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A Fluorescent Marking Technique for Individual Recognition of Terrestrial Salamanders

KIISA C. NISHIKAWA[1] AND PHILIP M. SERVICE,[1] *Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27514, USA.*

Repeated observation of the location and behavior of neighboring individuals within a population is essential for many types of ecological and behavioral research. Such observations require a method that permits both the census of a local population and the recognition of its individual residents. Conventional mark-recapture techniques are inadequate for amphibians (Ferner, 1979). Toe clipping requires handling for mark recognition, and a high proportion of marked animals is never recaptured (e.g., Merchant, 1972). Radioactive tagging yields recapture rates up to 100%, but the technique is time-consuming and expensive, and the tags do not uniquely identify individuals (e.g., Barbour et al., 1969; Madison and Shoop, 1972; Kleeberger and Werner, 1982).

Here we describe a relatively simple and inexpensive technique for marking terrestrial salamanders using dry fluorescent dust applied to the skin with pressurized air. Marks can be applied in the field without anaesthesia, recapture success is high, and individuals can be recognized with little or no handling, in light or in darkness.

Fluorescent dust has been used previously for mass-marking fishes (Phinney et al., 1967) and aquatic amphibians (Ireland, 1973; Taylor and Deegan, 1982). Ireland (1973) applied acetone solutions of fluorescent dust to the skin of salamander larvae (*Ambystoma annulatum* and *Eurycea multiplicata*) using a heated probe. Taylor and Deegan (1982) applied dry fluorescent dust to the skin of larval *Rana clamitans* and adult *Notophthalmus viridescens* using pressurized air. Because our technique is designed for individual recognition in terrestrial species, it differs substantially from previously described procedures and has yielded some different results.

Essential materials include several colors of fluorescent dust, canisters, spray gun (nozzle with ¼ inch inner diameter-6.4 mm), hose, single-stage regulator, and source of pressurized air. All of these supplies, as well as portable UV lights, can be obtained from Scientific Marking Materials, Inc., P.O. Box 24122, Seattle, Washington, 98124, USA. Four colors of inert, dry, fluorescent, granular (50–350 μm diameter) pigments are available from Scientific Marking Materials, and 10 colors of inert, dry, fluorescent, powdered (5 μm diameter) pigments, are available from Radiant Color Co., 2800 Radiant Ave., Richmond, California 94804, USA. We recommend having a separate canister for each color of pigment if more than one color is to be used during one marking session.

We reduced the inner diameter of the spray gun nozzle from ¼ to ⅛ inch by inserting a piece of ⅛ inch diameter aluminum or brass tubing (flared to ¼ inch at the proximal end) into the nozzle of the gun. This increased mark density, reduced mark size and improved control over mark location.

The canister-spraygun-hose assembly is attached to a single-stage low pressure regulator with a refillable 2 L nitrogen cylinder. The dry fluorescent dust is poured into the spray canisters and attached to the spray gun. The delivery pressure on the regulator should be 1.8–2.8 kg/cm² (25–40 psi). Several factors are important in determining the correct delivery pressure, especially the size and species of the specimen to be marked, the distance from which dust is to be applied to the skin, and the size of the dust particles used. Smaller or more delicate specimens require lower pressures of about 1.8 kg/cm² (25 psi). Smaller fluorescent dust particles require higher delivery pressures of about 2.8 kg/cm² (40 psi). For salamanders, the smaller particles were less effective than large particles, even when applied at a pressure of 2.8 kg/cm² (40 psi).

In order to obtain unique marks on individuals, it is necessary to apply the fluorescent dust from a distance of less than 1 cm from the surface of the skin. The resulting mark is 2–5 mm in diameter, and has a dense concentration of pigment. During marking, the specimen is placed in a dry enamel pan and restrained lightly with the free hand. Anaesthesia is not required. The spray gun is held within 1 cm of the location where the mark is to be applied. If the specimen is not restrained, the air in front of the fluorescent dust will blow the specimen away before the dust has penetrated the skin. After marking, the skin should appear to be roughened, but not torn, under the layer of fluorescent dust. If the pressure is too high and the skin tears, less dust is retained in the skin. Rinsing the animal with water after marking removes excess dust and tests for dust penetration. With practice, the entire marking procedure, from capture to release of specimen, takes only a few minutes.

For salamanders, we used five mark locations on each side of the body (anterior and posterior to foreleg, midbody, anterior and posterior to hindleg) for a total of 10 mark locations. With 5 colors of pigment and 10 mark locations, it is possible to uniquely mark 60,466,175 salamanders. In practice, however, it is difficult to apply more than 3 or 4 marks to small in-

TABLE 1. Results of mark-recapture studies on *Plethodon jordani* and *P. glutinosus* comparing the recapture success of toe clipping and fluorescent dust marking. Only the present study used fluorescent dust to mark specimens.

Species	# marked	% recaptured	$\bar{x}$ recaptures per individual	Effort[1] per area	Effort[2] per specimen	Source
P. glutinosus	417	33.6	0.50	—	—	Wells and Wells, 1976
P. glutinosus	68	22.1	0.35	—	—	Highton, 1956
P. glutinosus	25	87.5	3.17	0.90	3.6	present study
P. jordani	156	55.1	1.40	0.75	3.2	Madison, 1969
P. jordani	287	45.5	—	0.79	1.3	Merchant, 1972
P. jordani	45	80–100	2.05	0.90	2.0	present study

[1] Total number of hours spent searching per year divided by the area (m^2) of the study plot.
[2] Total number of hours spent searching per year divided by the number of salamanders marked during the study.

dividuals, and we never marked any specimen more than four times. Even if each specimen is marked only twice, the number of unique mark combinations is 1175. For salamander species that are frequently found in burrows, cracks or crevices, it may be desirable to mark individuals at least once at a location on the body that is on or anterior to the forelegs, so that the salamanders can be identified without being disturbed.

During the first 24 h after marking, the size of the mark decreases as the dust that has not penetrated the skin is lost. The small, dust-filled lesions are completely healed within two weeks and the fluorescent marks are easily seen for several months after marking. Eventually, pigment cells reinvade the area and the marks may become difficult to see in visible light. However, they may remain visible under UV light for up to two years after marking. It is important to note that fixation of specimens in formalin interferes with the fluorescence of the pigments, so identification of individuals should always take place before fixation.

In order to test the effectiveness of the technique, 18 adult *Plethodon jordani* were marked in the laboratory during June–July 1983. The marks of all the salamanders showed an initial decrease in intensity during the first week. Marks were checked again in March and November 1984. By March, three specimens had lost their marks entirely. All of the remaining 15 specimens were still clearly marked in November. The specimens were then fixed in formalin and preserved in 70% ethanol. After fixation, the fluorescent pigment was no longer visible in nine of the 15 specimens when examined under a dissecting microscope.

A total of 25 *P. glutinosus* (25–95 mm SVL) and 45 *P. jordani* (20–75 mm SVL) were marked between 25 May and 10 July 1983, on 10 × 10 mm plots in the Balsam and Great Smoky Mountains (Nishikawa, 1985). One-year old salamanders (10–20 mm SVL) were not marked because it was feared they were too fragile. The plots were searched on 15 nights between 24 May and 25 September, 1983. During this season of salamander activity, 80–100% of the marked salamanders were recaptured at least once, with an average of 2.5 recaptures per individual. For comparison, Table 1 gives the results of several mark-recapture studies for *Plethodon jordani* and *P. glutinosus*, in which toe clipping was used to mark the specimens. The highest published recapture rates for *Plethodon jordani* and *P. glutinosus* were 55.1% and 33.6%, with averages of 1.4 and 0.5 recaptures per individual, respectively (Madison, 1969; Wells and Wells, 1976).

Two years after our initial study (Nishikawa, 1985), each plot was searched on two nights between 30 June and 3 July 1985. Of 40 salamanders collected in or near the plots, seven were marked. It is impossible to estimate the proportion remaining marked after two years from these data because not all salamanders present in a given area are active on any particular night. However, these observations show that some fluorescent marks may last up to two years in the field.

To date, this technique has been used to mark five species of plethodontid salamanders: *Plethodon jordani, P. glutinosus, Desmognathus monticola, Aneides lugubris* and *Ensatina eschscholtzii*. Individuals ranging in size from 12–95 mm snout-vent length (SVL) have been marked successfully.

More recently, we have begun to study (with N. Staub) the home range size and territorial behavior of *Aneides lugubris*. Thus far, we have successfully marked more than 100 one-year old individuals (SVL 12–30 mm). Only three fatalities occurred during marking, when the fluorescent dust particles tore open the body cavity of the specimen through the axial musculature. We have had no other fatalities in any of our marking studies.

Previous studies using fluorescent dust to mass-mark aquatic amphibians have examined mark longevity for periods up to five months. Ireland (1973) reported that 50% of fluorescent marks applied to salamander larvae with a heated probe were no longer visible after 70 days, and only two of 110 larvae retained the mark for five months. Taylor and Deegan (1982) reported that all adult newts (*Notophthalmus viridescens*), marked with fluorescent dust using pressurized air, had retained their marks for five months, but they do not report on the maximum longevity of the fluorescent marks for these animals. Our results indicated that over 80% of fluorescent marks applied at close range with pressurized air were still visible one year after marking, and some marks were still recognizable two years after marking.

We observed no greater mortality among marked

animals than among unmarked animals in the laboratory. Phinney and Matthews (1969), Ireland (1973) and Taylor and Deegan (1982) also report no increase in the mortality of laboratory animals resulting from fluorescent marking.

Survival rates of toe clipped and fluorescently marked salamanders have not been compared experimentally. We note, however, that the recapture probabilities we report here for *Plethodon jordani* and *P. glutinosus* are significantly higher than any previous reports from toe clipping studies on these species. In fact, our recapture probabilities are higher than previously reported for any toe clipping study on any species of plethodontid salamander (Nishikawa, 1985).

Unlike toe clipping, our fluorescent marking technique yields recapture probabilities high enough for reliable censuses of salamander populations. Neighboring individuals can be observed simultaneously, permitting the study of territorial behavior and overlap in home range use (Nishikawa, 1985). Our technique should prove useful for field studies of territorial behavior, especially for nocturnal plethodontid salamanders.

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[1] Present address: School of Biological Sciences, University of Kentucky, Lexington, Kentucky 40506, USA.