USE OF PVC PIPES OF DIFFERENT DIAMETERS AS ARTIFICIAL REFUGES BY GREEN TREE FROGS (*HYLA CINEREA*) IN TEXAS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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With the sharp decline in amphibian populations worldwide, it is becoming increasingly important to evaluate use of artificial refuges by amphibian species. The objective of this study is to evaluate PVC pipes as artificial refuges for green tree frogs (*Hyla cinerea*) in Texas. This method has been widely used in the southeast US, but only rarely in Texas. I will analyze unpublished field data on green tree frogs provided by colleagues at Texas State University and assess possible differences in use of PVC pipes of different diameters (1.5-inch versus 2.0-inch diameters). Based on the data collected by Texas State University, green tree frogs do utilize PVC pipes as artificial refuges. Moreover, they do not show preference in either 1.5-inch (46.5% of captures) or 2-inch (53.5% of captures) diameter pipes. In addition, 64.3% of captures were juveniles, more of which were captured around the pond perimeter, suggesting that PVC pipes might serve as protection for juveniles post-emergence.

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CHAPTER I

INTRODUCTION

Global amphibian decline

Around the world, amphibian species are facing mass extinctions, and their populations are declining faster than both birds and mammals (Stuart et al., 2004). Although habitat loss is probably the primary reason for population extirpation, diseases such as chytridiomycosis (Voyles et al., 2009), parasitism (Johnson and Chase, 2004), the effects of toxic chemicals (Blaustein et al., 2003), increased levels of UVb light (Blaustein et al., 2003), and changing climatic conditions (Araújo et al., 2006; McMenamin et al., 2008) all negatively affect amphibian populations to one degree or another. Pollution and UVb radiation compound on other factors to further limit the already restricted area of viable habitat for amphibians. The chemicals used to manufacture herbicides and pesticides can act directly to kill amphibians via immunosuppression if applied in excess. In addition, studies suggest that aquatic acidification can reduce larval growth rates and body size, increase embryonic and larval mortality, as well as decrease overall recruitment and diversity of breeding assemblages in amphibian communities. (Alford, 1999) Depletion of stratospheric ozone, aquatic acidification and climate warming all contribute to UVb radiation, which can also reduce growth rates, increase immune system malfunction, cell mutation, or cause direct fatality. (Blaustein, 2003) Amphibians are particularly vulnerable to habitat fragmentation because of their low migration rates, high mortality when moving across roadways, and narrow habitat tolerances. (Cushman, 2006) Ultimately, the threats to amphibian species should be viewed synergistically, as they work together to drastically reduce the range in which amphibian species can thrive. Though overarching climactic and ecological changes are less feasibly reversed, there are ways we can restore viability to superficial habitat destruction, including restoration techniques such as the erection of artificial refuges. Because of the rising number of threatened and endangered species of amphibians, there is an emphasis being placed on evaluation of capture techniques such as pitfalls, drift fences, and cover boards (Mitchell et al., 2006) to survey amphibian species and estimate their abundances.

Capture techniques

Traditional techniques used to collect herpetofauna include searching/hand capture, pitfall traps, drift fences, single and double ended funnel traps, dip nets, and seine nets. Target species should determine the type of trap used, as different traps are more effective in the collection of certain species of herpetofauna. Dip nets and seine nets are used primarily in the capture of aquatic salamanders, newts, sirens, and other amphibious species. Combinations of other trapping techniques are used for species that inhabit terrestrial environments. Data obtained from terrestrial captures is often used to estimate species richness, abundance, and habitat use. (Mitchell, 1993) To construct a capture site, plastic buckets are placed in holes so that they are level with the ground to create a pitfall at the center of multiple arrays of mesh drift fences. Funnel traps may be lined up along the edges of drift fences and used in combination with pitfalls. While these capture methods have proven effective in the capture of most anurans, salamanders, lizards, and small snakes, the ability of tree frogs to evade capture both by climbing out of pitfall traps (Greenberg et al., 1994), moving over drift fences (Dodd, 1991), and to evade detection in densely-vegetated habitat, has led to the development of new capture techniques. Successful new techniques include a modified drift fence made from clear plastic sheeting

(Parris et al., 1999) and artificial refuges such as PVC pipe (Boughton et al., 2000), and bamboo (Kam et al., 1998). PVC pipes have shown to be highly effective in the study of tree frogs. Pipes are manufactured using the same sizes and materials, allowing experiments to be easily repeated. In addition, PVC trapping has been shown to minimize the effects of observer bias encountered in hand capture because there is a high concentration of target species in an easily searchable area. (Willson, 2010) Although PVC pipes have been widely used in the southeast US, to my knowledge, only one study in Texas used this method to evaluate tree frog assemblages (Glorioso and Waddle, 2014). In previous studies capture efficiencies have varied among pipes shapes, sizes, and/or locations, which indicate that tree frogs are selective in their use of PVC pipes. Selection appears to be species specific, varying depending on the time of year, diameter and shape of the pipe, and whether there is a cap on the end to retain moisture. (Zacharow et al., 2003) It is possible that tree frogs may be more inclined to use PVC pipes as an alternative refuge when their habitat is destroyed by fire or anthropogenic causes.

CHAPTER II

METHODS

Focal species

Hyla cinerea (the green tree frog) was selected as the focal species due to its high abundance in the study area. Like most tree frogs, *Hyla cinerea* have adhesive toe pads that allow them to stick to surfaces in their arboreal habitats. They are green with white dorsolateral stripes that extend from the mouth to mid body and often have white or yellow spots on their backs although coloration is highly variable depending on geographic location, conditions of light, moisture, temperature, and stress. (Conant et al., 1998) *Hyla cinerea* commonly inhabit water lily prairies, cypress ponds and dense thickets along the southeastern border of the United States from Texas to Delaware. They can be found clinging to twigs or grasses near the ground, or resting on low branches but few climb to higher parts of trees. They occupy tree holes or burrow in the ground during periods of inactivity in the cool weather. (Wright, 2002) Breeding season occurs from April to September, peaking in June and July. Males have single median pharyngeal sac that inflates when calling to attract mates. Males sometimes practice sexual parasitism and non-calling satellite males will intercept mates moving towards callers. (Perrill, 1978) Pond vegetation serves as substrate for eggs and habitat for tadpoles. Juveniles typically remain near the breeding site and will double in size as they reach sexual maturity after the first year. (Garton, 1975) Their diets consist of small insects and invertebrates, so they are often seen on windows where lights attract their prey and there is some debate about whether prey selection is indiscriminate or based on prey activity. (Freed, 19980) They are preyed upon by snakes, lizards and birds. Though this species is not currently listed, changing climactic conditions and habitat loss have effected populations causing them to expand northward into previously uninhabited states such as Illinois. (Tucker, 2006) Green tree frogs are common in the pet trade and have a lifespan of 2-3 years.

Study area

Colleagues from Texas State University collected this data at the Griffith League Scout Ranch property in Bastrop, Texas. The warmest month on average in Bastrop is August with an average high of 97 degrees Fahrenheit and an average low of 72 degrees Fahrenheit, while the coldest month is January with an average high of 63 degrees Fahrenheit and an average low of 38 degrees Fahrenheit. Average yearly rainfall is 37.7 inches, with May and October being the wettest months and July and August being the driest. (Weather.com, 2016) Bastrop lies within the Texas post oak savannah ecoregion and contains a diverse array of flowering plants, shrubs, grasslands and most notably, the unique pine-oak forest habitat named “The Lost Pines” ecosystem. According to pollen records, pine dominated forests have persisted in the sandy soils of this central Texas area since 18,000 years ago. The genetically unique Lost Pines region near Bastrop, Texas was burned in a series of devastating fires in 2011 (Brown et al., 2014) and studies focused on the regrowth of vegetation in the region revealed that areas which were more severely burned are still in the process of restoration. (Lee, 2015) Even though high severity fires have been shown to increase nutrient levels and productivity in aquatic environments (Spencer et al., 2003) and increase diversity of understory plants in terrestrial environments (Sánchez Alfaro et al., 2015), recently-burned habitats lack the canopy cover used by many species (Brown et al., 2014). Therefore it was a good time to test if the PVC pipes could be used as refuges of green tree frogs (*Hyla cinerea*), which are an abundant amphibian species in that location, and whether the green tree frogs do exhibit preferences among different refuge diameter sizes.

Trapping design

Data was collected from two ponds in the burned forest and two ponds in the unburned forest once a week from June through October, which encompasses the main breeding period of the green tree frog. Twenty PVC pipes of alternating 1.5 and 2 inch diameters were placed at each pond, ten around the perimeter and ten in the surrounding vegetation, for a total of eighty PVC pipes. All pipes were five feet tall and positioned at a 90-degree angle from the ground in which they were placed. Pipes that were placed in vegetation were tied to loblolly pines, Salix, Juniper and Yaupon. The data collected included the locale of capture, specifying the pond and the pipe, morphological measurements including snout to urostyle length, head width, and mass of each frog. Specimens exceeding 30 mm in snout to urostyle length were recorded as adults, marked, and tissue samples were collected. Environmental data such as time of day, temperature, relative humidity, wind speed and hydro-period were also recorded using a Kestrel upon the arrival of each capture site.

CHAPTER III

RESULTS

A total of 223 observations of *Hyla cinerea* were recorded from the first sampling event on June 21st, 2015 to the final sampling event on October 4th, 2015. Descriptive capture data of sample events and captures per pond can be found in Table 1. Additional data regarding pipe preferences can be found in Tables 2, 3 and 4. Chi square analysis was used to evaluate pipe preference of green tree frogs.

Table 1: Number of frogs per pond using PVC pipes

	Number of frogs per pond using PVC pipes							
	Pond1		Pond5		Pond13		Pond14	
Sampling event	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile
1			1		1			
2			2	1		16		
3			2	1		4		
4			1	2		7		
5			3	2				
6			2					
7	3	3	3	5	1	12		
8	3	1		5	1	7		1
9	4	5	2	10	3	9		
10	4	1	2	9	4	8		
11	5		2	4	2	3		
12	1	2	2	2	2	2		
13	2		3	3	2	7		
14	4	1	1	4	2	3		
15	1	1	8	5	3	2		
Total (out of 223)	27	14	34	53	21	80		1

Preference for pipe diameter

Table 2 details the numbers of adults and juveniles found in pipes of different diameters. 120 tree frog observations were recorded in pipes with 2 in. diameters (53.8% of our total captures) while 103 observations were recorded in pipes with 1.5 in. diameters (47.2% of total observations).

Table 2: Descriptive data on the use of pipes of different diameters

Adults and Juveniles in Pipes of Different Diameters			
	1.5 in.	2 in.	Total
Adults	37	40	77
Juveniles	66	80	146
Totals	103	120	

The difference between the number of individuals using pipes of different diameter was statistically not significant ($X^2 = 1.291$, df = 1, $P > 0.05$). When separated into adults ($X^2 = 0.117$, df = 1, $P > 0.05$) and juveniles ($X^2 = 1.342$, df = 1, $P > 0.05$) the difference was still not statistically significant.

Preference for pond or tree pipes of different diameters

Table 3 further divides the numbers of adults and juveniles found in pipes of different diameters by whether the pipe was attached to surrounding trees or positioned on the perimeter of the pond.

Table 3: Descriptive data on the use of pond vs. tree pipes of different diameters

Adults and Juveniles in Pond vs. Tree			
	1 in.	2 in.	Totals
Adults			77
Pond Pipes	21	22	43
Tree Pipes	16	18	34
Juveniles			146
Pond Pipes	48	46	94
Tree Pipes	18	34	52

There was no statistically significant difference in the use of pipes of different diameter both around the pond only ($X^2 = 0.007$, df = 1, $P > 0.05$) and in the surrounding vegetation only ($X^2 = 3.767$, df = 1, $P = 0.0523$). Green tree frogs did however show a preference for pond pipes over tree pipes regardless of diameter. ($X^2 = 11.664$, df = 1, $P < 0.05$). When further divided into juvenile and adult categories, there was no significant difference in use of pond pipes by adults ($X^2 = 1.052$, df = 1, $P > 0.05$) but juveniles showed preference for 2 in. pipes ($X^2 = 12.082$, df = 1, $P < 0.05$).

Preference for pipe diameter in burned and unburned areas

Table 4 further divides the numbers of adults and juveniles found in pipes of different diameters by whether the pipe was in a location that was burned (ponds ? & ?) or unaffected (ponds ? & ?) by the Bastrop fire in 2011.

There was no statistically significant difference in the use of pipes of different diameter in the burned areas ($X^2 = 2.510$, df = 1, $P > 0.05$) even when divided into adult ($X^2 = 0.048$, df = 1, $P > 0.05$) and juvenile ($X^2 = 2.778$, df = 1, $P > 0.05$) categories. However, green tree frogs did show statistically significant preference for 2 in. pipes in the unburned areas ($X^2 = 9.00$, df = 1, $P < 0.05$). Specifically juveniles showed statistically significant preference for 2 in. pipes ($X^2 = 12.938$, df = 1, $P < 0.05$), while adults did not ($X^2 = 0.286$, df = 1, $P > 0.05$).

Body size and pipe diameter

Table 5: Descriptive statistics on frog size and body mass of frogs in 1 and 2 in. pipes. SUL = Snout to urostyle length (mm); HW= head width (mm); body mass (g); N = number of captures.

Diameter Size	Variable	N	Mean (Range)
1.5 in.	SUL (mm)	103	29.08 mm (8.51-54.73 mm)
	HW (mm)	101	10.45 mm (0.5-17.44 mm)
	Body mass (g)	103	1.47 g (0.3-10.9 g)
2 in.	SUL (mm)	120	26.25 mm (16.7-54.99 mm)
	HW (mm)	110	10.59mm (6-18.86 mm)
	Body mass (g)	120	1.2 g (0.1-12.2 g)

Table 6: Box plot illustration of SUL data spread

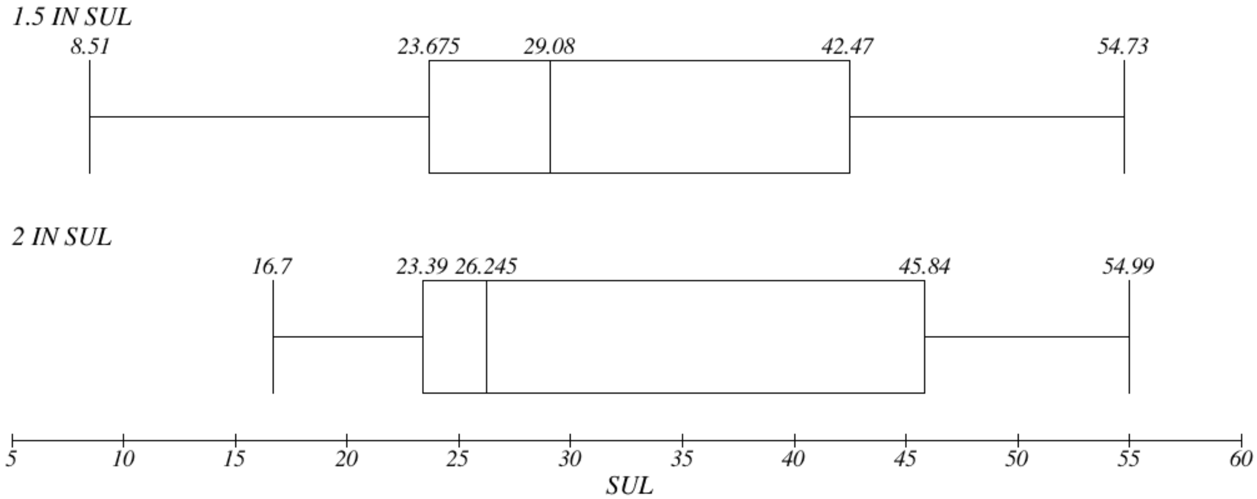
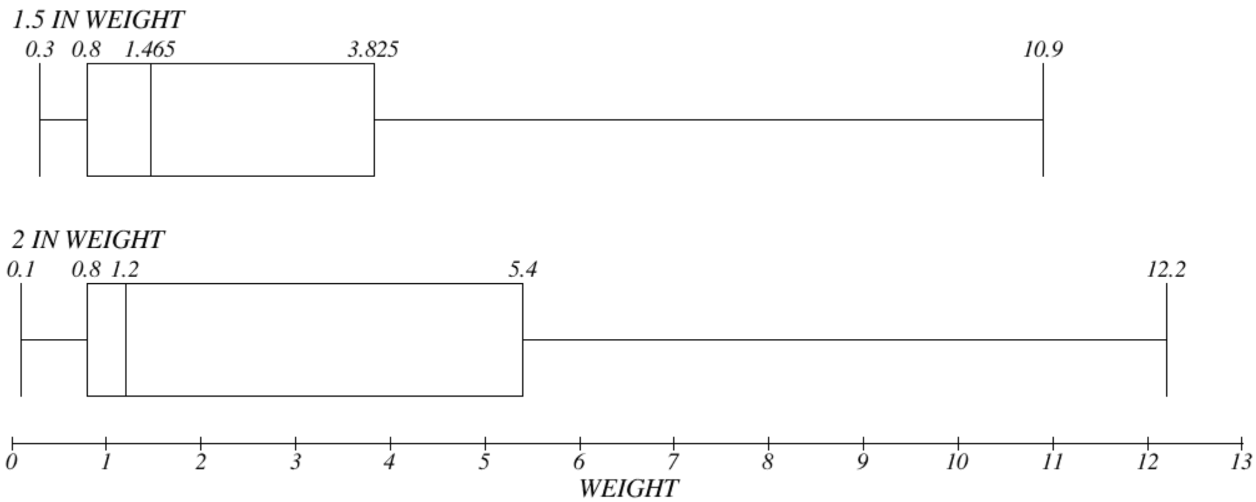


Table 7: Box plot illustration of Body weight data spread



CHAPTER IV

CONCLUSION

Based on the results of this study, green tree frogs do not show preference in pipes of 1.5 in. and 2 in. diameters. Moreover, body size does not differ significantly in pipes of 1.5 in. and 2 in. diameters. However, in unburned areas juvenile green tree frogs favor pipes with a 2 in. diameter to pipes with a 1.5 in. diameter. The results show that green tree frogs do favor pond pipes to tree pipes regardless of diameter, particularly if they are juveniles. Because more juvenile frogs were found in the pipes located at the water's edge, it is possible that juveniles could be using the PVCs as protection post-emergence. Evaluating pipe preference is a complex task because each facet of preference (ie. Shape, size, diameter, distance from the pond, etc.) warrant independent studies to produce definitive results. My recommendation for future studies is to increase the control in this experiment by reducing variation in tree species used for pipes tied to surrounding vegetation, to adjust the "pond pipes" as hydroperiod decreases in the warmer weather to ensure they stay at the water's edge, and increase the sample size with more regular and numerous site visits. However experimenters must keep in mind that increasing the control of these experiments may impact the outcome of the study because "lab settings" do not often coincide with the natural balance of ecosystems. In the future it may be useful to assess the microclimates within the pipes throughout the study to gain insight on pipe preferences, and seek a deeper understanding of juvenile dispersal post-emergence. Little is known about the behavior of juvenile Hyla cinerea and community structures that may or may not exist post emergence.

REFERENCES

Alford, R. A., & Richards, S. J., 1999. Global Amphibian Declines: A Problem in Applied Ecology. Annu. Rev. Ecol. Syst, 30, 133-65.

Araújo, M.B., Thuiller, W. and Pearson, R.G., 2006. Climate warming and the decline of amphibians and reptiles in Europe. Journal of Biogeography, 33:1712-1728.

Bastrop, TX Monthly Weather Forecast. 2016. Retrieved March 27, 2016, from https://weather.com/weather/monthly/l/USTX0082:1:US

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

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

Brown, D.J., Duarte, A., Mali, I., Jones, M.C. and Forstner, M.R., 2014. Potential impacts of a high severity wildfire on abundance, movement, and diversity of herpetofauna in the Lost Pines ecoregion of Texas. Herpetological Conservation and Biology:192-205.

Conant, R., and J. T. Collins. 1998. A Field Guide to Reptiles & Amphibians. Eastern and Central North America. Third Edition, Expanded. Houghton Mifflin Company, Boston. 532 pp.

Corn, P. S., & Bury, R. B. 1990. Sampling methods for terrestrial amphibians and reptiles. *General technical report PNW (USA)*.

Cushman, S. A., 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation, *128*, 231-240.

Dodd, C.K., Jr., 1991. Drift fence-associated sampling bias of amphibians at a Florida sandhills temporary pond. Journal of Herpetology, 25:296-301.

Freed, A. 1980. Prey Selection and Feeding Behavior of the Green Tree Frog (Hyla cinerea). *Ecology*, 61/3: 461-465.

Garton, J. S., & Brandon, R. A., 1975. Reproductive ecology of the green treefrog, Hyla cinerea, in southern Illinois (Anura: Hylidae). *Herpetologica*, 150-161.

Greenberg, C.H., Neary, D.G. and Harris, L.D., 1994. A comparison of herpetofaunal sampling effectiveness of pitfall, single-ended, and double-ended funnel traps used with drift fences. Journal of Herpetology, 28:319-324.

Johnson, P.T.J. and Chase, J.M., 2004. Parasites in the food web: linking amphibian malformations and aquatic eutrophication. Ecology Letters, 7:521-526.

Kam, Y.-C., Yen, C.-F. and Hsu, C.-L., 1998. Water balance, growth, development, and survival of arboreal frog eggs (*Chirixalus eiffingeri*, Rhacophoridae): Importance of egg distribution in bamboo stumps. Physiological Zoology, 71:534-540.

Lee, R. J., & Chow, T. E. 2015. Post-wildfire assessment of vegetation regeneration in Bastrop, Texas, using Landsat imagery. *GIScience & Remote Sensing*, *52*(5), 609-626.

McMenamin, S.K., Hadly, E.A. and Wright, C.K., 2008. Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. Proceedings of the National Academy of Sciences, 105:16988-16993.

Mitchell, J. C., Erdle, S. Y., & Pagels, J. F., 1993. Evaluation of capture techniques for amphibian, reptile, and small mammal communities in saturated forested wetlands. *Wetlands*, *13*(2), 130-136.

Parris, K.M., Norton, T.W. and Cunningham, R.B., 1999. A comparison of techniques for sampling amphibians in the forests of south-east Queensland, Australia. Herpetologica, 55:271-283.

Perrill, S. A., Gerhardt, H. C., & Daniel, R. (1978). Sexual parasitism in the green tree frog (Hyla cinerea). *Science*, *200*(4346), 1179-1180.

Sánchez Alfaro, R., Camarero, J.J., Serrano, F.R.L., Sánchez-Salguero, R., Moya, D. and Heras, J.D.L., 2015. Positive coupling between growth and reproduction in young post-fire Aleppo pines depends on climate and site conditions. International Journal of Wildland Fire, 24:507-517.

Spencer, C.N., Gabel, K.O. and Hauer, F.R., 2003. Wildfire effects on stream food webs and nutrient dynamics in Glacier National Park, USA. Forest Ecology and Management, 178:141-153.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. and Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. Science, 306:1783-1786.

Tucker, J. K. , J. T. Lamer and C. R. Dolan. 2006. Hyla cinerea. Herpetological Review 37(4): 488.

Vogt, R.C., Hine, R. L. 1982. Evaluation of techniques for assessment of amphibian and reptile populations in Wisconsin. In: Scott NJ Jr, editor. Wildlife Research Report 13, U.S. Department of the Interior, Fish and Wildlife Service, Washington DC 1982. p 201-217.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerratt, L.F. and Speare, R., 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science, 326:582-585.

Willson, J. D., & Gibbons, J. W., 2010. Drift fences, coverboards, and other traps. *Amphibian ecology and conservation: a handbook of techniques. Oxford University Press, Oxford*, 227-246.

Wright, A. H., 2002. *Life-histories of the Frogs of Okefinokee Swamp, Georgia: North American Salientia (Anura)* (Vol. 2). Cornell University Press.

Zacharow, M., Barichivich, W.J. and Dodd, C.K., 2003. Using ground-placed pvc pipes to monitor hylid treefrogs: capture biases. Southeastern Naturalist, 2:575-590.