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Population Densities of the Cuban Treefrog, *Osteopilus septentrionalis* and Three Native Species of *Hyla* (Hylidae), in Urban and Natural Habitats of Southwest Florida

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by

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ABSTRACT

The Cuban treefrog, *Osteopilus septentrionalis*, is an invasive species in Florida that may be negatively impacting adult and larval native treefrog species through competition via direct predation. The purpose of this study was to determine the abundance and distribution of *O. septentrionalis* in urban, semi-urban and natural habitats. The distribution and abundance of native treefrogs within the study area, *Hyla cinerea, Hyla femoralis* and *Hyla squirella,* were also estimated and compared to that of *O. septentrionalis.* Treefrogs were captured using PVC pipes with two internal diameters, 1.9 cm and 4.45 cm, hung on three tree types, *Pinus, Quercus* and *Sabal.* Distance to water, time of year and monthly rainfall were also considered as a potential influence of treefrog captures. *O. septentrionalis* was found in sites with both urban and natural habitats in frequencies far greater than those of native treefrogs. Results indicate that *O. septentrionalis* are captured more frequently in sites with urban habitat; however recapture rates and estimates of population size indicate that the population sizes of *O. septentrionalis* may actually be larger at sites with natural habitat. *O. septentrionalis* are found significantly more frequently in *Quercus*. *H. femoralis* and *H. squirella* were found significantly more frequently in PVC pipes with an internal diameter of 4.45 cm. A weak, although significant, positive correlation was found between distance to water and treefrog abundances for *O. septentrionalis* and *H. squirella.* Native treefrogs were found less frequently in PVC pipes that also contained *O. septentrionalis;* this result is perhaps the most interesting because it may indicate that native treefrogs will avoid habitat or refuges where *O. septentrionalis* are present. If *O. septentrionalis* is a real threat to species of treefrogs in Florida, these results should be of concern. Findings suggest that PVC pipes may give biased estimates of treefrog densities. Available refuge, temperature and precipitation all appear to have an effect on how attractive PVC pipes are to treefrogs.

Introduction

The invasion of natural communities by exotic species is a major threat to biodiversity, second only to habitat destruction (Williamson, 1999). This threat may arise through predation, competition for resources, disruption of trophic dynamics, habitat modification, hybridization with natives or as vectors for disease (Simberloff, 1997; Greenless, 2007). Habitats which are susceptible to invasion by exotics include those which have been disturbed by urban development, habitats with a tropical climate or island habitats. Florida possesses all of these characteristics, and therefore is particularly vulnerable to invasions; the southern peninsula of Florida is effectively an island as it is surrounded on the eastern, western and southern sides by water and on the northern side by frost. Additionally, habitats of Florida are favorable targets for both terrestrial and aquatic invaders because of the abundance of wetland habitats. The introduction of non-indigenous species is facilitated by shipping ports and airports in the cities of Miami and Tampa (Simberloff, 1999).

The Cuban treefrog, *Osteopilus septentrionalis*, Figure 1, is an invasive species in Florida which may be negatively influencing populations of native treefrogs. The native range of *O. septentrionalis* includes Cuba, Isla de Juventud, the Cayman islands and the Bahamas (Meshaka, 2001). Other than Florida, the exotic range of *O. septentrionalis* includes Puerto Rico, St. Croix, St. Thomas, St. Maarten (Powell et al., 1992; Meshaka, 2001), Anguilla (Townsend et al. 2000; Meshaka, 2001), Necker Island (Meshaka, 2001), Antigua (Lindsay and Cooper 2008) and Curacao (Van Buurt, 2007). The colonization of Key West, Florida by *O. septentrionalis* was first reported by Barbour in 1931. According to Barbour (1931) *O. septentrionalis* had been present on Key West long before his report and was most likely introduced to the island via passenger boats and ferries carrying shipments of Cuban produce. In 1952 there was a report of several *O. septentrionalis* on mainland Florida in Miami (Schwartz, 1952). *O. septentrionalis* was first reported in Hillsborough county, where the present study takes place, in 1996 (Meshaka, 1996). Oliver (1950) reported that the Cuban anole, *Anolis sagrei,* was most likely introduced to Tampa via shipments of lumber and vegetable produce imported from Cuba; it is likely that *O. septentrionalis* were also introduced in this way. Presently the distribution of *O. septentrionalis* reaches Duval County as is shown in Figure 2. It is believed that *O. septentrionalis* will continue to disperse along the Gulf coast of Florida into adjacent coastal states and eventually into Mexico; breeding populations may begin to colonize Georgia, however cool winter temperatures will limit dispersal further along the East coast of the United States (Meshaka, 2001; Soma, 2007). Meshaka (2001) predicts that *O. septentrionalis* will continue to disperse throughout the Caribbean as well as Central and South America.

Figure 1. *Osteopilus septentrionalis*. Photo by Teresa Piacenza.

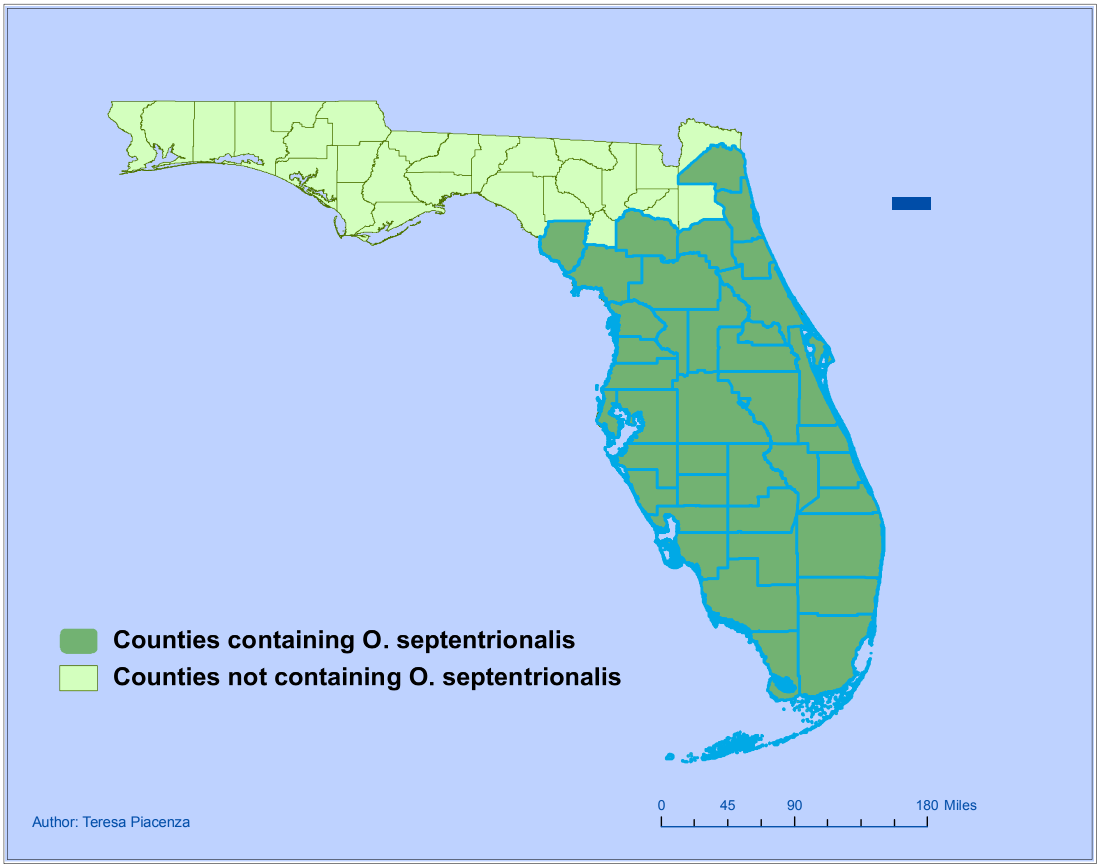

Figure 2. Current distribution of *O. septentrionalis* in Florida.

The successful colonization of Florida by *O. septentrionalis* can be attributed to several characteristics. Members of *O. septentrionalis* have high fecundity because of large clutch sizes, short larval periods and the ability to reach sexual maturity in a short period of time. Additionally *O. septentrionalis* are capable of breeding year round in counties including and south of Hillsborough (Meshaka, 2001). Known indigenous Florida predators of *O. septentrionalis* include *Thamnophis sauritus, Thamnophis sirtalis, Elaphe obsolete, Coluber constrictor* (Love, 1995; Meshaka and Ferster, 1995; Meshaka, 1997) and *Corvus brachyrhynchos* (Butterfield et al., 1997). However, a noxious skin secretion produced by *O. septentrionalis* may potentially be making them unpalatable to many other potential predators, which may contribute to successful colonization. *O. septentrionalis* also have the ability to tolerate a range of physical conditions; Meshaka (2001) reports that larvae are able to survive in breeding waters with temperatures varying from 12-41°C and adults will remain active at night during periods of low temperatures. Finally, little adaptation was required of *O. septentrionalis* while invading because of the striking similarities of habitat in their native and novel ranges (Butterfield et al., 1997; Meshaka, 2001) *O. septentrionalis* primarily inhabits mesophytic forests and also mangrove forests (Meshaka, 2001).

It is difficult to assess the threat that *O. septentrionalis* poses to populations of native Florida treefrogs. *O. septentrionalis* have been shown to compete with native anurans through direct adult (Meshaka, 2001; Wyatt, 2004) and larval predation (Smith, 2005a; Smith 2005b; Smith 2006). Dietary and habitat preferences of *O. septentrionalis* overlap with the preferences of native treefrogs, however these resources are not limited and therefore competition is of little consequence. Although it has been shown that *O. septentrionalis* can have a negative impact on individuals of native treefrogs, currently, it is unknown whether the presence of this invader is affecting populations of natives.

The purpose of this study is to determine how population densities of *O. septentrionalis* vary in natural and urban habitats using PVC refugia. It has been reported that *O. septentrionalis* will readily inhabit urban areas (Meshaka 2001). However, before now, an intense sampling effort in urban areas has not been performed. I was interested to know if *O. septentrionalis* would be found in similar densities in natural and urban habitats and also if these densities coincide with the densities of native treefrogs. Additionally I hope to determine if internal PVC pipe diameter, tree type or distance to water will influence treefrog capture rates. PVC refugia have been shown to be an effective method for capturing treefrogs (Boughon and Staiger, 2001; Zacharow et al. 2003; Bartareau 2004). This trap method is particularly useful in Florida; treefrogs are able to escape from poles if temperatures get too high or the environment becomes too dry, therefore eliminating trap mortality.

Methods

Study Sites

Nine sites including natural, urban and semi-urban habitats were chosen for this study, Figure 3. I chose the USF Tampa campus as the location for my three urban sites. The plant communities at these sites have been altered by urban development and are surrounded on all sides by roads or parking lots. Trout Creek Park, part of the Hillsborough County owned Wilderness Park, was used as a natural site. Trout Creek is composed of mesic and scrubby flatwood plant communities. The University of South Florida Ecological Research Area (USF Eco Area) was used as the location for three additional natural sites which are also composed of mesic and scrubby flatwood plant communities. The USF Eco Area is located on the Hillsborough River and is north and northeast of the USF Tampa Campus. The remaining two sites chosen for this study are located at the USF golf course and the USF Riverfront Park. The plant communities at these sites have been also altered by urban development, however, they are bordered by natural habitat. The USF golf course is bordered by a wetland. The USF Riverfront Park is bordered by a wetland on one side and by hardwood forest on the other. For the purpose of this study, these sites will be considered semi-urban. To further characterize each of the nine sites chosen, I estimated tree density using the T-squared sampling procedure (Besag 1973, Krebs 1999). Additionally, the land use descriptions for each site were obtained from the Federal Geographic Data Committee. Table 1 gives the area, tree density and land use description for each of the nine sites.

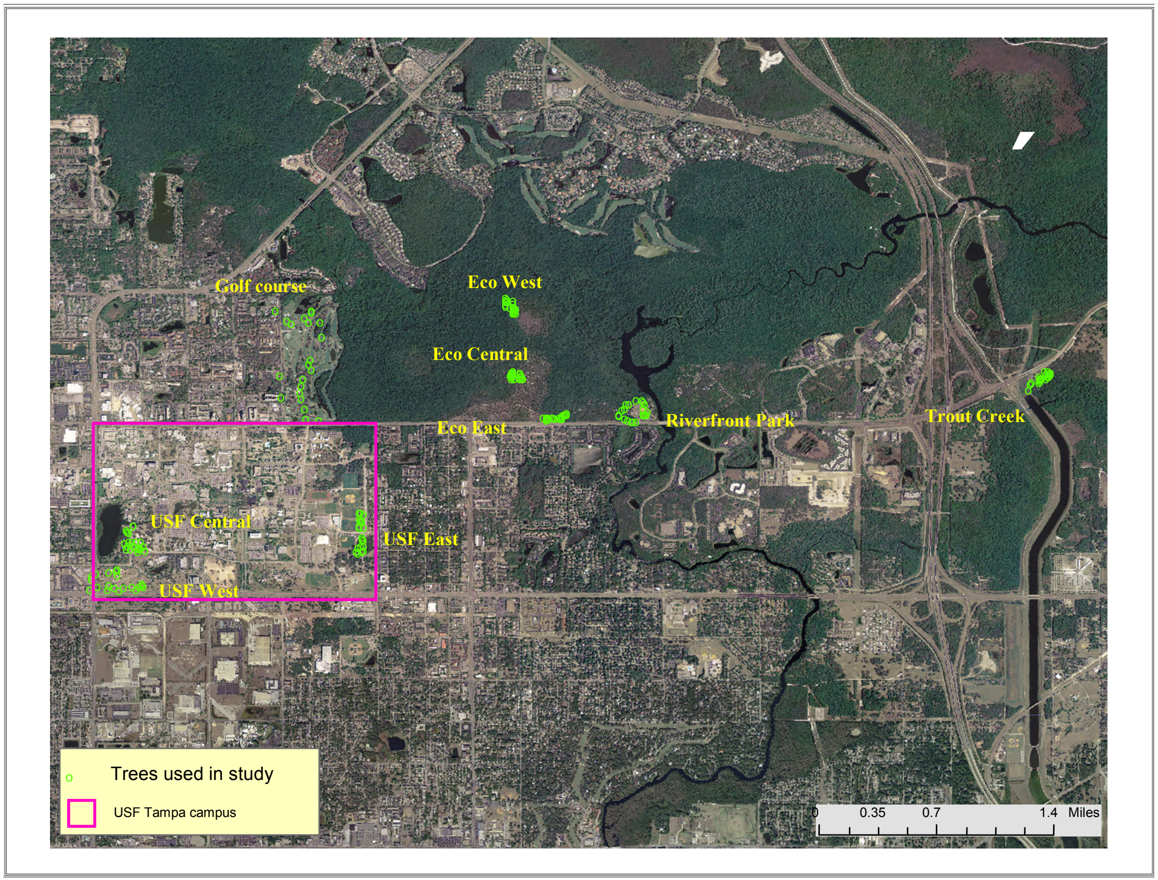

Figure 3. Aerial map of sites used in study.

site	area ($km^{2)}$	trees / $meters^2$	land use description
Eco Central	0.099	0.118	Upland forests, hardwood conifer mixed
Eco East	0.015	0.042	Upland forests, hardwood conifer mixed
Eco West	0.015	0.072	Upland forests, hardwood conifer mixed. Wetland hardwood forest, steam and lake swamps (bottom land).
Golfcourse	0.414	0.003	Urban and built-up, recreational
Riverfront Park	0.052	0.005	Urban and built-up, institutional
Trout Creek	0.033	0.074	Upland forests, hardwood conifer mixed. Wetland hardwood forest, steam and lake swamps (bottom land).
USF Central	0.046	0.005	Urban and built-up, institutional
USF East	0.025	0.020	Urban and built-up, institutional
USF West	0.107	0.003	Urban and built-up, institutional

Table 1. Area, tree density and land-use descriptions of study sites.

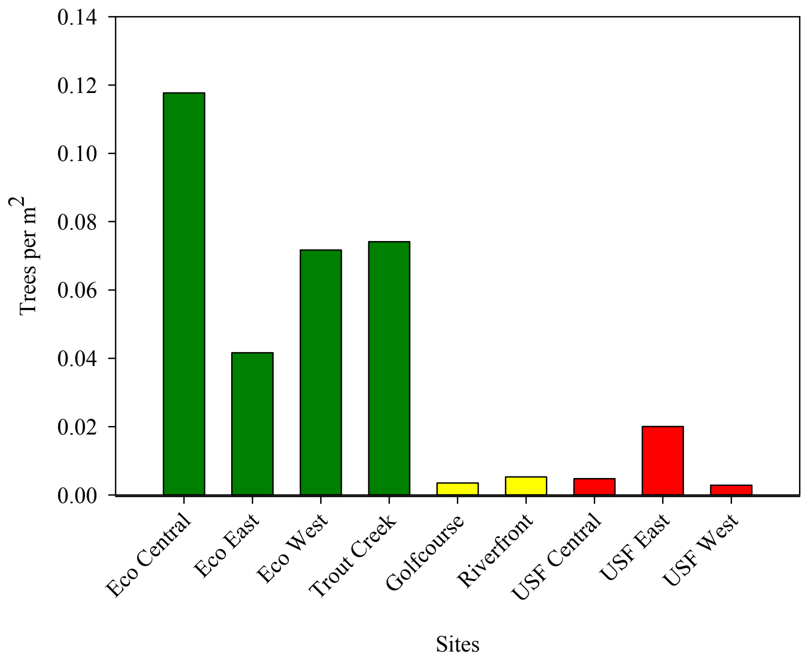

Figure 4. Tree densities of each study site.

Trapping Methods

To attract treefrogs I hung PVC pipes on trees over an aluminum nail which was placed approximately 2 meters above the ground. Two 76cm long poles with different internal diameters, 1.9cm and 4.45cm, were hung on each tree. I capped the bottom of each pipe so that they would hold water after a rain. I drilled two holes in each pipe, one 3 cm from the top to fit over the nail and one 8cm from the bottom so that the entire pipe wouldn't fill with water.

At each site I chose 20-21 trees, either *Pinus, Quercus* or *Sabal.* I determined the latitude and longitude of each tree using Trimble, GeoExplorer CE, GPS equipment. The numbers of each tree type used at each site are listed below in Table 2.

	Tree type		
	Quercus	*Sabal*	*Pinus*
Eco Central	10	0	10
Eco East	7	6	7
Eco West	7	7	7
Golfcourse	7	7	7
Riverfront Park	7	7	7
Trout Creek	7	7	7
USF Central	19	1	0
USF East	8	4	8
USF West	19	0	1

Table 2. Number of trees chosen at each site.

After installing the PVC pipes at each site I waited a minimum of 2 weeks before beginning data collection in order to give the treefrogs enough time to find the pipes. I collected data from October 2006 through September 2007. I checked pipes twice a month, usually every other week. There were some months that pipes were only visited once; because of this sampling error I used the average number of treefrogs caught each month in my data analysis.

I removed treefrogs by gently shaking them out of the pipes. All vertebrates found inside the pipes were identified by species and recorded. All new treefrog captures were marked using toe clips. A single toe, specifically the back right outermost toe, was clipped to distinguish recaptures from new captures.

Statistical Analysis

To compare the number of treefrogs captured at each site, on different tree types and in PVC pipes with different internal diameters, I ran a 3 way analysis of variance (ANOVA), using a type IV sums of squares (SS), for each species. A type IV SS was used to account for empty cells, i.e. the three genera of trees used in this study were not present at all nine sites. Before running the analysis I transformed the data by taking the square root of total captures. I chose this analysis in order to test for a significant difference in the number of treefrogs at each site, for each tree type, in PVC pipes with small or large internal diameters, and also for site by tree type interactions, site by PVC diameter interactions, tree type by PVC diameter interactions and finally site by tree type by PVC diameter interactions. Sites with less than two treefrog captures were not included in the analysis.

I estimated the population size of each treefrog species at each site using the Schnabel method (Schnabel 1938, Krebs 1999). In order to determine whether the population size of each species of treefrogs differed significantly at each site I used the χ^2 goodness of fit test, using the mean frequency as my expected value. Sites where zero treefrogs were captured were not included in the analysis.

To determine if *O. septentrionalis* are found more frequently in PVC pipes that also contain native treefrogs I ran a Pearson correlation test for each sites. The Pearson correlation test will indicate if there is any correlation between the number of *O. septentrionalis* and the number of native treefrogs found in a given PVC pipe on a given day. Specifically, a positive correlation will indicate that *O. septentrionalis* are found more often in PVC pipes where native treefrogs can also be found. Only PVC pipes that contain at least one treefrog on a given day were used for the analysis.

To test for a correlation between treefrog abundance and distance to water I used the Pearson correlation test for each species of treefrog. Distance to water was calculated by marking each PVC pipe using Trimble, GeoExplorer CE, GPS equipment. I converted these data into a vector point feature in ArcMap, ArcGIS9. I obtained a hydrologic map of Hillsborough County from the Florida Geographic Data Library and overlaid this feature on to my GPS data feature. Small ponds and ditches that were not shown on the hydrologic map were selected in ArcMap, ArcGIS and added to the data file. Using ArcMap, ArcGIS9 I spatially joined the 2 features in order to calculate the distance from each PVC pipe to the nearest body of water.

To test for a relationship between captures of each species of treefrog and the time of year the capture took place, I created a contingency table and performed a G-test. The G-test is similar to a χ^2 test, however it is less sensitive to sample size. This test will show if different species of treefrogs are behaving similarly in different times of the year.

I correlated monthly treefrog captures with monthly rainfall measurements, taken from the National Climatic Data Center, with the Pearson correlation test.

Results

Treefrog Density Estimates

During this study I captured 2,086 treefrogs including 1,305 recaptures. *O. septentrionalis* were captured the most frequently (n = 1,449) followed by *H. squirella* (n = 424), *H. cinerea* (n = 160) and *H. femoralis* (n = 53). The modified numbers of total captures, recaptures and % recaptures are shown in Table 3. In addition to treefrogs, seven other types of vertebrates were also found in PVC pipes. These are listed in Table 4.

	H. cinerea			*H. femoralis*			*H. squirella*			*O. septentrionalis*		
	total	recap	% recap	total	recap	% recap	total	recap	% recap	total	recap	% recap
Eco Central	0	0	0.00	16	12	0.72	4	2	0.50	57	28	49.56
Eco East	0	0	0.00	3	1	0.33	0	0	0.00	79	36	45.86
Eco West	4	2	0.43	9	5	0.53	0	0	0.00	16	4	25.00
Golf Course	87	57	0.65	0	0	0.00	11	8	0.76	153	112	73.11
Riverfront	3	1	0.40	0	0	0.00	8	6	0.75	49	31	63.27
Trout Creek	2	2	0.75	5	2	0.33	8	4	0.47	5	1	20.00
USF Central	1	0	0.00	0	0	0.00	94	31	0.33	228	164	71.87
USF East	0	0	0.00	0	0	0.00	38	22	0.57	93	62	66.67
USF West	0	0	0.00	0	0	0.00	78	28	0.36	137	89	65.20

Table 3. Total captures, recaptures and % recaptures of treefrogs.

Organism	Total captures
Osteopilus septentrionalis	1468
Hyla squirella	450
Hyla cinerea	162
Hyla femoralis	54
Anolis carolinensis	96
Anolis sagrei	196
Hemidactylus garnotii	84
Eumeces inexpectatus	6
Diadophis puctatus	1
Lampropeltis triangulum	1
Thamnophis sauritus	1

Table 4. Vertebrates found in PVC pipes during study.

The number of total captures and recaptures for each treefrog species at each site is shown in Table 3. *H. cinerea* were captured almost exclusively at the USF golf course and not at all at the natural sites Eco Central and Eco East or the urban sites USF East and USF West. *H. femoralis* were only captured at the natural sites and in very low numbers. The majority of *H. squirella* were captured at the urban USF campus sites and not at all at the natural sites Eco East or Eco West. *O. septentrionalis* were captured at all sites, most frequently at the urban USF campus sites and at the semi-urban USF golf course but less frequently at Trout Creek.

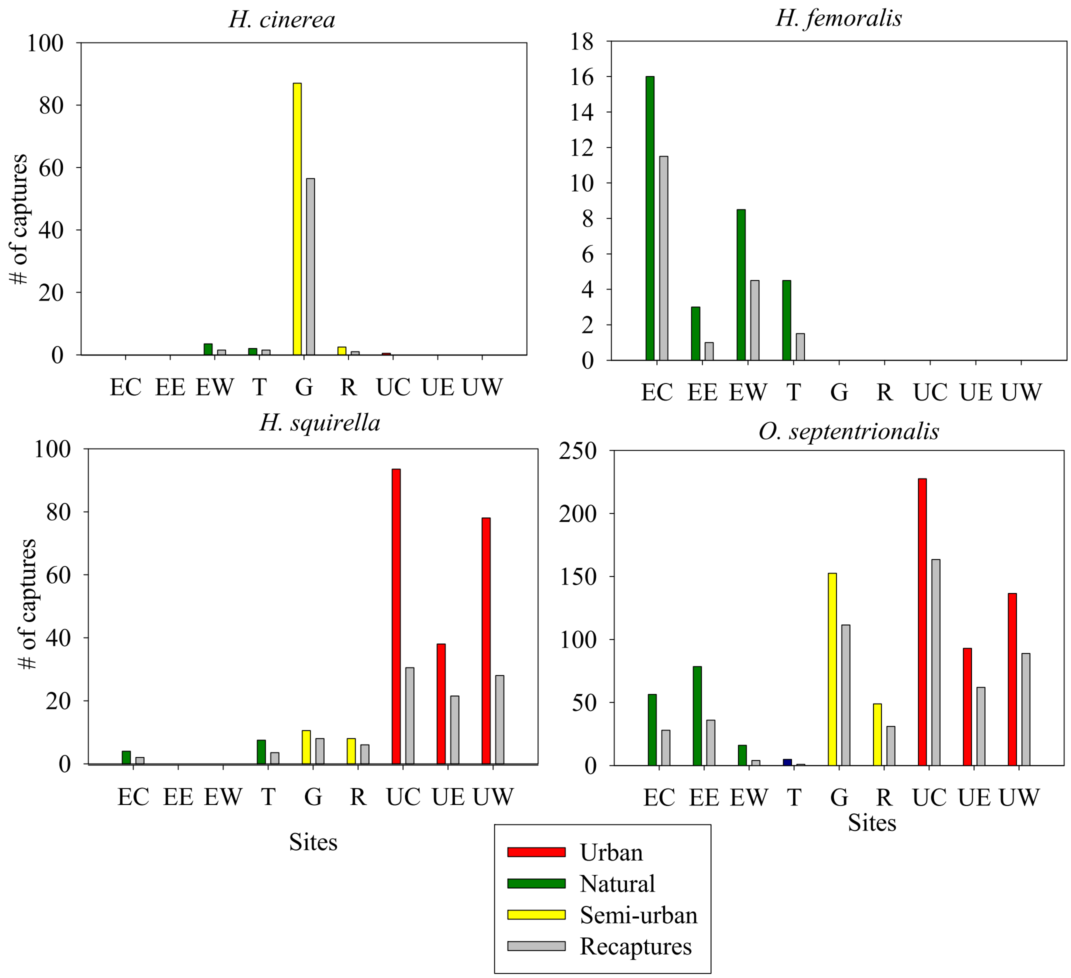

Figure 5. Total captures and recaptures of *H. cinerea, H. femoralis, H. squirella* and *O. septentrionalis.* EC = Eco Area Central, EE = Eco Area East, EW = Eco Area West, G = Golf course, R = Riverfront, T = Trout Creek, UC = USF Central, UE = USF East, UW = USF West.

Recapture rates for each species of treefrog at each site are shown in Figure 6. *H. cinerea* had high recapture rates at the USF golf course and at Trout Creek. *H. femoralis* had high recapture rates at Eco Area Central and Eco Area West. *H. squirella* had high recapture rates at USF East. The recapture rates of *O. septentrionalis* have an interesting pattern. Rates of recapture are high at all urban and semi-urban sites while are much lower at the natural sites.

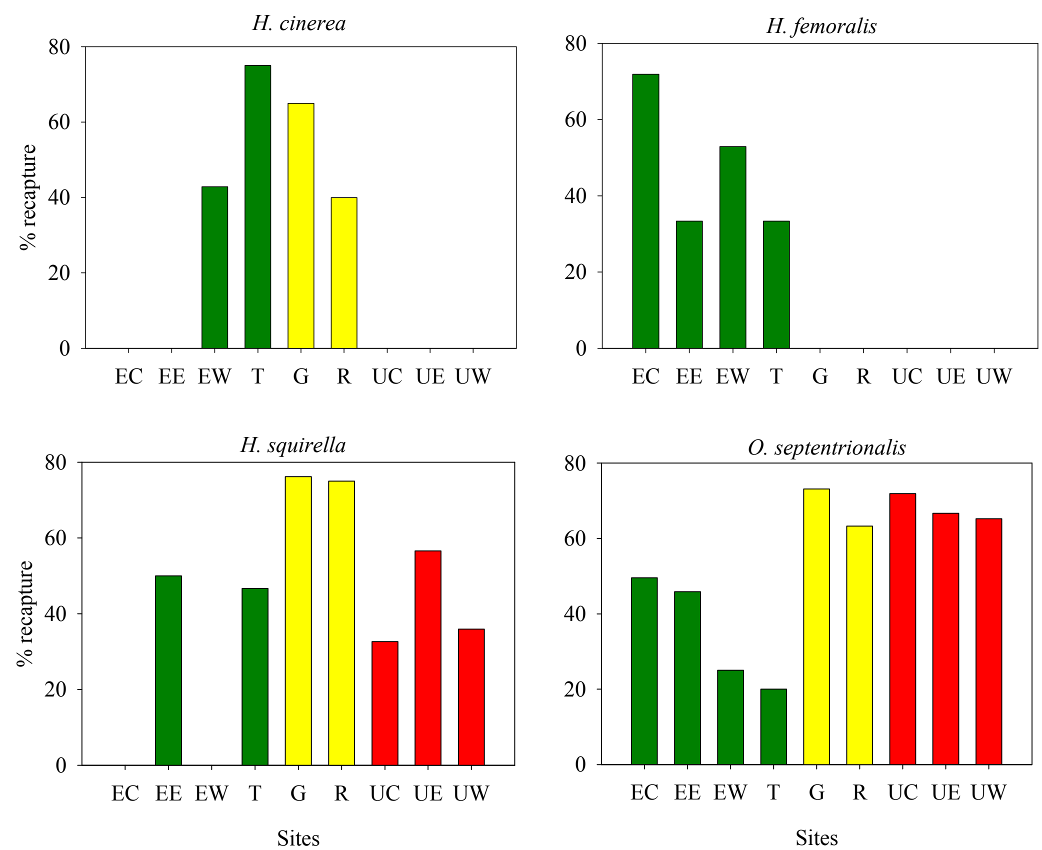

Figure 6. Recapture rates of *H. cinerea, H. femoralis, H. squirella* and *O. septentrionalis.* EC = Eco Area Central, EE = Eco Area East, EW = Eco Area West, G = Golf course, R = Riverfront, T = Trout Creek, UC = USF Central, UE = USF East, UW = USF West.

The results of the Schnabel population estimates are shown in Figure 7. The largest population estimate of *H. cinerea* was found at the USF Golf course. *H. femoralis* have a very small population estimate (n < 7) at all sites. The largest population estimates of *H. squirella* were found at USF Central, USF West and Riverfront Park . The largest population estimates of *O. septentrionalis* were found at the USF golf course, Eco Central and Eco East. A χ^2 goodness of fit test was used to compare the population sizes of each treefrog species at each site. Sites where treefrogs were not captured were not included. No significant difference in population size was found for *H. femoralis* (χ^2 =

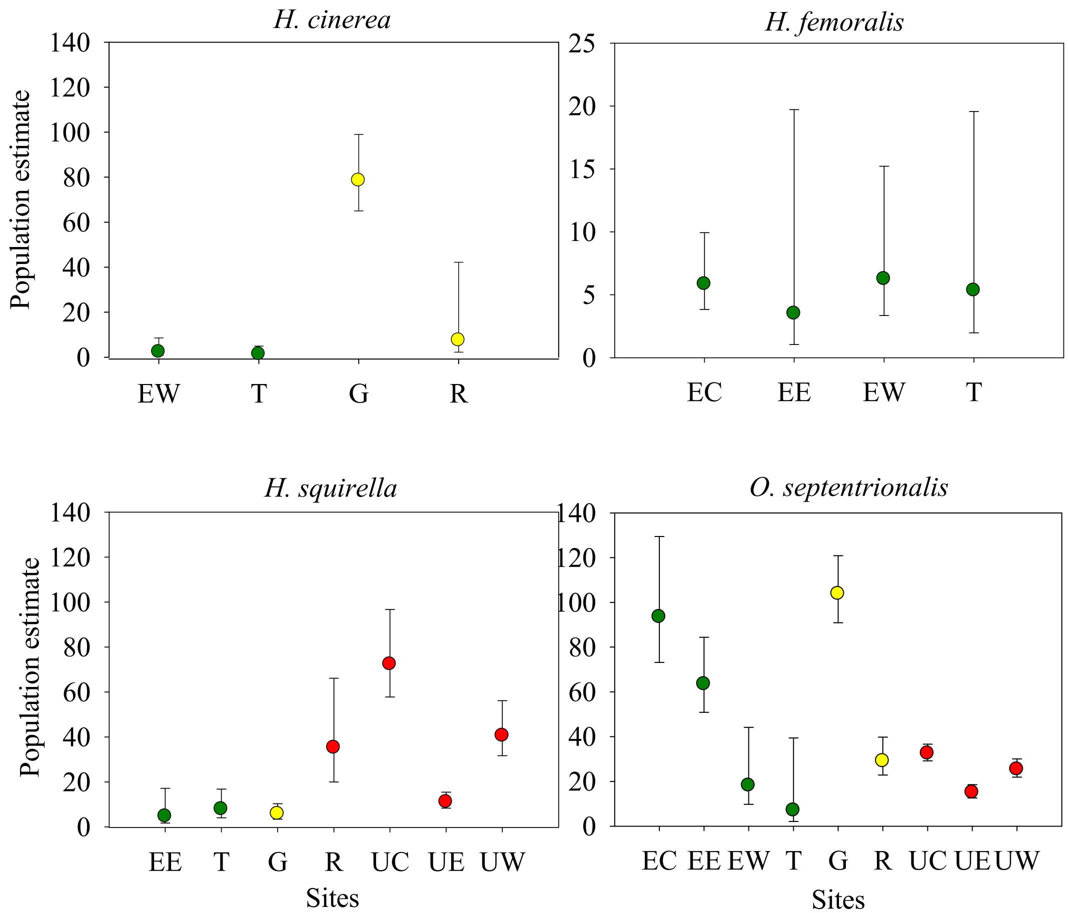

Figure 7. Population size estimation using Schnabel method for *H. cinerea, H. femoralis, H. squirella* and *O. septentrionalis*. Note the difference in scale. Error bars represent the upper and lower confidence limits using a 95% confidence interval. EC = Eco Area Central, EE = Eco Area East, EW = Eco Area West, G = Golf course, R = Riverfront, UC = USF Central, UE = USF East, UW = USF West.

0.085, df = 3, P = 0.80). A significant difference in population size was found for *H. cinerea, H. squirella* and *O. septentrionalis.*

Correlation of Natives and Osteopilus septentrionalis

The results of the Pearson correlation test indicate that captures of *O. septentrionalis* and native treefrog in PVC pipes are negatively correlated at all sites, Table 5. These results are significant at the 0.01 level for Eco Central, Eco West, Golf course, Trout Creek, USF East and USF West and at the 0.05 level for Riverfront Park.

Site	r =	P =
Eco Central	-0.585	0.01
Eco East	-0.086	0.367
Eco West	-0.846	0.01
Golf course	-0.412	0.01
Riverfront	-0.209	0.05
Trout Creek	-0.844	0.01
USF Central	-0.34	0.061
USF East	-0.412	0.01
USF West	-0.334	0.01

Table 5. Correlation of *O. septentrionalis* and native treefrogs

Effects of Site, Tree Type and PVC Diameter on Treefrog Captures

The results of the 3-way ANOVA can be found in Table 6. There was a significant difference in treefrog captures at each site for *H. cinerea, H. squirella* and *O. septentrionalis* but not for *H. femoralis.* A significant difference in treefrog captures among tree type was found for *H. squirella* and *O. septentrionalis* but not for *H. cinerea* or *H. femoralis*. *H. femoralis* and *O. septentrionalis* were found significantly more frequently in PVC pipes with an internal diameter of 4.45cm. There was no significant difference in treefrog captures in PVC pipes with different diameters for *H. cinerea* or *H. squirella.* The number of treefrog captures in PVC pipes of each internal diameter can be found in Table 7. The interaction between site and tree type was significant for *O. septentrionalis*; all other interactions were not significant.

		SS	df	MS	F	P
H. cinerea	site	6.44	4	1.61	5.06	**0.001**
	tree	1.23	2	0.61	1.93	0.149
	diameter	0.95	1	0.95	2.99	0.086
	site × tree	0.50	6	0.08	0.26	0.953
	site × diameter	0.98	4	0.25	0.77	0.546
	tree × diameter	0.11	2	0.05	0.17	0.843
	site × tree × diameter	0.47	6	0.08	0.25	0.961
	Error	46.13	145	0.32		
H. femoralis	site	0.59	3	0.20	1.30	0.278
	tree	0.34	2	0.17	1.13	0.326
	diameter	1.59	1	1.59	10.57	**0.001**
	site × tree	1.05	5	0.21	1.40	0.230
	site × diameter	0.12	3	0.04	0.28	0.844
	tree × diameter	0.20	2	0.10	0.67	0.514
	site × tree × diameter	0.21	5	0.04	0.28	0.926
	Error	21.67	144	0.15		
H. squirella	site	21.76	6	3.63	8.52	**0.000**
	tree	3.60	2	1.80	4.23	**0.016**
	diameter	0.51	1	0.51	1.20	0.274
	site × tree	5.34	10	0.53	1.26	0.257
	site × diameter	2.82	6	0.47	1.10	0.360
	tree × diameter	0.32	2	0.16	0.38	0.686
	site × tree × diameter	1.42	10	0.14	0.33	0.972
	Error	105.58	248	0.43		
O. septentrionalis	site	54.90	8	6.86	10.16	**0.000**
	tree	11.10	2	5.55	8.22	**0.000**
	diameter	2.52	1	2.52	3.73	**0.054**
	site × tree	15.40	13	1.18	1.75	**0.050**
	site × diameter	3.95	8	0.49	0.73	0.664
	tree × diameter	1.38	2	0.69	1.02	0.363
	site × tree × diameter	2.69	13	0.21	0.31	0.991
	Error	220.27	326	0.68		

Table 6. Site, tree type and PVC pipe effects on treefrog captures.

	PVC pipe diameter	
	1.91cm	4.45cm
H. cinerea	29	158
H. femoralis	7	48
H. squirrela	228	253
O. septentrionalis	767	881

Table 7. Number of treefrog captures in PVC pipes with internal diameters of 1.91cm and 4.45cm.

Distance to Water Correlation

The results of the Pearson correlation which compared total numbers of treefrogs captured in each PVC pipe with distance to water are shown in Table 8 and Figure 8. A significant positive correlation was found for *H. squirella* and *O. septentrionalis.*

	N	r	P
H. cinerea	182	-0.130	0.078
H. femoralis	182	-0.074	0.322
H. squirella	182	0.172	0.02
O. septentrionalis	182	0.151	0.041

Table 8. Correlation of treefrog captures and distance to water.

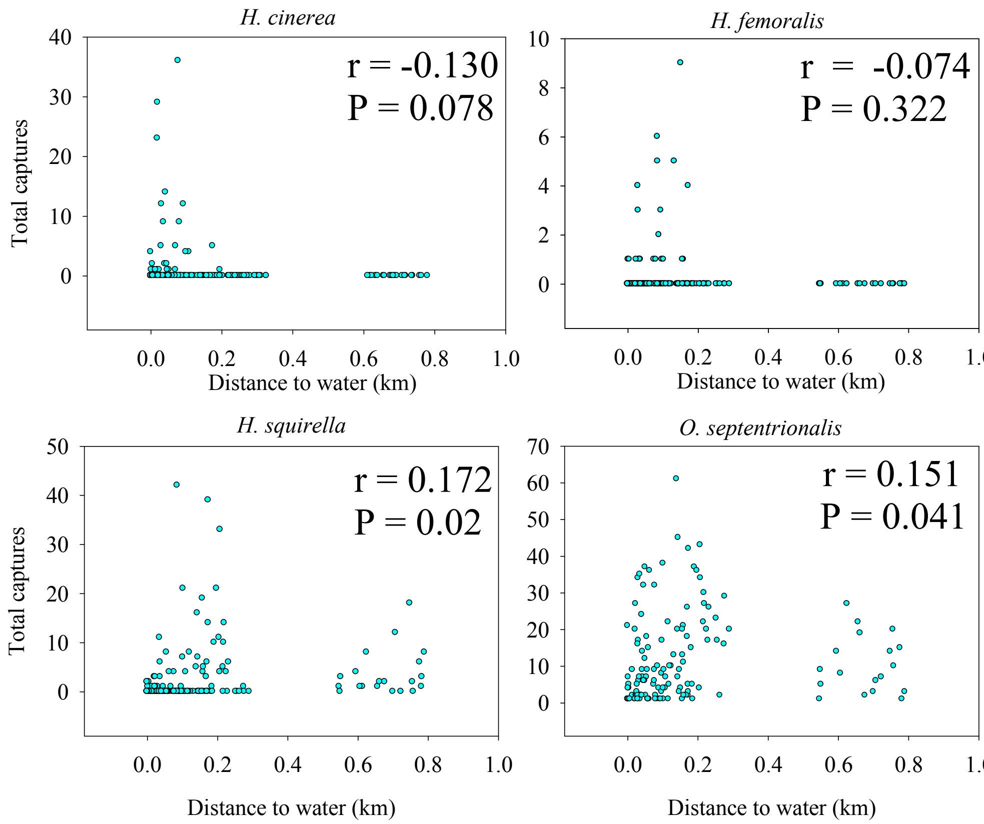

Figure 8. Correlation of treefrog captures and distance to water for *H. cinerea, H. femoralis, H. squirella* and *O. septentrionalis.*

Seasonal and Precipitation Effects on Treefrog Captures

Figure 9 shows the total rainfall and total treefrog captures for each month. Rainfall was the highest during the months of June through September. Small peaks in rainfall occurred in the months of December and April. There appears to be little variation in total captures of *H. cinerea* and *H. femoralis* each month regardless of rainfall. Total captures of *H. squirella* peaked in February and August. Total captures of *O. septentrionalis* peaked in the months of March through May. Monthly treefrog captures are plotted against monthly precipitation in Figure 10. A significantly negative correlation was found between total captures and monthly precipitation for *O.*

septentrionalis (r = -0.782, P = 0.003) as shown in Table 9. No correlation was found for *H. cinerea, H. femoralis* or *H. squirella.*

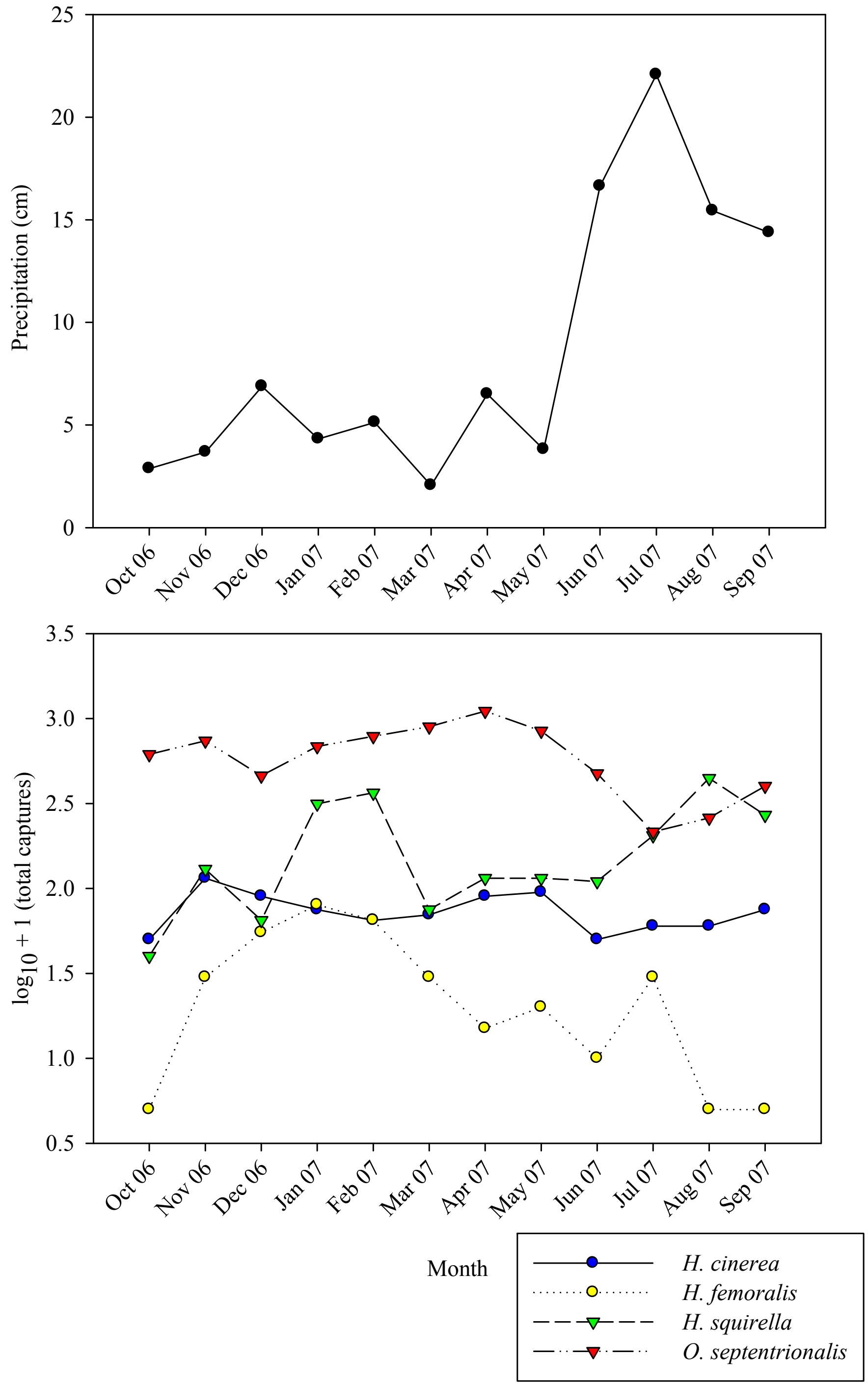

Figure 9. Monthly precipitation and total treefrog captures

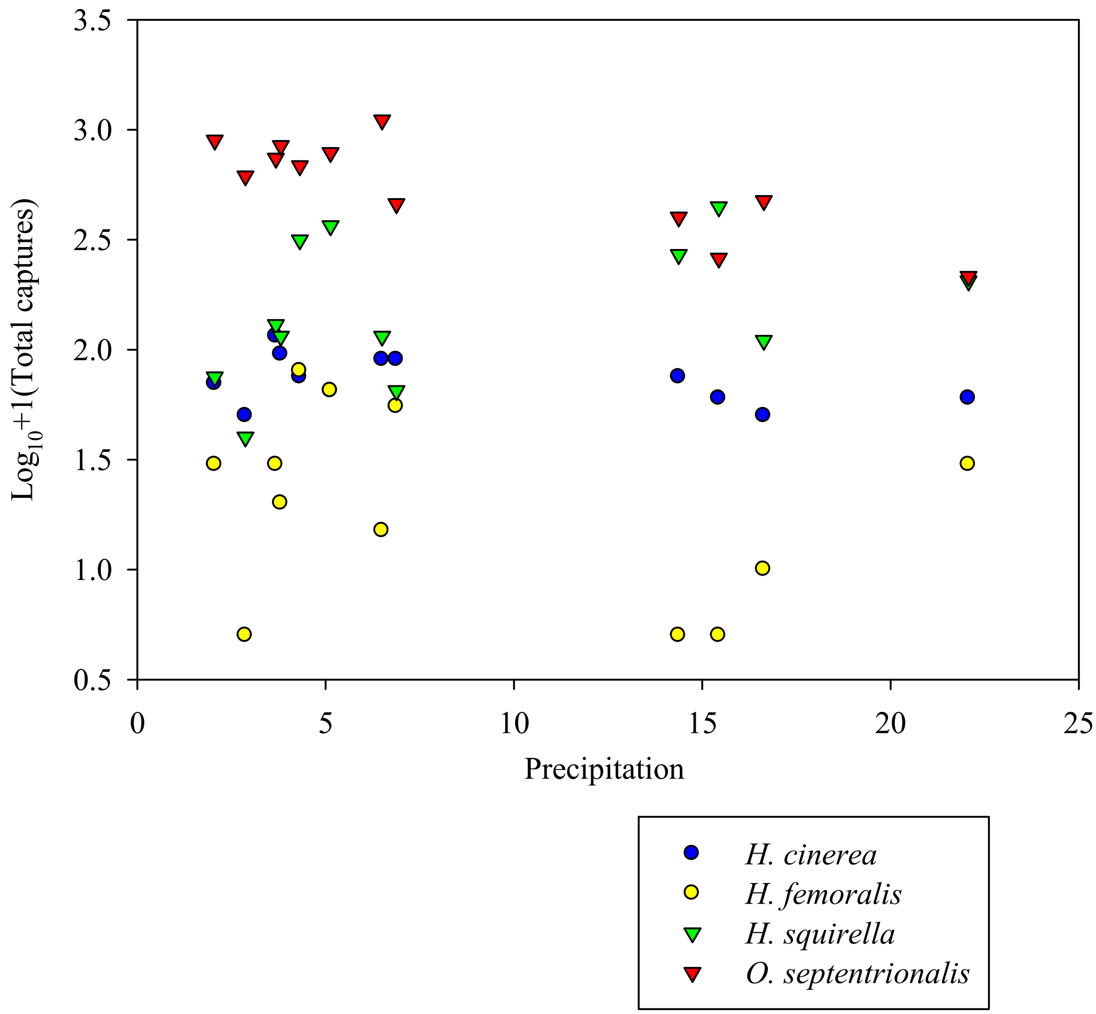

Figure 10. Total treefrog captures versus monthly precipitation.

	N	r	P
H. cinerea	12	-0.447	0.145
H. femoralis	12	-0.344	0.274
H. squirella	12	0.339	0.281
O. septentrionalis	12	-0.782	0.003

Table 9. Correlation of total captures and monthly precipitation.

To determine whether treefrog species and season are related to each other a G-test contingency table was created, Table 9. The G value exceeds the critical value at P = 0.001. This indicates that treefrog species and the time of year they are captured are not independent of one another. The deviations of each observed value from the expected value is shown in Figure 10.

	January-March	April-June	July-September	October-December
H. cinerea	42	42	27	51
H. femoralis	24	8	5	18
H. squirrela	151	68	184	47
O. septentrionalis	473	485	175	363

df = 1
$P < 0.001$
$\chi^2 = 233.54$

Table 10. Number of treefrog captures at different times of the year.

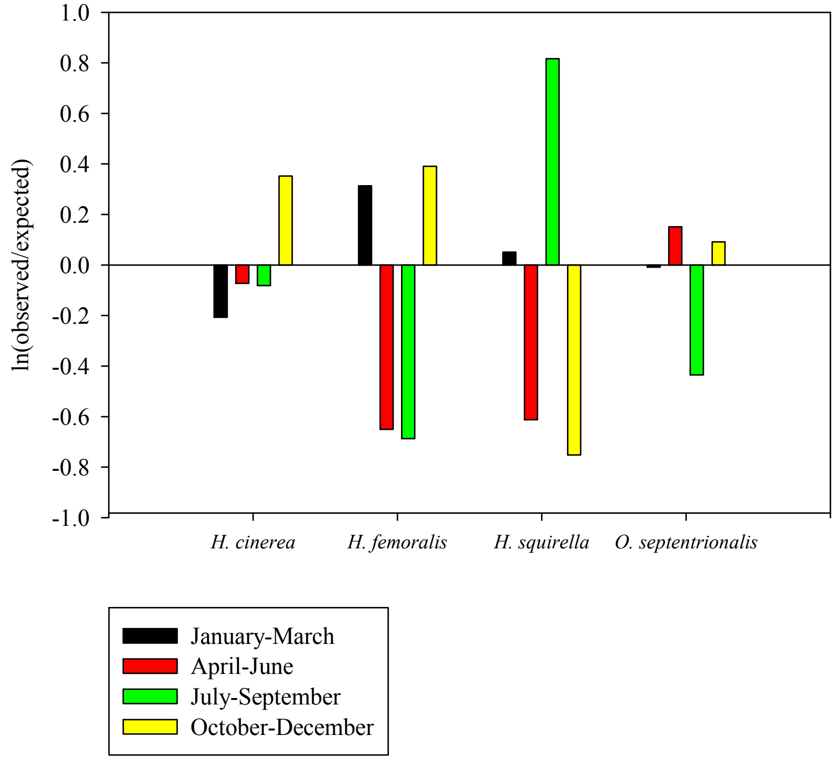

Figure 11. The deviation of observed treefrog captures from the expected treefrog captures at different times of year.

Discussion

Treefrog Density Estimates

The results show that *O. septentrionalis* have successfully colonized the urban, semi-urban and natural sites of this field study. Among all of the sites sampled, the distribution of *O. septentrionalis* overlaps with that of native treefrog species including *H. cinerea, H. femoralis* and *H. squirella.* Moreover, as you can see from Figure 4, *O. septentrionalis* is not merely present in habitats where native treefrogs reside; the frequencies of *O. septentrionalis* are greater than the frequencies of natives at every site. Total captures of *O. septentrionalis* are higher at the urban sites USF Central, USF East, USF West and the semi-urban USF golf course. One potential explanation for these results is that *O. septentrionalis* is largely present in urban and semi-urban habitats, while less so in natural habitats. However, another explanation for these results may be that the PVC pipes used in this experiment are attracting treefrogs in urban areas more so than they would in natural areas reflecting the lack of refuge in urban habitats. Urban habitats lack refuge that natural habitats provide and so PVC pipes may be more attractive in urban habitats. Therefore, low numbers of captures at natural sites may be misleading. Furthermore, PVC pipes may not be a good indicator of the actual treefrog abundances because the refuge provided by the PVC pipe may only be attractive when little other refuge is available. It is important to remember that this method of trapping is different from other methods of trapping, i.e. pitfall traps or live traps such as Sherman traps, because the organism has the ability to stay or leave. The number of captures may not be an estimate of abundance but a estimate of the number of organisms that choose to take refuge in a PVC pipe.

It is possible that very high recapture rates of treefrogs are an indicator of a small population size. Although total captures at a certain site may be high this may not be an indication of the actual population size if the same individual is being captured over and over again. Site fidelity has been observed in treefrogs in previous studies (Irvin et al., 2007; Pittman et al. 2008). Figure 5 shows that *O. septentrionalis* had high total captures at the urban sites USF Central, USF East, USF West and the USF golf course (semi-urban) and lower total captures at the natural sites Eco Central, Eco East, Eco West and Trout Creek. Figure 6 shows that *O. septentrionalis* has very high rates of recaptures (65-73%) at the sites with high total captures and lower rates of recaptures (20-50%) at the sites with lower total captures. An exception to this trend for *O. septentrionalis* is Riverfront Park. These results strengthen the idea that *O. septentrionalis* may not necessarily be more abundant in urban habitats than in natural habitats. Moreover, the Schnabel population estimate (Figure 7), which accounts for recapture rates, indicates that the largest populations of *O. septentrionalis* can be found at Eco Central, Eco East and the USF golf course; the smallest populations can be found at USF Central, USF East, USF West and Eco West. The Schnabel population estimate of Trout Creek may be misleading because of the low number of captures (n = 5). With the exception of Eco West and the USF golf course these results may indicate that *O. septentrionalis* can be found in natural habitats more frequently than in urban habitats.

The results for total captures and recaptures for the native treefrogs do not follow the same trend as *O. septentrionalis.* Although *H. squirella* have the highest total captures at the urban site USF Central, Figure 5, they have the lowest recapture rate, Figure 6. Additionally, the results of the Schnabel population estimate indicate that the largest population of *H. squirella* can be found at USF Central. *H. squirella* have previously been associated with urban areas; moreover they prefer to breed in temporary ponds and ditches (Ashton and Ashton, 1988). Within USF Central there is a small ditch that holds water for most of the year; the presence of this potential breeding pool may account for high captures of *H. squirella* at this site. *H. femoralis* are found most frequently and have high recapture rates at the natural site Eco Area Central, Figures 5 and 6. The Schnabel population estimates for *H. femoralis* may be misleading because of the low number of captures in this species. *H. cinerea* were captured almost exclusively at the USF golf course. I believe that this is because the golf course is bordered by a wetland which includes several permanent ponds. *H. cinerea* is the only treefrog in this study that prefers to breed in permanent ponds, and, not coincidentally the USF golf course is the only site used in this study that includes a permanent pond.

Correlation of Natives and Osteopilus septentrionalis

Native treefrogs were found less frequently in PVC pipes that also contained *O. septentrionalis,* Table 7. This result is perhaps the most interesting because it may be indicating that native treefrogs will avoid habitat or refuges where *O. septentrionalis* are present. Another possibility is that natives are taking refuge in PVC pipes containing *O. septentrionalis* but leave pipes to escape predation; or maybe natives were not able to escape quickly enough and were consumed by *O. septentrionalis.*

Effects of Site, Tree Type and PVC Pipe Diameter on Treefrog Captures

The results of the 3-way ANOVA show that there is an interaction between tree type and site for *O. septentrionalis*, Table 6. This indicates that the habitat location of *Quercus, Sabal* and *Pinus* may be influencing their ability to attract treefrogs. Moreover, this suggests that the sites may differ in ability to attract treefrogs due to differences in plant community, Table 2. No interaction was found between tree type and pipe diameter, Table 6. This suggests that pipe diameters do not differ in the ability to attract treefrogs when hung on different tree types.

All species of treefrog were found more frequently in PVC pipes with an internal diameter of 4.45 cm. These results were significant for *H. femoralis* and *O. septentrionalis.* This may be because of the larger volume of water large PVC pipes retain after a rain. Treefrogs are perhaps attracted to the pipes for the offered moisture and not necessarily the size. In addition it is possible that the small PVC pipes used are excluding very large individuals of *H. cinerea* or *O. septentrionalis*. Previous studies have shown that *H. cinerea* and *H. squirella* are captured more frequently in 1.91cm PVC pipes (Zacharow et al. 2003).

H. squirella and *O. septentrionalis* were found significantly more frequently in PVC pipes hung on *Quercus*, Table 6. Although not significant *H. cinerea* were found more frequently in *Sabal* and *H. femoralis* were found more frequently in *Pinus*. Boughton et al. (2000) examined the effects of tree type on treefrog capture rates using hardwood trees, including *Quercus,* and softwood trees, including *Pinus.* Their results indicate that *H. cinerea* and *H. squirella* were captured significantly more frequently on hardwood tree species.

Distance to Water Correlation

Although significant, the positive correlation of treefrog captures and distance to water for *H. squirella* and *O. septentrionalis* was very weak, Table 8 and Figure 8. However a positive correlation is interesting because it suggests more than one possibility. One explanation of these results is that as the distance to water increases the number of treefrogs also increases. Another explanation is that as distance to water increases the PVC pipes become more attractive to treefrogs. After a moderate rain, water that has collected in PVC pipes would remain for at least 2 weeks. The moisture offered by PVC pipes placed far from water may be what is attracting the treefrogs.

Seasonal and Precipitation Effects on Treefrog Captures

The results of the G-test indicate that the species of treefrogs examined in this study are behaving differently at different times of the year. The time of year with the highest frequency of captures was different for each species of treefrog as shown in Table 11. However the time of year with the second highest frequency, January through March, was the same for *H. cinerea, H. squirella,* and *O. septentrionalis. H. femoralis* were captured the most frequently during this time period. Additionally, the total number of treefrogs captured in January through March was greater than any other time period. This pattern suggests that PVC pipes are more attractive to treefrogs during cooler months. Previous studies have also concluded that treefrogs will seek refuge in PVC pipe and other types of shelter in cold weather (Goin 1958, Zacharow et al. 2003) During the months of July through September *H. cinerea, H. femoralis* and *O. septentrionalis* were captured the least frequently. *H. squirella* were captured the most frequently during this time period. These results are considered further in the discussion below.

A negative correlation between treefrog captures and rain fall does not necessarily indicate that *O. septentrionalis* are more abundant during dry months. It is more probable that PVC pipes become more attractive to *O. septentrionalis* in the drier months. This may be because PVC pipes collect water after a rain and retain moisture for a considerable time. As time after a hard rain increases total captures of *O. septentrionalis* also increase. In months with high rainfall *O. septentrionalis* may no longer rely on moisture offered by the PVC pipes and may find refuge elsewhere; this may explain dips in total captures in months with or after high rainfall, Figure 9. No pattern seems to exist between total captures and rainfall for *H. cinerea, H. femoralis* or *H. squirella.* Peaks in total captures during the winter months may be explained by temperature or rainfall.

Invasion of Florida by Osteopilus septentrionalis

The invasion of Florida by *O. septentrionalis* is interesting because it is an example of an island species who has invaded a mainland. It is generally accepted that islands are susceptible to invasion. Moreover species endemic to islands are vulnerable to predation and are also poor competitors as a result of evolving a habitat with few natural predators and competitors (Lazell, 2005). These generalizations do not apply to *O.*

septentrionalis; here is an island species who is an excellent competitor and evader of predators.

O. septentrionalis has dispersed beyond the insulated southern peninsula and into parts of Central and North Florida. 18 of the 36 non-native herpetofauna in Florida are also endemic to islands. However the majority of these species have not dispersed very far and most have remained within one county as can be seen in Figure 11. *O. septentrionalis, Anolis sagrei* and *Eleutherodactylus planirostris* do not, however, follow this trend and have successfully colonized large portions of southern, central and north Florida (Butterfield et al., 1997). *A. sagrei* and *E. planirostris* first colonized Florida in the late 1800's however *O. septentrionalis* was not reported on mainland Florida until 1952. The dispersal of *O. septentrionalis* has happened relatively quickly. Simberloff (1997) describes two methods of dispersal; diffusion and jump dispersal. Diffusion dispersal occurs as an organism slowly expands its distribution through natural emigration to neighboring habitats. Jump dispersal may occur when the dispersing organism is brought to a new location, far from the habitat in which it originally colonized. A good example of jump dispersal in treefrogs is individuals who will hitch rides on cars while taking refuge in door gaps (personal observation). Perhaps one more reason why *O. septentrionalis* is such a successful colonizer is that it is dispersing through Florida by both diffusion and jump dispersal.

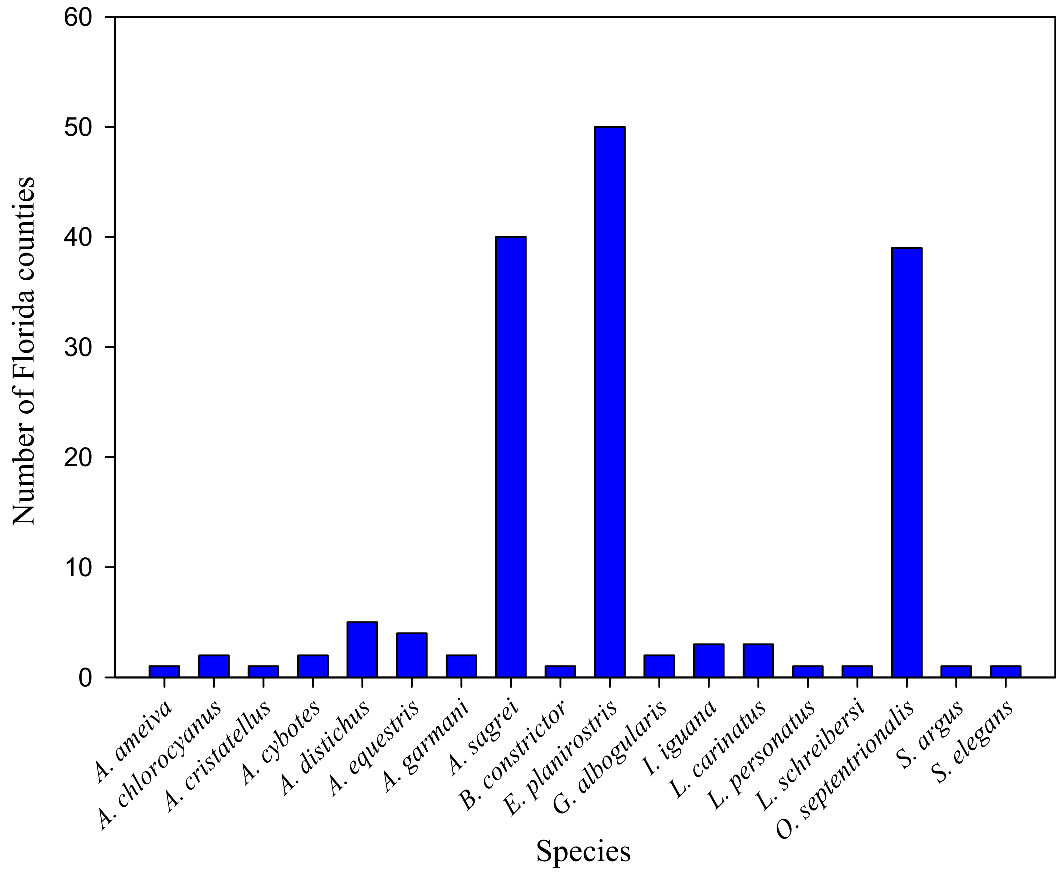

Figure 12. Dispersal of non-native herpetofauna, endemic to islands, in Florida counties. Data was obtained from *Strangers in Paradise* (Butterfield et al. 1997). The number of counties in which *O. septentrionalis* are present have been modified to the current 2008 distribution.

Conclusions

Perhaps the most important finding in this study is that PVC refugia, when used in estimates of treefrog density, are potentially producing biased results. Available refuge, temperature and precipitation all appear to have an effect on how attractive PVC pipes are to treefrogs.

If the presence of *O. septentrionalis* in Florida is a real threat to populations of native treefrogs then the results of this study are concerning. Future studies of *O. septentrionalis* using PVC pipe refugia should consider using PVC pipes of a 4.45cm internal diameter or larger which are associated with *Quercus* or other hardwood trees. Additionally the capture biases mentioned above should be considered in future estimates of treefrog densities.

A much longer term, and larger scale, study is needed to determine if the presence of *O. septentrionalis* is negatively influencing the populations of native treefrogs in Florida. Campbell et. al (2007) has proposed a removal study of *O. septentrionalis* from a public park in Hillsborough County. Their study may lead to a better understanding of the effects of the invasion by *O. septentrionalis.* However, due to the exceptional ability of *O. septentrionalis* to colonize as well as the continued immigration from native and novel ranges, this invader is undoubtedly taking permanent residence in Florida.

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