Biological Sciences

THE USE OF PVC PIPE REFUGIA TO EVALUATE SPATIAL AND TEMPORAL DISTRIBUTIONS OF NATIVE AND INTRODUCED TREEFROGS

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ABSTRACT: *As part of a monitoring program to study the distribution of native frogs and toads and the introduced Cuban treefrog (*Osteopilus septentrionalis*) invasion, PVC pipe refugia were installed along the edge of 24 marsh, cypress, riverine, and borrow pit wetlands at a natural area in Hillsborough County, Florida. From June 2005 through September 2007, observations of 7,237 treefrogs were recorded, which included 673 green treefrog (*Hyla cinerea*) observations, 945 pinewoods treefrog (*Hyla femoralis*) observations, 1,530 squirrel treefrog (*Hyla squirella*) observations, and 4,089 Cuban treefrog observations. Overall, Cuban treefrogs were the most frequently observed species regardless of wetland type and season. Observations of all treefrog species were typically higher during the dry seasons as compared to those for the wet seasons, suggesting that the pipe refugia provided shelter from the dry and cold conditions. Observations of green or pinewoods treefrogs in pipes were not correlated with Cuban treefrog observations. However, there was a negative correlation between the squirrel treefrog observations in the pipes and the observations of Cuban treefrogs, suggesting that squirrel treefrogs were affected by the presence of Cuban treefrogs.*

Key Words: PVC pipe refugia, green treefrog, *Hyla cinerea*, pinewoods treefrog, *Hyla femoralis*, squirrel treefrog, *Hyla squirella*, Cuban treefrog, *Osteopilus septentrionalis*, Florida

WE studied the spatial and temporal distribution of native frogs, as well as the spread of the introduced Cuban treefrog (*Osteopilus septentrionalis*), at an approximately 10,000-hectare natural area owned and managed by the Southwest Florida Water Management District (SWFWMD) in Hillsborough County, Florida (Fig. 1 in Guzy et al., 2006). The site includes a wellfield, the Morris Bridge Wellfield (MBWF) in operation since 1978 and managed by Tampa Bay Water since 1999, as well as public parks, the Flatwoods and Trout Creek Units of the Hillsborough County Wilderness Park system. It supports a mosaic of six major ecosystems, typical of landscapes in West-Central Florida. Flatwoods is the most prevalent ecosystem, while riverine forest, associated with the Hillsborough River and some of its tributaries, is the second most common ecosystem. Pine-mesic oak forest, isolated cypress domes, isolated freshwater marshes, and several borrow pits dominate the remainder of the site.

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During our initial study at the site during Summer 2004, substantial populations of Cuban treefrogs were found at half of the sites we studied (Guzy et al., 2006). Cuban treefrogs were not established at the site as of 1992 (Delis et al., 1996); however, they were observed in residential developments to the north and west of the site and at a few wellfield pumping stations in the northeast section of the site during Summer 2001. The Cuban treefrog invasion in Florida began in the Florida Keys (Barbour, 1931), and Cuban treefrogs have been established in peninsular Florida since at least 1951 (Schwartz, 1952). They have since spread throughout peninsular Florida and represent a threat to native amphibians (Meshaka, 2001). Cuban treefrogs reduce the populations of native frogs through competition and predation; their tadpoles consume and are superior competitors to native tadpoles (Ashton and Ashton, 1988; Dalrymple, 1994; Smith, 2005a; 2005b). Adult Cuban treefrogs eat native frogs (Dalrymple, 1994), including squirrel treefrogs (*Hyla squirella*) and green treefrogs (*Hyla cinerea*) (Wyatt and Forys, 2004). While introduced species are widely considered to be a threat to native biodiversity (Mack et al., 2000), there have been relatively few studies on the impacts of Cuban treefrogs in natural systems (Rice et al., 2009).

Using PVC pipe refugia to "trap" treefrogs has been shown to be a highly effective and efficient way to monitor hylid treefrogs, which use the pipes as diurnal hiding places; in addition, movement for breeding and foraging activity is not impeded (Lohoefener and Wolfe, 1984; Dodd and Scott, 1994; Domingue O'Neill and Boughton, 2009; Moulton et al., 1996; Boughton et al., 2000; Zacharow et al., 2003; Bartareau, 2004). In a pilot study conducted at our study site in Fall 2004, ground-placed PVC pipe refugia were effective at capturing Cuban treefrogs, as well as native treefrog species, including pinewoods treefrogs (*Hyla femoralis*), squirrel treefrogs, and green treefrogs (Buckingham et al., 2005). While some investigations have found treefrog capture bias associated with pipe diameter (Boughton et al., 2000; Zacharow et al., 2003; Bartareau, 2004), treefrogs exhibited no preference for refugia size in our pilot study (Buckingham et al., 2005).

The purpose of this investigation was to determine the spatial and temporal distributions of native and Cuban treefrogs within the SWFWMD-owned natural area using ground-placed PVC pipe refugia. We also investigated whether or not treefrog observations were affected by wetland type and season. In addition, we evaluated whether or not the presence of Cuban treefrogs in each pipe affected the presence of native treefrogs.

METHODS—Ten 5.1-cm PVC pipes (one meter in length) were placed in the ground at ten-meter intervals along the edge of 24 wetlands in early June 2005 (for a total of 240; Fig. 1). The edge of each wetland was usually represented by the waterward extent of the saw palmetto (*Serenoa repens*). The study wetlands included four types of wetlands: isolated freshwater marshes (n = 3); isolated, ephemeral cypress wetlands (n = 9); isolated, artificial borrow pits that are typically permanently inundated and contain fish (n = 5); and riverine wetlands that are bottomland hardwood hammocks or cypress strands with hydrological connections to the Hillsborough River (n = 7; Table 1). To account for the differences in wetland type sample size in this investigation, we

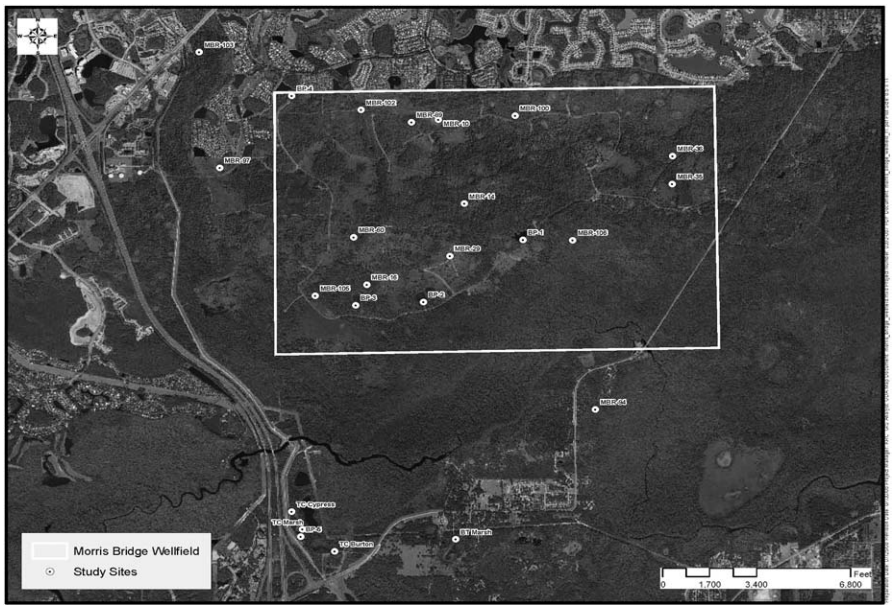

FIG. 1. Location of wetland study sites, Southwest Florida Water Management District lands, Hillsborough County, Florida.

used the proportions of observations [(number of observations of treefrog species in a wetland/number of total observations of treefrogs in wetland type)*100] for all wetland type analyses and comparisons.

From June 2005 through September 2007, pipes were checked at a frequency that ranged from once per week (July and August 2005), biweekly every month (June 2005, September 2005 through May 2006, August 2006 through October 2006), to once per month (June, July, November, and December 2006), to once every other month (January through September 2007). Each pipe was checked during daylight conditions between 0700 and 1800 hours. The numbers and species of treefrogs found in each pipe at each wetland were recorded during each monitoring event. To identify the treefrogs to species, frogs were removed from the pipe. After the frogs were identified, they were placed back into or within one meter of the pipe. Because of the differences in monitoring frequency over the course of this investigation, the data were converted to observations per event (number of observations of treefrog species in a wetland/number of total monitoring events during the season) for all seasonal analyses and comparisons.

During each monitoring event, water level data were collected from staff gauges installed at the center of each study wetland. Rainfall data collected at the MBWF pumping station were obtained from Tampa Bay Water. In West-Central Florida, ecological and hydrological data are typically compared by water year, which is defined as the period between October 1st of one year and September 30th of the next year and is designated by the calendar year in which it ends; therefore, our results were evaluated by water year. Water years in West-Central Florida are divided into a dry season (October through May) and a wet season (June through September). This investigation began in June 2005 and was conducted during the wet season of Water Year (WY) 2005 (June through September 2005), all of WY2006 (October 2005 through September 2006), and all of WY2007 (October 2006 through September 2007).

Data were analyzed using JMP® software (SAS Institute, 2002). Because individuals were not marked and treefrogs had the potential to be observed multiple times, nonparametric Wilcoxon Tests were used to determine the effect of wetland type and season on the number of treefrog

TABLE 1. Treefrog observations in PVC pipe refugia at 24 study wetlands, June 2005 through September 2007, Southwest Florida Water Management District lands, Hillsborough County, Florida.

Study Site	Wetland Type	Green Treefrogs (*Hyla cinerea*)	Pinewoods Treefrogs (*Hyla femoralis*)	Squirrel Treefrogs (*Hyla squirella*)	Cuban Treefrogs (*Osteopilus septentrionalis*)
MBR-10	Marsh	22	16	135	40
BT Marsh	Marsh	43	4	47	57
TC Marsh	Marsh	9	260	15	240
MBR-14	Cypress	9	71	71	328
MBR-16	Cypress	2	38	3	230
MBR-29	Cypress	7	33	53	184
MBR-35	Cypress	12	8	22	153
MBR-89	Cypress	78	30	247	55
MBR-94	Cypress	47	61	77	68
MBR-97	Cypress	23	61	8	67
TC Button	Cypress	90	13	97	242
TC Cypress	Cypress	23	54	218	130
MBR-36	Riverine	40	56	55	155
MBR-60	Riverine	14	16	145	163
MBR-100	Riverine	74	45	25	190
MBR-102	Riverine	76	19	175	126
MBR-103	Riverine	7	3	9	132
MBR-105	Riverine	15	3	37	281
MBR-106	Riverine	3	35	8	297
BP-1	Borrow Pit	10	16	13	211
BP-2	Borrow Pit	0	15	1	185
BP-3	Borrow Pit	3	13	11	226
BP-4	Borrow Pit	56	69	57	41
BP-5	Borrow Pit	10	6	1	288

observations. Significant effects were further explored with Tukey multiple comparison procedures ($p = 0.05$). A Wilcoxon Test, followed by a Tukey multiple comparison procedure ($p = 0.05$), was used to determine the effect of water year on the presence of surface water at the study wetlands from May through October. Multiple regression procedures yielding Pearson product-moment correlations were used to determine significant correlations ($p = 0.05$) between treefrog observations by pipe.

RESULTS—From June 2005 through September 2007, observations of 7,237 treefrogs in the PVC pipes located in the 24 study wetlands were recorded (Table 1). These observations included 673 green treefrogs, 945 pinewoods treefrogs, 1,530 squirrel treefrogs, and 4,089 Cuban treefrogs.

Over the course of the study, green treefrogs were observed in 23 of 24 wetlands (Table 1). Pinewoods and squirrel treefrogs were observed at all 24 wetlands. Either pinewoods or squirrel treefrogs were the most frequently observed species at four of the five wetlands (BP-4, MBR-102, MBR-89, and MBR-10) located along the northern MBWF boundary adjacent to the residential development (Fig. 1). From June 2005 through September 2007, Cuban treefrogs were observed at all wetlands and were the most frequently

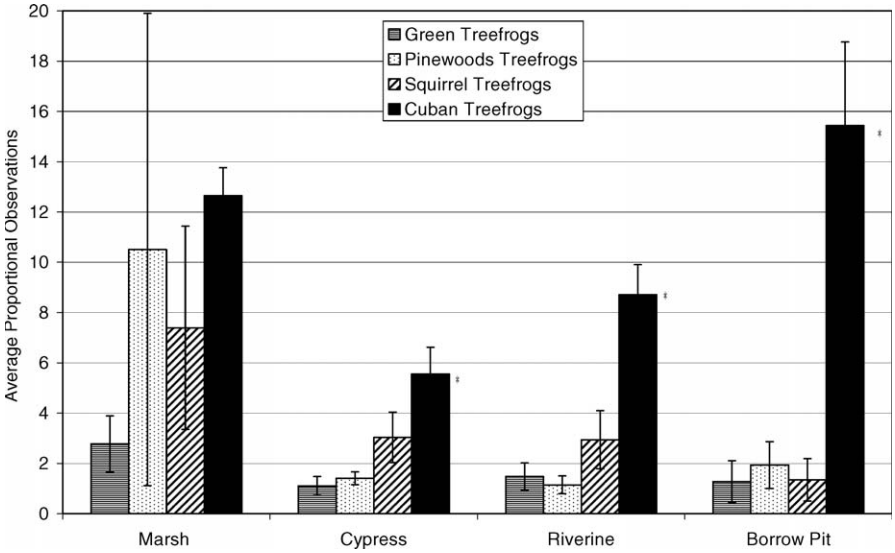

FIG. 2. The average proportional observations of treefrogs in marsh, cypress, riverine, and borrow pit wetlands, Southwest Florida Water Management District lands, Hillsborough County, Florida, June 2005 through September 2007. Error bars represent the standard error, an asterisk indicates significant differences ($p < 0.05$).

observed species at 17 of the sites (Table 1). Cuban treefrogs were observed at all study wetlands by August 2005.

There was no significant difference ($p > 0.05$) in the proportional observations for green, pinewoods, and squirrel treefrogs across the four wetland types (Fig. 2). However, Cuban treefrogs were observed more frequently ($p < 0.05$) at borrow pits as compared to cypress wetlands. Within marshes, there was no significant difference ($p > 0.05$) in proportional observations for the four treefrog species (Fig. 2). Cuban treefrogs were observed more frequently ($p < 0.05$) in cypress wetlands than green and pinewoods treefrogs (Fig. 2). In riverine wetlands and borrow pits, Cuban treefrogs were observed more frequently ($p < 0.05$) than green, pinewoods, and squirrel treefrogs (Fig. 2).

Over the course of the 27-month study, for the months for which we conducted monitoring events, the highest average monthly observations for Cuban, squirrel, and green treefrogs occurred in December 2005 (Fig. 3). For pinewoods treefrogs, average monthly observations were highest in January 2007.

Rainfall at the site during WY2005, WY2006, and WY2007 was 125.7, 99.3, and 114.1 cm, respectively. These values represent the 40^{th} percentile when comparing all water year rainfall data collected at the site to date, the lowest water year rainfall value since rainfall data have been collected at the site, and the 19^{th} percentile when comparing all water year rainfall data collected at the site to date, respectively.

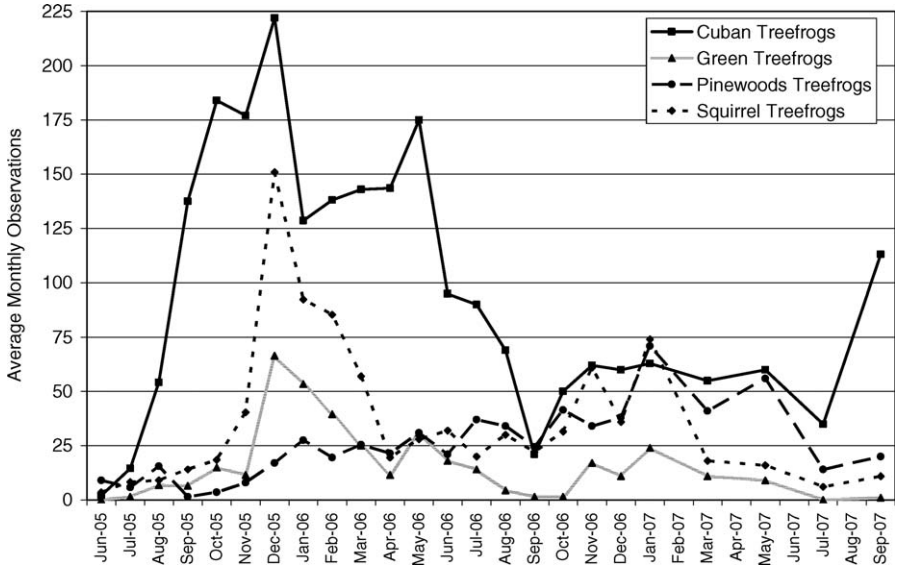

FIG. 3. The average monthly observations of treefrogs from June 2005 through September 2007, Southwest Florida Water Management District lands, Hillsborough County, Florida.

From May through October 2005, all wetlands had standing water (Table 2). However, more than half of the 24 wetlands had no standing water from May through October 2006 or only had standing water for approximately two weeks. Similar conditions occurred from May through October 2007 at seven of the 24 study wetlands (Table 2). Wetland hydroperiods from May through October 2005 (mean = 4.71 months) were significantly longer ($p < 0.05$) than those during 2006 (mean = 1.46 months) and 2007 (mean = 1.75 months).

For the wet season of WY2005, dry season of WY2006, wet season of WY2006, and wet season of WY2007, Cuban treefrog observations per event were significantly higher ($p < 0.05$) than those for green, pinewoods, and squirrel treefrogs (Fig. 4). For the dry season of WY2007, Cuban treefrogs were observed more frequently ($p < 0.05$) than green treefrogs. Green and Cuban treefrog observations per event during the WY2006 dry season were significantly higher ($p < 0.05$) than those for any other season (Fig. 4). Pinewoods treefrog observations for the dry season of WY2007 were significantly higher ($p < 0.05$) than those for the WY2005 wet season, the WY2006 dry season, and the WY2007 wet season. Squirrel treefrog observations for the WY2006 dry season were significantly higher ($p < 0.05$) than observations for the wet seasons of WY2005, WY2006, and WY2007 (Fig. 4).

Treefrogs were observed at least once in 239 of the 240 pipes in this study. Observations of green or pinewoods treefrogs in pipes were not significantly ($p > 0.05$) correlated with Cuban treefrog observations (Table 3). However, there

TABLE 2. Description of 24 study wetlands where the number and species of treefrogs were monitored using PVC pipe refugia, June 2005 through September 2007, Southwest Florida Water Management District lands, Hillsborough County, Florida.

Study Site	Wetland Type	Number of Months Water Present, May–October 2005	Number of Months Water Present, May–October 2006	Number of Months Water Present, May–October 2007
MBR-10	Marsh	4.5	0	0
BT Marsh	Marsh	4.5	1.5	2
TC Marsh	Marsh	4.5	0.5	2
MBR-14	Cypress	4.5	0	0
MBR-16	Cypress	4.5	0	0
MBR-29	Cypress	4.5	0	0
MBR-35	Cypress	3.5	0	0
MBR-89	Cypress	5	1	2
MBR-94	Cypress	4.5	0.5	0.5
MBR-97	Cypress	4.5	0	0.5
TC Button	Cypress	4.5	0.5	2
TC Cypress	Cypress	4.5	0.5	2
MBR-36	Riverine	4.0	1.5	2
MBR-60	Riverine	5	1.5	2.5
MBR-100	Riverine	3.5	0	2.5
MBR-102	Riverine	3.5	0.5	2
MBR-103	Riverine	6	3.5	3
MBR-105	Riverine	4	1.0	2
MBR-106	Riverine	5	1.5	2
BP-1	Borrow Pit	6	6	3
BP-2	Borrow Pit	6	6	3
BP-3	Borrow Pit	6	3	3
BP-4	Borrow Pit	6	6	3
BP-5	Borrow Pit	4.5	0	3

was a significant negative correlation ($p < 0.05$) between the observations of squirrel treefrogs in pipes and the observations of Cuban treefrogs (Table 3).

DISCUSSION—Although individual treefrogs were not marked and the same treefrogs could have been observed multiple times, the observational data collected during this investigation provided useful information regarding the distribution of native and Cuban treefrogs throughout our study area. While Cuban treefrogs were found at only half of the wetlands studied during Summer 2004 (Guzy et al., 2006), they were observed at all study wetlands two months into this investigation. In addition, Cuban treefrogs were the most frequently observed treefrog species at 17 of the 24 study wetlands. This indicates that Cuban treefrogs had most likely spread throughout the site by Summer 2005. Cuban treefrogs were the most frequently observed treefrog species at borrow pits and were observed more frequently at borrow pits than at the other wetland types, suggesting that this species makes higher use of artificial, created wetlands and disturbed areas compared to the native species

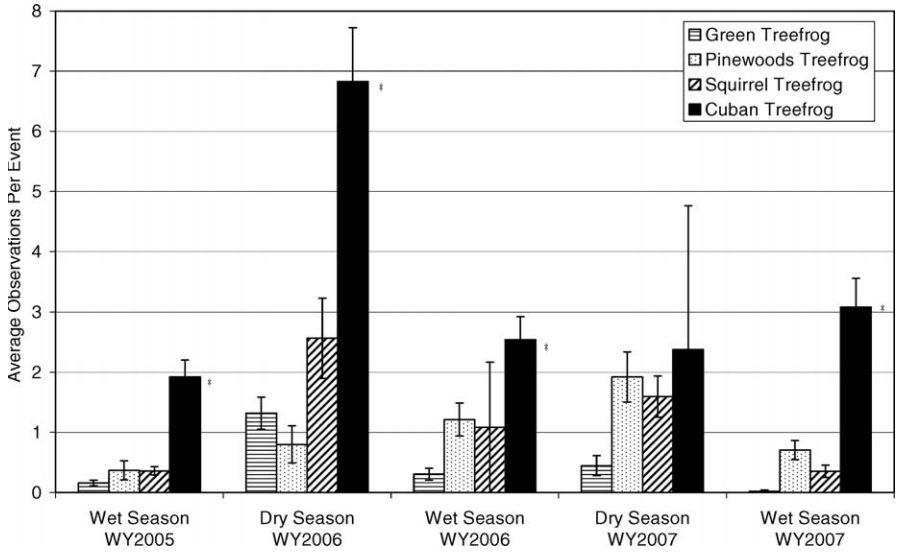

FIG. 4. The average observations per event of treefrogs during the Water Year 2005 wet season, Water Year 2005 dry season, Water Year 2006 wet season, Water Year 2007 dry season, and Water Year 2007 wet season, Southwest Florida Water Management District lands, Hillsborough County, Florida. Error bars represent the standard error, , an asterisk indicates significant differences ($p < 0.05$).

and compared to the other wetland types. The presence of fish at the borrow pits may not affect Cuban treefrog breeding success. Squirrel and pinewoods treefrogs typically breed in ephemeral wetlands, which usually do not contain fish (Jensen et al., 2008).

It is interesting to note that pinewoods or squirrel treefrogs were the most frequently observed species at four of the five wetlands located along the northern boundary of our study area, which suggests that the Cuban treefrog invasion may not have originated from the residential development to the north (Fig. 1). Cuban treefrogs may have spread throughout the area by hitchhiking on vehicles associated with recreation, wellfield operations, and land management activities traveling on the Loop Road; this is further supported by the large number of Cuban treefrog observations at wetlands located in the interior of the study site (e.g., MBR-14). In other parts of Florida, Cuban treefrogs have been known to disperse via vehicles (Meshaka, 1996).

PVC pipes are effective passive traps because they mimic natural shelters and provide a moist environment, shelter from the wind, and refuge from extreme temperatures and predators (Domingue O'Neill and Boughton, 2009; Boughton et al., 2000; Bartareau, 2004). This was most notable during the dry season months when observations of all treefrog species were typically higher than those for the wet seasons included in this study, indicating the frogs may have been seeking shelter from the dry and cold conditions during the non-

TABLE 3. Correlations (Pearson product-moment correlation coefficients) between native treefrogs and Cuban treefrogs observed in a PVC pipe, June 2005 through September 2007, Southwest Florida Water Management District lands, Hillsborough County, Florida.

Treefrog Species	Cuban Treefrogs	P Value
Green Treefrog	−0.1194	0.0654
Pinewoods Treefrog	−0.0663	0.3074
Squirrel Treefrog	−0.2002	0.0019

breeding season. Zacharow and coworkers (2003) found that pipe occupancy by squirrel treefrogs increased during the winter months and into the spring, squirrel treefrogs dispersed in April as they sought favorable microclimates or breeding sites, green treefrog captures were highest in November, and green treefrog captures dropped off substantially in April, suggesting that they left the vicinity of the pipes, perhaps to breed; this was similar to what we found in our investigation.

The frog reproductive season in our study area ranges from about mid-May through the end of October, although some species will breed earlier if water is present (Carmichael and Williams, 1991). As indicated by the amount of rainfall and the presence of surface water in the study wetlands, WY2005 was a fairly typical year. However, WY2006 was an extremely dry year, as indicated by the lowest rainfall measurement to date and the lack of, or short duration, of standing water in more than half of the study wetlands from May through October. Dry conditions continued into WY2007, with most of the rainfall occurring at the end of the wet season. These drought conditions could have contributed to the decrease in observations of green, squirrel, and Cuban treefrogs after the dry season of WY2006 since breeding could not occur in most wetlands during 2006 and 2007. Observations of pinewoods treefrogs did not decrease until the wet season of 2007; because they are adapted to living in flatwoods and sandhills (Jensen et al., 2008), pinewoods treefrogs may be more tolerant to drought conditions than Cuban, green, and squirrel treefrogs.

Groups of treefrogs were commonly observed in single PVC pipe refugia in this investigation, as well as in previous studies (Moulton et al., 1996; Boughton et al., 2000; Bartareau, 2004). The negative correlation between the presence of Cuban treefrogs in pipes and the presence of squirrel treefrogs in this study suggests that the presence of Cuban treefrogs affected squirrel treefrogs. However, this pattern was not observed for green or pinewoods treefrogs. In a PVC pipe refugia study conducted in Collier County, Florida, some of the variation in squirrel treefrog captures could have been attributed to a negative correlation with the coexistence of Cuban treefrogs (Bartareau, 2004). In the same study, Bartareau (2004) found no correlation between the coexistence of green treefrogs and Cuban treefrogs, which was similar to what we found. Meshaka (2001) found negative relationships between green, squirrel, and Cuban treefrogs and suggested that predation was responsible for the unstable coexistence of these species across a variety of coastal habitats.

Wyatt and Forys (2004) have suggested that although these artificial refuges mimic natural refuges by protecting treefrogs from many native predators, they may expose native treefrogs to increased predation by Cuban treefrogs because the PVC pipes are particularly attractive to this introduced species. Therefore, Cuban treefrogs could have been observed more frequently as compared to native treefrog species in this investigation because they were particularly attracted to PVC pipe refugia. Although predation on native treefrogs is most likely to be significant in human-modified environments where Cuban treefrogs are most abundant, researchers, including ourselves, could be increasing the incidence of predation through the use of PVC pipes refugia in more pristine areas where Cuban treefrogs are present (Wyatt and Forys, 2004).

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