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Interspecific Variation in Use of Polyvinyl Chloride (PVC) Pipe Refuges by Hylid Treefrogs: A Potential Source of Capture Bias

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Amphibian trapping techniques are designed to exploit various behaviors; as knowledge regarding the behavior of the target taxa increases, sampling techniques must be adapted accordingly. Refinement of the technique increases effectiveness and reduces capture biases that might influence detection probability. Equal detectability is a critical assumption of many mark-recapture models, and unequal, imperfect, or inconstant detectability will affect estimates of population size, species richness, and site occupancy (Mazerolle et al. 2007). Therefore, it is critical that researchers consider potential sources of variation when planning studies in order to reduce capture biases, and select models for data analysis that incorporate estimates of detection probability (Mazerolle et al. 2007).

In recent years, researchers have begun to take advantage of the propensity of hylid treefrogs to use cavities as diurnal shelters (McComb and Noble 1981; Ritke and Babb 1991; Walters and Kneitel 2004) by deploying artificial refuges that mimic natural cavities to sample hylid populations (Meshaka 1996; Moulton et al. 1996). Polyvinyl chloride (PVC) pipe refuges are perhaps the most popular method for studies of hylid population ecology and ongoing monitoring efforts (e.g., Boughton et al. 2000; Campbell et al. 2009; Hirai 2006; Johnson et al. 2008; Moulton et al. 1996; Pittman et al. 2008; Smith et al. 2006; Zacharow et al. 2003). The PVC refuges are more effective for hylid sampling than traditional drift-fence arrays, and passively attract (rather than trap) frogs. As this effectively eliminates trap mortality, there is no need to remove or close "traps" between sampling periods, making PVC sampling less labor-intensive than many trapping methods. Sampling can be conducted throughout the day without fear of heat stress to "captured" animals. This method is also potentially less biased than call surveys as it facilitates year-round sampling of both genders (but see Pittman et al. 2008), and it allows identification of individual frogs for mark-recapture studies.

However, a wide variety of factors affect use of PVC refuges by hylids and can result in capture bias and influence detection probability. Although vegetative cover does not influence captures of some hylids (Moulton et al. 1996), hylids may prefer refuges in close proximity to breeding sites (Boughton et al. 2000; Pittman et al. 2008). Arboreal placement of refuges and tree type (hardwood vs. pine) may also influence the composition of the hylid sample (Boughton et al. 2000; Johnson et al. 2008; Myers et al. 2007; Townsend 1989). Capture rates may also be influenced by refuge design (i.e., pipe diameter or length, T-shaped or straight, end caps to hold water), age of pipes (i.e., outgassing of new pipes), or other variables that affect the microclimate within the PVC refuge (Bartareau 2004; Boughton et al. 2000; Buchanan 1988; Moulton et al. 1996; Zacharow et al. 2003). Season, temperature, humidity, and rainfall may also influence refuge use (Johnson et al. 2008; Pittman et al. 2008; Zacharow et. al. 2003). Some field data suggest that interspecific variation in refuge use may exist (Boughton et al. 2000; Zacharow et al. 2003), but this potential source of bias has not been explicitly investigated.

In order to unequivocally evaluate interspecific variation in PVC refuge use, it is necessary to determine the location of each study frog during each sampling period, even when frogs choose an alternative refuge (i.e., they choose natural cover rather than a PVC refuge). This can only be achieved by controlled behavioral trials or radio telemetry field studies. However, size of available radio transmitters precludes telemetry of smaller hylid species for any length of time, and the arboreal nature of some hylids makes it difficult to pinpoint diurnal refuges (Johnson 2005; M. E. McGarrity, unpubl. data). Therefore, we conducted refuge choice trials to evaluate interspecific variation in refuge preference among four species of hylids in a controlled setting in order to provide insights on potential variation in detection probability of these species in the field. As previously mentioned, numerous factors can influence variation in PVC pipe refuge use, including weather and microclimate of the PVC refuge. By conducting a laboratory study, we were able to control for many of these sources of variation and focus on behavioral variation among species. We then analyzed original field data from several of our field sites and published data from one field site (Zacharow et al. 2003), and compared laboratory and field data to evaluate the relevance of trends seen in the laboratory study.

Methods.—We collected frogs for laboratory trials during June–July 2007 from multiple urban and rural sites in Alachua, Hillsborough, and Polk Counties, Florida, USA, using PVC refuges and hand collection. Collection sites included natural marshes and swamps, retention ponds, parks, roadside and agricultural ditches, paved roads, and buildings. We conducted laboratory experiments at the University of Florida/IFAS Gulf Coast Research and Education Center (Wimauma, Florida, USA) in rooms equipped with individual climate and lighting control systems. We maintained frogs in individual 0.75 L clear plastic containers with 3 mm air holes for a maximum of 18 days; containers were disinfected between frogs. We fed all frogs crickets *ad libitum* during captivity. We set temperature to 24°C during the day (the maximum the system would allow); temperature fell to a minimum of 21°C during the night. We used a 14-hour light cycle (0630–2030 h) to approximate the natural photoperiod in central Florida during the study (June/July).

We evaluated refuge preferences in a controlled setting for four species of hylids commonly encountered in PVC refuges in the southeastern USA: *Hyla cinerea* (Green Treefrog), *H. squirella* (Squirrel Treefrog), *H. femoralis* (Pine Woods Treefrog), and *Osteopilus septentrionalis* (Cuban Treefrog, nonindigenous). We conducted 30 individual trials per species to evaluate refuge preference; each frog participated in only one refuge choice trial. We placed each frog into a 38 L aquarium with a wet sand substrate and screen lid. Since we were unable to regulate humidity in the room, we used the wet sand substrate to increase relative humidity in the aquarium to approximately 65% to prevent desiccation and minimize variation. We offered the frog a choice of two refuges placed at opposite ends of the aquarium: a PVC pipe installed vertically in the sand (3.8 cm diameter, 20 cm tall) and an herbaceous plant (*Vinca* sp., ~20 cm tall). We alternated the placement order of the two refuges within the aquarium to control for potential directional bias in refuge choice. Although the plant was not a native Florida species, all hylid species in this study readily used it when it was the only refuge provided and it closely mimicked the structural complexity of small, native shrubs. We initiated trials at 2000 h and recorded refuge choice (i.e., PVC pipe, plant, other location) at 1000 h the following day, by which time frogs in natural habitats would likely have sought diurnal shelter. After completion of each trial, we marked indigenous hylids with visible implant elastomer to prevent re-capture (and re-testing), and released them at the site of capture. Since *O. septentrionalis* is an invasive, nonindigenous species in Florida, we euthanized these frogs immediately after trials by applying 20% benzocaine to the skin of the ventral surface followed by freezing for 48 hrs. Frog collection techniques, captive maintenance, experimental trials, and euthanasia procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida (protocol E870).

We evaluated frequency of refuge use in the field with data we collected during several previous studies using PVC pipe refuges. We deployed ground-based PVC pipe refuges in 100 × 100 m grids of 121 pipes each in flatwoods habitat at sites in the Lower Suwannee National Wildlife Refuge (LSNWR, Chiefland, Florida, USA; August 2003–July 2004), Wekiwa Springs State Park (WSSP, Apopka, Florida, USA; July 2006–February 2007), and Flatwoods Wilderness Park (FWWP, Thonotosassa, Florida, USA; August 2006–June 2007). We monitored these PVC refuges at 3–4 week intervals, and individually marked each frog by toe clipping (LSNWR) or using a combination of visible implant elastomer with toe clipping (WSSP, FWWP; Hoffmann et al. 2008). We also evaluated frequency of refuge use from data in a published study that used transects of ground-based PVC refuges to sample hylid populations (at weekly intervals) at sites between breeding ponds and mesic hardwood hammocks (Gainesville, Florida, USA; August 2001–April 2002; Zacharow et al. 2003). We calculated capture frequency (i.e., number of individually marked frogs captured once, twice, etc., *not* frequency of presence/absence of any frog in a given pipe) and mean number of captures per frog for each species at each site. Since frogs were individually marked, we were able to determine frequency of capture for each frog.

We evaluated variation among species in frequency of refuge use during controlled behavior trials using a Pearson Chi-square test (SYSTAT 10.2, SYSTAT Software Inc., Richmond, California, USA; $\alpha = 0.05$). We evaluated variation among

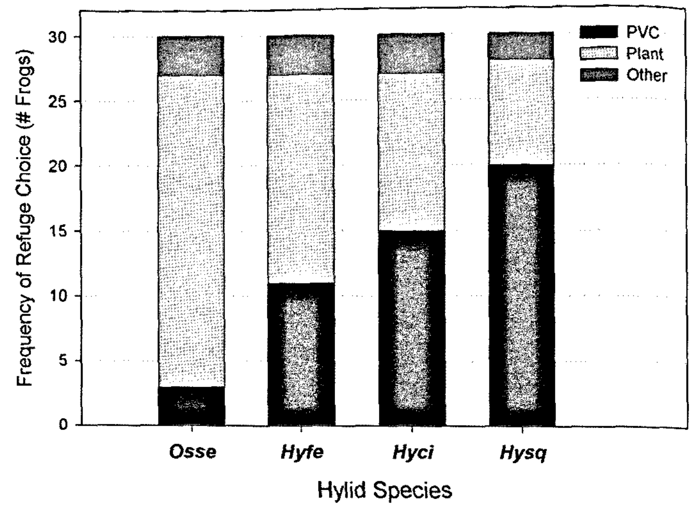

FIG. 1. Frequency of refuge choice for four hylid species (N = 30/species): *Osteopilus septentrionalis* (*Osse*; Cuban Treefrog), *Hyla femoralis* (*Hyfe*; Pine Woods Treefrog), *H. cinerea* (*Hyci*; Green Treefrog), and *H. squirella* (*Hysq*; Squirrel Treefrog). "PVC" indicates vertical PVC refuge, "Plant" indicates *Vinca* sp. plant, and "Other" indicates other (exposed) resting places in the aquarium.

species in frequency of refuge use at our field sites (number of captures per individually marked frog) using ANOVA with *a priori* contrasts ($\alpha = 0.05$).

Results.—Frequency of refuge use during controlled behavior trials differed significantly among species ($\chi^2_{0.05, 6} = 21.61$, $P = 0.001$; Fig. 1). *Osteopilus septentrionalis* chose to rest in the plant eight times as often as the PVC pipe, whereas *Hyla squirella* chose the PVC pipe more than twice as often as the plant. The remaining two species, *H. femoralis* and *H. cinerea*, did not exhibit an obvious refuge preference. Individuals of all species preferred to use PVC pipe and plant refuges as diurnal shelters, as opposed to more exposed areas of the aquarium (Fig. 1), and rarely chose to rest elsewhere (i.e., on sand substrate, on glass walls, or under edge of lid).

Frequency of capture in PVC refuges in the field also varied among species (Table 1). When data from our field sites (LSNWR, WSSP, FWWP) were subjected to ANOVA, mean number of captures (per individual frog) differed greatly among species (F = 12.173; $P < 0.001$). Mean number of captures of *H. squirella* and *H. femoralis* at our field sites were similar ($P > 0.1$), and were significantly higher than the mean number of captures per *O. septentrionalis* ($P < 0.001$). Mean number of captures of *H. squirella* differed by 33% (Table 1) between our study site and the site used by Zacharow et al. (2003); however habitat also differed greatly between these sites (respectively, flatwoods vs. breeding ponds and surrounding hardwood hammock). Across all sites (including Zacharow et al. 2003), native *H. femoralis, H. squirella,* and *H. cinerea* were at least 1.5 times more likely to be recaptured than *O. septentrionalis*.

Discussion.—We identified significant interspecific variation in artificial refuge use that may present a significant source of bias when using PVC refuge sampling. Our laboratory trial setup provided the advantage of controlling for climatic, microclimatic, seasonal, and other variables that might obscure interspecific variation in frog behavior in the field, and yielded insights to

TABLE 1. Frequency of capture at field sites for four hylid species: *Osteopilus septentrionalis* (Cuban Treefrog), *Hyla femoralis* (Pine Woods Treefrog), *H. cinerea* (Green Treefrog), and *H. squirella* (Squirrel Treefrog). Data are shown as the number of frogs captured *x* times at a given site, followed in parentheses by the percent of total frogs of that species captured *x* times. Sources correspond to abbreviations in study area descriptions (ZACH corresponds to Zacharow et al. 2003).

Number of captures / frog	*O. septentrionalis*		*H. femoralis*		*H. cinerea*	*H. squirella*	
1	65 (71%)	77 (71%)	205 (45%)	53 (52%)	106 (58%)	84 (60%)	70 (39%)
2	23 (25%)	22 (20%)	97 (21%)	14 (14%)	42 (23%)	17 (12%)	39 (22%)
3–5	3 (3%)	8 (7%)	118 (26%)	24 (24%)	28 (15%)	29 (21%)	45 (25%)
6–10	0 (0%)	1 (1%)	37 (8%)	9 (9%)	7 (4%)	10 (7%)	24 (13%)
Mean ± SD	1.32 ± 0.53	1.45 ± 0.87	2.37 ± 1.70	2.46 ± 2.04	1.95 ± 1.66	2.11 ± 1.75	2.82 ± 2.36
Range	1–3	1–6	1–8	1–10	1–10	1–9	1–11
Source	WSSP	FWWP	LSNWR	FWWP	ZACH	LSNWR	ZACH

guide future field studies. However, these laboratory trials were limited, in that they restricted the refuge choices available to the frogs, rather than mimicking the natural conditions where many natural refuge choices are available around each artificial PVC refuge. Therefore, we used data from field studies to evaluate the applicability of our laboratory results to more natural settings. Capture frequencies from PVC sampling field studies have been used to make comparisons of interspecific variation in site fidelity (Zacharow et al. 2003), and may also be good descriptors of fidelity to PVC refuges, or the propensity of a species to seek shelter in them. Theoretically, individuals of a species that prefers PVC refuges to more natural shelters will be recaptured in the PVC refuges more frequently. Factors including interspecific differences in physiology, seasonal habitat use, site fidelity, susceptibility to predation, and microhabitat preferences likely contribute to variation in PVC refuge use among species.

Capture frequencies from field studies are consistent with the results of our controlled behavioral trials. During behavioral trials, *Hyla squirella* showed an obvious preference for PVC pipes over plant refuges (Fig. 1). In the field, *H. squirella* had the highest documented number of captures per frog (Table 1; Zacharow et al. 2003), in keeping with the demonstrated tendency of this species to choose PVC refuges under controlled laboratory conditions. In addition, *H. squirella* were nearly seven times more likely to choose a PVC refuge during behavioral trials than *Osteopilus septentrionalis*, which preferred to seek refuge in the plant as opposed to the PVC pipes. Similarly, *O. septentrionalis* at our field sites were much less likely to be recaptured in PVC refuges than native hylids; only 28% of *O. septentrionalis* were captured more than once. This suggests that, although this species is well known to use PVC refuges in the field, they may use these shelters less frequently than other species, especially when alternative refuges are available. Overall, native hylids were 1.4–2.1 times more likely than *O. septentrionalis* to be recaptured in the field. Capture frequencies and mean number of captures per frog were similar for each species between field sites (Table 1), with the greatest amount of variation seen for *H. squirella* between sites in different habitat types.

Although artificial PVC refuges are the most effective method for sampling hylids currently available, sources of potential capture bias must be carefully considered in order to minimize bias, increase detectability, and reduce effects on population estimates inferred from these studies. We have identified interspecific differences in PVC refuge use as a major source of variation that could mask the difference between low abundance and low capture rate. We suggest that researchers consider behavioral differences, including diurnal refuge use, of all target species when planning PVC refuge sampling studies. When data for a given target species are not readily available in the literature, a small laboratory pilot study might be advisable to identify potential interspecific variation in refuge use and thus detectability. Furthermore, our results suggest that researchers should consider using mark-recapture methods rather than point counts to estimate population parameters, as interspecific variation in PVC refuge use may be misinterpreted as variation in relative abundance.

The implications of this interspecific variation in PVC refuge use are particularly important with regard to efforts to monitor and manage invasive species such as *O. septentrionalis*. This nonindigenous hylid is believed to impact native hylid populations through predation and competition (Knight et al. 2009; Meshaka 2001; Rice et al. 2003; Smith 2005). Management efforts to date largely consist of point-counts and removal of *O. septentrionalis* from PVC refuges used to monitor hylid populations. However, our results show that only a small portion of the population is likely to be captured using this method. The preference of *O. septentrionalis* for natural refuges, shown in this study, reduces detectability and suggests that even small numbers of *O. septentrionalis* in point-count PVC monitoring studies may be indicative of a significant infestation. Furthermore, the highly arboreal nature of this species (McGarrity and Johnson, *in press*) indicates a need for evaluation of optimal placement of PVC refuges (e.g., arboreal vs. ground-based). Until the PVC sampling method is further refined in order to maximize capture, when possible, managers should not solely rely on point-counts using ground-based PVC refuges to monitor and remove *O. septentrionalis*. Other techniques, including mark-recapture and dip netting of larvae or hand capture of breeding adults, should be considered to enhance monitoring and removal efforts that rely on PVC pipe refuges.

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AMPHIBIAN DISEASES

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Evaluation of Chytrid Infection Level in a Newly Discovered Population of *Anaxyrus boreas* in the Rio Grande National Forest, Colorado, USA

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The Western Toad, *Anaxyrus boreas*, ranges throughout the western United States, exclusive of the desert southwest (Goebel et al. 2009; Hammerson 1999). In the southern Rocky Mountains, *A. boreas* breeds in slow moving water, such as wetlands, marshes, ponds and lakes, in subalpine forest at high elevation, 2000–3500