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Comparison of three methods for marking a small floodplain minnow

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Abstract

Background: Evaluation of the movement patterns of small-bodied fish is often hindered by the lack of a suitable long-term mark. We evaluated several techniques for long-term group and individual identification of adult (40–70 mm total length [TL]) Oregon chub (*Oregonichthys crameri*). We marked Oregon chub with one of two different sized passive integrated transponder (PIT) tags (a 9 × 2.12 mm, 0.067 g PIT tag [PIT-tag] or a 8.4 × 1.4 mm, 0.033 g PIT tag [PICO-tag]), a red visible implant elastomer (VIE) tag, or a freeze brand. We monitored survival, tag retention, and mark quality over 150 days. In addition, we assessed the minimum length and weight thresholds to achieve 80% and 90% survival of PIT-tagged fish.

Results: Marking with a freeze brand, PICO-tag, or VIE tag had no effect on survival ($P > 0.05$). In contrast, marking with a PIT-tag was associated with significantly lower ($P < 0.05$) survival than in the control group. Survival was significantly higher ($P = 0.002$) for fish implanted with a PICO-tag than with the larger PIT-tag.
The initial minimum TL for 80% and 90% survival was 54 mm and 64 mm TL, respectively, for the PIT-tag treatment. The 90% survival threshold for PICO-tagged fish was 44 mm TL. The 80% survival threshold was outside the range of sizes used in our experiment (<40 mm TL). Similarly, the 80% and 90% survival weight thresholds for the PIT-tag treatment were 1.5 g and 2.4 g, respectively, and the 90% survival threshold for PICO-tagged fish was 0.9 g.
Tag retention was 94% and 95% in the PIT-tag and PICO-tag treatments, respectively; 80% of the freeze branded fish had easily recognizable tags after 150 days and 88% of the fish marked with VIE had easily recognizable tags after 150 days.

Conclusions: PICO-tags, VIE marks, and freeze brands are all feasible long-term marking techniques for Oregon chub with negligible effects on survival through 150 days. The selection of a particular technique should be based on the study design and objectives (e.g., individual versus group identification), cost, ease, speed of tagging, and survival.

Keywords: Freeze brand, Minimum size, Oregon chub, Passive integrated transponder tag, Retention, Survival, Visual implant elastomer

Background

The small- and large-scale movements of small bodied fish (<70 mm total length (TL)) are poorly understood. Such information is critical to assess population dynamics, physiology, and behavior, and inform conservation planning. Research to address individual movement and population dynamics has been hindered by the lack of a suitable long-term mark for individuals or groups of individuals that is both innocuous and has a high retention rate. Larger fishes are commonly marked with a variety of techniques including, but not limited to, passive integrated transponder (PIT) tags, visual implant elastomer (VIE) tags, and mutilation and scarring of dermal tissue through freeze branding. Each of these approaches, as well as the method of application, has limitations related to costs, ease of application, longevity or retention of the mark, and effects on the behavior or survival of the individual [1-5].

PIT tags offer a powerful tool for the unique identification of individual fish in a population. Until the recent development of smaller sized (e.g., 9 × 2.12 mm, 0.067 g and 8.4 × 1.4 mm, 0.033 g) PIT tags, their utility for use with small-bodied fish was limited because of their size

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and weight. Bridger and Booth recommended that fish should not be implanted with tags weighing more than 2% of their body weight [6]. However, Jepsen et al. argued that this rule be disregarded, and that appropriate size is driven by the objectives of the study, implantation method, and the species or life stage in question [2]. Although relatively few studies have determined the minimum size at tagging for small-bodied species [4,5,7-9], survival and retention appear to vary by species, implantation method, and tag size. Thus, minimum length and weight thresholds should be established for target species, or as new PIT-tags are developed [5,10], prior to implementation in field studies. Such information is critical when evaluating the potential effects of a tagging procedure on the study design.

VIE is a biologically compatible, brightly colored, fluorescent polymer that cures into a flexible tag that is visible after subcutaneous injection in unpigmented tissue. These tags have a number of benefits for marking small-bodied fish, as their application is not limited to fish of a certain size [11,12], retention is often high, up to 99% in small bodied fish after four months [13,14], and individuals can be identified by application of different colors at different places on the body [15,16]. However, the suitability of marking locations on the body and availability of colors limits the number of unique identifiers, and retention rate is influenced by the location of the tag on the body and varies among different fish species (e.g., [17-19]). Therefore, group marks at a single suitable location may be the only acceptable application of the VIE mark for some species or for studies requiring the tagging of large numbers of individual fish.

Freeze branding is another technique used to mark individuals. Freeze branding is the scarring of dermal tissue using liquid nitrogen and has been widely used to mark fish of all sizes [3]. Individuals or groups of individuals can be identified by freeze branding using different symbols on different parts of the body [3,20]. While high survival and retention is typical in small fish [1,3,21], brand retention and recognition is usually poor in salmon fry smaller than 50 mm fork length (FL) [22] and is likely influenced by dermal morphology. Thus, there is a need to quantify freeze brand retention and recognition rates in the species of interest prior to their implementation in field studies.

Oregon chub are a small floodplain cyprinid endemic to the Willamette Valley, Oregon, USA. The species was listed as endangered under the federal Endangered Species Act in 1993, and was downlisted to threatened in 2010. Oregon chub prefer off-channel habitats, such as sloughs, oxbows, stable backwaters, and low gradient streams with little to no flow. Oregon chub are thought to be poor swimmers [23] and genetic data suggests there is not a substantial genetic exchange among populations [24]. However, in certain reaches of the Willamette River tributaries, we have found Oregon chub to be well distributed and have documented the colonization of newly created habitats (unpublished observation, B. Bangs). Our objective was to determine the optimal method for marking Oregon chub for future evaluation of seasonal movement patterns, based on high tag retention, mark quality, and survival. We tested the effects of three marking techniques on the survival of Oregon chub. Fish were marked with two different sized PIT tags, a VIE mark, or a freeze brand. We monitored survival and tag retention over a period of 150 days. In addition, given that exposure to high temperatures typically exacerbates the effects of a stressor such as tagging [25], we tested whether exposure to a more natural thermal regime that incorporated large daily fluctuations in temperature would influence survival or tag retention in fish with the heaviest tag burden. The results provide insight into methods that may be used to mark Oregon chub and other small fishes.

Results

The initial mean length and weight of Oregon chub were not significantly different between treatments (ANOVA: $P = 0.788$, df = 6, F value = 0.527 and $P = 0.349$, df = 6, F value = 1.12, respectively) (Table 1). Most (72%) of the early (30 days) mortality for all treatments occurred by day 5. At day 30, we observed no mortality in the control and freeze brand treatments and very low mortality in the sham injection and VIE treatments, which were not significantly different from the control (Figure 1, Table 2). The survival probability of the PICO-tagged fish was significantly higher than that of fish in the PIT-tag (binomial test: $P = 0.009$, with alternative one-tailed hypothesis of higher PICO survival; n = 120 in each group) and PIT-tag + natural temperature regime (NTR) groups (binomial test: $P = 0.014$, with alternative one-tailed hypothesis of higher survival of PICO; n = 120 in each group). Within each of the PIT tag treatment groups (PICO-tag, PIT-tag, and PIT-tag + NTR), the survival of large fish (61–70 mm) was significantly higher than that of small fish (40–50 mm) (Generalized linear mixed model (GLMM):

Table 1 Mean (±SE) initial total length (TL; mm) and weight (g) for Oregon chub in each of the treatment groups

Treatment	Mean initial	
	Length	Weight
Control	54.39 (0.72)	1.64 (0.06)
Freeze brand	54.65 (0.71)	1.66 (0.07)
PICO	54.92 (0.71)	1.84 (0.08)
PIT-tag	55.27 (0.68)	1.68 (0.07)
PIT-tag + NTR	55.20 (0.74)	1.67 (0.07)
Sham	55.04 (0.71)	1.67 (0.07)
VIE	53.73 (0.75)	1.66 (0.07)

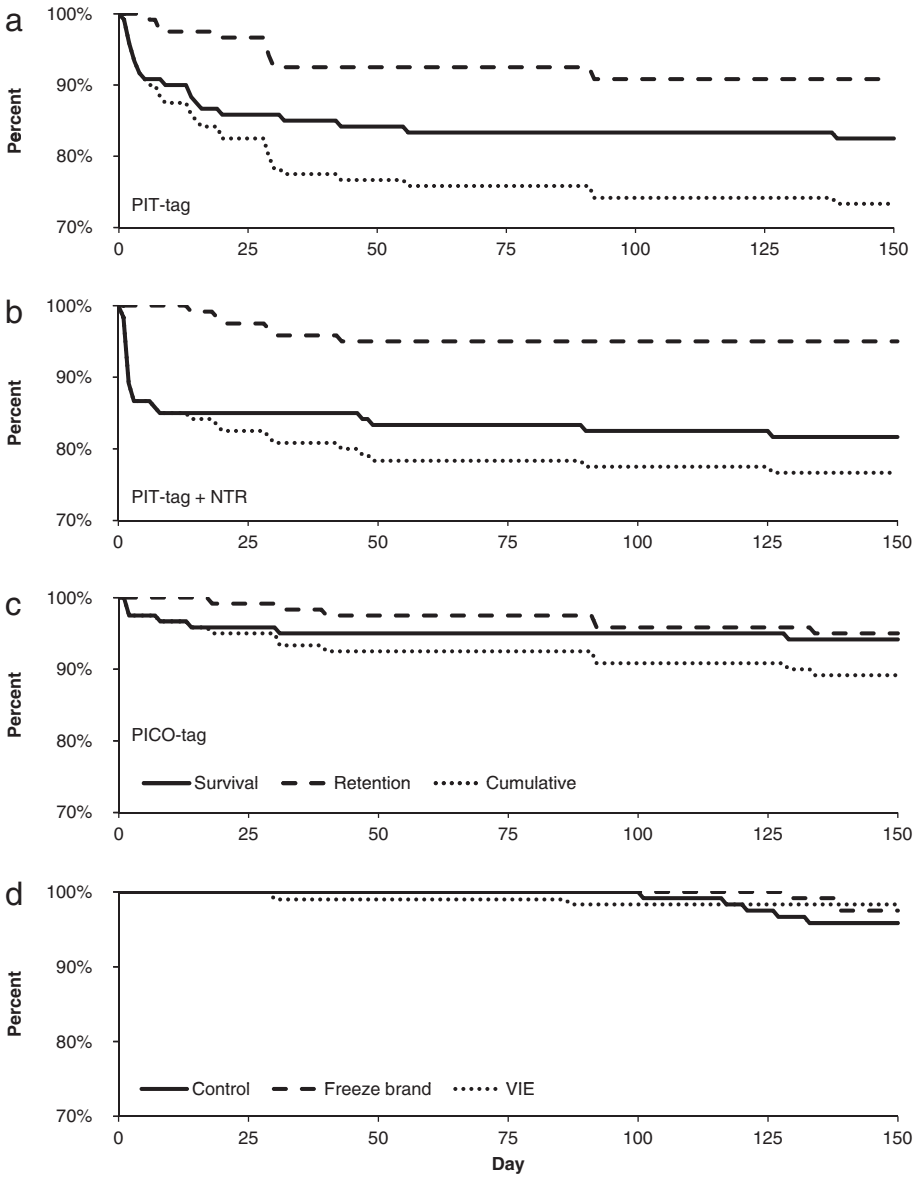

Figure 1 Daily survival, tag retention, and cumulative survival and tag retention of Oregon chub. Oregon chub were tagged with **a)** 9 × 2.1 mm PIT-tag, **b)** 9 × 2.1 mm PIT-tag + NTR, or **c)** 8.4 × 1.4 mm PICO-tag; **d)** daily survival of Oregon chub in the control, freeze brand, and VIE treatment groups. Data for each treatment group represent the sum of four replicate tanks (n = 30 fish/tank).

P = 0.012, Table 3) but was not different than that of medium fish (51–60 mm) (GLMM: P = 0.856, Table 3). Similarly, the survival of the small fish was significantly lower than that of medium fish (GLMM: P = 0.017, Table 3).

After 150 days, the survival probabilities for the freeze brand, PICO-tag, and VIE treatments were not significantly different from the control (binomial tests: P >0.05, with alternative one-tailed hypotheses of higher survival of control; n = 120 in each group; Table 2). Conversely, survival was significantly lower in the PIT-tag and PIT-tag + NTR treatments than in the control group (binomial tests: P <0.05, with alternative one-tailed hypotheses of higher survival of control; n = 120 in each group). Survival was significantly higher in the PICO-tag treatment than the PIT-tag and PIT-tag + NTR treatment groups (binomial test: P = 0.002 and P = 0.0008, respectively, with alternative one-tailed hypothesis of higher survival of PICO; n = 120 in each group). For all PIT tagged groups (n = 3) combined, large fish had significantly higher survival than small fish (GLMM: P = 0.002, Table 4); however, the survival of large fish was not different from that of medium sized fish (GLMM: P = 0.20, Table 4). Small fish had significantly

Table 2 Percent survival and percent tag/mark retention (±SE) for Oregon chub 30 and 150 days after treatment

	30 days					150 days			
	Survival			Retention				Retention	
Treatment	Mean	Range	*P* value	Overt mark	Tag	Survival	*P* value	Overt mark	Tag
Control	100	-	NA		-	96	-		-
Freeze brand	100	-	NA	98 (1.7)	100	97	0.788	81	98
PICO-tag	96 (2.2)	90-100	0.019		98 (1.0)	94	0.5		95
PIT-tag	86 (2.5)	80-90	<0.001		91 (3.2)	82	0.001		89
PIT-tag + NTR	85 (3.9)	77-90	<0.001		95 (2.8)	82	<0.001		95
Sham	98 (1.8)	93-100	0.123		-	NA	-		-
VIE	99 (0.8)	97-100	0.5	91 (4.1)	98 (1.7)	98	0.5	88	99

Data for each treatment group represents the mean of four replicate tanks (n = 30 fish/tank). *P* values reflect a one-way binomial significant test and were not adjusted for multiple comparisons. Overt marks were tags that were subjectively scored a 0.

lower survival than medium sized fish (GLMM: $P = 0.05$, Table 4).

The initial minimum TL for 80% and 90% survival was 54 and 64 mm, respectively, for the PIT-tag treatment (Figure 2a). The 90% survival threshold for PICO-tagged fish was 44 mm TL. The 80% survival threshold was outside the range of sizes used in our experiment (<40 mm). Similarly, the 80% and 90% survival weight thresholds for the PIT-tag treatment were 1.5 g and 2.4 g, respectively, and the 90% survival threshold for PICO-tagged fish was 0.9 g (Figure 2b). The equations for the 80% and 90% survival thresholds for weight and length are given in Additional file 1.

PIT tag retention was consistent across treatments over the first 30 days (Figure 1). At 30 days, tag retention was 95% in all three PIT tag treatment groups (Table 2). All individuals in the freeze brand treatment group had easily recognizable marks at 30 days, with 98% receiving a score of 0, and 2% receiving a score of 1. At 30 days, 90% of the fish marked with VIE tags had an easily recognizable tag (score = 0), 8% were somewhat recognizable (score = 1), and only 2% were unrecognizable (score = 2). At 150 days, PIT-tag retention was similar to retention at 30 days, and was 91%, 96%, and 95% in the PIT-tag, PIT-tag + NTR, and PICO-tag treatments, respectively. At 150 days, 80% of the freeze-branded fish had easily recognizable tags (score = 0), 18% were somewhat recognizable (score = 1), and only 2% were unrecognizable (score = 2). In fish marked with VIE, 88% had easily recognizable tags (score = 0), 11% were somewhat recognizable (score = 1), and 1% were unrecognizable (score = 2) at 150 days. One of the VIE tags was only observable under UV illumination, otherwise all remaining VIE tags were recognizable under ambient light.

Discussion

Our results suggest that PICO PIT-tags, VIE tags, and freeze branding are all effective methods for the long-term marking of small bodied fishes like Oregon chub. Tag retention was similar among the VIE, freeze brand, and PICO-tag treatments over 150 days. However, because we observed some scar tissue regeneration, fragmentation or loss of VIE, and shedding of PICO tags throughout the study, we do not assume perfect detection of marks after 150 days. PICO tags offer the greatest power to monitor fish, allowing unique identification of large numbers of individuals and allowing assessment of individual growth and time extant. Passive monitoring stations can be effective for PIT-tag interrogation and the greater detection range associated with larger tags enable the use of larger

Table 3 Statistical output for GLM (binomial distribution; link: logit) at day 30

Fixed effects	Estimate	SE	Z value	*P* value
Intercept: PICO, large fish	3.459	0.5483	6.309	<0.001
PIT-tag	−1.229	0.4983	−2.467	0.014
PIT-tag + NTR	−1.173	0.501	−2.341	0.019
Medium fish	−0.088	0.485	−0.181	0.856
Small fish	−1.078	0.427	−2.526	0.012
Small fish, when medium is intercept	−1.078	0.427	−2.526	0.012

Small, medium, and large size categories were 40–50, 51–60, and 61–70 mm TL, respectively.

Table 4 Statistical output for GLM (binomial distribution; link: logit) at day 150

Fixed effects	Estimate	SE	Z value	*P* value
Intercept: PICO, large fish	3.693	0.540	6.838	<0.001
PIT-tag	−1.426	0.489	−2.916	0.004
PIT-tag + NTR	−1.548	0.485	−3.192	0.001
Medium fish	−0.575	0.450	−1.279	0.201
Small fish	−1.286	0.419	−3.068	0.002
Small fish, when medium is intercept	−0.711	0.369	−1.960	0.05

Small, medium, and large size categories were 40–50, 51–60, and 61–70 mm TL, respectively.

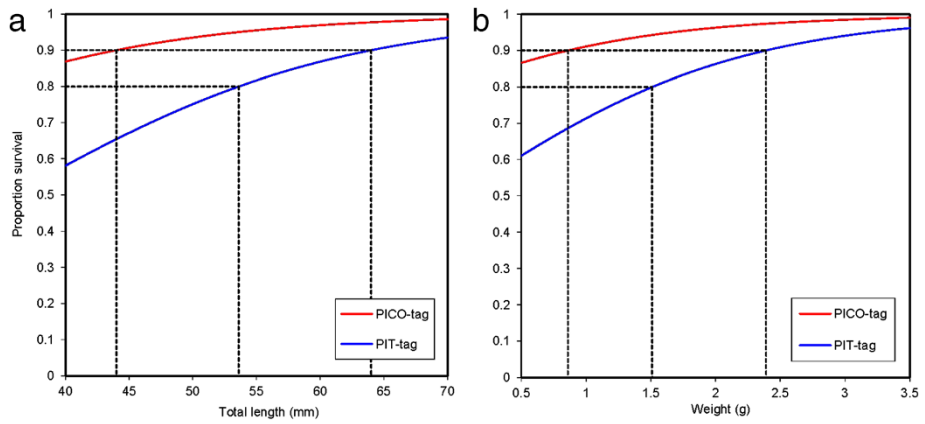

Figure 2 Minimum total length (TL) (a) and weight (b) required to achieve 80% and 90% survival in PICO- and PIT-tagged Oregon chub.

antennas that can span a greater area [26]. However, for use with Oregon chub in large, complex, and dynamic floodplain habitats, the utility of PICO tags may be limited due to the limited read range of the tag versus the size of antenna needed to adequately cover the area through which fish are moving. Similarly, to allow individual identification, each PICO-tagged fish must be captured and manually scanned, which could be cumbersome when handling large numbers of fish in the field. In contrast, VIE and freeze brands are highly visible during handling, and should allow rapid identification of marked individuals in the field. Unique identification with VIE and freeze brands is limited by the number of body locations available to mark, availability of colors or brand designs, and longevity. Because of these limitations, VIE or freeze brands appear to be the most feasible methods for group marking of large numbers of small-bodied fish. Depending on the objectives and study design, cost may factor in the decision to choose a particular tagging technique. Although PIT tags offer a number of advantages relative to VIE tags and freeze brands, the cost of the tag may be prohibitive for some studies.

The implantation technique and the relationship between tag weight and fish size are important factors in determining the effect of the tag on fish survival [2,10]. Ombredane suggested that survival after PIT tag implantation was influenced more by fish handling and tagging time than by the tag itself [27]. We observed no significant difference in survival between the sham treatments and the control treatments; however, survival differed significantly between the two sizes of PIT tags used in the current study. Although there were slight differences in the gauge of the needle used to implant these two tag types, the effect of needle size is likely negligible given that we did not insert the needle into the peritoneal cavity. The results are in contrast to previous studies which compared PIT tag implantation techniques in small-bodied fish and concluded that survival was higher using an incision technique [4,5]. However, as noted earlier, our method for implanting PIT tags allows us to minimize the chance of needle overinsertion, which has been attributed to internal organ damage and increased mortality with small fish [9]. McCormick and Smith used a similar technique to implant 11.5 × 2.1 mm PIT tags into marine damselfish (*Pomacentrus amboinensis*) as small as 5.2 g [28]. We urge researchers to assess the effect of PIT tag implantation method on survival of their target species prior to their implementation in field studies, as success appears to be species and/or size dependent and is influenced by the implantation technique.

We demonstrated that fish as small as 44 mm TL or 0.9 g can be PIT-tagged with smaller-sized PIT tags (PICO-tag) with 90% survival over 150 days, which is similar to other studies that assessed survival thresholds to determine the minimum size for PIT-tag implantation [5,8]. In one study, 95% survival was predicted at 52 mm for juvenile brown trout *Salmo trutta* (41–70 mm FL) implanted with 11.5 × 2.1 mm PIT tags and reared for four weeks [8]. In another study, 90% survival was predicted at 63 mm for adult Rio Grande silvery minnow *Hybognathus amarus* (46–89 mm standard length) implanted with 12.5 × 2.07 mm PIT tags and reared for 32 days [5]. Several studies have reported high survival when implanting 11 mm PIT tags in small fish of a similar weight as adult Oregon chub. For example, survival of 2–3 g juvenile gilthead seabream *Sparus auratus* was 85.7% over 66 days [7], survival of 2–3 g Nile tilapia *Oreochromis niloticus* was 83% over 49 days [4], and survival of 2.5–3 g Eurasian perch *Perca fluviatilis* was 93% over 126 days [9]. Moapa White River Springfish *Crenichthys baileyi* as small as 40 mm TL (1 g) implanted with 9 × 2 mm PIT tags had high survival (95.6%) and 100% retention over 41 days [10]. We demonstrated the ability to tag fish substantially smaller than in previous studies; however, we recognize that the two smaller PIT tags evaluated have a low

read range, limiting their ability to be utilized in passive interrogation.

We tagged and reared fish in a laboratory environment where temperatures were cooler than temperatures typically encountered during the spring and summer months when Oregon chub are studied in the field. Survival and tag retention is often inversely correlated with water temperature for PIT-tagged fish. For example, hybrid striped bass *Morone saxitilis* × *Morone chrysops* and bluegills *Lepomis macrochirus* that were tagged and reared in warmer water had significantly lower survival than those reared in cooler water [29,30]. However, exposure to a thermal regime similar to that experienced in the wild had no effect on long-term survival or tag retention in Oregon chub following PIT tagging in the present study. This suggests that our estimates of long-term survival and tag retention are applicable to the field. We suspect that the higher initial mortality in the PIT-tag + NTR group was due to stress associated with rapid temperature change following tagging (i.e., from 12.8°C to between 12.8°C and 20°C). Despite our attempt to mimic natural temperature fluctuations, we did not simulate other factors that may also affect survival and tag retention in the field conditions (e.g., pathogens, predators) so our estimates of survival and tag retention should be viewed as maximum estimates.

Our results with VIE tags in Oregon chub are consistent with other studies demonstrating high survival, tag retention, and visibility in small-bodied fish (e.g., [31-34]). However, success with VIE tags appears to vary with body location and species. For example, barbel *Barbus barbus* tagged at the base of the anal fin had high retention (82.6%) over two months [17], yet retention rates were low in the anal fin of largemouth bass *Micropterus salmoides*, blacktail shiner *Cyprinella venusta*, channel catfish *Ictalurus punctatus* [19], Colorado squawfish *Ptycholcheilus lucius*, and razorback suckers *Xyrauchen texanus* [1]. In Oregon chub, the tissue around the base of the anal fin is relatively thick, unpigmented, and translucent, with little chance of damaging organs by overinserting the tagging needle. Our marks were highly visible in and out of the water at 150 days (approximately five months). Furthermore, we have since observed these tags in the field 407 days after marking (unpublished observation, B. Bangs). VIE tag retention of juvenile brook trout *Salvelinus fontinalis* reared in an indoor hatchery and lake environment was 100% after 970 days [33]. The visibility of the tag in hatchery reared fish was >95% through 585 days, but decreased to 55–70% between 700 and 900 days. In the lake environment, visibility of the VIE tag was 50–70% through 400 days, and 0% at 959 days. Josephson et al. used blue filtered light and amber glasses in dark conditions to increase tag visibility, and observed 100% retention for lake reared fish at 959 days and 75% retention in hatchery fish at 970 days [33]. This suggests that UV illumination may increase tag visibility and recognition in long-term field studies.

Because VIE tags often make fish highly visible, several studies have evaluated the relationship between brightly colored marks and vulnerability of small-bodied fish to predation. No significant differences in predation between marked and unmarked small bodied or juvenile fish have been reported [1,19,35,36]. However, juvenile bluegill marked with highly visible fluorescent photonic dyes had significantly higher susceptibility to predation by largemouth bass than cryptically marked fish in a controlled laboratory environment [31]. The vulnerability to increased predation of VIE-marked fish should be considered prior to implementation of this tagging method in field studies.

The results from studies of survival, retention, and recognition of freeze brands used with small fish are inconsistent. Fingerling walleye (50–170 mm TL) had high survival and 95% freeze brand retention at 5 months in a rearing pond, and brands were observed in wild fish after 40 months [37]. Juvenile (65–160 mm FL) Chinook *Oncorhynchus tshawytscha*, Coho *Oncorhynchus kisutch*, and sockeye salmon *Oncorhynchus nerka* had high survival and tag retention at 14 months [38]; however, the author noted that retention in fish <55 mm (FL) was less than 3 weeks. Chinook, Coho, and sockeye salmon have high survival with freeze brands, but marks become faded and difficult to discern within four months [22]. In contrast, another study reported that initial mortality was high in Coho (8.3%), although survival was not significantly different from control fish and freeze brands became unrecognizable after 6 weeks [39]. Furthermore, in Oregon chub marked with two marks (i.e., VIE and freeze brand) in the field, we have observed high retention of freeze brands after 172 days, and poor retention after 407 days (unpublished observation, B. Bangs). Here, we inadvertently found that body placement affected the recognition of freeze brands on Oregon chub. Brands placed below the lateral line were more difficult to recognize than brands above the lateral line, because there were slight variations of freeze brand placement on each fish, and because of the differential Oregon chub pigment patterns on lateral surfaces. Even though we noted 98% retention through 150 days, 19% of these fish had poor quality brands. When brands were placed below the lateral line, the horizontal element of the "L" shape was sometimes absent. This may have been caused by inadequate pressure or duration against the branding terminal, build-up of ice on the iron, or a combination of these factors.

Conclusions

We evaluated several techniques for long-term group and individual identification of Oregon chub. We found that small bodied Oregon chub could be VIE-tagged or

freeze-branded with no appreciable effect on survival to 150 days. We predict 95% survival and retention from PICO-tagged Oregon chub ≥44 mm TL (0.9 g). PICO PIT-tags, VIE marks, and freeze brands are all feasible long-term marking techniques for Oregon chub, yet selection of a particular technique should be based on the species, size range, research objectives, cost, ease, and speed of tagging, and effects of the technique on survival, retention, and detection. Our results will facilitate future research on the population dynamics and behavior of Oregon chub by enabling us to monitor for movement within and between basins and within microhabitats.

Methods

We collected 840 Oregon chub from an abundant population in the Willamette Valley, Oregon, USA, with equal numbers of fish collected from each of the following size categories: 40–50, 51–60, and 61–70 mm TL. These fish were transferred to the Oregon State University Salmon Disease Laboratory (Corvallis, Oregon, USA) facility and initially held separately by size class in 100 L fiberglass tanks supplied with 12.8°C flow-through, pathogen-free well water. Fish were acclimated to the laboratory environment for 7 days prior to initiating the study.

At the beginning of the study, fish were separated randomly into seven treatment groups, with four replicates per treatment. The treatment groups included: 1) control; 2) fish that were tagged with a Biomark® (Boise, Idaho, USA) HTP9 full-duplex PIT tag (9 × 2.12 mm, 0.067 g) via injection into the peritoneal cavity and reared at 12.8°C, hereafter referred to as the PIT-tag treatment; 3) fish that were tagged with the same tag via injection into the peritoneal cavity and reared on a diurnal fluctuating temperature cycle (range: 12.8–20.0°C) to mimic the natural daily temperature cycle observed in the field, hereafter referred to as the PIT-tag + NTR treatment; 4) fish that were tagged with a Biomark® HTP8 PICO full-duplex PIT tag (8.4 × 1.4 mm, 0.033 g) and reared at 12.8°C, hereafter referred to as the PICO-tag treatment; 5) fish that received an injection into the peritoneal cavity but were not PIT tagged, hereafter referred to as the "sham" treatment; 6) fish that were marked with a freeze brand; and 7) fish that were marked with red VIE tags. For treatments 2 and 3, we used a 12-gauge Biomark N125 hypodermic needle attached to a MK10 implanter. For treatment 4, we used an 18-gauge Biomark N165 needle attached to a MK165 implanter. We pre-recorded PIT tag numbers prior to the tagging day and stored tags on a numbered foam cradle. We recorded the PIT tag number implanted in each fish in the PIT tag treatments. The method for implanting PIT and PICO tags differed from prior studies [40]. With a tag loaded and partially exposed in the needle, we placed the lancet nearly parallel to the epidermis and applied gentle pressure until the peritoneal cavity was breached. We then rotated the needle 180°, using the lancet to hold open the incision, and pressed the plunger to insert the tag. The tag was partially exposed after the needle was withdrawn, and we massaged the tag into the peritoneal cavity with a wetted thumb. This method resulted in higher survival in a preliminary study. To freeze brand fish, we used a 2 L modified Dewars flask fixed with an "L" shaped brass branding terminal (4 × 1 mm) extending from the reservoir and cooled with liquid nitrogen. We held the fish (left side) against the branding terminal for 2 s. To VIE tag fish, we implanted red fluorescent elastic polymer gel subcutaneously near the point of anal fin insertion with a 29-gauge needle on a 0.3 cc syringe. We injected the elastomer as the needle was being withdrawn, stopping before the needle bevel reached the dermal surface. We gently wiped over the insertion point with the thumb to remove excess elastomer. We anesthetized, weighed, measured, and handled control fish using the same protocol as the fish in the other treatments (described below), except we did not perform marking or implant tags.

We collected 10 fish at a time from the tank holding the appropriate size class, placed them in a 20 L bucket, and transferred the bucket to the workstation. We randomly assigned each fish to a treatment group and immersed it in tricaine methanesulfonate (MS-222; 30 mg/L), buffered with sodium bicarbonate (30 mg/L), and held it there until it was in stage III anesthesia. We recorded the weight (±0.01 g) and total length (mm) of each individual. The appropriate treatment was then applied and the fish were allowed to recover from the anesthetic in an aerated observation tank until they regained equilibrium and were actively swimming. After recovery, the fish were assigned randomly to one of the four replicate experimental tanks (25 L, n = 30 fish/tank) within the appropriate treatment group. The replicate tanks (n = 30 tanks total) were randomly distributed among the block of tanks to minimize the effect of tank location. We then reared the fish for a period of 150 days. The fish were fed a ration of 3% body weight once per day of 1.5 mm diameter Omega One™ (Painesville, Ohio, USA) Marine Pellets with garlic.

We inspected the fish in each tank daily, and we removed any dead fish and recorded their length, weight, PIT tag number, and tank number. We scanned tanks daily for expelled PIT tags. We tallied daily and cumulative mortality and tag loss for each replicate. After 30 days, we examined all fish to assess tag retention, wound healing, and growth. For fish tagged with VIE and freeze brands, we subjectively assessed the quality of the mark, where: 0 = a clearly identifiable freeze brand or VIE tag, 1 = partially healed or incomplete brand or VIE tag which was fragmented and/or visible only under UV light (VIE only), and 2 = no visible brand or VIE tag. In the PIT-tag treatments, we assessed the effects of

tagging method and fish size on survival. For fish in the PIT tag treatments, we scanned for the presence of PIT tags.

After 30 days, we combined replicates within treatment groups, and reared the control, PIT-tagged, VIE, and freeze brand treatments in 100 L tanks at 12.8°C for an additional 120 days. After a total of 150 days, we tallied the cumulative mortality and cumulative tag loss/detection for each treatment group. In addition, we compared the detection of VIE tags using ambient light and with the aid of ultraviolet light, which causes the VIE tag to fluoresce, potentially aiding detection.

We used a one-way analysis of variance (ANOVA) to assess whether initial fish length and weight differed between treatments. The model was fitted with the AOV function in the base stats package of program R. Visual inspection of the quantile-quantile plots suggested non-normality; however, estimates of coefficients and their standard errors are robust to the non-normality assumption of ANOVA [41]. More importantly, plots of residuals against fitted treatment means indicated excellent homogeneity of variance and no outliers. We tested for differences in survival between treatments with a one-sided binomial test using the prop test function in the base stats package of program R. We used one-sided tests because we hypothesized that fish with PICO-tags would have higher survival than those with the larger PIT-tags. We also hypothesized that fish in the control group would have higher survival than fish receiving treatments. Significance levels were not adjusted for multiple comparisons. We assessed differences in the final survival rates among treatments and size classes using a GLMM (binomial distribution, link: logit), with treatment and size class as fixed effects, and tagger and tank as the random effects. The model was fitted using the lmer function in the lme4 package for program R. Generalized linear models (GLMs) (binomial distribution; link: logit) were used to predict the expected length and weight of PIT- and PICO-tagged fish that will result in 80% and 90% survival, regardless of tank or tagger effects. These thresholds were selected because they fell within the observed range of survival probabilities. Note that the 'size' variable in the GLMM is an ordinal-scale category assigned to each fish upon death. The purpose of the GLM is to use continuous-scale measurements of size of each fish at the beginning of the study as a predictor of survival. Unlike the GLMM, the GLM ignores tank and tagger effects. All significance tests were assessed at the $\alpha = 0.05$ level.

Additional file

Additional file 1: Statistical analyses used to generate equations for 80% and 90% thresholds for length and weight.

Abbreviations

ANOVA: Analysis of variance; FL: Fork length; GLM: Generalized linear model; GLMM: Generalized linear mixed model; NTR: Natural temperature regime; PIT: Passive integrated transponder; TL: Total length; UV: Ultraviolet; VIE: Visual implant elastomer.

Competing interests

BioMark donated a portion of the PIT tags utilized in this study.

Authors' contributions

BB, PS, and SC designed the study, collected data, and drafted the manuscript. MF advised on the study design and completed statistical analysis. All authors read and approved the final manuscript.

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