punctures easily. Based on our trial of the glue-and-tape method, we conclude that applying this method to active-foraging, arboreal snakes that have prehensile tails or thin skin will result in dermal abrasion, which can develop into severe lacerations and infection. Although injuries sustained from the glue-and-tape transmitter attachment method were distinct from the expected injuries acquired during coelomic transmitter implantation, the end effects on behavioral normalcy may be equal.

The tape-glue-tape method appears to have greater potential as a non-invasive method for use in short-term telemetry studies. The transmitter package remained adhered to the snake until the first shed and did not cause injuries or obviously negatively affect behavior. However, given that the first two transmitters detached within a few days of attachment, the tape-glue-tape method had lower reliability. The tape-glue-tape method used on the similarly slender and arboreal Mexican Vine Snakes was also found to be effective (Madrid-Sotelo and Garcia-Aguayo 2008), although these authors recorded greater attachment longevity with a mid-body attachment site (48 days). It is possible that a mid-body attachment site increased the duration of transmitter attachment, or BTS may have a more frequent shed cycle than Mexican Vine Snakes. For BTS and perhaps other snakes, external transmitters appear to cause less dermal damage if a layer of tape separates the transmitter from the body of the snake—the tape-glue-tape method. Thus for short-term studies (2–5 weeks) that can accommodate some loss of study subjects, the tape-glue-tape method appears to be appropriate for use on slender, arboreal snakes when surgical implantation is not desired.

Acknowledgments.—We thank A. Collins, F. Erickson, V. Deem, R. Lee, and N. Van Ee for their help in maintaining our study animals. We also thank E. Muths and three anonymous reviewers for improving this manuscript. L. Bonewell provided essential logistical support. Animal maintenance followed protocols specified by Institutional Animal Care and Use Committee number FORT IACUC 2017-04 and general project Standard Operating Procedures. All data generated during this study are included in this publication. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Herpetological Review, 2018, 49(1), 34–36.

A New Blood Sampling Method for Smaller Anurans that Preserves Critical Features of Specimens

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In small anurans, obtaining adequate blood samples can be challenging (Rexer-Huber et al. 2012; Ward et al. 2015). A variety of methods have been developed for amphibian blood collection (Allender and Fry 2008; Forzán et al. 2012). Commonly used blood collection methods for anurans have historically included cardiac puncture (cardiocentesis), cerebral decapitation, or puncture of the facial, femoral, or sublingual vein (Hadfield and Whitaker 2005; Forzán et al. 2012). However, these methods either result in the near-total destruction of the specimen (e.g., decapitation) or have typically not been recommended for anurans smaller than 25 g (Wright 2001; Allender and Fry 2008; Forzán et al. 2012).

Here, we describe a lethal method of blood sampling for small- to medium-sized anurans that produces good blood yield while maintaining the integrity of specimens for museum

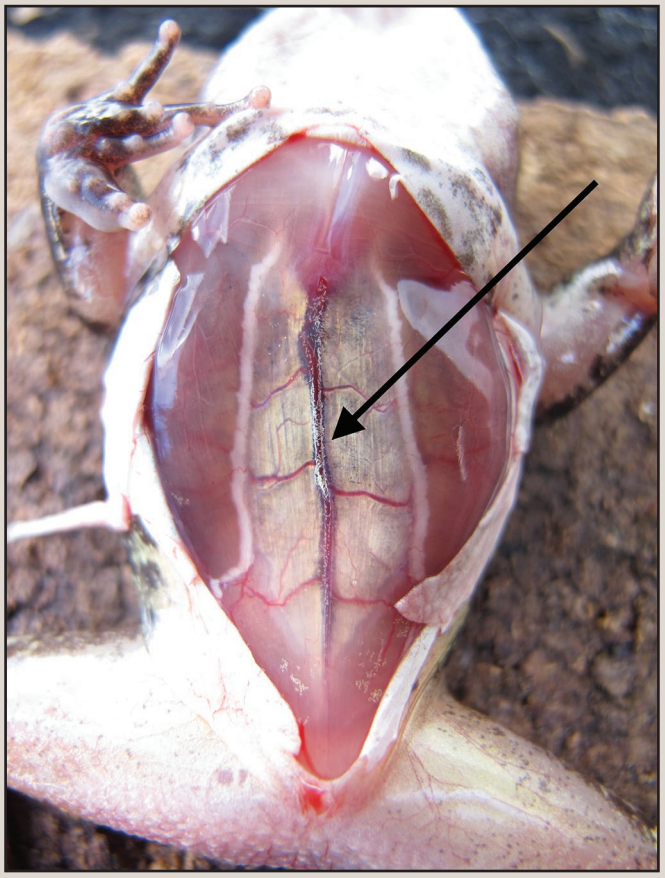

FIG. 1. Location of the abdominal vein, indicated by black arrow, in a euthanized male *Rana sylvatica*. Almost all of the ventral skin is removed from this specimen to more clearly depict the location of the vein. In contrast, only a single, small vertical incision is necessary to complete the blood draw procedure as described (see linked video).

collections. This method, which we call the lethal abdominal vein of anurans (LAVA) technique, can easily be used in either the field or laboratory setting. In brief, a frog is euthanized in a MS-222 solution and blood is immediately thereafter collected from the exposed abdominal vein (Fig. 1). The LAVA technique is advantageous when drawing blood from small frogs in that 1) it does not destroy critical features of specimens that may be needed for future study or for allocation in museum collections, 2) study animals are humanely euthanized prior to the procedure, and 3) it requires minimal training time and yet reliably produces blood samples.

MATERIALS AND METHODS

We used floating minnow traps to capture 59 adult male Wood Frogs (*Rana sylvatica*) from 12 ponds in south-central Connecticut, USA in March 2016 and April 2017. Following capture, we recorded the mass (g) and snout–vent length (SVL in cm) of each individual. We euthanized each frog via immersion in a buffered 2.5-g/L solution of MS-222 (Leary et al. 2013) and checked for responsiveness by pinching the skin and toes and by monitoring for any signs of respiration. All frogs used in this study were collected as part of a large multi-year project on the effects of suburbanization on Wood Frog morphology, behavior, and physiology. These specimens are retained in the Yale Peabody Museum collections.

FIG. 2. Example of blood collection from the abdominal vein of a recently euthanized *Rana sylvatica* using the LAVA technique.

We performed the LAVA technique within 10 seconds after confirming euthanasia. We thoroughly dried the abdominal skin with disposable wipes and used forceps and surgical scissors to cut through the skin directly over the abdominal vein. We then used a sterilized #15 scalpel (Dynarex, Orangeburg, New York, USA) to make a single, shallow incision, approximately 2–3 mm in length, along a posterior segment of the ventral abdominal vein. Immediately after the vein was broken, we dried the incision and surrounding area and used a new 75-µL heparinized microhematocrit capillary tube (Globe Scientific Inc., Paramus, New Jersey, USA) to collect blood directly from the abdominal vein (Fig. 2). We applied a gentle pumping pressure to the specimen's upper abdomen and chest with one finger to maximize the blood volume obtained. A video of the LAVA technique can be downloaded at https://scholarsphere.psu.edu/concern/generic_works/5x633dz848.

We collected blood from the incision until flow into the capillary tube slowed noticeably, between approximately 30 and 120 seconds after the time of incision. We immediately transferred blood samples from the capillary tubes to microcentrifuge tubes and kept tubes on ice until centrifugation (3500 rpm for 10 min), which occurred within 8 hours of collection. After centrifugation, we measured total plasma (supernatant) volume to the nearest 5 µL using a pipettor.

We used a linear regression analysis in R (R Foundation for Statistical Computing, Vienna, Austria) to test whether total plasma collected was influenced by mass or SVL. We log transformed the data to meet the assumptions of normality and used a = 0.05 as the threshold for statistical significance.

RESULTS

We successfully used the LAVA technique to collect blood samples from all 59 frogs in our study. Average frog SVL was 4.6 cm ± 0.04 (range: 4.0–5.1 cm) and mass was 9.6 g ± 0.2 (range: 7.0–13.8 g). Per frog, whole blood yielded, on average, 38 µL ± 2.8 (range: 10–100 µL) of plasma after centrifugation. Neither frog SVL ($F_{1,56}$ = 0.019, P = 0.891) nor mass ($F_{1,56}$ = 1.711, P = 0.196) influenced the plasma volume collected.

Discussion

Small anurans are notoriously difficult to restrain, anesthetize, and bleed (Wright 2001; Allender and Fry 2008; Forzán et al. 2012; Rexer-Huber et al. 2012; Ward et al. 2015;). Here, we introduce and demonstrate the LAVA technique of blood collection using *R. sylvatica* as small as 7 g. We show that an average of about 40 µL (and as much as 100 µL) of plasma can be obtained from whole blood that was collected using the LAVA technique. The LAVA technique is perhaps best suited to situations in which other blood collection techniques are inappropriate or inconvenient: for example, when specimens must be retained in good condition following blood extraction for museum collections or morphological studies. The LAVA technique described here results in only minimal alteration to the specimen by cutting a small slit through the skin on the abdomen; some very minor damage to the abdominal vein and the surrounding musculature is also possible.

Obtaining blood using the LAVA technique is also a simple procedure to master. An individual can complete the technique unassisted, and the amount of training required to successfully complete the procedure is low when compared to other techniques such as cardiocentesis. In the video link provided above the individual performing the blood draw had no prior experience with the LAVA technique.

When subjects are to be euthanized as part of a study, the LAVA technique may, in some instances, be preferable to other blood collection methods. The LAVA technique offers a humane solution for blood collection, as frogs are euthanized immediately prior to the manipulation and stress associated with blood sampling. The use of MS-222 as a method of euthanasia is generally approved by most institutional animal ethics committees (Leary et al. 2013), and thus ethics approval of the LAVA technique should be relatively straightforward.

As with all blood collection methods in the field, ensuring blood sample integrity is a concern. When using the LAVA technique, it is essential to thoroughly dry the specimen with cotton swabs or disposable wipes before and after the incision is made and prior to blood sampling to ensure that moisture from the skin does not contaminate blood samples. Only a shallow incision into the abdominal vein must be made, otherwise there is a risk of penetrating the body cavity (and expelling the fluids within). The capillary tube must then be held precisely at the incision site so that blood is drawn only directly from the vein. All our attempts at collecting blood directly from the vein using a syringe (even with a large-gauge syringe) were unsuccessful due to difficulty associated with accurately penetrating this narrow vein in these small frogs. The capillary tube produced the best results, by far. We did not test the LAVA technique on other amphibians (e.g., salamanders) or reptiles, but this basic approach could in principle be modified to suit other small herpetofauna.

The LAVA technique is an easily taught and relatively high-yield blood collection method for hard-to-sample frogs in studies in which euthanasia is appropriate. We suggest that this method be used whenever the maintenance of critical features of the specimens is key, such as when they will be deposited in museum collections or used in future morphological studies.

Acknowledgments.—We thank David Skelly, L. Kealoha Freidenberg, and Peter Smith for logistical support and advice and Adriana Rubinstein for field assistance. All procedures were approved by the Yale University IACUC (2013-10361 and 2015-10681). This material is based upon work supported by the National Science Foundation under Grant No. DGE1255832 awarded to D.A.S.O.

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