A RADIO TELEMETRY STUDY OF INVASIVE CUBAN TREEFROGS

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Abstract: *We evaluated the feasibility of using radio telemetry to study movements and microhabitat use of invasive Cuban treefrogs (*Osteopilus septentrionalis*) in Florida. We first conducted simple performance trials, and found no overt effects of radio belt attachment on locomotion in a test arena. We then attached radio transmitters to 17 frogs captured in bottomland forest and displaced them 200 m to sites in old-field or bottomland forest habitat, during the summer breeding season. We located each displaced frog daily for an average of 9 days each (range: 2–15d) and then evaluated their movements and the variables influential in predicting movement (e.g., relative transmitter weight, rainfall, release site, etc.). Radio tracked frogs predominantly chose arboreal refuges in the forest canopy, using the shrub and sub-canopy layer more frequently in old-field where there were few large trees. Frogs displaced to old-field moved rapidly and directly to forest habitats; in contrast, frogs displaced to similar forest habitat remained in forest and their movements were random. Frogs were more likely to move on nights with high relative humidity and temperature, low wind speed, and low moon fraction illuminated; frog sex, size, and relative transmitter weight did not significantly influence frog movement. Our study demonstrates that radio telemetry is a valuable tool for studying the ecology of large adult Cuban treefrogs. Additionally, our finding that displaced Cuban treefrogs are able to move rapidly to suitable forest habitat further attests to the resilience that has contributed to the success of this invasive frog.*

Key Words: Anuran, ecology, hylid, invasive, microhabitat, *Osteopilus*

Cuban treefrogs (Hylidae: *Osteopilus septentrionalis*) were introduced to the Florida Keys in the 1920s as stowaways in cargo shipments (Barbour, 1931), and have since become established in both urban and natural areas throughout peninsular Florida (Meshaka, 2001; McGarrity and Johnson, 2009). Although Cuban treefrogs are most commonly found in disturbed (e.g., urbanized) habitats in Florida, they are also able to invade natural habitats by hitchhiking on vehicles or dispersing across the urban/wildland interface (Wyatt and Forys, 2004; Johnson, unpublished data). This species is considered highly invasive, and is believed to affect native treefrog populations negatively via predation and competition, causing their decline in natural and urbanized areas (Knight et al., 2009; Meshaka, 2001; Rice et al., 2003; Smith, 2005; Johnson, unpublished data). In order to evaluate the potential degree of these

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impacts and develop management strategies for this invasive frog, a thorough understanding of its ecology is needed.

Although important data have been compiled regarding reproduction, diet, and certain population characteristics of this invasive frog in Florida (Babbitt and Meshaka, 2000; Meshaka, 2001; Rice et al., 2003; Wyatt and Forys, 2004), data on habitat preferences, microhabitat use, and movements are limited. Cuban treefrogs are known to use a variety of habitats in Florida, including some xeric natural habitats where they are frequently captured in PVC refuges used to sample treefrog populations (Hoffmann et al., 2008; Johnson, unpublished data). The relative frequency of hand-capture of Cuban treefrogs in various natural habitats during visual encounter surveys (along boardwalks and trails) suggests that this species prefers mesic, forested habitats (Meshaka, 2001). However, the inability to sample all microhabitats, strata, and habitat types with equal success and reliability is an inherent limitation of visual encounter surveys, and this monitoring technique cannot adequately describe habitat preferences (Crump and Scott, 1994). Habitat use and movements likely vary with frog size or sex, season, habitat type, or climatic conditions, and such variation may influence monitoring efforts. Although a deeper understanding of patterns of habitat use and movements of Cuban treefrogs in natural areas (and factors influencing these patterns) would benefit management efforts, these patterns have not yet been systematically evaluated.

Radio telemetry is commonly used to study the movements and habitat use of terrestrial and aquatic frogs. This technique provides unequivocal data on habitat and microhabitat use by facilitating relocation of individuals that might evade detection by other methods (e.g., visual encounter surveys), but precludes simultaneous monitoring of a large number of individuals. Only recently, investigators successfully used implanted radio transmitters to study the home range and habitat use of arboreal gray treefrogs (*Hyla versicolor*: Johnson et al., 2007; Golden, 2007). The success of these studies and of our own pilot study using transmitter belts, suggest that radio telemetry could be an effective method to provide much-needed data on the ecology of invasive Cuban treefrogs.

Here, we report on the feasibility of using radio telemetry to study Cuban treefrog habitat use and movements, and provide preliminary insights from our study. Since transmitter attachment has been shown to have limited, species-specific effects on frog locomotion (Blomquist and Hunter, 2007), we conducted locomotory performance trials to test for overt effects of radio-transmitter belt attachment before initiating field evaluations. We then displaced radio-belted frogs to sites in open or forested habitats, in order to evaluate (1) transmitter/belt use and explore (2) frog habitat/microhabitat preferences and (3) frog movements.

MATERIALS AND METHODS—*Frog collection*—We collected adult Cuban treefrogs for locomotory performance trials and field radio telemetry trials during late summer 2007, from a 200 m^2 grid of 100 ground-based polyvinyl chloride (PVC) pipe refuges (Boughton et al., 2000;

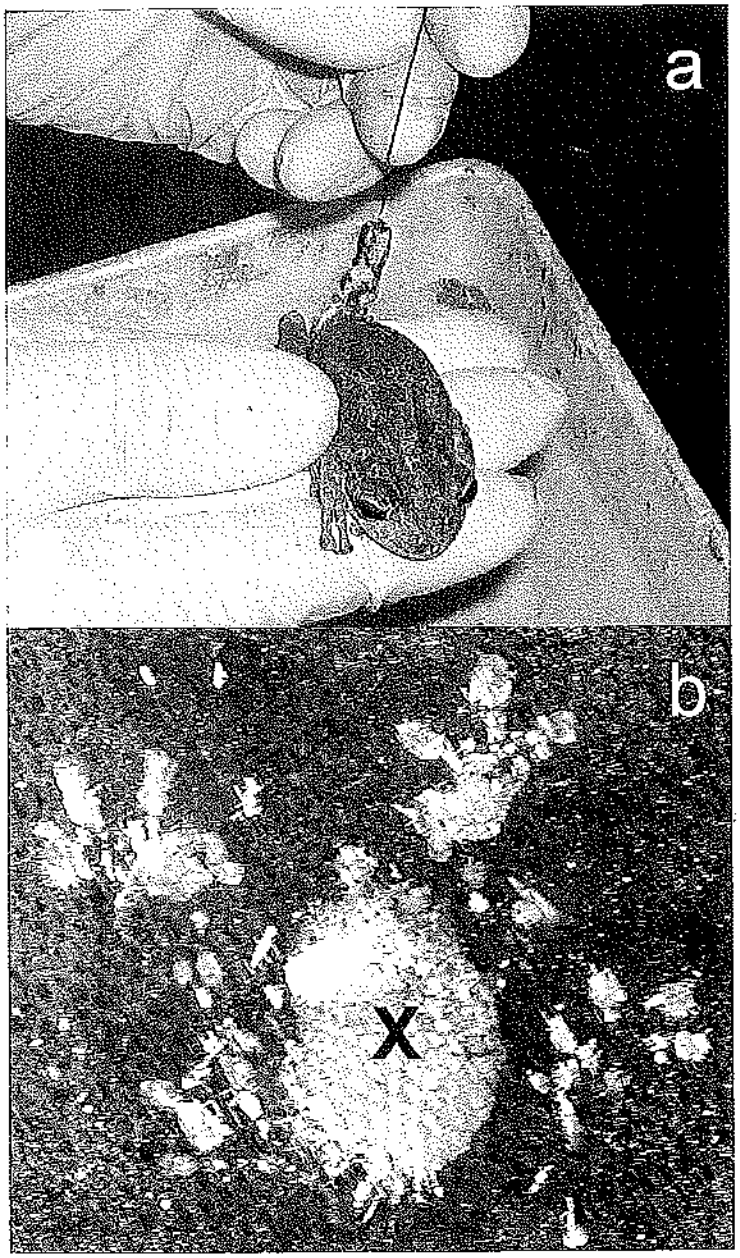

FIG. 1. We evaluated the effects of radio transmitter attachment on locomotory performance of Cuban treefrogs by conducting trials in a controlled setting. We quickly outfitted each frog with a radio transmitter belt, placed it into marking solution (a), and allowed it to jump for one minute; we measured jump distances to the center (X) of each paint mark (b).

Zacharow et al., 2003) installed in bottomland forest habitat at the English Creek Environmental Education Center (Bealsville, FL).

Radio transmitter attachment—We attached a radio transmitter (#R1625, Advanced Telemetry Systems, Inc.) to each frog using an elastic bead belt (Fig. 1a; Bartelt and Peterson, 2000; Richter et al., 2001; Muths, 2003). All frogs weighed > 7g, so that the transmitter belt (ca. 0.7g) did not exceed the recommended threshold of 10% of body weight (Richards et al., 1994). Due to the size of the transmitters and the marked sexual size dimorphism of Cuban treefrogs (Meshaka, 2001; Vargas-Salinas, 2006; McGarrity and Johnson, 2009), the majority of the frogs used in our study were female.

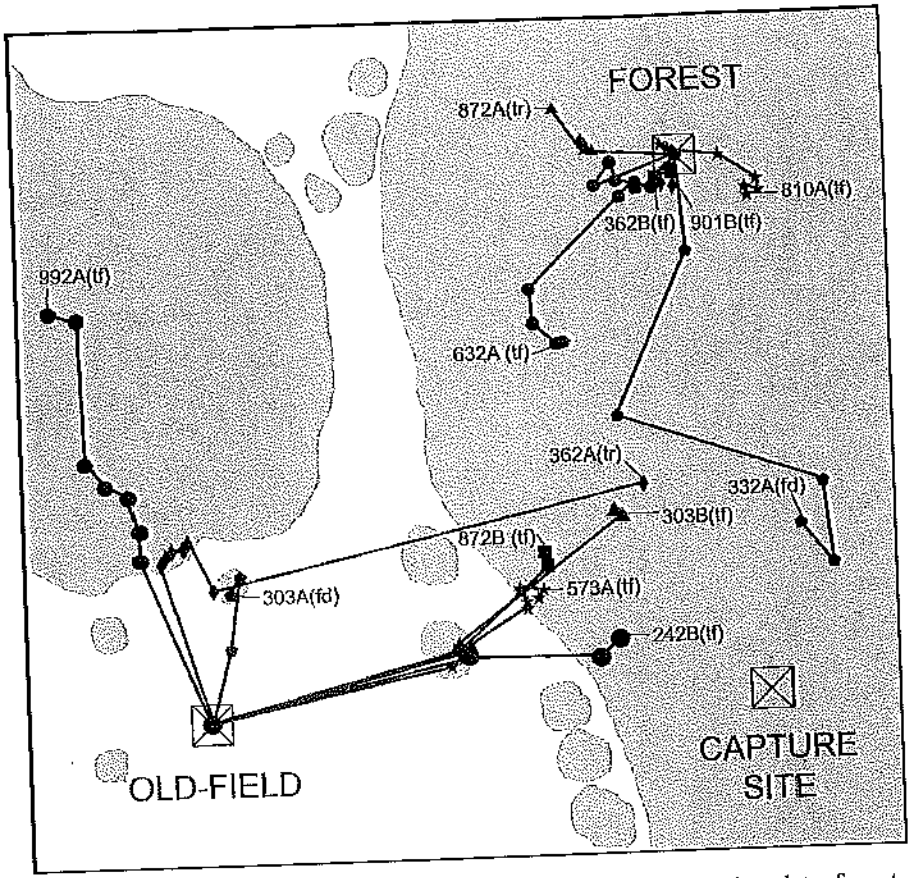

FIG. 2. Movement paths of Cuban treefrogs experimentally displaced to forest (n = 6) or old-field (n = 7) sites. Wooded areas and isolated trees in the old-field are shown in gray, and the old-field areas are shown in white. The alphanumeric labels correspond to individual frogs (see Table 1), and the letters in parentheses indicate final disposition – the transmitter failed (tf), the transmitter abraded the frog and was removed (tr), or the frog was depredated (fd). The lines between frog locations represent path segments, and depict the generalized (i.e., straight-line) movements of frogs between sequential known locations.

Locomotory performance trials—Radio belt attachment is known to affect movement of some frogs (Blomquist and Hunter, 2007); therefore, we tested for overt effects of transmitter attachment on locomotory performance of Cuban treefrogs using methods modified from Peplowski and Marsh (1997). We tested performance of Cuban treefrogs (n = 18) with and without a radio transmitter belt (in random order). We placed each frog into a tray of diluted non-toxic acrylic paint (Fig. 1a), and encouraged it to jump in an experimental arena for one minute; we allowed frogs to rest for at least 30 minutes between trials. After each trial, we measured the distance between paint-marked landing sites (Fig. 1b) to the nearest centimeter. We humanely euthanized all frogs upon completion of locomotory trials by application of 20% benzocaine gel to the venter, followed by freezing for 48 hours. We used paired t-tests (SPSS v.15.0, $\alpha = 0.05$) to evaluate effects of radio transmitter belt attachment on (1) average jump length and (2) number of jumps.

Field telemetry/displacement trials—We transported radio-belted Cuban treefrogs in covered plastic pots (method modified from Mazerolle and Vos, 2006) to release sites (Fig. 2) located in early-successional old-field (n = 7) or bottomland forest habitat (n = 6) approximately 200 m from the collection grid. We relocated frogs to instigate movement, and observed their habitat preferences and response to short-distance displacement. We located frogs daily (during the day) for 2–17 days (Table 1), and collected GPS position data at each location, recorded microhabitat characteristics, and approximated height of arboreal locations by triangulation.

TABLE 1. Movement data and path straightness index values (SI = straight line distance (SLD) / total distance traveled (TD); 0–1) for experimentally displaced Cuban treefrogs. (* indicates statistical significance at $\alpha = 0.05$).

Treatment	Frog	# Days Tracked	SVL (cm)	Wt (g)	Trans. % Wt.	Dist/Day (m)	Dist/Mvmt. (m)	SLD (m)	TD (m)	SI
Old-field	242B	4	54	10	7	17	51	149	152	0.98
	303A	3	48	7	10	21	21	48	62	0.77
	303B	9	62	16	4	20	44	166	176	0.94
	362A	12	50	9	8	21	36	178	251	0.71
	573A	12	49	7	10	12	18	121	161	0.75
	872B	3	58	10	7	47	47	135	142	0.95
	992A	13	58	11	6	11	25	161	172	0.94
	Mean	*8*	—	—	—	*21*	*35*	*137*	*159*	*0.86*
Forest	332A	12	50	8	9	19	45	142	227	0.63
	362B	2	59	14	5	5	5	12	16	0.75
	632A	15	50	7	10	10	14	80	149	0.54
	810AB	13	50	9	8	4	7	28	57	0.49
	872A	12	48	7	10	4	10	47	52	0.90
	901B	3	76	21	3	6	6	7	19	0.37
	Mean	*10*	—	—	—	*8*	*15*	*53*	*87*	*0.61*
t-Test:	t	—	—	—	—	−2.45	−2.53	—	—	−2.92
	p	—	—	—	—	*0.03**	*0.03**	—	—	*0.01**
	df	—	—	—	—	11	11	—	—	11

We used GPS position data to calculate daily distance, straight-line distance (start to finish), and total distance moved for each frog (White and Garrott, 1990), and used these measurements to calculate three movement descriptors: (1) path straightness index (SI = straight-line distance (SLD) / total distance traveled), (2) mean velocity (distance per day), and (3) mean distance per movement. We used a t-test (Zar, 1999; SPSS v15.0, $\alpha = 0.05$) to evaluate the effect of displacement site (forest vs. old-field) on these movement descriptors. We also calculated the compass bearing of the (1) initial movement and (2) mean bearing (overall path, start to finish) of each frog (White and Garrott, 1990). We used Rayleigh's Test (Batschelet, 1981; Zar, 1999; ORIANA v. 2.02e, Kovach Computing Services) to evaluate the directedness (i.e., random vs. significantly oriented) of movements and homing tendency.

We also conducted stepwise binary logistic regression (SPSS v. 12.0.1) to evaluate the influence of individual characteristics (sex, SVL), experimental conditions (release site, transmitter percentage of body weight), and environmental conditions of the preceding day (total rainfall, average air temperature, average relative humidity, average wind speed, moon phase) on the frogs' decision to move during the interval between locations. We obtained environmental data from the U.S. Naval Observatory Astronomical Applications Department (moon fraction illuminated; http://www.usno.navy.mil) and the Florida Automated Weather Network (all other data; FAWN's Wimauma, FL station; http://fawn.ifas.ufl.edu). We used a significance level of $\alpha = 0.05$ for entry of variables into the model and $\alpha = 0.10$ for removal and assessed goodness-of-fit with the Hosmer-Lemeshow test statistic.

RESULTS—*Locomotory performance trials*—Locomotory performance testing indicated that the effects of transmitter attachment were negligible under lab conditions. Attachment of the radio transmitter belt did not significantly affect jump length or number of jumps (all $P \gg 0.05$) in the testing arena. Therefore, we determined that it was justifiable to conduct field evaluations

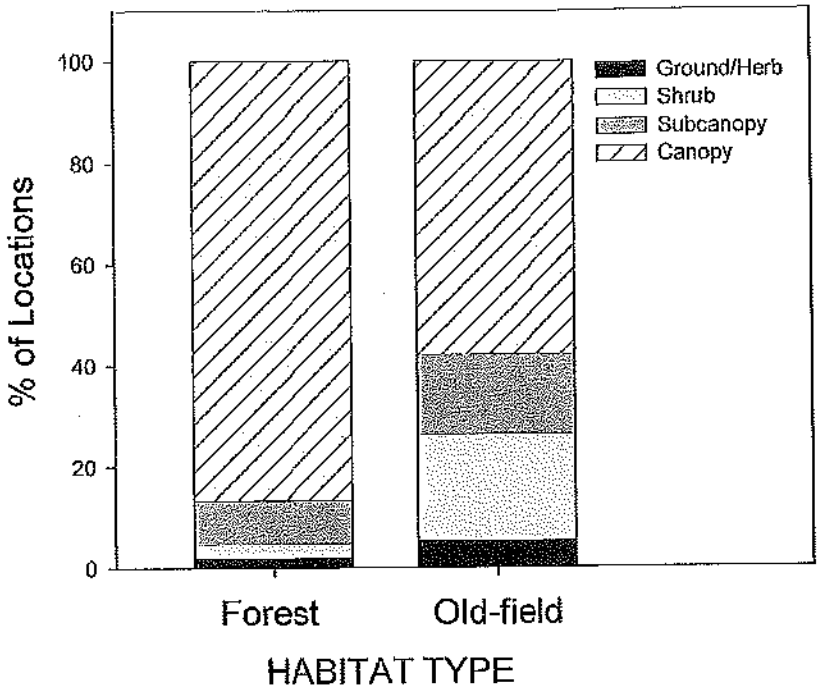

FIG. 3. Microhabitat used by Cuban treefrogs, shown as percent of locations in forest (N = 105) or old-field (N = 19). When frogs displaced to old-field reached the forest edge, their subsequent locations and associated microhabitat choices were considered to be in forest habitat and are included in this category. Microhabitat use was categorized as follows: ground/herb layer (0–1 m), shrub layer (1–2 m), sub-canopy (2–3 m), canopy (>3 m).

using radio telemetry as a method of studying movements and habitat preferences of Cuban treefrogs.

Field telemetry/displacement trials—We successfully used radio telemetry to document movements and habitat use of experimentally displaced Cuban treefrogs, obtaining 124 total locations (Fig. 2). We discontinued tracking when transmitters failed (n = 9), transmitters began to abrade frogs and were removed (n = 2; frogs humanely euthanized), or frogs were depredated by snakes (n = 2; depredated by *Coluber constrictor*, a common predator of Cuban treefrogs). Daytime refuges of displaced Cuban treefrogs (Fig. 3) were predominantly arboreal in the canopy layer (height > 3m) in both habitats, although frogs took refuge in the shrub and sub-canopy layer more frequently when in old-field than when in forest, likely due to the scarcity of large trees.

Frogs displaced to old-field moved more rapidly than frogs displaced to forest (Table 1); both mean velocity (t = 2.58, df = 9, *P* = 0.03) and distance per movement (t = 2.50, df = 10, *P* = 0.03) were significantly greater. Frogs displaced to old-field also moved farther from the release point (mean SLD = 137 m; range 48–178 m) than frogs displaced to forest (mean SLD = 53 m; range 7–142 m), even though on average, frogs displaced to old-field were tracked for fewer days (Table 1). Movement paths of frogs displaced to old-field were more directed (straighter) than were those of frogs displaced to forest (t = 2.81, df = 8, *P* = 0.02). Circular statistics confirmed that initial

TABLE 2. Binary logistic regression analysis of 124 movement decisions (tracking days; coded as 1 = move, 0 = no move) by 13 radio tracked Cuban treefrogs.

Predictor	β	*SE* β	Wald's χ^2	*df*	*P*	e^{β}
Constant	−47.435	13.478	12.387	1	—	—
Relative humidity (%)	0.244	0.069	12.730	1	0.000	1.277
Moon fraction illuminated	−1.282	0.638	4.037	1	0.044	0.277
Wind (kph)	−0.257	0.104	6.117	1	0.013	0.774
Air temperature (°C at 10m)	1.182	0.373	10.021	1	0.002	3.261
Goodness of Fit Test			χ^2	*df*	*P*	
Hosmer & Lemeshow			14.213	8	0.076	

Note: SPSS programming codes: LOGISTIC REGRESSION; METHOD = FSTEP(LR); CRITERIA = PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5). Cox and Snell $R^2 = 0.187$; Nagelerke $R^2 = 0.251$.

movements (Rayleigh's Test; z = 3.82, $P = 0.02$) and overall movement paths (z = 4.85, $P \ll 0.01$) of frogs displaced to old-field were highly oriented. Movements of frogs displaced to forest were randomly oriented, with no preferred direction of movement detected at any scale (all $P > 0.05$).

Environmental conditions exerted the greatest influence on frogs' decisions to move during the course of this study; the best-fitting regression model (Table 2) included relative humidity (%), temperature (°C at 10m), wind speed (kph), and moon phase (fraction illuminated; 0.00/new–1.00/full). After controlling for other factors, the odds that a frog would move during the interval between tracking periods increased greatly with increasing daily average air temperature and relative humidity, and decreased with increasing average daily wind speed and moon illumination (Table 2). Individual frog characteristics and experimental conditions were not significant predictors of frog movement.

DISCUSSION—Our findings demonstrate that radio telemetry is a valuable tool for studying the ecology of Cuban treefrogs, but is subject to certain limitations. We found no overt effects of transmitter attachment on locomotory performance in a test arena, and found that relative transmitter weight did not affect frog movements in the field. We removed transmitters from two frogs due to minor abrasions; therefore, we recommend that researchers use pilot trials to learn to fit radio belts properly and ensure that such injuries are minimized. Due to the relatively large size of the transmitters, our tracking efforts were limited to large adult frogs. In addition, due to the arboreal nature of these frogs, we were unable to replace transmitters before failure. We recommend that researchers consider these limitations when designing a radio telemetry study of arboreal frogs. However, we found that radio telemetry can provide valuable insights on the ecology of large adult Cuban treefrogs that may help to guide management efforts.

For example, we found that Cuban treefrogs displaced to old-field habitat rapidly moved to nearby hardwood hammock or bottomland forest; this novel finding attests to the resilience that has contributed to the success of these invasive frogs. This behavior suggests that they prefer closed-canopy, forested habitats over open areas, and corroborates data from previous visual encounter surveys (Meshaka 2001). This behavior is also consistent with the tendency of many amphibians to avoid or rapidly move out of open areas (Rosenburg et al., 1998; Rothermel and Semlitsch, 2002; Mazerolle and Desrochers, 2005; Mazerolle and Vos, 2006; Rittenhouse and Semlitsch, 2006). The highly directed paths of frogs displaced to old-field were consistent with a straight-line search, a strategy considered optimal in inhospitable or unfamiliar habitat (Zollner and Lima, 1999; Caldwell and Nams, 2006). However, frogs demonstrated an ability to identify and move toward an isolated tree in old-field (see Fig. 2), suggesting that they used visual cues rather than relying solely on straight-line searching. Cuban treefrogs were observed breeding in bottomland forest, suggesting that movements may have also been guided by auditory cues from calling conspecifics. Displaced frogs did not exhibit a strong homing tendency during the course of this study; however, radio telemetry provides an ideal method that will allow future investigation of the potential homing tendencies of these invasive frogs. Regardless of the mechanism used to locate a suitable refuge, the ability to do so rapidly may greatly increase the potential for survival of inadvertently displaced (i.e., hitchhiking) Cuban treefrogs, enhancing their invasion success. This ability to locate suitable habitat rapidly likely contributes to invasion success, and is a trait that may be shared by other invasive species; future avenues of research should include evaluation of this behavior in other invasive anurans.

Environmental factors exerted the greatest influence on Cuban treefrogs' decision to move during this study. Increasing humidity and temperature greatly increased the odds of frog movement, a finding that is also in keeping with the observations of Meshaka (2001) that Cuban treefrogs are most active on warm, humid nights. Rainfall was not a significant predictor of movement during this study; however, this study was conducted during a drought year and there were no major rainfall events during the study period. Increasing average daily wind speed greatly decreased the odds of frog movement, likely due to increased risk of dessication. Increasing moon illumination also greatly decreased the odds of frog movement, perhaps because of increased risk of predation on moonlit nights. These results not only lend support to previous findings, but also highlight additional factors that influence movement and may therefore affect the success of visual encounter surveys.

Our findings have important implications for techniques currently used to sample hylid communities that include invasive Cuban treefrogs. Cuban treefrogs showed a clear preference for arboreal refuges in the canopy layer (Fig. 3), a preference that has also been documented in native treefrogs (Boughton et al., 2000), and has obvious repercussions in studies relying on visual encounter surveys or ground-based PVC refuges. However, all frogs used in this study were captured in ground-based PVC refuges, suggesting that resident (rather than displaced) frogs may use habitat differently, or the presence of PVC pipe refuges may alter microhabitat use. Microhabitat use (and availability) varied between habitat types, and might vary seasonally, although evaluation of seasonal variation was beyond the scope of this study. Until these questions are addressed, we recommend that researchers studying Cuban treefrogs consider using tree-mounted PVC refuges in combination with ground-based PVC refuges, as a prudent measure that might increase sampling efficiency and detection probability.

Cuban treefrogs pose a serious threat to native frogs in Florida (Knight et al., 2009; Meshaka, 2001; Rice et al., 2005; Smith, 2005), yet attempts to manage invasions and minimize their impacts are few. Our findings suggest that displaced frogs, or frogs near an urban-wildland interface, may use multiple cues to rapidly identify, invade, and colonize forest habitats in natural areas. Furthermore, the observed preference for arboreal habitats suggests that visual encounter surveys and ground-based PVC refuges may underestimate the magnitude of such invasions. Radio telemetry can provide valuable information regarding movement patterns, habitat use, and home range size of Cuban treefrogs in (and between) urban and natural areas, that should help to guide monitoring and management efforts.

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