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Novel, Less Invasive Hylid Survey Device Performs Equally to Traditional Pipe Shelters in a Field-based Comparison

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Abstract.—**Detection of cryptic, arboreal amphibians requires specialized survey devices to account for taxa-specific climbing and hiding life histories. Hylid treefrogs are typically surveyed via tubes, wherein a polyvinyl chloride (PVC) pipe is placed upright in the ground or attached to a tree, which frogs then use as a shelter. Traditional survey methods typically require removal of amphibians for identification, adding time to data collection and stress to survey animals. As an alternative, we developed a novel shelter that includes a clear, acrylic tube nested within PVC pipe. Frogs can be identified through the clear tubing, eliminating the need for removal or handling. Here, we compare the efficacy of these novel devices to traditional PVC pipes in the field to determine any differences in device occupancy by hylid treefrogs. We placed 30 tubes of each type for 6 mo at a pond site in midwestern Virginia, USA. We found 23 frogs of two species (Gray Treefrogs, *Hyla versicolor*, and Spring Peepers, *Pseudacris crucifer*) in tubes. We found no significant difference in tube occupancy between device types. This demonstrates our novel design as a valid surveying method for these hylid species, particularly *H. versicolor*, though further research should examine the effectiveness of these devices for other species and habitats. The efficacy, ease of use, and minimally invasive nature of this trap design make it useful not only for hylid surveys but also for educational outreach and public engagement, which are key components for addressing ongoing amphibian population declines.**

Key Words.—*Hyla versicolor*; Hylidae; occupancy; *Pseudacris crucifer*; refugia; trap; tube; treefrog

Introduction

Surveillance of animals in their native habitats is critical for understanding population dynamics, life-history traits, and establishing baseline data for conservation (Elphick 2008; Schwartz et al. 2012; Nowakowski et al. 2017). For reptiles and amphibians, a multitude of survey methods exist, each specialized for the wide array of behaviors and life-history traits that these organisms exhibit (Heyer et al. 1994; McDiarmid et al. 2012). Treefrogs in particular require specialized methods, as they are challenging subjects to survey in situ due to their cryptic nature and climbing abilities, which allow them to escape ground survey methods such as drift fence and pitfall trap arrays (Corn 1994). Most frog surveys involve identification at breeding locations via male calls; however, this provides insight only into the presence or absence of a species at a given site, not necessarily information on the treefrog population dynamics at a locality (Zimmerman 1994; MacKenzie et al. 2002; Gooch et al. 2006; Roh et al. 2014). Therefore, the traditional method developed to survey populations of treefrogs outside of breeding ponds is to use polyvinyl chloride pipe (PVC) arrays (Moulton et al. 1996; Zacharow et al. 2003; Glorioso and Waddle 2014).

Tube arrays consist of PVC pipes placed in the ground or attached to trees within a landscape (Moulton et al. 1996; Boughton et al. 2000; Johnson 2005). The tubes mimic tree holes that treefrogs use during the day to avoid exposure to the elements, hide from predators, and have a safe place to rest before coming out at night to forage and/or breed (Boughton et al. 2000). To count and obtain proper identification of the animal, surveyors look down into the top of the pipe, and typically animals must be forcibly removed via shelter inversion and shaking (Boughton et al. 2000; Pittman et al. 2008). Some studies have employed the use of a Frog Plunger system in which the animal was pushed through the tube by a specially designed sponge (Johnson 2005; Boughton et al. 2000). Although this type of handling is often necessary for the survey process, we think that it has a greater potential for increasing stress of the animal, increasing the potential for injury, and increasing the possibility of disease spread if preventative measures are lacking.

We designed a novel shelter system to mitigate these possible effects. These novel shelters consist of a clear acrylic pipe sheathed inside opaque PVC (Fig. 1; McGrath et al. 2020). Surveyors simply have to pull up on the clear acrylic tube to see if an animal is inside. If a frog is found, surveyors can see the full animal, both dorsal and ventral views, and see distinguishing characteristics necessary for proper identification without removing or handling the animal. Our purpose was to test the efficacy of this novel design. We

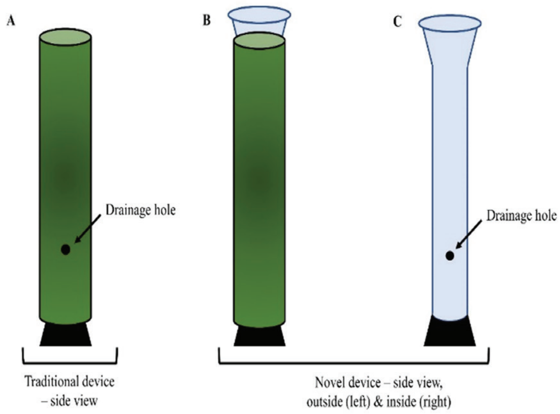

Figure 1. Schematic image comparing novel versus traditional survey device designs for frogs. (A) Traditional PVC pipe device as seen from the side with a black rubber plug (bottom) and drainage hole drilled into the side to allow for standing water in the bottom of the tube. (B) Novel device with the clear acrylic tube sheathed inside PVC pipe. Note the lack of drainage hole and rubber plug on the outer PVC pipe. (C) Clear acrylic tube unsheathed from PVC pipe with a drainage hole, rubber plug, and flared top.

compared occupancy of novel versus traditional style hylid shelters in the field to determine whether treefrogs display a preference for novel versus traditional PVC shelters. This will help to establish the validity of this novel, minimally invasive, tube within a tube design as a viable substitute for the traditional PVC shelters.

Materials and Methods

Tube design and construction.—Traditional PVC pipe survey shelters consisted of 60 cm long schedule-40 PVC pipe with a 2.54 cm inside diameter (Boughton et al. 2000). We placed a drainage hole 15 cm from the bottom and used 37 × 28 mm tapered rubber stoppers to plug the bottom of the tube. This created a reservoir of water for increased humidity (Boughton et al. 2000). Novel shelters had outer schedule-40 PVC pipes with a 3.175 cm inside diameter, which sheathed the 2.54 cm inside diameter clear acrylic tubes. Acrylic tubes had a drainage hole and rubber stopper in the same manner as the traditional shelters and had flared tops so that the acrylic tube rested on the PVC pipe without falling through (McGrath et al. 2020; Fig. 1). To flare the acrylic tubes, we used a heat gun (Wagner Furno 300, Wagner Systems Inc., Plymouth, Minnesota, USA) to heat one end of the tube until pliable and then pushed that end onto an upturned funnel until a flared effect was achieved. Once cool, the acrylic hardened and was quickly ready for use in a field application. Per a request from personnel of the U.S. Forest Service, we spray painted the outsides of the PVC pipes of both styles in camouflage colors (i.e., muted green and gray) to allow survey shelters to remain inconspicuous on public land. We secured shelters to trees using standard rubber bungee cords. We used rubberized bungee cords instead of nylon covered bungee cords because of their resistance to wear and dry-rot.

Survey methods.—We attached novel and traditional shelters to trees in a paired design and placed them near an ephemeral pond at Maple Flat Ponds, Virginia, USA (37.975971N, -78.996971W), an area with known hylid populations including Eastern Cricket Frogs (*Acris crepitans*), Spring Peepers (*Pseudacris crucifer*), Upland Chorus Frogs (*P. feriarum*), and Gray Treefrogs (*Hyla versicolor*; Mitchell and Buhlmann 1999). Observers performed surveys on a total of 30 traditional and 30 novel tubes starting in May 2017, continuing for an average of once per week until the end of October 2017. We placed one tube of each type on the same tree. We placed tubes on opposite sides of the tree from one another, equidistant from the centralized, ephemeral water source.

For traditional shelters, surveyors inspected the top of the tube to determine if a frog was present. If a frog occupied the shelter, it was removed from the device for identification and to collect snout-vent length (SVL) morphometric data. We collected SVL measurements to serve as a general proxy for capture rates, as we did not employ marking techniques in this survey that would account for individual frog recaptures in the same shelter. Surveyors took the tube off the tree and inverted it for removal of the animal. If a frog did not readily vacate the tube, the surveyor sprayed water into the tube from a small, plastic spray bottle and then the subsequent inversion would cause a water-slide type effect, and the frog was then slid into a clean plastic bag. We then identified the frog and collected measurements through the plastic bag to reduce stress to the animal and the likelihood of spreading disease. Afterward, we re-attached shelters to the tree and released frogs onto the tree, close to the opening of the tube they came from.

To survey for frogs in the novel device, surveyors pulled up the clear acrylic tube. If a frog occupied the shelter, surveyors performed identification and SVL measurement through the acrylic tube. To measure SVL, surveyors held a ruler to the outside of the tube over the area where the frog was sitting. Due to the reliability of data collection through the clear tube (unpubl. data), there was no need to remove the animal from the shelter to obtain data. After data collection and identification, the acrylic tube was re-sheathed within its PVC pipe with frogs still inside.

Data analysis.—We analyzed data in R (R Core Team, 2020, v 4.0.3) and codes can be found at https://github.com/smcgblaser/frog_tube_comparison_study. Data fit a nonparametric distribution, so we used the

Mann–Whitney U test with a value of $P < 0.05$ to determine significance. We compared the SVLs of each frog captured to evaluate if frogs were likely recaptured in the same tube across multiple surveys (Appendix Figure). We considered captures non-independent if the same SVL or a similar SVL within 1 mm was reported for frogs found within the same tube across multiple survey events. Therefore, we ran analyses twice, once with all captures and again with non-independent data captures (n = 2) removed. We report statistics for all captures (denoted as AS; all samples) and all independent captures (denoted as MD; minus data points).

Results

We found 23 frogs over the 29 weekly surveys conducted during the 6-mo survey period. There was a 1.32% chance that a tube of either design would have a frog in it, with a slightly higher chance of capturing a frog in a novel style tube (1.61%) versus a traditional tube (1.03%). We found two species, *Hyla versicolor* and *Pseudacris crucifer*, with *H. versicolor* accounting for 83% of observations and *P. crucifer* 17% (Fig. 2). We found an equal number of *P. crucifer* in each tube type (2 and 2) and a higher number of *H. versicolor* in the novel tubes (12 and 7), but the number of frog captures between shelter styles was not significantly different (AS: $W = 71$, df = 1, $P = 0.445$ MD: $W = 68.5$, df = 1, $P = 0.568$). Novel shelters were faster to survey, typically taking 10 s to determine occupancy, whereas traditional devices took 30 s or longer.

We encountered the first frog approximately one month after device placement. We had the most captures in September and October, with a 5-fold increase in frogs found in either tube type compared to summer months (June, July, August; Fig. 3). SVL data, being a proxy for independent captures, suggested the only data points of possible non-independence were for novel tubes 13 and 14 (Appendix Figure). SVL differed significantly between tube types for *H. versicolor* (AS: $W = 82$, df = 1, $P < 0.001$; MD: $W = 68$, df = 1, $P = 0.001$), but not for *P. crucifer* ($W = 2$, df = 1, $P = 1.00$; Fig. 4).

Discussion

We found that two species of hylid frogs were as likely to use our novel tube design as traditional PVC pipe shelters in a field-based comparison. There was no difference in shelter type used by *H. versicolor* or *P. crucifer*. Although overall capture rates were lower than rates from previous studies (e.g., Boughton et al. 2000; Johnson 2005; Pittman and Dorcas 2006), our capture rates between device designs were similar. This suggests our novel design is an effective survey method. Capture rates may have been low due to the centralized pond water source drying at the beginning of the survey (mid-June) and the water level remaining low throughout the rest of the survey period. The surrounding area had deeper ponds that retained more water, possibly attracting frogs away from our shelters. Future research using either shelter type should carefully consider species of interest, climatic fluctuations, and life-history traits before placement (Zacharow et al. 2003; Pittman et al. 2008; Glorioso and Waddle 2014). Also, we suggest that researchers allow roughly one month for frogs to colonize the shelters.

We found that SVL measurements differed between shelter types for *H. versicolor*. This could indicate a size-related preference for novel tubes in *H. versicolor* or may be an artifact of the methods used for collecting SVL data between shelter designs. Minute differences can be seen between *P. crucifer* SVLs between tube types (Appendix Figure), suggesting independent

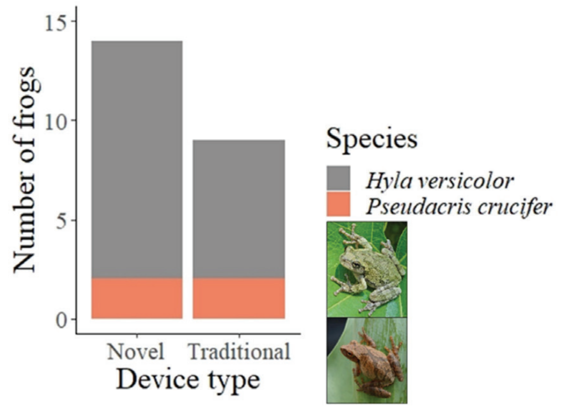

Figure 2. Cumulative number of frogs encountered in each tube type over the 6-mo survey period. Colors correspond to the species of frog and images are included underneath the legend, listed in the same order. (Photographs used with permission from © John White of the Virginia Herpetological Society).

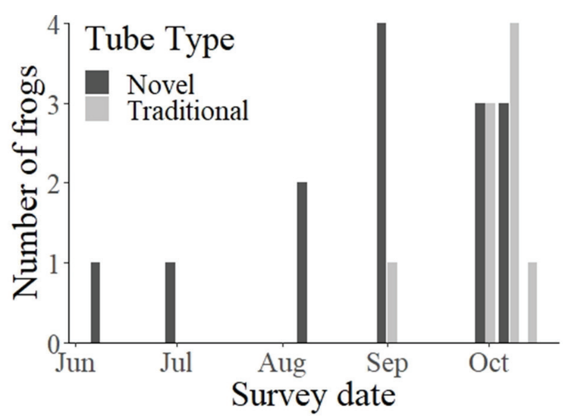

Figure 3. Hylid frogs found in clear (novel) and opaque (traditional) tubes by survey event. Note that tubes were placed in May and the first frogs in either tube type were encountered in June.

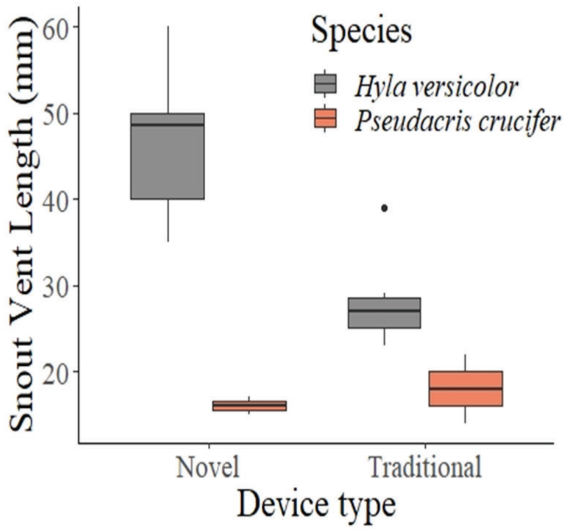

Figure 4. Boxplot comparing frog length for Gray Treefrogs (*Hyla versicolor*) and Spring Peeper (*Pseudacris crucifer*). Length of frogs found in the clear (novel) tubes measured through the clear acrylic pipe without handling, whereas frogs in opaque (traditional) tubes were removed and measured through a clean, clear plastic bag.

captures; however, we could not accurately determine size differences due to low capture rates for this species. If interested in specifically sampling *P. crucifer*, we suggest researching alternate shelter designs that might better attract this species. We did not remove frogs from the clear inner tubes to minimize stressing them during data collection, but frogs were forcibly removed from opaque tubes and then measured inside a clear bag. The difference between measuring SVL through a clear tube and through a clear plastic bag was not compared and would need to be determined if both device types are to be used simultaneously. If only the novel design is used, then SVL measurements would be standardized and therefore comparable. Future studies could use choice experiments to determine whether size-biased preference exists between trap designs.

Although we did not set out to quantify differences in survey time and organismal stress between tube designs, we did notice that it took much less time to check novel tubes versus traditional ones. For novel shelters, the tube could be viewed in approximately 10 s, whereas traditional tube checks typically took at least 30 s just to see into the opaque tube. If a frog was present, it took several minutes to detach the shelter from its tree, remove the frog, and reinstall the shelter. Frogs in the novel shelters barely moved or moved downward in the clear tubes when they were unsheathed, but frogs in the traditional shelters had to be vigorously shaken into the plastic bags due to their adhesion abilities (Green 1981). In some cases, a spray bottle helped to create a frog water slide to aid in removing the animal from the shelter (McGrath et al. 2020). Once in the plastic bag, frogs then had to be manipulated to obtain accurate measurements. We observed that frog secretions in response to manipulation often increased the time and effort needed to obtain an accurate SVL. After data collection and frogs being placed back on the tree from where they were removed, they generally remained still until researchers walked away. A detailed study focused on stress-related differences between shelter types would be beneficial, but we advocate that the novel devices inherently promote lower stress for the animal and the surveyor.

Surveys of cryptic amphibians are pivotal to better understand population dynamics, and in light of serious global amphibian disease spread, the need for methods that reduce organismal contact are ideal (Scheele et al. 2019). Our novel design provides an improvement from previous methods that both protects frogs from stress and simplifies surveys for the researcher. We encourage the use of this design in future mark-recapture studies because marked frogs can be identified by viewing them through the clear tube. We did not mark frogs in this study, but visual implant elastomer (VIE) or toe-clipping methods combined with our novel design could improve research into hylid population dynamics (Donnelly et al. 1994) and behavior.

Expanding beyond research, we believe our novel design is also valuable for educational outreach. The ease of use and degree of interaction with the frogs protected in the clear tubes are ideal for introducing hylids to large groups or less experienced observers who might not otherwise appreciate these charismatic amphibians. Viewing frogs in the field without directly handling them could serve many people, such as those with disabilities or benign herpetophobia, and children first learning to interact with nature. This study establishes the effectiveness of this novel design relative to traditional hylid survey techniques, and we hope others will continue to explore its benefits for research and educational outreach.

In summary, the current study establishes this novel shelter as a viable method for surveying wild hylid frogs. We also provide evidence that it is preferable to traditional methods, which are inherently more disruptive to the animal. Applications of these shelters in future hylid studies and community science engagement are expansive. Employing tools that better our understanding of amphibians in their natural habitat and at the same time protect the organism from undue harm are essential for the future of amphibian research, as these organisms face numerous threats.

Acknowledgments.—We conducted surveys under a scientific collection permit from the Virginia Department of Game and Inland Fisheries (Permit No. 059684) and with permission from the U.S. Forest Service. All practices adhered to Institutional Animal Care and Use Committee of James Madison University (protocol #A17-10). We would like to thank the Virginia Herpetological Society for funding this project. We also thank David McLeod for his support, and thanks go out to Nicholas Blaser and Jonathan Studio for assistance with tube construction.

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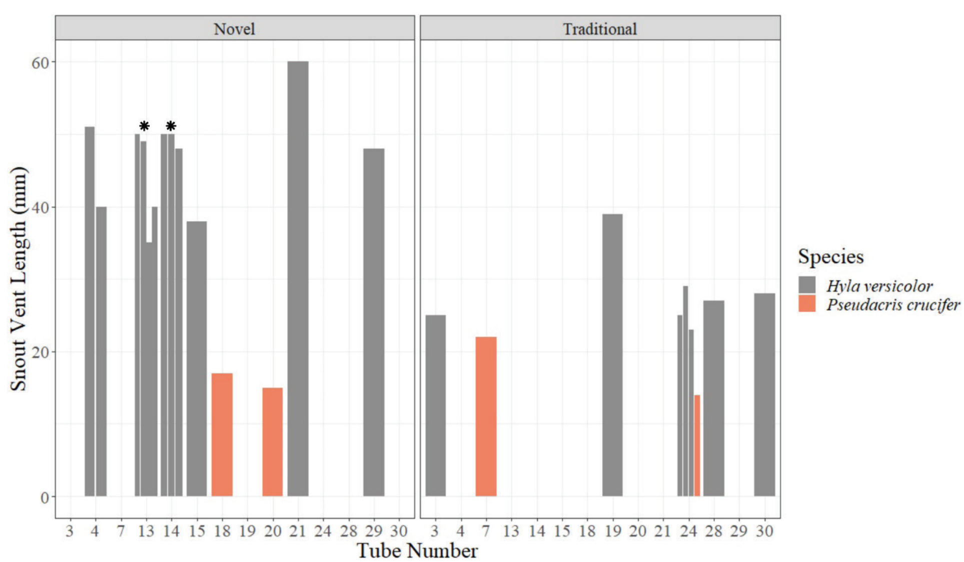
Sarah McGrath-Blaser graduated from James Madison University, Harrisonburg, Virginia, USA, with a M.S. in Biology in 2018 and is currently a Ph.D. student at the University of Florida, Gainesville, Florida, USA. Her research interests include amphibian monitoring, amphibian and reptile disease ecology, and using microbial ecology to understand how wildlife diseases spread. Sarah is pictured here in the field with a backpack full of frog tubes about to place them at the field site in Virginia. (Photographed by Oliver Hyman).

Althea Neighbors graduated with a B.S. in Biology from James Madison University in Harrisonburg, Virginia, USA, in spring of 2018. Her first research experience as an undergraduate student was with this experiment. Since graduating, she has worked for the Great Basin Institute (GBI), Reno, Nevada, USA, as a crew member, Research Associate, and Crew Leader. Her work in the Research Associate branch of GBI as a Field Technician for vegetation monitoring inspired a passion for botany. Her research interests include field botany, population ecology, and restoration ecology. (Photographed by Althea Neighbors).

Oliver Hyman is a Lecturer at James Madison University in Harrisonburg, Virginia, USA. He received his Ph.D. in biology from Arizona State University, Tempe, USA, studying the dynamics of infectious disease in chorus frogs. He is interested in answering questions related to biodiversity monitoring, species detection, species identification, and disease ecology using a combination of field, molecular, and bioinformatic techniques. Oliver is also interested in using DNA barcoding to integrate authentic research experiences into large enrollment introductory bio courses and demonstrate how tools from disparate fields like molecular biology, field biology, and bioinformatics can contribute to species conservation and undergraduate education. (Photographed by Sarah McGrath-Blaser).

Appendix



Appendix figure. Frog snout-vent length (SVL) measurements for each individual captured by tube type and species. Wider bars indicate only one frog was found in that given tube, whereas narrow bars correspond to measurements for multiple capture events in that given tube. Species are delineated by color. Asterisks (*) represent possibly replicated data points that were removed from duplicate analyses to determine significance.