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Efficacy of Visible Implant Elastomer Tags with Photographic Assist for Identifying Individuals in Capture-Mark-Recapture Studies using Larval Frogs

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***Abstract.*—Population demography studies often rely on marking individuals to estimate survivorship, population size, and dispersal. Ideally, marking methods allow researchers to identify individuals reliably over a given time frame (e.g., specific life stages); however, finding effective marking methods for larval amphibians can be particularly challenging. We evaluated efficacy of using a combination of visible implant elastomer tags and photo identification to identify individual larval anurans in the field. Initially, our pilot study in the laboratory using American Bullfrog (*Lithobates catesbeianus*) tadpoles showed that the probability of tag retention was higher for tags placed on the tail than tags placed on the body. We then used a combination of VIE tags placed on the tail and photo identification of tail spot patterns to identify tadpoles of Northern and California Red-legged Frogs (*Rana aurora* and *R. draytonii*) in the field at several sites across Oregon and California. We placed four VIE tags in the tail using unique sequences of colors for each individual. The retention rate of individual tags varied between sites from 82% to 98% with a mean of 92%. The proportion of individuals retaining all four tags varied significantly among sites, from 53% to 94% with a mean of 76.3%. Using photo identification of tail-spot patterns for tadpoles with missing tags, we successfully identified 98% of recaptured animals. Our work demonstrates that using VIE tags with photo assist can be highly effective for identifying individual tadpoles across a variety of conditions. The combined method is more reliable than using only VIE tags and requires less overall effort than using photo identification of markings exclusively.**

Key Words.—anurans; identification; marking; photograph; retention; tadpoles; technique

Introduction

Estimates of survivorship, population growth rate, and dispersal ability are crucial for understanding the ecology and conservation needs of different species. Capture-mark-recapture (CMR) techniques provide a useful tool to estimate demographic parameters (Lebreton et al. 1992; Nichols and Kendall 1995; Royle and Young 2008). Choosing an appropriate marking technique is a critical component of a CMR study, as tag loss, misidentification, or marking induced-mortality can bias parameter estimates (Arnason and Mills 1981; Pollock et al. 1990; McDonald et al. 2003). For amphibians, historically common marking techniques, such as toe clipping in adults and staining of larvae, may affect survival and growth rates (Skelly and Richardson 2010; Swanson et al. 2013). Passive integrated transponder (PIT) tags can be successfully used with adult frogs, but larval anurans are typically too small on which to safely use PIT tags (Pyke 2005).

Visible implant elastomer (VIE) tags (Northwest Marine Technologies, Shaw Island, Washington, USA) are a viable alternative for marking amphibians (Skelly and Richardson 2010) and have been used in studies of larval anurans (Belden 2006). Studies of both adult and larval frogs marked with VIE tags show no adverse effects on survival, growth rate, time to metamorphosis, movement, or adrenal response (Grant 2008; Antwis et al. 2014; Bainbridge et al. 2015; Sapsford et al. 2015). Tags such as VIE and similar tags that are injected under the skin (e.g., visually implanted alphanumeric tags or coded wire tags), however, may become lost or unreliable, potentially limiting their utility for CMR studies (Grant 2008; Hoffman et al. 2008; Martin 2011; Courtois et al. 2013). For marks based on combinations of VIE tags, marks are composed of multiple tags and mark reliability depends on each tag remaining in the individual in a way that the intended combination of tags can be reliably read.

There are several ways VIE tag reliability can be compromised. Tags can be completely lost through tag rejection. This occurs when a tag is expelled, commonly through the injection site, and may happen more frequently for tags in body areas that move

repeatedly (e.g., tadpole tails). In practice, VIE tag retention depends on tags neither being expelled nor migrating to a location where it cannot be detected upon inspection. Tags can also become unreliable when the tag is visibly retained within the body but migrates to a different position or merges with another tag.

The likelihood of tag loss or migration in anurans appears to vary considerably among species and may be related to tag location on the body. Brannelly et al. (2013) examined tag movement of VIE tags placed on the upper rear legs in adult Kihansi Spray Toads (*Nectophrynoides asperginis*) and found 50% of tags migrated leading to a possible misidentification rate of > 70%. In adult Alpine Tree Frogs (*Litoria verreauxii alpina*), Brannelly et al. (2014) found that individuals tagged in the upper rear leg with VIE were only correctly identified 18.4% of the time; however, Sapsford et al. (2015) found 84% of adult Common Mistfrogs (*Litoria rheocola*) retained three clearly visible VIE tags on the upper legs after one year.

The results are also variable in the few studies that have examined VIE tag retention in larval anurans. Grant (2008) found 50% of Wood Frog (*Rana sylvatica*) tadpoles marked on the tail with two VIE tags had lost one of the two tags after 20 d in a captive trial. Bainbridge et al. (2015), however, found retention rates of 95% and 88% at 10 d and through metamorphosis, respectively for Green and Golden Bell Frog (*Litoria aurea*) tadpoles marked with a single VIE tag on the body in the field. While there are techniques to account for mark loss in CMR analyses (e.g., Cowen and Schwarz 2006; Chilvers and MacKenzie 2010), the high rate of tag loss or migration found in some of the studies evaluating VIE tags in anurans calls into question the suitability of VIE tags for CMR studies.

Redundant VIE marking schemes or multiple identification methods used simultaneously may increase the probability of successfully identifying individuals; however, double-marking an animal can increase the associated handling stress or may be impractical due to body size, while other marking methods such as toe or tail clips and fin staining can reduce survival and/or recapture probability (Turner 1960; Travis 1981; Skelly and Richardson 2010; Swanson et al. 2013). Compared to toe or tail clips or staining, photographic identification is non-invasive. Photographic identification for CMR analyses has also been used effectively with Iberian Midwife Toad (*Alytes cisternasii*) tadpoles (Ribeiro and Rebelo 2011). Processing photographs from all captured and recaptured individuals quickly becomes time-intensive, though, when working with even moderate numbers of individuals. Even when using software to help select the best photograph matches, the time required to annotate photographs and sort through the most likely matches is considerable. Combining VIE tags with photograph identification has the potential to be both effective and efficient because only tadpoles with lost tags need to be identified using photographs.

In this study we evaluated the efficacy of the combined approach of using VIE tag color combinations and photograph identification to identify individual Northern and California Red-legged Frogs (*Rana aurora* and *R. draytonii*, respectively) tadpoles in the field at six sites in Northern California and Oregon. Prior to initiating the field study, we used American Bullfrog (*Lithobates catesbeianus*) tadpoles in a laboratory trial to determine whether marking tadpoles on the tail or on the body with VIE tags yielded higher mark reliability. We examined VIE mark reliability (i.e., the probability of an individual retaining a reliable full set of tags) in the laboratory trials and field study, and tag retention (i.e., the probability of any individual tag being retained and visible) in the field study. In addition to evaluating tag retention across sites, we also assessed how much our successful identification rate increased by using photographs to identify tadpoles with lost tags.

Materials and Methods

Study system.—Northern Red-legged Frogs are a pool-breeding Ranid frog native to the Pacific Northwest regions of the U.S. and southwest Canada. Juveniles and adults typically occupy upland habitats for the majority of the year. Adults emerge from over-wintering brumation and begin emigrating to breeding pools at the onset of the breeding season (Storm 1960). Adult male frogs usually arrive at breeding pools first, with adult females arriving afterward. Breeding pools can be a variety of aquatic habitats, including slow moving reaches and oxbows of large streams and rivers, or ephemeral and perennial ponds (Storm 1960). Females oviposit egg masses, attaching them to submerged and emergent vegetation, or to woody debris. Egg masses contain 500–1,000 individual embryos within a loose matrix (Stebbins and McGinnis 2012). After hatching, the larvae spend multiple weeks as hatchlings then tadpoles before transforming. Our study included populations within in the potential Northern–California Red-legged Frog hybridization zone (Schaffer et al. 2004). We did not observe substantial differences in breeding behavior or life-history traits in these populations compared to the populations in our study outside of the potential hybrid zone.

American Bullfrogs are native to the southeast regions of the U.S. In the western areas of the U.S. where our study occurred, this species has been introduced and has become a successful invader, co-occurring with and potentially influencing the growth and survival of red-legged frog larvae (Kiesecker et al. 2001). American Bullfrog life-history traits are similar

to that of red-legged frogs, though in many areas, bullfrog tadpoles can exhibit different developmental pathways to reproductive maturity making control difficult (Govindarajulu et al. 2005). We used American Bullfrog tadpoles in our initial VIE tagging trials because they were readily available, and as an invasive species, they were an ideal candidate for testing methodology without concern for impacting natural populations.

Laboratory experiment.—We followed VIE tagged American Bullfrog tadpoles in a controlled laboratory setting over 38–96 d to determine the reliability of VIE tags for CMR studies. We opportunistically collected Bullfrog tadpoles during management activities along the lower Mad River, near Blue Lake, California, USA. Prior to tagging, we held tadpoles in a 40 L tank partially filled (about 20 L) with non-chlorinated water. We placed detritus and invertebrates in the holding tank and in five additional tanks (about 30 L) used to hold tadpoles after they were tagged. We held the water volume in the additional tanks constant throughout the experiment at about 6 L of non-chlorinated water. We replaced 50% of water in each tank every 7–10 d. In addition to detritus and invertebrates, tadpoles were fed TetraFin® goldfish flake food (Spectrum Brands, Blacksburg, Virginia, USA) with ≥ 29% crude protein content, at minimum once every 3 d. We held all tanks under ambient lighting constant across all treatments. We did not influence water temperature in the tanks directly, but all tanks were held in the same temperature controlled environment at 18–20° C.

We selected tadpoles at random for marking by dip-netting from the holding tank. We marked 119 tadpoles in five batches, which varied in tag placement and the number of tags used (Table 1). Every tadpole within a batch was marked with the same color and number of tags with the exception of batch one that contained two groups of tadpoles with different marks. We used fluorescent yellow and purple in our marking schemes. We placed tags on the dorsal and ventral side of the body cavity in one batch, and along the tail in the other four batches.

Batch one included tadpoles that we marked with a single yellow tag on the right side of the tail, and with a single purple tag on the left side of the tail. Tadpoles in batch one with yellow tags were held in a separate tank than those with purple tags. Tadpoles in batch two we marked with a single yellow tag on the dorsal side of the body, and a single purple tag on the ventral side of the body. We marked tadpoles in batches three, four, and five on the tail. We placed two yellow tags on the left side of the tail for batch three, and three yellow tags on the left and right sides of the tail (six total tags) for batches four and five. Batches varied in size from 20 to 30 tadpoles, depending on the amount of time available to mark animals on the day we initiated each batch. We initiated batches at staggered intervals over the course of 58 d. We did not examine field retention of VIE tags in American Bullfrogs because the invasive nature of this species in our study area made live-release of bullfrog tadpoles into the field unethical.

Prior to tagging, we anesthetized tadpoles in an immersion bath using MS-222 (TMS, tricaine methanesulfonate) in solution at 0.02% concentration (Anholt 1998). We buffered anesthesia solutions with sodium bicarbonate to achieve a more neutral pH (about 7.2). We determined if tadpoles were fully anesthetized by gently prodding and watching for a startle or righting response. Once fully anesthetized, we injected the polymer subcutaneously using a 0.3 cc syringe with a 29-gauge needle. On the body, we placed tags slightly anterior to where the tail meets the body. On the tail, we placed tags where the fin and tail muscle tissue meet, and running parallel to the muscle bands. We placed marked tadpoles in a recovery bath of non-chlorinated water until movement of mouthparts, a startle response, and righting response indicated recovery. We observed a 100% recovery rate from anesthesia in all five batches.

During the course of this experiment, two batches (four and five) experienced significant mortality from an unknown cause. We removed all surviving tadpoles in these batches from the tanks and placed them in temporary holding tanks until the original tanks could be sanitized and vegetation removed. After sanitation we re-seeded these tanks with detritus and filled with non-chlorinated water. We observed no further mortality in these samples through the course of the experiment.

We sampled all of the tadpoles in each batch using a hand-held dip net. Each batch was sampled in order and on the same day. Sampling intervals varied but did not exceed one month. In our laboratory experiment, we measured mark reliability as the proportion of tadpoles that retained their full complement of tags that were readily detected during a complete yet expeditious inspection, attempting to mimic field conditions. Thus, we considered tadpoles with tags that split, migrated, or merged into one another as non-recovered identifications, reflecting a potential loss of information for field studies using unique color-coded identifications. We calculated the final mark reliability for each batch as the proportion of marked tadpoles that had all of their original tags intact during the last resampling session. We adjusted our recovery rates for mortality by removing the individuals that perished from the final calculation. We used logistic regression (glm function from the stats package in R; R Core Team 2017) to test whether individuals had greater odds of reliably retaining all tags (binary response variable) when tagged on the body vs. the tail (categorical predictor variable), and to test if the experiment duration (i.e., time from tagging to

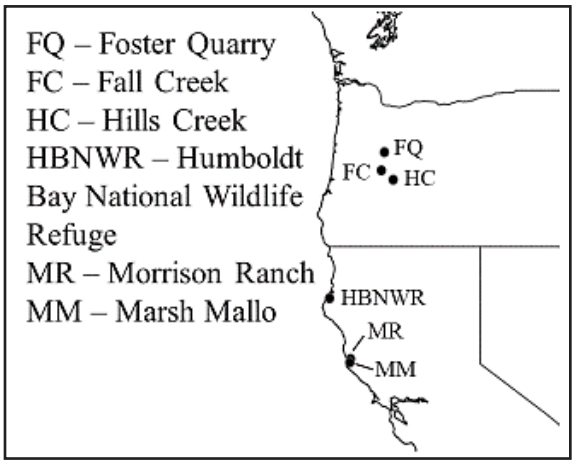

Figure 1. Locations of six sites in northern California and western Oregon, USA, for studying marking methods of red-legged frog tadpoles (*Rana* sp.).

last observation; continuous predictor variable) affected mark reliability rates. We performed all analyses using the R 3.3.3 statistical software program (R Core Team 2017).

Field study.—We marked 1,419 red-legged frog tadpoles captured at six field sites in Oregon and California with unique color-coded sequences of VIE tags from April through June 2017. The three sites in Oregon were on U.S. Army Corps of Engineers property in Linn and Lane counties (Fig. 1): Foster Quarry (FQ), Fall Creek (FC), and Hills Creek (HC). Of the three sites in Oregon, two were semi-permanent ponds (FQ and HC), which usually contain water year round but are subject to substantial drying. Both of these sites have areas with substantial submerged and emergent vegetation, as well as areas with considerable canopy cover contributing to detritus loads. The third site (FC) commonly has no measurable water by late August. This site also has little vegetation and canopy. In California, one site was in the Humboldt Bay National Wildlife Refuge (HBNWR) in Humboldt County, and two sites, Morrison Ranch (MR) and Marsh Mallo (MM), were on Mendocino Redwood Company property in Mendocino County (Fig. 1). The HBNWR site was a seasonally inundated wetland with dense emergent and submerged vegetation, but little to no overhead canopy cover. The breeding pool at MR was a relatively small, stock pond that remains filled with water year-round. It has no canopy cover, but does contain dense emergent vegetation. In contrast, MM is an ephemeral pond surrounded by dense conifer forest that contains a moderate amount of emergent and submerged vegetation, but lots of woody debris. All of our sites supported pure Northern Red-legged Frog populations, except for the two most southern sites, MR and MM, which potentially support Northern and California Red-legged Frog hybrid individuals (Shaffer et al. 2004).

At five of the sites (all except MM), we placed tagged tadpoles into 2 × 2 m enclosures consisting of permeable aquatic drift fences, *in situ*, to facilitate recapture of marked individuals while maintaining tadpoles in natural environments. There were one to four enclosures per site. The enclosures likely prevented some large vertebrate predators from entering, though we commonly observed potential tadpole predators including Rough-skinned Newts (*Taricha granulosa*), Northwestern Salamander (*Ambystoma gracile*) larvae, paedomorphs, and adults, as well as dragonfly larvae (order Odonata), predaceous diving beetles (family Dytiscidae), and giant water bugs (*Lethocerus* spp.) in and around our enclosures at most sites. We did not supplement the enclosures with substrates or detritus with the exception of FC, where our enclosures were placed in an area that was almost completely devoid of vegetation and detritus. Supplemental substrates at FC were taken from a different area of the same pond. At MM, FC, HBNWR, and MR, we also tagged and released tadpoles in the open pond.

We anesthetized all red-legged frog tadpoles prior to tagging using the same procedures as the laboratory experiment with American Bullfrogs. Only two of the 1,419 red-legged frog tadpoles we marked did not recover from anesthesia (99.9% anesthesia recovery rate). We marked red-legged frog tadpoles on the tail with four tags chosen from six different colors. Tags were injected into the tail where the tail muscles meet the tail fins. We placed two tags on the bottom left side of the tadpole, and two tags on the top right side (Fig. 2A). Thus each animal had a mark comprising four colored tags read in the following order: bottom left anterior, bottom left posterior, top right anterior, top right posterior. We used VIE in blue, red, pink, orange, yellow, and purple. All VIE colors fluoresced under ultraviolet light expect for purple, which was still easily visible under natural lighting. We chose these colors because they produced the greatest contrasts between colors. Using tags chosen from six colors allowed up to 1,296 unique tag combinations at each site. We did not use the same color combination for more than one tadpole per site.

We sampled tadpoles from each site on average every 3–8 d. We captured tadpoles using handheld dip nets and aquatic funnel traps. We tagged unmarked tadpoles and recorded recaptures of any previously marked tadpoles, noting the full complement of marks or the sequence of remaining tags for those animals that lost tags. On average, there were 19.6 d between the tagging date and the last capture of each recaptured tadpole (range, 1–70 d). We calculated two measures at each site: tag retention, measured as the ratio of the total number of tags

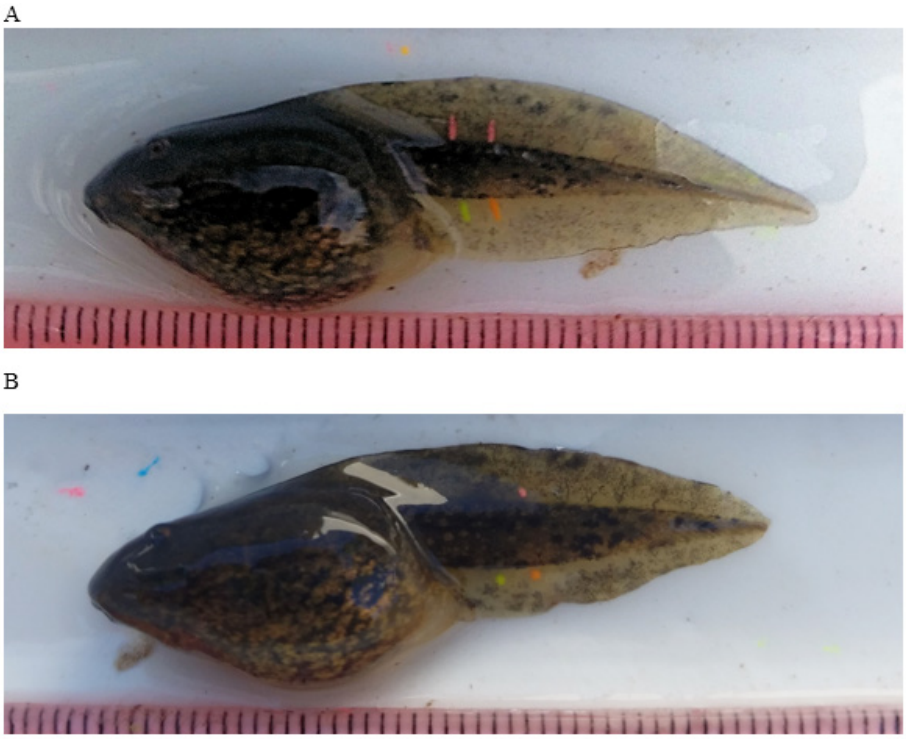

Figure 2. (A) Tadpole of Northern Red-legged Frog (*Rana aurora*) with the four-tag combination on the tail immediately after marking and while under anesthesia. (B) Same tadpole with one tag missing during recapture 12 d later. The tadpole was later identified based on the sequence of remaining tags and tail spot patterns in the photographs. (Photographed by Kelcy McHarry).

retained and visible to the total number of tags injected in recaptured individuals, and mark reliability, measured as the ratio of the number of individual tadpoles that lost no tags to the total number of marked animals recaptured at least once from the marked populations. We did not observe any cases of marks being compromised by tags merging or migrating relative to other tags prior to metamorphosis. To determine if an individual was more likely to lose multiple tags if it lost at least one tag, we estimated the expected proportion of tadpoles to retain all four of their tags assuming the chance of retaining each tag is independent within individuals. To calculate the expected proportion of tadpoles to retain all four tags, we raised the chance of retaining an individual tag (the ratio of the total number of tags retained to the total number of tags injected) to the fourth. We then used a Chi Square analysis to determine if the observed rate of retaining all tags differed significantly from the expected rates, as calculated based on rates of loss of individual tags as determined from our results. Because we found that retention rates varied significantly among sites, we performed separate Chi Square tests for each site.

We used logistic regression models (glm function from the stats package in R 3.3.3; R Core Team 2017) to explore the variables that might influence mark reliability, including: site (categorical predictor), tail length (continuous predictor), days between first and last capture (continuous predictor), and injuries (injured or not; binary predictor; Table 3). We evaluated models based on the Akaike Information Criterion (AIC; Akiake 1973), which measures model accuracy while penalizing excessive model complexity (additional parameters), with a lower AIC score indicating a preferred model. For our best performing model, we also tested the overall effect of site using the wald.test function in the aod package of R (Lesnoff and Lancelot 2012), which uses a Wald chi-squared test to evaluate significance of multiple coefficients jointly, given their variance-covariance matrix.

In addition to VIE marking, we also took pictures of all the tadpoles the first time we tagged them and again if they had lost any tags upon recapture (Fig. 2A, B). We did this as a secondary method of identification. Most red-legged frog tadpoles have unique spot patterns on their tails that allow them to be distinguished from one another. When a tadpole was recaptured missing one or more tags, there were often only a small number of possible individuals it could be based on the sequence and colors of remaining tags. In these cases, we sorted through photographs by hand to find matches. In a few cases, the list of possible individuals a tadpole with missing tags was quite long (> 20 individuals). In these cases, we used the I^3S program (Interactive Individual Identification System; Van Tienhoven et al. 2007) to aid with identification. The I^3S software uses photographs annotated with spot patterns marked to generate a list

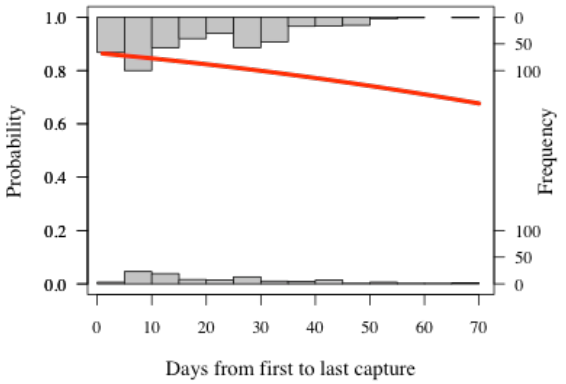

Figure 3. Relationship between days between first and last capture (days) and tag retention of frog tadpoles as a binary response. The graph shows the frequency of individuals that retained (probability = 1) or lost tags (probability = 0) grouped by days, as well as the binary fitted line for the relationship between days and tag retention (shown in red).

of closely matching previously annotated photographs. If tadpoles with missing tag(s) had few distinguishing marks and could not be identified by photograph, we attempted to determine the identity of tadpoles by process of elimination based on the possible tag combinations, original tagging date, tadpole size, and any identifying injuries we recorded (e.g., part of tail missing).

Results

Laboratory experiment.—The proportion of tadpoles recovered with all their tags intact varied across batches from 0.47 to 1.0 (mean = 0.857 ± 0.082 [SE]). Mark reliability was greater for tadpoles marked on the tail than those marked on the body (0.92 vs. 0.48; Wald $Z = 3.46$, $P = < 0.001$; Table 1). Length of time since tagging had no effect on mark reliability (Wald $Z = 1.37$, $P = 0.171$).

Field study.—Across all sites, 110 of the 560 recaptured tadpoles had lost at least one tag. Of the 110 tadpoles that lost at least one tag, only 13 could not be definitively identified using photographic identification and/or by process of elimination, resulting in successful identification rates from 0.91 to 1.00 across sites (mean = 0.98; Table 2). In the field, taking photographs of tadpoles while tagging tadpoles added only a few seconds of additional time per tadpole and because most of the tadpoles that lost tags in our study still had two or three tags intact, there was only a small subset of tadpole photographs to compare for identification.

For VIE tags, tag retention rates varied among sites from 0.82 to 0.98 (mean = 0.92 ± 0.03 [SE]; Table 2). Assuming independent tag loss within individuals, these tag retention rates corresponded to an expected probability of an individual retaining all four of its tags varying among sites from 0.45 to 0.94 (mean = 0.74 ± 0.075 [SE]; Table 2). The proportion of recaptured animals recovered with four clearly visible tags (mark reliability) at each site varied from 0.53 to 0.94 (mean = 0.76 ± 0.07 [SE]; Table 2), none of which differed significantly from expected recovery rates for those sites assuming independent tag loss ($\chi^2 = 0.030–3.59$, $P = 0.058–0.864$).

The top regression model for the likelihood of mark reliability (Table 3) included a weak negative effect of time since tagging (estimate = -0.02, Wald $Z = -2.58$, $P = 0.010$; Fig. 3), and a nonsignificant negative effect of tail length (estimate = -0.015, Wald $Z = -0.81$, $P = 0.419$). There was also an effect of site on mark reliability ($\chi^2 = 69.6$, $P = < 0.001$). While MM had only slightly higher mark reliability than FC (0.53 and 0.58 respectively; Table 2), mark reliability at all of the other sites was substantially higher (Table 2; Fig. 4). We found no effect of injuries on mark reliability.

Discussion

Our work shows that combining VIE tags with photograph identification can be a highly effective for tracking individual tadpoles in CMR studies and produces a much higher successful identification rate than VIE tags alone. Without photograph identification, we were able to identify on average only 76% of the red-legged frog tadpoles we recaptured based on VIE tags; however, by using photographs to identify tadpoles

Table 1. Tag position, number of tags placed in each individual (orientation on body), number of individuals tagged (n), tagging date, number of mortalities, mark reliability (proportion of individuals retaining all tags) adjusted for mortality for each batch of tagged tadpoles of the American Bullfrog (*Lithobates catesbeianus*). Mortality is considered collectively as number of animals observed dead, as well as animals not found during recapture. Mark reliability is calculated as proportion of animals recaptured as originally marked to animals available to be recaptured (recaptured as marked) / (number marked - mortality).

Batch	Position	Number of tags	n	Mark date	Mortalities	Mark reliability
1	tail	1 (right n = 6, left n = 19)	25	9 September	6	0.942
2	body	2 (one dorsal, one ventral)	23	11 September	2	0.476
3	tail	2 (left)	20	21 September	3	0.941
4	tail	6 (three right + three left)	30	25 September	27	1
5	tail	6 (three right + three left)	21	6 November	11	0.8

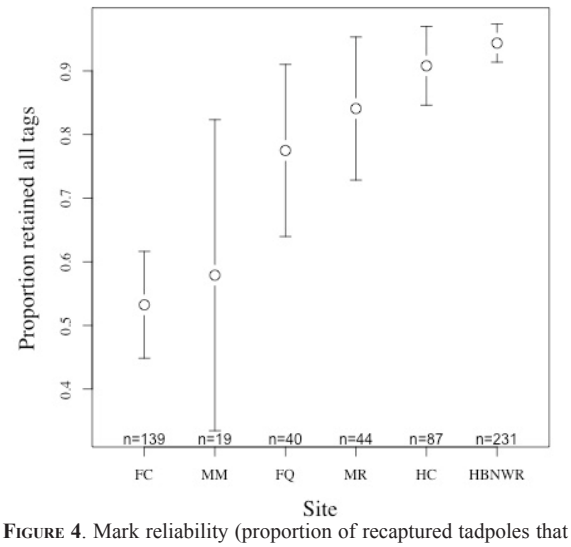

Figure 4. Mark reliability (proportion of recaptured tadpoles that retained all tags) and 95% confidence intervals by site: FC = Fall Creek, MM = Marsh Mallo, FQ = Foster Quarry, MR = Morrison Ranch, HC = Hills Creek, HBNWR = Humboldt Bay National Wildlife Refuge.

with lost tags, we were able to successfully identify to 98% of recaptured tadpoles. The only instances where photograph identification failed were when tadpoles did not have enough markings to distinguish individuals or when we missed taking a photograph of a tadpole.

Previous studies have also used a combination of identification methods to improve the success of VIE tagging for anuran CMR studies. When marking adult Treefrogs (family Hylidae), Hoffman et al. (2008) and Campbell et al. (2009) used a combination VIE tag-toe clipping scheme (VIE-C). In these studies, the researchers found that the two-mark approach of VIE-C not only provided a larger number of usable combinations for unique identifications, but also helped them interpret troublesome identifications when one of the two marks were partially lost. A primary advantage of photograph identification as a secondary identification method, as in our work, is that it is minimally invasive and should not affect survivorship or likelihood of recapture. Photograph identification is also highly reliable when distinguishing pigmentation patterns are present and requires minimal additional effort when used to identify individuals among smaller subsets of candidates.

Given the relatively high tag-retention rates but relatively low mark-reliability rates we observed for VIE tags alone, this technique may be more appropriate for batch marking larval anurans than for creating individual marks based on combinations of colored tags. For identifying individuals based on color combinations all tags (in our case four) must be retained, and the probability of retaining multiple tags will always be less than the probability of retaining one. In our study the likelihood of a tadpole retaining any given tag was 93%,

Table 2. Proportion of recaptured red-legged frogs (*Rana* sp.) that could be identified using a combination of VIE tags and photo identification (IDR, identification rate), retained all of their VIE tags and could be accurately identified (MR, mark reliability), and the retention rate of individual tags (TRR, tag retention rate), which is proportion of total tags placed (four/tadpole) that remained on recaptured individuals. Also included is the average tail length (ATL in mm) and average number of days (AND) between first and last capture for recaptured tadpoles for each site: FC = Fall Creek, MM = Marsh Mallo, FQ = Foster Quarry, MR = Morrison Ranch, HC = Hills Creek, HBNWR = Humboldt Bay National Wildlife Refuge. Abbreviations are ID = identification.

Site	IDR	VIE MR	VIE TRR	ATL	AND
FC	0.91	0.53	0.82	30.3	21.6
MM	1.00	0.58	0.88	43.1	11.8
FQ	0.98	0.78	0.91	36.6	20.5
MR	1.00	0.84	0.96	50.8	20.6
HC	1.00	0.91	0.97	35.4	6.40
HBNWR	1.00	0.94	0.98	37.2	23.8
Mean (SE)	0.98 (0.01)	0.76 (0.07)	0.92 (0.03)	36.5 (0.39)	19.6 (0.62)

which is comparable to the 95% retention rate found by Bainbridge et al. (2015) for tadpoles marked with a single VIE tag on the abdomen, and higher than the approximately 75% tag retention rate found by Grant (2008) when tagging tadpoles on the tail.

While we only measured mark reliability in our laboratory experiment with American Bullfrog tadpoles, we found higher mark reliability (92%) when tadpoles were tagged on the tail compared to the abdomen (48%). Our laboratory results differed qualitatively from previous studies that suggest that tag retention rates may be higher for tadpoles tagged on the body compared to the tail (Grant 2008; Bainbridge et al. 2015). Anecdotally, we found that tags located in the tail rarely migrated or merged with each other, while the tags placed in the abdomen were more likely to migrate and/or be obscured by pigmentation on the body of the

Table 3. Akaike's Information Criterion (AIC), delta AIC, and Akaike weights for the logistic regression models used to test the effects of site, days from first to last capture, tail length, and the presence of injuries on the likelihood of red-legged frog (*Rana* sp.) tadpoles retaining all of their tags in the field experiment.

Model	AIC	ΔAIC	Akaike Weight
Site + days + tail length	438.09	0.00	0.59
Site + days	438.95	0.86	0.39
Site + days + tail length + injury	444.79	6.70	0.02
Site + tail length	457.47	19.38	0
Site	464.68	26.59	0
Days	511.2	73.11	0
Tail length	536.5	98.41	0
Injury	560.39	122.3	0

tadpole. We also found that marking on the tail was considerably easier to accomplish, ultimately reducing handling time and associated stress. The higher mark reliability we found in American Bullfrog vs. red-legged frog tadpoles tagged on the tail could be due to differences between species or between laboratory and field conditions. The potential 52% misidentification rate of American Bullfrog tadpoles tagged on the abdomen contrasts with the results in Bainbridge et al. (2015). These differences among studies highlight that VIE tag reliability rates, and thus the efficacy of VIE tags, may vary among species, environments, or based on tagging location.

We found substantial variation in mark reliability among our study sites, with a nearly eight fold difference between HBNWR and FC in the proportion of recaptured individuals missing at least one tag. Some of the variation may be related to between site differences in tadpole size. The average tail length of FC tadpoles was 5.2 mm shorter than at any other site, and we did find a significant effect of tail size on mark reliability; however, our top models included both site and tail size as independent factors, indicating that additional factors were at play. Differences in activity levels among sites could influence mark reliability if tail muscle contractions increase the likelihood of tags migrating or being expelled. While we did not directly measure activity levels, our sites differed noticeably in their habitat components associated with tadpole activity levels- predator communities, benthic resources, and structural refugia (Eklöv and Halvarsson 2000; Thurgate 2006).

Taken as a whole, our work supports using a combination of VIE tags and photographic identification for amphibian CMR studies, especially when using tag color combinations to identify unique individuals. We found that the reliability of using VIE tags alone varies considerably across sites. Using photographic identification as the only method, however, would likely require a substantial time commitment not available in every study, and may be unsuitable for species with few distinctive markings. Using both VIE tags and photographs can concurrently increase the percentage of successfully identified animals while minimizing the time for processing and comparing photographs. Employing this combined method with red-legged frogs we were able to successfully identify nearly all of our recaptured animals.

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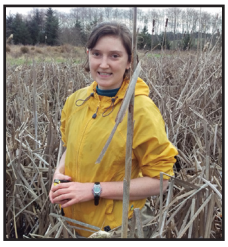

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