Transactions of the Missouri Academy of Science, Volume 48 (2021), pp. 17–21
doi:10.30956/MAS-33
Published online August 2, 2021

Assessing Summer Pond and Lake Inlet Use by Gray Treefrogs (*Hyla versicolor/chrysoscelis* complex) Using PVC Pipe Traps in Northwest Missouri

J. D. McGhee[1,2]

[1]Department of Natural Sciences, Northwest Missouri State University, Maryville, Missouri, USA
[2]Corresponding author. E-mail: jmcghee@nwmissouri.edu

Abstract: *The widespread decline in amphibian populations highlights the need for establishing rigorous monitoring methods for long-term population studies. In an attempt to launch a long-term monitoring study for a Gray Treefrog complex (*Hyla versicolor *LeConte* /chrysoscelis *Cope, hereafter treefrog) population in northwest Missouri, I tested the use of PVC pipe traps in a system of ponds and inlets along a lakeside habitat for three years. For each pond (3) and inlet (2), I established an array of 16 pipes so as to compare differences in use between pipe location, ponds and inlets, and sex ratio between sites. Pipes were checked twice a week during the summer for the presence of treefrogs. Treefrog usage of pipes between ponds and inlets were compared using a contingency table analysis, while an ANOVA was used to assess differences in sex ratios between sites ($\alpha = 0.05$). A single inlet was used by treefrogs more heavily than the other ponds or inlet ($G = 13.61$, $df = 3$, P $= 0.0035$), however, I found no differences in terms of pipe location within a pond or inlet. Mean sex ratio between water bodies varied but did not significantly differ. There appears to be little effect in terms of pipe placement within our 50 m buffer from the water's edge, but unique habitat effects at sampling locations may significantly affect detection rates or usage.*

Key words: *Monitoring*, *PVC pipe*, *Treefrog*

Introduction

Anuran populations are subject to a multitude of stressors that may act synergistically to contribute to their decline (Houlahan et al., 2000). These may include habitat degradation, invasive species, environmental contaminants and ultraviolet radiation, and chytrid fungus (Semlitsch, 2000; Blaustein et al., 2003; Kats and Ferrer, 2003; Rothermel et al., 2008). These stressors, in conjunction with the environmental variation inherent to a given habitat, act to create uncertainty in the vital rates of anuran populations. Specific to hylids, such as Gray Treefrogs and Cope's Gray Treefrogs complex (*Hyla versicolor,* LeConte*/chrysoscelis,* Cope), hereafter referred to as treefrogs, there is the added difficulty that populations are highly cryptic and difficult to sample outside of the breeding season (Johnson et al., 2008). Thus monitoring to assess presence/absence or annual vital rate change in these populations presents a conundrum for biologists (Lanoo et al., 1994; Biek et al., 2002). Yet robust monitoring methods and an understanding of the vital rates and population dynamics of these species are clearly needed and have been called for (MacKenzie et al., 2002; Storfer, 2003, Harper and Semlitsch, 2007). At least one way to begin to address this problem is to use common, well-tested monitoring methods to begin to assess populations. This study examines the effectiveness of a well-known monitoring method, the use of polyvinyl chloride (PVC) pipes, to assess a treefrog population's use of breeding ponds and lake inlets for a site in northwest Missouri.

Setting PVC pipes at breeding ponds has been a highly successful technique for capturing hylids, providing a high level of detectability and allowing the tracking of individuals (Boughton et al., 2000; Johnson et al., 2008; Pittman et al., 2008). This means PVC pipes are potentially an ideal sampling system for estimating hylid presence or absence in a sampling site (such as a pond or lake inlet), or for long-term monitoring to include the estimation of vital rates. Studies have examined the effectiveness of PVC pipe length, shape, and placement on trees (hardwood vs. conifer), humidity, rainfall, and air temperature, and terrestrial vs. aquatic usage of pipes (Boughton et al., 2000; Johnson et al., 2008; Pittman et al., 2008). However, with the exception of Johnson et al. (2007), who found very limited Gray Treefrog movement between ponds despite extensive movement outside of the breeding season, no studies have examined how effective PVC pipes are at detecting differential use of pond and/or inlet sites.

Hylid frogs express a high fidelity to breeding ponds which can be exploited with monitoring protocols to gain information throughout the year (Marsh and Trenham, 2001; Johnson et al., 2008). Marsh and Trenham (2001) point out, however, that while breeding ponds may be easy places to sample for breeding adults, exclusive use of these ponds for study may lead to biases in our understanding of amphibian population dynamics. Alternatively, relatively shallow lake inlets may be similar enough to ponds in terms of depth, and surrounding terrestrial habitat to allow for exploitation by treefrogs. Conversely, they may differ in terms of predator loads, as inlets will be more easily accessed by fish, which may have significant effects on treefrog populations (Smith et al. 1999).

Within ponds or inlets, treefrogs may space themselves from open water at varying degrees. Johnson and Semlitsch (2003) suggested a 60 m buffer around ponds to encompass breeding activity, but also reported that the majority of breeding activity (egg-laying in artificial pools) occurred within 15 m of a pond. Sampling within these buffer zones through the placement of PVC pipes could increase the likelihood of detection at a site. There may also be zones of preference even within these buffer zones. Pittman et al. (2008), for example, found that treefrogs tended to select pipes in close proximity to trees, and near the high-water line. An understanding of where to place pipes to a finer level of detail may allow us to determine where pipes are most productive within these broader buffer zones.

Treefrogs generally breed from early April to early July in Missouri, where males establish choruses to attract females who come in at night (Johnson, 2000). Males appear to stay near breeding sites where these choruses are established to a greater degree than females (Johnson et al., 2007). For this reason, sampling populations at breeding sites may result in biases toward males over females. This may in turn result in sex-related biases in vital rates, such as survival, that may be estimated using this PVC pipe monitoring system. Understanding sex ratio biases would allow for a correct estimation of these parameters.

In developing a PVC monitoring protocol, I wished to determine how treefrogs would use ponds and inlets on our field site at two scales: whether bodies of water (ponds and inlets) would be used differentially, and within these sites, whether pipes would be used differentially. In addition to this, I wanted to examine sex ratios in treefrog detections at each sampling site and between sites.

Methods

This study took place at Northwest Missouri State University and The Mozingo Outdoor Education Recreation Area (MOERA) in Nodaway County, Missouri during the summers of 2016 – 2018 (Lat-Lon: 40° 26′ 26″ N, 94° 46′ 16″ W). Nodaway County receives about 94cm of rainfall each year (U.S. Climate Data). Three ponds and two inlets of neighboring Mozingo Lake were sampled. Ponds and inlets were located an average (± SD) of 456.4 ± 214.66 m apart from each other. Ponds were small with the following approximate areas and depths: Pond A: 300 m^2, ~1 m deep; Pond B: 975 m^2, ~1 m deep; Pond C: 1950 m^2, ~1.5 m deep; Inlet D: 6000 m^2, ~1.5 m deep; Inlet E: 7480 m^2, ~1.5 m deep. Pond A would typically dry in August. Pond B dried up once in August 2018. Inlets D & E also receded significantly in August 2018. The ponds are not stocked with fish, nor periodically inundated with water from the lake, and would presumably have lower predation pressure from fish than inlet sites.

At each pond or inlet, within a 50m buffer from the water's edge, I randomly selected four trees to act as a centering point for four PVC pipes each. This placed the majority of pipes close to the water. The distance of the pipe furthest from the water ranged from 2.72 m to 16.25 m (mean ± SE: 6.79 ± 0.71) thus closely matching the 15 m buffer of highest breeding activity found by Johnson and Semlitsch (2003). Each array of four pipes were arranged such that a 0.6 m pipe was attached to the tree hanging vertically at about 1.5 m height, with the other 3 1-m tall pipes being hammered into the ground standing (ground-secured) upright at these locations: 2 m away from the tree towards the water, 5 m away from the tree towards the water, 2 m away from the tree in the direction opposite the water. Boughton et al. (2000) found that pipes attached to trees were most effective at 0.6 m in length attached at 2 m high and this is the reason for the size difference for "tree pipes". The trees pipes were attached to were Honey Locust (*Gleditsia triacanthos*), Eastern Red Cedar (*Juniperus virginiana*), Black Willow (*Salix nigra*), and Osage Orange (*Maclura pomifera*). A total of 16 pipes (four arrays) were placed at each pond or inlet. Pipes were 3.8 cm in diameter (Boughton et al., 2000; Pittman et al., 2008). Pipes were checked twice per week during each sampling season which started in early May and ended in late August. Johnson and Semlitsch (2003) found some evidence of breeding (oviposition in artificial pools) as late as mid-August in their study site in south-central Missouri.

If a treefrog was in a pipe, it was gently pushed using a metal rod into a plastic bag, measured for snout-vent length (svl) in mm, weighed to the nearest gram, and sexed based on throat coloration. A new bag was used for each individual captured except when multiple individuals used the same pipe. Treefrogs were marked with visible implant alphanumeric tags (VI Alpha© tags, Northwest Marine Technology, Inc, Shaw Island, Washington, USA) under the skin in the tibiofibular region of the leg. Clemas et al. (2009) found that VI Alpha tags were a reliable method for marking anurans. The injection needle for the tag was sterilized with isopropyl alcohol before and after each injection of a tag, the incision site was closed with New Skin® (Moberg Pharma North America), and the treefrog was returned to its pipe.

I used a contingency table analysis (Zar, 1999) to test the independence of pipe usage by site (Ponds A, B, C, Inlets D, E), pipe location (on tree, 2m past tree, 2m from tree towards pond, 5m from tree towards pond), and year (2016, 2017, 2018). As with Johnson et al. (2008), only unique pipe-frog captures were analyzed since treefrogs would be found in the same pipes in subsequent sampling periods. I used a chi-square test to determine if there was a significant bias in sex ratio across years for each site and I used a one-way ANOVA to test for average sex ratio differences between water bodies. For all statistical tests, $\alpha = 0.05$, and Bonferroni adjustments were made to account for multiple chi-square tests and subdivided contingency tables.

Results

Over the course of three field seasons, I marked 148 treefrogs in the three ponds and two inlets (62 in 2016, 29 in 2017, 57 in 2018). We identified the sex of 138 individuals (58 females and 80 males) for an overall sex ratio of 0.73 females per male. Mean monthly detection rates (the probability of detecting a treefrog in a PVC pipe) were (± SE) 0.12 ± 0.01 in May, 0.09 ± 0.01 in June, 0.14 ± 0.01 in July, and 0.15 ± 0.01 in August. Daily detection rates averaged (± SE) 0.12 ± 0.01 in 2016, 0.09 ± 0.01 in 2017, and 0.16 ± 0.01 in 2018. The proportion of pipes that were occupied by a treefrog at least once over the sampling period was 0.53 in 2016, 0.36 in 2017, and 0.61 in 2018. Recaptures were common, with many individuals being found in the same pipe (one male, K38, was found in the same pipe 38 times over a three year period).

The combination of categories for contingency table analysis (pipe location x site x year) allowed for a number of zeros to occur in the data set (Feinberg 1970). As a solution to this, I conducted a heterogeneity test to determine if the data could be pooled by year (Zar 1999). The outcome of the heterogeneity test suggested that this was reasonable ($G = 23.40$, $df = 24$, $P = 0.4966$). The pooled contingency table analysis did detect a difference between pond and inlet use ($G = 9.00$, $df = 3$, $P = 0.0267$), so I subdivided the contingency table to compare each individual site to all other sites (Bonferroni adjustment of $\alpha <= 0.01$) and found that this difference was driven by inlet D in which we detected significantly more treefrogs than either the ponds or inlet E ($G = 13.61$, $df = 3$, $P = 0.0035$, Table 1). I also compared each pipe location to all other locations (Bonferroni adjustment of $\alpha = 0.0125$), but found that pipe location had no significant effect on use by treefrogs (A: $G = 4.43$, $df = 3$, $P = 0.2183$; B: $G = 2.00$, $df = 3$, $P = 0.5720$; C: $G = 10.76$, $df = 3$, $P = 0.0131$; E: $G = 2.06$, $df = 3$, $P = 0.5597$). The overall sex ratio (female:male) in pond B (0.19) and inlet E (0.14) was significantly male-biased (B: $\chi^2 = 11.56$, $df = 1$, $P = 0.0006$; E: $\chi^2 = 9.94$, $df = 1$, $P = 0.0016$), but no other sites were significantly different from 1:1 (Bonferroni adjustment of $\alpha = 0.01$; A: $\chi^2 = 3.60$, $df = 1$, $P = 0.0578$; C: $\chi^2 = 0.04$, $df = 1$, $P = 0.8415$; D: $\chi^2 = 3.69$, $df = 1$, $P = 0.0549$). I also conducted the chi-square analysis limiting the data to the primary breeding months of May and June (Johnson 2000). Under this constraint, only pond B was male-biased (0.2 female:male; $\chi^2 = 8.00$, $df = 1$, $P = 0.0047$). Sex ratio in the other water bodies in May and June was not different from 1:1 (Bonferroni adjustment of $\alpha = 0.01$; A: $\chi^2 = 2.00$, $df = 1$, $P = 0.1573$; C: $\chi^2 = 2.57$, $df = 1$, $P = 0.1088$; D: $\chi^2 = 1.20$, $df = 1$, $P = 0.2733$; E: $\chi^2 = 5.44$, $df = 1$, $P = 0.0196$). Mean sex ratio between water bodies varied but did not significantly differ ($F_{2,12} = 0.5799$, $P = 0.5749$).

Table 1. The number of unique frog detections by pipe location from three ponds and two lake inlets of Mozingo Lake, Nodaway County, MO for May through August 2016–2018. Pipe locations refer to the placement of PVC relative to a randomly selected tree within 50 m of the pond's edge. Numbers represent the number of times a unique individual frog occurred in a pipe. Recounts of individual frogs only occur if it moved between pipes. Sex ratio refers to the number of females per male for each water body pooled across the three year period.

	Water Body				
Pipe Location	Pond A	Pond B	Pond C	Inlet D	Inlet E
2 m away of tree	10	9	19	32	19
on tree	2	6	6	10	6
2 m towards water	3	10	12	32	9
5 m towards water	1	6	1	29	6
Sex Ratio	0.25	0.19	0.92	1.65	0.13

Discussion

Treefrogs do not appear to use ponds with any greater affinity than lake inlets during summer at our site. This suggests that lake inlets should be incorporated into PVC pipe trap monitoring efforts. Inlet D was a notable exception to this general finding, with significantly more unique treefrog detections than its sister inlet or the other ponds. It is difficult to infer the reasons for this from a single site, but this inlet did seem to have a higher density of trees than other sites and this may have the effect of collecting moving treefrogs. Johnson and Semlitsch (2007) found that Gray Treefrogs might move as far as 200m during the breeding season, and it stands to reason that a higher density of trees would allow for more movement and more foraging sites, or simply more spatial area for frogs to use.

At ponds and inlets, at least within 16.25 m of water, pipe location made little difference in the amount of use it received from treefrogs. Johnson and Semlitsch (2003) found that most treefrogs occurred in PVC pipes within 60 m from the water, with the majority of breeding activity within 15 m of the water. Pittman et al. (2008) found during the peak of the breeding season, most frogs were detected in pipes 10.0–11.5 m from the water. Having placed pipes well within this range, I found no finer scale of differentiation. Similarly, I was unable to detect a difference in use between tree-mounted shorter pipes (60 cm), and 1-m pipes hammered into the ground. Boughton et al. (2000) found a preference by a suite of Hylid frogs, but primarily Squirrel Treefrogs (*Hyla squirella*) and Green Treefrogs (*Hyla cinerea*) for tree-mounted 60-cm pipes placed at 4 and 2 m above ground, relative to 60 cm pipes placed at the base of the tree. However, Zacharow et al. (2003) found that Green Treefrogs and Squirrel Treefrogs readily used ground-secured pipes 60-cm high. Likewise, Pittman et al. (2008) found that Cope's Gray Treefrogs readily used ground-secured pipes 1.5-m high. Ground-secured pipes appear to be used as readily as tree-mounted pipes, at least when placed close to water.

Between the ponds and inlets, two sites out of five demonstrated a significant male bias, with one site of five significantly male-biased in May and June, despite lower sample sizes. Under both tests, pond C was consistently male-biased, while inlet E was only significant under the broader time period with higher sample sizes. In addition, when considering average sex ratio (by year) across sites, variability prevented us from statistically distinguishing ponds from each other based on sex ratio. The combination of being unable to distinguish ponds and inlets by annual sex ratio, with only a single pond being significantly male-biased, and an inlet with a significant male-bias (when considering a broader time scale) is suggestive that inlets may potentially be used as breeding sites. This would strengthen Marsh and Trenham's (2001) argument that the attitude of treating shallow ponds alone as the center of the population for treefrogs is unwarranted, at least for this study area. Johnson et al. (2007) came to a similar conclusion when they found that the movement of treefrogs away from different breeding ponds overlapped and could allow individuals to shift between ponds. Treefrogs may be more opportunistic regarding potential breeding sites than previously believed. Johnson et al. (2007) also found that females tended to move further from breeding ponds than males, returning at night only after males have had time to establish choruses. Movement of females away from breeding ponds may be habitat-specific or driven by prey availability (Johnson et al. 2007). Regardless of the reasons for these differences in sex ratio, the presence of a male bias even at only some sites suggests that vital rate estimates, if the true value differs between sexes, may be skewed towards the male value. This bias should be measured on a site by site basis and accounted for in vital rate estimation or population modeling efforts in future studies.

Using PVC pipes to detect treefrogs in both ponds and lake inlets, with some freedom in pipe placement within a 16.25 m buffer of the water's edge will undoubtedly supplement long-term monitoring efforts for the study of treefrogs. This practice may be used to help understand variation in sex ratios in differing habitats, provide a starting place for regional monitoring in northwest Missouri, and develop better population models for these amphibians.

Acknowledgments

I would like to thank B. Lang, S. Olson, A. Athen, A. Slagle, H. Bassich, T. Sutton, R. Dawson, C. Bridgeman, and K. Nowack for their on-going help with field work for this project. Field data were collected under a Missouri scientific collector's permit (15836, 16715). Frogs were sampled under IUCAC number 200102.

Literature Cited

Biek, R. W., C. Funk, B. A. Maxell, and L. S. Mills. 2002. What is missing in amphibian decline research: insights from ecological sensitivity analysis. Conservation Biology 16: 728–734.

Blaustein, A. R., J. M. Romansic, J. M. Kiesecker, and A. C. Hatch. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. Diversity and Distributions 9: 123–140.

Boughton, R. J., J. Staiger, and R. Franz. 2000. Use of PVC pipe refugia as a sampling technique for hylid treefrogs. American Midland Naturalist 144: 168–177.

Clemas, R. J., J. M. Germano, R. Speare, and P. J. Bishop. 2009. Use of three individual marking methods in Australian frogs (Genus: Litoria) with notes on placement of visible implant alphanumeric tags. New Zealand Natural Sciences 34: 1–7.

Feinberg, S. E. 1970. The analysis of multidimensional contingency tables. Ecology 51: 419–433.

Harper, E. B., and R. D. Semlitsch. 2007. Density dependence in the terrestrial life history stage of two anurans. Oecologia 153: 879–889.

Houlahan, J. E., S. C. Findlay, B. R. Schmidt, A. H. Meyer, and S. L. Kuzmin. 2000. Quantitative evidence for global amphibian population declines. Nature 404: 752–755.

Johnson, J. R., and R. D. Semlitsch. 2003. Defining core habitat of local populations of the gray treefrog (*Hyla versicolor*) based on choice of oviposition site. Oecologia 137: 205–210.

Johnson, J. R., J. H. Knouft, and R. D. Semlitsch. 2007. Sex and seasonal differences in the spatial terrestrial distribution of gray treefrog (*Hyla versicolor*) populations. Biological Conservation 140: 250–258.

Johnson, J. R., R. D. Mahan, and R. D. Semlitsch. 2008. Seasonal terrestrial microhabitat use by gray treefrogs (*Hyla versicolor*) in Missouri oak-hickory forests. Herpetologica 64: 259–269.

Johnson, T. R. 2000. The Amphibians and Reptiles of Missouri. Jefferson City, Missouri: Missouri Department of Conservation.

Kats, L. B., and R. P. Ferrer. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions 9: 99–110.

Lanoo, M. J., K. Lang, T. Waltz, and G. S. Phillips. 1994. An altered amphibian assemblage: Dickinson county, Iowa, 70 years after Frank Blanchard's survey. The American Midland Naturalist 131: 311–319.

MacKenzie, D. L., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle, and C. A. Langtimm. 2002. Estimating site occupancy when detection probabilities are less than one. Ecology 83: 2248–2255.

Marsh, D. M., and P. C. Trenham. 2001. Metapopulation dynamics and amphibian conservation. Conservation Biology 15: 40–49.

Pittman, S. E., A. L. Jendrek, S. J. Price, and M. E. Dorcas. 2008. Habitat selection and site fidelity of Cope's gray treefrog (*Hyla chrysoscelis*) at the aquatic-terrestrial ecotone. Journal of Herpetology 42: 378–385.

Rothermel, B. B., S. C. Walls, J. C. Mitchell, C. K. Dodd, Jr., L. K. Irwins, D. E. Green, V. M. Vazquez, J. W. Petranka, and D. J. Stevenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Diseases of Aquatic Organisms 82: 3–18.

Semlitsch, R. D. 2000. Principles of management of aquatic breeding amphibians. Journal of Wildlife Management 64: 615–631.

Smith, G. R., J. E. Rettig, G. G. Mittelbach, J. L. Valiulus, and S. R. Schaack. 1999. The effects of fish on assemblages of amphibians in ponds: A field experiment. Freshwater Biology 41: 829–837.

Storfer, A. 2003. Amphibian declines: future directions. Diversity and Distributions 9: 151–163.

U.S. Climate Data. 2020. Climate Maryville – Missouri. https://www.usclimatedata.com/climate/maryville/missouri/united-states/usmo0565. Accessed 24 Oct 2020.

Zacharow, M., W. J. Barichivich, C. K. Dodd, Jr. 2003. Using ground-placed PVC pipes to monitor hylid treefrogs: Capture biases. Southeastern Naturalist 2: 575–590.

Zar, J. H. 1999. Biostatistical Analysis, 4th Edition. Prentice Hall, Upper Saddle River, NJ.