Recovery of Native Treefrogs After Removal of Nonindigenous Cuban Treefrogs, *Osteopilus septentrionalis*

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RECOVERY OF NATIVE TREEFROGS AFTER REMOVAL OF NONINDIGENOUS CUBAN TREEFROGS, *OSTEOPILUS SEPTENTRIONALIS*

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ABSTRACT: Florida is home to several introduced animal species, especially in the southern portion of the state. Most introduced species are restricted to the urban and suburban areas along the coasts, but some species, like the Cuban Treefrog (*Osteopilus septentrionalis*), are locally abundant in natural protected areas. Although Cuban Treefrogs are known predators of native treefrog species as both adults and larvae, no study has demonstrated a negative effect of Cuban Treefrogs on native treefrog survival, abundance, or occupancy rate. We monitored survival, capture probability, abundance, and proportion of sites occupied by Cuban Treefrogs and two native species, Green Treefrogs (*Hyla cinerea*) and Squirrel Treefrogs (*Hyla squirella*), at four sites in Everglades National Park in southern Florida with the use of capture–mark–recapture techniques. After at least 5 mo of monitoring all species at each site we began removing every Cuban Treefrog captured. We continued to estimate survival, abundance, and occupancy rates of native treefrogs for 1 yr after the commencement of Cuban Treefrog removal. Mark–recapture models that included the effect of Cuban Treefrog removal on native treefrog survival did not have considerable Akaike's Information Criterion (AIC) weight, although capture rates of native species were generally very low prior to Cuban Treefrog removal. Estimated abundance of native treefrogs did increase after commencement of Cuban Treefrog removal, but also varied with the season of the year. The best models of native treefrog occupancy included a Cuban Treefrog removal effect at sites with high initial densities of Cuban Treefrogs. This study demonstrates that an introduced predator can have population-level effects on similar native species.

Key words: Abundance; Capture–mark–recapture; Florida; Green Treefrog; *Hyla cinerea*; *Hyla squirella*; Introduced species; Occupancy; Squirrel Treefrog; Survival

FLORIDA, USA has the highest abundance of introduced species of animals in the contiguous 48 United States, and is second only to Hawaii, USA (Simberloff, 1997). Florida, especially the subtropical southern region, is prone to invasions by nonindigenous animals because of its favorable climate, relatively young geologic age, high rates of human disturbance, and insular geographic nature (Simberloff, 1997). Some ecological roles are unfilled by native species, and many invaders from the world's diverse tropical faunas can thrive in south Florida (Butterfield et al., 1997). The suitable climate and empty niches, coupled with the fact that Miami is an international hub for shipping and air cargo, has led to south Florida having the richest

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introduced herpetofauna in the United States (Wilson and Porras, 1983). In fact, 36 of the 40 nonindigenous reptiles and amphibians in Florida occur in Miami–Dade County (Meshaka et al., 2004).

The greatest concentrations of nonindigenous reptiles and amphibians in Florida are found in disturbed areas, where invasion has been facilitated by human alteration of natural habitats (Meshaka et al., 2004; Wilson and Porras, 1983). However, an increasing number of species are invading natural areas. We are only now beginning to understand the extent to which some species have invaded Everglades National Park (ENP), a large protected natural area encompassing the southern tip of the Florida peninsula. For instance, Cuban Treefrogs (*Osteopilus septentrionalis*) and Brown Anoles (*Anolis sagrei*) are now ubiquitous in the Long Pine Key (LPK) and Flamingo areas of ENP (Meshaka, 2001; Meshaka et al., 2000). Certainly urban and disturbed areas are not the only places where nonindigenous amphibians and reptiles will establish viable populations.

Cuban Treefrogs appear to be a particular threat to native fauna in protected areas throughout south Florida. Cuban Treefrogs occurred at all sites inventoried for amphibians in forested habitats along Main Park Road in ENP (~70 km, sites located up to 500 m from road; Rice et al., 2004). Cuban Treefrogs are larger than the native treefrog species in the Everglades: Green Treefrog (*Hyla cinerea*) and Squirrel Treefrog (*Hyla squirella*). All three treefrog species can occur in similar habitats, but studies have found that these native treefrog species were much less likely to occur at sites occupied by Cuban Treefrogs (Meshaka, 2001; Waddle et al., 2010). The direct cause of this exclusion of native treefrog species by Cuban Treefrogs remains unclear, but is likely a combination of the effects of direct competition for resources and predation by Cuban Treefrogs (Meshaka, 2001). In addition to competition for prey, and predation on adults, Cuban Treefrogs compete for breeding sites with native species, and tadpoles of the Cuban Treefrogs may decrease survival and disrupt development of native tadpoles (Babbitt and Meshaka, 2000; Knight et al., 2009; Smith, 2005). Despite all of this

evidence of a negative effect of Cuban Treefrogs, no study has shown the extent to which populations of native frogs have been reduced in natural areas.

The objectives of this study were to examine effects of Cuban Treefrogs on survival, abundance, and occurrence of native treefrog species in ENP. We used capture–mark–recapture and occupancy modeling to monitor treefrog populations captured in polyvinyl chloride (PVC) pipe refuges in two habitats. We first monitored populations of all treefrog species, and subsequently began removing Cuban Treefrogs while continuing to monitor native treefrog populations. The extent to which native populations recovered upon removal of Cuban Treefrogs was quantified. We hypothesized that survival, abundance, and occupancy rates of native treefrog populations would increase in the period following the removal of Cuban Treefrogs.

MATERIALS AND METHODS
Field Sites and Data Collection

Four study sites were selected in ENP. Two sites (LPK East and LPK West) were located in pine rockland habitat in the Long Pine Key area of ENP. The other two sites, Flamingo and Harney River, were located in mangrove habitat. The minimum distance between study sites was approximately 1.5 km, with most sites being greater than 10 km apart to reduce the chance of interaction between sites. Populations of Cuban Treefrogs at the beginning of the study were very dense at Flamingo relative to the other three sites, and the Flamingo site also represents the most disturbed site in the study because of its proximity to Main Park Road.

We set up white, Schedule 40, 5-cm-diameter, polyvinyl chloride (PVC) pipes at each site as treefrog refuges (Boughton, 2000). The pipes were cut to 1 m in length with a cap on the bottom to retain water. A small hole, 1 cm in diameter, was drilled approximately 10 cm from the bottom of the pipes to allow them to retain a small amount of rainwater. Pipes were hung on nails in trees so the top of the pipe was approximately 2 m from the ground. All pipes at LPK East and LPK West were hung on south Florida Slash

Pine (*Pinus elliottii* var. *densa*), and pipes at Flamingo and Harney were placed on either Red Mangrove (*Rhizophora mangle*) or Black Mangrove (*Avicennia germinans*) trees.

In 2001, the Flamingo site was set up as a test plot to determine the effectiveness of PVC refuges as a sampling method in ENP. Pairs of pipes were used for the first 38 refuges at these sites, one on the north and one on the south side of each tree. These sites were expanded later in 2001 by adding 61 single refuges, for a total of 99 refuges. LPK East and LPK West were set up with single pipes on 99 trees each in April 2002. The exception to this pattern was Harney. Because it was difficult to access this site, we mounted pipes on trees along an existing nonpublic boardwalk in 2001. At Harney, there were a total of 84 refuges hung 2 per tree in 42 trees in a linear fashion along the boardwalk. Each of the four sites was between 0.2 and 0.6 ha in area.

Refuges at all sites were checked biweekly, with a few exceptions associated with inclement weather or equipment failure. Pipes were checked in numerical order consistently throughout the study. We recorded animal presence/absence for all pipes. We also recorded whether or not the pipes contained water. All frogs captured were identified to species and measured snout–vent length (SVL) in millimeters. Newly captured frogs were administered a unique toe clip. In the case of recaptured animals, the clip was read and checked against previous data to ensure accuracy. If a recaptured individual had begun to regenerate toes, this tissue was removed for sound future identification. The frogs were then returned to the refuge from which they were captured. Juvenile or small individuals (<20 mm SVL) were not given a toe clip because of the difficulty of clipping smaller individuals and the probable inaccuracy of reading those clips. These captures consisted primarily of Squirrel Treefrogs at sites LPK East and LPK West, and were discarded from the data analysis. Individuals that escaped before clips could be read were also discarded from the analysis.

This capture–mark–release process continued on all species for 1 yr at the Flamingo and Harney sites and for 4 mo at the LPK East

and LPK West sites. In August 2002, removal sampling of Cuban Treefrogs began at each site. During this period, all Cuban Treefrogs encountered in the pipes were removed from the population and euthanized by administering Benzocaine to the abdomen (Chen and Combs, 1999). Green Treefrogs and Squirrel Treefrogs were captured and released as preremoval. All Cuban Treefrogs collected were fixed in 10% formalin and preserved in 70% ethanol.

Study sites generally were visited once per month during the preremoval period, and once every 2 wk after initiation of Cuban Treefrog removal. All pipe refuges were checked on each visit to a site, except in two instances, when a subset of pipes were checked one day and the remainder checked on the next visit: 4–5 February 2002 at Flamingo and 3 July and 12 July 2002 at LPK East. We excluded those four visits from survival analysis. When estimating site occupancy we combined 4–5 February 2002 at Flamingo, but accounted for missing observations in cell probabilities when modeling data for 3 July and 12 July 2002 at LPK East (MacKenzie et al., 2002).

Predictions

The hypothesis that Cuban Treefrogs adversely affected native treefrogs makes several predictions that can be examined using capture–mark–recapture techniques. Cuban Treefrogs might reduce survival of native frogs either through predation or through competitive exclusion from preferred habitats. If so, then survival of native frogs is predicted to increase after Cuban Treefrogs are removed. If Cuban Treefrogs are excluding native frogs from areas, then we predict capture probability, but not necessarily abundance, of native treefrogs would increase after initiation of Cuban Treefrog removal. If predation or competition with Cuban Treefrogs is occurring, then an increase in abundance of natives is predicted after Cuban Treefrog removal, as is an increase in the site occupancy rate of native treefrogs.

Survival Estimation

We estimated daily apparent survival rates of native treefrogs and Cuban Treefrogs with

the use of the Jolly–Seber model (Jolly, 1965; Seber, 1965) and programs SURVIV (White, 1983) and MARK (White and Burnham, 1999). Daily survival was estimated because season lengths were unequal. For each species–site combination, we created a set of 8–13 competing models. Survival and capture probability were fully time specific (φ_t, p_t) in the most general model. In other models we estimated effects of Cuban Treefrogs on survival of native Treefrogs by constraining survival as constant before versus after initiation of Cuban Treefrog removal (φ_{CTR}). Survival of Cuban Treefrogs was only estimated up to initiation of the removal period.

We also estimated effects of seasonal hydrologic conditions (s) on survival (φ_s). Four annual seasons were used: early wet season (June–July), late wet season (August–October), dry season (November–March), and transitional season (April–May). November is also a transitional month, but sample days during that time of year generally occurred in late October and next in mid-November. We therefore classified November as a dry month. Fit of the most-general model was assessed with the use of $\hat{c}$ (Burnham and Anderson, 2002), which we estimated via simulation in program SURVIV.

The best model of daily survival was identified with the use of Akaike's Information Criterion adjusted for small sample size (AIC_c) or for small sample size and over-dispersion ($QAIC_c$; Burnham and Anderson, 2002). The model with the lowest AIC_c or $QAIC_c$ was considered best. Akaike weights, w_i, were constructed to evaluate the relative support for each model (Burnham and Anderson, 2002). Parameter estimates were model averaged with the use of their Akaike weights (Burnham and Anderson, 2002). Model-averaged daily survival estimates were standardized to 2-wk survival estimates ($\hat{\varphi}_{2wk} = \hat{\varphi}_{daily}^{13}$). The SE of $\hat{\varphi}_{wk}$ was estimated with the use of the delta method (Seber, 1982).

A priori, we did not expect capture probability to be constant ($p.$) annually or within periods. Thus, we included only two models with a constant p in each model set. Model-averaged daily capture probabilities and their model-averaged variance–covariance matrices were imported into program CONTRAST (Sauer and Williams, 1989) to test for differences in capture probability before versus after initiation of Cuban Treefrog removal (p_{CTR}), and for differences in capture probability among seasons (p_s).

Abundance

Abundance of a species on a given sample day ($\hat{N}_{day}$) can be estimated by dividing the total number of individuals captured on that day (n_{day}) by the estimated capture probability for that day ($\hat{p}_{day}$; Lancia et al., 1996; Wood et al., 1998). We used this technique to estimate abundance of each species in each site on all visits for which survival was estimable, except the last visit when survival was estimable only for a subset of models. The SE of $\hat{N}_{day}$ for a given species at a given site was estimated as (Wood et al., 1998):

$$\text{SE}(\hat{N}_{day}) = \frac{n_{day}\left[\text{SE}\left(\hat{p}_{day}\right)\right]}{\left(\hat{p}_{day}\right)^2}.$$

Covariance between model-averaged $\hat{N}_{day\,i}$ and $\hat{N}_{day\,j}$ was estimated with the use of a technique developed by Jolly (1965) and implemented in program JOLLY (Pollock et al., 1990). Model-averaged daily estimates of N and their model-averaged variance–covariance matrices were analyzed with program CONTRAST to test for differences in abundance of native treefrog species before versus after initiation of Cuban Treefrog removal, and for differences in abundance among seasons.

Site Occupancy

Estimates of probability of occurrence (ψ) with individual PVC refuges used as sites were obtained with program SURVIV with the use of single-season models developed by MacKenzie et al. (2002). Models of ψ exist to estimate colonization and extinction rates among seasons (MacKenzie et al., 2003), as well as to estimate potential degree of interspecific interactions (MacKenzie et al., 2004). However, these latter models contain additional parameters. Data sparsity can cause model-convergence failure, particularly as model complexity increases. As such, we opted to create a series of relatively simple

TABLE 1.—Habitat type, total number of visits, dates of visits, and number and arrangement of polyvinyl chloride pipe refuges at each of the sites in this study.

Site	Habitat	Visits	Dates of visits	No. of refuges
Flamingo	Mangrove	42	23 July 2001–13 August 2003	99[a]
Harney	Mangrove	41	8 March 2001–15 August 2003	84[c]
LPK[b] East	Pine rockland	30	11 April 2002–14 August 2003	99[d]
LPK West	Pine rockland	29	11 April 2002–21 August 2003	99[d]

[a] Thirty-eight refuges at this site were paired (2 per tree), the remaining 61 were single.
[b] LPK = Long Pine Key.
[c] All refuges at this site were in pairs.
[d] All refuges at this site were one per tree.

single-species, single-season models for each species–area combination.

We used two seasons per year when estimating ψ: June–October (wet) and November–May (dry). Site occupancy models are closed population models and ψ is assumed constant within a season. For this reason we did not formally estimate ψ of Cuban Treefrogs. Additionally, at all but one site we excluded the second–sixth visits, a 2.5-mo interval, during the Cuban Treefrog removal period to give native treefrogs an opportunity to redistribute and stabilize at a new ψ level.

For each species–area combination we analyzed the entire series of single-species, single-season models in one SURVIV run. Thus, our multiple-season models did not include parameters for colonization or extinction. Although we were primarily interested in whether ψ varied, and in which covariates (Cuban Treefrogs, season, or neither) best accounted for that variation, we were also concerned about the effect of Cuban Treefrogs on detection probability (p). We estimated the effect of Cuban Treefrogs on ψ, or on p, of each native frog species by constraining ψ or p constant before versus after initiation of Cuban Treefrog removal. Those models were compared, with the use of AIC_c or $QAIC_c$, to other models in which ψ or p varied by season or by season and year. However, note that we first analyzed each season separately in program SURVIV. Only those single-season models that converged were allowed into our multiple-season model for a given species–area combination. We estimated model fit ($\hat{c}$; Burnham and Anderson, 2002) of each multiple-season most-general model via simulation in program SURVIV. Only frogs large enough to be toe-clipped ($\geq$20 mm and $\geq$0.5 g) were included in the ψ analysis. Frogs too small to be toe-clipped potentially were new recruits that would have violated the assumption of a closed population within a season.

RESULTS

Cuban Treefrog Removal

Total number of field visits ranged from 29 at LPK East to 42 at Flamingo (Table 1). A large number of Cuban Treefrogs (291) were captured, marked, and released during the preremoval stage at the Flamingo site (Table 2), but fewer than 10 Cuban Treefrogs were captured during the preremoval stage in each of the remaining three sites. During the removal period, the number of Cuban Treefrogs removed ranged from 10 at LPK West to 589 at Flamingo.

TABLE 2.—Number of individual Cuban Treefrogs marked during the marking period and number of individual Cuban Treefrogs removed during the removal period at each site.

Site	Marking period		Removal period	
	Dates of visits	No. marked	Dates of visits	No. removed
Flamingo	23 July 2001–25 July 2002	291	9 August 2002–13 August 2003	589
Harney	8 January 2002–18 July 2002	9	13 August 2002–15 August 2003	74
LPK[a] East	22 April 2002–2 August 2002	8	4 September 2002–21 August 2003	20
LPK West	22 April 2002–2 August 2002	4	4 September 2002–21 August 2003	10

[a] LPK = Long Pine Key.

TABLE 3.—Number of individually marked Cuban Treefrogs (*Osteopilus septentrionalis*) used in survival analyses for each site–species combination.

Site	Species	No. of visits	Dates of visits	No. of individuals
Flamingo	Green	20	30 September 2002–13 August 2003	127
Flamingo	Cuban	15	21 September 2001–9 August 2002	257
Harney	Squirrel	32	8 January 2002–15 August 2003	115
LPK[a] East	Squirrel	27	22 April 2002–21 August 2003	714
LPK West	Squirrel	28	22 April 2002–21 August 2003	167

[a] LPK = Long Pine Key.

Survival Estimation

We did not estimate survival for species–area combinations for which <90 individuals were marked during the entire study. In areas where ≥90 individuals of a species were marked we generally restricted ourselves to periods during which ≥10 animals were captured per visit. Five species–area combinations met that criterion (Table 3). Three of those five combinations enabled comparison of survival rates before and after initiation of Cuban Treefrog removal: Squirrel Treefrogs at Harney, LPK East, and LPK West. Only one Green Treefrog was captured at LPK East and four Green Treefrogs were captured at LPK West. An average of 3.6 Green Treefrogs were marked or recaptured per visit to Harney. Only 15 Green Treefrogs were marked at Flamingo prior to initiation of Cuban Treefrog removal and a total of 20 Squirrel Treefrogs were marked at that site during the study.

The two best models of Squirrel Treefrog survival at LPK East and Harney included a constant survival term. These two models had a combined $QAIC_c$ weight of 0.60 in LPK East and 1.0 in Harney. The best model of Squirrel Treefrog survival in LPK West included an effect of four hydrologic seasons ($QAIC_c$ weight = 0.79). Model-averaged estimates of 2-wk survival were highest in the dry season for Squirrel Treefrogs at both LPK sites (LPK East: $\hat{\phi}_{wet, pre-CTR}$ = 0.902, $\hat{\phi}_{dry}$ = 0.906 and $\hat{\phi}_{wet, post-CTR}$ = 0.900; LPK West: $\hat{\phi}_{wet, pre-CTR}$ = 0.893, $\hat{\phi}_{dry}$ = 0.947 and $\hat{\phi}_{wet, post-CTR}$ = 0.888; Fig. 1). Only one dry season occurred at these sites during this study, and that dry season followed initiation of Cuban Treefrog removal. Model-averaged estimates of Squirrel Treefrog survival during wet seasons differed little before versus after initiation of Cuban Treefrog removal. Model-averaged estimates of 2-wk survival also appeared to be higher during the dry season than the wet season for Green Treefrogs at Flamingo ($\hat{\phi}_{dry}$ = 0.937, SE = 0.017 and $\hat{\phi}_{wet}$ = 0.878, SE = 0.082).

The fourth-best model of daily survival of Squirrel Treefrogs in LPK East was the only survival model with substantial $QAIC_c$ weight to include an effect of Cuban Treefrog removal ($QAIC_c$ weight = 0.12). In that instance survival was higher during the removal period ($\hat{\phi}_{2 wk, pre-CTR}$ = 0.901 and $\hat{\phi}_{2-wk, post-CTR}$ = 0.904). Nevertheless, these results, in total, provide relatively little support for an effect of Cuban Treefrogs on survival probability of native treefrogs.

Capture Probability

Model-averaged capture probability (p_i) for Squirrel Treefrogs at LPK East did not vary over time (p = 0.60 for overall test, $\widehat{\overline{p}}_{LPKE}$ = 0.60, SE = 0.06). Mean model-averaged capture probability for Squirrel Treefrogs at LPK West appeared to be higher during the wet season than during the dry season (constant p_i rejected, p < 0.001; $\widehat{\overline{p}}_{wet}$ = 0.79, SE = 0.05 vs. $\widehat{\overline{p}}_{dry}$ = 0.56, SE = 0.03). We found no significant difference in $\widehat{\overline{p}}$ for Squirrel Treefrogs in LPK West before versus after initiation of Cuban Treefrog removal (p = 0.20; $\widehat{\overline{p}}_{pre-CTR}$ = 0.61, SE = 0.08 vs. $\widehat{\overline{p}}_{post-CTR}$ = 0.58, SE = 0.02). Program CONTRAST provides p values and not AIC values. We suspect results from the above seasonal test are more likely to approximate truth for Squirrel Treefrogs in LPK West based on the larger difference in $\widehat{\overline{p}}$ from the seasonal test, lack of significance of the Cuban Treefrog test, similar SEs in both tests, and the counterintuitive result from the Cuban Treefrog test. The best model of Squirrel
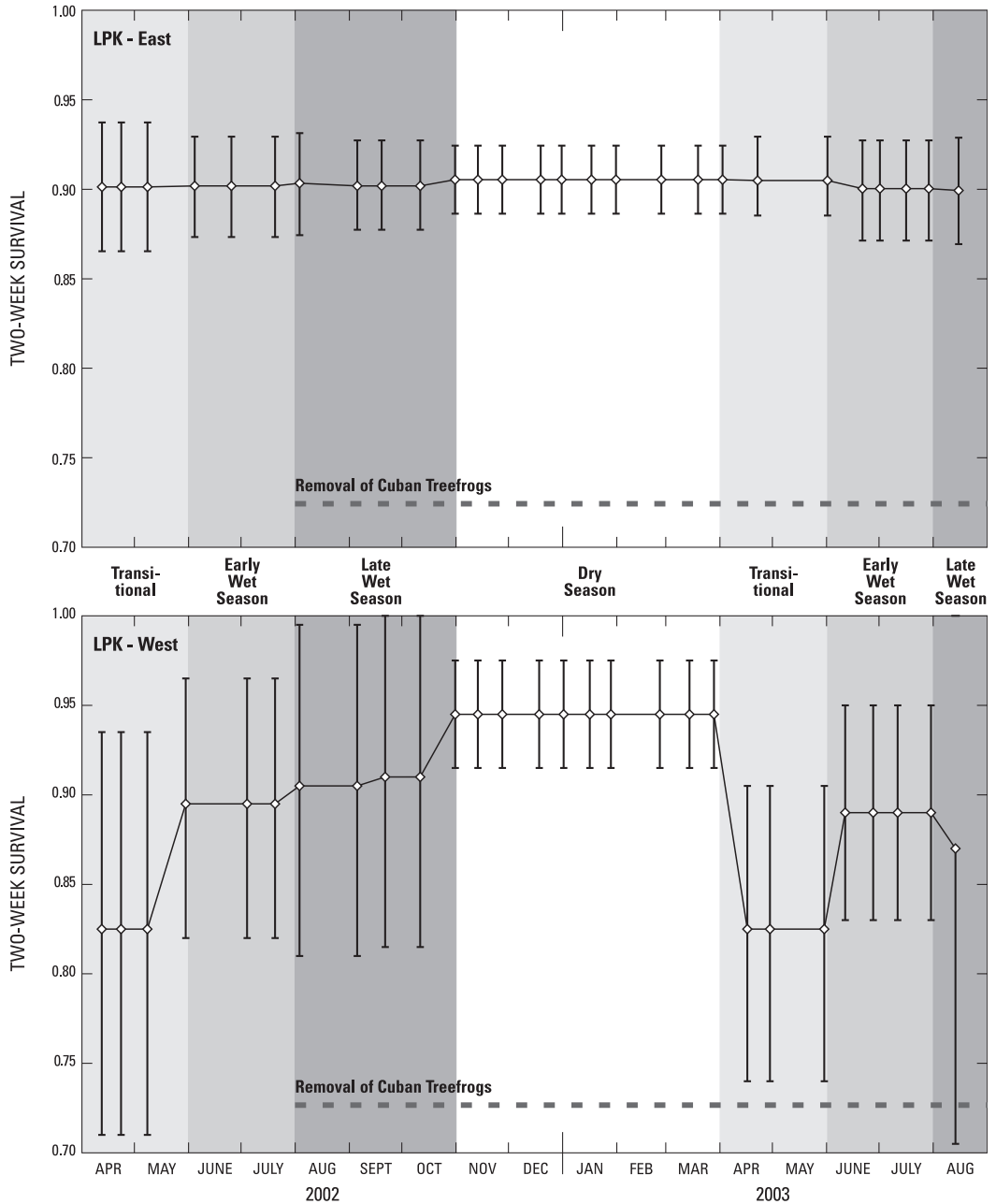
Fig. 1.—Model-averaged estimates with 95% confidence intervals of 2-wk survival of Squirrel Treefrogs (*Hyla squirrella*) at the pine rockland sites Long Pine Key (LPK) East and LPK West. Season was a significant factor at LPK West, but not at LPK East, and the removal of Cuban Treefrogs had little effect on survival at either of these sites.

Treefrog survival at Harney had a constant p_i of 0.31 (SE = 0.04) and a $QAIC_c$ weight of 1.0 in program MARK. Therefore, we did not analyze those capture probabilities in CONTRAST.

Model-averaged capture probability of Green Treefrogs at Flamingo was higher during the dry season ($\widehat{\overline{p}}_{wet}$ = 0.21, SE = 0.12 vs. $\widehat{\overline{p}}_{dry}$ = 0.39, SE = 0.05). Model-

averaged capture probability of Cuban Treefrogs did not vary over time at Flamingo ($\widehat{\overline{p}}_{\text{FLAM}} = 0.51$, SE = 0.07). The results above provide no support for an effect of Cuban Treefrogs on capture probability of native treefrogs.

Abundance

Mean abundance of Squirrel Treefrogs at LPK East appeared to be influenced by hydrology and Cuban Treefrogs, almost doubling in the wet season following initiation of Cuban Treefrog removal compared with the wet season the previous year ($\widehat{\overline{N}}_{\text{wet, pre-CTR}} = 109$, SE = 12; $\widehat{\overline{N}}_{\text{dry, post-CTR}} = 237$, SE = 14; $\widehat{\overline{N}}_{\text{wet, post-CTR}} = 200$, SE = 17). Abundance of Squirrel Treefrogs at LPK East was highest during the dry season.

Abundance of Squirrel Treefrogs at LPK West may have been influenced by hydrology, being higher during the dry season ($\widehat{\overline{N}}_{\text{wet}} = 35$, SE = 3; $\widehat{\overline{N}}_{\text{dry}} = 58$, SE = 5), or by Cuban Treefrogs ($\widehat{\overline{N}}_{\text{wet, pre-CTR}} = 12$, SE = 3; $\widehat{\overline{N}}_{\text{post-CTR}} = 54$, SE = 7). A test including both effects was also significant, but estimated abundances were similar in the wet and dry season following initiation of Cuban Treefrog removal ($\widehat{\overline{N}}_{\text{wet, pre-CTR}} = 10$, SE = 2; $\widehat{\overline{N}}_{\text{dry, post-CTR}} = 58$, SE = 5; $\widehat{\overline{N}}_{\text{wet, post-CTR}} = 53$, SE = 5).

The overall test of no variation in abundance of Squirrel Treefrogs at Harney was not rejected ($p = 0.24$). Nevertheless, all tests for effects of hydrology, Cuban Treefrogs, and their interaction on abundance of Squirrel Treefrogs at Harney were significant ($p < 0.01$). Hydrology appeared to have the strongest effect on abundance of Squirrel Treefrogs at Harney. Estimated abundance was higher during the dry season ($\widehat{\overline{N}}_{\text{dry}} = 41$, SE = 3; $\widehat{\overline{N}}_{\text{wet}} = 9$, SE = 2). However, estimated abundance of Squirrel Treefrogs was also higher during the Cuban Treefrog removal period ($\widehat{\overline{N}}_{\text{pre-CTR}} = 19$, SE = 7; $\widehat{\overline{N}}_{\text{post-CTR}} = 28$, SE = 6). Squirrel Treefrog abundance in the dry season was also estimated to be higher following initiation of Cuban Treefrog remov-

al ($\widehat{\overline{N}}_{\text{dry, pre-CTR}} = 31$, SE = 4; $\widehat{\overline{N}}_{\text{dry, post-CTR}} = 44$, SE = 4). Initially we did not estimate daily survival of Green Treefrogs at Flamingo prior to Cuban Treefrog removal because only 15 Green Treefrogs were marked during that period. However, the number of captures of Green Treefrogs increased substantially after initiation of Cuban Treefrog removal. We estimated daily survival of Green Treefrogs at Flamingo a posteriori during the preremoval period to obtain $\hat{p}$ for abundance estimation ($\hat{p} = 0.42$, SE = 0.13). Estimated abundance of Green Treefrogs increased from $\widehat{\overline{N}} = 7$ in the preremoval stage to $\widehat{\overline{N}} = 83$ in the dry season following onset of Cuban Treefrog removal and $\widehat{\overline{N}} = 24$ in the subsequent wet season. The estimate of seven Green Treefrogs in the preremoval stage was only for the 9 d on which ≥1 Green Treefrog was captured. No Green Treefrogs were captured on seven other field days during the preremoval period; however, Green Treefrogs were captured on all but the first visit after initiation of Cuban Treefrog removal (Fig. 2).

Site Occupancy

The best single-species, multiple-season models of ψ included an effect of Cuban Treefrogs on Squirrel Treefrogs at LPK East ($\hat{\psi}_{\text{pre-CTR}} = 0.85$, SE = 0.05; $\hat{\psi}_{\text{post-CTR}} = 1.00$, SE = 0.002; $w_i = 0.82$) and on Green Treefrogs at Flamingo ($\hat{\psi}_{\text{pre-CTR}} = 0.43$, SE = 0.14; $\hat{\psi}_{\text{post-CTR}} = 0.99$, SE = 0.01; $w_i = 0.48$). In both cases $\hat{\psi}$ was higher during the removal period. The best model of ψ for Squirrel Treefrogs at LPK West included a time effect only: ψ differed for each season and each year. However, model-averaged estimates of ψ suggested an increase for Squirrel Treefrogs at both LPK sites (Table 4), as well as for Green Treefrogs at Flamingo, following initiation of Cuban Treefrog removal. Naïve estimates of ψ (unadjusted for detection probability) also suggested possible increases in proportion of sites occupied for Squirrel Treefrogs at Flamingo during the Cuban Treefrog removal period, but the lowest-AIC$_c$ model for Flamingo did not include an effect of Cuban Treefrogs on probability of occurrence of Squirrel Treefrogs.
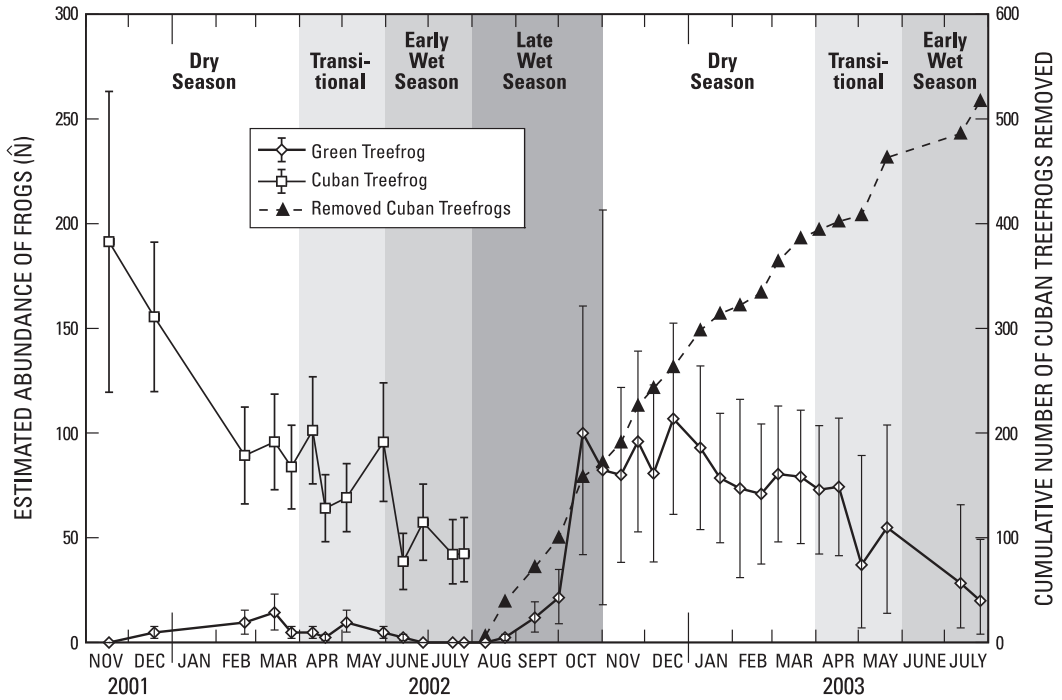
F.IG. 2.—Estimated abundance with 95% confidence intervals of Green Treefrogs (*Hyla cinerea*) and Cuban Treefrogs (*Osteopilus septentrionalis*) at Flamingo. Abundance of Cuban Treefrogs was no longer estimated after the initiation of Cuban Treefrog removal, but the cumulative number of Cuban Treefrogs removed is shown on the right *y*-axis.

DISCUSSION

Overall, our results indicate that Cuban Treefrogs have a negative effect on native treefrog populations in natural areas of southern Florida as evidenced by the apparent recovery of native treefrog populations upon initiation of Cuban Treefrog removal from our study sites. We predicted we would find an increase in survival, abundance, and occupan-

cy of native treefrog species upon removal of Cuban Treefrogs because of release from predation or competition. We also predicted that if Cuban Treefrogs were excluding native treefrogs from preferred refuges we would detect an increase in detection probability of native treefrogs upon removal of Cuban Treefrogs. Although we found evidence of an increase in abundance and occurrence of

TABLE 4.—Model-averaged estimates of site occupancy probability with (SE) by site, species, and season, estimated with the use of single-species, multiple-season models without extinction or colonization parameters. Dashes indicate models that were not run because species–site combinations did not allow estimation of a possible Cuban Treefrog effect, and NA represents models that failed to converge because of low sample size.

Site	Species	Before Cuban Treefrog removal				Removal period	
		Dry 2001	Wet 2001	Dry 2001–2002	Wet 2002	Dry 2002–2003	Wet 2003
Flamingo	Squirrel	–	NA	0.20 (0.08)	NA	0.23 (0.05)	NA
	Green	–	NA	0.57 (0.11)	NA	0.99 (0.01)	0.83 (0.11)
Harney	Squirrel	NA	0.17 (0.06)	0.78 (0.07)	0.23 (0.07)	0.78 (0.07)	0.20 (0.06)
	Green	0.72 (0.09)	NA	0.75 (0.08)	NA	0.75 (0.09)	NA
LPK[a] East	Squirrel	–	–	0.84 (0.06)	0.86 (0.05)	1.00 (<0.01)	1.00 (<0.01)
	Green	–	–	NA	NA	NA	NA
LPK West	Squirrel	–	–	0.58 (0.10)	0.63 (0.11)	0.95 (0.03)	0.79 (0.05)
	Green	–	–	NA	NA	NA	NA

[a] LPK = Long Pine Key.

native species upon Cuban Treefrog removal, we found only limited evidence of an increase in survival of native species. Our ability to estimate survival prior to Cuban Treefrog removal was hindered by the low sample size of native species at some of the sites. Increases in abundance and occurrence of native frogs without a concomitant increase in survival after initiation of Cuban Treefrog removal could be the result of increased recruitment or immigration of native frogs. It is also possible that survival of native frogs increased, but our models lacked sufficient power to detect this increase. We found no evidence that the detection rate of native treefrog species changed after commencement of Cuban Treefrog removal, indicating no support for the hypothesis that Cuban Treefrogs were excluding native treefrogs behaviorally. Based on our a priori predictions, we feel these results provide evidence to support the hypothesis that Cuban Treefrogs are negatively impacting native treefrog populations.

A weakness of our study design is the lack of simultaneously sampled reference or control sites for comparison to our Cuban Treefrog removal sites. Capture–recapture modeling requires large sample sizes of captured individuals to estimate population parameters efficiently (Williams et al., 2002). With a limited amount of time and money, we were unable to add additional sites to monitor as control sites. Admittedly, our design complicates interpretation of the effect of Cuban Treefrog removal on native treefrog population parameters, but it does not make our results inconsequential. The fact that treefrog populations tend to fluctuate seasonally in an annual cycle (Ackleh et al., 2010; Waddle, 2006) potentially confounds the effects of Cuban Treefrog removal. Rather than ignore that fact, we modeled the effect of hydrologic season in our capture–recapture and occupancy analyses and used information-theoretic model selection techniques to determine the relative weight of evidence for models including season or Cuban Treefrog removal. Our model-selection methodology does not help with distinguishing between a treatment effect and long-term population fluctuations that amphibian populations are known to undergo (Green, 2003; Pechmann and Wilbur, 1994),

but this could not be accomplished with a separate control site either.

The most likely means by which Cuban Treefrogs could reduce abundance and occurrence of native treefrog species is through predation and competition for limited resources. Although our study was not designed to determine whether predation was the cause of the observed effect, we were able to gather some evidence about the possible role of predation in the decline of native treefrog populations. Each of the Cuban Treefrogs removed during the study was sacrificed and dissected to search stomach contents for vertebrate prey. Native treefrog species were found in the stomachs of Cuban Treefrogs removed from each of the study sites, but only 3.52% of the Cuban Treefrogs examined had frogs (including the Eastern Narrow-Mouthed Toad, *Gastrophryne carolinensis*) in their stomachs (Glorioso et al., in press). Predation of egg and larval-stage native treefrog species by Cuban Treefrog larvae can occur (Babbitt and Meshaka, 2000) and may be a factor in our study, but we found no larvae at our sites during our study, so we have no way to evaluate this possibility. If predation explained these results then we would have expected to see increases in survival of native treefrogs after Cuban Treefrog removal. However, we were unable to capture enough individuals of the native species to estimate survival prior to removal of Cuban Treefrogs at Flamingo, the site with the highest abundance of Cuban Treefrogs and the largest increase in abundance and occurrence of a native species after commencement of Cuban Treefrog removal. We have no means to evaluate whether resource competition is a factor in this system, but we know that there is a degree of overlap in the prey of the treefrog species (Meshaka, 2001) and that there may be competition taking place among larvae (Smith, 2005).

Parameter estimates of population size, survival, and site occupancy for each of the three species were highest at the start of the dry season and decline gradually through the next wet season. Breeding for these species takes place early in the wet season and new recruits enter the adult population at the beginning of the dry season (Waddle, 2006). Population fluctuations during the yearly cycle

could explain some seasonal differences (Ackleh et al., 2010), but an alternative explanation concerns our trapping method. Treefrogs might prefer PVC pipe refuges during the dry season, where cover in proximity to water is limiting in these terrestrial habitats. Other studies have shown a strong seasonal pattern in the use of PVC refuges by treefrogs in southern Florida with a peak during the cooler dry season months (Donnelly et al., 2001; Zacharow et al., 2003). If some proportion of animals left our refuges during the wet season, then bias in our estimates could have been introduced and led to the decreased parameter estimates observed. Because our study did not capture animals outside the PVC refuges, we have no information to address these hypotheses concerning seasonal differences. However, because of the comparative effects of the Cuban Treefrogs on native treefrogs and given that the parameters changed for all species, we feel confident that this possible bias does not influence our conclusions about the effect of Cuban Treefrog removal on the recovery of the native treefrog species.

One possible criticism of our methodology is that our PVC refuges would not sample these treefrog species equally if the smaller native species avoid the refuges or the study area because of the presence of Cuban Treefrogs. Although no quantitative measure of trap avoidance can be derived from this study, lines of evidence suggest that the native treefrog species do not recognize Cuban Treefrogs as a predator and alter their behavior accordingly. Meshaka (2001) found both Green and Squirrel Treefrogs foraging alongside Cuban Treefrogs on buildings and sharing diurnal retreats, and surmised that these interactions suggest a lack of predator-avoidance behavior. In a laboratory experiment, Hoffman (2007) found that the native treefrog species did not avoid refuges that had been recently used by Cuban Treefrogs. In this study we found no support for a change in detection probability of the native species after the commencement of Cuban Treefrog removal from the sites. In addition, the three species were frequently found sharing PVC refuges in our study. Examining only data from traps with multiple captures, Green Treefrogs were found twice as often with

Cuban Treefrogs than with conspecifics, and there was no difference in the number of times Squirrel Treefrogs were found with either of the other two species (unpublished data). Although this is not conclusive proof that no avoidance behavior was taking place, it strongly suggests that that is the case. Collectively there is no evidence that the increases in abundance and occurrence probability of the native species we observed are a result of a behavioral change of the frogs rather than a population response.

We found evidence during this study that Squirrel Treefrogs are more vulnerable to negative effects of Cuban Treefrog introductions than Green Treefrogs. Negative effects of Cuban Treefrogs on the Green Treefrogs were only detected when the invasion had resulted in a complete change in dominance of the community, as occurred at Flamingo. However, we detected effects on Squirrel Treefrogs (the smaller species) at sites with abundance rates of Cuban Treefrogs around two orders of magnitude lower. Abundance of Squirrel Treefrogs increased from 109 to 200 at LPK East and 10 to 53 at LPK West with the removal of only 20 and 10 Cuban Treefrogs, respectively. Further, site occupancy rate estimates of Squirrel Treefrogs increased from 84% to 100% at LPK East and 58% to 95% in LPK West after Cuban Treefrog removal while accounting for the effects of changes due to wet–dry season. This result corroborates the findings of Waddle et al. (2010) that Squirrel Treefrogs are less likely than Green Treefrogs to co-occur at a site with Cuban Treefrogs. It is unknown if this pattern is because their smaller size makes Squirrel Treefrogs more vulnerable to predation by Cuban Treefrogs or if some other factor is involved.

The disturbed habitat at Flamingo produced densities of Cuban Treefrogs that far exceeded any observed densities of native Treefrogs. After removal of over 500 individuals, we found site occupancy rates of Cuban Treefrogs at over 80%, indicating minimal effects of our reduction. Any foreseeable management action involving removal of the species where it occurs at high density is presumably too costly at this point, but our removals did have an effect on reduction of

Cuban Treefrogs at sites where they occurred at low densities. A management plan using a combination of actions including removal of Cuban Treefrogs from low-density natural areas and a plan to avoid introductions in remote areas could be effective in reducing the threat to native species.

Few studies have shown effects of introductions of nonindigenous animals on native-species population parameters, including survival, population size, and occupancy. Most studies have concentrated on changes in distribution of native animals and/or effects at the individual level (Pearl et al., 2004; Walston and Mullin, 2007). In our study, we have shown that the introduction of a nonindigenous species can directly affect the abundance and occurrence of two native species. Further, we have shown complete change in dominance among potentially competing native and nonnative treefrogs. We have also shown the resilience of native populations in their ability to recover once the predator is removed. Given that some areas cannot be restored by removal of invasive species, careful study of the cascading effects of the change in community dominance are crucial. The effects on predator and prey populations could be significant in areas with high densities of an introduced species.

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