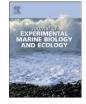
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Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe



# Experimental test of two marking methods on survival, growth, mark retention and readability on young-of-year pinfish (*Lagodon rhomboides*)

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## ARTICLE INFO

Article history: Received 18 July 2012 Received in revised form 15 November 2012 Accepted 16 November 2012 Available online 21 December 2012

Keywords: Fluorescent tag Food ration Freeze brand Mark-recapture Seagrass Tank experiment

# ABSTRACT

Ecological studies often require marking individuals or cohorts. However, different marks may have inherent advantages and disadvantages which should be considered before designing studies that use them. Visible implant elastomer (VIE) tags and liquid-nitrogen cold brands are two techniques commonly used with fishes, but their effects on growth and survival, and their retention rates and mark readability have not been explicitly tested on pinfish (*Lagodon rhomboides*), an ideal model organism due to its high abundance and tractability. We used a controlled mesocosm experiment to test for mark-induced differences in survival and growth rates, and growth-induced differences in mark retention and readability, between VIE-tagged and cold-branded juvenile pinfish. Neither VIE tags nor brands affected survival or growth in pinfish. Furthermore, growth did not affect retention or readability was better for VIE-tagged fish. Thus, both methods appear to satisfy the criterion of not affecting basic biological processes, an important assumption in all studies that use marking techniques, while also differing in other regards. We discuss some of the competing advantages and disadvantages of each that investigators must consider before the onset of a marking program.

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# 1. Introduction

Ecological studies often require marking individuals to examine processes such as survival, growth, population dynamics, and migration. Given that numerous types of tags and procedures are available to mark fish (e.g., visible implants elastomer, liquid nitrogen cold brands, binary-coded wire tags, fin clipping), and that each may have inherent advantages and shortcomings, investigators must carefully consider which type would be most suitable for their study organism. Wydoski and Emery (1983) established five criteria that marking procedures should meet: they should (1) distinguish individuals or batches of fish; (2) not affect the growth, survival, behavior or probability of being captured of the individual fish; (3) remain throughout the study, (4) be easy to apply and identify; and (5) be cost effective.

Visible implant elastomer tags (hereafter elastomer tags) and liquid nitrogen cold brands (hereafter brands) are two types of marks commonly used for fishes. An elastomer tag consists of a two-part, silicone based colored compound that is injected subcutaneously. The compound cures into a pliable solid after injection. Brands are made by holding a metal marker cooled with either liquid nitrogen or carbon dioxide (Bryant and Walkotten, 1980) in contact with the skin of the fish. The extreme cold temperature destroys melanophores, leaving an identifiable mark, which is later invaded by melanin-containing cells (Laird et al., 1975). Elastomer comes in a variety of colors and brands that can be customized to have unique codings (e.g., alpha-numeric sequences). Thus, both provide researchers with options to separate marks according to specific memberships (e.g., cohort, site) and combinations can be used to identify individuals, meeting the first criterion set forth by Wydoski and Emery (1983). They are both easy to apply (first part of criterion 4) and cost effective (criterion 5), while their effects on growth and survival (criterion 2), and retention (criterion 3) require additional attention.

In this study, we used a controlled, mesocosm experiment to examine the effects of elastomer tags and brands on growth and survival (criterion 2) of young-of-year pinfish (*Lagodon rhomboides*). We also tested whether growth affected mark retention (criterion 3) and readability (second part of criterion 4).

### 2. Methods and materials

#### 2.1. Study organism

The pinfish is a widely-distributed and highly-abundant sparid found in coastal and offshore waters along the U.S. Atlantic and Gulf

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coasts (Darcy, 1985; Nelson, 2002). Young-of-year use shallow habitats, both vegetated (e.g., seagrass, marsh) and non-vegetated (e.g., sand, mud), for foraging and refuge. Due to their high abundance, they are an important prey for piscivores located in habitats both nearshore (Stallings, 2010; Stallings et al., 2010) and offshore (Nelson et al., 2012). Their high abundance, widespread distribution, and tractability also make them attractive model organisms for ecological study, including research that requires marking individuals and/or cohorts.

#### 2.2. Experimental design

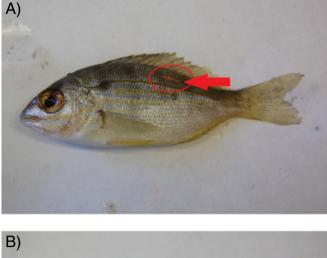
We used a controlled, orthogonal experiment with two treatments: (1) mark type and (2) food ration. There were three levels of the marking treatment (elastomer tag, brand and unmarked control) and two levels of the food ration treatment (high ration and low ration). The food ration treatment was included to both experimentally drive different growth rates (to test marking effects on growth) and to determine whether different growth rates affected tag retention.

Pinfish used in the experiment were captured using otter trawls pulled at 3.5 km/h through the seagrass bed located adjacent to the Florida State University Coastal and Marine Laboratory (FSUCML). Captured fish were placed in aerated coolers filled with water from the capture site, carried back to the FSUCML, and placed in flow-through holding tanks. The fish were allowed to recover for 24 h before being transferred to 18.9 L experimental mesocosms. To eliminate potential confounding effects of intraspecific interactions (e.g., competition, aggressive territorial behavior) we placed a single fish in each mesocosm arena.

After a brief two-hour acclimation period in the experimental mesocosms, individual fish were randomly assigned to one of the six levels of the mark\*ration treatment combinations: (1) elastomer tag high ration; (2) elastomer tag low ration; (3) brand high ration; (4) brand low ration; (5) unmarked high ration; and (6) unmarked low ration. Our experiment involved 15 replicates per mark\*ration treatment (N = 90).

Each fish was measured to the nearest mm standard length (SL), weighed to the nearest centigram, and placed in an aerated recovery tank for 1 min. Fish receiving elastomer tags were injected with orange-colored visible implant elastomer (VIE, Northwest Marine Technology, Inc.) on the left side between the lateral line and dorsal fin (Fig. 1A). Hypodermic needles (0.3 cc, 29 gauge) were used to inject the elastomer between the skin and muscle. Branded fish were marked with a copper brand cooled by insertion into liquid nitrogen, also between the lateral line and dorsal fin, with a horizontal line (Fig. 1B). The brand was held in contact with the fish for 5 s. Fish in the control group were held out of the water for 15 s to mimic the handling time associated with the two marking treatments. All fish were then returned to their respective experimental mesocosms.

Fish were fed daily (at approximately 0900). Those fed with highration diets received food equaling 16% of the individual fish's body mass per day and low-ration fish received 4%. The length and mass of each fish were re-measured at three and six weeks after the start of the experiment. The food rations were adjusted to account for changes in mass. While remeasuring, the tags/brands were independently examined by two investigators (CVM and CDS) and rated on a scale from 1-4 for visibility. A rating of 1 indicated that no tag/brand was visible. A rating of 2 (poor) indicated that the tag/brand was visible, but very hard to distinguish. Elastomer tags with a rating of 2 were often broken up into a series of dots or obscured by tissue, while brands with a rating of 2 often required manipulating the fish relative to ambient light until a faint brand appeared. A rating of 3 (good) indicated the tag/brand was easily seen, but was not as visible as one would expect on a freshly tagged/branded fish. A rating of 4 (excellent) indicated that the tag/brand appeared as though the fish had just recently received the mark. We considered tags with a rating of 3 or 4 to be easily readable (sensu Wydoski and Emery, 1983; criterion 4).





**Fig. 1.** Photos showing young-of-year pinfish marked with A) orange elastomer and B) a cold-brand. (photo credit: CVM).

#### 2.3. Statistical analysis

We calculated the percent growth of each individual fish as (final SL – initial SL)/initial SL + 100.<sup>2</sup>We used a *t*-test to determine whether the food ration treatment drove different growth rates for the unmarked controls. Analysis of variance (ANOVA) with Tukey's pairwise comparisons (data were log transformed to homogenize variance), was used to compare the percent growth across the three levels of marking treatment within each food ration treatment. Logistic regression was used to test whether 1) survival was different between controls and both elastomer and brands, 2) mark retention changed with percent growth for both marking treatments, 3) retention differed between elastomer tags and brands, 4) readability changed with percent growth for both marking treatments, and 5) readability differed between elastomer tags and brands. For the readability analyses, we used the lowest of the readability ratings for each fish given by the two independent observers.

#### 3. Results

Unmarked (control) fish fed with high-ration diet grew more (mean (se) percent growth (SL) = 75.37% (5.90)) than control fish fed with low-ration diet (mean (se) percent growth (SL) = 45.19% (2.92)); ( $t_{26}$  = 4.5042, P = 0.0001) (Fig. 2). Thus, the feeding treatment was successful.

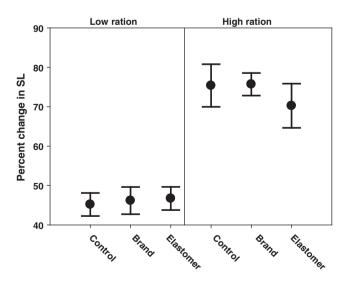
<sup>&</sup>lt;sup>2</sup> Results were qualitatively identical for length and mass due to the strong relationship between the two metrics in juvenile fishes, so we limit our report to length-based growth for simplicity.

Growth did not differ among the three levels of the marking treatment (unmarked control, elastomer tag, brand) within the high food treatment ( $F_{2,39} = 0.39, P = 0.68$ ) (Fig. 2). Mean (se) growth (SL) was 75.37% (5.41) for the control fish, 70.24% (5.61) for the elastomer tagged fish, and 75.69% (2.87) for the branded fish. None of the pairwise comparisons indicated that fish grew differently depending on the marking treatment (control-brand [-17.3, 16.6], elastomercontrol [-21.7, 11.5], elastomer-brand [-22.7, 11.8]). Growth also did not differ among the three levels of the marking treatment (unmarked control, elastomer tag, brand) within the low food treatment  $(F_{2,39}=0.06, P=0.94)$  (Fig. 2). Mean (se) growth (SL) for these was 45.19% (2.92) for the control fish, 46.72% (2.94) for the elastomer tagged fish, and 46.17% (3.45) for the branded fish. None of the pairwise comparisons suggested differences in growth (controlbrand [-11.9, 9.9], elastomer-brand [-10.3, 11.4], elastomer-control [-8.9, 11.9]).

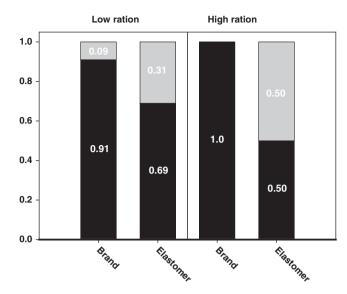
Survival was highest in the controls (93%), followed by those marked with elastomer (90%) and brands (80%). However, survival was not significantly different between controls and either elastomer (z = 0.46, P = 0.64) or brands (z = 1.45, P = 0.15).

Retention of brands was high, regardless of food ration, but slightly lower in fish fed with lower ration diet (91%) than those fed with high ration diet (100%) (Fig. 3). Logistic regression confirmed that this difference was not significant (z=0.62, P=0.54). Retention of elastomer tags was slightly higher in fish fed the low ration diet (69%) than those fed the high ration diet (64%), but this difference was also not significant (z=1.49, P=0.14) (Fig. 3). Because differences in growth rates did not affect retention for either brand or elastomer treatments, we pooled by mark type to test for differences in retention between brands and elastomer tags. Overall, mark retention was higher in branded fish (95.8%) than it was in elastomer tagged fish (66.7%; z=2.22, P=0.03; Fig. 3).

Readability of the tags was not affected by growth for either elastomer (z=1.69, P=0.09) or brands (z=0.41, P=0.68; Fig. 4). Among fish that retained their tags, we pooled observations that were easily readable (i.e., those rated 3 or 4 on the visibility scale) and used logistic regression to compare between elastomer tags and brands. The presence of easily readable marks was marginally higher for elastomer (proportion=0.75; z=1.91, P=0.05) compared to brands (proportion=0.43; Fig. 4).



**Fig. 2.** Growth as a function of the percent change in standard length (SL) of pinfish for the duration of the seven-week experiment. Pinfish were reared under low (4% of body mass) and high (16% of body mass) food rations and each received one of three levels of the marking treatment (control, brand, elastomer).



**Fig. 3.** Retention of two types of marks (brand, elastomer) crossed with two feeding rations that experimentally affected growth rates (see controls in Fig. 2). The proportion of marks retained is shown in black ( $\blacksquare$ ) and lost in light gray ( $\square$ ). Marks were blindly and independently observed by two observers (CVM and CDS).

## 4. Discussion

Using a mesocosm experiment, we found that neither growth rates nor survival of pinfish were affected by elastomer tags or cold brands, two commonly used marking techniques, compared to controls. Thus, either type of mark appears to satisfy Wydoski and Emery's (1983) second criterion. Both types of marks generally satisfied the third (retention) and fourth (readability) criteria, but there were some contextual differences that would likely affect investigators' choice of which technique to use. Our experiment therefore provides important insight on how researchers may proceed with marking studies involving

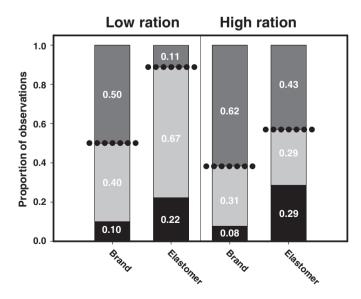


Fig. 4. Readability of two types of marks (brand, elastomer) crossed with two feeding rations that experimentally affected growth rates (see controls in Fig. 2). The proportion of marks ranked as excellent are shown in black (■), good in light gray (□), and poor in dark gray (□). Marks were blindly and independently scored by two observers (CVM and CDS). Marks deemed to be either excellent or good (proportion below horizontal bubble line) were easily identified while those deemed to be poor (proportion above bubble line) required careful and often lengthy examination.

pinfish while also highlighting the importance of examining basic assumptions and criteria that must be met before conducting any research that uses marked individuals.

The difference between mean growth of the controls indicated that our food treatment worked; we successfully induced different growth rates. That there were no effects of marking technique on either growth or survival of pinfish was consistent with studies on other fishes that used elastomer (Bushon et al., 2007; Frederick, 1997) and brands (Bryant and Walkotten, 1980; Bryant et al., 1990). However, our mesocosms did not include predators, one of the primary sources of mortality on juvenile fishes (Sogard, 1997). We therefore do not know whether antipredator responses would be affected by the different marks but we did not observe any behavioral differences between marked fish and controls. Researchers may also be concerned that the marks could attract attention from predators, especially brightly colored elastomer tags. However, field experiments have shown that mortality rates of fish tagged with elastomer did not differ from untagged controls (Hixon et al., 2012 Malone et al., 1999). Recapture rates of cold-branded pinfish stocked in field enclosures were relatively low (~53%) but the authors attributed the losses to escape rather than mortality, citing a lack of physiological indicators of stress after marks were applied combined with a lack of carcasses inside their cages (Spitzer et al., 2000).

Mark detectability has been shown to be positively related to the size of the fish at the time of tagging (Close, 2000; Dewey and Zigler, 1996), suggesting that fast-growing, juvenile fish are prone to tag loss. However, retention rates in this study were not related to the growth of the fish indicating that either type of mark could be used for fast-growing pinfish, at least within the time frame of our experiment (nearly two months). Retention of elastomer tags in other species has been shown to decrease as fish grow (e.g., Close, 2000), often due to the stretching and breakup of the tag (Zeller and Cairns, 2010; CD Stallings, pers obs). Indeed, a few elastomer tags in the current study appeared as broken lines instead of solid ones. In addition, any portion of the elastomer tag that protrudes from the skin can cause drag which eventually pulls it out of the fish. Although we took great care to minimize this problem during the tagging process, we observed two of 30 elastomer-tagged fish had protruding tags, one of which lost its tag and the other died. Brands, however, scar the fish making mark loss less common. Indeed, brands had a higher retention rate than elastomer tags in the current experiment, consistent with other findings (e.g., Haines et al., 1998). Occasionally, pinfish that grew especially large would have brands that were more difficult to read than on smaller fish, vet still identifiable. Unlike elastomer, which appears to become more difficult to read as a function of growth, reductions in the readability of brands may be a function of time as melanin-containing cells mask the scar.

There is often a need to run studies that use marks for longer periods than our seven-week mesocosm experiment. Several studies have found that brands fade rapidly, suggesting they are unsuitable for research lasting beyond a few weeks (Basavaraju et al., 1998; Bourgeois et al., 1987; Russell and Hales, 1992). However, Koenig and Coleman (1998) successfully used brands on postsettlement gag (Mycteroperca microlepis) in a study lasting 3.5 months and Bryant et al. (1990) reported brands on coho salmon (Oncorhynchus kisutch) were clearly visible six months after receiving the mark. One bet-hedging remedy against tag loss is to mark more than one location on the fish. Although marking multiple locations has been a successful method in some research (e.g., Weston and Johnson, 2008) it has also been shown to have negative effects on growth and survival (Hoey and McCormick, 2006). Retagging individuals within a study population is often necessary when the research lasts for an extended period of time. In an eight-year study, Hixon et al. (2012) monitored demographic rates of a damselfish that required multiple retagging events. That they followed individuals for over 3.5 years (i.e., their maximum natural age) suggests the retagging process did not have deleterious effects. Further research that explicitly tests the effects of multiple tagging locations and/or retagging on biological processes such as growth and survival is advised before administering a research program that uses such methods (Brennan et al., 2005).

From a logistical perspective, we noted that brands were cheaper and easier to apply, consistent with the observations of Haines et al. (1998). Indeed, Fay and Pardue (1985) were able to brand 360 rainbow trout (Oncorhynchus mykiss) per man-hour and Bryant et al. (1990) used a CO<sub>2</sub> freeze branding device to mark 2000 coho salmon within 2 h, productivity that is likely unachievable with elastomer tags. However, brands have some disadvantages for in situ use. Distinguishing the brands often required manipulating the fish until light reflected from the scar resulting in an increased handling time, a problem also reported by Laird et al. (1975). Cold branding also required a liquid nitrogen source to cool the brand to an effective temperature. In this study, we were forced to apply brands in an air-conditioned facility to prevent moisture in the air from condensing and freezing on the brand, which results in the formation of an insulating layer that prohibits a temperature low enough to adequately mark the fish. Thus, branding fish in the field in subtropical and tropical latitudes, where humidity is commonly high, may not be feasible. In addition, brands that are not easily readable could be confused as natural marks. Conversely, elastomer tags have some attractive properties that are beneficial for in situ use. We found that the readability of elastomer tags was marginally higher than brands (P = 0.05). However, we suspect this difference would be stronger in the field since we had the advantage of working in a laboratory setting where we were able to manipulate the fish relative to a consistent light source. If capturing and manipulating the fish are not feasible or desirable, the fluorescent colors of elastomer tags are advantageous since they are easier to see, especially if the color contrasts with the fish. Indeed, many studies that use observations of tagged fish by SCUBA divers have taken advantage of this property (e.g., Geange and Stier, 2009; Hixon et al., 2012; Johnson, 2006; Steele and Forrester, 2005).

Deciding on a mark type to use for juvenile fish can be challenging due to their fast growth and small size, both of which can cause issues with retention and application (Hoey and McCormick, 2006). Although this study focused on survival, growth, retention and readability, there are other factors that should be considered before deciding on a marking methodology including time required for application, cost, and the level of training required for those who are marking fish. We agree that more species-specific tests examining all of the aforementioned factors are necessary before conducting studies that rely on marking fishes (Buekers et al., 1995).

#### Acknowledgments

We thank M. Daniels and B. Henderson for their assistance in maintaining the seawater system at the FSUCML, A. Cruz, C. Gerrity and V. Ramos for their assistance with various lab-based activities, and F. Coleman for feedback on earlier versions of the manuscript. This study was conducted by CVM in partial fulfillment of the requirements of the Florida State University (FSU) Certificate Program in Marine Biology. Funding was provided by an Undergraduate Research and Creative Activity Award from FSU to CVM and from the U.S. National Oceanic and Atmospheric Administration (Northern Gulf of Mexico Cooperative Institute 191001-363558-01) to CDS. This study was conducted under the auspices of the FSU Animal Care and Use Committee (protocol # 9902) and adhered to NEPA guidelines. **[SS]** 

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