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Evaluation of a Visible Implant Fluorescent Elastomer Tag in the Greenling *Hexagrammos otakii*

In-Seok Park[1*], Young Ju Kim[1], Hyun Woo Gil[1] and Dong-Soo Kim[2]

[1]*Division of Marine Environment and Bioscience, Korea Maritime University, Busan 606-791, Korea*
[2]*Institute of Marine Living Modified Organism* (*iMLMO*), *Pukyung National University, Busan 608-737, Korea*

Abstract

The aim of this study was to assess visible implant fluorescent elastomer (VIE) tagging in greenling *Hexagrammos otakii*. The experiental fish were anesthetized individually and marked with orange, yellow, red, and green elastomer at the following five body locations, respectively: the adipose eyelid, the surface of the dorsal fin base, the inside surface of the pectoral fin base, the inside surface of the pelvic fin base, and the surface of the anal fin base. Control fish were anesthetized but not marked. During the 20-month trial, fish growth and retention, underwater visibility, and readability of the tags were determined. After 20 months, body length of marked greenling (43.2 ± 3.5 cm, mean ± standard deviation [SD]) did not differ from that of the control (41.4 ± 3.7 cm). Additionally, the body weight of marked greenling (527.4 ± 39.8 g, mean ± SD) did not differ from that of the controls (505.9 ± 31.7 g). Greenling retained >90% of the tags at the surface of the dorsal fin base. The anal fin base showed a higher tag retention rate than the inside surfaces of the pectoral fin and the pelvic fin bases ($P < 0.05$). Red and orange tags were identified more easily underwater than green and yellow tags. Green and yellow tags emitted fluorescence in response to a narrower range of light wavelengths. Thus, the VIE mark was easy to apply to greenling (< 1 min per fish) and was readily visible when viewed under an ultraviolet lamp.

Key words: *Hexagrammos otakii*, Tag readability, Tag retention, Visible implant fluorescent elastomer tag

Introduction

Identification of individual fish is essential for growth, migration, mortality, stock identification, and gear selectivity to trace particular fish populations. Although short-term tag retention may suffice for some experiments, the effect of a tag on fish survival, behavior, growth, recognition, and cost of the marking technique should be considered. However, traditional external tags (such as spaghetti or dart tags) are often lost quickly (Crossland, 1980; Bergman et al., 1992) and may affect growth or survival (Crossland, 1976; Tong, 1978; McFarlane and Beamish, 1990; Serafy et al., 1995). Furthermore, these types of tags can only be read by recapturing the fish.

Internally situated but externally readable devices such as acoustic tags are often limited by their expense. Problems associated with the physiological characteristics of the fish, reliability of identification, fouling of the tag by algae (Jones, 1987; Barrett, 1995), tag retention (Crossland, 1976; Parker, 1990), and external visibility have reduced the confidence of in situ studies of reef fish ecology.

Many fish species have transparent tissue suitable for tagging, including opecula, mandible, top of the head, body, and fins. However, sites to retain tags vary among species. Tagging sites in other body locations may also be used successfully. An example of a visual mark that has been successfully applied to several fish species is the visible implant fluorescent elasto-

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***Corresponding Author**
E-mail: ispark@hhu.ac.kr

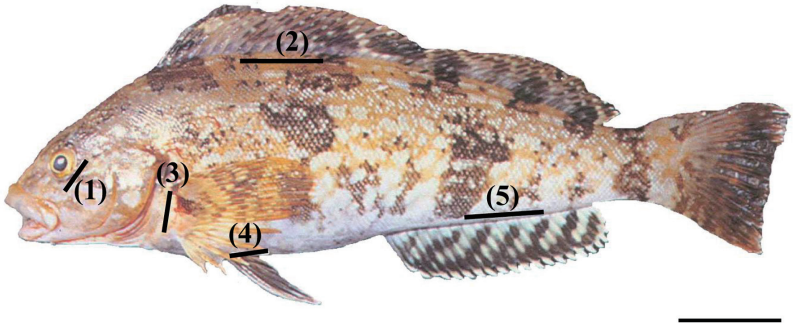

Fig. 1. Elastomer injection locations shown on the greenling *Hexagrammos otakii*: 1) the adipose eyelid, 2) the surface of the dorsal fin base, 3) the inside surface of the pectoral fin base, 4) the inside surface of the pelvic fin base, and 5) the surface of the anal fin base. Scale bar = 3 cm.

mer (VIE) tag (Northwest Marine Technology, Ltd., Shaw Island, WA, USA). It was developed primarily for tagging large batches of small or juvenile fishes. The VIE is comprised of a viscous liquid elastomer that sets into a pliable solid over a period of hours. The elastomer can be injected into transparent or translucent tissues to form a permanent, biocompatible mark. The compound fluoresces brightly when exposed to blue light (UV lamp) and viewed through an amber filter.

Tag size can easily be varied according to the requirements of the researcher and the size of the fish to be tagged. This system has been used to identify groups or cohorts of juvenile reef fishes (Frederick, 1997) and salmonids (F. Haw personal communication) but is also proving potentially effective for controlled laboratory studies of adults (Dewey and Zigler, 1996). As an externally visible but subdermally situated marking system, VIE tags are potentially able to eliminate many of the problems experienced with other methods and can be used to trace individual fish.

Greenling *Hexagrammos otakii* which is a benthic species in the family Hexagrammidae, is commonly found in coastal areas throughout the year in Korea and is a favorite seawater food fish due to its taste and tender meat. In this study, we determined the tag colors that resulted in the highest rates of detection, as well as the likelihood of tag or handling-related mortality and retention rates of VIE tags at particular body sites.

Materials and Methods

The fish used in this experiment were sub-adult greenling *Hexagrammos otakii* (mean body length ± standard deviation [SD], 21.0 ± 1.4 cm; mean body weight ± SD, 154.4 ± 13.8 g). The VIE tags were applied to treated and control fish on April 16, 2009. According to the method of Park et al. (2003) all fish were anaesthetized in 800 ppm lidocaine-hydrochloride/ $NaHCO_3$ at a water temperature of 18°C. Fish were sedated until they were completely immobile, individually removed from the anesthetic solution, rinsed in fresh water, and placed on a flat surface for tagging.

Fifty fish in triplicate groups were individually marked with orange, yellow, red, or green elastomer at five body locations (Fig. 1): 1) the adipose eyelid, 2) the surface of the dorsal fin base, 3) the inside surface of the pectoral fin base, 4) the inside surface of the pelvic fin base, and 5) the surface of the anal fin base. Control fish (n = 50) were anesthetized but not marked. We used the VIE Hand Injection Master Kit (Northwest Marine Technology, Ltd.). The elastomer and curing agent were properly mixed at a 10:1 ratio, and the elastomer was injected as a liquid. Each site of the injection volume was 4 mL. Tagged fish were placed in tanks according to tag color and body part marked.

The fish were held in 18 flow-through fiberglass-reinforced plastic tanks (2 × 2 × 0.5 m; water volume, 2,000 L) supplied with filtered seawater. The bottom of the tanks was coated with a black sheet to facilitate decoding of the tags. Flow rate was 100 L min^{-1} $tank^{-1}$, and mean water temperature was 20 ± 2.5°C respectively. A day-night cycle was established, and all tanks were covered with mesh to prevent fish jumping out. The fish were fed daily to satiation with dry commercial flounder feed (Agribrand Furina Korea Co., Seoul, Korea) throughout the 20-month trial.

Growth of fish and retention, underwater visibility, and readability of the tags were determined at 2-month intervals, and dead fish were removed daily. Only individuals marked with color on the dorsal fin could be distinguished more than 2 m away by eye and with a blue light (UV lamp) after 2 months. Tag retention rates were calculated according to the method of Zerrenner et al. (1997). The mark-retention data recorded from dead fish were used to calculate the percent retention up to the date on which they died, but were not used in subsequent calculations.

Data are expressed as means of triplicate experiments. Differences in survival and growth between the control and experimental groups were assessed by the t-test (Cody and Smith, 1991) and tag retention rates (%) among tagging sites were evaluated by one-way analysis of variance and Duncan's multiple range test (Duncan, 1955). A $P < 0.05$ was considered to indicate statistical significance.

Results and Discussion

Survival and growth of greenling *Hexagrammos otakii* during the 20-month trial are shown in Table 1. Tagging did not affect survival or growth of greenling. Among the 150 individuals in the control and experimental groups, 21 marked fish and 23 control fish died. After the 20-month trial, the body length of marked greenling (43.2 ± 3.5 cm, mean ± SD) did not differ from that of the control (41.4 ± 3.7 cm, mean ± SD). Additionally, the body weight of marked greenling (527.4 ± 39.8 g, mean ± SD) did not differ from that of the controls (505.9 ± 31.7 g, mean ± SD).

The primary causes of mortality in caged snapper tagged with the same system are internal damage and infection from gas bladder rupture, or infection from anatomical trauma caused by handling (Willis and Babcock, 1998). Greenling was less fragile; however, steps should be taken to minimize the possibility of capture-related injury. Therefore, skilled application of the elastomer injection was crucial for maintaining low mortality, as suggested by the decrease in mortality of marked fish during a laboratory experiment (Frederick, 1997). Jang et al. (2007) investigated the effectiveness of passive integrated transponder (PIT) tags in a small cyprinid *Pseudorasbora parva* (n = 110; mean body length ± SD, 17.4 ± 1.1 cm; mean body weight ± SD, 71.7 ± 0.2 g). Upon use of three types of tags, including small PIT tags (width, 11.0 mm; height, 2.1 mm; weight, 0.088 g), mid-sized PIT tags (width, 20.0 mm; height, 3.5 mm; weight, 0.188 g), and large PIT tags (width, 30.0 mm; height, 3.5 mm; weight, 0.298 g), survival rates of 100 individuals 30 days after tag insertion were as follows: large tag, 50.0%; middle tag, 57.5%; and small tag, 61.4%. Survival rates varied among the three tag types because death was due to surgical damage. However, we used the VIE tag which resulted in a high survival rate and no surgical damage to the fish.

Greenling retained > 90% of the tags at the surface of the dorsal fin base (Table 2), which is similar to previous VIE studies (Dewey and Zigler, 1996; Willis and Babcock, 1998). The anal fin base showed a higher tag retention rate than marking the inside surface of the pectoral fin and pelvic fin bases (P < 0.05). The high loss of the VIE at the inside surface of the pectoral and pelvic fin bases during tagging seemed to related to the benthic behavior of this species. Buckley et al. (1994) found that VIE tags in juvenile Sebastes spp. released on an artificial reef could be identified visually after 258 days during strip transects using ultraviolet underwater lights.

Tags of different colors were not equally identifiable underwater (Fig. 2). Only individuals marked with color on the

Table 1. Survival (%) and growth in greenling *Hexagrammos otakii* from 0 to 20 months after visible implant fluorenscent elastomer (VIE) tagging

Months	Group	Survival* (%)	Growth*	
			Body length (cm)	Body weight (g)
0	Cont.	100.0 ± 0.00	21.0 ± 1.43	154.4 ± 16.81
	Exp.	100.0 ± 0.00	21.0 ± 1.44	154.4 ± 16.80
2	Cont.	100.0 ± 0.00	26.9 ± 1.41	249.0 ± 19.71
	Exp.	98.7 ± 1.21	27.6 ± 1.50	255.7 ± 21.27
4	Cont.	99.3 ± 1.23	30.3 ± 1.61	311.5 ± 22.36
	Exp.	98.7 ± 1.22	31.4 ± 1.57	320.4 ± 19.93
6	Cont.	98.0 ± 0.03	33.0 ± 2.02	362.7 ± 24.13
	Exp.	97.3 ± 1.22	34.8 ± 1.89	375.4 ± 20.82
8	Cont.	95.3 ± 1.27	35.2 ± 2.13	408.0 ± 29.86
	Exp.	94.7 ± 1.21	37.1 ± 1.93	419.2 ± 25.65
10	Cont.	92.0 ± 0.00	37.1 ± 2.07	444.3 ± 30.73
	Exp.	92.7 ± 0.51	37.8 ± 2.66	440.9 ± 30.84
12	Cont.	91.3 ± 1.26	38.7 ± 2.24	467.6 ± 32.73
	Exp.	92.3 ± 0.62	38.2 ± 1.90	452.1 ± 32.00
14	Cont.	90.0 ± 0.02	39.0 ± 2.32	485.6 ± 35.91
	Exp.	88.7 ± 1.28	40.3 ± 2.72	497.7 ± 32.39
16	Cont.	87.3 ± 1.29	40.1 ± 3.15	490.0 ± 33.54
	Exp.	88.0 ± 0.02	42.3 ± 2.91	512.3 ± 35.74
18	Cont.	86.0 ± 0.01	41.2 ± 3.36	498.8 ± 29.13
	Exp.	87.3 ± 1.21	40.9 ± 3.12	520.1 ± 36.21
20	Cont.	85.3 ± 1.23	41.4 ± 3.77	505.9 ± 31.72
	Exp.	86.0 ± 0.07	43.2 ± 3.54	527.4 ± 39.80

Values are means ± SEM of triplication. Cont. and Exp. word means that each control group and experiment group.
*Growth and survival of all experimental and control groups were deducted with average. None of criteria measured were not significantly different between control and experimental group (P > 0.05).

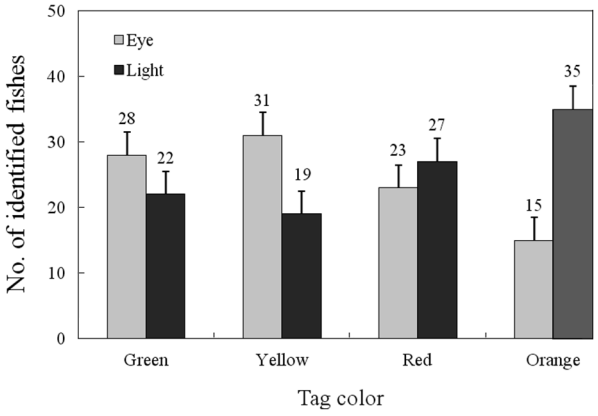

Fig. 2. Comparison of visible implant fluorescent elastomer (VIE) tag detection, identification between the four tag colors used two detection methods in greenling *Hexagrammos otakii*, using observations taken on 2 m above water visibility. Number of observations for each category is given above each bar. Tags site were dorsal fin base (eye, tags observed with the naked eye; light, tags observed using a blue UV lamp light to activate fluorescence. Error bars are 95% confidence).

dorsal fin were distinguishable at distances > 2 m by eye or with a UV lamp. Twenty-eight fish tagged with green were identifiable with the naked eye, and 22 were with a UV lamp. Thirty-one fish tagged with yellow were identifiable with the naked eye, and 19 with a UV lamp. Twenty-three fish tagged with red were identifiable with the naked eye, and 27 with a UV lamp. Fifteen fish tagged with orange were identifiable with the naked eye, and 35 with a UV lamp. These results are similar to those of a previous study (Willis and Babcock, 1998).

Significant differences were found among tag colors; red and orange were easier to detect than green and yellow using a UV lamp. Green and yellow could be detected easily with the naked eye. The method of detection (naked eye *vs.* UV lamp) did not affect overall recognition rates, although there was a significant interaction between method and color because the green and yellow tags were more difficult to see when the UV lamp was used (Fig. 2). This was likely because the green and yellow tags emit fluorescence in response to a smaller range of light wavelengths than that of brighter colors (G. Thomburgh personal communication).

Buckley et al. (1994) found that red monofilament micro tags were difficult to detect underwater because of the attenuation of short wavelength light. However, we could more easily detect red compared to green or yellow (Willis and Babcock, 1998). All dives in this study were limited to 2 m. Deeper water where natural light levels are lower may result in greater attenuation of red light (Willis and Babcock, 1998). Red tags are clearly recognizable at up to 5 m in clear water under direct sunlight (Willis and Babcock, 1998).

As mentioned by Willis and Babcock (1998), a variety of factors may influence the distance at which tags can be positively identified. It was often possible to recognize a fish as possessing a tag at > 6 m without being able to detect tag color. The direction and intensity of ambient light made a large difference; when the sun was low, and behind the observer, tag color could be correctly identified at a greater distance. The amount of suspended material was also a contributing factor, particularly in the effectiveness of fluorescence. Backscatter from large particles as well as strong ambient light decreases the effective range of a UV lamp beam, thereby decreasing the range of detection. The range of detection of orange tagged

Table 2. Retention rate (%) of visible implant fluorenscent elastomer (VIE) tags in each sites of greenling *Hexagrammos otakii* from 0 to 20 months after VIE tagging

Months	Tag retention (%)*				
	Adipose eyelid	Surface		Inside surface	
		Dorsal fin base	Anal fin base	Pectoral fin base	Pelvic fin base
0	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
2	92.8 ± 4.21^{a}	99.3 ± 1.83^{b}	98.9 ± 2.14^{b}	95.8 ± 3.71^{a}	97.1 ± 3.52^{b}
4	90.7 ± 3.52^{a}	99.0 ± 1.97^{b}	95.6 ± 1.90^{b}	91.4 ± 3.57^{a}	97.1 ± 3.57^{b}
6	86.1 ± 3.03^{a}	98.9 ± 2.11^{b}	95.6 ± 1.92^{b}	90.9 ± 2.98^{a}	95.6 ± 1.81^{b}
8	81.7 ± 2.91^{a}	98.9 ± 2.10^{b}	93.8 ± 2.51^{b}	87.4 ± 2.10^{c}	94.5 ± 2.72^{b}
10	71.9 ± 3.10^{a}	98.7 ± 3.29^{b}	93.8 ± 2.59^{c}	85.2 ± 1.82^{d}	90.6 ± 2.00^{c}
12	70.1 ± 2.71^{a}	98.7 ± 3.25^{b}	90.9 ± 2.07^{c}	85.2 ± 1.82^{c}	88.7 ± 2.56^{c}
14	68.0 ± 1.19^{a}	94.5 ± 2.72^{b}	89.3 ± 1.75^{c}	80.7 ± 1.94^{d}	88.7 ± 2.57^{c}
16	65.6 ± 2.15^{a}	93.1 ± 1.83^{b}	89.3 ± 1.78^{b}	80.7 ± 1.96^{c}	85.0 ± 2.08^{d}
18	65.0 ± 3.87^{a}	92.6 ± 2.27^{b}	85.0 ± 1.08^{c}	77.2 ± 2.01^{d}	82.4 ± 1.63^{c}
20	62.7 ± 3.62^{a}	92.6 ± 2.28^{b}	84.8 ± 1.31^{c}	75.4 ± 2.42^{d}	78.6 ± 1.53^{d}

*Values (means ± SEM of triplication) with different superscripts in raw indicate significant differences ($P < 0.05$). Each site of tags retention was induced four tags colors with average. Tag retention rate (%) is based on the original number of tagging fish ($n = 50$).

fish using a UV lamp and amber filter appears to be limited primarily by the water clarity and the power of the light used (Willis and Babcock, 1998). We found that the VIE mark was easy to apply, requiring < 1 min per fish, and was readily visible when viewed under an UV lamp in greenling, a benthic species.

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