

research can be important in understanding ecosystem dynamics. Many studies involving small mammals involve mark-recapture methods, which provide estimates of population density, survival, recruitment, growth rates, and movement (Hayes et al. 1996; Williams et al. 2001, 314, 418, 497, 672). Mark-recapture techniques for small mammals involve placing an identifiable marker such as an ear tag, a passive integrated transponder (PIT) tag, fur clipping, toe clipping (a form of mutilation marking), and hair dye on captured individuals (Silvy et al. 2012, 244–48). For short-term studies, hair dye marking may be favored because it is low-cost, can be applied easily, and is of limited duration. Also, the replacement of permanent marks such as ear tags, PIT tags, and toe clipping with temporary hair dye marking can avoid impacts on small mammals such as bodily damage, infection, behavioral changes, and stress (Ascensão et al. 2012; Silvy et al. 2012, 244–48; Smyth and Nebel 2013; Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

Many dyes have been used on mammals in the field, including colored paint, ink, felt-tipped pen, fluorescent pigment, printer's ink, model airplane paint, and human hair dye products (Silvy et al. 2012, 244). Studies on the effectiveness of hair dyes in field studies have been contradictory, with large variations in reported results associated with hair dye fading based on age of dye, age of guard hairs, molt, or natural behavior of the animal (Melchior and Iwen 1965; Parker et al. 2012). Because we were unable to identify any study that strictly quantified hair dye intensity over time for small mammals, our goal was to document the longevity of hair dye marks on abundant wild-caught white-footed mice (*Peromyscus leucopus*) in the field. Also, we wanted to determine if estimates of population size based on mark-recapture analysis using hair dye were similar to those using ear tag marks.

We tested the longevity of an easily obtainable, relatively low-cost, ready-to-use hair dye marker, the Animal ID Marker hair dye by Muromachi Kikai (<http://muromachi.com/en/archives/english/2149/>). The Animal ID Marker is available as a fine-tipped marker, is advertised to last 6–12 wk in the laboratory, is nontoxic and devoid of hazardous materials, and is approved and regulated by the Japanese Ministry of Health, Labor and Welfare for use in food, drugs, and cosmetics.

MATERIALS AND METHODS

Our study took place at the Millersville University Biological Preserve in Millersville, PA. Two transects, each with 10 Sherman live traps (22.5 × 9 × 7.5 cm; H. B. Sherman Traps, Tallahassee, FL, <https://www.shermantraps.com/>),

RESEARCH NOTE

Documenting the longevity of an animal marker hair dye on small mammals

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Abstract: Permanent marks such as ear tagging and passive integrated transponder tags have been successfully used in small-mammal mark-recapture research. However, permanent marks can influence small mammal behavior and promote infection. Hair dyes may be favored for short-term mark-recapture studies because they are low-cost, can be applied easily, and are less damaging to marked individuals. Our objective was to determine the longevity of an animal hair dye marker placed on small mammals. Our study was conducted at the Millersville Biological Preserve in Millersville, PA and consisted of two transect lines, each with 10 Sherman traps checked twice a week over 3 months. We captured 27 individuals and marked each of them once with hair dye. Hair dye longevity was monitored via photodocumentation of recaptured individuals. Results showed that the hair dye can be used to distinguish individuals for up to 60 days in short-term mark-recapture studies of small mammals.

Keywords: hair dye, longevity, mark-recapture, small mammals

Many small mammals (e.g., rodents, shrews, bats) are considered keystone species or ecosystem engineers because they contribute to ecosystem structure and function (Dickman 1999; Delibes-Mateos et al. 2011). Thus, small mammal

were set for small mammal capture. Although a grid design is optimal for population size estimation, our goal was to maximize capture rates to monitor markings; therefore, we used transect arrangements for traps, as they are more efficient than grids for maximizing total captures (Pearson and Ruggiero 2003). We spaced traps 10 m apart, flagged them, and baited them with sunflower seeds. When evening temperatures dropped below 8°C, we placed cotton balls inside traps to aid thermoregulation of captured individuals. We baited traps in the evenings (≈ 1800–1900 hours) and checked them the next morning (≈ 0700 hours). Starting in September 2016, we opened traps and checked them twice a week on separate evenings for 13 weeks, ending in December 2016 (520 trap nights). We kept trap doors closed when traps were not in use.

Upon first capture, we ear-tagged individuals with #1005-1 MONEL tags from the National Band and Tag Company, Newport, KY (<https://nationalband.com/>). We then dried each mouse with a towel and uniquely marked individuals with numbers on the chest using a blue Animal ID Marker (figure 1). Ravieh et al. (2017) marked the dorsal surfaces of diurnal Columbian ground squirrels (*Urocyon columbianus*) and found that predation by visual predators (e.g., raptors and canids) was rare for marked squirrels. Therefore, we felt that marking the ventral surfaces of nocturnal *P. leucopus* would not impact their survival. The Animal ID Marker required no preparation, which allowed quick and easy marking of individuals. We marked all captured individuals only once and monitored them via recapture events during the study period. We photographed and weighed all captured and recaptured individuals and released them in the same locations where they were captured. This research was approved by the Millersville University Animal Care and Use Committee, conducted under the authority of Pennsylvania Game Commission Wildlife Collecting Permit 143, and accomplished in accord with the guidelines of the American Society of Mammalogists (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

Based on the photorecord for each capture and recapture event of *P. leucopus*, we used the histogram analysis tool in the ImageJ software 1.47 (Schneider et al. 2012) to document the change in hair color intensity over time. This was done by contrasting the mean gray-scale intensity of the Animal ID Marker dye with the unmarked undercoat pattern for each captured and recaptured individual (figure 1). We used linear regression in EXCEL to model the mean intensity of hair dye contrast over time to determine when hair dye intensity contrast would reach zero (i.e., when hair dye gray-scale intensity matched that of the unmarked undercoat pattern).

We used Program MARK (Cooch 2009) to estimate the size of our *P. leucopus* population based on mark-recapture trapping history from both

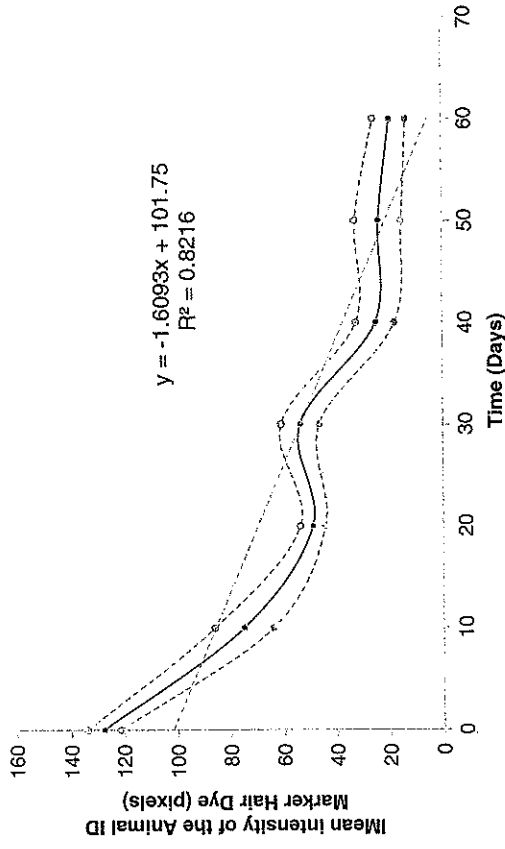


Figure 1. Mean Animal ID Marker dye intensity with 95% C.I. on the ventral pelage of *Peromyscus leucopus* ($n = 12$) over time on the Millersville Biological Preserve, Millersville, PA, from September to December 2016. Based on the regression equation for the trend line, dye intensity after one marking would reach 0 at approximately 63 days or 9 weeks.

hair dye and ear tags separately. We compared closed capture models to identify the model of best fit based on the lowest AICc score, the highest AICc weight score, and whether the top model was $>\Delta AIC$ from the other models. Models with $>2\Delta AIC$ from the top model have considerably less support for best fit, and models >10 have essentially none (Burnham and Anderson 2003, 70, 72).

RESULTS AND DISCUSSION

Twenty-seven individual *P. leucopus* were captured and marked, and we recorded 116 recapture events during the study period, with 12 *Peromyscus* being recaptured more than twice. The Animal ID Marker dye allowed identification of individual *P. leucopus* using unique marking patterns. Based on the results of our linear regression model (figure 1), we found that hair dye intensity remained on individual *P. leucopus* long enough for short-term field studies lasting <9 wk. This time frame would allow estimates of small mammal population size and density, and hair dye longevity can be extended by remarking recaptured individuals. Melchior and Iwen (1965) and Keith (1968) found that picric acid saturated in alcohol and Nyanzol A and D could be visible on

Table 1. Identification of the model of best fit to estimate population size for a *Peromyscus leucopus* population in Millersville University, Millersville, PA

Hair dye models	AICc	ΔAIC	AICc weight	Number of parameters
Model (b)	463.62	0.00	0.99	3
Model (0)	484.50	20.88	0.00	1
Model (h)	497.65	34.02	0.00	83
Model (t)	501.49	37.87	0.00	27

Ear tag models	AICc	ΔAIC	AICc weight	Number of parameters
Model (b)	466.39	0.00	0.99	3
Model (0)	487.98	21.59	0.00	1
Model (t)	504.06	37.66	0.00	27
Model (h)	507.69	41.30	0.00	83

Notes: Based on separate mark-recapture data using Animal ID Marker hair dye and ear tags for individual identification. The closed model of best fit for both hair dye and ear tags (i.e., lowest AICc, highest AICc weight, and $\Delta AIC > 2$ from the other models) was the model for animal behavior change after first trapping (b). Other models incorporated equal capture probability among individuals (c), capture probability change based on time (t), and separate capture probabilities for each mouse (h).

mammals in the field from 2 wk to 9 months. However, picric acid is an explosive and thus may be unsafe in field studies, and Nyanzol A and D is difficult to purchase commercially and requires extensive preparation (Melchior and Iwen 1965).

The model of best fit (a closed-capture model that assumed that animals behaved differently after being trapped the first time) based on mark-recapture events of *P. leucopus* was the same for hair dye marking trapping history and trapping history based on ear tag marks (table 1). The population size estimate based on hair dye marking was 38.28 (SE 12.41) and was indistinguishable from the population size estimate based on ear tag marks, 38.27 (SE 12.40).

For all *P. leucopus*, we used number markings to identify individuals, but some of these markings blurred and faded over time (5 individuals had blurred dye, 2 of which became unrecognizable). To mitigate blurring, marks used in the field should consist of clear and obvious characters, such as Roman numerals, instead of letters or Arabic numerals. However, we recommend

further testing to identify unique markings that maintain their distinctiveness over time. Though ear tags aided in the identification of some individuals with faded Animal ID Marker dye, we had three situations where the ear tag was lost, but we were still able to identify the individual based on its unique dye marking. Because the Animal ID Marker provides a relatively small number of unique marks that fade over time, it may have limited use in determining both population abundance across seasons and the survival of individuals. However, our results indicate that this technique may be quite effective tracking the persistence and movement of individual small mammals, such as rodents, shrews and bats, in the short term.

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WORKS CITED

- Ascensão, F., A. Clevenger, C. Grilo, J. Filipe, and M. Santos-Reis. 2012. Highway verges as habitat providers for small mammals in agrosilvopastoral environments. *Biodivers. Conserv.* 21 (14): 3681–97.
- Burnham, K., and D. Anderson. 2003. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. Springer Science and Business Media, New York.
- Cooch, E. 2009. Program MARK: A Gentle Introduction. Available at <http://www.phidot.org/software/mark/docs/book/>.
- Delibes-Mateos, M., A. Smith, C. Slobodkinoff, and J. Swenson. 2011. The paradox of keystone species persecuted as pests: A call for the conservation of abundant small mammals in their native range. *Biol. Conserv.* 144 (5): 1335–46.
- Dickman, C. 1999. Rodent-ecosystem relationships: A review. Ecologically-based management of rodent pests. *ACIAR Monogr.* 59: 113–33.
- Hayes, J. P., M. Adam, R. Anthony, and J. Witt. 1996. Comparison of the effectiveness of Sherman and modified Fitch live-traps for capture of small mammals. *Northwest. Nat.* 77: 40–43.
- Keith, L., E. Meslow, and O. Rongstad. 1968. Techniques for snowshoe hare population studies. *J. Wildl. Manage.* 32 (4): 801–12.
- Melchior, H., and F. Iwen. 1965. Trapping, restraining, and marking Arctic ground squirrels for behavioral observations. *J. Wildl. Manage.* 29 (4): 671–78.
- Parker, L., R. Lopez, N. Silvy, D. Davis, and J. Cathey. 2012. Alternative methodology for handling and marking meso-mammals for short-term research. *Wildl. Biol. Practice* 8: 20–25.

- Pearson, D., and L. Ruggiero. 2003. Transect versus grid trapping arrangements for sampling small-mammal communities. *Wildl. Soc. Bull.* 31: 454–59.
- Raveh, S., D. Heg, F. Dobson, D. Coltman, J. Gorrell, A. Balmer, S. Rösöli, and P. Neuhaus. 2011. No experimental effects of parasite load on male mating behaviour and reproductive success. *Anim. Behav.* 82 (4): 673–82.
- Schneider, C., W. Rasband, and K. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7): 671–75.
- Sikes, R., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J. Mammal.* 97 (3): 663–88.
- Silvy, N. J., R. R. Lopez, and M. J. Peterson. 2012. Techniques for marking wildlife. In *The Wildlife Techniques Manual*, vol. 1, *Research*, ed. N. J. Silvy. Johns Hopkins University Press, Baltimore, 230–57.
- Smyth, B., and S. Nebel. 2013. Passive integrated transponder (PIT) tags in the study of animal movement. *Nat. Educ. Knowl.* 4 (3): 3.
- Williams, B., J. Nichols, and M. Conroy. 2001. *Analysis and Management of Animal Populations*. Academic Press, San Diego.

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