TESTING THE INFLUENCE OF CUBAN TREEFROGS (*Osteopilus septentrionalis*) ON NATIVE TREEFROG DETECTION AND ABUNDANCE

By

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For Ron
Rest in Peace

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The Cuban treefrog *(Osteopilus septentrionalis*) is a nonindigenous species in Florida that anecdotally replaces native treefrog species where it becomes established. However, few empirical data are available to verify this claim; thus, I sought to determine the effects of *O. septentrionalis* on native treefrog populations. I removed *O. septentrionalis* from natural areas and monitored the response of native treefrog populations using polyvinyl chloride (PVC) pipe refugia. In conjunction with my field study, I conducted laboratory experiments to determine the frequency at which treefrogs use PVC pipe refugia and to test the null hypothesis that *O. septentrionalis* does not affect behaviorally or chemically exclude native treefrogs from refugia.

In my first laboratory study, I compared the variation by species in frequency of refugia used when treefrogs were presented with the choice of either a PVC pipe refugia or a plant. I found that the native squirrel treefrog (*Hyla squirella)* rested in the pipe twice as often as on the plant, and *Osteopilus septentrionalis* used the pipes infrequently. The other two natives tested, green treefrog (*H. cinerea*) and pinewoods treefrog (*H. femoralis*), did not strongly favor either the PVC pipe refugia or the plant.

In my other laboratory studies, I tested if *Osteopilus septentrionalis* interferes with the use of PVC pipe refugia by native treefrogs. First, I paired a native treefrog (*Hyla sp.*) with an *O.*

septentrionalis in an aquarium with only one pipe, and then observed which frog(s) rested inside the PVC pipe refugium 14 hours later. I found that *O. septentrionalis* did not behaviorally exclude native treefrogs from refugia. In another study, I presented treefrogs with a choice of a new PVC pipe refugium and one that had been recently used by *O. septentrionalis* to determine if treefrogs avoided refugia recently used by this predator. I found that *Hyla cinerea* and *H. femoralis* did not avoid refugia that were recently used by *O. septentrionalis*. These laboratory studies suggest that native treefrog detection in PVC pipe refugia is not altered by the presence of similar sized *O. septentrionalis.*

In my field experiment, I established paired grids of PVC pipe refugia to attract treefrogs at three study sites at Wekiwa Springs State Park, Apopka, Florida, and at three study sites at Flatwoods Park, Hillsborough Wilderness Park, Tampa, Florida. One grid of each pair was used as a treatment grid where I removed *Osteopilus septentrionalis,* and the other served as a control. I monitored the community of native treefrogs within each grid, and hypothesized that if *O. septentrionalis* reduces the abundance of native treefrogs, then at sites where this species was removed native treefrogs should become more abundant. However, capture rates were low and few *O. septentrionalis* were removed to potentially decrease its abundance. Therefore, I was not able to determine if its removal positively impacts native treefrog abundance. However, my data did indicate a negative correlation between the abundance of *O. septentrionalis* and *Hyla femoralis*. More research is needed to determine the cause of this relationship.

CHAPTER 1
INTRODUCTION

Nonindigenous Species

Nonindigenous species are those that have been transported to an area in which they do not naturally occur (Austin 1975, Kiesecker and Blaustein 1997). Some nonindigenous species arrive as stowaways on cargo, plants, and with human travelers; some are released or escape from laboratories, aquaculture operations, or the global trade in live organisms; some have been released deliberately by humans as crops, game, unwanted pets, bait, biological controls, or for aesthetics; and others have moved in on their own aided by roads and canals (Elton 1958, Simberloff 2000, Kiesecker 2003). Some nonindigenous species rapidly colonize new areas (Austin 1975), becoming widespread (stage IVa), dominant (stage IVb), or both (stage V) (Colautti and MacIssac 2004). Without natural controls nonindigenous species may alter the ecosystem into which they have been introduced. They may directly affect natural communities by preying on native species that do not have adequate defenses against invading species. Nonindigenous species may also compete with native species for food and essential microhabitat (Simberloff 2000, Meshaka 2001). They may also produce toxins and harm native predators that try to consume them (Phillips et al. 2003). Nonindigenous species can harm natives indirectly by introducing new diseases, or altering nutrient cycles, fire regimes, and other aspects of the environment that then create less suitable habitat for native species (Simberloff 2000). The result is an increase in abundance of the nonindigenous species and a decrease in abundance of natives, and even the possible extinction of native species (Elton 1958, Smith 2005a). Wilcove et al. (1998) ranked competition and predation by nonindigenous species as the second greatest threat to imperiled plants and animals in the United States, surpassed only by habitat loss due to humans.

Not all species introduced into new areas become established, and not all that do become established cause problems. Some are quickly preyed upon, out-competed, parasitized, or infected with native diseases (Elton 1958). When individuals die without reproducing, or when there is no recruitment, a recently introduced species cannot persist. Those that do establish themselves tend to have similar characteristics. These include reduced parasite load, high reproductive output, broad feeding niche, ability to thrive in disturbed ecosystems, tolerance of a wide variety of conditions, introduction in high numbers, and introduction to areas where predators are absent (Willamson and Fitter 1996, Crossland 2000, Torchin et al. 2003, Lockwood et al. 2005).

Nonindigenous Frogs

The ability of nonindigenous frogs to become established outside their native range is demonstrated by two well known examples, the cane toad (*Bufo marinus*) and the bull frog (*Rana catesbeiana*). *Bufo marinus*, native to South America north to southern Texas, was introduced into Australia in 1935 in an effort to control beetles that damaged sugar cane crops. This toad did not control the beetles as planned, but became a nuisance when it began to reproduce explosively and consume and poison native fauna. *Bufo marinus* produces strong toxins (Bufotoxins) throughout its life cycle that can result in mortality of native predators at such high frequency that the toad now threatens native populations (Phillips et al. 2003, Crossland 2000). *Rana catesbeiana*, native to the eastern United States, was translocated to the western United States and is now associated with the decline of native lowland frogs (McAuliffe 1978, Kiesecker 2003). This frog consumes a variety of prey, including tadpoles and adults of other frogs. The yellow-legged frog (*R. muscosa*), a rare species impacted by *R. catesbeiana,* is not naturally sympatric with this nonindigenous frog, and when prey upon by *R. catesbeiana* it does not recognize this larger frog as a predator, and does not behave defensively in its presence

(Kiesecker and Blaustein 1997, Kupferberg 1997). Also, *R. catesbeiana* has larger tadpoles that out-compete other tadpoles (Kiesecker 2003).

Numerous characteristics, several of which are shared between *Rana catesbeiana* and *Bufo marinus*, are suspected to contribute to their successful invasion of new areas. These include large body size, broad feeding niche, high fecundity, ability to thrive in developed areas, and toxicity (e.g. *B. marinus*). The Cuban treefrog (*Osteopilus septentrionalis*) displays all of these traits (Schwartz 1952, Lee 1969, Meshaka 2001). Perhaps then it is no coincidence that this frog may also have negative impacts on native fauna where it has been introduced.

Native to Cuba, Isla de Juventud, the Cayman Islands, and the Bahamas (Meshaka 2001), *Osteopilus septentrionalis* was first observed in the United States on Key West, Florida, in 1931 (Barbour 1931), and on the Florida mainland in 1951 (Schwartz 1952). Since then, this frog has expanded its range northward throughout most of peninsular Florida and isolated locations in the Coastal Plain (Welker 2004, Krysko et al. 2005, Johnson 2004, 2007). *Osteopilus septentrionalis* thrives in disturbed areas, but also invades natural habitats (Meshaka 2001, Meshaka and Babbitt 2005). Although *O. septentrionalis* consumes mostly invertebrates (Heflick 2001, Meshaka 2001), it is large enough that it also preys upon and may compete with native treefrogs (Barbour 1931, Lee 1969, Meshaka 2001). Additionally, Smith (2005a) found that southern toad *(Bufo terrestris*) and green treefrog (*Hyla cinerea*) tadpoles metamorphosed later and at a smaller size in the presence of introduced *O. septentrionalis* tadpoles. Breeding *O. septentrionalis* can also interfere with the breeding of native frogs, as males have been observed amplexing with other species (Smith 2004). Anecdotal accounts of native treefrogs being replaced by *O. septentrionalis* are common, yet the severity of the effects of competition and predation on the community of native treefrogs remains to be demonstrated. Whereas some studies of gut contents suggested that predation on other treefrogs is infrequent (Meshaka 2001, S. A. Johnson unpubl. data), another study (Heflick 2001) suggests that *O. septentrionalis* preys heavily on *H. cinerea* in some urban areas. Because of the stresses *O. septentrionalis* exerts on native communities in Florida, this frog might negatively impact native treefrog populations.

Three Native Treefrogs

Similar habitat affinities and behavior of other treefrogs in the family Hylidae make them especially vulnerable to the effects of *Osteopilus septentrionalis*. These frogs are mostly arboreal and have adhesive disks on the tip of each digit that allow them to climb (Dickerson 1908, Wells 2007). This adaptation allows treefrogs to utilize microhabitats not available to other frogs. Treefrogs are nocturnal foragers, and are relatively small (Dickerson 1908). Native treefrogs can easily be preyed on by *O. septentrionalis* and have been found repeatedly in diet studies of *O. septentrionalis* (Meshaka 2001, Heflick 2001, S. A. Johnson unpubl. data). Thus, native treefrogs are active in similar habitats at the same time as *O. septentrionalis*, and may be more likely than other frogs to be influenced by interactions with this nonindigenous species.

Hyla cinerea is a medium-sized treefrog with mature females measuring 41-63 mm and males 37-59 mm (Wright and Wright 1949). This species occurs from Virginia to Florida, west to Texas, and north along the Mississippi River to southern Illinois (Wright and Wright 1949, Hardy 1972, Redmer and Brandon 2005). *Hyla cinerea* is common throughout central Florida, and is often observed on buildings at night. This species consumes arthropods, including Cicadellidae, Acrididae, Lepidoptera larvae, Chrysomelidae, and Arachnida (Brown 1974). Its breeding season ranges from April to August (Wright and Wright 1949). Eggs are laid near the surface of permanent, stagnant water bodies and hatch within 2–3 days into tadpoles that metamorphose in about 60 days (Hardy 1972). Adult *H. cinerea* remain near wetlands on emergent vegetation (Wright and Wright 1949). Habitats include marshes, cypress swamps, artificial wetlands, and edificarian areas (Hardy 1972, Delis et al. 1996).

Hyla squirella is a small frog; mature females measure 23-37 mm and males 23-36 mm (Wright and Wright 1949). The range of *Hyla squirella* is similar to that of *H. cinerea*, but does not extend as far north along the Mississippi River Valley (Mitchell and Lannoo 2005, Redmer and Brandon 2005). Its diet of consists of small arthropods including Elateridae, Gryllidae, Formicidae, and small crustaceans and spiders (Duellman and Schwartz 1958). During the spring and summer breeding season adults migrate to ephemeral ponds (Wright 1932, Goin 1958, Mitchell and Lannoo 2005). Breeding sites include roadside ditches, wooded ponds, and grassy pools (Wright 1932). Eggs hatch in about two days, and tadpoles metamorphose within 25-55 days (Wright 1932). Adult frogs are generally found away from wetlands, seeking shelter under bark, in bromeliads, and along the axils of palmettos (Wright 1932, Goin 1958). This frog occurs in urbanized areas and all major habitat types in Florida (Duellman and Schwartz 1958, Delis et al. 1996).

The pinewoods treefrog (*Hyla femoralis*) is also a small frog; mature females measure 23-40 mm and males 24-37 mm (Wright and Wright 1949). *Hyla femoralis* occurs along the coastal plain southward from Virginia to Louisiana (Mitchell 2005). It feeds on a variety of arthropods, including Tetrigidae, Gryllidae, Carabidae, Elateridae, Scarabaeidae, Formacidiae, Vespidae, Attidae, Hymenoptera, and Lepidoptera (Duellman and Schwartz 1958, Carr 1940). Adults breed in temporary wetlands from June to October (Duellman and Schwartz 1958), eggs hatch in three days, and tadpoles metamorphose within 50-75 days (Wright and Wright 1949). As adults, these frogs are generally associated with pine forests (Duellman and Scwartz 1958).

Hyla femoralis is sensitive to habitat degradation and is not often found in edificarian areas (Delis et al. 1996).

PVC Pipe Sampling

Treefrogs are not easily captured outside their breeding season using standard amphibian trapping techniques. Because of their arboreal nature, they do not normally utilize cover boards and cannot be found by raking through forest debris. Additionally, their toe pads allow them to climb over typical drift fence arrays (Dodd 1991), and therefore fences must be modified with plastic barriers (Murphy 1993). As an alternative to these trapping techniques, some researchers have been developing refugia to attract treefrogs.

The use of refugia has progressed over the last fifty years. Goin (1958) used tin cans resting upside down on stakes to attract treefrogs. While these cans attracted numerous treefrogs, a single pipe found nearby on the ground attracted nearly twice as many frogs as all six cans combined (Goin 1958). McComb and Noble (1981) used wooden nest boxes of varying sizes to sample reptiles and amphibians, and found treefrogs to use boxes more frequently than natural tree cavities. Stewart and Pough (1983) used bamboo refugia to study the coqui (*Eleutherodactylus coqui*), and Moulton et al. (1996) introduced the use of polyvinyl chloride (PVC) pipes to capture treefrogs.

With the use of PVC pipes established, researchers turned their attention to identifying and improving on sources of bias. To design a PVC pipe refugium that would capture all species and size classes of treefrogs present in central Florida, Boughton et al. (2000) examined the effects of pipe diameter and length, the use of caps to retain water, placement on hardwoods versus pines, and height at which pipes were placed; Zacharow et al. (2003) studied the effects of PVC pipe diameter of ground-placed pipes; and Bartareau (2004) examined the effects of pipe diameter in oak scrub habitat. Borg et al. (2004) modified pipe refugia to curtail entrapment and mortality of non-target flying squirrels (*Glaucomys* spp.), a source of bias that reduced refugia use by hylids.

Information regarding detection probability of frogs using this method and the effects of species interactions are lacking. Although trends in species composition are apparent in the literature, variation in the frequency of PVC pipe refugia use, and thus detection probability, have not been compared across species encountered using this method. It is also not known how behavioral interactions among treefrogs affect use of the refugia, or if these interactions vary depending on gender, size, and species of frogs. These factors also need to be addressed to identify potential biases in sampling with PVC pipe refugia.

Objectives

I examined the use of PVC pipe refugia as a method for sampling treefrogs, especially when *Osteopilus septentrionalis* is present, and to examine the potential influence of *O. septentrionalis* on native treefrog populations. Using laboratory studies, I determined the frequency of use of PVC pipe refugia when vegetative refugia were present for four species of treefrogs (*Hyla cinerea, H. squirella, H. femoralis,* and *O. septentrionalis*), and tested the null hypothesis that this frequency did not vary among species. I tested the hypothesis that *O. septentrionalis* affects the probability of pipe use by two native treefrog species (*H. cinerea* and *H. squirella*). I tested the null hypotheses that *O. septentrionalis* does not affect the use of PVC pipe refugia of native treefrogs by behavioral exclusion and that native treefrogs do not avoid refugia previously used by *O. septentrionalis.* I also designed a field study to examine potential ecological effects of *O. septentrionalis* on native treefrogs (*H. cinerea, H. squirella,* and *H. femoralis*). I tested the hypothesis that if *O. septentrionalis* does reduce the abundance of native treefrogs, then native treefrogs should be more abundant at sites where *O. septentrionalis* was removed.

CHAPTER 2
VARIATION IN PVC PIPE REFUGIA USE AMONG TREEFROGS

Introduction

The use of cavities provides many benefits to frogs, such as predator avoidance, thermoregulation, breeding sites, and osmoregulation (Stewart and Rand 1991, Schwarzkopf and Alford 1996, Walsh and Downie 2005). For example, in the southeastern United States, treefrogs are known to use tree cavities as diurnal shelters presumably to avoid desiccation (McComb and Noble 1981, Ritke and Gabb 1991, Walters and Kneitel 2004). Researchers have taken advantage of this behavior to study the ecology of treefrogs by deploying artificial refugia that mimic natural cavities. As early as the 1950s, Goin (1958) used aluminum cans inverted on wooden stakes to compare the non-breeding habits of two treefrog species. More recently, researchers have used polyvinyl chloride (PVC) pipes to attract treefrogs for study.

The use of PVC pipe refugia is becoming commonplace as a method to monitor and sample treefrogs (Moulton et al. 1996, Boughton et al. 2000, Zacharow et al. 2001). This method is superior to call surveys for studies focused specifically on treefrogs because PVC pipe refugia can be used to sample both genders during both the breeding and non-breeding seasons. PVC pipe refugia allow for capture and identification of individual frogs as well. Refugia are also better suited than drift fence arrays to the study of treefrogs, as treefrogs may traverse these barriers (Dodd 1991). Furthermore, refugia passively attract (rather than trap) treefrogs (Moulton et al. 1996). As treefrogs are not restrained and can enter and leave at will, use of this method eliminates trap mortality and allows researchers to check PVC pipe refugia infrequently. Because of this, there is no need to remove PVC pipe refugia between sampling periods, which reduces the amount of labor needed for repeat sampling or long-term monitoring.

Variation in design and placement of PVC pipe refugia can bias sampling and may alter the probability of detection of some treefrog species or size classes. Differences in pipe design such as pipe diameter, length, shape (straight or T-shaped), or if the pipe is capped at one end can alter the species composition and size class of treefrogs sampled (Boughton 1997, Zacharow et al. 2003, Bartareau 2004). Location of PVC pipe refugia in terms of distance to buildings, height above ground, tree type (i.e., hardwood of softwood), arboreal or ground placement, and distance to water can also affect the sample (McComb and Nobel 1981, Boughton 1997, Zacharow et al. 2003). Since frogs change their behavior according to temperature, humidity, and rain, detection also varies seasonally (Buchanan 1988, Boughton 1997, Zacharow et al. 2003). Regardless of pipe configuration or placement, some species are not effectively sampled with this method. Although present at their study site, Boughton et al. (2000) did not observe spring peepers (*Pseudacris crucifer*) and only rarely found barking treefrogs (*Hyla gratiosa*) in their PVC pipe refugia. Despite our understanding about the effects of pipe design and placement, variation in frequency of refugia use among species easily sampled with this method has not been examined.

The propensity to use cavities may vary among treefrogs due to differences in their natural history and physiology. Treefrogs that remain near wetlands or have evolved morphological adaptations to prevent desiccation or predation may not frequently seek out refugia. Treefrogs vary in the amount of cutaneous lipid secretions they produce, and in their repertoire of associated body wiping behaviors that aid in water retention and thus inhibit desiccation (Barbeau and Lillywhite 2005).

I examined variation in frequency of PVC pipe refugia use by four species of treefrogs in order to better understand potential variation in detection probability, and thus occurrence, of these species in the field. I conducted a laboratory study in which frogs were given the choice of resting in a PVC pipe refugium or on a plant. By using a known number of frogs in a laboratory setting, I was able to examine not only how many frogs rested within PVC pipe refugia, but also how many frogs chose to rest elsewhere. I tested the null hypothesis that treefrog species did not vary in their frequency of refugia choice.

Methods

Collection and maintenance of frogs

I sampled four species of treefrogs (three natives and one nonindigenous) commonly encountered in PVC pipe refugia studies in central Florida: green treefrog (*Hyla cinerea*), squirrel treefrog (*H. squirella*), pinewoods treefrog (*H. femoralis*), and Cuban treefrog (*Osteopilus septentrionalis*). I opportunistically collected treefrogs in Alachua, Hillsborough, and Polk Counties, Florida. Collection methods included use of PVC pipe refugia and hand capture during breeding events, rainstorms, and at night. I caught frogs at retention ponds, natural ponds, swamps, roadside and agricultural ditches, suburban streets, county parks, and on buildings.

I transferred these frogs to growth rooms at the University of Florida Gulf Coast Research and Education Center in Hillsborough County. The growth rooms were equipped with individual heating, ventilation, and air conditioning (HVAC) systems, and had full spectrum fluorescent lights suspended 0.66 m above 1-m high benches. These lights were set to mimic the natural photoperiod during the study by automatically turning on and off at 0630 h and 2030 h, respectively. Temperature in the growth rooms was set to 24°C during the day and dropped to 21°C during the night. Due to the limitations of the growth rooms, I was not able to raise the temperature higher to more closely imitate the natural temperature regime. So that frogs would not be foraging in absolute darkness, a high-intensity desk lamp with a 40-watt bulb was set to shine into a corner of each growth room.

I housed frogs individually in 0.75-L Sterilite containers with 3-mm air holes drilled in the lids. I held frogs separately during the entire length of their captivity. I fed the frogs crickets, misted their containers daily, and replaced their paper towel substrate as needed.

Laboratory Trials

Frogs were maintained in captivity and tested following protocols approved by the Institutional Animal Care and Use Committee of the University of Florida (protocol number E870). Frogs were collected from 2 June 2007 until 23 July 2007, and trials were conducted from 18 June 2007 until 24 July 2007. No frog remained in captivity longer than 18 days. After completion of each trial I marked the native frogs with Visual Implant Elastomer (VIE) and released these treefrogs at their site of initial capture. All *Osteopilus septentrionalis* were euthanized humanely, as this is a nonindigenous species in Florida and cannot be legally released.

For each species, I conducted 30 overnight trials, whereby individual frogs were offered a choice between a plant and a PVC pipe refugium as a diurnal refuge. During each trial, a frog was individually placed into a 37.8-L aquarium with moist sand substrate, screen lid, 3.8 cm diameter PVC pipe that stood 20 cm tall near one end of the tank, and an approximately 20-cm tall vinca (*Catharanthus roseus*) planted at the other end of the tank. To ensure the tank would remain humid throughout the night, I saturated the substrate until standing water was observed. Trials began at 2000 h and ended the next day at 1000 h, when frogs in natural habitats would no longer be active and would have sought diurnal shelter. At the completion of each trial I recorded the location of the frog as being inside the pipe, on the plant (i.e., on leaves, stems, or at the base of the plant), or elsewhere (on the side of the tank or on the sand substrate). I measured snout-vent length (SVL) to the nearest mm and weighed each frog to the nearest 0.1 g. Each individual was used for only one trial, and lab conditions were the same for all trials. I used SYSTAT 10.2

to conduct a Pearson chi-square test ($\alpha = 0.05$) to test the null hypothesis that the frequency of refugia use by treefrogs did not vary among species.

Results

Frequency of refugia use varied significantly by species ($\chi^2 = 21.61$, df = 6, $P = 0.001$; Fig. 2-1); thus, I rejected the null hypothesis that these values did not differ among species. *Osteopilus septentrionalis* used the plant eight times as often as the PVC pipe refugia. *Hyla squirella* used the pipe refugia over twice as often as the plant. The frequency of pipe use did not vary considerably from that of plant use for *H. femoralis* and *H. cinerea.* Frogs of all species rarely chose to rest elsewhere within the aquarium, with three or fewer individuals per species resting away from the PVC pipe refugia or the plant (Fig. 2-1).

Discussion

Refugia choice varied significantly among the four species of treefrogs I tested. *Hyla cinerea* and *H. femoralis* were similar in their choice of refuges and appeared to show no preference in their use of PVC pipe refugia or plants. In contrast, *H. squirella* and *Osteopilus septentrionalis* showed a preference for one refuge type over the other. *Hyla squirella* preferred the enclosed shelter of a PVC pipe refugium over resting on a plant, whereas *O. septentrionalis* rarely rested in a location other than on the plant. Frogs of all four species preferred a sheltered hiding place rather than resting exposed on the wall of the aquarium or the sand substrate.

My results are similar to other observations of refugia use by the native species. Capture and recapture rates of *Hyla squirella* in artificial refugia have been reported as higher than those of *H. cinerea* and *H. femoralis* (Goin 1958, Boughton et al. 2000, Zacharow et al. 2003). Goin (1958) opportunistically observed *H. squirella* using artificial retreats more often than natural retreats, and noted that retreats of *H. squirella* were often more enclosed than the retreats of *H. cinerea.* Boughton et al. (2000) captured more *H. squirella* than *H. cinerea* using PVC pipe refugia, but also caught very few *H. femoralis*. *Hyla squirella* was the only species of treefrog found by Walters and Kneitel (2004) to use red-cockaded woodpecker (*Picoides borealis*) cavities. Similar numbers of individual *H. cinerea* and *H. squirella* were caught in PVC pipe refugia by Zacharow et al (2003), but *H. squirella* was more frequently recaptured. *Hyla squirella* occurred more often than *H. cinerea* in PVC pipe refugia during a study by Bartareau (2004). However, McComb and Nobel (1981) found *H. cinerea* to use natural cavities and wooden nest boxes more frequently than *H. squirella*. These observations are similar to my results as *H. squirella* used the PVC pipe refugia more often than *H. cinerea* and *H. femoralis*, but differed in that *H. femoralis* did not often occur in refugia during these field studies.

Variation in the use of refugia among treefrogs may be due to differences in their natural history and physiology. *Hyla cinerea* exhibits relatively high evaporative water loss, and engages in vigorous head wiping to stimulate secretion of cutaneous lipids that limit desiccation (Barbeau and Lillywhite 2005). Frogs of this species breed in permanent wetlands, and are often found in vegetation within and around wetlands (Wright and Wright 1949, Redmer and Brandon 2005). As they typically remain near permanent water, these frogs may rely on wetlands for moisture and may be less likely to seek out refugia primarily for water retention (Goin 1958). In contrast, *Hyla squirella* breeds in temporary ponds, and Goin (1958) hypothesized that when this frog disperses to upland habitats it seeks refugia that are more sheltered in order to prevent water loss. In addition, Barbeau and Lillywhite (2005) reported that *H. squirella* exhibits a higher number of body wipes before entering a water conservation posture than other Florida treefrogs. *Hyla femoralis* also breeds in temporary wetlands and appears to spend much of its time in tree canopies, though specific adaptations to avoid desiccation are unknown (Wright and Wright 1949, Mitchell 2005). The nonindigenous *Osteopilus septentrionalis* typically breeds in temporary wetlands and has skin co-ossified to the dermal bones in its skull, an adaptation that reduces the amount of vascular tissue in the skin on its head and thus the rate of water loss from this area (Duellman and Treub 1986, Meshaka 2001). *Osteopilus septentrionalis* is relatively large and secretes a slimy toxin when disturbed (Meshaka 2001), so it may be less reliant on refugia for protection against predators than native treefrogs that lack this protection.

Researchers using PVC pipe refugia to sample treefrog populations should take this variation in refugia use, and thus detection probability, into account when using this method to study frog communities. A dominance of *Hyla squirella* in a sample may not necessarily indicate that this species is more abundant than other treefrogs in an area, and a lack of *Osteopilus septentrionalis* in a sample may not indicate absence of this species.

The low detectability of *Osteopilus septentrionalis* obfuscates interpretation of the occurrence of this frog when PVC pipe refugia are used in monitoring programs. As this species does not frequently use the refugia, managers cannot be certain if *O. septentrionalis* is absent when it is not observed. When this species is observed, there may be several times more individuals present than sampled using this method. However, as this species uses PVC pipe refugia considerably less often than native treefrogs, managers should be concerned when it is the dominant frog found in the PVC pipe refugia (see Chapter 3). Researchers need to consider using mark-recapture methods rather than counts when comparing abundance across species.

Management of this nonindigenous species is complicated by the strong aversion of *Osteopilus septentrionalis* to PVC pipe refugia. These frogs should be removed when observed; however, my results indicate that only a small portion of the frogs can be captured using this method. To attract a larger percentage of the population of *O. septentrionalis*, researchers need to investigate the optimal placement and configuration of PVC pipe refugia. Until a better management strategy is available, managers should not rely on PVC pipe refugia alone to catch these frogs. Other techniques, such as dip netting larvae and hand capturing breeding adults, must be used to supplement removal efforts. Otherwise, frogs that do not use the pipes will not be captured.

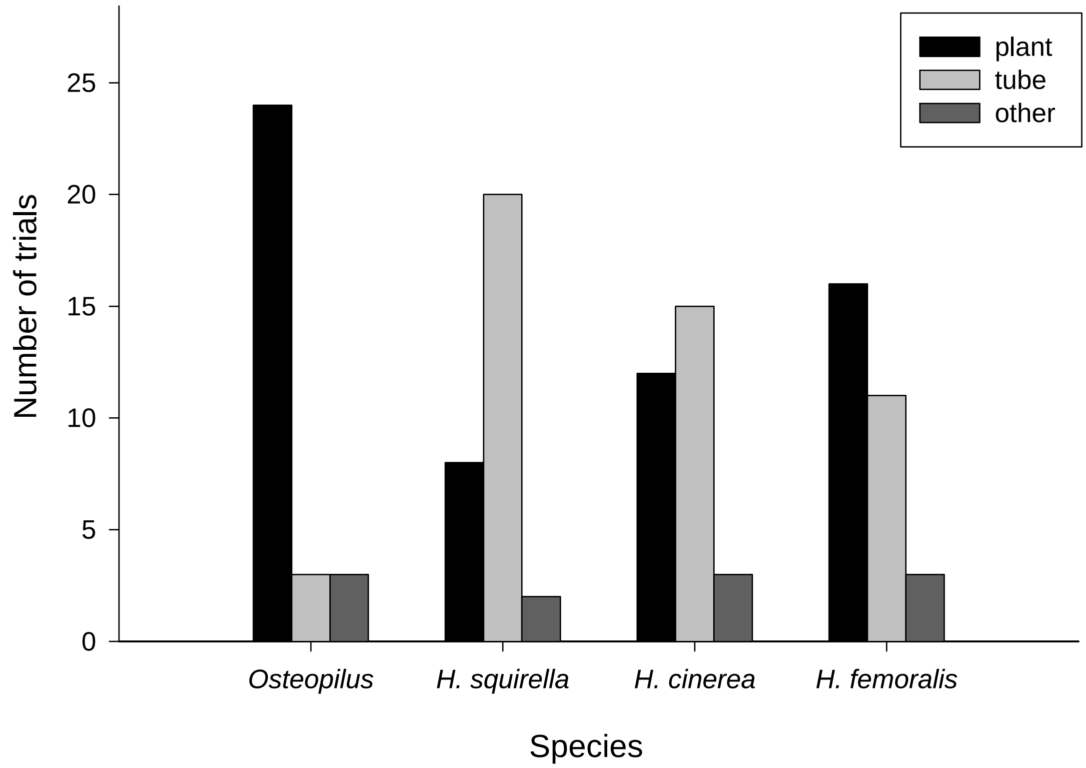

Figure 2-1. The frequency of refugia type used by individuals of four treefrog species after 14 hours in an aquarium with a plant and a PVC pipe. Other resting sites included the side of the tank, the corner where the lid met the tank, and the sand substrate.

CHAPTER 3
TESTING FOR INTERFERENCE COMPETITION BETWEEN *OSTEOPILUS SEPTENTRIONALIS* AND NATIVE TREEFROGS AT PVC PIPE REFUGIA

Introduction

Competition within and among species is an important ecological interaction that can limit the distribution and abundance of coexisting species (Gause 1934, Jaeger et al. 2002). In exploitative competition, competition reduces the availability of a resource (Greenlees 2007). In interference competition, the competitor reduces the access to resources (Greenlees 2007).

Nonindigenous species can harm native species through competition. When competing taxa co-occur naturally, competition is often reduced through resource partitioning and character displacement (Pacala and Roughgarden 1982, Jaeger et al 2002). However, when a species is introduced into new area competition can be intense because the native fauna have not evolved mechanisms to accommodate competition by nonindigenous species (Greenlees 2007). If the introduced species is a superior competitor, it may cause declines, or even local extinction, in the native species.

The Cuban treefrog (*Osteopilus septentrionalis*) is a nonindigenous species in Florida. It is known to consume native treefrogs and compete with their tadpoles (Allen and Neil 1953, Meshaka 2001, Smith 2005), and the establishment of this species in an area has been associated with a decline in native treefrogs (Meshaka 2001). This species was first reported on Key West in 1931 and is now established throughout most of peninsular Florida (Barbour 1931, Meshaka 2001, Krysko et al. 2005). As *O. septentrionalis* continues to spread, an understanding of how it impacts native communities, especially treefrogs, is essential to the eventual development of management strategies for this nonindigenous frog.

The use of PVC pipe refugia is becoming a common method of sampling treefrogs (Moulton et al. 1996, Boughton 1997, Hirai 2006). Additionally, this method has the potential to assist in the control of *Osteopilus septentrionalis* in both developed and natural settings. PVC pipe refugia attract treefrogs that can then be captured for individual identification and marking (Moulton et al. 1996), or removed and euthanized, as in the case of *O. septentrionalis*. As this technique becomes more widespread it is increasingly important to test the underlying basic assumptions of this method. The assumption that PVC pipe refugia occupancy by *O. septentrionalis* does not interfere with occupancy by native species through interference competition, is a potential source of bias when sampling native treefrogs and has not yet been evaluated.

Territorial behavior exhibited by a nonindigenous species may result in competition with native species for refuge sites when this resource is limiting. Although most reported territorial aggression in anurans is related to courtship (Duellman and Trueb 1986, Lutz 1960), several treefrogs have been reported to exclude other frogs from refugia. Both male and female coquis (*Eleutherodactylus coqui*) defend diurnal retreat sites, using aggressive calls and biting the heads of intruders (Stewart and Rand 1991). Male Mexican leaf-frogs (*Pachymedusa dacnicolor*) use prepollical spines on their wrists to defend burrows from conspecifics (Wiewandt 1971). Resident male squirrel treefrogs (*Hyla squirella*) are known to force invaders out of short PVC pipe refugia Buchanan (1988). *Osteopilus septentrionalis* is often found in aggregations (Meshaka 1996), and territoriality has not yet been reported in this species.

In addition to the potential for behavioral interactions, there is also the potential for chemically mediated effects between nonindigenous and native species. When threatened, *Osteopilus septentrionalis* produces a milky toxin with a distinct odor (Meshaka 2001). This toxin irritates mammalian mucous membranes (Meshaka 2001) and may also affect amphibians. Tadpoles and adult frogs often use chemical cues to detect predators (Stauffer and Semlitsch

1993, Kiesecker et al. 1996, Chivers, et al. 1999), and it has yet to be determined if secretion residue left by *O. septentrionalis* may be detected and avoided by native treefrogs. As detecting chemical cues is important in amphibian ecology (Stauffer and Semlitsch 1993, Kiesecker, et al 1996, Chivers, et al. 1999), there is the potential that if native treefrogs do avoid this residue, then *O. septentrionalis* resting on a substrate might chemically interfere with the frogs that later use that area.

I conducted two laboratory experiments to examine possible interference competition between *Osteopilus septentrionalis* and native treefrogs for refugia sites. I tested the null hypothesis that *O. septentrionalis* does not affect the detection probability of native treefrogs in PVC pipe refugia via behavioral exclusion and that native treefrogs do not avoid pipes previously used by *O. septentrionalis* (chemical exclusion).

Methods

Behavioral Exclusion

To determine if *Osteopilus septentrionalis* behaviorally excludes native treefrogs from PVC pipe refugia, I conducted 30 trials for each of two native species: green treefrog (*Hyla cinerea*) and squirrel treefrog (*H. squirella*). I paired a native frog with an *O. septentrionalis* in an aquarium with a single PVC pipe refugium; as a control, I paired frogs with conspecifics. To prevent predation by *O. septentrionalis,* I paired frogs of similar size such that frogs did not differ more than 20% in snout-vent length (SVL). At 2000 h, I placed each pair into a 37.85-L aquarium with a screen lid, moist sand substrate, and a 20-cm tall and 3.81-cm diameter PVC pipe refugia planted vertically in the sand substrate in the center of the enclosure. At 1000 h the following morning, I recorded the location of each frog as either in or out of the PVC pipe refugium. Laboratory conditions, described below, were held constant and did not vary from those of the growth room in which the frogs were housed.

Chemical Avoidance

To determine if native treefrogs avoid PVC pipe refugia that have recently been used by *Osteopilus septentrionalis*, I tested treefrogs by placing them in an aquarium with one pipe that had been used recently by *O. septentrionalis* and one new, clean pipe. In control trials, I placed each treefrog in an aquarium with two clean pipes. To create the treatment pipes, I housed 30 large (> 45 mm SVL) *O. septentrionalis* in a 113-L plastic enclosure with a moist sand substrate, small dish of water, screen lid, potted vinca plant (*Catharanthus roseus*), and 20 PVC pipe refugia. On nights before a trial, I replaced the refugia and the plant in this container with 15 new, clean refugia so that the *O. septentrionalis* would rest in these pipes. At 2000 h the next night, I removed *O. septentrionalis* from these now used pipes, and placed these pipes opposite new, clean pipes in 37-L aquaria with sand substrate and screen lids. One frog was then introduced to each aquarium. At 1000 h the location of the frog was recorded. To control for possible directional bias, the side of the enclosure on which each treatment pipe was placed was alternated among tanks. To discourage smaller frogs from resting between the lid and lip of the aquarium, I filled this space with a rope that was secured with non-toxic glue. A high-intensity desk lamp with a 40-watt bulb was placed in the growth room where the tanks were housed to provide light so that the frogs would not be in complete darkness at night. Laboratory conditions, described below, were held constant and did not vary from those of the growth room in which the frogs were housed.

Collection and Maintenance of Captive Frogs

I opportunistically collected treefrogs by use of PVC pipe refugia and by hand. Collection occurred primarily at night, particularly during breeding events and rainstorms. Frogs were collected from 10 May 2007 through 26 August 2007 in Alachua, Hernando, Hillsborough, Manatee, Orange, Pinellas, Polk, Sarasota, and Seminole counties, Florida. I caught frogs at retention ponds, along the edges of natural wetlands, in roadside and agricultural ditches, on suburban streets, in forested county parks, on walls of buildings, and in parking lots.

I transferred these frogs to growth rooms at the University of Florida's Gulf Coast Research and Education Center in Hillsborough County. I maintained frogs in captivity in accordance with University of Florida Institutional Animal Care and Use Committee (protocol number E870). Growth rooms were equipped with individual heating, ventilation, and air conditioning (HVAC) systems set to 24°C. The rooms also had full-spectrum fluorescent lights set to automatically turn on at 0630 h and off at 2030 h to mimic the natural photoperiod. A high-intensity desk lamp with a 40-watt bulb shining into a corner provided light so that the frogs would not be in complete darkness at night. I housed frogs individually in 0.75-L Sterilite containers with 3-mm air holes drilled in the lids during the entire length of their captivity. I fed them crickets, misted their containers daily, and replaced their paper towel substrate as needed.

I conducted experiments from 15 May 2007 through 31 August 2007. After completion of each trial I marked native treefrogs with Visual Implant Elastomer (VIE) and released them at their initial capture site. All *Osteopilus septentrionalis* were humanely euthanized, as this is a nonindigenous species in Florida.

Statistical Analysis

To test null the hypothesis that *Osteopilus septentrionalis* does not behaviorally exclude native frogs from PVC pipe refugia, I compared the probability of refuge use by native frogs in the presence of *O. septentrionalis* with the probability of refuge use when paired with a conspecific. Probabilities were calculated using a maximum likelihood model base on frequency of refugia use by pairs of frogs. To calculate probabilities when two native frogs were paired, each was randomly assigned post hoc identification as frog A or frog B. Four combinations of outcomes were possible: (1) both frogs were in the pipe (AB), (2) only native frog A was in the pipe (A0), (3) only *Osteopilus septentrionalis* or the native frog B was in the pipe (0B), and (4) neither frog was in the pipe (00). The probabilities of these outcomes are as follows:

$$P_{AB} = P_A \times P_B$$
$$P_{A0} = P_A \times (1- P_B)$$
$$P_{0B} = (1- P_A) \times P_B$$
$$P_{00} = (1- P_A) \times (1- P_B)$$

I used Program R, version 2.5.1, to calculate the maximum likelihood estimates of the probability that native treefrog (frog A when paired with a conspecific) was in the pipe (P_A), and that the *O. septentrionalis* or frog B was in the pipe (P_B). I used the program to calculate the likelihood ratio -2 (log $L_{\text{reduced model}}$ - $L_{\text{full model}}$) for each species to compare the probability of the native frog being in the pipe when paired with an *O. septentrionalis* to the probability of native frog A being in the pipe when paired with a conspecific (native frog B). I used the likelihood ratios in a chi-square analysis to test the null hypotheses that the native treefrogs are equally likely to occupy the pipe in the presence of *O. septentrionalis* as with a conspecific.

To analyze the outcome of my chemical avoidance experiment, I tested the null hypothesis that there was no difference in occupancy of clean PVC pipe refugia and those that had recently housed *Osteopilus septentrionalis.* To do this I used Microsoft Excel to conduct a chi-square test for each species to determine if the frogs were more likely to use one type of pipe (used by *O. septentrionalis* vs. clean) over the other. I used the results of the control trials as expected values for this analysis.

Results

Behavioral Exclusion

The probability of *Hyla squirella* occupying PVC pipe refugia in the presence of *Osteopilus septentrionalis* was not significantly different from the probability of *H. squirella* occupying the refugia in the presence of a conspecific (LR = 1.69, P =0.193). These probabilities were also not significant for *H. cinerea* (LR = 0.130, *P* = 0.718; Table 3-1). Therefore, I failed to reject the null hypothesis that native treefrogs were equally as likely to rest in a PVC pipe refugium with an *O. septentrionalis* as with a conspecific.

Chemical Avoidance

Native treefrogs did not demonstrate significant avoidance of PVC pipe refugia that had been recently used by *Osteopilus septentrionalis* (χ^2 =0.86, df = 2, *P* = 0.65 for *Hyla squirella*, and χ^2 =1.54, df = 2, *P* = 0.46 for *H. cinerea*; Table 3-2). Although *O. septentrionalis* used to treat the pipes were often found sharing refugia in their holding enclosure, with 30 frogs congregated in approximately half of the 15 available clean pipes, *O. septentrionalis* in experimental trials rested in used PVC pipe refugia significantly less frequently than in control pipes or other locations (on glass or sand substrate; χ^2 =7.11, df = 2, *P* = 0.03). Therefore, I failed to reject the null hypothesis that native treefrogs were equally likely to use a PVC pipe refugium that had recently been used by *O. septentrionalis* as new, clean refugia, but did reject the null than *O. septentrionalis* would use these pipes equally.

Discussion

Neither experiment indicated that *Osteopilus septentrionalis* interferes with native treefrog refugia use and detection during studies using PVC pipe refugia. The frequency of pipe use by native treefrogs was not altered by the presence of *O. septentrionalis* or by any residue that might have been left on pipes recently used by this nonindigenous frog. However, *O. septentrionalis* avoided refugia that had recently been used by its batracophagic conspecifics. These results support the underlying assumption that the presence of *O. septentrionalis* does not reduce the detection probability of native treefrogs, and thus competition for refugia is unlikely. However, the results indicate that as it avoids refugia recently used by conspecifics, *O. septentrionalis* may be underrepresented in samples using this method. With this assumption in place, it is possible to use PVC pipe refugia to compare native treefrog populations prior to the invasion of *O. septentrionalis* to populations of treefrogs sampled after this nonindigenous species has become established. However, researchers should keep in mind the possibility that *O. septentrionalis* may be underrepresented.

My results are inconsistent with those of Buchanan (1988), who found that *Hyla squirella* was territorial and excluded conspecific invaders from the PVC pipe refugia in the lab. I often observed *H. squirella* sharing refugia with conspecifics or *Osteopilus septentrionalis,* although this inconsistency may be due to the fact that I did not allow one frog to become established in the aquarium before introducing a second frog. Stewart and Rand (1991) found that while intruder *Eleutherodactylus coqui* were often evicted from refugia, resident frogs were rarely displaced from their refugia by invaders. Thus, it may be important for a frog to establish claim over a refugium before defending it, which might explain the difference between my results and those of Buchanan (1988). Still, *H. squirella* has been observed in the wild sharing a PVC pipe refugium with up to five adult conspecifics (Johnson, unpubl. data). Future research studies should be conducted in which the native frog is allowed to reside in the aquarium for sufficient time to establish residency prior to the introduction of *O. septentrionalis*, and in which frogs in the field are examined for territorial behavior.

The avoidance of PVC pipe refugia that had recently been used by *Osteopilus septentrionalis* by conspecifics and not by native treefrogs suggests that these frogs may be avoiding a potential predator that natives do not recognize. The ability to recognize and respond appropriately to predators is extremely important to the fitness of an individual as those that fail to do so are likely to be eaten (Chivers and Smith 1994, Kiesecker and Blaustein 1997, Griffin et al. 2000). Although native frogs readily respond to native predators, they may not recognize and respond to nonindigenous predators (Kiesecker and Blaustein 1997, Pearl et al. 2003). I did not investigate the possibility that native treefrogs attracted to the pipes might be more likely to be preyed upon by *Osteopilus septentrionalis*, thus making the artificial refugia used in research efforts into population sinks. If *O. septentrionalis* residing inside a PVC pipe refugium consume native treefrogs attracted to that refugium, native populations might be underrepresented in surveys using this sampling technique. I recommend this topic for further research.

Table 3-1. Number of each species of native treefrog (*Hyla squirella* and *H.* cinerea) that chose to rest in the PVC pipe refuge when paired with either *Osteopilus septentrionalis* or a conspecific

	Both (AB)	Native only (A0)	*O. septentrionalis* or conspecific only (0B)	Neither (00)
H. squirella with *O. septentrionalis*	6	13	4	7
H. squirella with conspecific	12	2	9	7
H. cinerea with *O. septentrionalis*	3	7	12	18
H. cinerea with conspecific	5	3	5	17

Table 3-2. Total number of treefrogs resting in each location after 14 hours in an enclosure with a choice of two PVC pipe refugia during experimental trials*

	Experimental	Control	Neither
O. septentrionalis	3	11	16
H. squirella	11	9	10
H. cinerea	5	9	16

*Experimental pipes had several *Osteopilus septentrionalis* resting in them during the day of the trial and were placed in the aquaria just before the start of the trial. Control pipes are new pipes, and Neither indicates the frog was not in a pipe.

CHAPTER 4
REMOVAL OF *OSTEOPILUS SEPTENTRIONALIS* FROM FLATWOODS HABITAT IN CENTRAL FLORIDA

Introduction

Some nonindigenous species may directly affect communities of native species by preying on or competing with native species, or indirectly by introducing new diseases, altering nutrient cycles, fire regimes, and other aspects of the environment that then create habitat less suitable for native species (Simberloff 2000). Wilcove et al. (1998) ranked competition and predation by nonindigenous species as the second greatest threat to imperiled plants and animals in the United States, surpassed only by habitat loss caused by humans.

The potential for the nonindigenous Cuban treefrog (*Osteopilus septentrionalis*) to negatively affect native treefrogs has been evident since its discovery on Key West, Florida (Barbour 1931). In a letter to Barbour (1931), A. G. Elbon reported that this frog was common on the island, had been there since before he moved to the island, and that it fed almost exclusively on smaller frogs. Twenty years later, Schwartz (1951) documented *O. septentrionalis* on mainland Florida and the rapid development of its eggs. Shortly after, Allen and Neill (1952) observed *O. septentrionalis* feeding on breeding frogs, and warned that the presence of this frog might affect native frogs. Lee (1969) repeated this warning, although he cautioned that there were no data to substantiate this potential threat at that time.

Recently, studies have begun to confirm the adverse effects of *Osteopilus septentrionalis* on native frogs. *Osteopilus septentrionalis* tadpoles have been found to consume and reduce the growth rate of native tadpoles (Smith 2005a, Smith 2005b). Although *O. septentrionalis* has been observed consuming native frogs since the time of its introduction, adults appear to prey mainly upon insects and less frequently on other frogs (Lee 1969, Wyatt and Forys 2004, Meshaka 2001). These and other stresses may add up to a population-level effect on native treefrogs. Before we can consider costly management plans to conserve native frogs, we need to know if *O. septentrionalis* truly does cause declines in native frogs. It is also necessary to examine possible management strategies, such as the removal of *O. septentrionalis* using PVC pipe refugia.

I conducted a large-scale field study to determine if native treefrogs respond to the removal of *Osteopilus septentrionalis*. I tested the hypothesis that if *O. septentrionalis* reduces the abundance of native treefrogs, then native treefrogs should be more abundant at sites where *O. septentrionalis* was removed. I did not seek to identify the mechanism by which *O. septentrionalis* could be affecting native treefrogs.

Methods

Sampling

At Wekiwa Springs State Park and Flatwoods Park in Central Florida, I selected three study blocks based on accessibility, similarity of habitat, and proximity to development (and thus probability of containing an established *O. septentrionalis* population). In each block I constructed a pair of 50- by 50-m grids of 1-m tall, 3.81-cm diameter PVC pipes installed vertically at 5-m intervals. Every three weeks, I checked each grid once daily for three consecutive days. I monitored each grid over two sampling periods to estimate initial treefrog abundance. I then randomly selected one experimental grid of each pair at which I removed all *O. septentrionalis* I encountered. Frogs were not removed from the other grids, which were used as controls.

During each sampling period, I removed frogs from PVC pipe refugia by gently coaxing them into a plastic bag using a “plunger” constructed from a sponge cut to the inside diameter of the PVC pipe and attached to the end of a dowel. I identified each frog to species, measured snout-vent length (SVL) to the nearest mm, and weighed to the nearest 0.1 g. I also marked each frog for future identification using a unique combination of toe-clipping and the injection of visual implant elastomer (VIE) in up to two toes. I released all native frogs and *Osteopilus septentrionalis* caught within control grids, and euthanized all *O. septentrionalis* captured within the experimental grid after the initial two sampling periods to estimate initial population sizes.

Wekiwa Springs State Park

Wekiwa Springs State Park is located in Apopka, Florida, less than 20 km north of Orlando. The park is managed by the Florida Park Service and consists of 2,800 ha surrounded by urban development to the south, Rock Springs Run State Preserve to the northeast, and urban development and agriculture to the west (Fig. 4-1).

During June 2006, I established my original grids at the park. I installed Grids A and B in the southeastern corner of the park in a small mesic flatwoods opening surrounded by sandhill to the south, hydric hammock to the north and west, and an urban/wildland interface to the east (Fig. 4-1). Grids C, D, E, and F were in a large tract of mesic flatwoods within the interior of the park. I checked these grids for treefrogs during 19-21 July 2006. *Osteopilus septentrionalis* were captured in Grids A and B, and these grids remained in their original position throughout the study. However, during this first sampling period it became apparent that the grids located in the interior of the park (C, D, E, and F) did not contain *O. septentrionalis* in detectable numbers, if at all. Therefore, I removed these four grids on 8 August 2006, and established new Grids G, H, I, and J in mesic flatwoods in the southwestern corner of the park. These new grids were surrounded on two sides by an urban/wildland interface, and seemed likely to contain *O. septentrionalis*. Indeed, during the next survey period, all grids contained *O. septentrionalis*. Removal of *O. septentrionalis* from experimental grids (A, H, and J) began on 12 October 2006, after the completion of two survey periods.

On 18 November 2006, I discovered mowed firebreaks approximately 15 m wide cut through Grids G and I, both of which were control grids. This disturbance had the potential to alter the distribution of the frogs within each grid and introduce edge effects. Therefore, I removed these grids on 3 January 2007. To replace the grids destroyed by mowing, I established new Grids K and L in scrubby flatwoods just east of the previous grids and new Grids M and N between Grids H and I. After sampling these new grids for two survey periods, and observing less than one frog per grid, I decided I would be unable to obtain adequate sample sizes to conduct this study. On 10 May 2007 all grids were removed from Wekiwa Springs State Park.

Flatwoods Park

Flatwoods Park, located northwest of Tampa, Florida, is part of the Hillsborough Wilderness Park System, which contains over 2,200 ha (Fig. 4-2), and is managed by the Southwest Florida Water Management District as the Morris Bridge Wellfield. The park is a popular cycling location, and an 11.2-km paved bicycle loop encircles the center of the park. The park consists predominantly of flatwoods habitat, and is surrounded by ongoing urban development to the north and other parks in the Hillsborough Wilderness Park System to the east and south.

On 11 July 2006, I installed six grids at Flatwoods Park (Fig. 4-2), all of which were located north of the bicycle loop in mesic flatwoods habitat. Grids A and B were located in the western side of the park, Grids C and D were central, and Grids E and F were near the eastern corner of the park. The urban/wildland interface was visible from the majority of the six grids. I began monitoring the grids on 8 August 2006 and began to remove *Osteopilus septentrionalis* from experimental Grids A, D, and E on 30 September 2006. Monitoring and removal continued through 29 June 2007, at which time the study was concluded and the grids were removed.

Statistical Analysis

I used SYSTAT, version 10.2, to perform a paired t-test to test if the total number of *Osteopilus septentrionalis* varied by grid type, and thus determine if a significant number of these invasive frogs were removed from experimental grids. I also visually inspected the number of *O. septentrionalis* captured at each grid over time to determine if fewer frogs of this species were observed in experimental grids throughout the study than in control grids. Using program MARK, version 4.3, I conducted a robust closed capture model with constant emigration rates to estimate abundance of native treefrogs in each grid over time. I plotted the estimated abundances of each grid, and visually inspected estimates for patterns. I expected that if removal of *O. septentrionalis* caused native treefrog abundance to increase, then estimates of abundance of native treefrogs in experimental grids would increase after removals began, while the abundance in control grids would drop due to continued effects of *O. septentrionalis.* As another indication of fitness of native treefrogs within each grid, I used SYSTAT to perform a Pearson's chi-square test to test the null hypothesis that equal numbers of gravid female *H. femoralis* were captured in control and experimental grids from 7 May 2007 through 8 August 2007. Finally, as a final attempt to determine if *O. septentrionalis* affected native treefrog abundance, I used a Pearson's Correlation Coefficient to determine if the number of *O. septentrionalis* and native treefrogs in each grid were negatively correlated.

Results

Flatwoods Park

Weather conditions were dry during the year leading up to the experiment (Fig 4-3; Florida Automated Weather Network 2007), and capture rates of frogs at all sites throughout the experiment were lower than anticipated. *Hyla femoralis* occurred regularly in PVC pipe refugia and was observed on a total of 1,517 occasions. I captured fewer *Osteopilus septentrionalis*, which I found a total of 532 times. *H. squirella* was only observed 14 times, and *H. cinerea* only once. Therefore, my analysis focused on *H. femoralis* and *O. septentrionalis.*

The total number of individual *Osteopilus septentrionalis* observed in control grids did not differ significantly from that of the experimental grids (t = -0.77, df =2, P = 0.52). Due to low capture rates of this species, I was unable to estimate their abundance using MARK. I plotted and inspected counts of *O. septentrionalis*, which did not vary visibly between experimental and control grids, and it appears that my removal had no effect on the abundance of this species. However, these counts did vary seasonally, with more frogs being observed in summer and early fall than in winter and spring (Figures 4-4, 4-5, 4-6).

Based on visual observation of abundance estimates of *Hyla femoralis* over time, I did not find compelling trends of increased abundance in experimental grids and decreased abundance in control grids. It does not appear the removals affected the abundance of this native species. Abundance of this frog in experimental Grid A was often, though not consistently, higher than in its partner control grid (Figure 4-7). On occasions when the estimated abundance of Grid A differed most from that of Grid B, standard errors were high. Populations of *H. femoralis* in Grids C and D appeared to be small, and on many days no frogs of this species were observed. The low capture rates in these grids created highly variable estimates with high standard errors (Figure 4-8). Only in Grid E did abundance of *H. femoralis* appear to increase with the removal of *O. septentrionalis* (Figure 4-9). Other indicators of fitness did not vary by grid type. The ratio of gravid females observed during the 2007 breeding season did not differ significantly according to grid type (χ^2 = 12, df = 10, P = 0.285). All *H. femoralis* observed were adults at first capture, and did not experience sufficient growth (snout-vent length and weight) to enable me to rule out error as a source of variation in measurements. However, there were less *H. femoralis* in grids with higher *O. septentrionalis* abundances (Figure 4-10). A negative correlation was found between total number of individual *O. septentrionalis* and *H. femoralis* (log transformed data; Pearson correlation = -0.820; Figure 4-11).

Wekiwa Springs State Park

At Wekiwa Springs State Park, removals of *Osteopilus septentrionalis* were only conducted during two sessions at the western grids, and the eastern grids experienced extremely low recapture rates (Appendix A). Thus my analysis focused on results from the Flatwoods Park site.

Discussion

These results suggest that *Osteopilus septentrionalis* was not removed in sufficient numbers to cause a decline in abundance and thereby exert an effect on *Hyla femoralis*. The number of observations of *O. septentrionalis* did not differ significantly between control and experimental grids, so it is unclear if the abundance of *H. femoralis* would have increased had I been able to remove more of this nonindigenous species. More research will need to be conducted to determine if removal of *O. septentrionalis* is an appropriate strategy to manage native treefrogs.

The low capture rates experienced at the Flatwoods Park site during the study period may have been caused by a combination of factors. First, although 2006 started off fairly wet, no precipitation was recorded in March at the nearby Hillsborough River State Park, located within 1 km from Flatwoods Park, and the months of April and May were also somewhat dry (P. Potts, Hillsborough River State Park, pers. comm.). In addition, Flatwoods Park is also the site of the Morris Bridge Wellfield, an active, long-standing site of groundwater pumping by Tampa Bay Water. This dry spell, combined with the potential effects of groundwater pumping, likely affected treefrog communities at Flatwoods Park. Secondly, numbers of frogs at the grid locations may have been extremely low. Squirrel treefrogs (*Hyla squirella*) chose PVC pipes over other refugia more often than other treefrogs and thus are most likely to be observed (see Chapter 2); however few individuals of this species were observed in the grids. Green treefrogs (*H. cinerea*) are associated with wetlands (Goin 1958) and although I have often observed this species a distance from permanent water, it might not have occupied the grid areas in detectable numbers. Third, frogs living at the grid sites might simply not be using the PVC pipe refugia. Recaptured frogs were often encountered in or near refugia of initial capture after avoiding detection for multiple days or sampling sessions. This suggests that either they returned to the same area after absence from the grid or, perhaps more likely, that they made use of other available refugia within the grid. Other researchers have observed higher capture rates in PVC pipe refugia positioned $\geq$ 2 m above the ground (Boughton et al. 2000), and it is possible that frogs within the study area utilized taller structures as refugia and did not encounter the PVC pipes. Finally, native frog populations in the grids may have already experienced substantial reductions subsequent to the establishment of *Osteopilus septentrionalis* within the park. Additionally, this nonindigenous species seems to prefer natural refugia over PVC pipe refugia, and may have been underrepresented during sampling for this study (see Chapter 2).

Seasonal changes are known to occur in the activity of *Osteopilus septentrionalis* (Meshaka 2001) and in the use of PVC refugia by some native treefrogs (Boughton 1997, Zacharow et al. 2003); thus, it is not surprising that the use of PVC pipe refugia by *O. septentrionalis* varied seasonally during the study period. The observed behavioral variation of *O. septentrionalis* in conjunction with low capture rates served to confound potential effects of removal, as seasonal variation in PVC pipe refugia use by this species was asynchronous to its removal. Captures of this species peaked near the beginning of the study, before and during the beginning of removals. Shortly after, this species became scarce in both experimental and control grids, and thus removal was infrequent.

Despite the lack of significant observable effects of *Osteopilus septentrionalis* removal, it is noteworthy that *Hyla femoralis* was observed less frequently at grids where this species was observed more frequently. While this correlation is not necessarily indicative of a cause and effect relationship, it lends credibility to anecdotal evidence that *O. septentrionalis* may be replacing native treefrogs in areas where it has become established. However, further research is needed to assess the potential effects of this invasive frog to actually cause declines of native treefrogs.

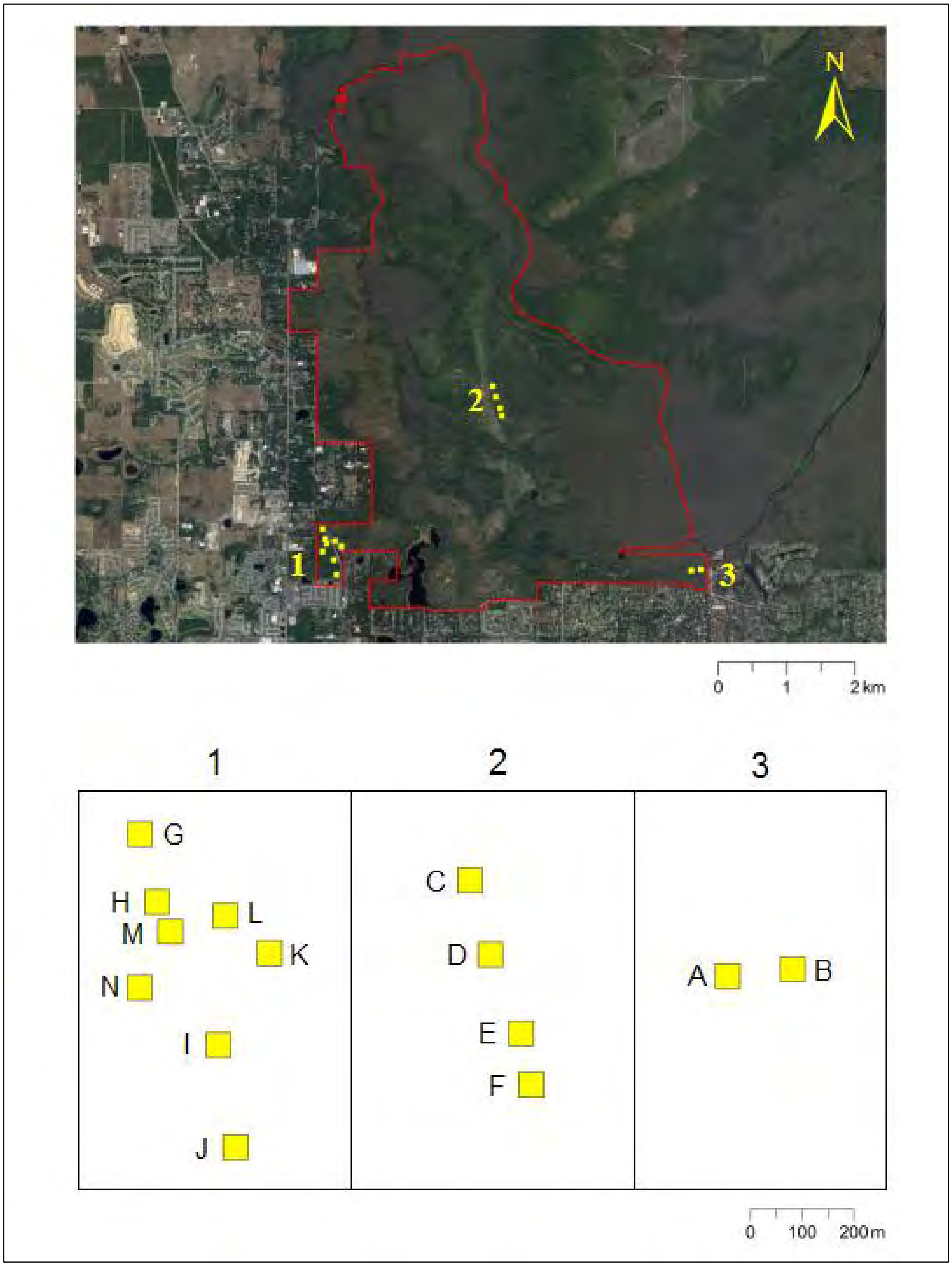

Figure 4-1. Location of each grid at Wekiwa Springs State Park (outlined in red). Above is an image of the area, and maps 1-3 below details the location of each grid relative to the other grids.

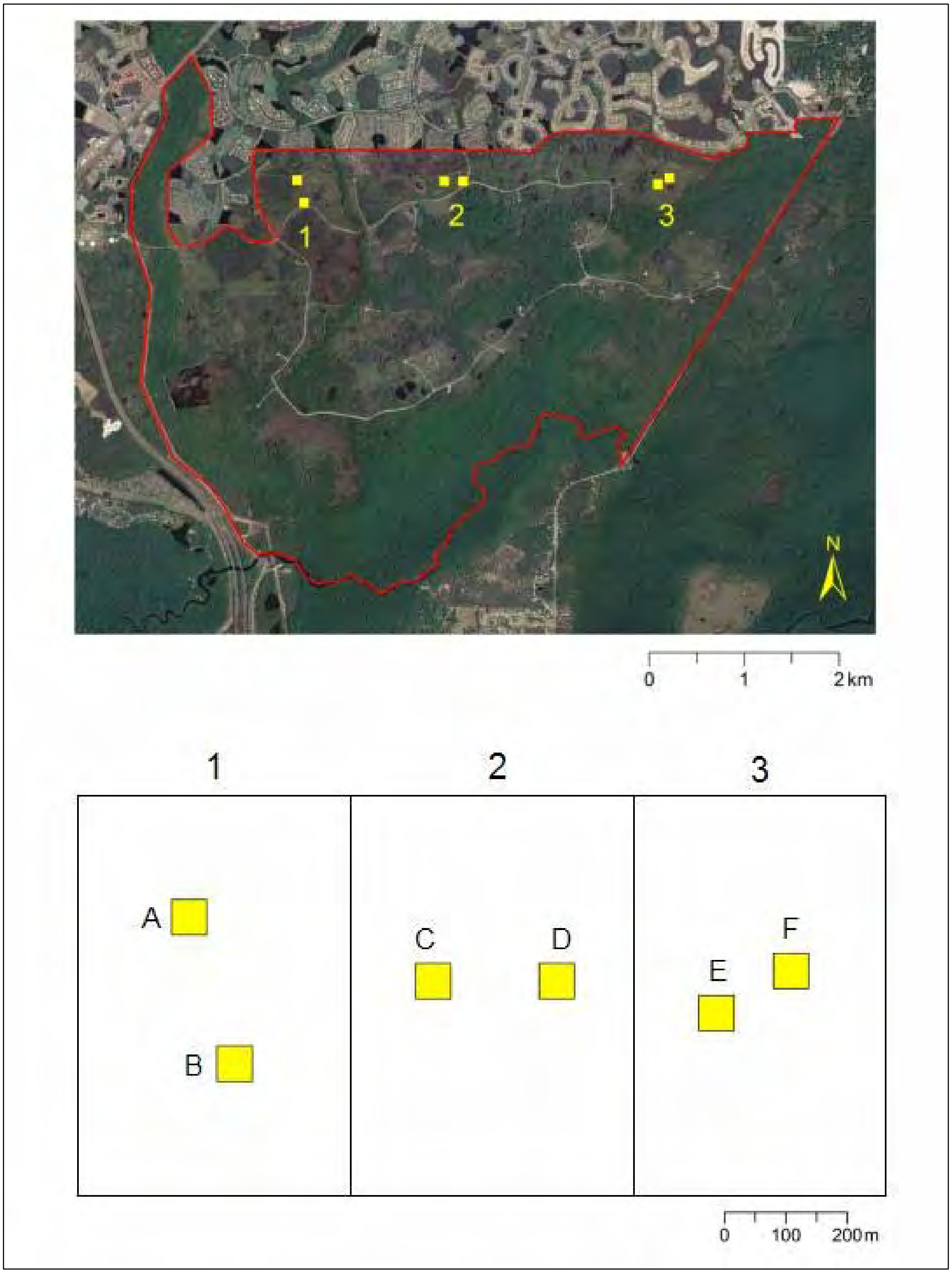

Figure 4-2. Location of each grid at Flatwoods Park (outlined in red). Above is an image of the area, and maps 1-3 below details the location of each grid relative to the other grids.

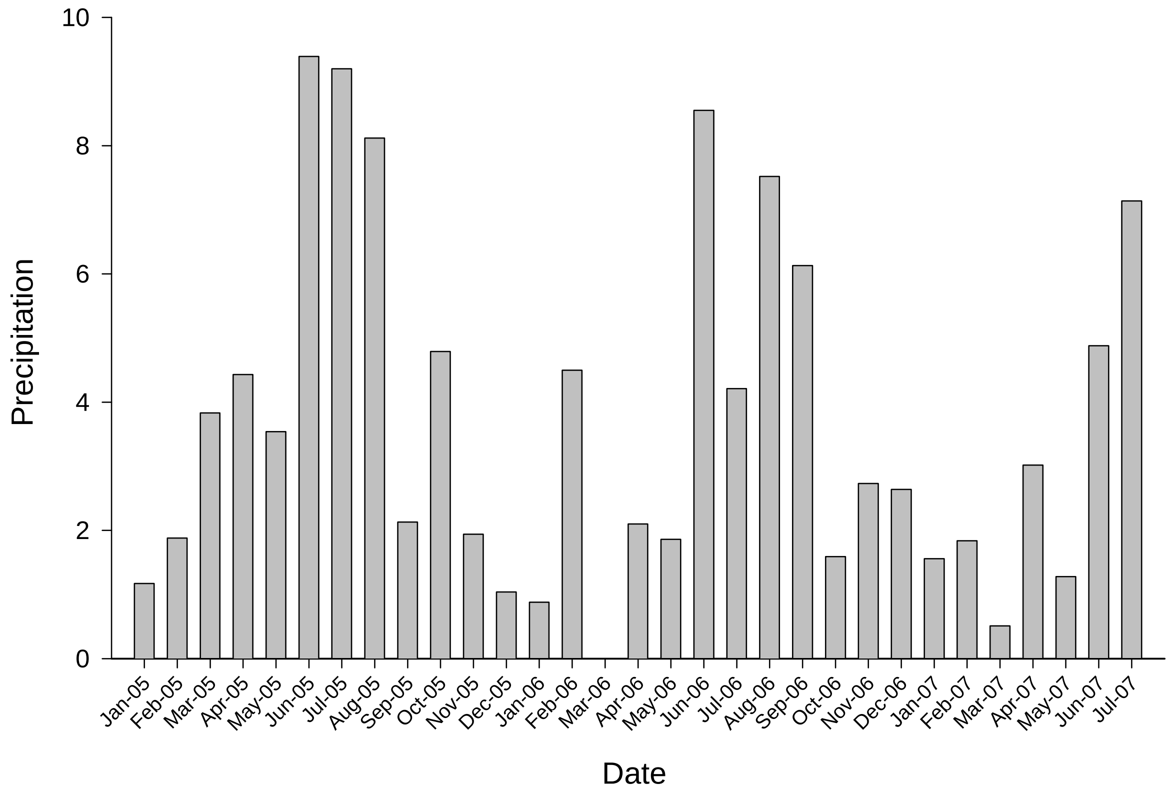

Figure 4-3. Total monthly precipitation at the Dover weather station in Hillsborough County (adapted from Florida Automated Weather Network, 2007). This weather station is located near Flatwoods Park.

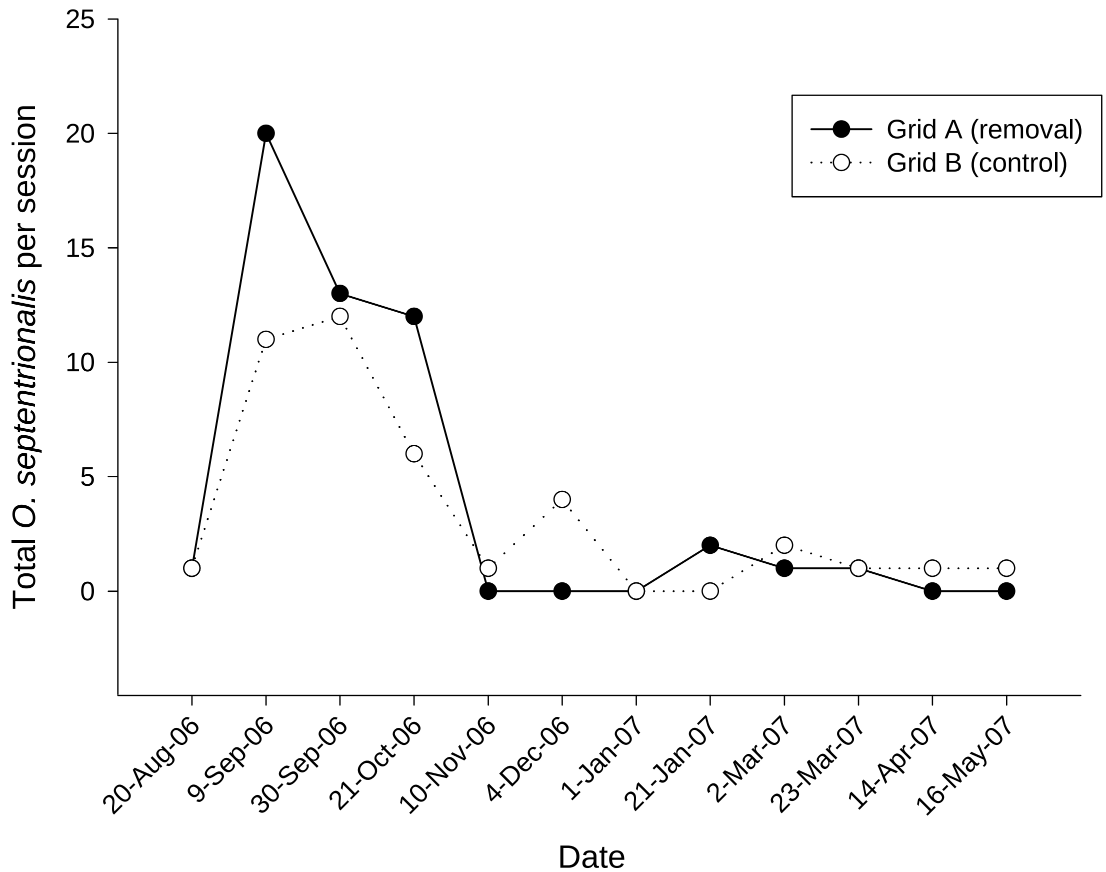

Figure 4-4. Seasonal trend in total individual *Osteopilus septentrionalis* observed per sampling session at Grids A and B at Flatwoods Park.

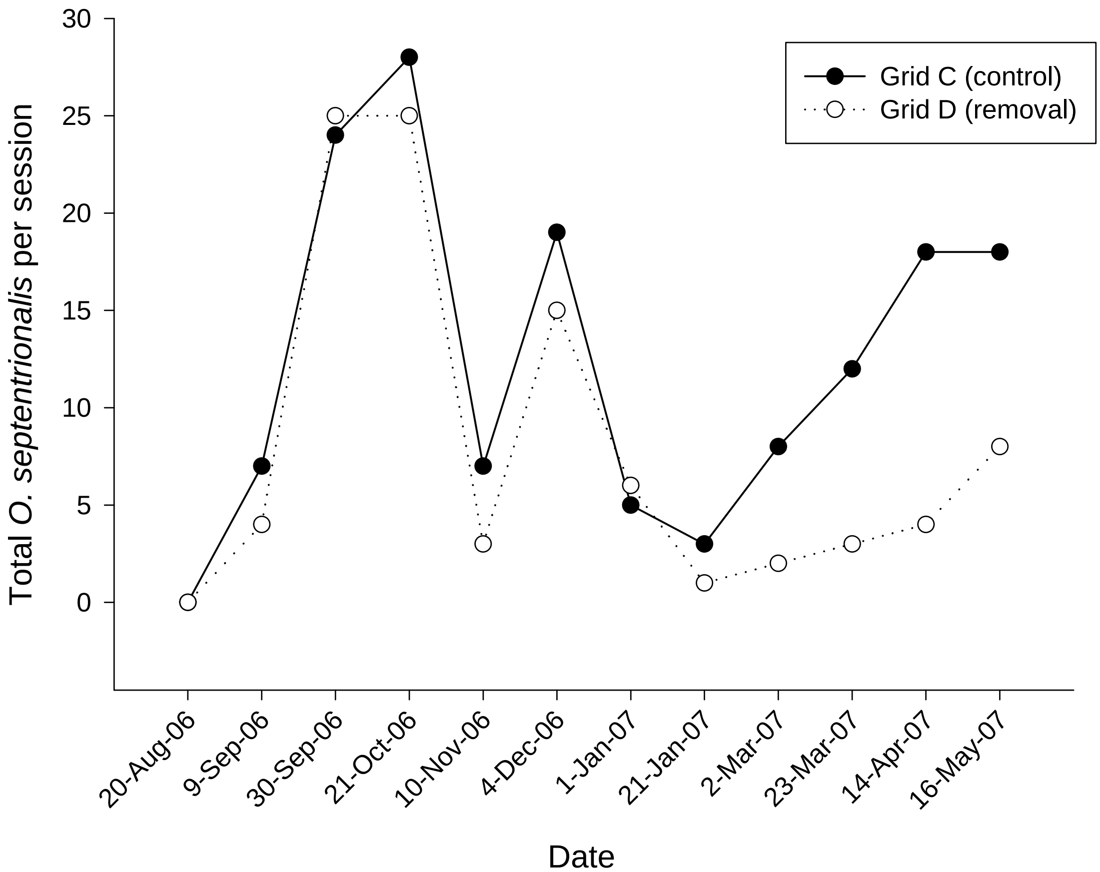

Figure 4-5. Seasonal trend in total individual *Osteopilus septentrionalis* observed per sampling session at Grids C and D at Flatwoods Park.

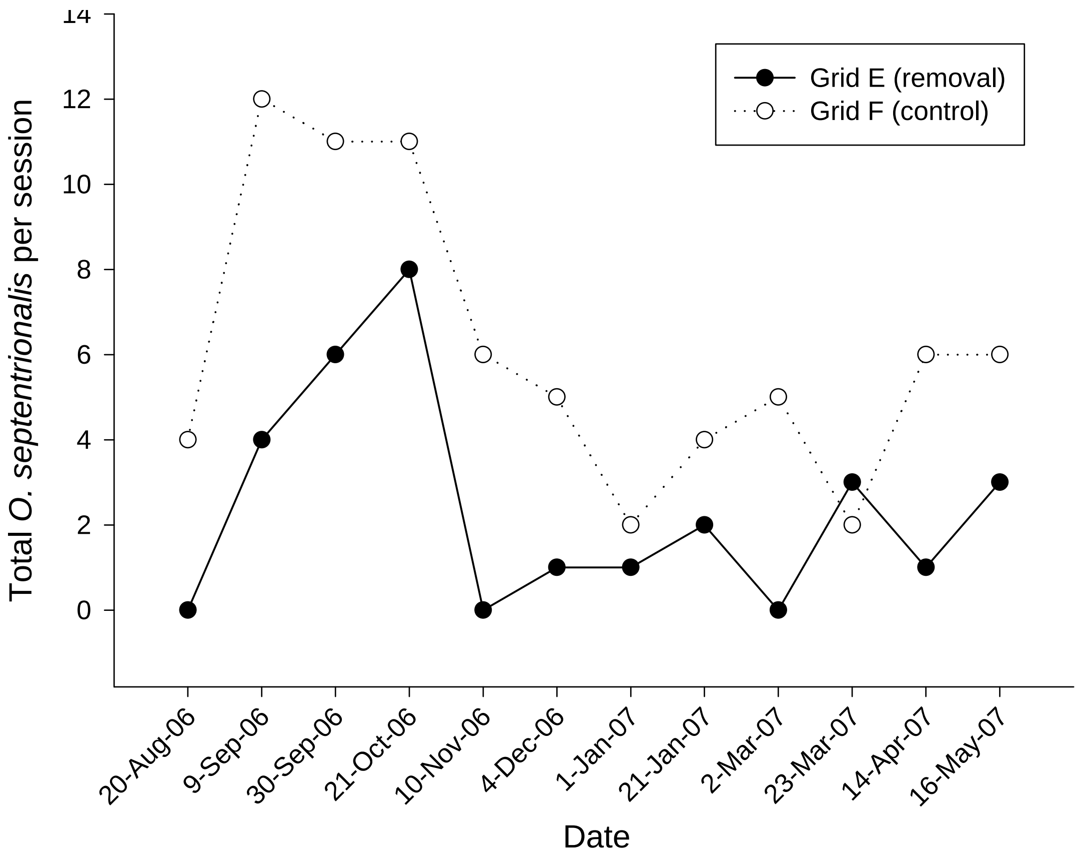

Figure 4-6. Seasonal trend in total individual *Osteopilus septentrionalis* observed per sampling session at Grids E and F at Flatwoods Park.

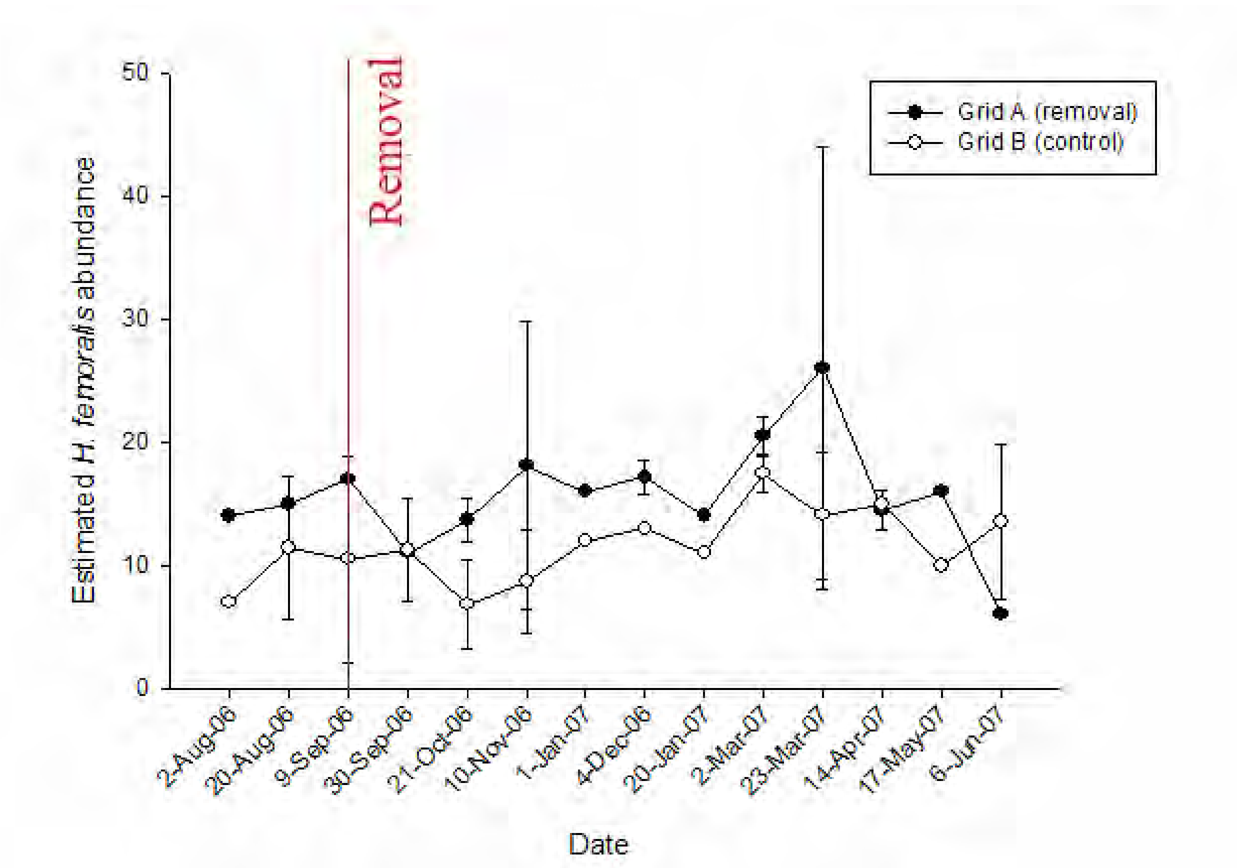

Figure 4-7. Estimated abundance of *Hyla femoralis* (± SE) at Grids A and B at Flatwoods Park. *Osteopilus septentrionalis* were removed from Grid A starting on 9 September 2006.

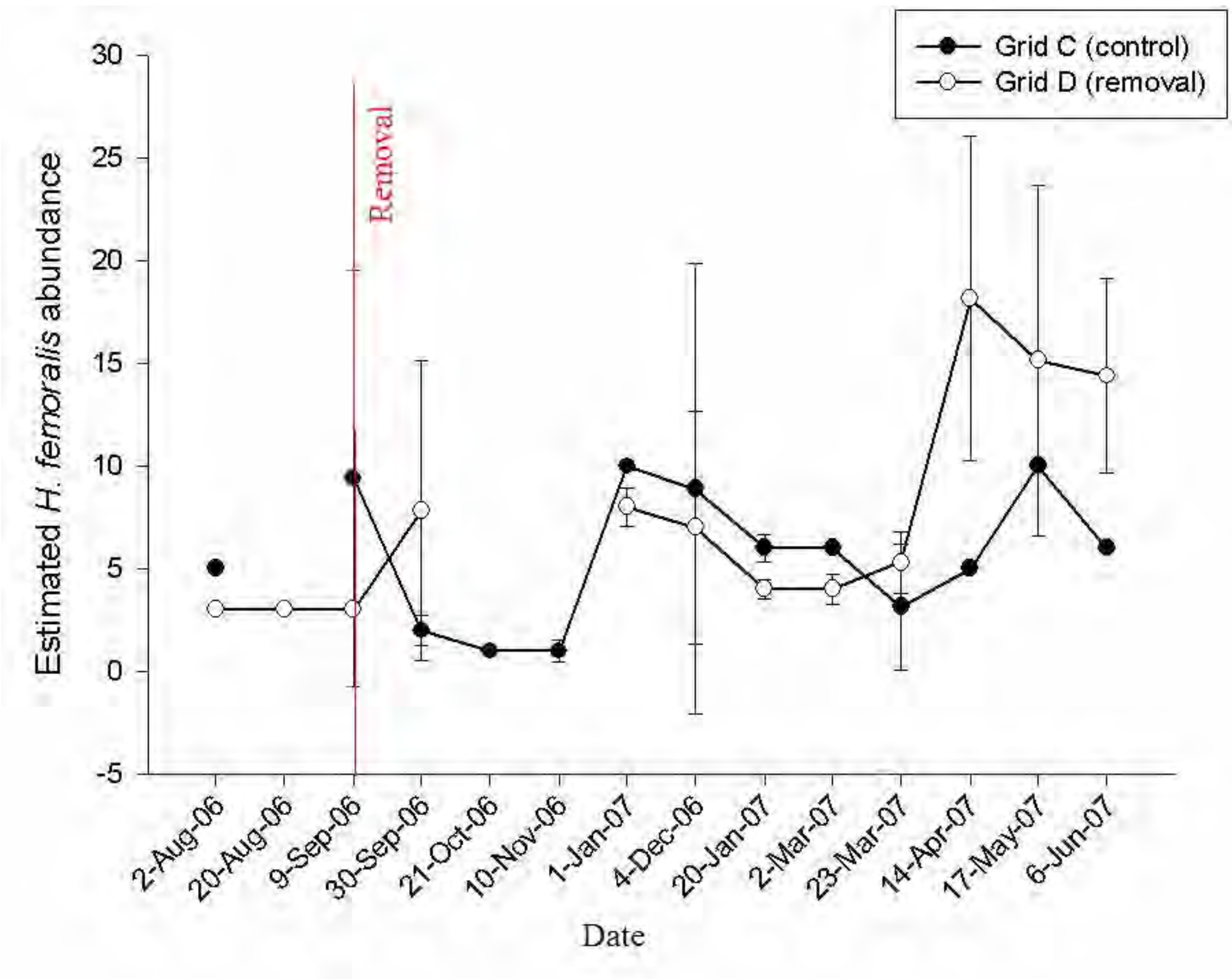

Figure 4-8. Estimated abundance of *Hyla femoralis* (± SE) at Grids C and D at Flatwoods Park. *Osteopilus septentrionalis* were removed from Grid D starting on 9 September 2006. Missing values were unrealistically estimated as over 500 individuals, due to low capture and recapture rates, and are not shown on this figure.

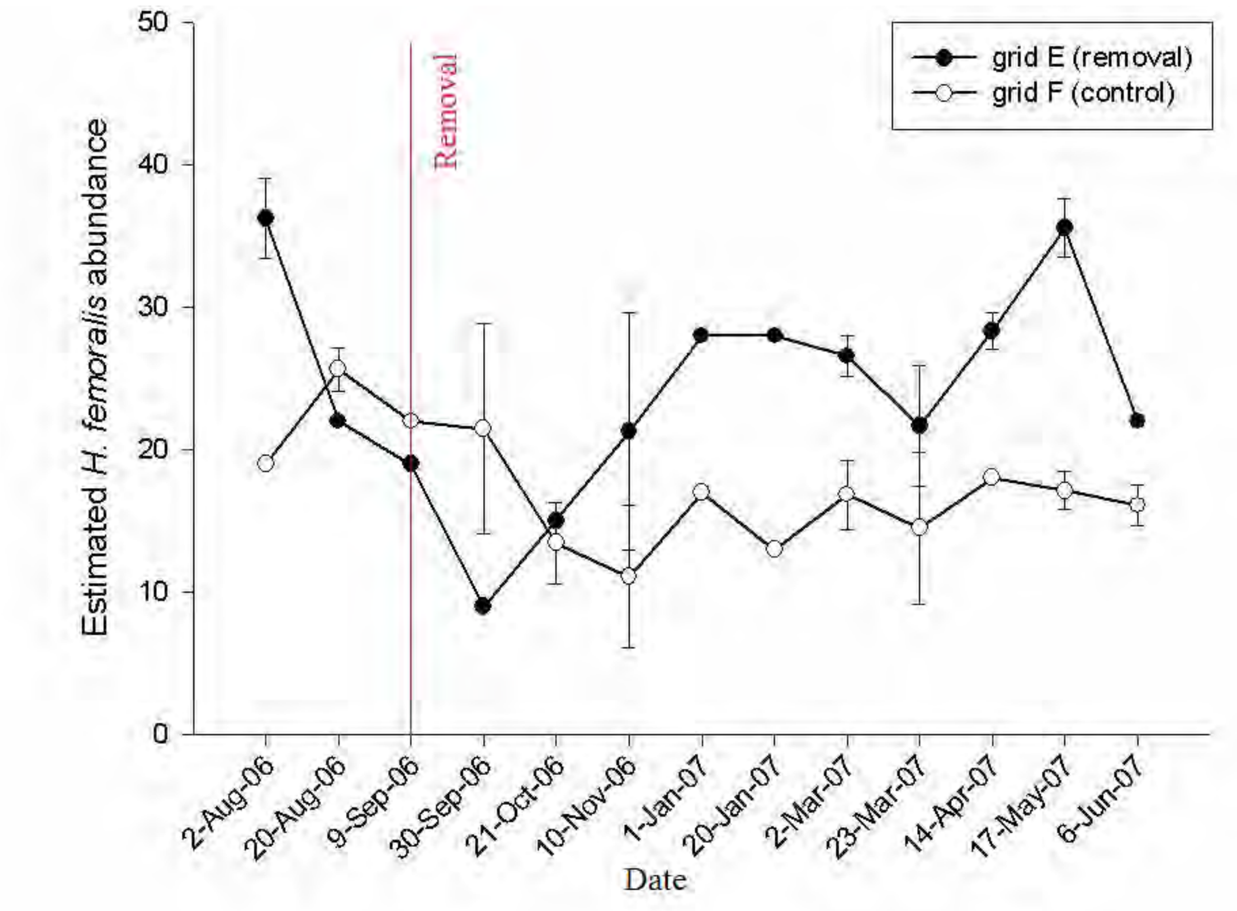

Figure 4-9. Estimated abundance of *Hyla femoralis* (± SE) at Grids E and F at Flatwoods Park. *Osteopilus septentrionalis* were removed from Grid E starting on 9 September 2006.

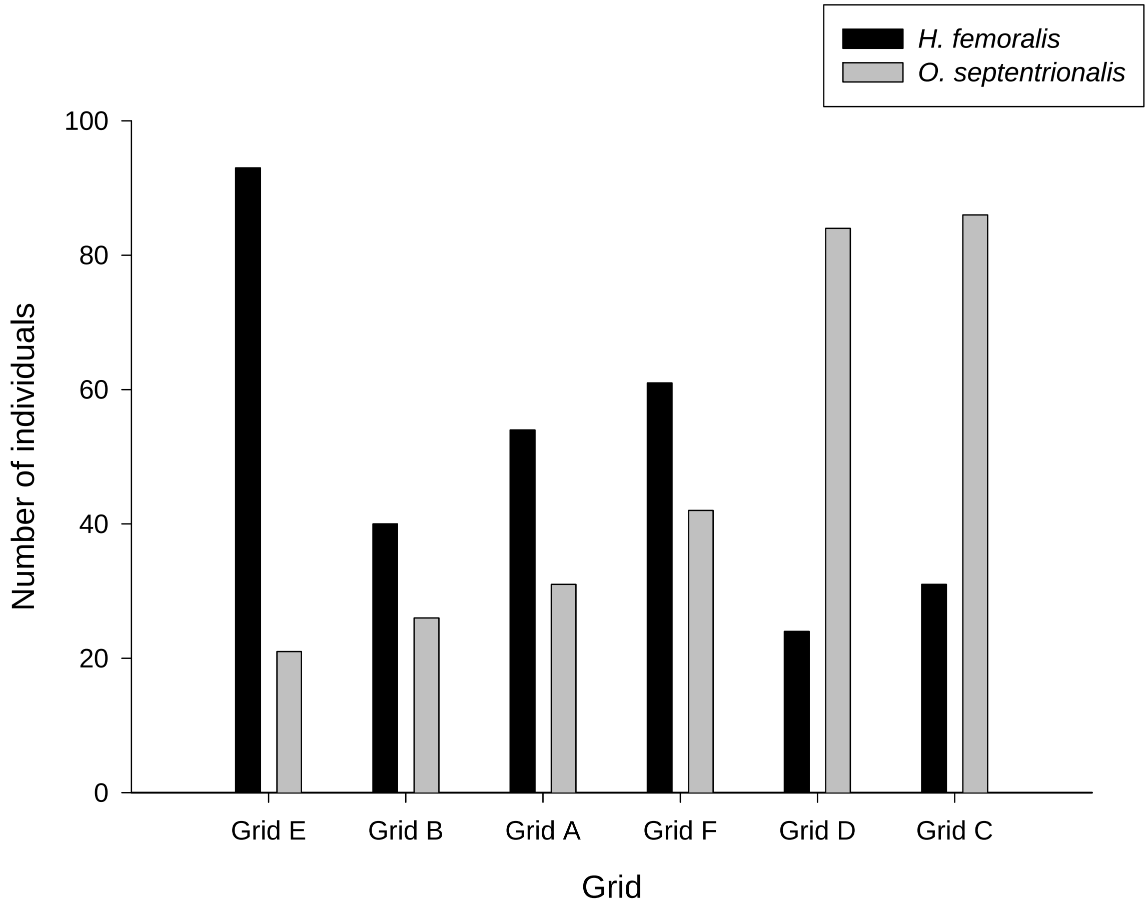

Figure 4-10. Total individual *Hyla femoralis* and *Osteopilus septentrionalis* per grid over the course of the study. Grids are arranged in order of increasing *O. septentrionalis*.

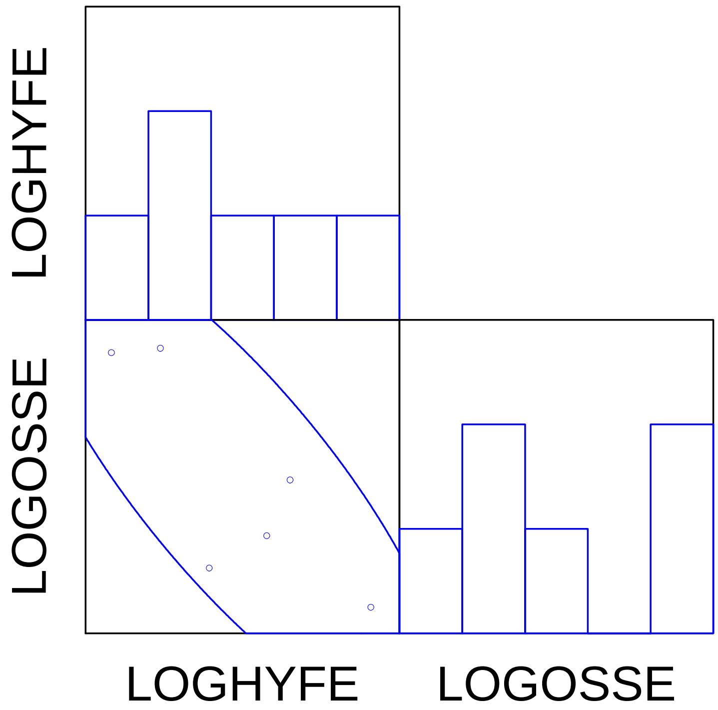

Figure 4-11. Scatterplot matrix of the log of the total number of individual *Hyla femoralis* (LOGHYFE) and *Osteopilus septentrionalis* (LOGOSSE). The Pearson correlation coefficient of these two logs was -0.820.

CHAPTER 5
CONCLUSIONS

Summary

For decades it has been speculated that predation and competition by the nonindigenous Cuban treefrog (*Osteopilus septentrionalis*) have caused declines in native treefrogs in Florida (Allen and Neil 1953, Lee 1969). With the use of PVC pipe refugia, treefrog populations can now be studied outside of the breeding season and these concerns can be addressed. However, the use of PVC pipe refugia is a technique that is still developing, with new biases identified and new designs reported in recent years (Zacharow et al. 2003, Johnson 2005). If we are to rely on this method for the study of treefrogs then we must be aware of associated biases and assumptions. I addressed two factors that contribute to the assumption that treefrogs sampled by PVC pipe refugia accurately represent the population. With these assumptions in mind, I tried to examine the effects of *O. septentrionalis* on native treefrogs.

The results from the first laboratory study (Chapter 2) suggest that treefrog species vary considerably in their frequency of occupying PVC pipe refugia. While the squirrel treefrog (*Hyla squirella*) often chose PVC pipe refugia over a plant for cover, *Osteopilus septentrionalis* did so rarely. The pinewoods treefrog (*H. femoralis*) and the green treefrog (*H. cinerea*) showed no preference for the PVC pipe refugium or the plant. In some field studies, *H. squirella* was the most common frog observed (Boughton et al. 2000, Zacharow et al. 2003, Borg et al. 2004). This could be because either there are more *H. squirella* present in that area or due to its affinity for PVC pipe refugia. The low frequency of pipe use by *O. septentrionalis* is alarming as it is the only frog found in pipes in some areas (pers. obs.). My results suggest that PVC pipe refugia are poor indicators of the abundance of this frog compared to other species, and that there may be many more in an area than observed using this sampling technique. Researchers using PVC refugia should be aware of this sampling bias when interpreting their results, especially when individuals are not marked.

The hypothesis that *Osteopilus septentrionalis* does not interfere with the detection of native treefrogs using refugia was supported by results of my laboratory experiments (Chapter 3). I did not find evidence of behavioral or chemical exclusion of native treefrogs by *O. septentrionalis*. This implies that native treefrogs sympatric with *O. septentrionalis* can be compared to those where *O. septentrionalis* is absent; populations before and after establishment of this nonindigenous species can be compared, as can populations before and after their removal. However, although I determined that *O. septentrionalis* does not behaviorally exclude native treefrogs when they reach the PVC pipe refugia simultaneously, I did not test to see if they displayed territorial behavior as residents when an invader frog attempts to use the refugia. Also, I did not determine if native frogs that use the PVC pipe refugia in the field are more likely to be preyed upon than those occurring away from artificial refugia. It is possible that the population may be underestimated if frogs in PVC pipe refugia are consumed before they can be counted. While native treefrogs do not avoid PVC pipe refugia that had recently been used by *O. septentrionalis*, frogs of this nonindigenous species did avoid refugia that had been used by their conspecifics. These frogs may be avoiding their batracophagic conspecifics, whereas native treefrogs may be unaware of this potential predator, and may be more susceptible to predation. These are two more questions that need to be addressed to be sure that *O. septentrionalis* does not interfere with the detection of native frogs.

Capture rates were low during the field study and an insufficient number of *Osteopilus septentrionalis* was caught to examine the effects of its removal on native treefrogs. However, the results of this study did reveal a negative correlation between the frequency of observations of the nonindigenous frog and the native *Hyla femoralis*. This finding relates back to the original question of the severity of predation and competition by *O. septentrionalis* on the natives, and suggests that this nonindigenous frog might cause declines in native treefrog populations. Further research is needed to examine other possible causes of this correlation and to determine the effects of removing this frog, and to determine if removal using PVC pipe is a suitable management strategy for *O. septentrionalis*.

The detection of native frogs in PVC pipe refugia does not appear to be affected by the presence of *Osteopilus septentrionalis*, but populations of native treefrogs do appear to be negatively affected. These findings support the anecdotes that *O. septentrionalis* might be replacing native treefrogs in central Florida.

Recommendations

As *Osteopilus septentrionalis* seems to negatively affect populations of native treefrogs, I recommend that these nonindigenous frogs be removed when observed. This species appears abundant in areas where they are established, and management of this species may require great effort.

As I had low capture rates of this frog in flatwoods habitat, I recommend that PVC pipe refugia be placed where these frogs are more likely to occur. These areas should include near wetland and around buildings, but further research should be conducted to determine where PVC pipe refugia would be most effective in attracting this nonindigenous frog.

Other methods, such as road cruising during rainstorms or hand capture at breeding sites, should supplement removals using PVC pipe refugia. As I found *Osteopilus septentrionalis* to use PVC pipe refugia less frequently than native treefrogs, these additional methods may be necessary to remove individuals that do not use PVC pipe refugia.

Left unmanaged, *Osteopilus septentrionalis* will continue to spread. As it invades new areas it may cause native treefrogs to decline throughout Florida and other areas in the southeastern United States.

APPENDIX
TOTAL CAPTURES AT WEKIWA SPRINGS STATE PARK

Table A-1. Total individual frogs captured in Wekiwa Grid A during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	5	0	0	18
11-Aug	8	1	0	9
4-Sep	11	0	0	5
25-Sep	7	2	0	6
14-Oct	7	0	0	2
6-Nov	2	0	0	1
20-Nov	6	1	0	1
6-Jan	4	5	0	1
28-Jan	15	4	0	5
18-Feb	2	4	0	2

Table A-2. Total individual frogs captured in Wekiwa Grid B during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	4	0	1	16
11-Aug	7	0	0	4
4-Sep	6	0	0	10
25-Sep	5	0	0	12
14-Oct	3	1	0	7
6-Nov	2	0	0	6
20-Nov	4	0	0	7
6-Jan	3	5	0	15
28-Jan	10	0	0	3
18-Feb	6	0	0	1

Table A-3. Total individual frogs captured in Wekiwa Grid C during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	0	11	0	0

Table A-4. Total individual frogs captured in Wekiwa Grid D during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	0	9	0	0

Table A-5. Total individual frogs captured in Wekiwa Grid E during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	0	5	0	0

Table A-6. Total individual frogs captured in Wekiwa Grid F during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
21-Jul	0	8	0	0

Table A-7. Total individual frogs captured in Wekiwa Grid G during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
4-Sep	1	1	0	8
25-Sep	1	1	0	23
14-Oct	2	2	0	18
6-Nov	1	1	0	17
20-Nov	0	2	0	2

Table A-8. Total individual frogs captured in Wekiwa Grid H during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
4-Sep	0	1	0	1
25-Sep	0	3	0	20
14-Oct	1	1	0	22
6-Nov	0	3	0	4
20-Nov	0	0	0	8

Table A-9. Total individual frogs captured in Wekiwa Grid I during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
4-Sep	0	1	0	2
25-Sep	1	0	0	14
14-Oct	0	0	0	7
6-Nov	0	1	0	6
20-Nov	0	0	0	1

Table A-10. Total individual frogs captured in Wekiwa Grid J during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
4-Sep	1	1	0	3
25-Sep	1	3	0	5
14-Oct	1	2	0	8
6-Nov	0	4	0	2
20-Nov	0	4	0	4

Table A-11. Total individual frogs captured in Wekiwa Grid K during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
28-Jan	0	1	0	1
18-Feb	0	0	0	0

Table A-12. Total individual frogs captured in Wekiwa Grid L during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
28-Jan	0	0	0	2
18-Feb	0	0	0	1

Table A-13. Total individual frogs captured in Wekiwa Grid M during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
28-Jan	0	1	0	0
18-Feb	0	0	0	2

Table A-14. Total individual frogs captured in Wekiwa Grid N during each sampling session.

Date	*H. cinerea*	*H. femoralis*	*H. squirella*	*O. septentrionalis*
28-Jan	0	0	0	1
18-Feb	0	1	0	1

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BIOGRAPHICAL SKETCH

Kristine E. Hoffmann grew up in Sterling, Massachusetts, where she lived with her parents, two brothers, and her twin sister, Carolyn. She graduated with honors from the University of Massachusetts in 2005 with a B.S. in biology and a minor in wildlife and fisheries conservation, and entered the University of Florida that fall. She hopes to teach biology and conservation at a junior college, continue field and lab work on amphibians and reptiles, and to help support her sister, who recently entered graduate school for costume design at New York University.