Habitat Selection and Ontogenetic Shifts in Treefrogs (Family: Hylidae)

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Abstract

Few studies have concentrated on the life history characteristics of arboreal anurans away from breeding ponds, and sampling of these species is difficult due to their ability to evade visual detection and other traditional herpetological census techniques. This study utilizes polyvinyl chloride (PVC) pipes to test for habitat selection of hylid treefrogs at the Natural Areas Teaching Lab in the University of Florida Campus (Gainesville, Florida). Variables such as habitat type, trap elevation, and PVC pipe diameter were used to test selection and ease of capture in relation to abundance, phylogeny, and ontogeny. A separate analysis sought to determine the prevalence of predation on native treefrog species by the exotic Cuban Treefrog (*Osteopilus septentrionalis*). The findings of this study reveal that habitat type and elevation are the primary factors affecting the distribution and capture of hylid treefrogs. PVC pipe diameter and age-class have no significant effect on the location and distribution of treefrogs in this study. PVC pipe refugia are an effective means of sampling treefrog species, but should only be used when targeting select taxa. Results indicate that *O. septentrionalis* are preying upon on native treefrog species, and should be removed upon detection. The results of this study should be combined with similar work to develop appropriate criterion for sampling and conservation practices of hylid species.

Introduction

Recent paradigm shifts have begun to incorporate the importance of life history characteristics into our understanding of the natural history of an organism (Jameson 1956 and Semlitsch 2008). Increased insight of life history strategy will no doubt further our knowledge of both evolutionary history, and proper conservation and management practices. Of particular interest is the concept of ontogeny, which not only greatly influences the morphologic and metabolic repertoire of an organism, but the behavioral response to environmental stressors. Ontogenetic shifts in habitat exploitation have served, in some organisms, as a means to reduce competition between offspring and parent (Jameson 1956). Appropriate studies of behavioral shifts during ontogeny are considerably lacking in the literature, and information about reptiles and amphibians is almost absent. Frogs of the family hylidae provide an excellent model organism to address these questions as multiple species tend be sympatric, species tend to congregate and concentrate in large densities, and the literature has established adult size criterion (Wright and Wright 1949).

Relatively little information is known about the life history characteristics of hylid treefrogs away from breeding ponds (Ritke and Babb 1991). Treefrogs are rarely represented in herpetological surveys that employ the use of drift fences because they can easily escape by climbing out of pitfalls, and transverse fences (Dodd 1991, Greenberg, Neary, and Harris 1994, Gibbons and Bennett 1974, Lohoefner and Wolfe 1984, McComb and Noble 1981, and Murphy 1993). Visual detection methodologies also prove difficult, as treefrogs are generally small and often prefer densely covered habitats (Duellman and Trueb 1985). The use of polyvinyl chloride (PVC) pipes has provided a simple means to passively capture treefrog species

(Boughton et al. 2000, Domingue O'Neill and Boughton 1996, Greenberg, Neary, and Harris 1994, Moulton et al. 1996, and Zacharow, et al. 2002).

Five arboreal hylid species have been documented at the study site. They are *Hyla femoralis, H. cinerea, H. squirella, Pseudacris crucifer,* and *Osteopilus septentrionalis*. *Pseudacris crucifer* has an expansive geographic distribution, ranging from southeastern Manitoba to eastern Texas (Wright and Wright 1949). *Osteopilus septentrionalis* was first reported in Florida in the 1930s (Barbour 1931), and is now widespread throughout the state. This treefrog has thrived in habitats influenced by anthropogenic disturbance, and has spread as far north as South Carolina. This hylid is known to prey on multiple native species, and has even been reported to prey on other treefrogs (Granatosky et al. *in press,* and Meshaka 1996). Little research has yet focused on whether arboreal anurans make up a substantial portion of the *O. septentrionalis* diet. *Hyla squirella, H. cinerea*, and *H. femoralis* share similar ranges along the Atlantic and Gulf coastal regions of the southeastern United States (Garton and Bennett 1974, and Wright and Wright 1949). These treefrog species share a similar distribution throughout Florida, and are more-or-less limited by the presence of fresh water and high humidity levels (Duellman and Trueb 1985). While proximity to water plays an integral role in habitat utilization and selection, relatively little is known about taxon specific or age-based habitat exploitation (Boughton et al. 2000).

Adult *H. squirella* reach sexual maturity after 23 millimeters; adult *H. cinerea* 37 millimeters; adult *H. femoralis* 25 millimeters, and adult *P. crucifer* at 19 millimeters (Wright and Wright 1949). No established measurement of sexual maturity has been established for *O. septentrionalis*.

To date, few studies have addressed the possibilities of ontogenetic shifts of habitat use in arboreal anurans. Dispersal has been defined as a movement of an individual from one habitat to another, or simply away from the parental group (Jameson 1956 and Semlitsch 2008). Dispersal does not only affect the individual, but also influences inclusive fitness, genetic viability, and population dynamics. There are many hypotheses for reasons of dispersal, and most assume that it is a mechanism used to reduce the potential of inbreeding depression. Other hypotheses assume that dispersal reduces the possibility of parental/offspring competition. While few studies have addressed the possibility of ontogenetic dispersal in treefrogs, theoretically it is likely that there exists some form of age specific habitat distribution (Jameson 1956).

Hyla cinerea is found primarily on reeds and stems near a source of water. This species tends to be more restricted to water than other treefrog species (Garton and Bennett 1974). *Hyla squirella* is less dependent on water, and is considered to be more arboreal than other hylids. This species prefers open, wooded habitats, and is commonly found in Upland-Pine and mixed hammock systems (Duellman and Trueb 1985, Goin 1958, Lee 1969, and Wright and Wright 1949). *Osteopilus septentrionalis* is a more versatile species, but tends to do better in habitats with some form of human disturbance (Granatosky et al. *in press*, and Meshaka 1996). In order to address the question of taxon specific habitat selection, a study site with multiple habitat types would prove beneficial. Therefore, the Natural Areas Teaching Lab (NATL) within the University of Florida Campus (Gainesville, Florida) would provide a perfect study area to address multiple questions related to habitat selection in hylid treefrogs. Within NATL there are four primary habitat types, in which treefrogs have been reported. The four habitats are hammock, Upland-Pine, Old-Field succession, and wetland.

In previous studies that have utilized PVC pipe refugia to capture treefrogs, not all species and age classes have been equally represented. Although studies using PVC pipes have proven successful, it is likely that different species and age-classes have different requirements for pipe refugia (Boughton et al. 2000, Domingue O'Neill and Boughton 1996, and Moulton et al. 1996). The main objectives of this study require that ease of capture be equal for all species and age-classes. This study seeks to determine whether characteristics such as habitat type, pipe location and diameter, ontogeny, and phylogeny influence the distribution of treefrog species in NATL. By varying pipe location and characteristics, this project aims to determine and establish an effective sampling protocol to account for variation of habitat utilization in treefrog distribution. Primarily, this study seeks to determine whether treefrogs would utilize PVC pipes as habitual refugia, and if so whether pipe diameter or elevation influences use. This study also seeks to determine if age or phylogeny influences habitat use or pipe selection. Finally, as a separate hypothesis, this work aims to determine the prevalence of predation on native treefrogs by *O. septentrionalis*.

Materials and Methods

Ninety-six PVC pipes measuring 2.5 feet in length were divided into three primary groups based on the internal diameter of the pipe. These pipes consisted of ½ inch, ¾ inch, and one inch diameters. These 96 pipes were evenly distributed across 16 randomly selected sites (see Figure 1), among the four primary habitat types (hammock, Upland-Pine, Old-Field Succession, and wetland) found within NATL. These pipes and were further subdivided into two subgroups based on elevation. One group represented an arboreal habitat, which was placed six feet off the ground nailed to a tree, while the other was placed around the base of the same tree.

Six pipes were placed around each one of the 16 selected sites, and pipes were divided based on first elevation, and then diameter (see Figure 2).

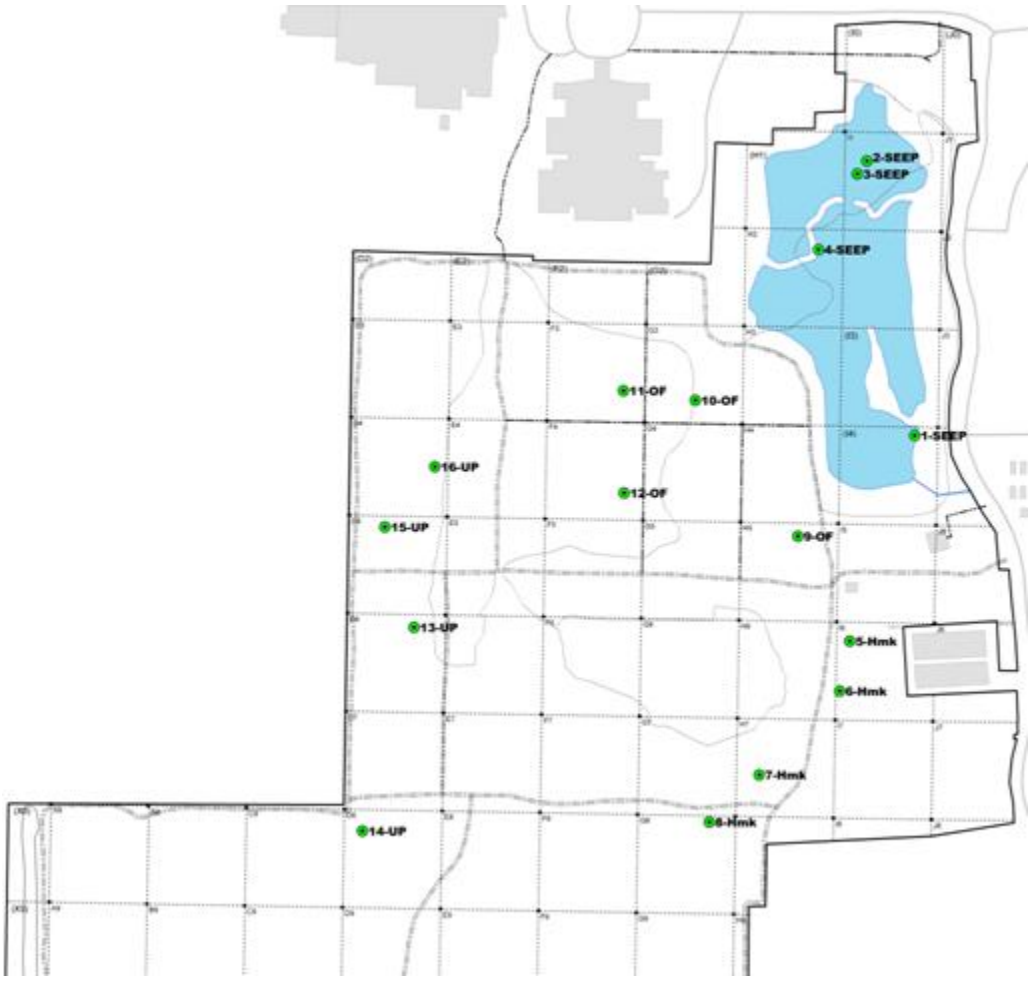

Figure 1. The distribution of the 16 randomly selected sampling sites in the Natural Areas Teaching Lab on the University of Florida Campus (Gainesville, Florida). Four trapping sites were selected in each of the four habitat types (hammock, Old-Field successional, Upland-Pine, and wetland).

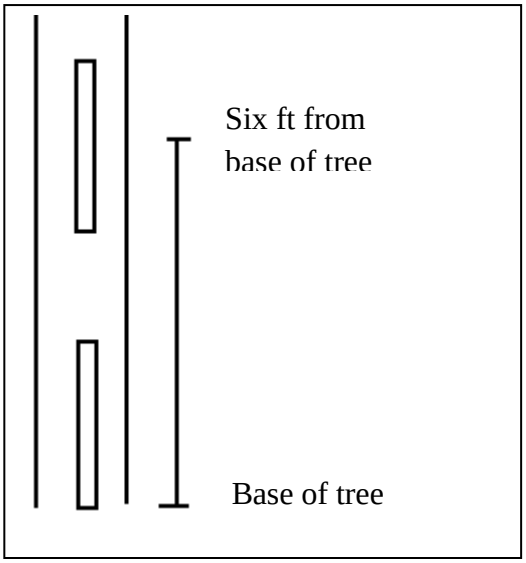

Figure 2. The basic setup of each trapping station consisted of six PVC pipes either placed at the base of the tree or six feet above the base. PVC pipes were further subdivided based on diameter (i.e., ½ inch, ¾ inch, or 1 inch).

Hammock ecosystems were defined as shaded, thick stands of hardwood and sparse pine, while wetlands included some form of permanent water retention. Old-field succession was characterized by the presence of annual weeds and sparse hardwoods, but because this ecosystem has been cleared relatively recently, large dense hardwoods and pines have yet to become established. Upland-Pine or sandhill ecosystems were identified by well-drained soils and widely spaced longleaf pines with sparse understory vegetation.

Traps were open continuously from August 1 – October 30, 2010. A 30-day acclimation period allowed treefrogs to locate and habitually use pipe refugia. Trapping effort totaled 10,272

trap-days, where one trap-day equaled one trap open for a 24-hour period. Traps were checked once per week during the trapping session.

Upon capture, species were identified, measured for length, and released. When possible, *O. septentrionalis* were captured, removed, and humanely euthanized for later study. Any treefrogs seen perched on or next to pipe refugia were captured by hand and added to species totals.

Capture data was reported for all species combined and on an individual basis. Relative species composition and total captures were calculated in Microsoft Excel 2007. Analysis of variance tests (ANOVA) were used to examine the possibility of random selection of habitat type, pipe diameter, and elevation. Dunn's pairwise comparison test was used to determine the main effects when the ANOVA detected a significant ($P < 0.05$) difference. All ANOVA and Dunn's pairwise comparison tests were conducted using Sigmastat 2.0 (Jandel Corporation 1995). In situations in which data did not pass a test of equal variance or normality, data was ranked transformed. *Hyla cinerea* and *H. squirella* data allowed for separate analysis based on age. Pearson's chi-square tests were used to determine whether capture of juveniles and adults was distributed randomly. In the event that observed values were too small, Yates' chi-square test was employed to determine significance ($P < 0.05$). All Pearson's chi-square tests and Yates' chi-square tests were calculated on quantpsy.org, an interactive calculation tool for chi-square tests of goodness of fit and independence (Preacher 2001).

For all captured *O. septentrionalis*, specimens were removed from NATL and humanly euthanized. Each specimen was assigned a unique identification number and then dissected. Stomach contents were analyzed for the presence of any identifiable animal remains. All

stomach contents were identified down to taxonomic order, except in the case of vertebrates which were classified to the species level. All analyses were conducted in Microsoft Excel 2007.

Results

This study successfully captured four of the five species of hylids reported from NATL. Of the captures, *H. squirella* was the most commonly observed species, followed by *H. cinerea*, and *O. septentrionalis*. Only one *P. crucifer* was captured during the study (see Table 1). Adults and juveniles of both *H. squirella* and *H. cinerea* were captured, but distribution of age classes was more uniform in *H. cinerea* (see Table 1). Based on data collected from this study, *H. squirella* and *H. cinerea* make up a substantial portion of the hylid species within NATL, with the exotic *O. septentrionalis* contributing the rest of the variation in treefrog species composition (see Figure 3).

Treefrog Species	Adult	Juvenile	Total
Hyla cinerea	66	44	110
Hyla squirella	105	17	122
Osteopilus septentrionalis	33	n/a	33
Pseudacris crucifer	1	0	1
			266

Table 1. Total number of captures, species composition, and number of juveniles and adults collected.

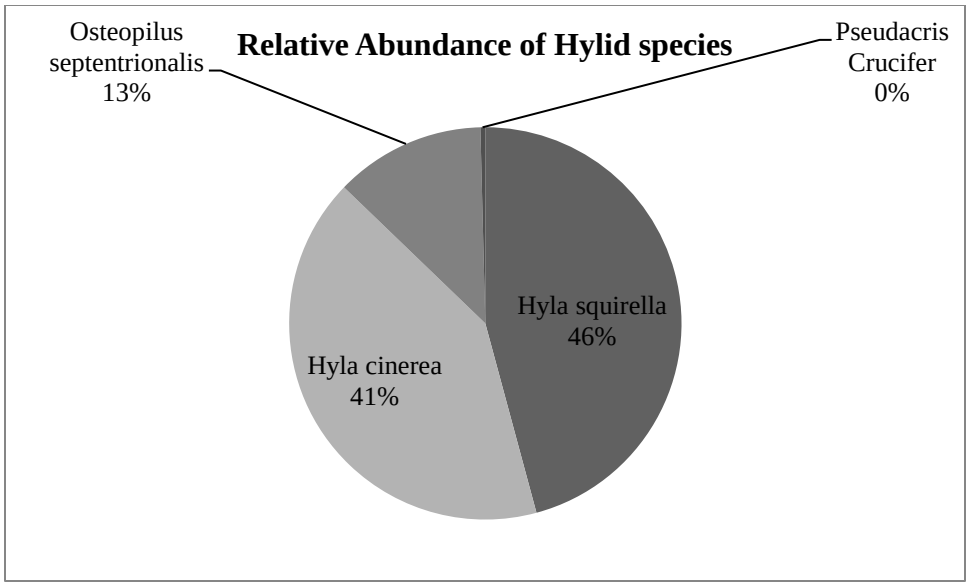

Figure 3. The relative abundance of treefrog species captured during the extent of this study at the Natural Areas Teaching Lab on the University of Florida Campus (Gainesville, Florida).

Analysis of variance results indicate that there is no statistically significant ($P = 0.257$) difference between treefrog ease of capture based on habitat type when all species are pooled. *Pseudacris crucifer* was excluded from all statistical tests due to the low number of captures. On an individual species basis, *H. cinerea* tends to be most commonly observed in hammock (43.6%) and wetland ecosystems (33.6%) with no significant difference in capture detected between the two environments ($P = 0.846$). However, capture rate is significantly different ($P < 0.05$) between these two ecosystems compared to that of the Old-Field succession and Upland-Pine study areas. In contrast, *H. squirella* reveals no significant difference ($P = 0.223$) between capture rate across any of the study areas. Observation of *O. septentrionalis* differs significantly ($P = 0.045$) between the different habitat types, but a test for the main effects reveals that this

significance is only detected between capture in hammock (0.3%) and Upland-Pine (42.4%) ecosystems.

When comparing the species composition within each habitat type separately, significant differences ($P = 0.004$) were observed. Although there was no significant difference ($P > 0 .05$) between capture of *H. cinerea* and *H. squirella*, there was a significant ($P < 0.05$) difference in capture between these species and *O. septentrionalis*, which comprised only 1.1% of the treefrog abundance in the hammock study area. No significant difference ($P > 0.05$) was detected between the capture rates of the three treefrog species within the Old-Field successional, Upland-Pine, and wetland study areas.

Pooled capture data reveals that treefrog observation differs significantly ($P < 0.001$) based on pipe elevation, with the ground pipes encompassing 77.0% of all captures. In *H. squirella*, there is a significant difference ($P = 0.008$) between ground and elevated captures, with 75.4% of all captures occurring in a pipe placed in the ground. The same significant difference ($P = 0.002$) was detected for the capture of *H. cinerea*, with 73.6% of observations being from pipes on the ground. A similar difference in capture rates of *O. septentrionalis* revealed the same significance ($P < 0.001$), with 93.9% of all captures observed on the ground. When comparing the use of ground pipes between species, no significant difference ($P =0.529$) was detected.

Pooled observations revealed no significant difference ($P = 0.348$) in treefrog capture between the three different pipe diameters. When comparing capture within species based on pipe diameter, no significant difference ($P > 0.05$) was observed. A similar lack of significance ($P > 0.05$) was detected when comparing intra-specific variation in ease of capture based on the three pipe diameter sizes.

To test for the possibility of nonrandom juvenile and adult distribution of *H. cinerea* and *H. squirella* amongst each habitat type, PVC pipe elevation, or PVC pipe diameter, both species were analyzed separately. Within observations of *H. squirella*, Yates' chi-square test and Pearson's chi-square test revealed no significant ($P > 0.05$) difference between adult and juvenile ease of capture in relation to PVC pipe elevation ($\chi^2 = 0.171$, df = 1), habitat type ($\chi^2 = 0.623$, df = 3), or PVC pipe diameter ($\chi^2 = 4.018$, df = 2). Analysis of *H. cinerea* data reveal no significant ($P > 0.05$) difference adult and juvenile ease of capture in relation to PVC pipe elevation ($\chi^2 = 1.319$, df = 1), habitat type ($\chi^2 = 6.705$, df = 3), or PVC pipe diameter ($\chi^2 = 2.366$, df = 2).

Diet analysis consisted of the dissection of 32 *O. septentrionalis* ranging in snout-vent length (SVL) from 24.2 millimeters to 51.3 millimeters. Of these specimens, 11 (34.4%) contained some form of animal remains in their stomachs. Insects made up a substantial portion of the contents examined, with Orthoptera being the most common (Figure 4). Only one anuran was collected during diet analysis, a juvenile *H. cinerea* (21.3 mm SVL), taken from an *O. septentrionalis* (49.5 mm SVL).

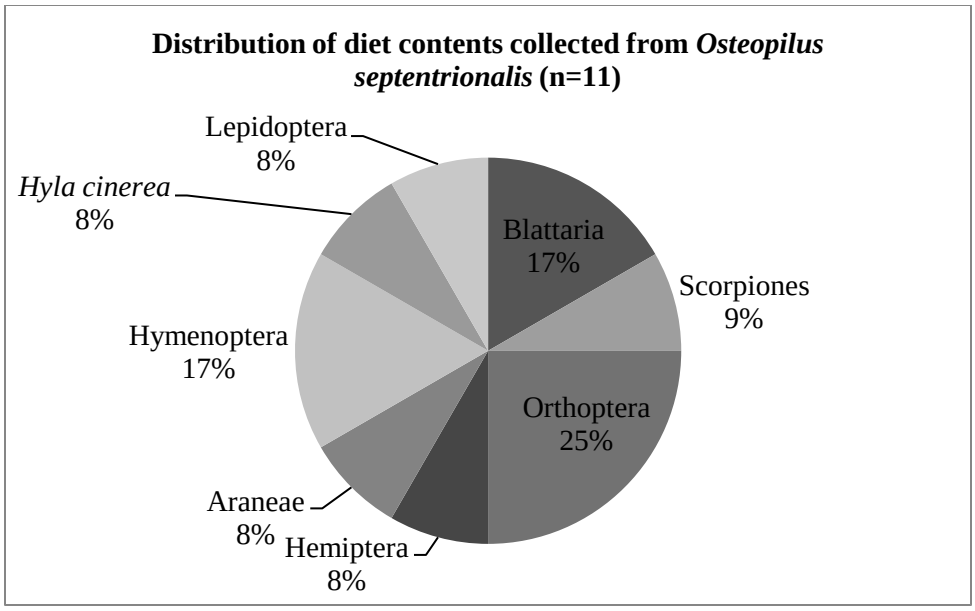

Figure 4. Relative diet contents of *Osteopilus septentrionalis* (n=11). One juvenile *Hyla cinerea* (21.3 mm SVL) was discovered in the stomach of an adult female (49.5 mm SVL).

Discussion

The findings of this study reveal considerable information about the biology and habitat use of hylid treefrogs. The methodology developed in this study proved to be an effective means of passively trapping arboreal anurans. The study period consisted of approximately three months of trapping and resulted in the capture of 266 individual treefrogs, spanning all age-classes and four species. The observation of *P. crucifer* was extremely low, and there were no captures of *H. femoralis*. This observation is in accordance with previous studies (Boughton et al. 2000, and Domingue O'Neill and Boughton 1996) in which the capture of these two species was extremely rare. This observation supports the hypotheses that the probability of capture is not equal amongst all species, and that PVC pipe refugia should not be used in certain situations depending on the research goals. This methodology proved to be a highly effective means of capturing *H. cinerea* and *H. squirella* and, to a lesser degree, *O. septentrionalis*. It is not clear

whether this is a result of actual selection of PVC pipe refugia, or simply a result of relative abundance. *Osteopilus septentrionalis* numbers were low at the beginning of the study (personal observation), and therefore may account for the relatively lower capture rates.

Overall, the pooled results indicate that there is no one habitat type that treefrogs tend to prefer. This is not surprising since the study was intentionally conducted in the nonbreeding season (Ritke and Babb 1991), a time in which treefrogs do not congregate around water sources. As predicted, all species were detected within the wetland study area, and species abundance did not vary significantly within this habitat type. *Hyla squirella* was the most cosmopolitan of all the species. Results indicate that this species is not limited by any environmental conditions (i.e., moisture and vegetative composition), and distribution is probably only limited by the ability to find shelter or prey. *Hyla cinerea* capture, in contrast, did significantly differ between habitat types, with capture being most common within hammock and wetland study areas. This finding agrees with previous studies (Boughton et al. 2000, Domingue O'Neill and Boughton 1996, and Wright and Wright 1949), in which the distribution of *H. cinerea* was found to be limited by the presence of water. The hammock environment has moist air, and additional study accounting for the humidity in a particular ecosystem might account for the variability in the distribution of *H. cinerea*.

The abundance of *O. septentrionalis* only differed significantly between the comparison of the hammock to Upland-Pine ecosystem. *Osteopilus septentrionalis* tend to thrive in relatively disturbed environments (Meshaka 1996), and the low disturbance factor of the hammock ecosystem in NATL may account for the low number of captures. If this explanation is true, then it should be expected that *O. septentrionalis* populations would be most dense in the Old-Field succession study area, as this habitat type is prone to the most disturbance. Since this

hypothesis was rejected, however, it is likely that there are unaccounted variables affecting the distribution of *O. septentrionalis,* and further studies should be conducted in order to understand dispersal and habitat selection in these exotic pests.

What was perhaps most interesting about this study was the overwhelming amount of captures from ground compared to elevated pipes. This finding agrees with other studies (Boughton et al. 2000), and seems to be a result of the amount of moisture that escapes from elevated pipes. Constructing traps with some sort of water collection device may change capture rate in elevated pipes.

Pipe diameter also had no significant effect on the probability of capture. This was surprising, as it has been shown that treefrogs tend to prefer the security of tight refugia (Lee 1969, and Wright and Wright 1949). This prediction is still controversial because, although behaviorally this assumption makes sense, the findings of studies that utilize PVC pipe refugia have come up with mixed results (Boughton et al. 2000). An intense literature review may be the best means of identifying the actual effects of PVC pipe diameter on treefrog capture.

There was no significant effect of age-class on the distribution and habitat use of the treefrogs observed in this study. The concept of dispersal in herpetofauna is still unknown, and tendencies to disperse seem to be a result of phylogeny, rather than an all-encompassing trend (Jameson 1956 and Semlitsch 2008). It may be that treefrogs of different size classes are able to coexist as a result of an ontogenetic shift in diet, rather than habitat utilization. Further studies concentrating on an intensive diet analysis may reveal information about age-specific behavioral adjustments in hylid treefrogs.

The results of the diet analysis of the captured *O. septentrionalis* revealed that 92% of animal remains removed from the stomach were invertebrates. Only one anuran was collected

during diet analysis, and this was a juvenile *H. cinerea* (21.3 mm SVL). The individual in which the *H. cinerea* was found was a large female measuring 49.5 mm SVL. The size of this individual may be the reason that other anurans were a part of its diet. While the results indicate that the predation of native treefrogs by *O. septentrionalis* was low, multiple studies have now shown that these exotic amphibians are preying upon native herpetofauna (Meshaka 1996).

The findings of this study reveal that habitat type is the primary factor affecting the distribution and capture of hylid treefrogs. PVC pipe refugia are an effective means of sampling treefrog species, but should only be used when targeting select taxa. It does not appear that treefrogs are selecting habitat based on the diameter of the refugium. Results do indicate that treefrog species tend to prefer PVC pipes on the ground, rather than those that are elevated. This may be a result of moisture in the pipe rather than a preference for a particular elevation. There appears to be no ontogenetic shifts in behavior as a means to reduce competition between adults and juveniles, although further studies should be conducted to explore the possibility of dietary shifts. There is no doubt that *O. septentrionalis* are preying upon native treefrog species, and therefore they should be removed upon detection. The results of this study should be combined with similar work to develop appropriate criterion for sampling and conservation practices of hylid species.

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