

in pipe-traps inserted upright into the ground, placed open-ended or capped at varying heights on trees, and in 'T' configurations (see Bartareau 2004; Boughton et al. 2000; Moulton et al. 1996). However, in more northerly locations (Michigan; J. Ball, pers. comm.; New York, Tregger 2004) similar pipe-trap designs have not succeeded in capturing large numbers of treefrogs. The large number of captures reported in this study indicate that this new pipe-trap design may be more appropriate than previous designs for sampling populations of hylid frogs in areas outside of the southeastern U.S. Additional data regarding the factors that drive the use of artificial refugia would be beneficial to the development of pipe-traps designed to optimize captures of particular species in specific locations. For example, the height at which a pipe-trap is placed, the internal diameter and the depth of water retained within the pipe-trap could easily be adjusted to fit the specific requirements of the hylid species under investigation. While the use of pipe-trap refugia to generate estimates of treefrog density or abundance may be complicated by biases of pipe diameter and design configuration, artificial pipe-trap refugia may be especially useful for delineation of terrestrial core habitat used by hylid frogs during both the breeding and non-breeding seasons when traditional methods of capturing amphibians are inadequate.

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LITERATURE CITED

- BARTAREAU, T. M. 2004. PVC pipe diameter influences the species and sizes of treefrogs captured in a Florida coastal oak scrub community. *Herpetol. Rev.* 35:150–152.
- BOUGHTON, R. B., J. STAIGER, AND R. FRANZ. 2000. Use of PVC pipe refugia as a sampling technique for hylid treefrogs. *Am. Mid. Natur.* 144:168–177.
- CONANT, R., AND J. T. COLLINS. 1998. *A Field Guide to Reptiles and Amphibians of Eastern and Central North America*. 4th ed. Houghton Mifflin Co., New York, New York. 616 pp.
- DOLE, J. W. 1971. Dispersal of recently metamorphosed leopard frogs, *Rana pipiens*. *Copeia* 15:221–228.
- DODD, C. K. JR. 1991. Drift fence-associated sampling bias of amphibians at a Florida sandhill temporary pond. *J. Herpetol.* 25:296–301.
- GIBBONS, J. W., AND D. H. BENNETT. 1974. Determination of anuran terrestrial activity patterns by a drift fence method. *Copeia* 1974:236–243.
- , AND R. D. SEMLITSCH. 1982. Terrestrial drift fences with pitfall traps: an effective technique for quantitative sampling of animal populations. *Brimleyana* 7:1–16.
- GOIN, C. J., AND O. B. GOIN. 1957. Remarks on the behavior of the squirrel treefrog, *Hyla squirella*. *Ann. Carnegie Mus.* 35:27–35.
- LOHOEFENER, R., AND J. WOLFE. 1984. A 'new' live trap and a comparison with a pit-fall trap. *Herpetol. Rev.* 15:25–26.
- MARTIN, F. D., L. D. WIKE, AND M. H. PALLER. 2003. PVC pipe samplers for hylid frogs: a cautionary note. World Wide Web <http://sti.srs.gov/fulltext/ms2004128/ms2004128.pdf>. Accessed 06 June 2004.
- MOULTON, C. A., W. J. FLEMING, AND B. R. NERNEY 1996. The use of PVC pipes to capture hylid frogs. *Herpetol. Rev.* 27:186–187.
- MURPHY, C. G. 1993. A modified drift fence for capturing treefrogs. *Herpetol. Rev.* 24:143–145.
- SHOOP, C. R. 1965. Orientation of *Ambystoma maculatum*: Movements to and from breeding ponds. *Science* 149:558–559.
- TREGGER, N. 2004. Evaluation of PVC pipe as a trap and evaluation of transmitter harness for hylidae species. World Wide Web <http://www.schoharie-conservation.org/scholarships/html/TreeFrogs.html>. Accessed 06 June 2004.

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A New Method of Temporarily Marking Lizards

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Labeling individual animals with unique and distinct markers is necessary in many ecological studies, including population density assessments, estimates of home range or territory areas, and behavioral observations. Researchers have developed many techniques for identifying individuals, but few allow convenient field identification of individuals from a distance. The ability to distinguish among individuals without repeatedly disturbing them is especially important in studies of behavioral ecology where the goal is to determine natural patterns of behavior. The most appropriate marking techniques for these studies are therefore quickly and easily implemented, clearly distinguishable from a distance, and not harmful to the marked individual (Murray and Fuller 2000; Niefeld et al. 1994). I have developed a new method for temporarily marking small lizards that meets these criteria.

Individually marking reptiles presents a special challenge for field workers in that reptiles frequently shed their skin and with it any external markers. The bead-tagging method of Fisher and Muth (1989) avoids this problem by sewing unique combinations of beads into the most proximal region of a lizard's tail muscle. This is a permanent marker that works well for lizards that have a snout-vent length (SVL) of greater than 40–45 mm, but one that is difficult and often impossible to use for smaller lizards and for those with very slender tails. Other common methods of marking lizards have shortcomings as well. Many researchers use toe-clipping to distinguish among individuals (Ferner 1979). However, there are several problems with using this method in short-term behavioral studies. First, it may take a substantial amount of time for a toe-clipped lizard to recover from the injury caused by the clipping, which may further inhibit normal behavioral and movement patterns. Second, lizards sometimes naturally lose toe joints (Hudson 1996; Middelburg and Strijbosch 1988), which can make identifications difficult. Finally, toe clips cannot easily be seen without handling the animal. Other researchers have used non-toxic paint on the dorsum or colored acrylic polymers inserted under the skin, but these are also difficult to administer and distinguish on small lizards. For studies that can be completed in less than the average interval between natural sheddings of the species, I recommend the use of queen bee marking kits for smaller lizards. These kits, available from The Bee Works of Orillia, Canada (www.beeworks.com) for US \$17.50 each, contain cardboard dots in five colors (white, blue, yellow, green, and orange) numbered 1–99 in each color. Each kit also includes phial glue and an applicator for adhering the dots to the animal.

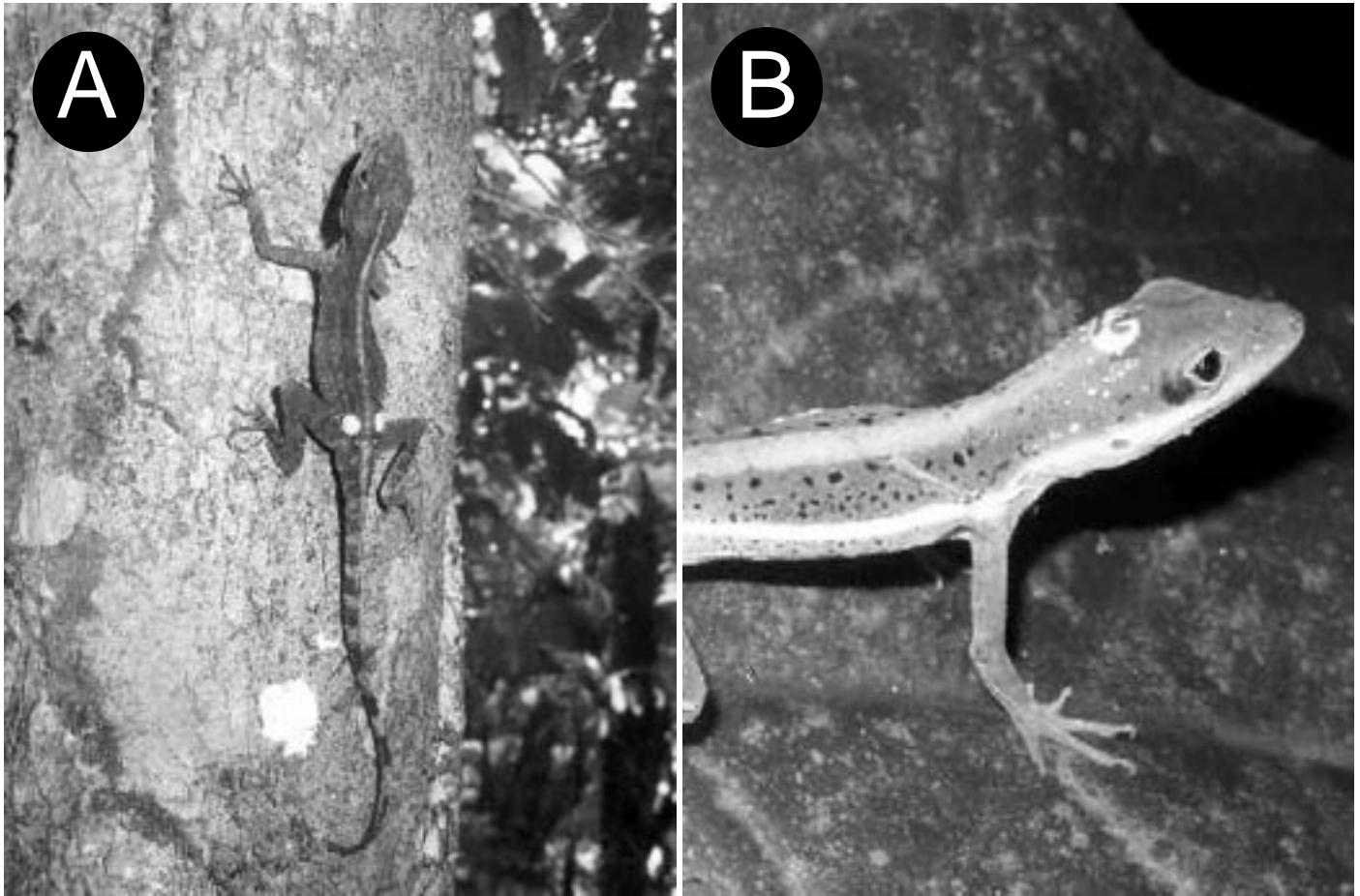


FIG. 1. A) Female *Anolis gundlachi* with two bee tags attached to its lower dorsum. B) Female *Anolis krugi* with bee tag (numbered 21) attached to its head.

We have used bee tags for marking four species of diurnal lizards: *Anolis cristatellus*, *A. gundlachi*, *A. krugi*, and *Sceloporus undulatus*. These four species have different habitat requirements and different scale types, and bee tags have proven to work well for each. *Anolis gundlachi* and *A. krugi* occur in the montane rainforests of Puerto Rico (Rand 1964; Schoener and Schoener 1971), *A. cristatellus* occurs in lowland dry forests and disturbed areas in Puerto Rico (Rand 1964; Schoener and Schoener 1971), and *S. undulatus* is found in North American woodland areas and glades (Stebbins 1954). During our behavioral studies of these species, we found that bee tags were dependable markers for approximately three weeks in the natural habitats of these species. We marked males and large females with the bead-tagging method described above (Fisher and Muth 1989), and smaller females and juveniles with bee tags. Bee tags are remarkably easy to attach; after catching a lizard we handled it for approximately two minutes to measure its SVL, take a small tissue sample from its tail, attach a bee tag combination, and allow the glue to dry. In contrast, almost twice that time was needed to attach a bead tag. Another primary advantage of the bee tag technique is its flexibility; the dots can be glued on almost any part of the dorsal surface of the lizard (Fig. 1). In our studies, we glued 2-3 bee tags on the head, the upper dorsum, or the lower dorsum of the lizards using the colors, numbers, and location of the tags to identify individuals. While the colors and position of the tags are often visible with

the naked eye, close-focus binoculars were needed to read the numbers on the tags. When a tag was occasionally missing from a lizard, we were almost always able to identify the individual by the remaining tags on that individual, or by a process of elimination in which other lizards of known identification were ruled out.

Marking lizards with bee tags provided short-term results comparable to those of the bead tagging technique (Fisher and Muth 1989). Of the 166 lizards of the four species we have marked using the bee tag method, we were able to conduct behavioral observations on 141, or 85%, in a three-week period. Likewise, of the 75 lizards marked with permanent bead tags for the same studies, we were able to observe 66, or 88%. It therefore appears that these two methods have similar "success" rates. There are many reasons we may fail to repeatedly observe a lizard other than loss of its tags, including death by predation or disease. It is also logical to assume that some lizards may be captured in a rare foray from their normal home range into the study area. However, we did observe one lizard (a female *A. krugi*) shed its entire skin and eat its dots immediately after being marked with bee tags, and the dots of one female *A. cristatellus* were found in its feces. These occurrences appear to be rare in the field, but when *Anolis* lizards were placed in the animal care facility at Washington University almost every individual shed and ate its bee tags.

In many circumstances, marking small lizards with queen bee marking kits is preferable to other marking techniques, as it is

inexpensive, relatively non-intrusive, and an easily visible way to identify particular individuals. The technique is also useful for larger lizards when temporary highly visible markers are preferred to permanent ones, particularly in studies in which the researcher wishes to avoid disturbing the focal individuals as much as possible.

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LITERATURE CITED

- FERNER, J. W. 1979. A review of marking techniques for amphibians and reptiles. *SSAR Herpetol. Circ.*, No. 9. 41 pp.
- FISHER, M., AND A. MUTH. 1989. A technique for permanently marking lizards. *Herpetol. Rev.* 20:45–46.
- HUDSON, S. 1996. Natural toe loss in southeastern Australian skinks: Implications for marking lizards by toe-clipping. *J. Herpetol.* 30:106–110.
- MIDDELBURG, J. J. M., AND H. STRIBOSCH. 1988. The reliability of the toe-clipping method with the common lizard (*Lacerta vivipara*). *Herpetol. J.* 1:291–293.
- MURRAY, D. L., AND M. R. FULLER. 2000. A critical review of the effects of marking on the biology of vertebrates. In L. Boitani and T. K. Fuller (eds.), *Research Techniques in Animal Ecology: Controversies and Consequences*, pp. 15–64. Columbia University Press, New York.
- NIETFELD, M. T., M. W. BARRETT, AND N. SILVY. 1994. Wildlife marking techniques. In T. A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, pp. 140–168. Wildlife Society, Bethesda, Maryland.
- RAND, A. S. 1964. Ecological distribution in anoline lizards of Puerto Rico. *Ecology* 45:745–752.
- SCHOENER, T. W., AND A. SCHOENER. 1971. Structural habitats of West Indian *Anolis* lizards II. Puerto Rican uplands. *Breviora* 375:1–39.
- STEBBINS, R. C. 1954. *Amphibians and Reptiles of Western North America*. McGraw-Hill Book Company, Inc., New York.

A Refined Method for Culturing Reptilian Cells with Comments on Aggregations of Reptilian Melanomacrophages

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Many herpetologists are using cell culture to answer questions ranging from cell and tissue function to studies of the aging process. This paper reports a culture technique improved from that of Rund et al (1998), especially a method of concentrating cells such as melanomacrophages (MMs). We became interested in these cells with the discovery that they formed aggregations in spleen, liver, and other organs in mud turtles, *Kinosternon flavescens*, and that these aggregations increase in number and size with turtle age (Christiansen et al, 1996). Aggregations of MMs have been reported in fishes (Aguis, 1985), amphibians (Sichel et al, 1997), and some reptiles (Duncker, 1968) among others. The basics of our methods were similar to those of Clark and Karzon (1967), Clark et al (1970), and Koment and Haines (1982). Ulsh et al (2000) used autologous turtle serum instead of fetal bovine serum to facilitate culture of reptilian lymphocytes.

In our studies of the advantages these cells provide turtles in low temperature environments (Johnson et al, 1999), we observed that our cultures became overrun with fibroblast-like cells. Our need to obtain nearly pure cultures of MMs for this study as well as our need for nearly pure fibroblast-like cell cultures for our current studies of aging and the aging process in reptiles, necessitated development of the purification techniques we report here. In addition, our MM cultures have provided evidence for a potential reason for the hepatic aggregations.

Culture Technique.—Liver and spleen from Western Painted Turtles (*Chrysemys picta bellii*) were used for preparation of MMs and these tissues along with skin and lung provided cells for fibroblast cultures. The turtles were collected and sacrificed under scientific collecting permit SC255 0101 and others issued to JLC by procedures approved by the Drake University Animal Care Committee as reported in Rund et al. (1998). Approximately 10 mm cubes of tissue were placed in RPMI-1640 (Sigma-Aldrich, Inc., St Louis, Missouri) containing penicillin, streptomycin, and neomycin (100 IU/ml: 100 mg/ml; 200 mg/ml, respectively, all from Sigma-Aldrich, Inc.). Tissues were rinsed three times in 40 ml RPMI-1640 with antibiotics to remove clotted blood and debris. Washed tissues were transferred to petri dishes and crossed scalpels were used to divide them into 2–3 mm cubes. The cubes of friable organs were pushed through a 1.0 mm stainless steel screen with a spoonula but skin was shredded further with crossed scalpels. The tissue fragments, cell aggregates, and individual cells