Use of a Fluorescent Marking Technique on Small Terrestrial Anurans

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The choice of a marking technique for research should be based on the objective of the study, effectiveness of the technique, ease of application, and the potential risk to the organism. While toe-clipping is convenient and inexpensive, and thus remains a common technique for marking adult anurans (Donnelly et al. 1994), there is some evidence that it may affect behavior or survivorship (Clark 1972; Golay and Durrer 1994; but see Lemckert 1996). The use of a marking technique that potentially affects the organism's behavior or survival is usually unacceptable; thus, alternatives to toe-clipping should be considered. Marking amphibians with fluorescent pigments appears to be a promising alternative (Donnelly et al. 1994; Windmiller 1996). A pressurized application of fluorescent powder was applied successfully to aquatic and terrestrial salamanders (Ireland 1973, 1991; Nishikawa and Service 1988; Taylor and Deegan 1982). However, the effectiveness, details of application, and risks of this technique have not been quantified for other amphibians.

I recently used a pressurized fluorescent marking technique on small terrestrial leaf-litter frogs (*Eleutherodactylus podiciferus*; Leptodactylidae) in Costa Rica to evaluate its applicability. Inert fluorescent powder was applied to a hind leg of frogs using a pressurized source of air similar to that described in Nishikawa and Service (1988). However, Nishikawa and Service (1988) used a small 2-liter source of pressurized N_2, whereas I used a source of pressurized air delivered by a SCUBA tank. The nozzle of the spray gun was modified to accommodate a variety of interchangeable nozzle openings. The 7/64 inch (2.8 mm) opening was optimal, as it did not clog the nozzle yet produced a concentrated point. The yellow, fluorescent, granular powder (50–350 mm) produced a mark 3–4 mm in diameter on the hind leg of the frog when delivered at a pressure of 100 psi, approximately 0.5 cm from the skin.

I compared the use of fluorescent pigments in the field to toe-clipping with respect to i) ease of application and disturbance to frogs, ii) risk to frogs (i.e., survival), iii) longevity of the mark, and iv) logistics and cost. I caught and marked 68 adult *E. podiciferus* (10–24 mm SVL) in 1996, alternating between the fluorescent powder technique, and toe-clipping a single foot digit (digit IV). Clipped toes were disinfected with a dab of Bactine® to reduce the likelihood of infections (Martin and Hong 1991). Juveniles (< 10 mm SVL) were judged to be too fragile to mark with either method. Frogs were marked during two Periods: Period I, 2–8 July: 22 yellow-marked and 23 toe-clipped frogs were released; Period II, 20–23 July: 11 yellow-marked and 12 toe-clipped frogs were released. To determine whether marks were disappearing, a description of each individual's natural markings (modified from Savage and Emerson 1970) were noted, as well as SVL. Frogs were recaptured during Period II and Period III (13 August).

The pressurized powder technique was more difficult to apply, and seemingly more harmful to the frogs than toe-clipping. Applying the pigment required holding the frog tightly on a flat surface and then releasing a blast of air. If the frog was not properly immobilized, legs were occasionally dislocated by the blast (N = 5; 15%). Also, frogs were occasionally blown away, literally. One frog died due to the fluorescent powder procedure, and 30–50% of the frogs appeared to be stunned to various degrees by the blast of air. Recovery from the blast, measured by a return to a righting position and the ability to jump when touched, was often incomplete several minutes later. *Eleutherodactylus podiciferus* may have been particularly susceptible to the blast of air due to its small size. Toe-clipping was easier and quicker to apply than the pressurized powder, although it would not have been possible to clip the smaller, inner toes (I–III) on the younger individuals (<18 mm SVL) due to their small size. Fingers are also very small and would be difficult to clip for the same reason (pers. obs.). None of the toe-clipped frogs suffered visibly from the clipping procedure.

Eleven of the 68 marked frogs were recaptured. Six were toe-clipped and 5 bore yellow fluorescent spots (Period II: 2 toe-clipped and 3 yellow from Period I; Period III: 2 toe-clipped from Period I, 2 toe-clipped from Period II, and 2 yellow from Period II). No toe-infections or other effects of either technique were observed on recaptured individuals. I was unable to detect any significant difference in survivorship between the two marking methods, because of low recapture rates.

Yellow spots were still clearly visible on 3 of 5 recaptured frogs with these marks. The other two had faded to a light gray spot during the three weeks since the application, but were still visible, particularly under UV light. Nishikawa and Service (1988) found that fluorescent marks on salamanders would last 1–2 years in the laboratory. Toe-clips were still clearly visible on the two individuals marked in Period I and caught in Period III, suggesting that they do not regenerate within a 5–6 week period. No unmarked individuals fit the profile of a previously marked individual (using natural variation and SVL); thus, no lost yellow marks or regenerated toe-clips could be confirmed among the recaptured individuals.

I agree with Ireland (1991) that the equipment was rather cumbersome to transport into the field, weighing 3.2 kg excluding the source of air (a SCUBA tank and the regulator). It was also expensive, costing US$ 340 in 1996 (including UV lamp, but excluding the price of the SCUBA tank and the regulator). Toe-clipping required a pair of surgical scissors and a bottle of Bactine® (weight: 120 g; cost: $12).

It appears that toe-clipping is easier, less harmful, more convenient, and less expensive to apply to *E. podiciferus* than pressurized fluorescent powder. Fluorescent marking has the advantage that marked organisms may be identified by day, or by night using a UV lamp, without further handling. Nishikawa and Service (1988) also found higher rates of recapture per unit effort of salamanders that had been marked with fluorescent powder relative to other studies that used toe-clipping. Fluorescent powder may

therefore still present an interesting alternative technique for marking larger frogs, which can be properly immobilized and which are less likely to suffer from the blast of air during the application of the powder. I concur with Donnelly et al. (1994) who recommended that researchers test a variety of techniques on their organism prior to adoption for field or laboratory studies, because effectiveness and application will vary with body size and life history.

Acknowledgments.—Financial support was generously provided by the Guani Family Fellowship in Conservation Biology, a pre-doctoral NSF fellowship, and OTS-Las Cruces. Special thanks to the Guani family and R. Carnahan for their logistical support; to the Gamboa family for the permission to work on their land; to T. Gavin, K. Adler, and M. Donnelly for helpful comments on earlier drafts of this manuscript; to the Costa Rican government for permission to study; and to the staff at Cornell's Metal Shop for the modifications made to the spray gun.

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Female *Ambystoma opacum* (Marbled Salamander) with eggs. USA: South Carolina: Beaufort Co. Illustration by Michael G. Frick.