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Reducing Small Mammal Mortality in Treefrog Polyvinyl Chloride (PVC) Pipe Studies

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***Abstract*.—Polyvinyl chloride (PVC) pipe refugia have been used to study hylid treefrogs for decades. These inexpensive and easy-to-build refugia effectively mimic damp tree cavities but pose a hazard for small mammals that fall into pipes and cannot climb up smooth surfaces. Borg et al. (2004) found that by installing escape ropes, Southern Flying Squirrels (*Glaucomys volans*) could escape, but this paper remains rarely cited. In this study, we revisit this technique and evaluate its ability to reduce small mammal mortality. We checked pipe refugia for treefrogs and dead mammals during the 2018 and 2019 activity seasons (April-October) and documented a 10.2% treefrog capture rate and a 2.4% mammal mortality rate. We then installed escape ropes before the 2021 activity season and documented similar treefrog capture (8.2%) but no mammal mortality. Accounting for different check efforts pre- and post-rope installation, the mortality reduction was significantly reduced. Escape ropes did not significantly influence treefrog capture rates, but mammal mortalities appeared to reduce treefrog detection. We offer several suggestions to reduce small mammal mortality and recommend wider use of escape ropes.**

Key Words.—artificial refuge; by-catch; hylid; non-target capture; refugia; sampling bias; small mammal

***Resumen*.—Los tubos de cloruro de polivinilo (PVC) se han utilizado como refugio artificial para estudiar las ranas arborícolas de la familia Hylidae durante décadas. Estos refugios son baratos, fáciles de construir, y efectivos para mantener un ambiente húmedo similar a las cavidades húmedas de los árboles, pero representan un riesgo de mortalidad para pequeños mamíferos que caen en los refugios y no pueden trepar las superficies lisas. Borg et al. (2004) encontraron que, mediante la instalación de cuerdas de escape, las ardillas voladoras del sur (*Glaucomys volans*) podían escapar los refugios, pero este trabajo es raramente citado. En este estudio, revisamos esta técnica y evaluamos su capacidad para reducir la mortalidad de pequeños mamíferos. También hicimos una revisión de la literatura asociada. Monitoreamos refugios de tubos de PVC y cuantificamos capturas de ranas arborícolas y mamíferos muertos durante los meses de abril a octubre (temporada de actividad) de 2018 y 2019. Documentamos una tasa de captura de ranas arborícolas del 10,2% y una tasa de mortalidad de mamíferos del 2,4%. En la temporada de 2021, instalamos cuerdas de escape en los refugios. Documentamos una tasa similar de captura de ranas arborícolas (8,2%) pero la mortalidad de mamíferos fue nula. Teniendo en cuenta los diferentes esfuerzos de monitoreo antes y después de la instalación de las cuerdas, la reducción de la mortalidad fue estadísticamente significativa. Las cuerdas de escape no influyeron significativamente en las tasas de captura de ranas arborícolas, pero la mortalidad de los mamíferos pareció reducir la detección de ranas arborícolas. Ofrecemos varias sugerencias para reducir la mortalidad de pequeños mamíferos y recomendamos un uso más amplio de cuerdas de escape.**

Palabras Clave.—captura incidental; captura no objetivo; hílidos; pequeño mamíferos; ranas arborícolas; refugios; refugio artificial; sesgo de muestreo

Introduction

Non-target species mortality, also known as by-catch, receives most attention as it relates to commercial harvest and fisheries (e.g., Gilman et al. 2005), poaching (e.g., Becker et al. 2013), legal fur-trapping (e.g., Andreasen et al. 2018), and control of nuisance or invasive species (e.g., Hoare and Hare 2006). Harm to non-target species can be lethal, such as occurs for many species of sea turtles within different fisheries (Biju Kumar and Deepthi 2006). Wildlife studies are also prone to such non-target mortality events but receive little research attention (Borg et al. 2004; Edwards and Jones 2014; Surtees et al. 2019). Even less research effort has focused on how non-target capture and mortality within survey traps could affect detection of target species, possibly introducing bias in the study of the target species or community.

Polyvinyl Chloride (PVC) pipes have been used to sample Hylid treefrogs in the Americas (Johnson 2005; Pittman et al. 2008; Ferreira et al. 2012), Africa (Trimble et al 2014), Europe (do Vale et al. 2018), and Asia (Hirai 2006); however, these pipe studies are especially prone to mortality of small mammals, reptiles, and arthropods because they cannot climb up slick, vertical PVC pipe surfaces. The pipes attract frogs because they simulate a humid and water-filled tree cavity but do not trap them as they can leave refugia via their sticky toe pads. The pipes are capped at the base to collect rainwater, which fills part-way up the pipe, creating a humid environment in the section of pipe without water (Johnson 2005). The pipes can also attract arboreal small mammals (e.g., flying squirrels) which fall in, are unable to escape, and often die (Borg et al. 2004). Borg et al. (2004) solved the problem of flying squirrel mortality in PVC pipe studies by installing escape ropes, but the technique has not been adopted widely. These non-target mortality events are likely under-reported, and these losses may have negligible impacts on common species populations. This, however, does not alleviate the ethical issues related to preventable wildlife deaths and the introduction of detectability bias into studies when target treefrog species are likely avoiding pipes with decomposing small mammal carcasses. The primary objectives of our study were to: (1) compare mammal mortality before and after escape rope installation; (2) compare treefrog pipe presence and total abundance before and after escape rope installation; (3) compare treefrog capture rate pre- and post-escape rope installation and among pipes with and without mammal mortality; (4) provide a literature summary of PVC treefrog studies and their discussion of non-target mammal mortality; and (5) offer modifications to this technique that would further reduce mortality of small mammals and improve study efficiencies. Ultimately, we hope our work encourages a greater adoption of the technique offered by Borg et al. (2004) to prevent wildlife mortality and sample bias within treefrog refugia studies globally.

Materials and Methods

***Study site and species*.**—We conducted our study along the Illinois River near its confluence with the Mississippi River at Pere Marquette State Park (38°59’57.2»N, 90°31’33.4»W, Jersey County, Illinois, USA). Habitat was dominated by upland deciduous forest, riparian woodlands, and ephemeral streams in valleys. The woodlands are composed of native hickory (*Carya* spp.) and oak trees (*Quercus* spp.). Common understory shrubs and trees include dogwoods (*Cornus* spp.), Redbud (*Cercis canadensis*), Common Hackberry (*Celtis occidentalis*), elm (*Ulmus* spp.) and non-native and invasive bush honeysuckle (*Lonicera* spp.). The climate of the region is characterized by wet springs with severe weather, hot and humid summers (average high = 30.4° C), mild and less humid autumns, and winters with periodic light snow (average low = –7.0° C). The average monthly temperature between 1991–2020 was -2.4° C in January, 11.9° C in April, 22.7° C in July, and 13.0° C in October, and average annual precipitation was 107.3 cm (https://www.weather.gov/wrh/climate?wfo=lsx).

We focused on the gray treefrog complex (Fig. 1), which includes the Eastern Gray Treefrog (*Hyla versicolor*) and Cope’s Gray Treefrog (*Hyla chrysoscelis*), which are morphologically identical but have different calls (Dorcas and Gibbons 2008). They possess adhesive toe pads and can cling to slippery surfaces. Their range extends from Canada to Texas (USA), with an active season from April to October, and most breeding activity occurring late spring through early summer, followed by a non-breeding period in the fall before overwintering (Johnson 1987).

***Transect and pipe refugia description*.**—We sampled three areas, each with a cluster of three 200-m long transects (n = nine transects). We placed 10 refugia on each transect (i.e., two pipes were located at 0, 50, 100, 150, and 200 m on two trees near the interval) for a total of 90 pipe refugia. Each transect started at the edge of a prominent feature, such as an ephemeral stream, wetland, ridge, or road and ran perpendicularly toward the forest interior. We followed the Johnson (2005) design and haphazardly affixed pipes to different tree species with a minimum size of 15.24 cm diameter at breast height (Fig. 2). We cut schedule 40 PVC pipe into 61 cm lengths, 4 mm thick with an inside and outside diameter of approximately 40 and 48 mm, respectively. Collar and water-holding pipes were also 4 mm thick

Figure 1. Gray treefrog complex (*Hyla versicolor/chrysoscelis*) examples from the study showing the wide range of pattern and coloration (Jersey County, Illinois, USA). (Photographed by Nicole Morris, left, and Emma DeVeydt, center and right).

but had an inside and outside diameter of approximately 51 and 60 mm, respectively; collars and capped water holding pipes were approximately 8 and 15 cm long, respectively.

Escape ropes.—We installed escape ropes following the methods of Borg et al. (2004) during one month of the treefrog dormancy season in 2021. We drilled a hole approximately 10 mm in diameter at the top end of each pipe to fit the escape rope. We tied a single overhand knot at one end of a 70-cm long piece of rope and the remainder reached the bottom of the capped water holding pipe (Fig. 2). We used braided synthetic nylon rope with a diameter of 6.35 mm. The approximate cost of escape ropes was $0.45 USD per pipe at the time of writing.

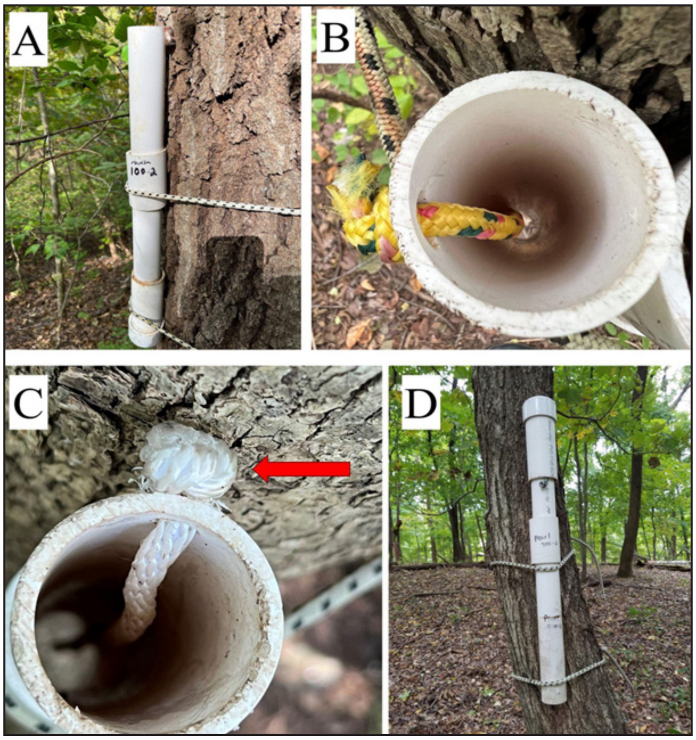

Figure 2. (A) Polyvinyl Chloride (PVC) pipe refugia used to passively sample treefrogs following the design of Johnson (2005). (B) Escape rope to prevent mammal mortality with water-holding pipe removed to allow light for picture. (C) Escape rope knot against the tree to prevent the escape rope from dislodging and being removed, which further prevents non-target mammal mortality. (D) Closing pipes for the season, so water cannot collect, with the water-holding pipe inverted and placed on top of the refugia pipe. (Photographed by Nicole Morris).

Sampling.—We surveyed transects monthly during the activity season (April to October) in 2018, 2019 and 2021 (no sampling was done in 2020 due to the COVID-19 pandemic). Due to resource constraints, we conducted fewer surveys in 2018. Before checking inside the refugia, we recorded if the escape rope had been removed or partially dislodged. Next, we checked for treefrogs and non-target vertebrates. Because treefrogs sometimes moved into the water-holding pipes when checking the main pipe, we gently stirred the water-holding pipe with a stick to ensure no treefrogs escaped detection. We did not identify dead mammals to species and we always emptied the contents of the water-holding pipe onto the forest floor if a mammal mortality was discovered.

Statistical analysis.—Following methods of Borg et al. (2004), we used Two-way Contingency Tables and: (1) compared proportions of mammal mortality before and after escape rope installation (using the total pipe checks to account for effort); (2) compared treefrog pipe presence (the total number of occurrences where at least one treefrog was discovered in a pipe refugia across surveys) and capture abundance (total number of combined treefrog individuals discovered using pipes across surveys) before and after rope installation; and (3) determined if a dead mammal found within the pipe influenced treefrog presence. In 2021, we occasionally found the escape ropes partially dislodged or completely removed from the refugia. In these instances, we removed these data from the analyses. Also, we excluded data when pipes contained active wasp nests (no treefrogs co-occurred with wasps), were disturbed, or were found on the ground. In all analyses we accounted for effort by including the number of pipe checks within our contingency tables. We compared the mean number of days between checks of pipes with ropes to checks without ropes using a *t*-test. We used R to perform statistical analyses with a significance level of $\alpha = 0.05$.

Results

We made 966 valid pipe checks across the entire study. Before escape ropes in 2018 and 2019, we conducted six rounds of monthly checks (total = 540 pipe checks). The 2018 (July – August) and 2019 (July – October) check periods spanned 39 and 106 d, respectively (the maximum total days between the earliest and latest pipe check). In 2018 we did not record the pipe opening date; however, in 2019 the earliest pipe opening date was 4 June 2019 and the last pipe check of the season was 24 October 2019, representing a 142 d sampling period. The mean number of days with 95% confidence intervals (CI) in between pipe checks in 2018 and 2019 was 33.71 ± (CI) 2.14 d. For post-escape rope installation (2021), we made 426 valid pipe checks from five rounds of surveys. The mean number of days between pipe checks in 2021 was 38.22 ± (CI) 2.70 d. Thus, with ropes, the mean number of days between checks was significantly greater than without ropes ($t = -2.57$, df = 84, $P = 0.012$).

Mammal pipe mortality and treefrog associations.—Without ropes, we discovered 13 mammal mortality events, sometimes with two dead individuals in the same pipe, and zero mortalities in 2021 with escape ropes installed. Accounting for pipe check effort, we found a significant difference in mammal mortalities and pipe check effort between pre- and post-escape

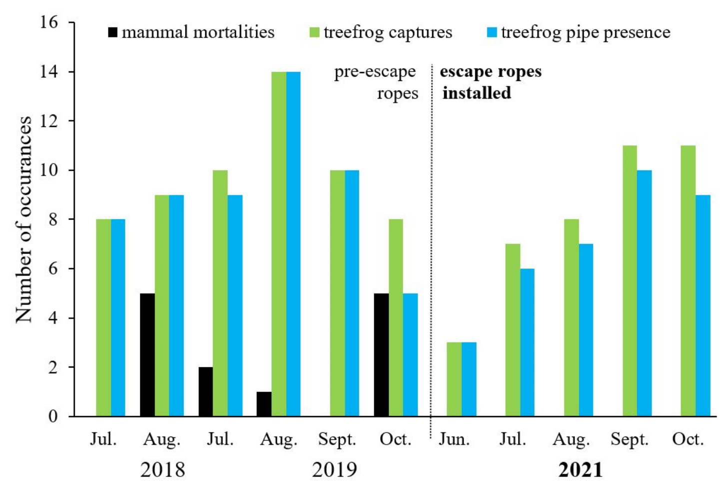

Figure 3. Monthly captures of gray treefrog complex (*Hyla versicolor/chrysoscelis*) species, pipe presence (i.e., two frogs within the same pipe counted as one presence occurrence but two capture occurrences), and mammal mortality occurrences (i.e., one occurrence means at least one dead mammal was found during a single pipe check) from monthly pipe checks (nine 200 m transects with 10 PVC pipe refugia each) from 2018–2019 (pre-escape rope installation) compared with 2021 (escape ropes installed) at Pere Marquette State Park, Jersey County, Illinois, USA.

rope installation (X^2 = 10.72, df = 1, P = 0.001). We never found a treefrog present in pipes with mammal mortalities but the difference in treefrog presence between pipes with dead mammals and without was not significant (X^2 = 1.35, df = 1, P = 0.225). Of the 10 instances where a pipe had a dislodged rope, there was one mammal mortality event. In addition, we discovered both skinks (*Plestiodon* spp.) and Gray Ratsnakes (*Pantherophis spiloides*) alive inside PVC refugia with escape ropes, along with insects and other arthropods in our larger study area.

Treefrog presence and total captures.—We documented 90 total occurrences of at least one treefrog using a pipe across all surveys (treefrog pipe presence occurrences) and 99 treefrog captures across all pipe checks. Of this total, we detected 55 pipe presence occurrences and 59 treefrog captures within the pre-escape rope period from 2018–2019. In 2021 we documented 35 treefrog pipe presence occurrences and 40 individual captures (Fig. 3). When accounting for differences in sampling effort, by removing invalid checks, we found no significant difference between pre- and post-escape rope installation and treefrog pipe presence (X^2 = 0.236, df = 1, P = 0.627) and total capture frequencies (X^2 = 0.671, df = 1, P = 0.413).

Discussion

Polyvinyl Chloride (PVC) pipes and similar sampling devices (e.g., bamboo pipes) have been used for more than two decades to aid in the sampling of hylid treefrogs for various research objectives (Glorioso and Waddle 2014), including treefrog conservation efforts (Hirai 2006; Suriyamongkol et al. 2021). They are relatively inexpensive and easy to build, and this passive sampling technique has no real mortality risk to the treefrogs, but other wildlife, particularly small arboreal mammals, are at a much greater risk of mortality if they fall into these bottom-capped pipes. Our study adds knowledge to the gap in non-target by-catch within wildlife studies and reinforces the finding of Borg et al. (2004) that escape ropes are effective at reducing small mammal by-catch in PVC pipe studies for frogs. We found zero mammal mortalities after escape rope installation and previously observed a 2.4% mortality rate. Although mortality rate was low before escape rope installation, mortalities can quickly accumulate over larger studies using many PVC refugia. This would be approximately 35 mortality events, and an event could potentially include multiple individuals, if we extrapolated a similar survey effort and mortality rate across our entire 24 transect study area. We also had additional transects not used in our analyses due to some inconsistencies in earlier data collection, which appeared to routinely cause Southern Flying Squirrel (*Glaucomys volans*) mortality during mid to late spring (Ross Albert, pers. obs.). Thus, our pre-escape rope mortality rate could be an underestimation. Additionally, these escape ropes do not appear to impact PVC pipe use by treefrogs or their capture probability, further supporting results of Borg et al. (2004).

We did not specifically identify mammal species in our study, but we suspect a variety of species fell into the PVC pipes. Morphological observations indicate that the species were deer mice (*Peromyscus* sp.), Southern Flying Squirrels, Eastern Gray Squirrels (*Sciurus carolinensis*), and Eastern Fox Squirrels (*Sciurus niger*). For the larger squirrel species, we suspect young may have been entering pipes as adults likely are too large to enter the pipes. Squirrel mortality is a particular concern because they breed twice a year and young may fall into open pipes when treefrogs are inactive in winter and early spring (December–January; Brown and Yeager 1945). Therefore, we recommend closing pipes during treefrog inactive seasons by inverting the capped pipe (closed pipe using the Johnson 2005 design) or inverting the entire pipe as with the Boughten et al. (2000) design that has a drilled drain hole so that the entire pipe cannot hold water. The only other study conducted on this topic was in hardwood depressions surrounded by pine trees (*Pinus* sp.) in Georgia, USA, and found mortalities of Southern Flying Squirrels (Borg et al. 2004). Their study incorporated controls within the same sampling season and found no mortality events, which was significant, in pipes with installed escape rope (Borg et al. 2004). Borg et al. (2004) also found that flying squirrel mortalities within pipes significantly reduced treefrog occupancy for those pipes when compared to pipes without mortalities. Although we never found a treefrog in a pipe with a dead mammal, our result was not significant in this comparison. We suspect having more mammal mortality events by increasing the sample size for this comparison would have led to a significant impact of dead mammals on treefrog pipe occupancy as found in Borg et al. (2004).

Studies with low occupancy rates of the target treefrog species should employ escape ropes to avoid biased detection results. Detection bias due to by-catch is rarely discussed in wildlife literature, but Edwards and Jones (2014) report on mammal mortality and capture rate in a drift fence and pit-fall trap study and suggested that predator exclusion interventions may have improved capture of target amphibians and reptiles. Similarly, our addition of escape ropes likely improved treefrog capture rate by eliminating mammal mortalities as decomposing carcasses likely deter treefrog use of refugia. Consequently, not detecting an individual in a refugia due to a dead mammal could influence the interpretation of results, especially if target species occupancy is already relatively low. These limitations on reliable knowledge are especially detrimental when dealing with threatened and endangered treefrog species, possibly leading to misguided management recommendations or conservation actions. We suggest more wildlife biologists investigate the effects of non-target species captures on target species capture results and inferences in their own wildlife studies and be more transparent in non-target species captures.

Because PVC refugia are widely used as a method to study arboreal herpetofauna, and from our own experience, we suspect mammal mortality goes unreported. Of all the papers we reviewed, only one study in subtropical North America (Hoffmann et al. 2018) studying interspecific competition between multiple native hylid treefrog species and Cuban Treefrogs (*Osteopilus septentrionalis*) reported using an escape rope. The only publication we found that mentioned mammal mortality as a result of surveying was a general anuran study that used pitfall traps and PVC refugia in West Africa where mortalities were discovered only in the pitfall traps (Mraz et al. 2018). In our systematic literature review (Appendix Table), we found no studies mentioning the use of escape ropes or other mortality mitigation strategies, suggesting that many treefrog studies do not use escape ropes in refugia. Within the same review, there was no mention of mammal mortalities, and we suspect mammal mortalities are under reported. Even if mortalities are not discovered in these studies, this is useful information to readers and the interpretation of results (e.g., Suriyamongkol et al. 2021).

Conclusions and recommendations.—Escape ropes can significantly reduce small mammal mortality in treefrog PVC refugia. Thus, we recommend adding escape ropes as preventative measures to all PVC refugia studies to ensure the lowest mammal mortality possible. We suggest that permitting agencies overseeing PVC studies in areas with threatened or endangered small mammals require implementation of escape ropes to prevent mortalities. In addition to significantly reducing mammal mortality, escape ropes likely reduce mortality for additional non-target vertebrates and invertebrates, and it appears the only means of escape for heavy-bodied taxa was the installed rope. We strongly encourage the use of escape ropes in PVC treefrog studies or similar wildlife studies in which smooth surfaces trap non-target animals, especially when pipes are affixed to tree trunks or hung from branches where there is an increased likelihood of non-target species becoming trapped. When escape ropes cannot be installed, we recommend appropriate by-catch data be collected to explore the possibility of non-target mortality/catch influencing the detectability of target species (e.g., using non-target mortality as a covariate when modeling detection within occupancy models; MacKenzie et al. 2017). To improve the design, we suggest escape rope knots at the top of the PVC pipe should be positioned directly against the trunk of the tree to further prevent animals from dislodging the rope from the pipe and be installed before pipe deployment to study areas. Finally, PVC refugia studies should close pipes during the inactive season to further prevent incidental mortalities. Ultimately, we hope our work reinforces a greater adoption of escape ropes.

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Appendix Table. Twenty-seven novel treefrog or salamander studies using PVC refugia/pipes that cited Boughten et al. (2000; a popular PVC technique paper) and had pipes attached to trees that were reviewed to determine the prevalence of mammal mortality being reported and if other mitigation strategies were being employed. Many other studies cited Boughten et al. (2000) but were not reviewed because they were not novel treefrog studies or their PVC refugia were not attached to trees or used open-ended pipes, suggesting limited chance of non-target mortalities. The literature review was restricted to 2005–2021 in Google Scholar, after the escape rope technique was first published (Borg et al. 2004).

Region of study	Number of studies	Source
Africa	2	Cabuy 2014; Trimble 2014
Central America	3	Laurencio et al. 2009; Brinkman 2016; Curlis et al. 2020
Central Pacific	1	Benard and Mautz et al. 2016
Europe	1	do Vale et al. 2018
North America	19	Muenz et al. 2006; Meyers et al. 2007; Piacenza 2008; Gloriosa et al. 2010; Granatosky 2010; Haggerty 2010; Windes 2010; Rice et al. 2011; O'Hare 2012; Haggerty and Crisman 2015; McDonald 2017; Smiley-Walters et al. 2017; O'Hare and Madden 2018; Rivera et al. 2019; Ceilley et al., unpubl. report; Howell et al. 2021; Hutto and Barrett 2021; McGhee 2021; McGrath-Blaser et al. 2021
South America	1	Ferreira et al. 2012