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Note

# Evaluation of a Visible Implant Fluorescent Elastomer Tag in the Soft-shelled Turtle, *Pelodiscus sinensis*

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Survival, tag retention and tag readability were compared among the control and three treatment groups of soft-shelled turtles, *Pelodiscus sinensis* Crother, 2000 (mean body weight ± SD: 182.6 ± 13.7 g), marked with visible implant fluorescent elastomer (VIFE) tags for 16 months. Mortality 4 to 16 months after tagging was attributed to collection and handling stress rather than to the tagging itself. Tags applied to the web surface between the fourth and fifth dactyl of the hindfoot appeared to have the highest retention rates, while adipose eyelid tagging had high tag readability but a high loss rate. We conclude that in soft-shelled turtles, the most suitable region for VIFE tagging is on the web surface between the fourth and fifth dactyls of the hindfoot.

Key words: *Pelodiscus sinensis*, Soft-shelled turtle, Survival, Tag readability, Tag retention, VIFE tag

## Introduction

The identification of individual animals is essential to fisheries research on growth, migration, mortality, stock identification, and gear selectivity of a particular aquatic population (Konstantinov, 1978; Park and Lee, 2001), although short-term tag retention may suffice for some experimental laboratory studies. In fishery management or research tagging experiments, the effect of the tag on animal survival, behavior, growth, and recognition, and the cost of the marking technique should be considered (Mcfarlane et al., 1990; Zerrener et al., 1997; Park and Lee, 2001). The ideal tag should provide positive identification throughout the life cycle and allow for an unlimited number of combinations, rapid application, and readability without damaging the individual, all at a low cost. In addition, the tag must not influence the behavioral or physiological characteristics of the study animal (Kincaid and Calkins, 1992; Park and Lee, 2001). Many aquatic species have transparent tissues suitable for tagging, including the opercula, mandible, top of the head, body, and fins. However, sites that reliably retain tags vary among species. Tagging sites in other parts of the body may also be used successfully (Park and Lee, 2001).

The visible implant fluorescent elastomer (VIFE) tag (Northwest Marine Technology, Shaw Island, WA, USA) is a visual marking technique that provides an externally visible internal marker for fish and other aquatic animals. The tag consists of a fluorescent two-part elastomer, which is injected into a transparent tissue of the animal using a hypodermic syringe. Within hours, the material cures into a pliable solid inside the tissue. The elastomer houses fluorescent pigments in a cohesive, well-defined biocompatible mark that is easily detectable under UV illumination (Uglem et al., 1996). The soft-shelled turtle *Pelodiscus* (=*Trionyx*) *sinensis* Crother 2000 (Trionychidae) is considered a nutritious food and is a commercially important aquaculture species of high demand in Korea, China, and Japan. A technique for farming this species has been developed in southern Korea (Jung et al., 2006; Park et al., 2006). VIFE tags have been used successfully to identify fishes, amphibians and crustaceans (Anholt et al., 1998; Willis and Babcock, 1998; Jerry et al., 2001). To the best of our knowledge, however, it has not yet been applied to reptiles. We therefore evaluated the subdermal injection of VIFE tags at several body locations of soft-shelled turtles over a

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period of 16 months.

## Materials and Methods

Healthy sub-adult soft-shelled turtles (mean body weight ± SD: 182.6 ± 13.7 g) were obtained from the Daegu Turtle Farm, Daegu, and the Chungcheongbuk-do Inland Fisheries Research Institute, Chungju, South Korea. The animals were acclimated to their environment for 4 weeks prior to the start of the experiment. Treatment and control animals were given VIFE tags on 16 May 2002, following the methods of Park et al. (2006). In brief, all turtles were anesthetized with 1,000 ppm lidocaine-hydrochloride/ $NaHCO_3$ at a water temperature of 25°C. They were sedated until completely immobile (total loss of reflex activity), and then removed from the anesthetic solution individually, rinsed in freshwater, and placed on a flat surface for tagging.

We individually marked 150 soft-shelled turtles in three groups ($n$=50 per group) with orange elastomer at one of three body locations: the web surface between the fourth and fifth dactyls of the hindfoot (Web Surface 1 Group), the web surface between the fourth and fifth dactyls of the forefoot (Web Surface 2 Group), and the adipose eyelid (Adipose Eyelid Group). In addition, 50 control turtles were anesthetiszed, but not marked. The water temperature was maintained at 25 ± 1°C and the photoperiod was a natural light-dark cycle. The turtles were fed to satiation once daily in the evening throughout the 16-month trial. Uneaten food was removed after 3 h, and one-fourth of the water in the tank was replaced with fresh water at 25 ± 1°C each day. The turtles were reared and maintained in accordance with the guidelines set forth by the National Institute of Safety Research, South Korea. The survival, retention, and readability of the tags were determined every 4 months, and dead turtles were removed daily. Tag retention rates were calculated using the methods of Zerrener et al. (1997), who used retention data from dead animals to calculate percent retention until the date of death, but not subsequently. Data are expressed as the means of triplicate experiments for all samples. Treatment effects were evaluated using one-way analysis of variance (ANOVA). For significant treatment effects, Duncan's test was used to analyze the significance of the difference among the means of each treatment (Duncan, 1995). In addition, regression analysis was conducted using the general linear model in the Statistical Analysis Systems package version 6.12 (SAS Institute, Cary, North Carolina, USA).

## Results

The survival, retention, and readability of the tags after 16 months are summarized in Table 1. Survival within the first 4 months of tagging was slightly lower in the VIFE-tagged groups (94.3-96.0%) than in the control group (98.9%). However, one-way ANOVA demonstrated that the differences in survival due to treatment were not significant ($p$=0.45). After 4 months, no mortality occurred in the control group. The Web Surface 1 Group experienced a great deal of tag loss up to 4 months after tagging, but stabilized after 4 months. The Web Surface 2 Group showed great loss for up to 8 months before stabilizing, and the Adipose Eyelid Group lost tags continuously throughout the 16-month period. Thus, placement of

Table 1. Cumulative survival, retention and readability of visible implant fluorescent elastomer tags in soft-shelled turtle, *Pelodiscus sinensis* over a 16-month tagging period. Values (means ± SEM of triplication) with different superscripts in same variable indicate significant differences ($p$<0.05). Web surface 1, web surface between the fourth and fifth dactyls of the hind foot; web surface 2, web surface between the fourth and fifth dactyls of the fore foot. *Tag retention (%) is based on the original number of tagged soft-shelled turtle ($n$=50). **Percentage of readable tags among remaining tagged soft-shelled turtle at each inventory

| Tagging site | Variable (%) | Months after tagging | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 4 | 8 | 12 | 16 |
| Control | Survival | 100.0±0.0 | 98.9±0.3 | 98.9±0.3 | 98.9±0.3 | 98.9±0.3 |
| Web surface 1 | Survival | 100.0±0.0 | 96.0±1.0$^a$ | 96.0±2.0$^a$ | 94.0±2.0$^a$ | 92.0±3.0$^b$ |
| | Tag retention* | 100.0±0.0 | 90.7±2.1$^a$ | 88.3±1.5$^a$ | 87.3±2.5$^a$ | 87.0±3.0$^a$ |
| | Tag readability** | 100.0±0.0 | 100.0±0.0$^a$ | 97.7±1.5$^a$ | 93.3±3.5$^b$ | 85.3±4.5$^c$ |
| Web surface 2 | Survival | 100.0±0.0 | 94.3±1.5$^a$ | 92.3±3.5$^b$ | 92.0±3.0$^b$ | 91.7±3.5$^b$ |
| | Tag retention* | 100.0±0.0 | 88.3±2.5$^a$ | 83.3±3.5$^b$ | 80.3±4.5$^b$ | 80.0±5.0$^b$ |
| | Tag readability** | 100.0±0.0 | 100.0±0.0$^a$ | 95.3±3.5$^a$ | 88.7±5.5$^c$ | 81.3±3.5$^d$ |
| Adipose eyelid | Survival | 100.0±0.0 | 94.3±2.5$^a$ | 92.3±5.5$^b$ | 90.0±5.0$^b$ | 89.7±5.5$^b$ |
| | Tag retention* | 100.0±0.0 | 83.7±3.5$^b$ | 78.3±7.5$^b$ | 73.7±6.5$^c$ | 69.7±5.5$^c$ |
| | Tag readability** | 100.0±0.0 | 100.0±0.0$^a$ | 100.0±0.0$^a$ | 96.7±2.5$^a$ | 94.0±2.0$^b$ |

the VIFE tag at the web surface between the fourth and fifth dactyls of the hindfoot yielded the highest retention rates. Adipose eyelid tagging was valuable in terms of tag readability, but experienced the highest loss (69.7% 16 months after tagging). The values of 'Survival×Tag retention×Tag readability' for Web Surface 1 Group, Web Surface 2 Group, and Adipose Eyelid Group were 0.68, 0.60, and 0.59, respectively in 16 months after tagging in Table 1.

## Discussion

Tags that were not readable with the naked eye were easily readable under UV light in reduced natural light. Similar to other studies that have reported high retention of VIFE tags (Dewey and Zigler, 1996; Willis and Babcock, 1998), the orange elastomer was highly visible in the adipose eyelid, with 94.0–100% readability wed under UV light (Table 1). In contrast to these results, Kincaid and Calkins (1992) and Mourning et al. (1994) reported the lowest tag readability of visible implants (VI) in the eyelid, noting that at 120 d, 30% of the VI tags were unreadable in rainbow trout and 11% were only readable under UV light. Zerrener et al. (1997) noted that VI tags became unreadable during a tagging experiment either because the transparency of the adipose eyelid tissue decreased or because the tags shifted. We found that the VIFE tags were easy to apply, required less than 1 min per turtle, and were readily visible when viewed under a UV lamp. Buckley et al. (1994) found that VIFE tags in juvenile reef fish, *Sebastes* sp., could be detected visually in situ for up to 258 d using underwater UV lights. In response to concerns about amphibian declines, Jung et al. (2000) evaluated and validated amphibian monitoring techniques using VIFE tags in Shenandoah and Big Bend national parks, USA. Godin et al. (1995) found that to identify populations of the shrimp *Penaeus vannamei*, individuals could be tagged internally using an externally visible elastomer. Basic considerations for the use of marks in fisheries management or research are the effects of the tags on animal survival, behavior, growth, permanency, and recognition, and the cost of the marking technique (Mcfarlane et al., 1990; Park and Lee, 2001). VIFE tags are non-toxic medical -grade fluorescent internal elastomer tags that have been used successfully to identify fishes, amphibians, and decapod crustaceans (Willis and Babcock, 1998; Jerry et al., 2001; Bailey, 2004). However, their use as a method for identifying Trionychidae has not been tested. The mortality of tagged turtles during our study was attributed to collection and handling stress, rather than to the tagging itself. The highest loss of tags (69.7% 16 months after tagging) from the adipose eyelid seemed to result from a behavioral characteristic of this species; these turtles frequently pull their heads inside their shell. We conclude that VIFE tags are a reliable, non-intrusive approach for identifying turtles, and that the most suitable tagging region is the web surface between the fourth and fifth dactyls of the hindfoot.

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