diversity

MDPI

Article

Lack of Behavioral and Chemical Interference Competition for Refuges among Native Treefrogs and Invasive Cuban Treefrogs (*Osteopilus septentrionalis*)

Kristine E. Hoffmann [1], Monica E. McGarrity [2] and Steve A. Johnson [1,*]

1 Department of Wildlife Ecology and Conservation, University of Florida, P.O. Box 110430, Gainesville, FL 32611, USA; kristine.hoffmann@maine.edu

2 Texas Parks and Wildlife Department, 4200 Smith School Rd., Austin, TX 78744, USA; Monica.McGarrity@tpwd.texas.gov

* Correspondence: tadpole@ufl.edu; Tel.: +1-352-846-0557

Received: 30 March 2018; Accepted: 26 July 2018; Published: 1 August 2018

Abstract: The introduction of a novel competitor can dramatically alter community dynamics, and competition-mediated impacts often result from biological invasions. Interference competition can be especially problematic as a source of methodological bias for studies seeking to evaluate population and community-level impacts of invasive species. We used polyvinyl chloride (PVC) refugia to conduct laboratory trials to determine whether behavioral or chemical cues of invasive Cuban treefrogs (*Osteopilus septentrionalis*) interfere with artificial refuge use by conspecifics or treefrogs native to Florida (USA). We found no evidence of behavioral or chemical competition for refuges by Cuban treefrogs or native treefrogs. The inability of native treefrogs to avoid chemical cues from Cuban treefrogs, despite living sympatrically with the invasive treefrogs for 10–20 years, has important implications for predation risk.

Keywords: invasive species; anuran; *Hyla*; hylid; interference competition; sampling bias; PVC pipe

1. Introduction

Competition within and among species can limit their distribution and abundance [1,2]. In natural systems, competition may lead to resource partitioning and character displacement [2,3]. Similarly, introduction of a novel species can greatly alter population and community dynamics through competition and, in some cases, cause trophic shifts, changes in population size-structure, and declines or local extinctions of native species [4–6]. Competitor introductions may also result in niche shifts within the native community [7,8]; however, such shifts may merely delay, rather than prevent, the decline of the native species [9].

Studies suggest that Cuban treefrogs (*Osteopilus septentrionalis*) are causing declines of native treefrog populations in natural and urbanized areas of their introduced range in Florida, USA [10–12]. Predation is usually implicated as the primary cause of declines, but competition among Cuban treefrogs and native species for food and space may also play a role. For example, in laboratory experiments, Cuban treefrog tadpoles were superior competitors than tadpoles of several native species of frogs [13,14].

In contrast to interference competition exhibited in larval anuran communities, exploitative competition for prey in post-metamorphic life-stages is less likely to negatively impact native treefrogs because there is minimal dietary overlap between adult Cuban and native treefrogs and arthropod prey are often abundant [10]. Territorial aggression is well known in anurans [15,16], and several species behaviorally exclude other frogs from refuges [17–19]. Chemical cues play important roles in amphibian ecology [20–24], and chemical-mediated interference may also affect refuge use but has yet to be studied in Cuban treefrogs. However, this species has the ability to respond to cues from man-made chemicals [25]. When threatened, Cuban treefrogs produce a milky, odorous skin secretion that is extremely irritating to mammalian mucus membranes ([10], pers. obs.), but the chemical composition of the secretion and its effects on amphibians are unknown. Thus, potential exists for chemically-mediated competitive interactions between Cuban treefrogs and native treefrogs.

In addition to the direct ecological ramifications of Cuban treefrog establishment outside its native range as described above, behavioral and/or chemical interference for refuge use with native species are also of concern. This is especially true for studies that use PVC refuges to sample treefrogs because of the potential for sampling bias that could result from chemical or behavioral competition among frog species [26,27]. PVC pipe refuges are a popular, and widely used method to study treefrogs, with the benefit that they function as passive "traps" (frogs enter and leave at will), attracting treefrogs that can be captured for individual identification and marking or euthanasia for management purposes (i.e., invasive Cuban treefrogs). As this technique becomes more widespread it is increasingly important to test the basic assumptions of this method. Although interference competition is a potential source of bias with this sampling method, the assumption that Cuban treefrogs do not behaviorally interfere with refuge occupancy by native species (or conspecifics) has not yet been evaluated. Furthermore, residual chemical secretions from Cuban treefrogs could potentially be detected (i.e., in artificial refuges) and avoided by other treefrogs, thus resulting in sampling bias.

Cuban treefrogs are native to Cuba, the Cayman Islands, and the Bahamas, but have been introduced and become established in many areas of the sub-tropics and tropics, including Florida and elsewhere [10,28]. Since its introduction into the Florida Keys in the early 1900s [29], this species has spread throughout peninsular Florida [30–32] and is continuing to expand its range to include coastal regions of the Southeast [33,34]. The Cuban treefrog is considered highly invasive and has the potential to alter native faunal communities. Cuban treefrogs prey on a diversity of native amphibians and reptiles [10,35–39]. In turn, they are preyed upon by a variety of native predators [40–43], and therefore have the potential to influence community structure through trophic subsidy [44].

We conducted laboratory preference trials using Cuban and two species of native treefrogs to test our hypothesis that behavioral exclusion and chemical residues in artificial PVC refuges previously used by large Cuban treefrogs would affect refuge use by conspecifics and native treefrogs. The results of our findings should help guide and inform interpretation of studies of treefrogs in which PVC pipe refuges are used as a sampling method.

2. Materials and Methods

2.1. Treefrog Collection and Maintenance

We opportunistically collected treefrogs by hand and in PVC pipe refugia, primarily during breeding events and rainstorms, from sites in Alachua, Hernando, Hillsborough, Manatee, Orange, Pinellas, Polk, Sarasota, and Seminole Counties in Florida, USA, from May through August 2007. Frogs were collected under permit EXC 06-07a, issued by the Florida Fish and Wildlife Conservation Commission. Cuban treefrogs had occurred sympatrically with native treefrogs at these locations for at least 5–15 years, depending on the site. We maintained frogs and conducted trials in a plant growth room at the University of Florida's Gulf Coast Research and Education Center (Wimauma, FL, USA). We set temperature and photoperiod to approximate environmental conditions in central Florida during the study period (temperature = 24 °C, photoperiod = 06:30–20:30). We housed frogs individually in 0.75 L Sterilite containers with a moist paper towel substrate, misted containers daily, and fed frogs crickets *ad libitum* prior to trials. Afterwards, we marked native treefrogs with Visible Implant Elastomer (VIE) to prevent resampling the same individuals, and then released them at their initial capture site; we euthanized all Cuban treefrogs in accordance with Florida law prohibiting the release of nonindigenous species. We euthanized Cuban treefrogs by liberal application of a 20%

benzocaine ointment to their venter and then placed them in a freezer for 24 h. These methods were approved by the University of Florida's IACUC under animal ethics protocol E870.

2.2. Behavioral Interference Trials

We conducted laboratory refuge choice trials to evaluate potential behavioral interference among Cuban treefrogs, green treefrogs (*Hyla cinerea*), and squirrel treefrogs (*H. squirella*). These three species occur together throughout the invaded range of the Cuban treefrog in the Southeast—they all inhabit natural areas as well as suburban neighborhoods, and readily use PVC pipe refugia. Moreover, Cuban treefrogs are well-documented as predators of native green and squirrel treefrogs [11,32,36,39]. At 20:00 we placed paired frogs in a 37.85 L aquarium (L × D × H = 50.8 × 27.9 × 33.0 cm) with a screen lid, a moist sand substrate, and one vertical PVC refuge (20 cm tall, 3.81 cm diameter) located in the center of the enclosure; at 10:00 the following morning we recorded the location of each frog. We conducted 30 trials each of a Cuban treefrog/green treefrog pair, Cuban treefrog/squirrel treefrog pair (Figure 1., panel A.1), and conspecific control pairs (Figure 1., panel A.2). For each pairing, we ensured that the snout-to-vent length (SVL) of the smaller frog was at least 70% of the SVL of the larger frog to prevent predation. Size (SVL) ranges of treefrogs used in behavioral interference trials were: Cuban treefrog—20–66 mm, green treefrog—21–55 mm, and squirrel treefrog—21–40 mm.

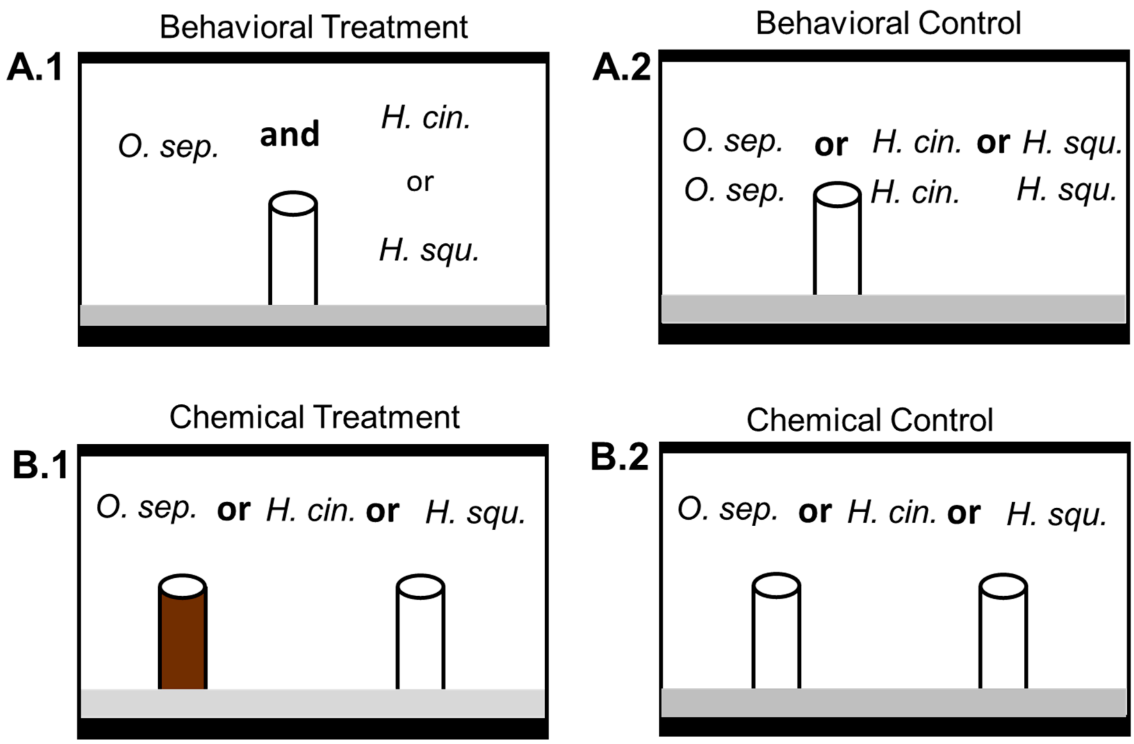

Figure 1. Experimental design for behavioral (panels **A.1** & **A.2**) and chemical interference (panels **B.1** & **B.2**) testing of treefrogs. *O. sep.* = invasive Cuban treefrog, *H. cin.* = native green treefrog, *H. squ.* = native squirrel treefrog. The dark PVC refuge in **B.1** depicts a "residue" pipe. See text for further explanation.

We developed a custom function in Program R (v2.5.1) [45] to compare the probability of refuge use by native frogs in the presence of Cuban treefrogs (and vice versa) with the probability of refuge use with a conspecific present. There were four possible trial outcomes—(AB) both frogs in the pipe, (A0) only frog A in the pipe, (0B) only frog B in the pipe, or (00) neither frog in the pipe; conspecifics were randomly designated 'A' or 'B'. We used Program R to calculate the maximum likelihood estimates of the probability that frog A (P_A) or frog B (P_B) was in the pipe, and then calculated the probabilities of the four possible trial outcomes using a multinomial model, as follows:

$$P_{AB} = P_A \times P_B \quad (1)$$

$$P_{A0} = P_A \times (1 - P_B) \quad (2)$$

$$P_{0B} = (1 - P_A) \times P_B \quad (3)$$

$$P_{00} = (1 - P_A) \times (1 - P_B) \quad (4)$$

We used Program R to calculate the likelihood ratio [LR = − 2 (log $L_{\text{reduced model}} - L_{\text{full model}}$)] of these maximum likelihood estimates to compare refuge use in conspecific pairings (L_{control}) to refuge use in heterospecific pairings ($L_{\text{experimental}}$). This ratio test compares the probability of obtaining our data for the conspecific and heterospecific models, indicating if one model has a significantly higher probability, which would imply differences in behavior.

2.3. Chemical Interference Trials

We conducted chemical interference trials with the same three species. Prior to each trial, we prepared "residue refuges" by housing 30 large (> 45 mm SVL) Cuban treefrogs jointly in an enclosure with only 15 PVC refuges for 24 h to allow them to transfer their chemical skin secretions to the refuges. We ensured that at least one Cuban treefrog used each residue refuge. We performed 30 treatment (i.e., residue refuge vs. clean refuge; see panel B.1, Figure 1) and 30 control trials (i.e., two clean refuges, see panel B.2, Figure 1). Size (SVL) ranges of treefrogs used in chemical interference trials were as follows: Cuban treefrog—17–78 mm, green treefrog—16–52 mm, and squirrel treefrog—20–43 mm. At 20:00 we removed Cuban treefrogs from the residue pipe and positioned this pipe with a clean pipe in an experimental trial aquarium. We then immediately placed a single frog into the aquarium (outfitted as in the behavioral interference trials) or into an aquarium with two clean vertical PVC refuges. At 10:00 the following morning we recorded the location of each frog. We controlled for possible directional bias by alternating the placement of refuges (i.e., compass direction). We conducted chemical and behavioral trials over a 3.5-month period from 15 May through 31 August 2007, and each frog was only used once in a trial.

We used a separate Fisher's exact test for each species to compare their use of the two clean refuges offered during control trials (expected) to their use of refuges during experimental trials (observed), when one of the refuges offered had previously been used by large Cuban treefrogs (i.e., residue refuge). We created a three by two contingency table for each species with the counts of frogs in experimental trials in the residue tube, clean tube, or neither as our observed values compared to the number of frogs for our control trials in the first clean tube, the other clean tube, or neither. We used these tables for our Fisher's exact tests.

3. Results

3.1. Behavioral Exclusion

Neither Cuban treefrogs nor natives demonstrated significant avoidance of artificial refuges when another similarly-sized treefrog was present (Table 1). The presence of a Cuban treefrog did not influence the likelihood that a native treefrog would use the PVC refuge (LR = 1.69, $p = 0.19$ for squirrel treefrogs and LR = 0.13, $p = 0.72$ for green treefrogs); both species of native treefrogs were observed sharing the refuge with Cuban treefrogs on multiple occasions. Likewise, the presence of a native treefrog did not influence the likelihood that a Cuban treefrog would use the refuge (LR = 2.89, $p = 0.09$ for squirrel treefrogs and LR = 2.13, $p = 0.14$ for green treefrogs). For all three species, the probability of occupying a PVC refuge did not differ significantly in the presence of a heterospecific or conspecific.

Table 1. Results of behavioral interference trials: number of observations of each outcome for heterospecific or conspecific pairings. Heterospecific pairing outcomes were compared to conspecific pairings to evaluate interference (LR—likelihood ratio). The outcomes A0 and 0B indicate that only one frog was found using the refuge.

Treefrog Pairing			Neither	A Only	B Only	Both	Behavioral Interference on Species A	
(A)	(B)	N	(00)	(A0)	(0B)	(AB)	LR	*p*
Squirrel	Cuban	30	8	12	4	6	1.69	0.19
	Squirrel	30	7	2	9	12	—	—
Green	Cuban	30	13	4	7	6	0.13	0.72
	Green	30	17	5	3	5	—	—
Cuban	Squirrel	30	8	4	12	6	2.89	0.09
	Green	30	13	7	4	6	2.13	0.14
	Cuban	30	11	3	13	3	—	—

3.2. Chemical Interference

None of the three species of treefrogs demonstrated significant avoidance of residue refuges (Table 2). Although many more Cuban treefrogs rested in the clean pipe or other locations (i.e., on the sand substrate or on enclosure walls) than in the residue pipe, the observed variation in the control trials (where both pipes were clean) was too high for the Fisher's exact test to indicate avoidance ($p = 0.65$). Native treefrogs had more even ratios of clean to residue pipe use, with two more squirrel treefrogs resting in the residue pipe than the clean pipe ($p = 0.68$) and insignificantly more green treefrogs resting in the clean pipe than the residue pipe ($p = 0.18$).

Table 2. Results of chemical interference trials: number of observations of each outcome for each treefrog species tested. We used the raw counts of frogs found in each location in a separate contingency table for each species. The *p*-values are the result of a Fisher's exact test for count data.

Treefrog Species	Experimental (Observed)			Controls (Expected)			
	Residue PVC	Clean PVC	Neither	Clean PVC A	Clean PVC B	Neither	*p*
Cuban	3	11	16	4	7	19	0.65
Squirrel	11	9	10	9	13	8	0.68
Green	5	9	16	11	5	14	0.18

4. Discussion

It is important for ecologists to evaluate indirect effects of invasive species on native species, as inter- and intraspecific interactions have the potential to influence both invasion success and impacts on native species. None of the three species we tested demonstrated a statistically significant avoidance of PVC refuges previously inhabited by invasive Cuban treefrogs, suggesting that they did not detect the residue left by Cuban treefrogs or did not recognize it as indicative of a threat. Nonetheless, chemical cues play important roles in amphibian territorial behavior [46,47], and are used by juvenile toads to detect and avoid cannibalistic conspecifics and other predators [20,48,49]. The skin secretions of Cuban treefrogs serve to dissuade potential predators [10], but as demonstrated in our study, likely play a limited role in avoidance of cannibalism among conspecifics as well as predation on native treefrogs.

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 [50–53]. Although native frogs readily respond to native predators, they may not recognize and respond to nonindigenous predators [52,54]. The ability to detect and avoid an introduced predator must be selected for over time; however, frogs used in our study were collected from areas where Cuban treefrogs had only been established for ~20 years or less (approximately 15 frog generations).Furthermore, we observed variation in use of clean PVC pipes by frogs in our chemical interference control trials (e.g., almost twice as many Cuban treefrogs used Pipe B as Pipe A even though both were clean) that may have affected outcome of our statistical tests. Therefore, future studies are needed to determine whether the ability of native species to detect chemical cues of invasive Cuban treefrogs may evolve over time, and to further examine variation in PVC refuge use among species.

Cuban treefrogs did not interfere, behaviorally or chemically, with refuge use by native treefrogs, nor did native treefrogs interfere with refuge use by Cuban treefrogs. These results support an underlying assumption of the PVC sampling method, that the presence of one species (i.e., Cuban treefrogs) does not reduce the detection probability of other species. With this assumption in place, it is possible to use PVC pipe refugia to conduct community-level studies to compare native treefrog populations between areas with and without Cuban treefrogs, or to evaluate the effects of management efforts. However, squirrel treefrogs are known to occasionally exclude conspecifics from natural retreat sites [17], but it was beyond the scope of our study to determine if this native species might affect PVC refuge use by green treefrogs.

In addition to using PVC pipes to study treefrog ecology, our findings support the notion that the pipes remain an important tool to manage invasive Cuban treefrogs, as alluded to above. In a captive setting there is considerable variation in the propensity of native treefrogs and invasive Cuban treefrogs to seek shelter in PVC pipes. As compared to three native species, Cuban treefrogs were more likely to choose a plant than a pipe in which to hide when given a choice [55]. Nonetheless, Cuban treefrogs readily seek shelter in ground-based PVC pipes in nature and in a study in central Florida this species represented 43% (145 of 335 individuals) of captures of unmarked individuals—three native species comprised the other captures [56].

Unfortunately, our research also suggests that PVC refuges could conceivably function as population sinks for native treefrogs that are unable to detect and avoid pipes used by predatory Cuban treefrogs (see [39]). We used size-matched pairs of frogs in the behavioral exclusion tests for our study in order to prevent predation, but did not investigate variation in predation risk among species and size classes. We speculate that if large Cuban treefrogs residing in a PVC refuge regularly consume other treefrogs attracted to that refuge, smaller individuals might be underrepresented in PVC refuge samples and the refuges themselves could act as sinks for native treefrogs. We suggest that future research should address this important topic.

Author Contributions: K.E.H. and S.A.J. conceived of and designed the experiments as part of Hoffmann's MS thesis at the University of Florida. K.E.H. and M.E.M. conducted the experiments. K.E.H. analyzed the data. All authors contributed to manuscript writing.

Funding: The University of Florida's Department of Wildlife Ecology and Conservation partially supported Kristine E. Hoffmann's graduate assistantship while the research was conducted.

Acknowledgments: We thank K. Krysko, and M. Moulton for comments on the study design, R. Dorazio for insights and help with analysis, and J. Rechcigl, J. Price, P. Elliott, and Plant Adoption Landscapes for facilitating this study. We also thank E. Brown, T. Blunden, M. Dykes, C. Esmel, R. Haring, Y. Kornilev, E. Roznik, C. Sunquist, and J. Watson for field assistance with frog collection. All research was conducted in compliance with University of Florida IACUC protocol E870. The University of Florida's Department of Wildlife Ecology and Conservation partially supported Kristine E. Hoffmann's graduate assistantship while the research was conducted.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Gause, G.F. *The Struggle for Existence*; Williams & Wilkins Company: Baltimore, MD, USA, 1934; p. 163. ISBN 0-486-49520-5.
2. Jaeger, R.G.; Prosen, E.D.; Adams, D.C. Character displacement and aggression in two species of terrestrial salamanders. *Copeia* **2002**, *2002*, 391–401. [CrossRef]
3. Pacala, S.W.; Roughgarden, J. Resource partitioning and interspecific competition in two two-species insular *Anolis* lizard communities. *Science* **1982**, *217*, 444–446. [CrossRef] [PubMed]

4. Freed, L.A.; Cann, R.L.; Bodner, G.R. Incipient extinction of a major population of the Hawaii akepa owing to introduced species. *Evol. Ecol. Res.* **2008**, *10*, 931–965.
5. Janssen, J.; Jude, D.J. Recruitment failure of mottled sculpin Cottus bairdi in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. *J. Great Lakes Res.* **2001**, *27*, 319–328. [CrossRef]
6. Gallardo, B.; Clavero, M.; Sanchez, M.I.; Vila, M. Global ecological impacts of invasive species in aquatic ecosystems. *Glob. Chang. Biol.* **2016**, *22*, 151–163. [CrossRef] [PubMed]
7. Campbell, T.S. Analyses of the Effects of an Exotic Lizard (*Anolis sagrei*) on a Native Lizard (*Anolis carolinensis*) in Florida, Using Islands as Experimental Units. Ph.D. Thesis, University of Tennessee, Knoxville, TN, USA, 2000.
8. D'Amore, A.; Kirby, E.; McNicholas, M. Invasive species shifts ontogenetic resource partitioning and microhabitat use of a threatened native amphibian. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2009**, *19*, 534–541. [CrossRef]
9. Bohn, T.; Amundsen, P.A.; Sparrow, A. Competitive exclusion after invasion? *Biol. Invasions* **2008**, *10*, 359–368. [CrossRef]
10. Meshaka, W.E., Jr. *The Cuban Treefrog in Florida: Life History of a Successful Colonizing Species*; University Press of Florida: Gainesville, FL, USA; p. 191. ISBN 0-8130-2109-X.
11. Rice, K.G.; Waddle, J.H.; Miller, M.W.; Crockett, M.E.; Mazzotti, F.J.; Pervival, H.F. Recovery of native treefrogs after removal of nonindigenous Cuban treefrogs, *Osteopilus septentrionalis*. *Herpetologica* **2011**, *67*, 105–117. [CrossRef]
12. Waddle, J.H.; Dorazio, R.M.; Walls, S.C.; Rice, K.G.; Beauchamp, J.; Schuman, M.J.; Mazzotti, F.J. A new parameterization for estimating co-occurrence of interacting species. *Ecol. Appl.* **2010**, *20*, 1467–1475. [CrossRef] [PubMed]
13. Smith, K.G. An exploratory assessment of Cuban treefrog (*Osteopilus septentrionalis*) tadpoles as predators of native and nonindigenous tadpoles in Florida. *Amphib. Rept.* **2005**, *26*, 571–575. [CrossRef]
14. Knight, C.M.; Parris, M.J.; Gutzke, W.H.N. Influence of priority effects and pond location on invaded larval amphibian communities. *Biol. Invasions* **2009**, *11*, 1033–1044. [CrossRef]
15. Duellmann, W.E.; Trueb, L. *Biology of Amphibians*; McGraw-Hill Book Company: New York, NY, USA, 1986; p. 670. ISBN 0-8018-4780-X.
16. Lutz, B. Fighting and an incipient notion of territory in male tree frogs. *Copeia* **1960**, *1960*, 61–63. [CrossRef]
17. Buchanan, B.W. Territoriality in the Squirrel Treefrog, *Hyla squirella*: Competition for Diurnal Retreat Sites. Master's Thesis, University of Southwestern Louisiana, Lafayette, LA, USA, 1988.
18. Stewart, M.M.; Rand, A.S. Vocalization and the defense of retreat sites by male and female frogs, *Eleutherodactylus coqui*. *Copeia* **1991**, *1991*, 1013–1024. [CrossRef]
19. Wiewandt, T.A. Breeding biology of the Mexican leaf frog. *Fauna* **1971**, *2*, 29–34.
20. Kiesecker, J.M.; Chivers, D.P.; Blaustein, A.R. The use of chemical cues in predator recognition by western toad tadpoles. *Anim. Behav.* **1996**, *52*, 1237–1245. [CrossRef]
21. Stauffer, H.P.; Semlitsch, R.D. Effects of visual, chemical, and tactile cues of fish on the behavioural responses of tadpoles. *Anim. Behav.* **1993**, *46*, 355–364. [CrossRef]
22. Chivers, D.P.; Kiesecker, J.M.; Wildy, E.L.; Blenden, L.K.; Kats, L.B.; Blaustein, A.R. Avoidance response of post-metamorphic anurans on cues of injured conspecifics and predators. *J. Herpetol.* **1999**, *33*, 472–476. [CrossRef]
23. Chivers, D.P.; Wildy, E.L.; Kiesecker, J.M.; Blaustein, A.R. Avoidance response of juvenile Pacific treefrogs to chemical cues of introduced predatory bullfrogs. *J. Chem. Ecol.* **2001**, *27*, 1667–1676. [CrossRef] [PubMed]
24. Schulte, L.M.; Yeager, J.; Schulte, R.; Veith, M.; Werner, P.; Beck, L.A.; Lotters, S. The smell of success: Choice of larval rearing sites by means of chemical cues in a Peruvian poison frog. *Animal Behav.* **2011**, *81*, 1147–1154. [CrossRef]
25. Johnson, S.A.; McGarrity, M.E.; Staudhammer, C.M. An effective chemical deterrent for invasive Cuban treefrogs. *Hum. Wild. Interact.* **2010**, *4*, 112–117.
26. Moulton, C.A.; Flemming, W.J.; Nerney, B.R. The use of PVC pipes to capture hylid frogs. *Herpetol. Rev.* **1996**, *27*, 86–187.
27. Boughton, R.G.; Staiger, J.; Franz, R. Use of PVC pipe refugia as a sampling technique or hylid treefrogs. *Am. Mid. Nat.* **2000**, *144*, 168–177. [CrossRef]

28. Lever, C. *Naturalized Reptiles and Amphibians of the World*; Oxford University Press: Oxford, UK; p. 318. ISBN 978-0-19-850771-0.
29. Barbour, T. Another introduced frog in North America. *Copeia* **1931**, *1931*, 140. [CrossRef]
30. Krysko, K.L.; Enge, K.M.; Townsend, J.H.; Langan, E.M.; Johnson, S.A.; Campbell, T.S. New county records of amphibians and reptiles from Florida. *Herpetol. Rev.* **2005**, *3*, 85–87.
31. McGarrity, M.E.; Johnson, S.A. Geographic trend in sexual size dimorphism and body size of *Osteopilus septentrionalis* (Cuban treefrog): Implications for invasion of the southeastern United States. *Biol. Invasions* **2009**, *11*, 1411–1420. [CrossRef]
32. Beard, K.H.; Johnson, S.A.; Shiels, A.B. Frogs (coqui frogs, greenhouse frogs, Cuban tree frogs, and cane toads). In *Ecology and Management of Terrestrial Vertebrate Invasive Species in the United States*; Pitt, W.C., Beasly, J.C., Witmer, G.W., Eds.; CRC Press: Boca Raton, FL, USA, 2018; pp. 163–192. ISBN 978-1-4984-0482-3.
33. Rödder, D.; Weinsheimer, F. Will future anthropogenic climate change increase the potential distribution of the alien invasive Cuban treefrog (Anura: Hylidae)? *J. Nat. Hist.* **2009**, *43*, 1207–1217. [CrossRef]
34. Glorioso, B.M.; Waddle, J.H.; Muse, L.J.; Jennings, N.D.; Litton, M.; Hamilton, J.; Gergen, S.; Heckard, D. Establishment of the exotic invasive Cuban treefrog (*Osteopilus septentrionalis*) in Louisiana. *Biol. Invasions* **2018**, 1–7. [CrossRef]
35. Bartareau, T.M.; Meshaka, W.E. *Osteopilus septentrionalis* (Cuban treefrog). Diet. *Herpetol. Rev.* **2007**, *38*, 324–325.
36. Glorioso, B.; Waddle, J.H.; Crockett, M.E.; Rice, K.G.; Precival, H.F. Diet of the invasive Cuban treefrog (*Osteopilus septentrionalis*) in pine rockland and mangrove habitats in South Florida. *Caribb. J. Sci.* **2012**, *46*, 346–355. [CrossRef]
37. Hoffmann, K.E.; Johnson, S.A. *Osteopilus septentrionalis* (Cuban treefrog). Diet. *Herpetol. Rev.* **2008**, *39*, 339.
38. Maskell, A.J.; Waddle, J.H.; Rice, K.G. *Osteopilus septentrionalis*. Diet. *Herpetol. Rev.* **2003**, *34*, 137.
39. Wyatt, J.L.; Forys, E.A. Conservation implications of predation by Cuban treefrogs (*Osteopilus septentrionalis*) on native hylids in Florida. *Southeast. Nat.* **2004**, *3*, 695–700. [CrossRef]
40. Love, W.B. *Osteopilus septentrionalis* (Cuban treefrog). Predation. *Herpetol. Rev.* **1995**, *26*, 201–202.
41. Meshaka, W.E., Jr. Theft or cooperative foraging in the barred owl? *Fla. Field Nat.* **1996**, *24*, 15.
42. Meshaka, W.E., Jr.; Ferster, B. Two species of snakes prey on Cuban treefrogs in southern Florida. *Fla. Field Nat.* **1995**, *23*, 97–98.
43. Meshaka, W.E., Jr.; Jansen, K.P. *Osteopilus septentrionalis* (Cuban treefrog). Predation. *Herpetol. Rev.* **1997**, *28*, 147–148.
44. Rodriguez, L.F. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biol. Invasions* **2006**, *8*, 927–939. [CrossRef]
45. R Core Team. *R: A Language and Environment for Statistical Computing 2017*; R Foundation for Statistical Computing: Vienna, Austria, 2017.
46. Forester, D.C.; Wisnieski, A. The significance of airborne olfactory cues to the recognition of home area by the dart-poison frog *Dendrobates pumilio*. *J. Herpetol.* **1991**, *25*, 502–504. [CrossRef]
47. Waldman, B.; Bishop, P.J. Chemical communication in an archaic anuran amphibian. *Behav. Ecol.* **2004**, *15*, 88–93. [CrossRef]
48. Flowers, M.A.; Graves, B.M. Juvenile toads avoid chemical cues from snake predators. *Anim. Behav.* **1997**, *53*, 641–646. [CrossRef]
49. Pizzatto, L.; Child, T.; Shine, R. Why be diurnal? Shifts in activity time enable young cane toads to evade cannibalistic conspecifics. *Behav. Ecol.* **2008**, *19*, 990–997. [CrossRef]
50. Chivers, D.P.; Smith, R.J.F. Fathead minnows, *Pimephales promelas*, acquire predator recognition when alarm substance is associated with the sight of unfamiliar fish. *Anim. Behav.* **1994**, *48*, 597–605. [CrossRef]
51. Griffin, A.S.; Blumstein, D.T.; Evans, C.S. Training captive-bred or translocated animals to avoid predators. *Cons. Biol.* **2000**, *14*, 1317–1326. [CrossRef]
52. Kiesecker, J.M.; Blaustein, A.R. Population differences in responses of red-legged frogs (*Rana aurora*) to introduced bullfrogs (*Rana catesbeiana*). *Ecology* **1997**, *78*, 1752–1760. [CrossRef]
53. Savidge, J.A. Extinction of an island forest avifauna by and introduced snake. *Ecology* **1987**, *68*, 660–668. [CrossRef]

54. Pearl, C.A.; Adams, M.J.; Schuytema, G.S.; Nebeker, A.V. Behavioral responses of anuran larvae to chemical cues of native and introduced predators in the pacific northwestern United States. *J. Herpetol.* **2003**, *37*, 572–576. [CrossRef]
55. Hoffmann, K.E.; Johnson, S.J.; McGarrity, M.E. Interspecific variation in use of polyvinyl chloride (PVC) pipe refuges by hylid frogs: A potential source of capture bias. *Herpetol. Rev.* **2009**, *40*, 423–426.
56. Campbell, T.S.; Irvin, P.; Campbell, K.R.; Hoffmann, K.; Dykes, M.E.; Harding, A.J.; Johnson, S.A. Evaluation of a new technique for marking anurans. *Appl. Herpetol.* **2009**, *6*, 247–256. [CrossRef]