

Fluorescent Powder Pigments as a Harmless Tracking Method for Ambystomatids and Ranids

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Many amphibian species require both aquatic and terrestrial habitats to fulfill their biphasic life cycle. Research often focuses on processes and life stages occurring in aquatic habitats because the high concentration of animals facilitates the logistics of research and strong density dependence in larvae suggests that populations may be regulated by this life stage (Wilbur 1980). However, adult amphibians use extensive amounts of terrestrial habitat (Semlitsch and Bodie 2003), populations worldwide are declining due to habitat loss (Stuart et al. 2004), and new evidence suggests that the juvenile or adult life stages may be important in regulating populations (Biek et al. 2002). Efforts to study amphibians in terrestrial habitats have increased greatly in the last decade, but limitations in finding, capturing or tracking animals away from breeding sites still restrict our ability to answer basic ecological questions in terrestrial habitats.

Fluorescent powdered pigments (hereafter referred to as powder) have been used to track a variety of organisms including small mammals, reptiles and larval amphibians (Blankenship and Bryan 1990; Fellers and Drost 1989; Ireland 1973; Lemen and Freeman 1985). More recently, powder has been used to track amphibians in terrestrial habitats (Birchfield and Deters 2005). The primary assumption of all tracking studies is that the tracking method does not affect the animal (White and Garrott 1990; Millsbaugh and Marzluff 2001). Experimentally testing this assumption is an important step to validate this technique. While reports of negative effects of powder tracking are rare, the inhalation of powder was reported to cause moderate levels of histiocytic pneumonia in deer mice, *Peromyscus maniculatus* (Stapp et al. 1994), and powder can persist in the environment for long periods of time (Halfpenny 1992). Studies examining the effects of powder on amphibians are limited (but see Berger 2000; Eggert 2002).

We experimentally tested both short-term (i.e., powder present on the skin) and long-term effects of powder on both recently metamorphosed Wood Frogs, *Rana sylvatica*, and Spotted Salamanders, *Ambystoma maculatum*. We chose the rate of water loss as the short-term response because water regulation is a critical process for amphibians in terrestrial habitats and because amphibian skin affords virtually no protection from desiccation (Ray 1958; Thorson 1955). The large surface area to volume ratio in recently metamorphosed juveniles causes water loss to be a greater threat to juveniles than adults (Thorson 1955). In addition, the small particle size of the powder coating the skin may alter the flow of water across the skin and thus presents a possible mechanism for how the powder could affect an amphibian. We chose survival

and growth as our long-term responses because these variables provide an indication of the general health of an amphibian and relate to demographic processes.

Materials and Methods.—Wood Frog egg masses were collected from the Daniel Boone Conservation Area in Warren County, Missouri, USA, on 6 March 2004. After hatching, tadpoles were reared until metamorphosis in outdoor 1000-liter cattle tank mesocosms stocked with 1 kg leaf litter and zooplankton inoculum. Larval Spotted Salamanders were collected (using dip nets) from the Baskett Wildlife Research Area in Boone County, Missouri, USA, on 25 August 2004 and maintained in aquaria with aerated pond water until metamorphosis. Newly metamorphosed frogs and salamanders were housed in aquaria with moist sphagnum moss at the University of Missouri and fed crickets *ad libitum*.

Two long-term experiments tested for differences in growth and survival of Wood Frogs and Spotted Salamanders covered and not covered with powder. For the experiment on frogs, 70 individuals were randomly assigned to six treatment groups based on powder color on 6 June 2004: blue (N = 10), green (N = 10), orange (N = 10), yellow (N = 10), red (N = 10), and a no powder control (N = 20). For the experiment on salamanders, 20 individuals were randomly assigned to two treatment groups on 20 September 2004: red (N = 10) and a no powder control (N = 10). Treatment consisted of dipping each individual in fluorescent powder pigments (Radiant Color, Richmond, California, USA) until completely covered. Control animals were handled in a similar manner, except they were not dipped in powder. Animals were randomly assigned to an individual 17 × 12 × 9 cm plastic container that contained moist sphagnum moss with a fiberglass window screen lid. All animals were fed approximately 18% of their body weight in small crickets each week, split between two feedings. Every two weeks for a six-week period all animals were weighed and the powder treatment was re-applied.

Two short-term experiments tested for differences in the rate of water loss between animals covered and not covered with powder. Similar procedures were followed for both the wood frog experiment on 12 August 2004 and the spotted salamander experiment on 9 November 2004. Animals were placed in a plastic container containing 0.5 cm of carbon-filtered water for approximately 12 hrs prior to the beginning of the experiment to ensure all animals were fully hydrated. The dehydration chamber consisted of a square chamber (5 × 5 × 5 cm) constructed of metal window screen (similar to Pough et al. 1983) that was suspended, exposing all sides to air and did not prevent animals from using water conserving postures. Animals were assigned to a powder treatment or control group according to the treatment that individual received during the long-term experiments, thus 20 frogs (i.e., N_{control} = 10 and N_{red} = 10) and 18 salamanders (i.e. N_{control} = 10 and N_{red} = 8) were tested. Using the same animals in both the short-term and long-term experiments did not bias our results because the data from the experiments were analyzed separately and because any potential carryover effects from the long-term experiments should increase the likelihood of detecting an effect in the short-term experiments. Each chamber was weighed, an animal was randomly assigned to the chamber, and the combination of the animal and chamber was weighed every 30 minutes for 120 minutes until animals lost approximately 15% of their body mass. Thus animals were not exposed to lethal dehydration levels: 30–35% for ranids

(Thorson and Svihla 1943); and 36–40% for ambystomatids (Pough and Wilson 1970; Ray 1958).

Changes in mass over six weeks for the long-term experiments and over 120 minutes for the short-term experiments were analyzed using repeated measures analysis of variance. Only animals without missing observations (i.e., individuals that survived the entire experiment) were included in the analysis of mass. Analysis of variance was used to test for the effects of the powder treatment on survival, with number of days alive as the response variable. All weights were obtained using a Mettler AT261 Delta Range electronic balance with readability of 0.01mg.

Results.—Growth between animals covered and not covered with powder did not differ for either Wood Frogs ($F = 0.19$, d.f. = 5,57, $P = 0.97$; Fig. 1a) or Spotted Salamanders ($F = 0.24$, d.f. = 1,17, $P = 0.63$; Figure 1b). A significant increase in mass occurred throughout the six weeks for both Wood Frogs ($F = 354.77$, d.f. = 3,55, $P < 0.0001$) and Spotted Salamanders ($F = 213.00$, d.f. = 3,15, $P < 0.0001$). No interactions between the powder treatments and time occurred (all $P \geq 0.40$). The number of days alive did not differ between frogs covered and not covered with powder ($F = 0.58$, d.f. = 5,64, $P = 0.71$), but seven frogs died throughout the course of the long-term experiment. Survival was 100% in the long-term salamander experiment, but one escaped and one from the red treatment died after the completion of the long-term experiment but prior to the short-term experiment. We believe the mortality occurred when animals were not feeding readily, because these individuals were the smallest at the initiation of the experiments.

Water loss between the control and powder treatments did not differ for either Wood Frogs ($F = 0.34$, d.f. = 1,18, $P = 0.57$; Fig. 2a) or Spotted Salamanders ($F = 0.49$, d.f. = 1,16, $P = 0.49$; Fig. 2b). Continuing the experiment until all animals had lost approximately 15% of their body mass produced a significant decrease in mass for both the frogs ($F = 590.04$, d.f. = 4,15, $P < 0.0001$) and the salamanders ($F = 442.29$, d.f. = 4,13, $P < 0.0001$). No interactions between powder treatments and time occurred (all $P \geq 0.34$). Survival was 100% in both short-term experiments.

Discussion.—We did not detect any short-term or long-term effects of powder on either Wood Frogs or Spotted Salamanders. Growth and survival over a six-week period, as well as rates of water loss, were unaffected by being covered with powder. We

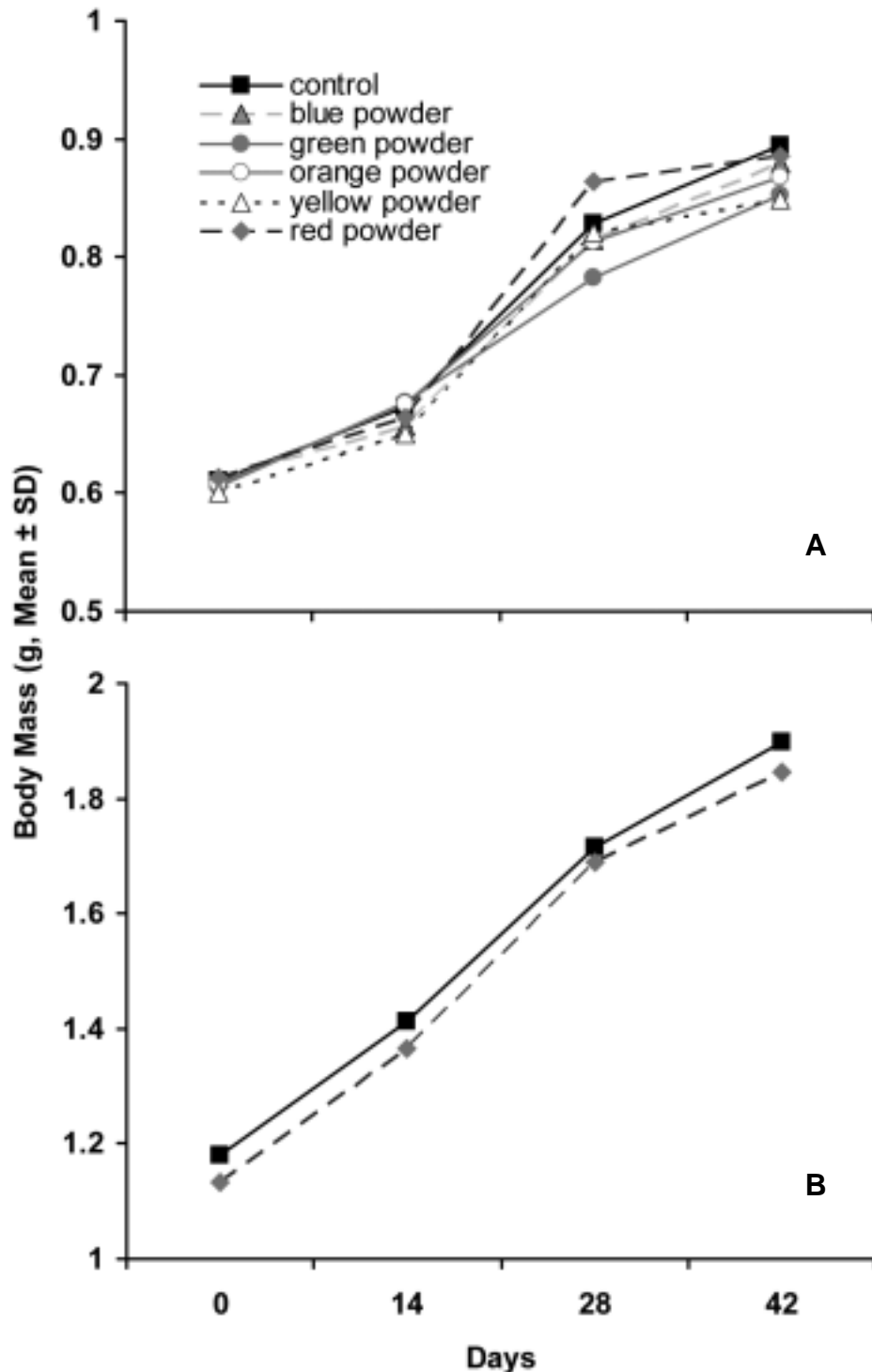


FIG. 1. Mean growth for each powder treatment during the long-term experiments for Wood Frogs (A) and Spotted Salamanders (B) at two week time intervals.

suggest that being covered with powder is similar to being covered with other natural items, such as soil or organic debris, and conclude that powder is a harmless method for tracking ambystomatids and ranids in terrestrial habitats.

All experiments provided conditions that may cause the powder to be more stressful than animals would experience when powder is used to track amphibians in the field. First, animals in the laboratory had limited opportunity to remove the powder by rub-

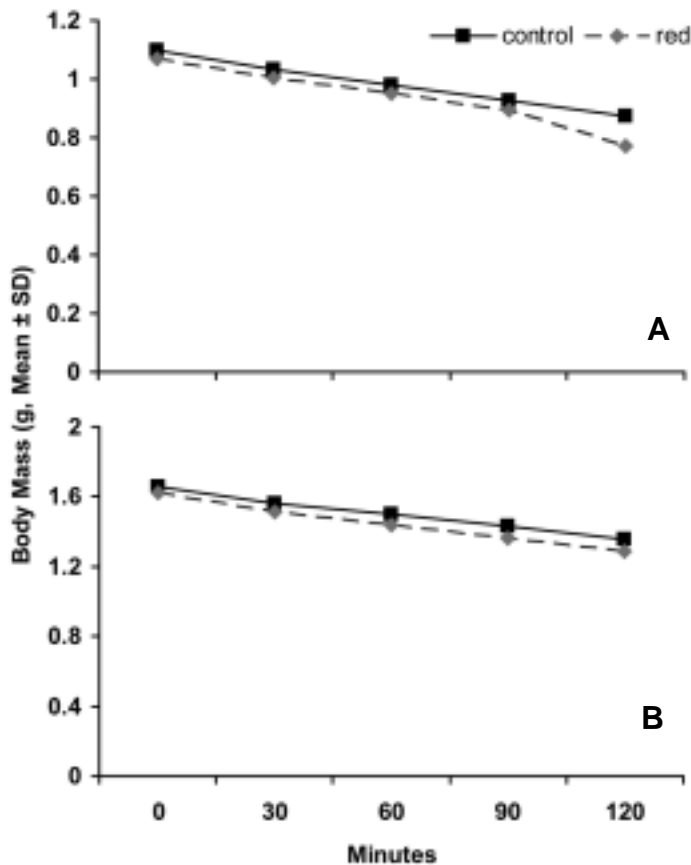


FIG. 2. Mean water loss for each powder treatment during the short-term experiments for wood frogs (A) and spotted salamanders (B) at 30 min time intervals.

bing against vegetation or hopping around. This was especially true in the dehydration experiments where small enclosures restricted hopping movements and substrate was not provided. Animals in these experiments remained completely covered with powder for the entire 120 minutes. Although we did not observe any behavior that suggested animals were purposely attempting to remove the powder, animals in the long-term experiments lost the powder quickly, with powder visible on approximately 52% of the animals at 24 h after powder application and no powder visible on any of the animals at 3 days after powder application. Second, animals in the long-term experiments were exposed to the powder on three occasions. When powder is applied in the field, animals are often only covered with powder once, because the individual is not recovered after being released. We found no evidence that repeated exposure to the powder is harmful to amphibians.

Three tracking methods are primarily used for directly following amphibians in terrestrial habitats: radio-telemetry, thread-trailing, and fluorescent powder pigments. Each method has advantages and disadvantages. For example, although radio-telemetry allows a researcher to track an individual for the longest time period (e.g., 1–4 months depending on transmitter size) and longest distances (e.g., Bartelt et al. [2004] tracked *Bufo boreas* > 200 m), the cost of radio-telemetry is the greatest (US \$150 per transmitter plus additional costs for receiving equipment) and risk to the animal can be the greatest (see Rittenhouse 2002 for mortality caused

by transmitter implantation). Tracking amphibians with powder often provides the shortest movement paths of all the tracking methods; however, several benefits can make powder the preferred tracking method in many instances. Powder tracking results in a detailed description of the movement path (e.g., Birchfield 2002; Eggert 2002). Powder can be used on juveniles or species too small for other tracking devices and is relatively inexpensive (e.g., US \$12 per one-pound can). However, a possible side effect to amphibians is the potential increase in visibility to predators that use color to locate prey. Although the optimum tracking method will vary based on the research objectives of a study, tracking amphibians with powder is an underutilized tracking technique that does not appear to detrimentally affect growth, water regulation or survival in the laboratory of ambystomatids or ranids. This technique might therefore be particularly useful when studying rare or endangered species.

Acknowledgments.—We thank E. Harper for raising the Wood Frogs to metamorphosis. J. Crawford provided thoughtful comments on the manuscript. Animals were captured under Missouri Department of Conservation Wildlife Collector's permits 12220 and 12227 and maintained under University of Missouri Animal Care and Use protocol 3368. Funding was provided by NSF grant DEB 0239943 to R. Semlitsch.

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Herpetological Review, 2006, 37(x), xxx–xxx.
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A New Technique for Measuring Body Color of Lizards in the Field

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Measurement of color in reptiles can play a fundamental role in research areas as diverse as behavior, physiology, evolution, and ecology. For example, studies incorporating color have investigated the behavioral role of dewlap color in *Anolis* lizards (Macedonia et al. 2003; Thorpe and Stenson 2003), thermoregulation in desert reptiles (Norris 1967), sexual selection in chuckwalla (Kwiatkowski and Sullivan 2002), and predator evasion in agamid lizards (Stuart-Fox et al. 2004). These studies rely on the ability to accurately quantify body color.

There are a number of small portable spectrometers available that measure reflectance or radiance. Previous studies using spectrometers to measure the color of lizards and/or their backgrounds in the field typically used sunlight as the source of illumination (Macedonia et al. 2002, 2003). This approach can cause problems if the measurements are not taken under similar and constant conditions, as weather conditions, nearby vegetation, different microhabitats, seasons, and the time of day can result in drastically different radiance spectra of the animal or background being measured (Endler 1990, 1993). Consequently, moment-to-moment fluctuations

in lighting conditions can cause errors and alter the radiance or reflectance output given by a spectrometer (Endler 1990, 1993). Thus, a method that eliminates or greatly reduces inconsistencies in lighting would provide far more consistent and repeatable measurement of reflectance or radiance. In particular, the exclusion of ambient light, which can change over short time periods because of movement of clouds or overhead vegetation (Endler 1993), would increase the constancy of reflectance spectra. Yet such a method has limitations when used in the field, because of the difficulty of removing all sources of ambient light. Until now these limitations have meant that study subjects have been returned to the laboratory to take controlled reflectance readings in a darkened room (Macedonia et al. 2002; Stuart-Fox et al. 2003, 2004). However, removing lizards from their natural habitat may result in stress responses, which could lead to skin-color changes, as reported in species that have the ability to rapidly lighten or darken their skin color, such as some agamids (Christian et al. 1996) and iguanians (e.g., Cooper and Greenberg 1992). When investigating background-color matching, such color changes may not be indicative of the lizard's typical body color.

We have designed and tested an opaque probe cover for use with a portable spectrometer that allows accurate and controlled measurement of reflectance in the field. We tested the reliability of this new method during a study of background-color matching in the painted dragon, *Ctenophorus pictus*. In this article we describe an effective method for measuring reflectance of virtually any species of lizard in the field with the aid of this probe cover. In fact, the methods described would have applicability across a wide range of animal taxa. Our methods are inexpensive, easily constructed, and portable.

Cover for Optical Fiber Probe.—Measures of reflectance were taken using an Ocean Optics USB2000 Miniature Fiber Optic Spectrometer® (Dunedin, Florida) and an illumination source was provided by a PX-2 Pulsed Xenon Lamp® (Dunedin, Florida), connected to the spectrometer by a standard reflection probe (200 µm diameter). The spectrometer and light source were then connected to a laptop computer via a USB cable for reflectance calculations using the Ocean Optics software package, OOIBase32.

We designed an opaque cover that attaches to the optical fiber probe, ensuring that only the light from the xenon lamp illuminates the target area. This cover was constructed from a large plastic drinking straw that fit snugly over the probe (Fig. 1). The straws were wrapped in layers of black duct tape to block out ambient light. One end of the straw was then cut to make a 45° angle at a distance of 1 cm from the end of the probe to the surface sampled (following Endler 1990). Once the probe was cut to size, several layers of duct tape were placed around the cut end of the probe to create a base (10 × 10 cm), which further reduced light from entering the receiving end of the probe. This base can be made to any size and/or shape to suit different-sized lizards. The resulting probe cover is flexible and after being placed on a lizard can be peeled backwards to ensure the probe is positioned in the desired location. The lizard is restrained by wrapping the base of the probe around the body until the measurement was made but we also recommend restraining the lizard while measurements are being taken (e.g., Rose et al. 2006). Additionally, a transparent probe cover was constructed to test the accuracy of the opaque-probe cover using the same methods described for the opaque cover, the only