (Fig. 4). Examination of the caudal fin rays with an ultraviolet light resulted in 17% more snapper identified with VIE marks. Approximately 12–19% more fish had visible VIE marks in them after 6 months when 2 marks were implanted in the caudal fin compared to 1 (Fig. 4). Growth rates in these groups averaged 0.62 mm/day from August to February (6 months).

3.3. Tagging rates and mortality

Maximum tagging rates for tagging operations in 1999 were 150–200 fish/h/operator. Coded-wire tagging was faster than VIE tagging and rates reported reflect VIE application rates. In October 2000, maximum tagging rates were again 150–200 fish per hour. Each fish received a CWT then two VIE marks in the caudal fin. There were two VIE taggers for every CWT tagger. Each year, overall tagging mortality was between 0.1 and 0.5%. While the effects of harvesting, anesthetizing, and tagging collectively caused mortality, we found overexposure
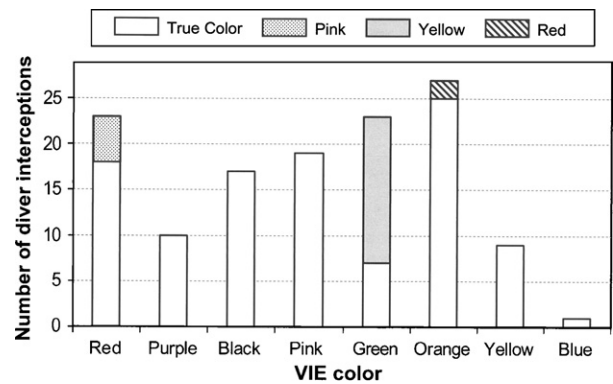

Fig. 5. Results from diver identification of visible implant elastomer (VIE) tags in juvenile red snapper. Data are from dives that occurred from day 1 to day 38 after release. Colors on the *x*-axis represent the true color of the VIE observed. Other colors observed (stacked above) represent misconceptions of the true tag color.

to MS-222 to be the most common and obvious cause of mortality.

3.4. In situ assessment

For the 1999, 2000, and 2001 release studies, SCUBA divers were able to identify tagged snapper and individual colors of VIE in snapper on artificial reefs. In the 2000 study, our reefs were spaced 50 m apart and snapper released on adjacent reefs were frequently found on neighboring reefs. This made it possible for divers to observe all three VIE colors (red, orange, and yellow) in fish over a reef at once. While yellow VIE was the dimmest of the three colors, we found no cases of color confusion in this study. In the 2001 study, water visibility was usually 2–3 m and divers were able to identify release treatments repeatedly without handling the fish. Eight VIE colors identified our experimental treatments and yellow was most commonly confused for green (70% of the time), although green was never confused with yellow. Red tags were confused as pink (22% of the time), and orange VIEs were confused as red (7% of the time). Black, purple, and blue were never confused (Fig. 5). However, when any two of the colors pink, red, or orange were seen along side of each other, their colors were not confused. During night dives (*n* = 3 divers), red VIE was seen as pink, but green was not confused with yellow. Tag loss was first reported in underwater counts 15 days after tagging and by 93 day after tagging 26% of the observed snapper were apparently missing tags.

4. Discussion

Overall, we found CWTs and VIE tags to be useful for stock enhancement studies with juvenile red snapper. This study has shown how tag implant location and tagging techniques can strongly influence the outcome of the study. In particular, while several implant locations for CWTs were shown to result in high retention, tagging into some implant locations (dorsal musculature, caudal peduncle, and cheek musculature) was logistically difficult and could substantially slow tagging speeds

and required more care to achieve high tag retention. For VIE tags, body location and the number of marks applied (one versus two marks in the caudal fin) significantly influenced mark retention and visibility. While high VIE retention was achieved in the head region and caudal fin (up to 6 months), visual observation by divers required the tags be located in the semi-transparent caudal fin, observable from either side of the body.

The tagging rates achieved in our large-scale tagging operations were similar to those reported in other studies. With two VIE tags applied to each fish our rates were still 150–200 fish/h/operator, which compares reasonably with other studies (Dewey and Zigler, 1996; Bailey et al., 1998; Astorga et al., 2005; Brennan et al., 2005). Furthermore, we required fish to be fully anesthetized before VIE tags could be injected in the fins, and often tagging rates were slowed by the anesthetization process. We found red snapper juveniles very sensitive to MS-222 and concentrations over 70 ppm would cause the fish to sedate faster, but sedation recovery times were longer and sometimes led to mortality. Overall most mortality during tagging was primarily attributed to MS-222 overdose (via prolonged exposure to the anesthesia). Future studies should identify the relative effects of accumulated stress by transport from source tanks, anesthetization (duration and concentration), tagging (duration of exposure times to air, and tag types, e.g. Sulikowski et al., 2005), and recovery times before transport. Future studies should also identify more lenient anesthetics for red snapper.

Growth rates of tagged fish in this study (0.52–0.69 mm/day) were similar to growth rates observed in wild age-0 snapper in the Gulf of Mexico (0.52–0.62 mm/day) (Szedlmayer and Conti, 1999) indicating no apparent influence of these tags on growth. Furthermore, Astorga et al. (2005) found no differences in growth rates between groups of VIE tagged and non-tagged juvenile gilthead seabream *Sparus auratus L.* Other studies with fishes and crustaceans (Russell and Hales, 1992; Malone et al., 1999; Kneib and Huggler, 2001; Davis et al., 2004) found no influence of these tag types on growth rates.

Coded-wire tags have shown to be suitable for application with small fish (e.g. salmon [Salmonidae, Jefferts et al., 1963; Blankenship, 1990], anchovies *Stolephorus purpureus* [Leary and Murphy, 1975], striped bass *Morone saxatilis*, and blue tilapia *Tilapia aurea* [Klar and Parker, 1986], largemouth bass *Micropterus salmoides* [Fletcher et al., 1987; Buckmeier, 2001], walleye *Stizostedion vitreum* [Peterson and Key, 1992], and striped mullet *Mugil cephalus* [Leber et al., 1996]). Our study found high retention of CWTs implanted in the nape, cheek musculature, nose cartilage, dorsal muscle, and caudal peduncle of juvenile snapper. We used the nape as our target site for larger-scale CWT tagging because tag application was relatively easy, fast, and offered high retention. Both the cheek and nose cartilage also showed good potential for CWT implant sites especially because they are located in the head region, and thus useful for fishery tag recovery programs that collect heads and carcasses. Large-scale application of tags in the nose cartilage would require development of head molds to guide tag placement (e.g. see Cook et al., 1990), but once developed, faster tagging rates (greater than 800 fish/h/operator [see Schurman and Thompson, 1990; Leber et al., 1998]) could be achieved than cheek implan-

tation without head molds (usually about 500 fish/h/operator [see Ingram, 1993; Buckmeier, 2001; Brennan et al., 2005]). Scales covering the cheek, caudal peduncle and dorsal musculature made tagging more difficult and ultimately slowed tagging speed. Furthermore, injections into the dorsal musculature and caudal peduncle were performed with the tail of the fish facing the needle, which was awkward. In this study, CWT retention after 6 months remained above 97% and high rates such as these are not uncommon (Blankenship, 1990; Leber et al., 1996). Furthermore, CWTs implanted in intramusculature have shown very little tag loss just days after tagging (Buckmeier, 2001), and retention rates are typically valid over the long-term (e.g. Brennan et al., 2005).

We used VIE tags in this study for external identification of small-scale stocking treatments. Our experimental designs were primarily restricted by the number of fish available and, at most, eight VIE codes were needed. Hundreds of VIE codes are possible using different body locations and colors (Frederick, 1997; Curtis, 2006), but even under controlled conditions (e.g. fish in aquaria) beforehand knowledge of tag combinations and experience are necessary to correctly identify codes. The highest retention of VIE marks were achieved in the caudal fin and the nose bridge (100% after 4 and 6 weeks, respectively). The anal fin muscle and ventral caudal peduncle of juvenile snapper did not prove to be good target locations for VIE material because pigmentation occurred over the tag reduced visibility.

While our preliminary studies with VIE tag placement showed several sites with high tag retention, other issues besides retention were also important considerations. Our release experiments required good tag visibility by underwater divers. For this to occur, we required that tags (1) be retained at high rates, (2) had maximum visibility regardless of fish orientation, and (3) have distinguishable colors at depth.

In this study VIE tags showed high retention up to 6 months. With snook, Brennan et al. (2005) showed good mark retention up to 1 year after tagging, but as in Astorga et al. (2005) and this study, VIE material fragmented over time became difficult to see. We stress that while VIE tags can are useful for short-term studies, after 6 months in our study they became essentially indistinguishable and were not useful. Underwater visibility of the tags by divers was an important component of this study. Juvenile snapper released in 1999 had VIE tags in the nose bridge, but divers could only see the tags when they were above the fish, and pigmentation obscured tag visibility. Divers found that VIE tags were most visible in the caudal fin and tags could be identified from either side of the fish. Bonneau et al. (1995) were able to successfully identify experimental treatments of trout marked with VIE in various body locations including on the top of the head, but this may in part have been because of the shallow water habitat of the trout and most observations were diagonally above the fish. In our study, divers were in 17–20 m deep water and juvenile snapper typically oriented alongside and above the artificial reefs which made observations of the head mark difficult.

Willis and Babcock (1998) noted that orange and red colors were confused by divers and green and yellow were also confused with a 5–20% error rate. The lack of color misidenti-

fications in our 2000 study is probably because the three colors were seen at once and relative differences could be compared. Color confusions by divers were common in our 2001 study however, with green being confused for yellow 70% of the time (Fig. 5). Whenever fish with yellow VIE were seen alongside fish with green VIE, confusion never occurred. Also when divers saw fish with yellow VIE alone they never confused it with green, probably because yellow looked very "yellow", but green was diffuse and looked somewhat "yellow" at depth. Although the VIE material did break up into smaller particles, it was still easily visible. Red was sometimes identified as "pink", and orange sometimes identified as "red", but this only occurred when one color was available to identify. Inter-reef migration was less common in our 2001 study than in our 2000 study, most likely due to the greater distance between reefs (about 1 km versus 50 m, respectively). Resultantly, only two colors were observed at a given reef site and were typically the VIE colors stocked there. Thus, without a comparison of similar colors, divers guessed at the VIE color and often misidentified them. To avoid this, divers should carry actual VIE samples to compare with VIE colors observed in fish. These fairly high rates of color confusion occurred with simple color codes (up to eight codes). Other studies (e.g. Curtis, 2006) using a combination of color and body location as unique codes (up to 1024 codes) and found diver misperception of colors to be the primary cause (beyond tag loss and visibility) of code errors.

We found diver-perceived VIE tag retention estimates to be much lower than laboratory-based tag retention studies (about 74% tag retention by divers at 3 months after tagging compared to over 98% in the laboratory). This is probably due to the difficulty of visually identifying tags in moving fish in water with diffuse lighting (17–20 m deep). Several factors contribute to this including (1) fish movement while divers are trying to identify the tag, (2) greater distance of the fish from the diver (e.g. see Willis and Babcock, 1998, for quantified analysis), and (3) water visibility and light absorption at depth. Interestingly, we found that during night dives VIE visibility in fish was very good, especially for fluorescent tags and green VIE was not confused with yellow. The artificial lighting from dive lights caused colors to be more visible due to less diffusion of light from the light source to the tag. Studies of visual census conducted during the day, in water with good visibility, and at shallower depths (e.g. see Frederick, 1997) may not experience color confusion as in this study.

Although we did not investigate the effect of VIE tags on mortality it is possible VIE marks could increase predation risk (e.g. Catalano et al. (2001) have found VIE to increase predation risk in largemouth bass). Furthermore, colored tags may influence social interactions and consequences. Further study on the abovementioned issues is warranted.

5. Conclusion

Overall we found that CWTs and VIE tags were useful for our experimental stock enhancement studies. The combined use of CWTs and VIE tags allowed us to mark small fish over the long-term, and also allow short-term benign evaluation of experimental treatments. Body implant location and tagging techniques significantly influenced tag retention and tag visibility in juvenile snapper. In this study, multiple body implant locations for VIE tags were identified with high VIE retention. The caudal fin was identified as an optimal location for VIE tags because of high tag retention and visibility by underwater divers. There was some unexpected confusion of VIE colors by divers, but this could be resolved by divers carrying samples of appropriately colored VIE material (e.g. mounted to data clipboards, etc.). Night dives offer potential for more accurate color identification because of decreased color diffusion when artificial lights are closer to the tagged fish. Estimates of relative performance of stocked experimental groups were achieved, but actual population estimation using these tags would be difficult.

References

Astorga, N., Afonso, J.M., Zamorano, M.J., Montero, D., Oliva, V., Fernández, H., Izquierdo, M.S., 2005. Evaluation of visible implant elastomer tags for tagging juvenile gilthead seabream (*Sparus auratus* L.): effects on growth, mortality, handling time and tag loss. Aquacult. Res. 36, 733–738.

Bailey, R.E., Irvine, J.R., Dalziel, F.C., Nelson, T.C., 1998. Evaluations of visible implant fluorescent tags for marking Coho salmon smolts. N. Am. J. Fish. Manage. 18, 191–196.

Blankenship, H.L., 1990. Effects of time and fish size on coded wire tag loss from Chinook and Coho salmon. In: Parker, N.C., Giorgi, A.E., Heidinger, R.C., Jester Jr., D.B., Prince, E.D., Winans, G.A. (Eds.), Fish Marking Techniques. American Fisheries Society Symposium 7, Bethesda, MD, pp. 237–243.

Blankenship, H.L., Leber, K.M., 1995. A responsible approach to marine stock enhancement. In: Schramm Jr., H.L., Piper, R.G. (Eds.), Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society Symposium 15, Bethesda, MD, pp. 376–387.

Blaylock, R.B., Leber, K.M., Lotz, J.M., Ziemann, D.A., 2000. The U.S. Gulf of Mexico Marine Stock Enhancement Program (USGMSEP): the use of aquaculture technology in "responsible" stock enhancement. Bull. Aquacult. Assoc. Can. 100 (3), 16–22.

Bonneau, J.L., Thurow, R.F., Scarnecchia, D.L., 1995. Capture, marking, and enumeration of juvenile bull trout and cutthroat trout in small, low-conductivity streams. N. Am. J. Fish. Manage. 15, 563–568.

Brennan, N.P., Leber, K.M., Blankenship, H.L., Ransier, J.M., DeBruler Jr., R., 2005. An evaluation of coded wire and elastomer tag performance in juvenile common snook under field and laboratory conditions. N. Am. J. Fish. Manage. 25, 437–445.

Buckley, R.M., West, J.M., Doty, D.C., 1994. Internal micro-tag systems for marking juvenile reef fishes. Bull. Mar. Sci. 55 (2–3), 848–857.

Buckmeier, D.L., 2001. Coded wire tag insertion sites for small fingerling black bass. N. Am. J. Fish. Manage. 21 (3), 696–698.

Catalano, M.J., Chipps, S.R., Bouchard, M.A., Wahl, D.H., 2001. Evaluation of injectable fluorescent tags for marking centrarchid fishes: retention rate and effects on vulnerability to predation. N. Am. J. Fish. Manage. 21 (4), 911–917.

Cook, S.B., Davin Jr., W.T., Heidinger, R.C., Jester Jr., D.B., 1990. Head mold design for coded wire tagging of selected spiny-rayed fingerling fishes. In: Parker, N.C., Giorgi, A.E., Heidinger, R.C., Jester Jr., D.B., Prince, E.D., Winans, G.A. (Eds.), Fish Marking Techniques. American Fisheries Society Symposium 7, Bethesda, MD, pp. 281–285.

Curtis, J.M.R., 2006. Visible implant elastomer color determination, tag visibility, and tag loss: potential sources of error for mark-recapture studies. N. Am. J. Fish. Manage. 26 (2), 327–337.

Davis, J.L.D., Young-Williams, A.C., Hines, A.H., Zmora, O., 2004. Comparing two types of internal tags in juvenile blue crabs. Fish. Res. 67, 265–274.

Dewey, M.R., Zigler, S.J., 1996. An evaluation of fluorescent elastomer for marking bluegills in experimental studies. Prog. Fish-Cult. 58, 219–220.

FitzGerald, J.L., Sheehan, T.F., Kocik, J.F., 2004. Visibility of visual implant elastomer tags in Atlantic salmon reared for two years in marine net-pens. N. Am. J. Fish. Manage. 24, 222–227.

Fletcher, D.H., Haw, F., Bergman, P.K., 1987. Retention of coded wire tags implanted into cheek musculature of largemouth bass. N. Am. J. Fish. Manage. 7, 436–439.

Frederick, J.L., 1997. Evaluation of fluorescent elastomer injection as a method for marking small fish. Bull. Mar. Sci. 61 (2), 399–408.

Godin, D.M., Carr, W.H., Hagino, G., Segura, F., Sweeney, J.N., Blankenship, L., 1996. Evaluation of a fluorescent elastomer internal tag in juvenile and adult shrimp *Penaeus vannamei*. Aquaculture 139, 243–248.

Hale, R.S., Gray, J.H., 1998. Retention and detection of coded wire tags and elastomer tags in trout. N. Am. J. Fish. Manage. 18, 197–201.

Hilborn, R., 2004. Population management in stock enhancement and sea ranching. In: Leber, K.M., Kitada, S., Blankenship, H.L., Svasand, T. (Eds.), Stock Enhancement and Sea Ranching. Blackwell Publishing, Oxford, UK, pp. 201–209.

Ingram, B.A., 1993. Evaluation of coded wire tags for marking fingerling golden perch, *Macquaria ambigua* (percichthyidae), and silver perch, *Bidyanus bidyanus* (teraponidae). Aust. J. Mar. Freshwater Res. 44 (6), 817–824.

Jefferts, K.B., Bergman, P.K., Fiscus, H.F., 1963. A coded wire identification system for macro-organisms. Nature 198, 460–462.

Jenkins, W.E., Denson, M.R., Bridgham, C.B., Collins, M.R., Smith, T.I.J., 2004. Retention of oxytetracycline-induced marks on sagittae of red drum. N. Am. J. Fish. Manage. 22 (2), 590–594.

Klar, G.T., Parker, N.C., 1986. Marking fingerling striped bass and blue tilapia with coded wire tags and microtaggants. N. Am. J. Fish. Manage. 6, 439–444.

Kneib, R.T., Huggler, M.C., 2001. Tag placement, mark retention, survival and growth of juvenile white shrimp (Litopenaeus setiferus Perez Farfante, 1969) injected with coded wire tags. J. Exp. Mar. Biol. Ecol. 266, 109–120.

Leary, D.F., Murphy, G.I., 1975. A successful method for tagging the small, fragile Engraulid, *Stolephorus purpureus*. Trans. Am. Fish. Soc. 104 (1), 53–55.

Leber, K.M., Arce, S.M., Sterritt, D.A., Brennan, N.P., 1996. Marine enhancement potential in nursery habitats of striped mullet, *Mugil cephalus*, in Hawaii. Fish. Bull. 94, 452–471.

Leber, K.M., Brennan, N.P., Arce, S.M., 1998. Recruitment patterns of cultured pacific threadfin, *Polydactylus sexfilis* (Polynemidae), released along sandy marine shores in Hawaii. Bull. Mar. Sci. 62 (2), 389–408.

Leber, K.M., 2002. Advances in marine stock enhancement: shifting emphasis to theory and accountability. In: Stickney, R.R., McVey, J.P. (Eds.), Responsible Marine Aquaculture. CABI Publishing, New York, pp. 79–90.

Malone, J.C., Forrester, G.E., Steele, M.A., 1999. Effects of subcutaneous micro-tags on the growth, survival, and vulnerability to predation of small reef fishes. J. Exp. Mar. Biol. Ecol. 237, 243–253.

Munroe, J.L., Bell, J.D., 1997. Enhancement of marine fisheries resources. Rev. Fish. Sci. 5 (2), 185–222.

Olsen, E.M., Gjøsæter, J., Stenseth, N.C., 2004. Evaluation of the use of visible implant tags in age-0 Atlantic cod. N. Am. J. Fish. Manage. 24, 282–286.

Perez-Enriquez, R., Taniguchi, N., 1999. Genetic structure of red sea bream population off Japan and southwest Pacific, using microsatellite DNA markers. Fish. Sci. 65, 23–30.

Peterson, M.S., Key, J.P., 1992. Evaluation of hand-tagging juvenile walleyes with binary coded-wire microtags. N. Am. J. Fish. Manage. 12, 814–818.

Prentice, E.F., Flagg, T.A., McCutcheon, C.S., 1990. Feasibility of using implantable passive integrated transponder (PIT) tags in salmonids. In: Parker, N.C., Giorgi, A.E., Heidinger, R.C., Jester Jr., D.B., Prince, E.D., Winans, G.A. (Eds.), Fish Marking Techniques. American Fisheries Society Symposium 7, Bethesda, MD, pp. 317–322.

Pruder, G.D., Ziemann, D.A., Leber, K.M., Lotz, J.M., Hawkins, W.E., Conquest, L.D., 1999. U.S. Gulf of Mexico marine stock enhancement five year program: 1999–2004. Program management for planning, execution, review, and evaluation. Bull. Natl. Res. Inst. Aquacult. Suppl. 1, 89–99.

Russell, D.J., Hales, P.W., 1992. Evaluation of techniques for marking juvenile barramundi, Lates calcarifer (Bloch), for stocking. Aquacult. Fish. Manage. 23, 691–699.

Saillant, E., Cizdziel, K., O'Malley, K.G., Turner, T.F., Pruett, C.L., Gold Jr., J.R., 2004. Microsatellite markers for red drum, *Sciaenops ocellatus*. Gulf Mex. Sci. 22 (1), 101–107.

Schurman, G.C., Thompson, D.A., 1990. Washington Department of Fisheries' mobile tagging units: construction and operation. In: Parker, N.C., Giorgi, A.E., Heidinger, R.C., Jester Jr., D.B., Prince, E.D., Winans, G.A. (Eds.), Fish Marking Techniques. American Fisheries Society Symposium 7, Bethesda, MD, pp. 232–236.

Smoker, W.W., Bachen, B.A., Freitag, G., Geiger, H.J., Linley, T.J., 2000. Alaska ocean ranching contributions to sustainable salmon fisheries. In: Knudson, E.E., Steward, C.R., MacDonald, D.D., Williams, J.E., Reiser, D.W. (Eds.), Sustainable Fisheries Management: Pacific Salmon. CRC Press LLC, Boca Raton, FL, pp. 407–422.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Company, New York.

Stoettrup, J.G., Sparrevohn, C.R., Modin, J., Lehmann, J.K., 2002. The use of releases of reared fish to enhance natural populations: a case study on turbot *Psetta maxima* (Linne, 1758). Fish. Res. 59 (1–2), 161–180.

Stone, R.B., Pratt, H.L., Parker Jr., R.O., Davis, G.E., 1979. A comparison of fish populations on an artificial reef in the Florida Keys. Mar. Fish. Rev. 41, 1–11.

Sulikowski, J.A., Fairchild, E.A., Rennels, N., Howell, W.H., Tsang, P.C.E., 2005. The effects of tagging and transport on stress in juvenile winter flounder, *Pseudopleuronectes americanus*: implications for successful stock enhancement. J. World Aquacult. Soc. 36 (1), 148–156.

Szedlmayer, S.T., Conti, J., 1999. Nursery habitats, growth rates, and seasonality of age-0 red snapper, *Lutjanus campechanus*, in the northeast Gulf of Mexico. Fish. Bull. 97 (3), 626–635.

Taniguchi, N., 2004. Broodstock management for stock enhancement programs of marine fish with assistance of DNA marker (a review). In: Leber, K.M., Kitada, S., Blankenship, H.L., Svsand, T. (Eds.), Stock Enhancement and Sea Ranching. Blackwell Publishing, Oxford, UK, pp. 329–338.

Willis, T.J., Babcock, R.C., 1998. Retention and in situ detectability of visible implant fluorescent elastomer (VIFE) tags in *Pagrus auratus* (Sparidae). N. Z. J. Mar. Freshwater Res. 32, 247–254.

Woods, C.M.C., Martin-Smith, K.M., 2004. Visible implant fluorescent elastomer tagging of the big-bellied seahorse, *Hippocampus abdominalis*. Fish. Res. 66, 363–371.