INVESTIGATING A MANAGEMENT PROGRAM FOR INTRODUCED GREEN TREEFROGS AT GREAT SMOKY MOUNTAINS NATIONAL PARK

by

JENNIFER ASPER

(Under the Direction of John C. Maerz)

ABSTRACT

The mandate of Great Smoky Mountain National Park (GSMNP) is to preserve its resources in ways that will leave them essentially unaltered by human influences, which includes the management of nonnative species. Non-native Green treefrogs (*Hyla cinerea*) were introduced and have established large population throughout Cades Cove, GSMNP. We used capture-mark-recapture to estimate the size of the breeding population at the putative introduction site, and call surveys to estimate native anuran and *H. cinerea* occupancy among wetlands. We also used mesocosms to test the effects of wetland type on larval performance. Finally, using data from these studies and literature, we used Individual Based Modeling (IBM) to evaluate likely scenarios for the invasion of *H. cinerea* into Cades Cove. Models suggest that facilitated “dispersal”, possibly via tourists, likely plays a role in the spread of *H. cinerea* throughout Cades Cove and therefore effective management strategies may require understanding visitor behavior.

INDEX WORDS: *Hyla cinerea*, Green treefrog, population management, Individual Based Modeling, population estimation.

INVESTIGATING A MANAGEMENT PROGRAM FOR INTRODUCED GREEN TREEFROGS AT GREAT SMOKY MOUNTAINS NATIONAL PARK

by

JENNIFER ASPER

BS, Messiah College, 2009

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

INVESTIGATING A MANAGEMENT PROGRAM FOR INTRODUCED GREEN TREEFROGS AT GREAT SMOKY MOUNTAINS NATIONAL PARK

by

JENNIFER ASPER

Major Professor: John C. Maerz

Committee: Brian Irwin
Clint Moore

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
May 2015

DEDICATION

I would first like to dedicate this thesis to my dad, Vernon Asper. From a very young age he taught me to "look and think". He encouraged me to explore answers for myself, rather than brushing off my childish questions or just giving me the answer. This allowed me to begin thinking about the world as a scientist before I even knew I wanted to pursue science as a career. Thank you, dad, for being the spark that sent me on this path.

Secondly, I would like to dedicate my thesis to my soon-to-be husband Andrew Spatz. It takes patience to deal with a girl who is simultaneously finishing a Masters and planning a wedding. You have been supportive even when I have been irrational. Thank you for being there for me.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, John Maerz, whose knowledge and passion have been crucial in the development and realization of this project. I would also like thank Vanessa Kinney Terrell who keeps our lab together and provided a great amount of field assistance, including driving to the Smokies and back home in a single day to make sure I could get all of my supplies to the site.

Thanks to all of my field help on night surveys and mesocosm set up. These people were such a help and sure beat being alone up there: Chris Engel, Kevin Fouts, Evan Gerry, Andrew Ward, Jonathan Cox, Lindell Asper, Vernon Asper, Andrew Spatz, Holly Scott, Andy Troutman, Andy Wszalek, Dawn Dextraze, Gar Secrist, Kendra Straub, and Bernie.

Thanks to the maintenance department at Great Smoky Mountains National Park for allowing me to set up my mesocosm experiments inside the fence at the Settling Lagoons. Not having to deal with bear interference save a lot of stress, I am sure. Thanks also to the law enforcement rangers and dispatch at the Park for making sure I made it out of the Cove after my late night sampling runs.

Thanks to Abby Sterling and Kelly Phillips for their contributions to the Capture-Mark-Recapture analysis in chapter 2. Thanks to Brian Crawford and Paige Howell for helping me figure out some occupancy modeling challenges. You are all very busy and I appreciate that you are willing to take the time to help a friend and peer in need.

Lastly, thanks to Great Smoky Mountains National Park and, in particular, Paul Super, for making sure I had the housing, funding, and access to conduct this research. I could not have done it without people wanting me there and helping me to know where to start looking. Living in the park was a true pleasure and I hope I can work there again someday soon.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

CHAPTER 1

INTRODUCTION

The Organic Act established the mandate of the National Park Service to preserve its exceptionally diverse resources in ways that will leave them essentially unaltered by human influences (Organic Act U.S.C. 1916). This mandate allows for maintaining human influenced areas (e.g. pastures for grazing livestock) if "such use is not detrimental to the primary purpose for which such park…was created" (Organic Act U.S.C. 1916). Great Smoky Mountains National Park (GSMNP) has identified priority research areas that pertain to the Park's ability to follow this mandate. One of these priority areas is the ecology and control of non-indigenous species. This priority is supported by executive order 13112 which states that federal agencies are "to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause" (Clinton 1999). As a significant component of global change, the human-mediated spread of organisms not native to the Park is a particular challenge, especially to the preservation of the Park's native species and given the high volume of tourists that visit the Park.

GSMNP has high species richness for many taxonomic groups and is known for its diversity of amphibians. Salamanders account for much of the amphibian richness within the Park, but Cades Cove is managed as a priority area for anurans. Cades Cove is also maintained as an agrarian landscape, emulating conditions when it was settled in the 1800s. However, Cades Cove is surrounded by forested mountains, which provide a geographic barrier not conducive to many species associated with the Cove Valley.

Green treefrogs (*Hyla cinerea*) are native to the southeastern United States, but are not native to GSMNP. GSMNP is approximately 225 km north of northern “natural” range of *H. cinerea* (Figure 1.1)(Lanoo 2005), though there are increasing reports of *H. cinerea* in north Georgia, western North Carolina, and eastern Tennessee since 2000. Many of these reports are anecdotal, however, the North American Amphibian Monitoring Protocol (NAAMP) reported *H. cinerea* in Tyner, TN, about 135 km from Cades Cove and in Salem, Georgia about 145 km from Cades Cove (USGS 2014b). Between 1998 and 2001, the United States Geological Survey (USGS) carried out an inventory of amphibian species within GSMNP and failed to detect *H. cinerea* despite extensive effort at sites currently occupied by the species today (Dodd 2003). USGS also maintained a NAAMP route through the Cove, and no *H. cinerea* were detected at any of the ten stops along the route during the last two surveys conducted in June 2007 (within *H. cinerea* breeding season) and in May 2008 (outside of the breeding season)(USGS 2014a). In 2011, *H. cinerea* were first reported calling by Park staff in Cades Coves at the wastewater Settling Lagoons adjacent to the campground, and in 2012, a “small” chorus of *H. cinerea* was confirmed at the Settling Lagoons.

The source of the introduction is not known, but Park biologists assume that the introduction occurred near the Cades Cove campground or the Settling Lagoons. In 2008 and 2009, management of the horse barns adjacent to the Settling Lagoons and of the Cades Cove store changed to an operator based in Florida, and involved the movement of heavy machinery and frequent movement of horse trailers between Florida and GSMNP (Molly Schroer, NPS personal communication). This is one potential route for the introduction of *H. cinerea* to Cades Cove.

Within its native range, adult *H. cinerea* can occur across a range of aquatic habitats, however, reproductive success of this species is typically associated with more permanent water bodies and longer hydroperiod wetlands, particularly those with more open canopies surrounded by pasture or prairie uplands (Gunzburger and Travis 2004, Babbitt et al. 2009). Therefore natural and man-made wetlands of longer hydroperiod found within the agrarian landscape of Cades Cove offer potentially suitable habitat to sustain and expand the presence of *H. cinerea* within Cades Cove. Because several of these wetlands are managed, in part, for native anurans including several priority amphibian species, GSMNP personnel have expressed concern over the potential impacts of the *H. cinerea* population on these focal organisms and have stated a need to evaluate potential management scenarios to eliminate or control the distribution and abundance of *H. cinerea* in the Cove.

The Ecology and Management of Invasions

The different stages of invasion (introduction, establishment, spread, and impact) are generally governed by different processes, and therefore, the management approaches depend on the stage of invasion (Sakai et al. 2001). Because survival during transport may be low, successful introductions are often the result of large numbers introduced individuals or repeated introduction events (Sakai et al. 2001). Therefore, management to prevent invasions focuses on reducing the number and size of potential introduction events. Once established, invaders often undergo a lag phase before their populations increase and spread (Sakai et al. 2001). The causes of lag phases are not well understood, but may be the result of Allee effects or stochastic extinctions, both of which are common when population sizes are small (Sakai et al. 2001). During the establishment and lag phase, management efforts shift to include eradication; however, locating small populations, particularly over larger geographic areas, may be challenging for many species. This approach also assumes that a species is a known or anticipated invader under surveillance prior to the species spread, which is often not the case (Sakai et al. 2001). Once a species has spread, it can often achieve local densities or biomass sufficient to alter ecosystem processes and native community structure.

When humans facilitate the movement of species over geographic barriers, invasive species may undergo ecological or evolutionary release from coevolved natural enemies (predators, parasites, and pathogens), giving them the potential to achieve high biomass and outcompete native species for access to resources (Blossey and Notzold 1995, Torchin and Mitchell 2004, Strayer et al. 2006, Everman and Klawinski 2013, Saul et al. 2013). In addition, invasive species may introduce new traits or "novel" weapons including novel allelopathic compounds or natural predators that can impact native species and alter ecosystem processes (Callaway and Ridenour 2004, Callaway and Maron 2006). Management of invasive species at this stage shifts toward control (reducing invader abundance rather than eradication) and alleviating or remediating the impacts of invaders on native species and processes (Sakai et al. 2001). Control and remediation can often be more expensive and logistically challenging than management alternatives used in earlier stages of invasion, and require some compromise on impacts to native systems since few management strategies are species specific, but it is the status under which many invasive species are managed.

Amphibian Ecology and Invasions

Though there is no evidence that introduced amphibian species have caused extinctions of native amphibians, invasive species can negatively impact native amphibian populations (Collins 2010). In particular, larval non-native amphibians can pose a threat to other larval species due to competition for resources (Vogel and Pechmann 2010) or they can threaten native species through hybridization (Sakai et al. 2001, Fitzpatrick et al. 2010). Large-scale invasions can also have a significant effect on other taxa, such as invertebrates, and can alter ecosystem processes (Beard and Pitt 2005, Sin et al. 2008, Choi and Beard 2012, Everman and Klawinski 2013, Kalnicky et al. 2014).

Notable anuran invasions with significant impacts include the Puerto Rican Coquí (*Eleutherodactylus coqui*), Cuban Treefrog (*Osteopilus septentrionalis*), Cane Toad (*Rhinella marina*), and the Bullfrog (*Lithobates catesbeianus*). Since the 1980s, Puerto Rican Coquí invasions have affected invertebrate populations and nutrient cycling in Hawaii (Beard and Pitt 2005, Sin et al. 2008, Choi and Beard 2012, Everman and Klawinski 2013, Kalnicky et al. 2014). Since its introduction to Florida in the 1920s, the Cuban treefrog has negatively impacted native treefrog species (Meshaka 2001, Rice et al. 2011) and has had economic impacts by causing utility outages when they use of plumbing or electrical boxes as refugia (Johnson et al. 2010). Cane toad invasions of

Australia have been linked to declines in other native anurans and amphibian predators as well as evolutionary shifts in the morphology and ecology of some native species (Brown et al. 2014, Llewelyn et al. 2014, Pearson et al. 2014, Shine 2014a, b, Shine and Phillips 2014, Bleach et al. 2015). Bullfrogs were introduced to the Northwestern United States intentionally, are capable of rapid spread, and are detrimental to both native amphibians and other fauna (Adams et al. 2003).

The success of nonnative amphibians and their potential to impact native species are likely to depend on a suite of well-known factors that affect amphibian community and population dynamics. For aquatic breeding amphibians, larval performance (survival, percent reaching metamorphosis, development rate) is an important determinant of species distributions and abundance. Larval performance is largely governed by hydroperiod, which affects the potential for catastrophic larval mortality, predator and competitor abundance, and wetland vegetation and canopy structure, which affect resource availability and thermal regulation (Skelly 2002, Rubbo and Kiesecker 2004, Maerz et al. 2005, Brown et al. 2006, Williams et al. 2008, Maerz et al. 2010, Stoler and Relyea 2011, Cohen et al. 2012, Earl and Semlitsch 2012, 2015). Some species of amphibians are more closely associated with deeper, permanent wetlands while others prefer ephemeral, shallow wetlands (Denton and Richter 2013). Forested wetlands generally have low productivity for amphibians because of shade and poorer quality deciduous litter inputs. In contrast, open canopy water bodies are generally more productive due to abundant light and dense herbaceous vegetation that can be higher in nutrient quality. Because they dry periodically, shorter hydroperiod wetlands tend to have lower predation rates due to fewer numbers of predatory species (i.e. no fish). Open, short hydroperiod wetlands can be highly productive environments for some amphibians because of high herbaceous production and predator die off during dry periods (no fish, invertebrate die off, etc), which creates productive larval environments when wetlands refill. More permanent water bodies tend to have high densities of predators including fish, and therefore, tend to support more predator resilient species with longer larval periods such as bullfrogs (*Lithobates catesbeianus*) (Denton and Richter 2013). Some species utilize a wide variety of breeding habitats, and they accommodate differences in resource availability, predation risk and hydroperiod with highly plastic larval morphology and life histories. Those species accelerate or reduce development rates in response to growth potential or risk, and can trade off a larger size at metamorphosis for accelerated development when conditions are no longer favorable. Species that are adapted to exploiting a wider range of water bodies often depend on shorter hydroperiod wetlands that support periodic booms in juvenile production (Semlitsch 2000, 2002, Petranka 2007, Denton and Richter 2013). Alternatively, some species have evolved resilience to predators and strong competitive abilities, and therefore, tend to breed in larger more permanent water bodies. These species may show reduced plasticity in larval development and thus have more restricted distributions among potential breeding sites.

In addition to wetland type, amphibian populations are affected by the availability and connectedness of suitable breeding sites within the landscape (Semlitsch 2002, Petranka 2007). Source-sink and metapopulation dynamics have strong effects on local extinction and rescue effects and, consequently, patterns of occupancy and abundance among potential breeding sites. In addition to the availability of productive water bodies, the capacity for high terrestrial survival and dispersal among wetlands affects occupancy rates and population viability (Petranka 2007). Because amphibian populations depend on source-sink/metapopulation dynamics to sustain high abundance and occupancy rates, introductions of small populations to single or isolated sites is likely to limit invasion success.

Anurans and Cades Cove Wetlands

There has been one intensive effort to monitor amphibians in GSMNP within the last 15 years. From 1998 to 2002, the USGS conducted thorough amphibian surveys throughout the park (Dodd 2003). These surveys included visual encounter surveys, intensive monitoring of selected plots, small grid-plots, and call surveys (Dodd 2003). In addition, the USGS North American Amphibian Monitoring Program (NAAMP) conducted call survey routes through the Cove starting in 2005, with the most recent surveys being in June 2007 and March 2008 (USGS 2014a). While *H. cinerea* was not detected historically or during any of these efforts, 13 species were detected in Cades Cove, including: *Acris crepitans, Anaxyrus americanus, Anaxyrus fowleri, Gastrophryne carolinensis, Hyla chrysoscelis, Pseudacris crucifer, Pseudacris feriarum, Lithobates catesbeianus, Lithobates clamitans, Lithobates palustris, Lithobates sylvaticus, Scaphiopus holbrookii,* and one report of *Lithobates pipiens* (Dodd 2004).

In Cades Cove there are 9 natural wetlands of significance in addition to three manmade wastewater Settling Lagoons, and a few episodically flooded grass pools (Dodd 2003). The wetlands can generally be classified as closed canopy, deciduous or open, herbaceous wetlands. The open wetlands include Abrams Creek Spring, Abrams

Creek Oxbow, Gourley Pond, Shields Pond, Hyatt Lane Pond, and a suite of wetlands including a beaver pond near Sparks Lane. The forested wetlands include Gum Swamp, Methodist Pond, and Stupkas Sinkhole Pond. Finally, there are three manmade wastewater Settling Lagoons which were constructed in 1972 adjacent to the Cades Cove Campground that are open canopy, permanent water bodies with high nutrient inputs and emergent vegetation around the perimeter. The majority of wetlands within Cades Cove have relatively short hydroperiods, often ponding in the winter or spring and drying by late summer. Besides the Settling Lagoons, only the lower portion of the Abrams Creek Spring wetland and the Beaver wetland near Sparks Lane exhibit any degree of permanency in the Cove. The network of wetlands within Cades Cove is relatively isolated as the Cove is surrounded by mountains with only one additional small set of wetlands 10 km east and one major creek flowing into the Cove (Figure 1.2).

Many of the water bodies in Cades Cove are important anuran habitat within the park. Gum Swamp is a priority wetland for GSMNP and is one of the most important amphibian breeding sites in the park. It is a breeding site for some priority species such as *Scaphiopus holbrookii*, as well as a high diversity of other amphibians including *Anaxyrus americanus, Hyla chrysoscelis, Lithobates sylvaticus, Lithobates palustris, Lithobates clamitans, Pseudacris crucifer, Pseudacris feriarum,* and *Pseudacris triseriata* (Dodd 2003, GRSM 2013). Gum Swamp is entirely forested and is dominated by deciduous litter inputs. The swamp holds water in most years, but dries up in late summer or fall in most years, including July of 2014 during this study.

Methodist Pond is located near the parking lot for the Methodist Church along the Cades Cove loop road. It is considered one of the most important breeding ponds in the park (Dodd 2003). It is primarily forested and filled with leaf litter detritus with only a section of herbaceous vegetation. It held water for the entire study period in 2013 and 2014. The pond is a breeding location for *Lithobates clamitans*, *Lithobates catesbeianus, Hyla chrysoscelis,* and *Pseudacris crucifer* (GRSM 2013).

The Stupkas Sinkhole Pond is located near the road leading to the Abrams Creek trailhead at the far west end of Cades Cove. It is a small, circular sinkhole that is entirely forested with no herbaceous vegetation. The sinkhole holds water during wet years, but can dry up in dryer seasons, such as summer 2014. The only anuran species documented at the site previously was *Lithobates sylvaticus,* but we also detected *Pseudacris crucifer, Anaxyrus americanus, Hyla chrysoscelis* and *Lithobates clamitans* (GRSM 2013).

Sparks Lane has three wetlands near Abrams creek that are partially shaded, but full of herbaceous vegetation. Two wetland areas have intermediate to short hydroperiods, but one of the wetland areas is a relatively new beaver pond still with living trees and a mixed understory of herbaceous vegetation. The beaver pond has relatively permanent water. Species detected there include *Pseudacris crucifer, Lithobates clamitans,* and *Hyla chrysoscelis* (GRSM 2013).

Hyatt Lane has ditches along the side and a section of river cane that is a breeding site for species less sensitive to short hydroperiods. It does not hold water for the entire breeding season and has a good deal of herbaceous vegetation. West of the road in the field is a pond (we refer to this as Hyatt Lane Pond) with partially open canopy of small trees. There is a record in the Park's inventory of species of *Hyla chrysoscelis* calling there, and we detected *Gastrophryne carolinensis, Anaxyrus americanus, Pseudacris crucifer, Lithobates catesbeianus,* and *Lithobates clamitans* (GRSM 2013).

Gourley Pond is a unique wetland and is considered a priority for amphibian monitoring in GSMNP and one of the most important amphibian breeding sites (Dodd 2003). It is an open pond with extensive herbaceous cover, and the wetland is hydrologically dynamic, ponding and drying repeatedly in some years. The wetland usually ponds each spring and dries up early, generally by May. It is the only site with a record of the Southern Leopard Frog (*Lithobates sphenocephalus*). It is also a breeding location for *Lithobates sylvaticus, Anaxyrus americanus, Anaxyrus fowleri, Hyla chrysoscelis, Pseudacris crucifer, Pseudacris feriarum, Lithobates clamitans,* and *Lithobates palustris* (GRSM 2013).

Abrams Creek has a spring located just off the creek in the western portion of the Cove along a large oxbow and extending up toward the cemetery to the north. The lower portion of the wetland holds water throughout the breeding season, but the more open wet meadow area (towards the cemetery) only holds water in years with high rainfall amounts. *Gastrophryne carolinensis* are known to breed at the site in those wet years. The spring portion of the wetland is an open canopy with shrubby and herbaceous vegetation, and the oxbow area is predominantly herbaceous. Other species known to breed at this site are *Pseudacris crucifer, Pseudacris feriarum, Lithobates catesbeianus, Lithobates clamitans, Lithobates sylvaticus, Lithobates palustris, Anaxyrus americanus* and *Hyla chrysoscelis* (GRSM 2013).

Shields Pond is an open, herbaceous wetland in the middle of a field. The wetland is situated at the end of a flooded ditch that connects to Abrams Creek at Hyatt Lane. The ditch is generally open canopy with shrubs and sapling trees throughout, while Shields Pond has extensive grassy wetland vegetation and only a few shrubs. Shields Pond is a known breeding location for a number of species, including *Hyla chrysoscelis*, *Gastrophryne carolinensis*, *Anaxyrus americanus*, *Lithobates clamitans*, and *Pseudacris crucifer* (GRSM 2013). The wetland only holds water in early spring or possibly into mid-summer in especially wet years.

The wastewater treatment Settling Lagoons are unique because they are the only man-made wetland in Cades Cove, constructed around 1972, and the only permanent water body. They are used as a breeding location for most species that are present in the park. The lagoons have an open canopy, are very sunny, are very high in nutrients due to the wastewater and are surrounded by herbaceous vegetation.

Hyla cinerea Biology

The biology of a particular species can be an important determinant of the types of landscapes and specific habitats in which the species' might establish or become invasive. In Georgia, *Hyla cinerea* mating and egg-laying occur between mid-April and mid-August and the species calls mostly from shortly after dark to midnight (Jensen 2008). Vegetation plays a role in *H. cinerea* calling as the males usually perch on shrubs, trees, or other emergent vegetation to call (Lanoo 2005, Jensen 2008). Like many anurans, not much is known about the terrestrial habits of individuals when they are not at the breeding sites, though it has been suggested that they winter terrestrially, presumably in forested habitats (Jensen 2008).

Female green treefrogs lay clutches of ~700 eggs several times each season, and they commonly use manmade structures as refugia (Lanoo 2005). Within their native range, *H. cinerea* are common at any relatively permanent wetland with emergent vegetation (Jensen 2008). They are associated with these types of wetlands because, unlike many anurans, *Hyla cinerea* cannot accelerate development and metamorphose at a smaller size in response to pond drying (Jensen 2008). The Settling Lagoons in Cades Cove have all the characteristics of an *H. cinerea* breeding site, as do several of the open and shrubby wetlands around the Cove.

Given the legal mandates that guide the actions of the NPS, the status of *H. cinerea* in Cades Cove needs to be examined to determine the potential impacts and management strategies for this introduced species. Given that anuran invasions are not unprecedented, this also allows for further insight into the mechanisms behind anuran population establishment. In order to determine phase of establishment (pre-establishment, early establishment, or well-established), and what management strategies will be most effective (prevention, eradication, or management), we must first determine the size of the breeding population and how it is distributed in the park. Second, it is important to know what ecological and anthropic factors regulate the distribution and abundance. Cades Cove is a managed, open agrarian landscape that includes a mixture of natural water bodies as well as man-made water bodies, including impounded wetlands, ditches, and wastewater settling ponds. Additionally, *H. cinerea* often use human structures including vehicles as refugia and therefore are frequent stowaways on vehicles. Given the high volume of traffic from within the range of *H. cinerea* that pass through the park, it is reasonable that the species could have been introduced in this manner. With more information about the current distribution and abundance of the species and about the narrative of how the introduction occurred and spread, park managers can better determine what management strategies will be most effective for minimizing the negative impacts of this introduced species.

Literature Cited

Adams, M. J., C. A. Pearl, and R. B. Bury. 2003. Indirect facilitation of an anuran invasion by non-native fishes. Ecology Letters **6**:343-351.

Babbitt, K. J., M. J. Baber, D. L. Childers, and D. Hocking. 2009. Influence of agricultural upland habitat type on larval anuran assemblages in seasonally inundated wetlands. Wetlands **29**:294-301.

Beard, K. H., and W. C. Pitt. 2005. Potential consequences of the coqui frog invasion in Hawaii. Diversity and Distributions **11**:427-433.

Bleach, I., C. Beckmann, C. Both, G. Brown, and R. Shine. 2015. Noisy neighbours at the frog pond: effects of invasive cane toads on the calling behaviour of native Australian frogs. Behavioral Ecology and Sociobiology **69**:675-683.

Blossey, B., and R. Notzold. 1995. Evolution of increased competitive ability in invasive and nonindigenous plants - a hypothesis. Journal of Ecology **83**:887-889.

Brown, C., B. Blossey, J. Maerz, and S. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions **8**:327-338.

Brown, G. P., B. L. Phillips, and R. Shine. 2014. The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. Proceedings of the Royal Society B-Biological Sciences **281**:7.

Callaway, R. M., and J. L. Maron. 2006. What have exotic plant invasions taught us over the past 20 years? Trends in Ecology & Evolution **21**:369-374.

Callaway, R. M., and W. M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment **2**:436-443.

Choi, R., and K. Beard. 2012. Coqui frog invasions change invertebrate communities in Hawaii. Biological Invasions **14**:939-948.

Clinton, W. 1999. Invasive Species. Pages 6183-6186 Executive Order 13112. Federal Register.

Cohen, J. S., J. C. Maerz, and B. Blossey. 2012. Traits, not origin, explain impacts of plants on larval amphibians. Ecological Applications **22**:218-228.

Collins, J. P. 2010. Amphibian decline and extinction: What we know and what we need to learn. Diseases of Aquatic Organisms **92**:93-99.

Denton, R. D., and S. C. Richter. 2013. Amphibian communities in natural and constructed ridge top wetlands with implications for wetland construction. Journal of Wildlife Management **77**:886-896.

Dodd, C. K. 2004. The Amphibians of Great Smoky Mountains National Park. The University of Tennessee Press, Knoxville, TN.

Dodd, C. K., Jr. 2003. Monitoring amphibians in Great Smoky Mountains National Park. U S Geological Survey Circular **1258**:i-vi, 1-117.

Earl, J. E., and R. D. Semlitsch. 2012. Reciprocal subsidies in ponds: does leaf input increase frog biomass export? Oecologia **170**:1077-1087.

Earl, J. E., and R. D. Semlitsch. 2015. Effects of tannin source and concentration from tree leaves on two species of tadpoles. Environmental Toxicology and Chemistry **34**:120-126.

Everman, E., and P. Klawinski. 2013. Human-facilitated jump dispersal of a non-native frog species on Hawai'i Island. Journal of Biogeography **40**:1961-1970.

Fitzpatrick, B. M., J. R. Johnson, D. K. Kump, J. J. Smith, S. R. Voss, and H. B. Shaffer. 2010. Rapid spread of invasive genes into a threatened native species. Proceedings of the National Academy of Sciences of the United States of America **107**:3606-3610.

GRSM. 2013. Species List. Great Smoky Mountains National Park, Gatlinburg, TN.

Gunzburger, M. S., and J. Travis. 2004. Evaluating predation pressure on green treefrog larvae across a habitat gradient. Oecologia **140**:422-429.

Jensen, J. B. 2008. Amphibians and reptiles of Georgia. Athens, University of Georgia Press.

Johnson, S. A., M. E. McGarrity, and C. L. Staudhammer. 2010. An effective chemical deterrent for invasive Cuban treefrogs. Human-Wildlife Interactions **4**:112-117.

Kalnicky, E. A., M. W. Brunson, and K. H. Beard. 2014. A social–ecological systems approach to non-native species: Habituation and its effect on management of coqui frogs in Hawaii. Biological Conservation **180**:187-195.

Lanoo, M. J. 2005. Amphibian declines: the conservation status of United States species. Berkeley, University of California Press.

Llewelyn, J., L. Schwarzkopf, B. L. Phillips, and R. Shine. 2014. After the crash: How do predators adjust following the invasion of a novel toxic prey type? Austral Ecology **39**:190-197.

Maerz, J. C., C. J. Brown, C. T. Chapin, and B. Blossey. 2005. Can secondary compounds of an invasive plant affect larval amphibians? Functional Ecology **19**:970-975.

Maerz, J. C., J. S. Cohen, and B. Blossey. 2010. Does detritus quality predict the effect of native and non-native plants on the performance of larval amphibians? Freshwater Biology **55**:1694-1704.

Meshaka, W. E., Jr. 2001. The Cuban treefrog in Florida: life history of a successful colonizing species. University Press of Florida.

Pearson, D. J., J. K. Webb, M. J. Greenlees, B. L. Phillips, G. S. Bedford, G. P. Brown, J. Thomas, and R. Shine. 2014. Behavioural responses of reptile predators to invasive cane toads in tropical Australia. Austral Ecology **39**:448-454.

Petranka, J. W. 2007. Evolution of complex life cycles of amphibians: bridging the gap between metapopulation dynamics and life history evolution. Evolutionary Ecology **21**:751-764.

Rice, K. G., J. H. Waddle, M. W. Miller, M. E. Crockett, F. J. Mazzotti, and H. F. Percival. 2011. Recovery of native treefrogs after removal of nonindigenous cuban treeforgs, *Osteopilus septentrionalis*. Herpetologica **67**:105-117.

Rubbo, M. J., and J. M. Kiesecker. 2004. Leaf litter composition and community structure: translating regional species changes into local dynamics. Ecology **85**:2519-2525.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology & Systematics **32**:305.

Saul, W.-C., J. M. Jeschke, and T. Heger. 2013. The role of eco-evolutionary experience in invasion success. NeoBiota:57–74.

Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management **64**:615-631.

Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. Conservation Biology **16**:619-629.

Shine, R. 2014a. The ecological, evolutionary, and social impact of invasive Cane Toads in Australia. Invasive Species in a Globalized World: Ecological, Social, and Legal Perspectives on Policy:23.

Shine, R. 2014b. A review of ecological interactions between native frogs and invasive cane toads in Australia. Austral Ecology **39**:1-16.

Shine, R., and B. L. Phillips. 2014. Unwelcome and unpredictable: the sorry saga of cane toads in Australia. Austral Ark:83.

Sin, H., K. H. Beard, and W. C. Pitt. 2008. An invasive frog, *Eleutherodactylus coqui*, increases new leaf production and leaf litter decomposition rates through nutrient cycling in Hawaii. Biological Invasions **10**:335-345.

Skelly, D. K. 2002. Experimental venue and estimation of interaction strength. Ecology **83**:2097-2101.

Stoler, A. B., and R. A. Relyea. 2011. Living in the litter: the influence of tree leaf litter on wetland communities. Oikos **120**:862-872.

Strayer, D. L., V. T. Eviner, J. M. Jeschke, and M. L. Pace. 2006. Understanding the long-term effects of species invasions. Trends in Ecology & Evolution **21**:645-651.

Torchin, M. E., and C. E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment **2**:183-190.

U.S.C. 1916. National Park Service Organic Act. 16 U.S.C. §1. U.S. Department of the Interior, United States of America.

USGS. 2014a. North American Amphibian Monitoring Protocol (NAAMP), Route Data Summary. Cades Cove.

USGS. 2014b. North American Amphibian Monitoring Protocol (NAAMP), Species Detection Map.

Vogel, L. S., and J. H. K. Pechmann. 2010. Response of Fowler's Toad (*Anaxyrus fowleri*) to competition and hydroperiod in the presence of the invasive Coastal Plain Toad (*Incilius nebulifer*). Journal of Herpetology **44**:382-389.

Williams, B. K., T. A. G. Rittenhouse, and R. D. Semlitsch. 2008. Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia **155**:377-384.

Figures

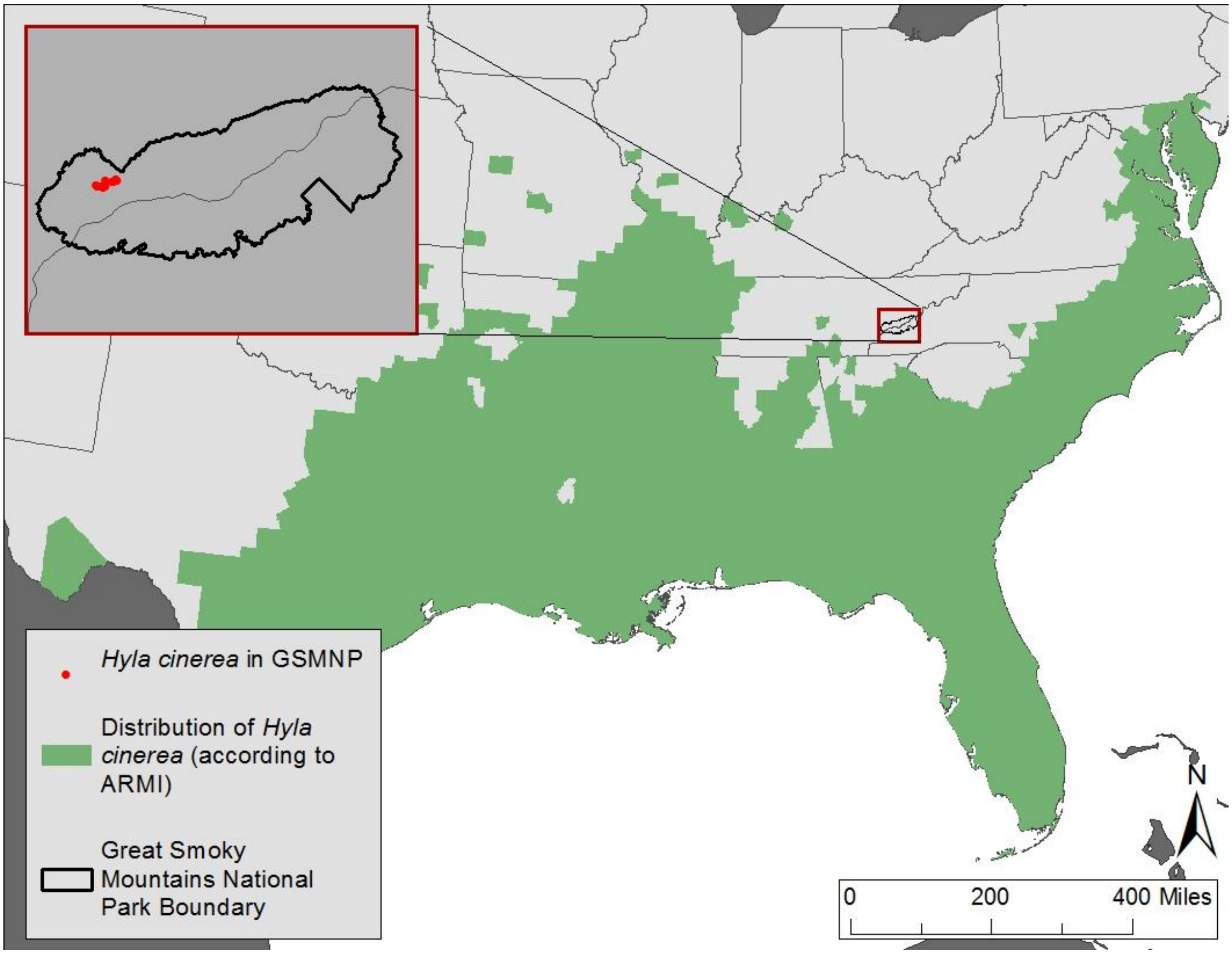

Figure 1.1. Distribution for Green treefrog (*Hyla cinerea*) according to the IUCN. The red circles within the callout box indicate the locations within GSMNP at which we have confirmed *H. cinerea* presence.

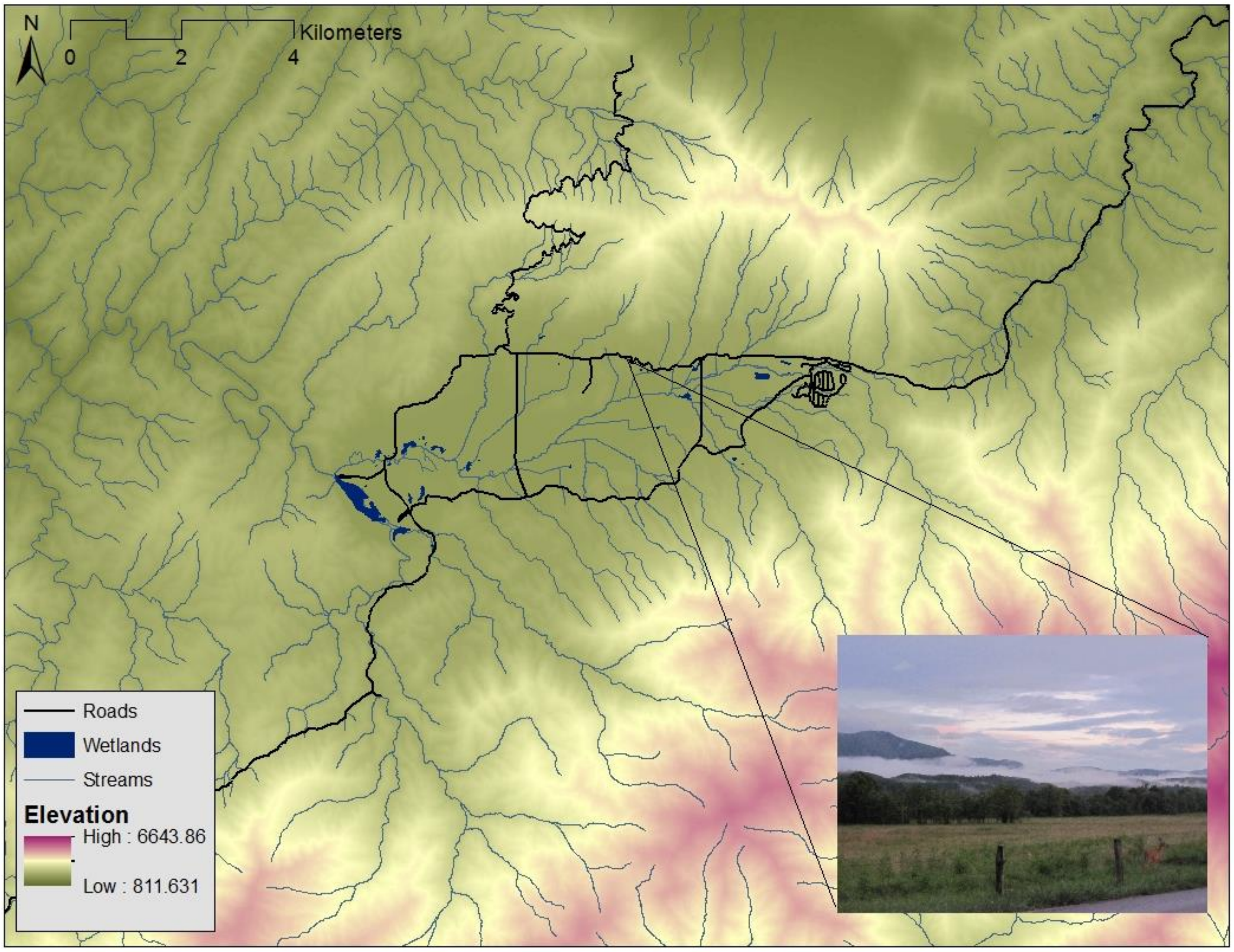

Figure 1.2. Cades Cove elevation, wetlands, and streams, illustrating the geographic and wetland isolation of the area.

CHAPTER 2

ABUNDANCE AND OCCUPANCY OF NON-NATIVE *HYLA CINEREA* IN CADES COVE, GREAT SMOKY MOUNTAINS NATIONAL PARK

Asper, J.R. and J.C. Maerz. To be submitted to undetermined journal.

Abstract

The mandate of Great Smoky Mountain National Park (GSMNP) is to preserve its diverse resources in ways that will leave them essentially unaltered by human influences. Given the introduction of *Hyla cinerea* into the park the objectives of this study were to estimate the current size of the *H. cinerea* breeding population at the Cades Cove Settling Lagoons, the putative introduction site, and to determine whether *H. cinerea* occupy other wetlands throughout Cades Cove in order to inform management decisions. Our results demonstrate that the invasion of *H. cinerea* in Cades Cove is far more advanced that was previously assumed as the Settling Lagoons have a large population and 5 other wetlands are occupied.

Introduction

The mandate of the National Park Service is to preserve its exceptionally diverse resources in ways that will leave them essentially unaltered by human influences (Organic Act U.S.C. 1916). Great Smoky Mountains National Park (GSMNP) has identified priority areas of research into issues that pertain to the Park's ability to follow this mandate and to protect its unique natural resources. One of these priority topics is the study of the ecology and control of non-indigenous species. This priority is supported by executive order 13112 which states that federal agencies are "to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause" (Clinton 1999). As a significant component of global change, the human-mediated spread of organisms not native to the Park is a particular challenge, especially to the preservation of the Park's native species.

GSMNP has a high level of diversity for many taxonomic groups and is globally known for its diversity of amphibians. While salamander diversity dominates the amphibian species present within most of the Park, the Cades Cove area is managed as the Park's priority area for anurans (frogs and toads). Cades Cove is maintained as an agrarian landscape, emulating conditions when it was settled in the 1800s. However, Cades Cove is surrounded by forested mountains, which provide a geographic barrier not conducive to many species associated with the Cove Valley.

Green treefrogs (*Hyla cinerea*) are native to the southeastern United States, but are not native to GSMNP. GSMNP is approximately 225 km north of the northern "natural" range of *H. cinerea* (Lanoo 2005), though since 2000 reports of *H. cinerea* are increasing in north Georgia, western North Carolina, and eastern Tennessee. Many of these reports are anecdotal, however, the North American Amphibian Monitoring Protocol (NAAMP) reported *H. cinerea* in Tyner, TN, about 135 km from Cades Cove and in Salem, Georgia about 145 km from Cades Cove (USGS 2014b). Between 1998 and 2001, the United States Geological Survey (USGS) carried out an inventory of amphibian species within GSMNP and failed to detect *H. cinerea* despite extensive effort at sites currently occupied by the species today (Dodd 2003). These surveys included visual encounter surveys, intensive monitoring of selected plots, small grid-plots, and call surveys (2003). USGS also maintained a NAAMP route through the Cove and no *H. cinerea* were detected at any of the ten stops along the route during the last two surveys conducted in June 2007 (within the *H. cinerea* breeding season) and in May 2008 (outside of the breeding season) (USGS 2014a). In 2011 Green treefrogs were first reported calling by Park staff in Cades Coves at the wastewater Settling Lagoons adjacent to the campground, and in 2012, a "small" chorus of *H. cinerea* was confirmed at the Settling Lagoons. Within its native range, adult *H. cinerea* can occur across a range of aquatic habitats, however, reproductive success of this species is typically associated with more permanent water bodies and longer hydroperiod wetlands, particularly those with more open canopies surrounded by pasture or prairie uplands (Gunzburger and Travis 2004, Babbitt et al. 2009). Therefore, the agrarian landscape of Cades Cove, with a limited number of natural and man-made wetlands of longer hydroperiod, offers potentially suitable habitat to sustain and expand the presence of *H. cinerea* within Cades Cove. Because several of these wetlands are managed, in part, for native anurans including several priority amphibian species, GSMNP personnel have expressed concern over the potential impacts of the *H. cinerea* population on native amphibians and other priority species and need to evaluate potential management scenarios to eliminate or control the distribution and abundance of *H. cinerea* in the Cove.

When dealing with a potentially invasive species, management actions will depend on an accurate estimate of a species' abundance and distribution. Invasion management generally targets three phases related to the status of the species: (1) prevention of species that have not yet arrived, (2) eradication of species that occur in low number and are concentrated at one or few locations, and (3) control to limit population size, distribution, and further spread once a species is established at high numbers or over larger geographic areas (Sakai et al. 2001). Limiting population size and spread may limit rather than eliminate the impact of an invasive species, and may also require compensatory management actions to assist native species or natural processes. Thus, correctly estimating the stage of the invasion can help managers prioritize strategies in the context of limited resources to determine more feasible outcomes.

The objectives of this study were to estimate the current size of the *H. cinerea* breeding population at the Cades Cove Settling Lagoons and to determine whether *H. cinerea* occupy other wetlands throughout Cades Cove. The Settling Lagoons are the purported introduction site of *H. cinerea* and the only location where the species has been reported. GSMNP staff wanted an estimate of *H. cinerea* population size at the Settling Lagoons to determine the feasibility of eradicating the species from that location via the hand removal of adults over several consecutive years. This strategy also depends on whether the species has already dispersed beyond the Settling Lagoons, and the feasibility of detecting and eradicating breeding populations emerging at other sites.

Methods

Study Area

Cades Cove is the most visited area within Great Smoky Mountains National Park, which is the most visited national park in the United States (NPS 2014). Cades Cove is located in the northwestern portion of the park and is managed as an agrarian landscape with pastures and old structures that emulate the way it was when it was originally settled. This is a relatively unique landscape bounded on all sides by forested mountains that are generally not suitable habitat for many species that occur within the geographically isolated cove. Cades Cove also includes a campground and a number of other buildings that require waste management. Therefore, around 1972 GSMNP constructed a series of wastewater Settling Lagoons adjacent to the campground (*Figure 2.*) (Paul Super, personal communication). These Settling Lagoons are a unique feature to the Cove in that they are permanent water bodies with an open canopy and high nutrient inputs. For this study, we estimate adult breeding population size at four replicate sections along the edges of the two largest Settling Lagoons. The margins of these ponds had low herbaceous vegetation including several graminoid species, and taller shrubby or reedy areas that have vegetative species such as *Juncus spp., Rubus spp.,* and *Typha spp.*

Most other wetlands within Cades Cove have shorter hydroperiods and are forested except a beaver wetland along Abrams Creek and an impounded spring-fed wetland along Abrams Creek near the Cades Cove Visitor Center at the western end of the Cove (*Figure 2.2*). Other important wetlands throughout the Cove include Methodist Pond, which receives frequent visitors, Gum Swamp, which is a high priority habitat for amphibians, and Gourley Pond, which is a short-hydroperiod, herbaceous wetland that is considered a priority habitat for rare species within the park including Southern Leopard frogs (*Lithobates sphenocephalus*). A number of historic structures and trails make most wetlands easily accessible to visitors. For this study we estimate occupancy at these other sites.

Estimation of Hyla cinerea Population Size at Settling Lagoons

We used capture-mark-recapture (CMR) modeling to estimate the breeding population size at the Cades Cove Settling Lagoons. We designated four, 25 meter sections of two of the three Settling Lagoons (*Figure 2.Figure 2*). Two sections had taller shrubby and herbaceous vegetation, and two had lower herbaceous vegetation. On June 19 and 20, and July 5 and 6, 2013, two people systematically searched all vegetation using flashlights and hand captured frogs. Animals were held in Ziploc bags with sufficient air until the entire section was thoroughly sampled, and then we determined each individual's sex, measured their snout to vent length (SVL) and marked them uniquely using toe tip clips (no more than one toe tip per foot). All of the investigators wore neoprene gloves to avoid the spread of disease, and the frog toes were clipped using sharp cuticle clippers sprayed with Bactine (antiseptic spray with benzlkonium chloride and lidocaine). Each individual was released in their capture section. There is no evidence that there is a notable effect of toe-clipping on survival or capture probability, particularly over the short time interval of our study (Grafe et al. 2011), and our high recapture rate was consistent with this assumption. In addition, many of the frogs were caught while calling or in amplexus, and most males were observed calling again within minutes of handling and release.

CMR Statistical Methods

Because of the narrow window of time that we sampled our population, we used a closed population CMR model to estimate capture probability and population size. We evaluated ten closed population CMR models to estimate abundance and detection probability including models that allowed initial capture probability and recapture probabilities to be equal or to vary in relation to each other (Table 2.1). The ten models allowed for all biologically relevant permutations of the model given the variables we were interested in. Capture probability can vary among individuals based on individual-level factors (Pollock 1982). Because calling males are more conspicuous, we included sex as a group factor in some models. We ran models in Program MARK (White and Burnham 1999) using the RMark (Laake 2013) interface in program R (R Core Team 2013) and evaluated our competing models using Akaike Information Criteria with a correction for small samples (AIC_c) (Burnham and Anderson 2002). We intended to use averaging of models within two ΔAICc to estimate capture and recapture probabilities and total population size within the four sampled sections; however, the top model in our analysis ultimately received 100% of the model weight. Because 100% of the perimeter of all three ponds was occupied by calling male *H. cinerea*, we used the ratio of our sampled area to the total pond perimeter to estimate the total population size at the Settling Lagoons.

We also simulated data to determine the sampling effort we would need to have optimal precision on our population estimates for future monitoring of *H. cinerea*. We used the abundance and capture probability estimates from our best model or set of models as starting values for the simulation, and varied the number of capture occasions for each iteration. We ran each iteration in RMark (Laake 2013) with 1000 replications, and used two, four, six, eight, and nine capture occasions, and estimated population size and coefficient of variation across all values for number of capture occasions (*k*). To draw conclusions about the best number of sampling occasions, we visually inspected the plot to determine where the coefficient of variation decreased and then levelled off.

Occupancy of Hyla cinerea and native anurans in Cades Cove

We used call surveys in a robust occupancy design (Pollock 1982) to estimate detection probabilities and occupancy rates for *H. cinerea* and native anurans among nine wetlands including the Settling Lagoons (Figure 2.2). Call surveys took place on each of three consecutive nights each month in mid-May, June, and July in 2013 and mid-May, June, July, and August in 2014. We generally followed the United States Geological Survey (USGS) Amphibian and Reptile Monitoring Initiative (ARMI) call survey protocol for North American Amphibian Monitoring Program (USGS 2012). Specifically, we waited for five minutes after arriving at our observation location for each wetland, and recorded the presence and intensity of anuran choruses for all species detected during that period.

Call Survey Statistical Methods

We modeled detection and occupancy using a Robust Design occupancy model in program unmarked (Fiske and Chandler 2011), an extension of program R (Team 2013). The Robust Design uses primary periods in which the population is considered open (survey months) with secondary periods where the population is considered closed (the three consecutive nights within a month) (Conroy and Carroll 2009). We used the *colext* function in program unmarked, which is a dynamic occupancy tool that allows for change in occupancy between primary periods (Kéry and Chandler 2012). Particularly, if occurrence (z) is related to the Bernoulli variable with parameter Ψ.

$$z_{i1} = Bernoulli(\Psi_{i1})$$

Then we can model occurrence for each sampling event using the following equation:

$$z_{it} \sim Bernoulli(z_{i,t-1} \emptyset_{it} + \left(1 - z_{i,t-1}\right) \gamma_{it})$$

We had seven primary occasions (three in 2013 and four in 2014) each with three secondary sampling occasions. For each species detected in Cades Cove, we constructed a series of models that had initial occupancy (Ψ), colonization (γ) and extinction (ε), either constant or varying with forest cover (deciduous or herbaceous), and detection probability (*p*) either constant or varying by sampling occasion (month). We ranked the models using Akaike Information Criteria (AIC). Using the top ranked model, we derived estimates for occupancy and detection probability.

Results

Estimation of Hyla cinerea Population Size at Settling Lagoons

We captured 302 individual *H. cinerea* (279 males and 23 females) during our four nights of sampling. Of those captured, 66 (22%) were recaptured at least once. The top model for abundance received 100% of the model weight and included capture probability varying among sampling nights, abundance varying between sexes; however, the capture probability for males (0.176, 95%CI=0.1-0.3) was only slightly greater than for females (0.171, 95%CI=0.07-0.35) (Table 2.1).

The top model estimate of male and female *H. cinerea* abundance was 517$\pm$ 96.6 (95% CI=390-791), and 43 $\pm$14 (95% CI=28-93) for the four, 25 m sections sampled (Table 2.2). Using these estimates, we estimated there were ~4,956 $\pm$ 979 *H. cinerea.* However, our estimate of females assumes a closed population. In reality, males were likely resident at wetlands throughout the breeding season, but female occurrence at breeding sites was more temporally diffuse such that most females were not present at breeding for 1-2 night intervals and not concurrently. Thus, there was temporary emigration of females within our closed capture period, which would result in an underestimation of female abundance when using a closed population model. A number of other studies indicate that adult sex ratios of amphibians are generally 1:1 (Greer and Byrne 1995, Teixeira et al. 2002). Therefore, if we consider our estimate of the male population more robust than the estimation of females and assume a similar adult female population size, then we estimate the *H. cinerea* breeding population Settling Lagoons was ~9,151 $\pm$ 1710. Our simulations of survey estimates required to generate precise population abundance estimates indicated that increasing to more than four occasions would only show diminishing returns on effort, and beyond six capture occasions the improvement of estimates relative to added effort would be negligible (Figure 2.3).

Occupancy of Hyla cinerea and native anurans in Cades Cove

We detected a total of seven species during our two seasons of summer sampling. These species included: Green Treefrog (*Hyla cinerea*), Cope's Gray Treefrog (*Hyla chrysoscelis*), Eastern Narrow-Mouthed Toad (*Gastrophryne carolinensis*), Green Frog (*Lithobates clamitans*), Bullfrog (*Lithobates catesbeianus*), Spring Peeper (*Pseudacris crucifer*), and American Toad (*Anaxyrus americanus*). A summary of which species were detected at which sites is available in Table 2.3. *Hyla cinerea* was detected at six of the nine sites, all of which had a least a partially open canopy and emergent herbaceous vegetation. Multiple-male choruses were detected at five sites (Figure 2.4), while only a single male *H. cinerea* was detected and verified calling at Methodist Pond on a single night in July 2013 (Figure 2.5). In addition, we opportunistically documented isolated male calling from an isolated oak tree in the field surrounding Hyatt Lane Pond, from shrubs and cane along the road side and ditch between Hyatt Lane and Shields Pond, and from riparian trees along Abrams Creek near the ford at Sparks Lane.

The top model for *H. cinerea* had initial occupancy invariant, colonization and extinction varying with forest cover, and detection probability varying with sampling event (Table 2.4, *Table 2.5*). The second ranked model was similar to the first with the exception that initial occupancy also varied with forest cover. The top models for other species had forest cover as a significant variable for one of the top two models (Table 2.6). For the early spring breeders, *A. americanus* and *P. crucifer*, the top model varied detection probability by sampling event. The second ranked model was more than two ΔAIC points below the first. The lack of other influential factors is likely a result of the limited number of sampling occasions during those species' breeding seasons. The null model was the top model for *G. carolinensis* and *L. catesbeianus* occupancy; however, the model that had extinction and colonization varying with forest cover and initial occupancy and detection probability as invariant was within two ΔAIC. The top model for *H. chrysoscelis* had initial occupancy invariant, extinction and colonization varying with forest cover, and detection probability varying with event. The top model for *Lithobates clamitans* had initial occupancy, colonization and extinction invariant with detection probability varying by event.

Discussion

Our results demonstrate that the invasion of *H. cinerea* in Cades Cove is far more advanced than was previously assumed. We are confident that *H. cinerea* were not established in Cades Cove before 2002 (Dodd 2003), and the species was not detected in 2007 (USGS 2014a); though we cannot evaluate whether the 2007 survey effort was adequate for detecting a small *H. cinerea* population. *H. cinerea* was first detected in 2011 and described as having a "small" breeding population in 2012. However, by 2013 we estimate that the breeding population of *H. cinerea* at the Settling Lagoons was between 4,500 and 9,200 adults. It is unlikely that a population of this size could have resulted from the introduction of a small number of individuals in the span of only three years. Given both the timing and the low probability of successful introductions, it is likely that multiple introductions and/or a large number of *H. cinerea* were introduced. Moreover, the occurrence of moderate to large male breeding choruses at four other sites distributed across the full extent of Cades Cove makes it seemingly improbable that those frogs were established from dispersers from a single introduction to the Settling Lagoons only two to three years prior. For example, a large breeding chorus was documented in both 2013 and 2014 ~5 km from the Settling Lagoons at the Abrams Creek oxbow and spring. This distance is further than frogs are known to disperse in a single season (Garton and Brandon 1975, Roble 1979, Semlitsch and Bodie 2003, Pellet et al. 2006). It is unlikely this was sufficient time for limited dispersal from a founding population at the Settling Lagoons to have established and grown to its current size.

While the source and true invasion history of *H. cinerea* to Cades Cove is not known, the timing and current pattern suggest several possibilities. The Settling Lagoons are adjacent to horse barns, and in 2008 and 2009, the Park contracted with a new operator based in Florida for both the horse concession and the Cades Cove store (Molly Schroer, NPS personal communication). The transition included the movement of heavy machinery as well as regular movement of equipment and horse trailers between Florida and Cades Cove. *H. cinerea* are abundant in Florida and common around farms where they frequently use manmade structures and objects as refugia. The regular movement of animals as “hitchhikers” on equipment and trailers could result in large numbers and repeated introductions of individuals adjacent to the Settling

Lagoons. In addition, it is possible that tourists traveling to the park have introduced frogs as "hitchhikers" on campers and other vehicles.

The current distribution of *H. cinerea* within Cades Cove is consistent with the species' habitat use within its native range and suggests *H. cinerea*'s distribution within the Cove may be limited by the distribution of wetland types. Breeding choruses of *H. cinerea* were only detected at wetlands that had at least a partially open canopy and shrubby or herbaceous emergent vegetation, and long or permanent hydroperiods. We hypothesize this pattern is related to variation in wetland productivity relative to the breeding phenology of *H. cinerea* within the Park. The majority of wetlands within Cades Cove have relatively short hydroperiods and are dry by mid-to-late summer in most years. *H. cinerea* breeding within the Cove does not begin until early to mid-June, and typically did not start in earnest before early July. *H. cinerea* tadpoles require a minimum of 25 days, but may often require at least 40 days to complete metamorphosis. Wetlands with partially open or open canopies and dense herbaceous cover are more productive and probably support more rapid larval development. As a result, population growth is likely dependent upon those wetlands that are highly productive and that, in most years, remain ponded through July.

Native anuran species show different occupancy rates and distributions within Cades Cove. Some species, such as *H. chrysoscelis* and *P. crucifer*, were detected at every wetland we surveyed, suggesting these species utilize the entire variety of sites present within the Cove for breeding. In contrast, species such as *L. catesbeianus* were only detected at wetlands that were known to hold water year-round. This is consistent with the fact that the species has a multiple-year larval period that requires perennial water for successful development (Lanoo 2005). We feel it is important to mention that no native species we documented in this study was limited to breeding at a single wetland. Therefore, if warranted, management strategies to eliminate *H. cinerea* from a breeding site that might have non-target effects on native species at the same site could be buffered by breeding at other sites in Cades Cove. However, *Gastrophryne carolinensis*, which is a GSMNP priority species, was detected breeding at only three wetlands, all of which were now occupied by *H. cinerea* breeding choruses. Larval competition occurs between *G. carolinensis* and *H. squirella*, which is a sister species to *H. cinerea*. Though *G .carolinensis* appears to be a superior competitor to *Hyla*, *Hyla* were less vulnerable to predation and may gain an indirect competitive advantage in some contexts (Walls et al. 2002). Therefore, *H. cinerea* has the potential to impact the limited number of breeding sites for *G. carolinensis*, and management efforts to eliminate *H. cinerea* might also impact *G. carolinensis*.

While the population of *H. cinerea* in Cades Cove is much larger and more established than previously thought, there are patterns that can inform management decisions. *H. cinerea* breeds in sites that are open and sunny and they require a hydroperiod of up to least 40 days starting in early June. Only a few wetlands within the Cove satisfy these conditions of being permanent, herbaceous wetlands that are ideal for *H. cinerea* reproduction. As such, permanent wetlands are expected to offer managers the largest potential of influencing the *H.* cinerea population in order to minimize impacts on native species. In particular, we predict that management strategies targeted at the Settling Lagoons could limit the *H. cinerea* population. The Settling Lagoons are one of three sites within the Cove known to hold water year round.

Given the consistent chorus at the Settling Lagoons, we believe that this is the largest and most established population within the Cove, and may be functioning as a significant source habitat supporting choruses at other wetlands. Since they are man-made ponds, management strategies targeting the lagoons may have a lesser impact on natural ecosystems and will thus be politically more palatable. The other two sites where breeding choruses were present periodically and the hydroperiods were relatively permanent were the Beaver Pond and the Abrams Creek spring. It is more difficult to think of efficient management options for these two sites if management targeted at the Settling Lagoons was insufficient. The relatively new beaver dam could be deconstructed to allow water to flow, limiting the potential for successful *H. cinerea* tadpole development. At Abrams Creek spring, hand removal of breeding adults might be enhanced using PVC refugia as a “trap”, though we suspect this would be an inefficient and labor intensive action. All management interventions would likely require years of continued effort to account for juvenile cohorts that have not been recruited into the breeding populations, and is premised on the assumption that future introductions are limited. It would be valuable for park managers to consider management actions as part of their decision on whether to continue to monitor or study the *H. cinerea* invasion.

Literature Cited

Babbitt, K. J., M. J. Baber, D. L. Childers, and D. Hocking. 2009. Influence of agricultural upland habitat type on larval anuran assemblages in seasonally inundated wetlands. Wetlands **29**:294-301.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. 2 edition. New York, Springer.

Clinton, W. 1999. Invasive Species. Pages 6183-6186 Executive Order 13112. Federal Register.

Conroy, M. J., and J. P. Carroll. 2009. Quantitative conservation of vertebrates. Chichester, West Sussex, UK ;, Wiley-Blackwell.

Dodd, C. K., Jr. 2003. Monitoring amphibians in Great Smoky Mountains National Park. U S Geological Survey Circular **1258**:i-vi, 1-117.

Fiske, I., and R. Chandler. 2011. unmarked: an R package for fitting hierarchical models of wildlife occurrence and abundance. Journal of Statistical Software **43**:1-23.

Garton, J. S., and R. A. Brandon. 1975. Reproductive ecology of the Green Treefrog, *Hyla cinerea*, in southern Illinois (Anura: Hylidae). Herpetologica **31**:150-161.

Grafe, T. U., M. M. Stewart, K. P. Lampert, and M. O. Rodel. 2011. Putting toe clipping into perspective: a viable method for marking anurans. Journal of Herpetology **45**:28-35.

Greer, A. E., and M. Byrne. 1995. Sex ratio and frequency of osteological abnormalities in the Australian hylid frog Litoria aurea from two apparently unpolluted localities in Sydney, New South Wales. Australian Zoologist **30**:43-47.

Gunzburger, M. S., and J. Travis. 2004. Evaluating predation pressure on green treefrog larvae across a habitat gradient. Oecologia **140**:422-429.

Kéry, M., and R. Chandler. 2012. Dynamic occupancy models in unmarked. Cran R Project Vignettes.

Laake, J. L. 2013. RMark: an R interface for analysis of capture-recapture data with MARK. AFSC Processed Rep 2013-01. Alaska Fish. Sci. Cent., NOAA, Natl. Mar. Fish. Serv, 7600 Sand Point Way NE, Seattle WA 98115.

Lanoo, M. J. 2005. Amphibian declines: the conservation status of United States species. Berkeley, University of California Press.

NPS. 2014. Annual park ranking report for recreation visitors. Department of the Interior.

Pellet, J., L. Rechsteiner, A. K. Skrivervik, J. F. Zurcher, and N. Perrin. 2006. Use of the Harmonic Direction Finder to study the terrestrial habitats of the European tree frog (*Hyla arborea*). Amphibia-Reptilia **27**:138-142.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management **46**:752-757.

Roble, S. M. 1979. Dispersal movements and plant associations of juvenile Gray Treefrogs, *Hyla versicolor* Le Conte. Transactions of the Kansas Academy of Science (1903-) **82**:235-245.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology & Systematics **32**:305.

Semlitsch, R. D., and J. R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology **17**:1219-1228.

Team, R. C. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Teixeira, R. L., J. A. P. Schineider, and G. I. Almeida. 2002. The occurrence of amphibians in bromeliads from a southeastern Brazilian restinga habitat, with special reference to Aparasphenodon brunoi (Anura, Hylidae). Brazilian Journal of Biology **62**:263-268.

U.S.C. 1916. National Park Service Organic Act. 16 U.S.C. §1. U.S. Department of the Interior, United States of America.

USGS. 2012. North American Amphibian Monitoring Protocol (NAAMP).

USGS. 2014a. North American Amphibian Monitoring Protocol (NAAMP), Route Data Summary. Cades Cove.

USGS. 2014b. North American Amphibian Monitoring Protocol (NAAMP), Species Detection Map.

Walls, S. C., D. G. Taylor, and C. M. Wilson. 2002. Interspecific differences in susceptibility to competition and predation in a species-pair of larval amphibians. Herpetologica **58**:104-118.

White, G. C., and K. P. Burnham. 1999. Program MARK. Survival estimation from populations of marked animals, Bird Study 46 Supplement, 120-138.

Figures

Figure 2.1. Settling Lagoons at Cades Cove with the red lines indicating the four, 25 meter transects used for CMR sampling.

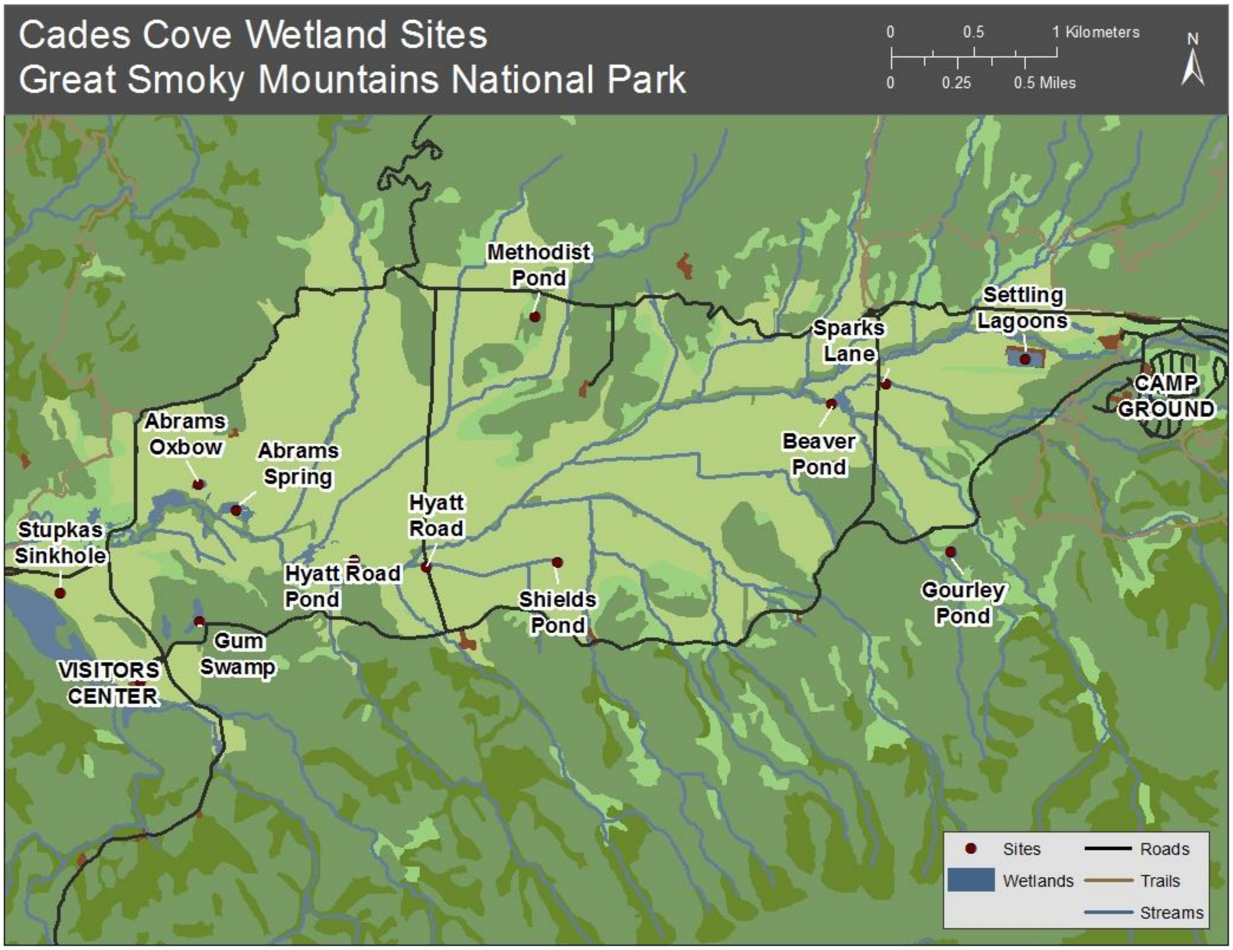

Figure 2.2. Locations used in 2013 and 2014 call surveys in Cades Cove, Great Smoky Mountains National Park. Also shown is the Cades Cove Campground.

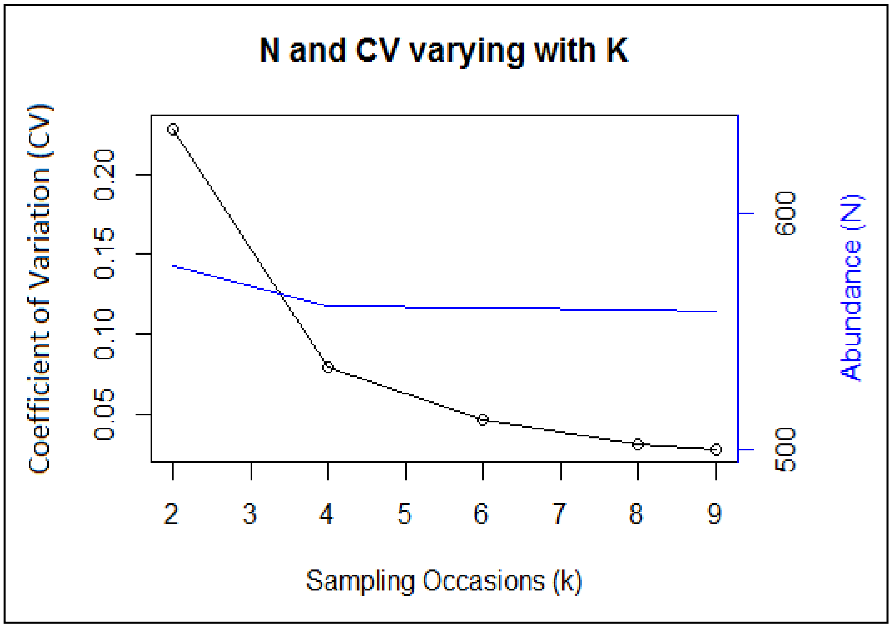

Figure 2.3. Simulation results: Abundance (N) and Coefficient of Variation (CV%) varying with capture occasions (K) in a closed CMR model for the abundance estimation of *H. cinerea* at GSMNP.

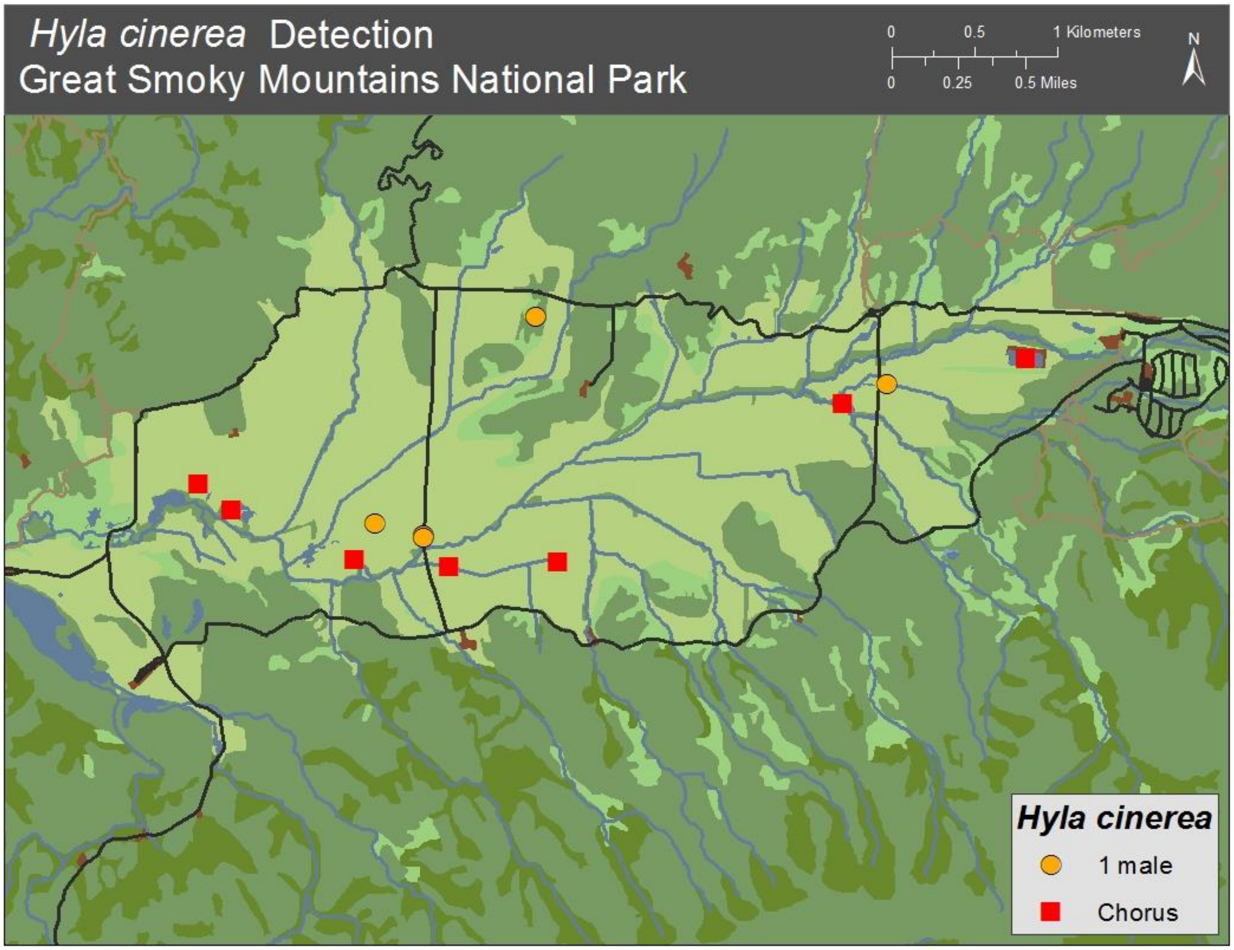

Figure 2.4. Locations where *H. cinerea* choruses and single individuals were detected.

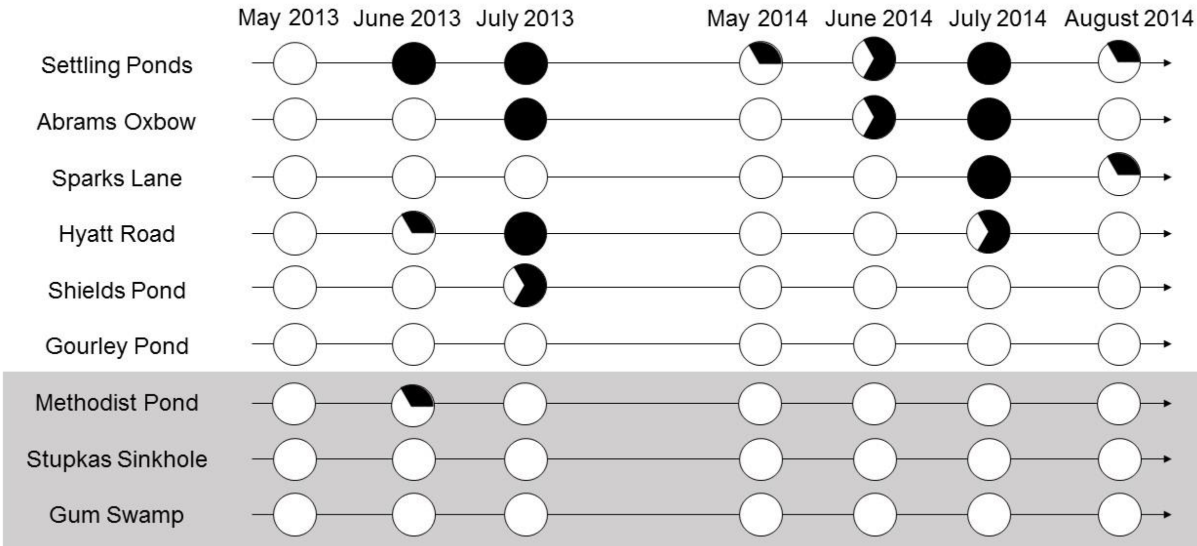

Figure 2.5. Detections of *H. cinerea* at each site during each month. The full black circles represents primary occasions that had detections in all three secondary occasions. The 1/3 and 2/3 full circles represent 1 or 2 of the secondary occasions having detections. The bottom three sites (with the shaded box behind them) are the shaded sites with forested canopy.

Tables

Table 2.1. Model structure, number of parameters, AICc, Delta AICc (=AICi-min(AICc)) and AIC weight for closed population CMR Models for estimating *H. cinerea* abundance at GSMNP. Capture probability (p) was either held constant (~1), varied by sampling event (~time), varied by sex (~Sex), or varied by sex and recapture probability (~Sex+c). Recapture probability (c) was either held equal to initial capture probability () or allowed to vary on its own (~1). Population abundance (N) was either held constant (~1) or allowed to vary by sex (~Sex).

Model	Parameters	AIC_c	Delta AIC_c	Weight
p(~time)c()N(~Sex)	6	-1497.16	0	1
p(~time)c()N(~1)	5	-1395.14	102.0256	0
p(~1)c()N(~Sex)	3	-1392.57	104.5942	0
p(~Sex)c()N(~Sex)	4	-1390.56	106.6049	0
p(~Sex + c)c()N(~Sex)	5	-1388.64	108.5258	0
p(~Sex)c()N(~1)	3	-1378.7	118.4606	0
p(~Sex + c)c()N(~1)	4	-1377.2	119.9623	0
p(~1)c(~1)N(~1)	3	-1322.64	174.5261	0
p(~1)c(~1)N(~1)	3	-1322.64	174.5261	0
p(~1)c()N(~1)	2	-1279.91	217.2488	0

Table 2.2. Abundance (N) and capture probability (p) estimates from the top closed population CMR model for the population of *H. cinerea* at GSMNP. The unequal population abundance estimates reflects the assumption of closure for females, rather than the reality of temporary emigration and limited availability for capture.

Parameter	Estimate	Standard Error	Lower 95% CI	Upper 95% CI
N (fem)	43.037	14.254	28.716	93.236
N (male)	517.332	96.609	389.963	790.905
1:p (fem)	0.171242	0.0682933	0.074466	0.346679
2:p (male)	0.175907	0.0438214	0.10557	0.278516

Table 2.3. Sites at which each detected anuran species was heard calling in Cades Cove, Great Smoky Mountains National Park during 2013 and 2014 summer sampling seasons. Also indicated is the “Forest Cover” of the site, whether the site is considered shaded and deciduous (1) or open canopy and herbaceous (0).

	Forest Cover	*Hyla cinerea*	*Gastrophryne carolinensis*	*Anaxyrus americanus*	*Hyla chrysoscelis*	*Pseudacris crucifer*	*Lithobates catesbeianus*	*Lithobates clamitans*
Settlings Ponds	0	•	•	•	•	•	•	•
Abrams Oxbow Cemetery	0	•	•		•	•	•	•
Shield Pond	0	•		•	•	•		
Hyatt Road	0	•	•	•	•	•		•
Gourley Pond	0				•	•		
Sparks Lane	1	•			•	•	•	•
Methodist Pond	1	•			•	•	•	•
Stupkas Sinkhole	1			•	•	•		•
Gum Swamp	1			•	•	•		•

Table 2.4. Models for estimating *H. cinerea* occupancy and detection probability at GSMNP ranked using AIC. Parameters is the number of estimate parameters for the model (i); Delta AIC_c is AIC_i – min(AIC); Weight is the rounded Akaike weights. In the Model description F represents the parameter varying by forest and E is the parameter varying by sampling event. “psi” is initial occupancy, “gam” is extinction, “eps” is colonization, and “p” is detection probability.

Model	Parameters	AIC	ΔAIC	Weight	Negative Log Likelihood
psi(.)gam(F)eps(F)p(E)	11	98.78966	0.0000	0.6783	38.395
psi(F)gam(F)eps(F)p(E)	12	100.7429	1.9533	0.2554	38.371
psi(.)gam(.)eps(.)p(E)	9	103.5761	4.7864	0.0620	42.788
psi(.)gam(F)eps(F)p(.)	6	109.6854	10.8957	0.0029	48.843
psi(F)gam(F)eps(F)p(.)	7	111.5108	12.7211	0.0012	48.755
psi(.)gam(.)eps(.)p(.)	4	114.6792	15.8895	0.0002	53.340

Table 2.5. Model estimates for the top model for *Hyla cinerea* colonization (col), extinction (ext), detection probability (p) and initial occupancy (psi).

Parameter	Estimate	Standard Error
col(forest1)	-7.465	25.309
col(Int)	-1.204	1.219
ext(forest1)	11.532	78.529
ext(Int)	-2.127	1.184
p(eventJune2013)	1.559	1.332
p(eventJuly2013)	3.978	1.328
p(eventMay2014)	-0.658	1.594
p(eventJune2014)	1.283	1.167
p(eventJuly2014)	3.990	1.354
p(Int)	-1.594	0.819
psi(Int)	0.048	1.403

Table 2.6. Top models for occupancy, extinction, colonization, and detection probability for anuran species detected in Cades Cove, Great Smoky Mountains National Park during summer 2013 and 2014. In the Model description F represents the parameter varying by forest and E is the parameter varying by sampling event. “Psi” is initial occupancy, “gam” is extinction, “eps” is colonization, and “p” is detection probability.

Species	Top Model	Model Weight	ΔAIC	Negative Log Likelihood	Parameters
Anaxyrus americanus	psi(.)gam(.)eps(.)p(E)	0.679	0	17.527	9
	psi(F)gam(F)eps(F)p(E)	0.106	3.711	16.382	12
Gastrophryne carolinensis	psi(.)gam(.)eps(.)p(.)	0.421	0	20.805	4
	psi(.)gam(F)eps(F)p(.)	0.242	1.107	19.358	6
Hyla cinerea	psi(.)gam(F)eps(F)p(E)	0.678	0	38.395	11
	psi(F)gam(F)eps(F)p(E)	0.255	1.953	38.371	12
Hyla chrysoscelis	psi(.)gam(F)eps(F)p(E)	0.532	0	55.794	4
	psi(.)gam(.)eps(.)p(E)	0.272	1.338	58.463	6
Lithobates catesbeianus	psi(.)gam(.)eps(.)p(.)	0.386	0	46.136	4
	psi(.)gam(F)eps(F)p(.)	0.302	0.489	44.380	6
Lithobates clamitans	psi(.)gam(.)eps(.)p(E)	0.537	0	28.298	9
	psi(.)gam(F)eps(F)p(E)	0.270	1.377	26.986	11
Pseudacris crucifer	psi(.)gam(.)eps(.)p(E)	0.767	0	66.517	9
	psi(.)gam(F)eps(F)p(E)	0.141	3.394	66.214	11

CHAPTER 3

AN EXPERIMENTAL TEST OF THE POTENTIAL FOR DECIDUOUS CANOPY COVER TO LIMIT *HYLA CINEREA* LARVAL RECRUITMENT WITHIN CADES COVE, GREAT SMOKY MOUNTAINS NATIONAL PARK

Asper, J.R. and J.C. Maerz. To be submitted to undetermined journal.

Abstract

The objectives of this study were to determine whether canopy openness and associated herbaceous or deciduous plant resources create larval growth environments that limit the current and potential future distribution of invasive *Hyla cinerea* within Cades Cove, Great Smoky Mountains National Park. We used a “common garden” of mesocosms containing leaf litter from one of three herbaceous of one of three forested wetlands to test the hypothesis that *H. cinerea* larval development rate and survival would be greater in open canopy environments with herbaceous detritus compared to shaded environments with deciduous leaf litter. We predicted that the combination of deciduous leaf litter and shade would reduce larval development such that *H. cinerea* tadpoles would likely fail to achieve metamorphosis within the period that many Cades Cove wetlands remain ponded. As expected, our median *H. cinerea* development rate 17% slower in shaded mesocosm containing deciduous leaf litter compared to open mesocosms containing herbaceous leaf litter; however larval survival did not differ among treatments. No tadpoles in the shaded, deciduous treatments achieved a

metamorphic stage within the period when most wetlands in Cades Cove were ponded. *H. cinerea* tadpoles did not show differences in stage specific body size, suggestion little plasticity in larval *H. cinerea* development. These results suggest that short hydroperiod and low growth conditions in forested wetlands limit the capacity for those wetlands to support *H. cinerea* population. This is consistent with the current distribution of *H. cinerea* within Cades Cove, which is limited to open, herbaceous water bodies with relatively long permanent hydroperiods. Our results suggest limited capacity for *H. cinerea* invasion to spread to forested wetlands with Cades Cove.

Introduction

The different stages of invasion (introduction, establishment, spread, and impact) are generally governed by different processes, and therefore, the types of management approaches depend on the stage of invasion (Sakai et al. 2001). Once introduced, the population growth and spread of nonnative species is difficult but important to forecast. Invaders often undergo a lag phase before the population increases and spreads. Management during this phase is best targeted at limiting the growth and spread of species and, potentially, eradicating populations while they remain small. Once invasive species have achieved higher abundance, management may shift towards limiting invader abundance and population growth (Sakai et al. 2001). Important in both these stages of invasion and management is identifying source and sink habitats. Identifying source habitats and factors that limit population growth and the distribution of invasive species can lead to management strategies that use source-sink dynamics or metapopulation dynamics to control invader abundance. Moreover, identifying source habitats and factors that limit population growth can reduce management costs by concentrating efforts on source habitats.

Wetland-breeding amphibians are long recognized as models systems for the importance of source-sink and metapopulation dynamics. Amphibian populations are affected by the availability and connectedness of suitable breeding sites within the landscape (Semlitsch 2000, 2002, Petranka 2007). Source-sink dynamics have strong effects on local extinction and colonization and, therefore, patterns of occupancy and abundance among potential breeding sites. Many species occupy breeding sites that are, in many years, sinks subsidized by dispersal from more productive breeding sites within the landscape (Petranka 2007).

Productive breeding sites are those that allow for rapid larval growth to an optimal size at metamorphosis. Larval performance is largely governed by hydroperiod, which affects the potential for catastrophic larval failure, predator and competitor abundance, and wetland vegetation and canopy structure (Skelly 2002, Skelly et al. 2002, Rubbo and Kiesecker 2004, Maerz et al. 2005, Brown et al. 2006, Williams et al. 2008, Maerz et al. 2010, Stoler and Relyea 2011, Cohen et al. 2012, Earl et al. 2012, Earl and Semlitsch 2015). More permanent water bodies tend to have high densities of predators including fish, and therefore, tend to support more predator resilient species with longer larval periods such as bullfrogs (*Lithobates catesbeianus*) (Denton and Richter 2013). Forested wetlands generally have low productivity because of shade and poorer quality deciduous litter inputs. In contrast, open canopy water bodies are generally more productive due to high light and dense herbaceous vegetation that can be higher in nutrient quality. The combination of high productivity and low predator abundance make open, short hydroperiod wetlands particularly productive environments for some amphibians (Semlitsch 2000, 2002, Petranka 2007). Some anuran species utilize a wide variety of breeding habitats, and they accommodate differences in resource availability, predation risk, and hydroperiod with highly plastic larval morphology and life histories. Those species accelerate or reduce development rates in response to growth potential or risk, and can trade off a larger size at metamorphosis for accelerated development when conditions are no longer favorable. Alternatively, some species have evolved resilience to predators and strong competitive abilities, and therefore, tend to breed in larger, more permanent water bodies. These species may show reduced plasticity in larval development and thus have more restricted distributions among potential breeding sites (Semlitsch 2000, 2002, Petranka 2007, Denton and Richter 2013).

Within the past decade, Green treefrogs (*Hyla cinerea*) were introduced to Cades Cove, Great Smoky Mountains National Park (GSMNP). In 2013-2014, we determined that *H. cinerea* occupy six wetlands distributed across the full extent of Cades Cove; however, occupancy of breeding adult *H. cinerea* was clearly limited to open herbaceous wetlands with relatively permanent to extended hydroperiod (Asper, Chapter 2). Within their native range, adult *H. cinerea* are known to occur across a range of aquatic habitats, however, reproductive success of this species is typically associated with more permanent water bodies and longer hydroperiod wetlands, particularly those with more open canopies surrounded by pasture or prairie uplands (Gunzburger and Travis 2004, Jensen 2008, Babbitt et al. 2009). These types of wetlands with adequate hydroperiod and appropriate vegetative qualities are naturally limited within Cades Cove, potentially limiting the distribution, abundance, and impact of *H. cinerea* within Cades Cove. In addition, because some of the longer hydroperiod, open canopy sites within Cades Cove are manmade they may be amenable to management to limit *H. cinerea* breeding success.

The objectives of this study were to determine whether shade and associated deciduous plant resources create larval growth environments that would limit breeding success for *H. cinerea* at many sites within Cades Cove. We hypothesized that *H. cinerea* larval performance (survival rate, percent reaching metamorphosis, and development rate) would be greater in unshaded environments with herbaceous detritus compared to shaded environments with deciduous leaf litter. Moreover, we predicted that the combination of deciduous leaf litter and shade would slow larval growth and development such that larval *H. cinerea* would likely fail to reach metamorphosis within the period that many Cades Cove wetlands remain ponded.

Methods

We used mesocosms in a ‘common garden’ to test the effects of shade and vegetation type on *H. cinerea* larval performance. We had two main treatments: unshaded mesocosms with herbaceous litter or shaded mesocosms with deciduous leaf litter. For each main treatment, we collected herbaceous or deciduous plant litter from three sites, three open herbaceous wetlands (Abrams Creek Oxbow, Shield’s Pond, and Gourley Pond) and three deciduous forest sites (Methodist Pond, Gum Swamp, and Stupkas Sinkhole Pond). Abrams Creek Oxbow and Shield’s Pond are two open, herbaceous wetlands where we documented *H. cinerea* breeding the previous year

(Asper, Chapter 2). *H. cinerea* have not been detected at Gourley Pond, but the site has a relatively short hydroperiod not typical of H. cinerea breeding sites. Nonetheless, it has an open canopy and dense herbaceous growth in the wetland. *H. cinerea* were not detected at Gum Swamp and Stupkas Sinkhole Pond, but a single male *H. cinerea* was detected calling at Methodist Pond on single night in 2013 (Asper, Chapter 2). Methodist Pond is forested, but has a gap in the canopy and patch of dense herbaceous vegetation under the canopy gap within the wetland. We collected plant material from each wetland in March 2014 by trimming senesced herbaceous vegetation within the ponded areas of wetlands or collecting deciduous leaf litter from the ground. We placed the litter in cotton pillow cases sealed with zip ties, and transported the leaf litter to the University of Georgia where we air dried the litter at 60°C.

We set up 18 mesocosms within the fenced area around the Settling Lagoons near the Cades Cove campground. Our design was nine replicates of each main treatment (open-herbaceous vs. shaded-deciduous), with three replicates per treatment containing plant litter from each of six sites. We arranged mesocosms in three blocks of six mesocosms each (Figure 3.1). Half of each block was covered with a tent of 70% shade cloth. Within each block we randomly stocked the unshaded mesocosms each with herbaceous litter from one of the open, herbaceous sites, and we randomly stocked shaded mesocosms each with leaf litter from one of the forested sites. Mesocosms were oval-shaped polypropylene cattle watering tanks that were approximately 0.3 m x 0.6 m x 0.9 m and had a capacity of 273 L of water. We added 300 g of dried vegetation from the assigned source site to each mesocosm, which was consistent with ambient biomass among our sites and with other published studies. For each mesocosm, we strained 1 L inoculate of water and soil from the respective source site through a mesh strainer to remove debris, and added it to the mesocosm. The mesocosms were allowed to age for 3.5 weeks before the tadpoles were added, allowing time period for initial microbial, fungal, and algal growth. We covered each mesocosms with a mesh cover to keep other frogs and potential predators from entering mesocosms and to keep metamorphs from escaping.

To collect *H. cinerea* eggs, we collected adult *H. cinerea* from the Settling Lagoons and placed them in extra mesocosms containing water and a limited amount of vegetation. We covered the mesocosms and left the animals overnight. Once the animals bred, we removed them and released them back at the Settling Lagoons. We collected the egg masses from the mesocosms and placed them individually in large Ziploc bags filled with water from the mesocosm. We transferred each egg masses to an individual aquaria containing dechlorinated tap water and an air bubbler. Seven days after hatching, we transported 30, haphazardly selected tadpoles to each experimental mesocosm, a density based on other mesocosm experiments (Morin 1983). We checked mesocosms daily for *H. cinerea* metamorphs. We removed any metamorphic frogs from the mesocosm, weighed and measured them (wet mass and snout-vent length = SVL), and released them at the Settling Lagoons. After 40 days (August 6, 2014), we terminated the experiment. We collected all living tadpoles from each mesocosm, and determined their body size, mass and developmental stage (Gosner 1960).

For each mesocosm, we determined the percentage of larvae still alive; the percent that had reach a metamorphic stage (Gosner stage >39), the percent that metamorphosed (Gosner stage 46); and median Gosner stage achieved. We used a nested analysis of variance to test the effects of our main treatment (open-herbaceous versus shaded-deciduous) and site on the percent larval survival, percent metamorphic, and median Gosner stage. Site was a nested factor within the main treatment.

Because many tadpoles did not have time to reach metamorphosis, we could not evaluate main treatment or site effects on size at metamorphosis, which is a common and important metric of larval amphibian studies. Instead, we examined whether there were difference in Gosner stage-specific larval size that would suggest larvae in different treatments might metamorphose at different sizes. For our analysis of tadpole SVL, we used all tadpoles in our analysis; however, because the relationship between Gosner stage and mass (g) was not linear beyond stage 40, we only used data on those tadpoles that were Gosner stage 40 or less in analysis of wet mass. We modeled both measures of tadpole size as a function of our main treatment (open-herbaceous versus shaded-deciduous), Gosner stage, and the interaction between main treatment and Gosner stage. To avoid over parameterizing our model, we did not include site as a nested factor in this analysis, and because tadpoles varied widely in Gosner stage within mesocosms, we used individual tadpoles as our unit of replication for these analyses.

Results

Of the 540 individuals stocked into the mesocosms, we recovered 363 (67%) at the end of the experiment. Survival rate was highly variable among treatments and within some treatments. A total of 83 individuals achieved a metamorphic Gosner stage

(> 39), and 13 completed metamorphosis (Gosner stage = 46) within the 40 day period. There was no difference in larval survival to 40 days between the open-herbaceous and shaded-deciduous treatment. However, consistent with our hypotheses, a higher percentage of tadpoles reared in the open-herbaceous treatment achieved a metamorphic Gosner stage (41% in herbaceous vs. 3% in deciduous) within the 40 days and achieved a higher median Gosner stage (38 in herbaceous vs. 31 in deciduous; 17.3% more developed) (Table 3.1 and Table 3.2). While the individuals in the open-herbaceous treatment developed 17% more quickly, there was no difference in tadpole wet mass or SVL relative to Gosner stage between the open-herbaceous and shaded-deciduous treatments (Figure 3.3, Table 3.3). Both SVL and wet mass increased with Gosner stage (Table 3.3, Figure 3.3). There was no main treatment effect on Gosner stage-specific wet mass. There was a statistically significant main treatment effect on Gosner stage-specific SVL; however, this effect was negligible and not likely biologically meaningful (Table 3.3, Figure 3.3).

Discussion

Our experiment confirmed the negative effects of shade and deciduous leaf litter on larval *H. cinerea* growth and development, suggesting those conditions could limit the current distribution of the species in Cades Cove via effects on the larval growth environment. Even in an “optimistic” mesocosm environment that lacked predators, the developement time required for *H. cinerea* tadpoles in the deciduous treatment well exceeded the time period when sites in Cades Cove with those conditions typically remain ponded. It took at least 36 days for *H. cinerea* tadpoles to metamorphose in the herbaceous treatment, which is within the larval period range reported for the species in its native range (Lanoo 2005). In Cades Cove, this would typically result in the earliest emergence of *H. cinerea* metamorphs in mid July through early August. In most years, most wetlands within Cades Cove are dry by this period. In 2013, which was an exceptionally wet year, most wetlands were dry by the first week of August, and in 2014, which was an average year, most wetlands were dry by mid July. We believe that even in an exceptional year when forested sites might remain ponded into the fall, the slow development rate of *H. cinerea* under the low productivity conditions for forested wetalnds would result in tadpoles emerging too late to grow and survive the winters at this latitude.

Based on our field observations and personal communications with GSMNP staff, only three relatively open, herbaceous sites within Cades Cove are permanently or perennially ponded for the time required for *H. cinerea* to complete its larval development: the Settling Lagoons, the newly formed Sparks Lane Beaver Pond, and Abrams Creek Spring. Indeed, these three sites support the only stable and large breeding choruses of *H. cinerea* we previously detected in Cades Cove (Asper, Chapter 2). Other sites where *H. cinerea* bred in 2013 but not 2014 likely support successful larval development in exceptionally wet years. As a result, the more ephemeral, open wetlands may contribute to positive population growth episodically, but likely function in most years as sink habitats if breeding occurs at all. Though our experiment did not include a treatment of conditions at the Settling Lagoons, it can be assumed that the nutrient levels from sewage are very high, and we know from direct observation that the site is highly productive for *H. cinerea*.

The tadpoles reared in shaded-deciduous litter showed slow development in treatments from all three source sites. The majority of individuals were still very early in development after 40 days. However, there were differences in larval development rates among the three shaded-decidous source site treatments. Specifically, tadpoles in the shaded treatments with plant litter from Methodist Pond were more developed than tadpoles in the other two forested source sites and intermediate between those sites and the three open-herbaceous source sites. Methodist Pond was our one forested site with a natural canopy gap with an stand of dense herbaceous plants under the gap. We hypothesize that tadpoles developed faster in this shaded treatment becaue plant litter from Methodist Pond included a mixture of hardwood and herbaceous material. These results are consistent with other studies that have examined the effects of herbaceous versus deciduous litter and light effects on tadpole growth and development (Earl et al. 2012, Earl and Semlitsch 2015). Methodist Pond has a longer hydroperiod than most of the forested wetlands in Cades Cove, so it is possible that the site might support limited larval development. Methodist Pond is the only forested site where *H. cinerea* has been detected within Cades Cove. In 2013, we detected a single male calling on a single night; however, the species has not been detected at the site in numerous surveys since.

One important result from our work is that it suggest that *H. cinerea* invasions of GSMNP are likely limited to Cades Cove. Because Cades Cove is managed as an agrarian landscape, it contains open, herbaceous wetlands in a landscape that would naturally be forested. Our results suggest that the shaded, deciduous conditions of wetlands within a forested landscape would likely exclude the successful establishment of *H. cinerea*. Most of GSMNP including the majority of its wetlands remain forested and therefore likely unsuitable for *H. cinerea*.

In summary, we have shown that the effects of shade and deciduous litter associated with many of the forested wetlands limit *H. cinerea* larval performance such that only a few wetlands within Cades Cove can likely support successful *H. cinerea* larval development and supporting positive population growth. All forested sites and many herbaceous sites have hydroperiods that are too short to support larval development in most years. We believe that three wetlands have sufficient hydroperiod and productivity to support *H. cinerea* populations, and management actions could target these sites. The Settling Lagoons are a highly productive site for *H. cinerea*, and they are a manmade features that could be drained periodically during the larval period or treated chemically to prevent kill *H. cinerea* larvae. Altering the hydrology of the Sparks Lane Beaver Pond would shorten the hydroperiod might also limit *H. cinerea* larval success. Management strategies for the Abrams Creek Oxbow are more challenging. The site has a perrenial source of water including a spring. It may be difficult to affect the larval environment of *H. cinerea* at that site, requiring actions targeted at other life stages.

Literature Cited

Babbitt, K. J., M. J. Baber, D. L. Childers, and D. Hocking. 2009. Influence of agricultural upland habitat type on larval anuran assemblages in seasonally inundated wetlands. Wetlands **29**:294-301.

Brown, C., B. Blossey, J. Maerz, and S. Joule. 2006. Invasive plant and experimental venue affect tadpole performance. Biological Invasions **8**:327-338.

Cohen, J. S., J. C. Maerz, and B. Blossey. 2012. Traits, not origin, explain impacts of plants on larval amphibians. Ecological Applications **22**:218-228.

Denton, R. D., and S. C. Richter. 2013. Amphibian communities in natural and constructed ridge top wetlands with implications for wetland construction. Journal of Wildlife Management **77**:886-896.

Earl, J. E., K. E. Cohagen, and R. D. Semlitsch. 2012. Effects of leachate from tree leaves and grass litter on tadpoles. Environmental Toxicology and Chemistry **31**:1511-1517.

Earl, J. E., and R. D. Semlitsch. 2015. Effects of tannin source and concentration from tree leaves on two species of tadpoles. Environmental Toxicology and Chemistry **34**:120-126.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**:183-190.

Gunzburger, M. S., and J. Travis. 2004. Evaluating predation pressure on green treefrog larvae across a habitat gradient. Oecologia **140**:422-429.

Jensen, J. B. 2008. Amphibians and reptiles of Georgia. Athens, University of Georgia Press.

Lanoo, M. J. 2005. Amphibian declines: the conservation status of United States species. Berkeley, University of California Press.

Maerz, J. C., C. J. Brown, C. T. Chapin, and B. Blossey. 2005. Can secondary compounds of an invasive plant affect larval amphibians? Functional Ecology **19**:970-975.

Maerz, J. C., J. S. Cohen, and B. Blossey. 2010. Does detritus quality predict the effect of native and non-native plants on the performance of larval amphibians? Freshwater Biology **55**:1694-1704.

Morin, P. J. 1983. Predation, competition, and the composition of larval anuran guilds. Ecological Monographs **53**:119-138.

Petranka, J. W. 2007. Evolution of complex life cycles of amphibians: bridging the gap between metapopulation dynamics and life history evolution. Evolutionary Ecology **21**:751-764.

Rubbo, M. J., and J. M. Kiesecker. 2004. Leaf litter composition and community structure: translating regional species changes into local dynamics. Ecology **85**:2519-2525.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annual Review of Ecology & Systematics **32**:305.

Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management **64**:615-631.

Semlitsch, R. D. 2002. Critical elements for biologically based recovery plans of aquatic-breeding amphibians. Conservation Biology **16**:619-629.

Skelly, D. K. 2002. Experimental venue and estimation of interaction strength. Ecology **83**:2097-2101.

Skelly, D. K., L. K. Freidenburg, and J. M. Kiesecker. 2002. Forest canopy and the performance of larval amphibians. Ecology **83**:983-992.

Stoler, A. B., and R. A. Relyea. 2011. Living in the litter: the influence of tree leaf litter on wetland communities. Oikos **120**:862-872.

Williams, B. K., T. A. G. Rittenhouse, and R. D. Semlitsch. 2008. Leaf litter input mediates tadpole performance across forest canopy treatments. Oecologia **155**:377-384.

Figures

Figure 3.1. Mesocosm set-up showing 2 treatments (i.e., herbaceous or deciduous) with 3 experimental blocks of 3 tanks each.

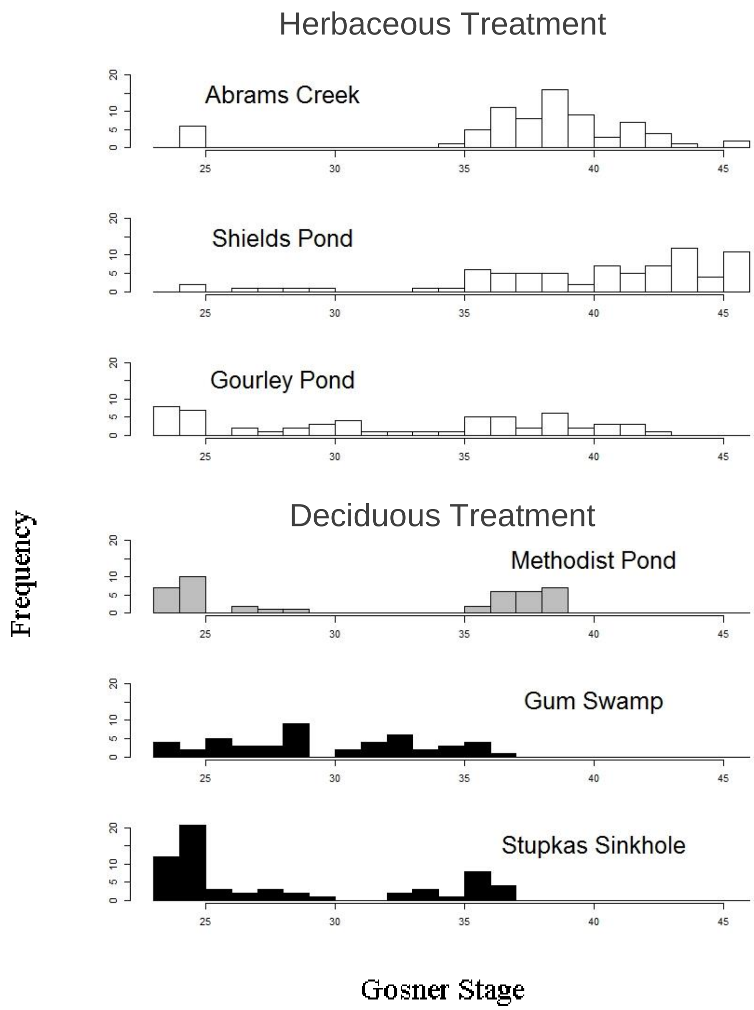

Figure 3.2. Distribution of individuals by Gosner stage for each treatment and plant source wetland. White bars are herbaceous sites, while grey and black bars are deciduous leaf litter sites. The grey bars for Methodist Pond signify that this site had a mixture of deciduous and herbaceous plan material, but grouped with the deciduous sites for statistical analysis.

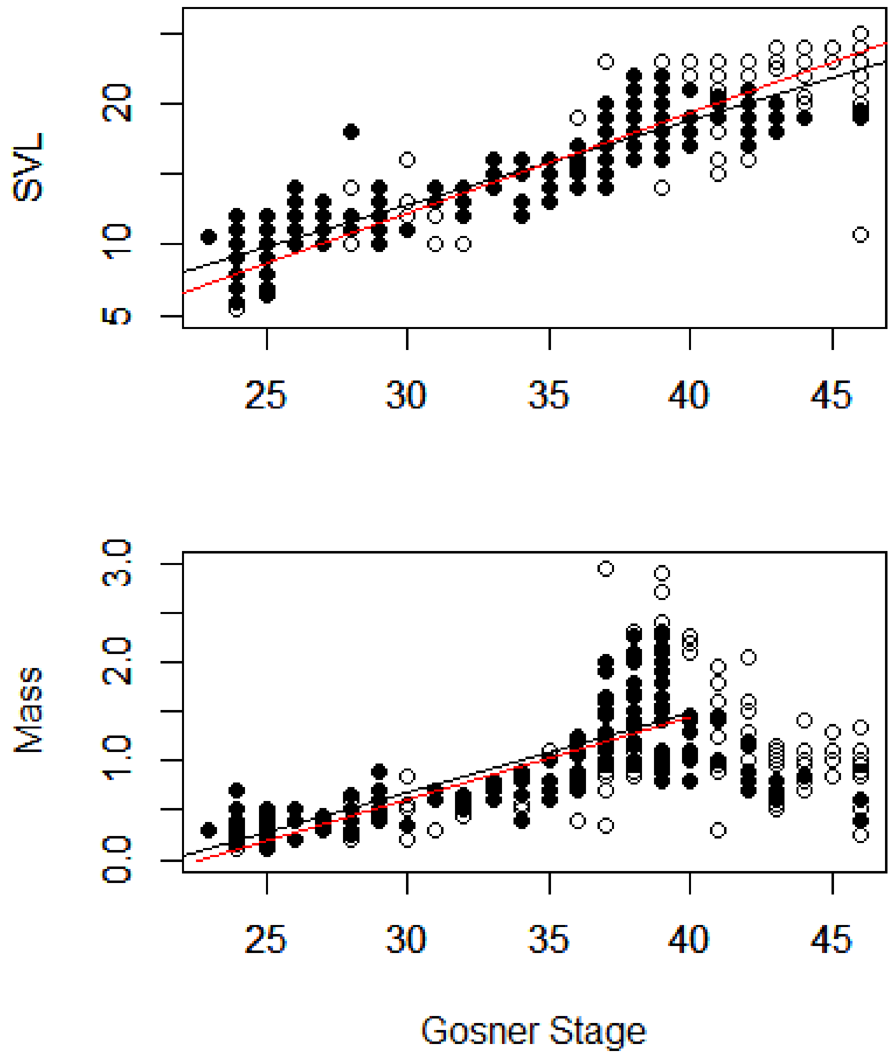

Figure 3.3. Gosner Stage as it varies with mass and SVL for decidous sites (black) and herbaceous sites (white). With trendlines for all points for SVL and Gosner Stages less than 40 for Mass (shaded = black, sunny = red)

Tables

Table 3.1. Mean tadpole performance among mesocosm treatments. For our main treatment, open-herbaceous treatments are shown on the left [no shading], and shaded-deciduous sites are shown on the right [grey shading]. Results are also broken down by the nested factor of source site for the plant material. There were three replicate mesocosms per source site.

	Abrams Oxbow	Gourley Pond	Shield Pond	Methodist Pond	Gum Swamp	Stupkas Sinkhole
Mean Gosner Stage	38.239	32.908	40.510	32.814	32.110	28.145
Median Gosner Stage	39.000	33.833	41.000	33.167	32.333	28.667
Maximum Gosner Stage	43.667	39.667	46.000	34.667	36.000	33.667
Percent Survival	0.811	0.644	0.856	0.467	0.533	0.722
Count Metamorphic (Gosner Stage > 39)	14.000	5.000	17.667	2.333	0	0
Percent Metamorphic	0.467	0.167	0.589	0.078	0	0
Count Metamorphosis (Gosner Stage 46)	0.667	0	3.667	0	0	0
Percent Metamorphosis	0.022	0	0.122	0	0	0
Mean SVL	17.471	14.779	19.205	13.922	13.872	11.675
Median SVL	18.000	15.833	19.333	14.167	13.833	11.667
Mean Mass	1.091	0.965	0.903	1.008	0.771	0.480
Median Mass	1.158	1.017	0.900	1.025	0.767	0.483

Table 3.2. Results of nested ANOVA of the main treatment (open-herbaceous vs. shaded-deciduous) and plant litter source site effects on median Gosner stage, percent larval survival, and percentage of larvae that achieved a metamorphic Gosner stage.

Variable Factor	MS	df	F	P
Median Gosner stage				
Intercept	21632	1	866.24	<0.001
Main treatment	193	1	7.74	0.017
Source site (nested)	29	4	1.17	0.374
Error	25	12		
Percent larval survival				
Intercept	8.134	1	119.25	<0.001
Main treatment	0.173	1	2.54	0.137
Source site (nested)	0.044	4	0.66	0.632
Error	0.068	12		
Percent metamorphic				
Intercept	0.302	1	15.408	0.002
Main treatment	0.302	1	15.408	0.002
Source site (nested)	0.076	4	3.871	0.030
Error	0.020	12		

Table 3.3. Results of GLM of the main treatment (open-herbaceous vs. shaded-deciduous) on Gosner stage-specific wet mass and SVL.

Variable Factor	MS	df	F	P
Wet mass (g)				
Intercept	26.486	1	226.782	<0.001
Gosner stage	54.430	1	466.059	<0.001
Main treatment	0.077	1	0.657	0.418
Gosner X Main treatment	0.042	1	0.358	0.550
Error	0.117	275		
Snout-vent length (mm)				
Intercept	368.93	1	146.924	<0.001
Gosner stage	1269.85	1	1269.849	<0.001
Main treatment	17.12	1	6.819	0.010
Gosner X Main treatment	15.81	1	6.297	0.013
Error	2.51	362		

CHAPTER 4

USING INDIVIDUAL BASED MODELING (IBM) TO EVALUATE INVASION SCENARIOS AND INFORM MANAGEMENT OF GREEN TREEFROGS (*HYLA CINEREA*) IN CADES COVE, GREAT SMOKY MOUNTAINS NATIONAL PARK

Asper, J.R. and J.C. Maerz. To be submitted to undetermined journal.

Abstract

The mandate of Great Smoky Mountain National Park (GSMNP) is to preserve its diverse resources in ways that will leave them essentially unaltered by human influences. Invasive species are a logistical challenge to the park mandate. Park managers need to know the extent of invader distributions, and to evaluate the potential effectiveness of management options. Knowledge on how invasive species are introduced and spread are important for evaluating whether and which management options might be effective. The Green Treefrog (*Hyla cinerea*) was reportedly introduced recently to Cades Cove within GSMNP, and currently occurs throughout the Cove and at higher abundance at a few sites. We used Individual Based Models (IBM) to model scenarios of the introduction and subsequent population growth and spread of *H. cinerea* in Cades Cove to evaluate the plausibility of the purported narrative of invasion and inform potential management actions. We evaluated scenarios of different initial introduced population sizes (15, 50, 100, 150), mean dispersal distance (50 m, 150 m, and 250 m), and random displacement of animals around the landscape (a

simulation of human-facilitated movement) on population growth at the purported introduction site and spread to additional wetlands throughout Cades Cove, and we compared our model results to the current distribution pattern and abundance of *H. cinerea*, Assuming 'natural' larval and adult survival rates, we found that initial population sizes of 15 and 50 individuals were seldom if ever able to achieve densities comparable to current populations within a 15 year period; but a larger initial population of 250 animals could reach comparable densities. We found that increased mean dispersal distance increased the numbers of additional wetlands colonized; however, no simulation that included natural dispersal could colonize the number of wetlands currently occupied by *H. cinerea*, and never reached the farther wetlands where large breeding populations currently occur. However, when we included a moderately sized initial population and random displacement of individuals, we could produce patterns of occupancy and abundance more consistent with the current distribution of *H. cinerea* in Cades Cove. Our results suggest that the original narrative of a small single introduction to a single site within Cades Cove within the past 3-5 years is unlikely. We know that an earlier introduction was unlikely, so our results suggest a larger initial introduction or multiple introductions, and tourist or staff facilitation of *H. cinerea* spread around Cades Cove is a more likely invasion scenario. Our results would suggest the need to address future introductions and the ongoing spread of *H. cinerea* on vehicles could be an important component of a management plan.

Introduction

The mission of the National Park Service is to preserve its exceptionally diverse resources in ways that will leave them essentially unaltered by human influences (Organic Act U.S.C. 1916). Great Smoky Mountains National Park (GSMNP) has identified priority areas of research into issues that pertain to the Park's ability to follow this mandate and to protect its unique natural resources. One of these priority topics is the study of the ecology and control of non-indigenous species. This priority is supported by executive order 13112 which states that federal agencies are "to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause"(Clinton 1999). In 2011 a new anuran species, *Hyla cinerea* (Green treefrog), was first reported calling by Park staff in Cades Coves at the wastewater Settling Lagoons adjacent to the campground. *H. cinerea* are not native to GSMNP, with the nearest natural populations occurring ~225 km from the Park (Lanoo 2005). From 1998 to 2002, a thorough survey was done for all amphibians within GSMNP and no evidence of *H. cinerea* was detected (Dodd 2003).

Due to the introduction of *H. cinerea* into Cades Cove, GSMNP personnel have expressed concern over potential management options given the distribution and abundance of *H. cinerea* in the Cove. To address this, we modeled possible scenarios for the introduction and spread of *H. cinerea* in Cades Cove with the aim of identifying elements of scenarios that best simulate current distribution and abundance of the species in Cades Cove. Specifically, we used knowledge from prior experiments on larval growth and development (Asper, Chapter 3) in conjunction with literature values for vital rates to evaluate scenarios of different initial introduced population sizes, mean dispersal distances, on population growth at the purported introduction site and spread to additional wetlands throughout Cades Cove. We also evaluated the effects of random displacement of animals around the landscape, which served to simulate the potential effects of human-facilitated movement on population growth and spread. We compared our model results to the current distribution pattern and abundance of *H. cinerea* within Cades Cove (Asper, Chapter 2).

Individual Based Modeling

Many different methods exist for exploring population dynamics in relation to invasions. To investigate the likelihood of different invasion scenarios, and consequently what management strategies could be effective, we used an Individual or Agent Based Model (IBM/ABM). In this type of model "individuals or agents are described as unique and autonomous entities that usually interact with each other and their environment locally" (Railsback and Grimm 2012). By using these models, it is possible to program in known interactions between individuals and their environments and then observe emergent trends (2012). In particular, IBMs allow for adaptive behavior, which many mathematical models do not (Railsback and Grimm 2012). The models allow for individual reactions to environment at every level from interactions between individuals to spatial interactions with the landscape and for the inclusion of detailed processes that might not be possible in simpler models (2006, 2012). The use of "Overview, Design Concepts, and Details" (ODD) methods and model calibration, parameterization, and sensitivity, uncertainty, and robustness testing are also well developed for IBM approaches (Grimm et al. 2006).

IBMs are a particularly useful way to look at spatially explicit population processes where functional connectivity of patches influences dynamics. It is often easier to look at how the landscape is physically structured (structural connectivity) rather than looking at how a landscape is connected for a particular species (functional connectivity). This is problematic when the functional connectivity is much more relevant to the question at hand. Often the connectivity of a landscape is explored through methods such as the Graph-Theoretic approach, which models habitat patches as nodes and uses measures such as Euclidean distances between them and as such has a limited ability to evaluate the true connectedness. Since it only looks at measures such as distance between suitable patches, it often overlooks the biology of a species including the influence of things such as reproductive rates and population size on the ability of a species to disperse (Uden et al. 2014).

For the case of the introduced Green Treefrog (*Hyla cinerea*), an Individual Based Model allows us to integrate knowledge or assumptions about how individuals behave, and manipulate assumptions or rates to determine whether emergent patterns from models agree with patterns we observe in the field. Once a realistic model is developed and its sensitivity understood, it could be used to simulate the effects of management strategies on the distribution and abundance of *H. cinerea* within the actual landscape.

Methods

IBMs can be complicated, so the use of "Overview, Design Concepts, and Details" (ODD) methods are a standardized way of presenting the model (Grimm et al. 2006). Because the ODD can be long and overly detailed, the ODD has been included as an appendix and we only summarize the model in this chapter (APPENDIX A). In short, our model had frogs as agents that undergo three seasonally-specific events each year: immigration to wetlands, reproduction, and emigration from wetland to overwintering habitat. This simplified version of anuran annual movements was programmed using the 'generous', higher values for hylid vital rates (survival, dispersal, etc.) gleaned from literature. In this regard, we were trying to maximize the population growth and dispersal capacities of the frogs while remaining within the range of natural vital rates. We recognize that some of our vital rates may not be the most probable, and that it is possible that conditions within this introduced landscape might be greater than those within the natural range of the species (see Discussion).

The individual based model was programed with NetLogo (Wilensky 1999), and the landscape for the model was created using ArcGIS (ESRI 2011). The landscape included all of the wetlands in the Cades Cove area. The landscape was also simplified to two land cover types, forested or open, and these land cover classifications created habitat specific vital rates for both larval success and survival during terrestrial stages. Each individual frog goes through a series of steps (Figure 4.1). During the emigration season and upon initial emergence as a metamorph, individuals disperse at random distances and directions into either forested or non-forested locations, with survival probabilities based on the specific land cover. We assumed that *H. cinerea* overwinter in forest habitat, so individuals that did not select forested habitat have a much larger chance of death. We assume adults are faithful to overwintering sites, so they emigrate back to that site after each breeding season. During the immigration season, individuals have a probability of either returning to their natal wetland (the wetland from which they metamorphosed), or a new wetland. The probability of switching to a new wetland was based on proximity to that wetland and the size of the male chorus at that wetland. Females only reproduced at a wetland if a male was present. Female reproductive success at the wetland used published fecundity rates with hydroperiod and forest cover also scaling the production. Wetland hydroperiod was scaled annually using annual precipitation data for Cades Cove for the past 15 years, and we used annual hydroperiods to affect the probability of larval survival and the number of successful clutches a female could produce.

We ran multiple simulations of model conditions, and varied conditions to explore different questions related to initial numbers of frogs introduced, mean dispersal distance, and the probability of random displacement of individuals around Cades Cove. We simulated initial population sizes of 15, 50, and 150 individuals and dispersal modeled as a random exponential decay function with a mean dispersal distance of 100 m (90% of individuals move between 0 m and 232 m) running the model 15 times for each size. Next, we held the initial population size at 15 individuals and we simulated mean dispersal distances of 50 m, 150 m, or 250 m (90% move between 0 m and 107 m, 324 m and 589 m respectively. Published dispersal distances of hylid frogs range between 30 and 480 m from the breeding ponds (Garton and Brandon 1975, Roble 1979, Semlitsch and Bodie 2003, Pellet et al. 2006). In our final simulations, we allowed each individual to have a small chance of being randomly displaced to a new location within Cades Cove. This was to simulate the potential effects of human-facilitated movement of frogs from the Settling Ponds and campground to other areas within Cades Cove. We used initial population sizes of either 15 or 50 individuals and a mean "natural" dispersal distance of 100m. We varied the probability of random displacement between 0.002, 0.001, or 0.0005 and varied whether individuals were displaced to random locations along a road within Cades Cove or were displaced to a randomly selected parking lot associated with a frequent tourist stop within Cades Cove. For each set of scenario conditions, we ran 15, 15-year simulations. The reported introduction period for *H. cinerea* was about three years, but we allowed for the maximum period since the most extensive amphibian survey of Cades Cove between 1998 and 2001 (Dodd 2003).

Results

The estimated abundance at the Settling Lagoons, the assumed introduction location, in 2013 was between 4,000 and 10,600 individuals (Asper, Chapter 2). At a mean dispersal distance of 100 m, an initial founding population of 15 individuals, the simulated population reached a mean of only 1,622 adult individuals after 15 years. (Table 4.1*Table 4.*). The maximum breeding population size ever achieved at the Settling Lagoons reached 4,049 adults in only 1 of 15 simulations, and only after 15 years. For founding populations of 50 and 150 individuals, the mean adult population sizes after 15 years were 6,470 and 13,368 respectively and the maximum adult population sizes achieved at the Settling Lagoons were 10,306 and 18,412 respectively.

Increasing mean dispersal distance had a small effect on the simulated mean population size at the Settling Ponds. With an initial population of 15, the largest adult population sizes at the Settling Lagoons were 2,578, 5,211, and 6,911 for a mean distance of 50 m, 150 m, and 250m respectively (*Table 4.1Table 4.*).

Increasing the "natural" dispersal ability of individuals had a small, positive effect on the number of wetlands occupied by individuals at the end of 15 years. At a mean dispersal distance of 100m, only one additional wetland beyond the Settling Ponds was colonized, regardless of whether the initial population was 15, 50, or 150 individuals (*Table 4.1*). This wetland was always the wetland most proximate to the Settling Lagoons. However, when the initial population was 15, the number of additional wetlands increased from one to three and four with an increase in mean dispersal distance from 50 m to 150 m and 250 m respectively (*Table 4.1*, Figure 4.2). However, in most cases, wetlands "colonized" within the 15 year simulation were colonized by single or a few individuals and no breeding populations ever became established (*Table 4.2*). Therefore, all our scenarios of natural dispersal failed to colonize all the wetlands and result in breeding choruses at the multiple wetlands where choruses are known to occur today.

Random displacement

When we allowed for random displacement of frogs from the Settling Lagoons to random locations along roads around the Cove, we observed wetland colonization patterns more similar to the current distribution of *H. cinerea* within Cades Cove (Table 4.3). With a random displacement probability of 0.0005, an initial population of 15, and a mean “natural” dispersal distance of 100 m, the simulated population colonized a maximum of three additional wetlands outside the Settling Lagoons at a maximum distance of 5 km, and the wetlands colonized were not always the ones most proximate to the Settling Ponds. At a displacement rate of 0.002 and 0.001, the population colonized a maximum of five additional wetlands, also at a maximum distance of 5 km, and again not always the ones most proximate to the Settling Ponds. Whether frogs were displaced to random locations along the road or to randomly selected parking lots within the Cove had no effect on the number of wetlands colonized during a simulation (*Table 4.3*). When the initial population was 50 individuals rather than 15 individuals, and the mean dispersal distance was 100 m, individuals were more consistently able to reach four or five wetlands other than the Settling Lagoons, and the population size at the Settling Lagoons got well above 5,000 on average for all three levels of random displacement (0.002, 0.001, and 0.0005) (Table 4.4). Again, however, though frogs colonized more of the wetlands in the simulations of random displacement, the simulated abundances at sites other than the Settling Lagoons were generally small after 15 years (*Table 4.2*, Figure 4.3).

Discussion

At the start of our project, The GSMNP staff’s operating narrative for the introduction of *H. cinerea* to Great Smoky Mountains National Park was that a single, small population was introduced to the Settling Lagoons in 2010 or 2011. However, by 2013, the breeding population at the Settling Lagoons was well over 5,000 individuals and breeding choruses were detected at five additional wetlands across the full extent of the Cove (Asper, Chapter 2). Our simulations suggest the original narrative is improbable. Our simulated population could not achieve population sizes close to the actual population size with a founding population of 15 individuals and a 15 year window for growth. Intuitively, our models suggest that a larger initial population could achieve a breeding population comparable to the actual population at the Settling Lagoons; however, this would require a period of ~15 years. We know that *H. cinerea* were not documented in the park between 1998 and 2001 during intensive surveys of the area including the Settling Lagoons (Dodd 2003), and were not detected during call surveys in 2008 (USGS 2014b). Moreover, the simulated populations, even with the largest dispersal distance considered plausible, was unable to colonize the number of wetlands as far from the Settling Lagoons as we observed.

Our simulations suggest that the initial introduction of *H. cinerea* occurred earlier, required a single large or multiple smaller introductions, or that larval or adult survival rates are significantly higher in Cades Cove than published rates from within *H. cinerea*'s natural range. We are confident that *H. cinerea* was not present in Cades Cove prior to 2002. The surveys conducted between 1998 and 2001 (Dodd 2003) were conducted by many highly qualified biologists, several of whom lived in residence adjacent to the Settling Lagoons (Dodd, personal communication). *H. cinerea* is highly detectable and it is unlikely it would have been missed. For example, during our 2013 and 2014 surveys, we detected, from the road about 150 meters away, a single male calling at Methodist Pond on a single night (Asper, chapter 2), which was the only time the species has ever been detected at that site. Therefore, we are confident the earliest potential introduction was 2002, which our models suggest would require an initial population of >150 individuals or significantly higher larval survival rates to achieve the current breeding population size at the Settling Lagoons. We were told that *H. cinerea* were not detected during a call survey in a June 2007, which is during the *H. cinerea* breeding season. Assuming, *H. cinerea* were absent in 2007, then our model suggests that the initial introduction of *H. cinerea* would have to have been very large to achieve the current breeding population size, would require multiple introductions of individuals between 2007 and present, or exceptional larval survival rates at the Settling Lagoons. We recently learned that, in 2008 and 2009, the park shifted operations of the Cades Cove store and horse barns adjacent to the Settling Lagoons to an outfit based in Florida (Molly Schroer, NPS personal communication). This involved the transfer of large machinery and equipment, and frequent movement of horse trailers between Florida and Cades Cove each year. This event would create the opportunity for repeated introductions of *H. cinerea* to Cades Cove [in addition to any potential introductions by tourists traveling from the southeastern U.S.].

One reason our simulations may have under-predicted natural colonization of other wetlands was our assumption about dispersal distance. We found that simulating extreme mean dispersal distances could produce results similar to observed patterns; however, those distances were greater than published reports of dispersal distances by frogs (Garton and Brandon 1975, Roble 1979, Semlitsch and Bodie 2003, Pellet et al. 2006). However, Pellet et al. (2006) reported that adult European treefrogs fitted with harmonic transponders moved between 18 and 440 m during post breeding emigration. Of the five frogs not “lost” in their study, three remained within 50 m from their release points at the breeding site, but two frogs moved between 660 m and 860 m in under two days [one on a single night]. Though a small sample size, these results suggest that some individual treefrogs are capable of moving several kilometers within a few days. Other studies have shown invasive species, such as the Cane Toad (*Rhinella marina*) in Australia, have evolved increased dispersal ability after introduction (Brown et al. 2014); however, that change appears to have required decades since introduction, and Cane Toads were intentionally introduced in very large numbers. We believe the evolution of increased dispersal ability is unlikely in the short time period of this invasion. Modeling a wider range of mean dispersal distances would, intuitively, increase the likelihood that *H. cinerea* would colonize a larger number of wetlands; however, it is still unlikely that any reasonable natural dispersal would result in colonization of many wetlands (and the growth of those breeding populations) within the short time period of this invasion.

We did not explore the sensitivity of our model to the vital rates we used. While we used rates at the higher end of those reported in the literature, we did not consider in this study the potential effects of larval or adult survival rates significantly higher than those typical for anurans (Wells 2007). In particular, higher larval survival at the Settling Lagoons could lead to a dramatic increase in population growth rate. Other studies demonstrate that amphibian populations are highly sensitive to larval survival, which can vary by orders of magnitude within and among breeding sites. By increasing larval survival from 5% to 10% at the settling ponds, it is likely the *H. cinerea* population would reach an adult breeding population comparable to current density estimates within 5 years (though we did not estimate this). In turn, and increase in population growth at the Settling Lagoons would increase the numbers of dispersing individuals, which in turn should increase the rate, number and distance of colonizing other wetlands. It is reasonable to speculate that larval survival at the Settling Lagoons was higher than the natural rates reported from other studies. The Settling Lagoons are nutrient rich and lack fish and many other predators of larval amphibians (though we did not estimate invertebrate predator abundance). In some regards they are similar to mesocosms, which support unnaturally high rates of larval survival (e.g., Asper, Chapter 3). Therefore, future iterations of our model will examine a range of larval vital rates at the Settling Lagoons and other sites to identify if and what larval survival rates result in population abundances and distribution similar to current patterns.

We believe it is unlikely that *H. cinerea* adult survival is higher within GSMNP compared to the natural range. It is true that many invasive species experience an ecological release from natural enemies when they are dispersed over large distances by humans (Torchin and Mitchell 2004). This enemy release often results in faster growth, higher fecundity, and increased survival, allowing invaders to rapidly grow and persist at higher densities than they do within their native range. Therefore, we cannot rule out the possibility that *H. cinerea* have experienced some ecological release that improves adult survival or fecundity. However, Cades Cove contains a wide range of native amphibians that are sympatric with H. cinerea across much of the southeastern U.S., and it is likely they share many natural enemies. Moreover, GSMNP is significantly farther north than the natural range of *H. cinerea* and its close relatives (*H. gratiosa* and *H. squirella*), and is mountainous landscape. Winter conditions are harsher than conditions within the natural range of *H. cinerea*, and we would predict should reduce juvenile and adult survival rates.

We believe that a more likely scenario for the rapid colonization of multiple wetlands throughout Cades Cove is the deliberate or inadvertent movement of frogs from the campground area to other areas within the Cove. *H. cinerea* is known to be associated with human modified landscapes and structures. *H. cinerea* are common around farm and fish ponds, open habitats including developed sites, and frequently seek refugia on manmade structures and objects (Lanoo 2005). They are common hitchhikers on vehicles and equipment, and therefore easily moved accidently. Moreover, they are charismatic frogs that might be collected and later released by tourists. *Hyla cinerea* are routinely observed at the information kiosk and parking lot entering Cades Cove, creating ample opportunity for hitchhikers and the collection of adults. Cades Cove gets thousands of cars daily and over two million visitors every year (Truett et al. 2002), creating the opportunity for tourists to regularly introduce frogs from outside and within the park, and supplementing natural dispersal. Our models indicated that adding a random displacement probability increased the likelihood that *H. cinerea* would colonize the majority of wetlands where they are observed today including the wetlands that are farthest from the Settling Lagoons. Our simulated rates that resulted in patterns similar to the observed distribution of *H. cinerea* within the Cove were between 0.001 and 0.002 (a 0.1-0.2% chance any individual would be translocated); which would translate to translocating an average of ~5-9 adult individuals from the Settling Lagoons-Campground area per year. This rate seems feasible; however, we note that our model still required ~15 years to colonize the number of wetlands we observe. It seems unlikely, though we have no data to conclude that a higher rate of animals is translocated each year.

Because of the potential importance of human activities facilitating the continued introduction and spread of *H. cinerea* within Cades Cove, Park managers may want to include efforts to limit the introduction and movement of animals in management strategies. This can be done with education programs for the public and more thorough checks of concession equipment brought into the park. Management strategies for the individuals already present can include targeting the large population of *H. cinerea* at the Settling Lagoons. The most effective strategy for the Settling Lagoons would be to reduce or eliminate larval survival, which could be achieved through seasonal draining of lagoons or the use of a poison such as Rotenone™. One application of our model results suggests that the continued growth or spread of *H. cinerea*, and perhaps the viability of the population within the Cove could be strongly affected by eliminating reproduction at the Settling Lagoons.

This modeling effort illuminated a few areas where more research would be useful. First, very little is known about the movement and wintering habits of this species. If more is known about their ability to move, such as through tracking individuals post mating or after metamorphosis, then the model could be better informed. Second, a genetic study could be used to test invasion scenarios and evaluate the relative importance of different processes explored within our model. For example, genetic data could be used to identifying the likely source locations for the founder population or populations. This could be used to determine whether the invasions started through the movement of large numbers of individuals by concessionaires, or whether there were numerous introductions suggesting repeated small introductions on tourist vehicles. Genetic data could also be used to look at patterns of genetic similarity among breeding choruses. If *H. cinerea* invasions were the result of a few introductions from a single source, then we would expect to see low allelic richness among all breeding choruses compared to richness levels reported for populations within the native range. In addition, if the Settling Lagoons are the single source population for other choruses, then we would expect to find the highest allelic richness at the Settling Lagoons, and only subsets of those alleles at other sites within Cades Cove. However, if there are alleles unique to each breeding site, this would imply multiple introductions to the cove. Further, if *H. cinerea* have spread through the cove through natural dispersal by individuals, then we would expect allelic richness and similarity to decline as a function of distance from the settling ponds (isolation by distance hypothesis). This pattern would suggest that larval production at the Settling Lagoons is the major factor driving growth and spread of *H. cinerea* within Cades Cove. However, if allelic richness or similarity were not related to distance, but instead was random or greatest at sites most proximate to visitor amenities, this would suggest human-facilitated spread of individuals is an important process driving this invasion. Our model suggests that the simple scenario of a single small introduction, even a long time ago, likely does not explain the current size and distribution of the *H. cinerea* population. It is more likely that some combination of higher larval survival at the Settling Lagoons, greater dispersal ability, human facilitated movement of animals around Cades Cove, and the continued introduction of new animals via tourist and concessioners are all contributing to the rapid growth and spread of the *H. cinerea* population. As such, managers may wish to consider tourist education as part of the management strategy for this introduced species.

Literature Cited

Brown, G. P., B. L. Phillips, and R. Shine. 2014. The straight and narrow path: the evolution of straight-line dispersal at a cane toad invasion front. Proceedings of the Royal Society B-Biological Sciences **281**:7.

Clinton, W. 1999. Invasive Species. Pages 6183-6186 Executive Order 13112. Federal Register.

Dodd, C. K., Jr. 2003. Monitoring amphibians in Great Smoky Mountains National Park. U S Geological Survey Circular **1258**:i-vi, 1-117.

ESRI. 2011. ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, CA.

Garton, J. S., and R. A. Brandon. 1975. Reproductive ecology of the Green Treefrog, *Hyla cinerea*, in southern Illinois (Anura: Hylidae). Herpetologica **31**:150-161.

Grimm, V., U. Berger, F. Bastiansen, S. Eliassen, V. Ginot, J. Giske, J. Goss-Custard, T. Grand, S. K. Heinz, G. Huse, A. Huth, J. U. Jepsen, C. Jorgensen, W. M. Mooij, B. Muller, G. Pe'er, C. Piou, S. F. Railsback, A. M. Robbins, M. M. Robbins, E. Rossmanith, N. Ruger, E. Strand, S. Souissi, R. A. Stillman, R. Vabo, U. Visser, and D. L. DeAngelis. 2006. A standard protocol for describing individual-based and agent-based models. Ecological Modelling **198**:115-126.

Lanoo, M. J. 2005. Amphibian declines: the conservation status of United States species. Berkeley, University of California Press.

Pellet, J., L. Rechsteiner, A. K. Skrivervik, J. F. Zurcher, and N. Perrin. 2006. Use of the Harmonic Direction Finder to study the terrestrial habitats of the European tree frog (*Hyla arborea*). Amphibia-Reptilia **27**:138-142.

Railsback, S. F., and V. Grimm. 2012. Agent-Based and Individual-Based Modeling: A Practical Introduction. Princeton University Press, Princeton, NJ.

Roble, S. M. 1979. Dispersal movements and plant associations of juvenile Gray Treefrogs, *Hyla versicolor* Le Conte. Transactions of the Kansas Academy of Science (1903-) **82**:235-245.

Semlitsch, R. D., and J. R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology **17**:1219-1228.

Torchin, M. E., and C. E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. Frontiers in Ecology and the Environment **2**:183-190.

Truett, L. F., S. M. Chin, and E. C. P. Chang. 2002. Strategic plan for coordinating rural Intelligent Transportation System (ITS) transit development in Great Smoky Mountains National Park Oak Ridge National Laboratory, Oak Ridge, TN.

U.S.C. 1916. National Park Service Organic Act. 16 U.S.C. §1. U.S. Department of the Interior, United States of America.

Uden, D. R., M. L. Hellman, D. G. Angeler, and C. R. Allen. 2014. The role of reserves and anthropogenic habitats for functional connectivity and resilience of ephemeral wetlands. Ecological Applications **24**:1569-1582.

USGS. 2014b. North American Amphibian Monitoring Protocol (NAAMP), Species Detection Map.

Wells, K. D. 2007. The ecology & behavior of amphibians. Chicago, The University of Chicago Press.

Wilensky, U. 1999. NetLogo.

Figures

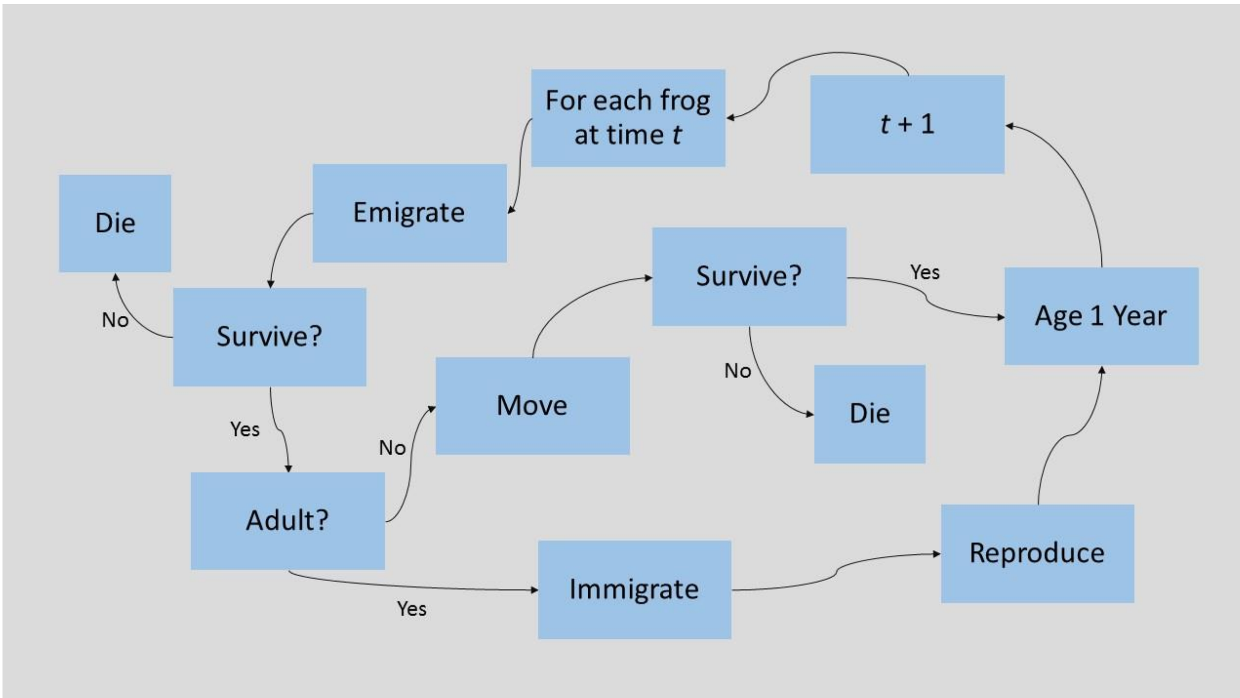

Figure 4.1. For each frog at time t, the above schedule is observed.

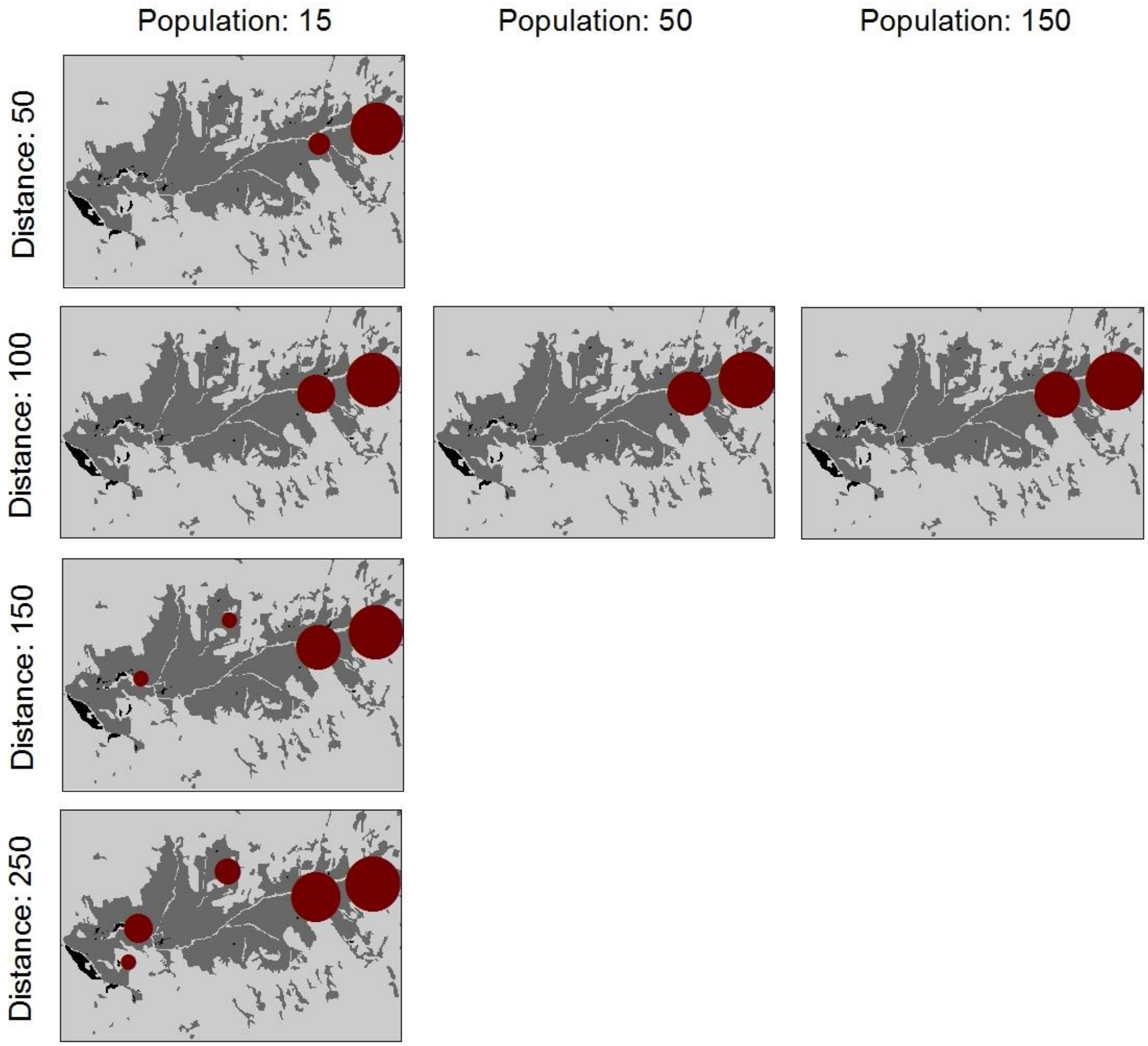

Figure 4.2. Maps illustrating the results of the IBM simulations varying initial population size and dispersal distance.

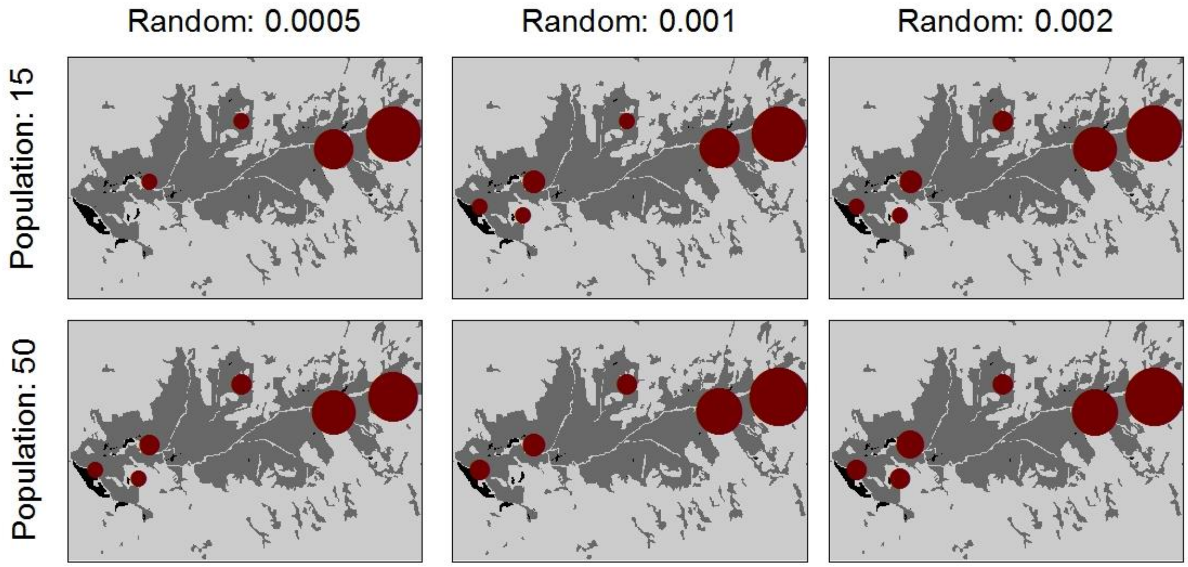

Figure 4.3. Maps illustrating the results of the IBM simulations varying random dispersal probability. For all simulations illustrated in this figure the dispersal distance (for normal dispersal movements) was held at a mean of 100 m.

Tables

Table 4.1. Results for Individual Based Model runs with varying *H. cinerea* introduction size (with mean dispersal distance of 100 m) and dispersal distance (with an initial population size of 15). Values shown include: maximum population reached after 15 years, mean population reached with standard error, number of years to reach 5000 individuals, number of simulations where the population at the Settling Lagoons exceeded 5000 individuals (out of 15 simulations), and the maximum number of wetlands in addition to the Settling Lagoons that were colonized.

Introduction Size	15	50	100
Max Population (15 years)	4,049	10,306	18,412
Mean Population (15 years)	1,622±248	6,470±465	13,368±764
Years to Reach 5000	N/A	14.9	13.13
Simulations exceeding 5000	0	13	15
Max Number of Additional Wetlands Colonized	1	1	1

Dispersal Distance	50m	150m	250m
Max Population (15 years)	2,578	5,211	6,911
Mean Population (15 years)	1,048±162	2,530±321	4,236±407
Years to Reach 5000	N/A	15	15
Simulations exceeding 5000	0	1	5
Max Number of Additional Wetlands Colonized	1	3	4

Table 4.2 Results showing which wetlands were occupied, how many repetitions had the wetland occupied out of the 15 repetitions (reps) and the maximum population size achieved for each wetland (max pop) under each scenario. In parentheses after the wetland name is the edge-to-edge distance in kilometers from the wetland to the Settling Lagoons (the wetland of origin). The results vary either initial population (left), dispersal distance (middle) or random dispersal probability to parking lots (right).

Dispersal distance Initial population Random displacement		**100 15 0**	**100 50 0**	**100 150 0**	**50 15 0**	**150 15 0**	**250 15 0**	**100 50 0.002**	**100 50 0.001**	**100 50 0.0005**	**100 15 0.002**	**100 15 0.001**	**100 15 0.0005**
Settlings Ponds (0)	reps	15	15	15	15	15	15	15	15	15	15	15	15
	max pop	4,049	10,306	18,412	2,578	5,211	6,911	9,356	10,211	815	3,787	3,043	3,259
Beaver Pond (0.97)	reps	15	15	15	5	15	15	15	15	15	15	15	15
	max pop	70	242	456	3	303	1,094	289	297	181	168	75	63
Gourley Pond (1.17)	reps	0	0	0	0	0	0	0	0	0	0	0	0
	max pop	0	0	0	0	0	0	0	0	0	0	0	0
Methodist Pond (2.85)	reps	0	0	0	0	4	13	12	5	6	4	1	2
	max pop	0	0	0	0	1	6	2	2	2	2	1	1
Shield Pond (2.95)	reps	0	0	0	0	0	0	0	0	0	0	0	0
	max pop	0	0	0	0	0	0	0	0	0	0	0	0
Abrams Spring (4.62)	reps	0	0	0	0	3	14	14	15	13	14	11	6
	max pop	0	0	0	0	1	10	7	3	2	3	3	1
Abrams Oxbow (4.89)	reps	0	0	0	0	0	0	0	0	0	0	0	0
	max pop	0	0	0	0	0	0	0	0	0	0	0	0
Gum Swamp (5.08)	reps	0	0	0	0	0	3	4	0	4	4	1	0
	max pop	0	0	0	0	0	1	2	0	1	1	1	0
Stupkas Sinkhole (5.87)	reps	0	0	0	0	0	0	7	3	1	2	1	0
	max pop	0	0	0	0	0	0	2	2	1	1	1	0
Total Wetlands Occupied		**2**	**2**	**2**	**2**	**4**	**5**	**6**	**5**	**6**	**6**	**6**	**4**

Table 4.3 Results for Individual Based Model runs with varying random dispersal with *H. cinerea* moving randomly to parking lots and along roads with an initial population of 15 and mean dispersal distance of 100 m). Values shown include: maximum population reached after 15 years, mean population reached (standard error values are in parenthesis), number of years to reach 5000 individuals, number of simulations where the population at the Settling Lagoons exceeded 5000 individuals (out of 15 simulations), and the maximum number of wetlands in addition to the Settling Lagoons that were colonized.

Random displacement (Roads)	0.002	0.001	0.0005
Max Population (15 years)	3,965	2,914	3,820
Mean Population (15 years)	1,820±244	1,731± 213	2,120±233
Years to Reach 5000	N/A	N/A	N/A
Simulations exceeding 5000	0	0	0
Max Number of Additional Wetlands Colonized	3	3	2

Random displacement (Lots)	0.002	0.001	0.0005
Max Population (15 years)	3,787	3,043	3,259
Mean Population (15 years)	2,134±236	1,762±166	1,663±220
Years to Reach 5000	N/A	N/A	N/A
Simulations exceeding 5000	0	0	0
Max Number of Additional Wetlands Colonized	5	5	3

Table 4.4. Results for Individual Based Model runs with varying random dispersal with *H. cinerea* moving to parking lots with an initial population of 50 and mean dispersal distance of 100 m. Values shown include: maximum population reached after 15 years, mean population reached (standard error values are in parenthesis), number of years to reach 5000 individuals, number of simulations where the population at the Settling Lagoons exceeded 5000 individuals (out of 15 simulations), and the maximum number of wetlands in addition to the Settling Lagoons that were colonized.

Random displacement (Lots)	0.002	0.001	0.0005
Max Population (15 years)	9,356	10,211	8,518
Mean Population (15 years)	5,615±504	7,809±459	5,913±374
Years to Reach 5000	15	14.8	15
Simulations exceeding 5000	9	14	9
Max Number of Additional Wetlands Colonized	5	4	5

CHAPTER 5

CONCLUSIONS

Given the legal mandates of Great Smoky Mountain National Park (GSMNP) to preserve its diverse resources in ways that will leave them essentially unaltered by human influences as well as to prevent and mitigate harm from invasive species, the park needed a better understanding of the status of Green Treefrogs (*Hyla cinerea*) in Cades Cove. In order to understand the establishment phase of the species, we needed to know the size of the breeding population and distribution among wetlands within the Cove. Secondly, we needed to know what ecological and anthropic factors regulate the distribution, given that Cades Cove is a managed, open agrarian landscape including natural and anthropic water bodies. Lastly, we wanted to know if the original narrative for the introduction, that it was a single, small introduction that happened relatively recently is a feasible narrative. Using this information about the current distribution and abundance of the species and about the narrative of this introduction, park managers can better determine what management strategies will be most effective for minimizing the negative impacts of this introduced species.

The first section of this thesis looked at the current population abundance and distribution within the park. We estimated the breeding population at the primary known breeding location, the Settling Lagoons to be between 5000 and 9000 adult individuals. Additionally, we detected *H. cinerea* at six of the nine wetlands in Cades Cove. Thus, the population was larger and had spread to more wetlands than initially assumed. Patterns emerged in the detection of *H. cinerea* showing correlation of their presence with open, herbaceous wetlands

The second section of this thesis looked into the suitability of the different wetlands in Cades Cove for *Hyla cinerea* larval survival and development. We showed that only a few wetlands within Cades Cove can produce metamorphs regularly. Shaded sites appear to require a longer time frame than is available in Cades Cove. The sunny/herbaceous sites are often limited by hyroperiod. Three wetlands consistently hold water long enough for the larval period of *H. cinerea* and have vegetative conditions that are more suitable for larval development. If management of this introduced species is necessary, these sunny, herbaceous sites that have the optimal conditions are likely the best target.

Using the numbers from the first two sections of the thesis, the third section used Individual Based Modeling (IBM) to explore the different narratives for the population spread. We were able to program in the behaviors and survival rates from literature, expert opinion, and experimental results and then adjust population introduction size, frog dispersal ability, and random dispersal probability (due to anthropic interference) in order to see what narratives are possible. The model showed that the scenario of a single small introduction, even a long time ago, does not explain the current size and distribution of the *H. cinerea* population. The initial introduction was likely either very large or introductions happened continually, possibly by concessioners, tourists, or some combination. It is also possible that the movement of individuals around the Cove is also influenced by tourists. As such, managers may wish to consider tourist education as part of the management strategy for this introduced species.

Since the models showed what was unlikely rather than exactly how the introduction did occur, further examination into this question would be beneficial. Genetic data could be used to identifying the likely source locations for the founder population or populations, which could be used to determine whether the invasions started through the movement of large numbers of individuals by concessionaires, or whether there were numerous introductions suggesting repeated small introductions on tourist vehicles. This would be useful for developing policies or management strategies for movement of equipment into the park to minimize continued introductions. Genetic data could also be used to determine whether *H. cinerea* productivity at the Settling Lagoons is the major factor driving growth and spread of the population within Cades Cove and the degree to which human-facilitated spread of individuals is an important process affecting this invasion.

Potential impacts of this introduction to other anurans are not fully known and warrant further exploration. First, potential competitive impacts of *H. cinerea* larvae on other anuran larvae could be detrimental to species of concern, particularly *Gastrophryne carolinensis*, which was the only species that bred exclusively in wetlands where *H. cinerea* populations have established. Secondly, we detected one individual that is likely a hybrid of *Hyla cinerea* and *Hyla chrysoscelis* (Cope's Gray Treefrog) (Figure 5.1). The individual was noticed due to its distinct call that was unlike anything else calling that evening. The individual has a call that is somewhere between the honking of a *H. cinerea* and the trill of *H. chrysoscelis*. It also bears physical characteristics of both species. This indicates that hybridization could be a concern, which is not unprecedented as a harmful impact of invasive amphibian species. Further monitoring for incidences of hybridization, would show whether or not this is an issue of concern for GSMNP. Continued call surveys could both monitor the occupancy of *G. carolinensis* within the Cove and monitor for additional instances of hybridization, though other survey types would better detect hybrid individuals less distinct than our one specimen.

Another concern is *H. cinerea's* potential influence on invertebrates through predation. Other anuran invasions, such as the Coquí in Hawaii, have had a significant impact on native communities (Beard and Pitt 2005, Choi and Beard 2012). We do not believe, however, that this is an issue of concern for *H. cinerea*, because, where the Coquí was introduced to a largely frogless environment in Hawaii, *H. cinerea* has a diet is very similar to *H. chrysoscelis* which is very common and widespread within Cades Cove, thus *H. cinerea* is not a novel predator to the invertebrate communities.

The abundance and distribution of *H. cinerea* in Cades Cove complicates eradication of the species from the park. Only a few wetlands within the Cove have the hydroperiod and vegetative qualities that we showed are ideal for *H. cinerea* development. As such, management strategies may be able to focus on limiting breeding potential for adult *H. cinerea* at these locations. Additionally, as *H. cinerea* is possibly either being introduced repeatedly by tourists or moved around the Cove by tourists, education programs may be a beneficial component of management. Continued monitoring of the population would help determine if the population distribution changes and if management strategies are effective.

Literature Cited

Beard, K. H., and W. C. Pitt. 2005. Potential consequences of the coqui frog invasion in Hawaii. Diversity and Distributions **11**:427-433.

Choi, R., and K. Beard. 2012. Coqui frog invasions change invertebrate communities in Hawaii. Biological Invasions **14**:939-948.

Figures

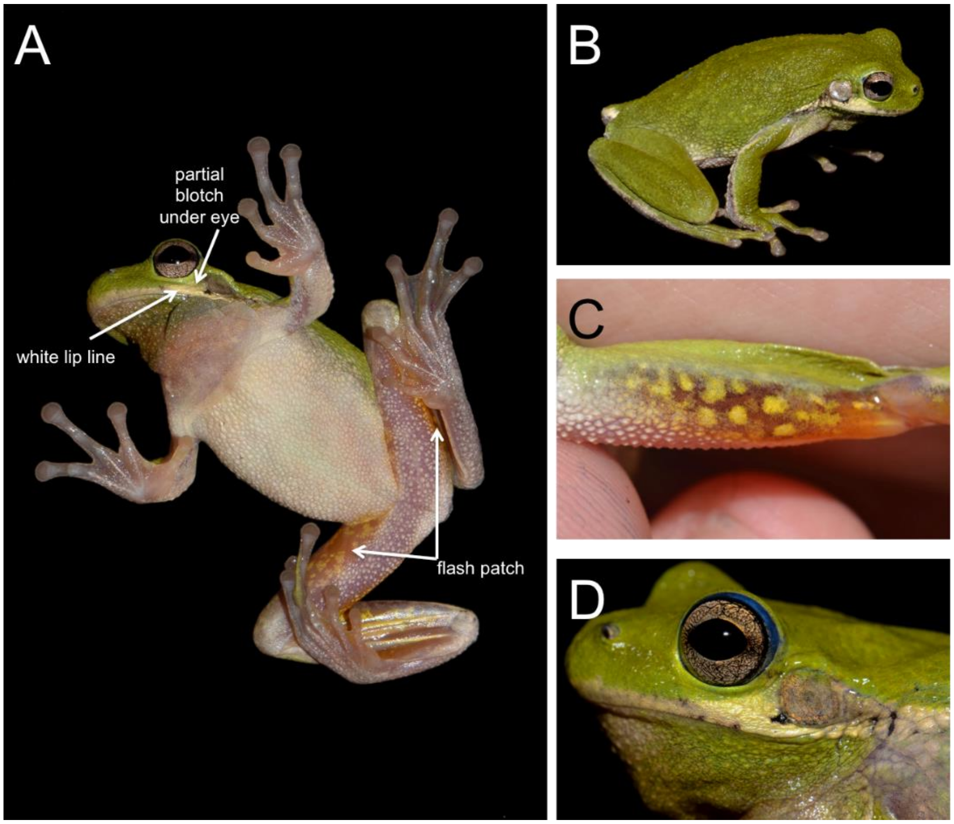

Figure 5.1. Male hybrid between native Gray treefrog and invasive Green treefrog captured at Cades Cove Settling Lagoons in July 2014. (A) is a full ventral image of the animal indicating the possession of a flash patch and blotch under the eye, which are Gray treefrog characteristics, and a white lip line, which is a Green treefrog characteristic. (B) is a profile of the animal showing the uniform green coloration, which is characteristic of Green treefrogs, and the granular [bumpy] skin that is characteristic of Gray treefrogs. (C) is a close up of the male's right thigh showing the flash patch, which is a characteristic of Gray treefrogs and is absent from Green treefrogs. (D) is a close up of the male's head showing the blotch under the eye, which is a Gray treefrog characteristic, and a white lip line, which is a Green treefrog characteristic

APPENDIX A

ODD (Overview, Design Concepts, and Details)

Purpose:

The purpose of this model is to examine different scenarios for the invasion of *Hyla cinerea* in Cades Cove, Great Smoky Mountains National Park. First we explored the scenario of a recent small introduction, then the possibility of tourist interference. Once a narrative is found that accurately recreates the invasion on a timeline similar to the one that has been observed in the park, different management strategies can be explored, such as eliminating the Settling Lagoons (thought to be the source population for the invasion) as a breeding site.

Entities, State Variables, and Scales

1. Entities and state variables
    a. Frogs
        i. State Variables:
            1. my-age (age of individual, from a Poisson distribution)
            2. my-sex (sex of individual, 50/50 male and female)
            3. winter location (home-x, home-y)
            4. natal-wetland, my-wetland (breeding wetland)
            5. wetland-here (the wetland the frog is currently on)

6. dist (distance frogs can travel in a movement (mean of an exponential distribution from which actual movements are drawn))
7. my-dist (value that the frogs draw from the distribution)
8. Values that determine how the frogs move/survive (forest-prob, fores-type, angle, mort-prob, etc.)
9. tourist_chance (the probability an individual frog will be randomly moved by tourists)

b. Land Patches

   i. State Variables:

      1. landcover-type (forest or non-forest)
      2. wetland-name (each wetland has different values)
      3. wetland stat (numerical value for wetland-ID, necessary for the raster based landscape)
      4. road-stat (if the patch is road, this value is > 0)
      5. lot-stat (if the patch is a parking lot, this value is > 0)
      6. water-here (whether or not water is in the wetland, based on weather)
      7. wet-num (the groupings for how likely the wetlands are to hold water)
      8. ring50, ring100, ring200, etc (each concentric ring from a wetland has a percent forest or not forest…this simplifies the movement step)

c. Global Variables

    i. State Variables:

        1. landcover (weather it is forest or not, mostly used for aesthetic purposes)
        2. wetlands (wetland spatial file)
        3. roads (road spatial file)
        4. weather (determined by the weather patterns observed in the park)
        5. weather-num (each wetland has a different value that determines how the frogs can perform there)
        6. values that determine if a patch exists to go to and if it matches the forest draw (patch-x, patch-y, patch-type)
        7. reporting variables (wetland-frog-count, wetland-survival, best)
        8. Patch Sets for each wetland

2. Scale

    a. The spatial scale of the model will be the extent of Cades Cove at Great Smoky Mountains National Park using a real landscape imported from ArcGIS.

    b. The temporal scale will be multiple years….the model will be created so that it can run for as many years as desired (depending on the question at the time). It is currently set to run no more than 15 years. Each tick is one year.

Process overview and scheduling

On each tick (one year) the following will occur in this order (Figure A.1):

***Frog Emigration*:**

In this sub-model frogs move from wetlands out to wintering locations.

- For this version all frogs are starting at the Settling Lagoons (where the invasion is believed to have started).
- Adult movement
    - The adults will leave the wetlands in a random direction and a distance (based on exponential distributions with means of 50, 100, 150, or 250 meters) either finding their initial winter location (age = 2), returning to their last winter location.
- Metamorph movement:
    - The frogs will move in a random direction and a distance eventually settling
    - Once the individual reaches 2 years of age, they will find a winter location and begin acting like adults.

***Frog Immigration*:**

In this sub-model frogs move from their wintering locations to wetlands.

- Adult Males will have a probability of going back to the wetland where they last successfully bred or to the nearest pond (based on natal_prob, which is 0.5 for all runs).
- Adult females will either go to the wetland where they last successfully bred or to the pond with the most frogs (based on natal_prob, which is 0.5 for all runs). If the pond

with the most frogs is too far away (more than twice their move dist) they will go to whatever pond is nearest.

- Juveniles will move again in the same manner as emigration (since they cannot breed yet).

Frog Reproduction

In this sub-model frogs reproduce.

- In order to expedite this step, if a male is present at the wetland all females that are there are able to reproduce. This is done in bulk so that they all reproduce at the same time. Reproduction is scaled based on wetland and on wetland survival rates (Table A.1.2)
- Both males and females will set the wetland as their new “my-wetland” if they successfully breed (i.e. the opposite sex is present).

Wetland Hydroperiod:

The weather effects the hydroperiod, and thus the breeding ability of frogs.

- In the initial scenarios, testing the narrative, weather was held to exactly what the historical weather patterns showed (Figure A.).
- For each seasonal time step, the hydroperiod for the wetlands will be determined by two factors:
    - The category of the wetland within the network of wetlands (defined in the code based on field experience)
    - The odds of a wet or dry year, based on weather data for the area (mm per year for the last 15 years).

- Wetlands each have a predetermined value for how likely they are to produce metamorphs, this value is multiplied by the weather number to determine how many clutches the pond can produce in a given year.

Design Concepts

- Basic Principles
    - This model addresses the principles of frog dispersal through the movements of the frogs. It also looks at reproduction effects on the population size and distribution. It demonstrates weather's effect on overall population dynamics through changing wetland water levels.
- Emergence
    - The model showed that, under the current narrative of the invasion, the frogs could not move in such a way, even with generous parameter values, to reach the furthest wetlands where the frogs have been observed. It also shows that the patterns of frog presence in the riparian corridors. Wetlands that were not surrounded by forest had smaller chance of becoming occupied.
- Adaptation
    - Frogs have a higher probability of going to their natal pond. Since the individuals were born at the natal pond they are assured that under some conditions the wetland is suitable for breeding. They can, however, also

go to other wetlands based on proximity, presence of other frogs, or wet/dry status of the wetland.

- Objectives
- Learning
    - Frogs change what wetland they use for breeding based on whether or not they successfully have bred at that wetland before. This makes individuals that were born at a wetland of poor quality be able to learn that the wetland is not suitable and go to a different wetland to breed.
- Sensing
    - Frogs can sense whether or not a wetland is dry and not go to it breed. This is mostly to simulate the frogs going to the wetland and finding no water and then going to another wetland to breed. They can sense which wetland within a certain distance contains the most frogs (in real life this would be the chorus size). Frogs can also sense whether or not there are individuals of the opposite sex at the same wetland.
- Interaction
    - The only interaction in the model is for breeding. A female can only produce a clutch if there is a male present at the wetland. Both males and females change their “my-wetland” based on the presence of an individual of the opposite sex.
- Stochasticity
    - Stochasticity is a large part of this model. When moving, the probability of juveniles stopping and settling for the winter or moving on is based on a

random distribution. The probability of returning to the natal pond or a different pond is based on a probability. The distance individuals move each time they move is based on an exponential distribution.

- Collectives
    - There are no collectives.
- Observation

Initialization

The model landscape was based on the section of Great Smoky Mountains National Park that contains Cades Cove. The study area included all of the wetlands in the Cades Cove area that are known to have breeding populations of anurans. The area is surrounded by mountains that are thought to be a geographic barrier to frog movement and thus a reasonable natural feature to delineate the study area.

In ArcGIS, we created a layer that was used to estimate the chance of an individual moving to a forested or not forested patch at different distances from the wetlands. The type of patch a frog moved to was then used to determine the chance that a particular individual survives. We did this using a vegetation layer and a wetlands layer. To create the layer, we first reclassified a vegetation layer provided by GSMNP into forest and non-forest (1 and 0 in the raster) (Table A.1.1). After creating this layer, we ran a Euclidean Distance tool from the wetland polygon, then reclassified that distance layer into distance groups so that there would be a group for 0m to 50m , 50m to 100m, 100m to 200m, 200m to 400m, 400m to 800m, 800m to 1600m and over

1600m. Lastly, we ran zonal statistics to determine the percent forest cover in each of the rings around each wetland. These numbers were then converted into an ASCII file format to be used in NetLogo.

The initial patch values are forest or non-forest and wetland-name (or “none” if the patch is not a wetland) and the wetlands have a forest cover percent for the rings around it (at 50, 100, 200, 400, 800, 1600, and >1600 meters) calculated using ArcGIS (ESRI 2011). Each wetland name is grouped as a patch set. The landscape is generated first with the “Setup-landscape” button and then the frogs are generated with the “Reset” button. The initial number of frogs is based on a slider and the age of individual frogs is drawn from a random Poisson distribution with a mean of 5 (though the initial ages are probably not greatly important in the long run).

Input Data

There is no input data

Submodels

- **Frog Emigration**

  In this submodel the frogs move from the wetlands out to the woods for their winter hibernation. Literature says that most hylid frogs don’t move more than between 30 and 240 meters from the ponds (Garton and Brandon 1975, Roble 1979, Semlitsch and Bodie 2003, Pellet et al. 2006). So in the testing of the model, the average movements should not exceed those distances. To allow for frogs movements that exceed our expectations, we varied the average move distance from an exponential distribution, allowing for “super frog” outliers either using a mean of 50, 100, 150, or 250.

The frogs move differently based on their sex and age. Adults draw their move distance from an exponential distribution with a mean that is equal to the “dist” value. Juveniles move based on twice the “dist” value. When a wintering location is not set (individuals under age 2), individuals move to a random patch in the radius of the distance value they draw from the distribution. If they are at age 2, they set that location as a winter location, recording the location as “home-x” and “home-y”. If the settling location has been defined in a previous step, they go to that location. During this stage, they have a chance of surviving. Juveniles have a 0.5 percent chance if they land on non-forest and 0.6 chance if they land on forest. Adults have a 0.75 chance of surviving if they land on forest and 0.55 percent chance if they land on non-forest.

- **Frog Immigration**

In the immigration submodel adult frogs move back to a wetland to breed. Juveniles move again like they did in the emigration submodel. If a juvenile is reaching adulthood (age 2), they choose to either go to their natal pond or to the nearest pond based on the natal_prob (0.5). Adult males (age > 2) either move to the wetland where they most recently bred (in the case where they have not yet bred this is the natal pond) or to the nearest pond to the wintering location. Adult females either move to the wetland where they most recently bred (in the case where they have not yet bred this is the natal pond) or to the pond with the most frogs (i.e. the largest chorus) if it is within twice the “dist” value. If the pond with the most frogs is not within twice the move “dist” she will go to the nearest pond. The pond with the maximum number of frogs in it is determined by identifying which patch set of wetland patches has the most total frogs. This is reported in the “max-wetland” reporter.

- **Frog Reproduction**

Once at the pond, the frogs go through the frog reproduction submodel. First, the frogs check to see if there are individuals of the opposite sex at the pond. If there are, all females hatch 400 multiplied by the individual wetland larval survival number (Table A.1.2) of new frogs multiplied by a weather value ((wetland-num + weather-num)/2). Wetland Number is either 3 (Settling Lagoons, Abrams Creek Spring, and Beaver Pond), 1 (Stupkas Sinkhole, Methodist Pond, and Gum Swamp), or 0.5 (Gourley Pond, Abrams Creek Oxbow, and Shield Pond). Weather number is either 0 (dry year), 1 (normal year), or 2 (wet year). The offspring have a 50/50 sex ratio and set their “natal-wetland” and “my-wetland” to the wetland in which they are born. Then the males and females change their “my-wetland” to the wetland they just successfully bred at. The “hatch” can happen more than one time each year depending on what category the wetland is in (1, 2, or 3) and what the weather number is (explained below).

  - **Weather Number:**
    - For each seasonal time step, the hydroperiod for the wetlands will be determined by two factors:
      - The previously determined ranking of the wetland within the network of wetlands

- The odds of a wet or dry year are based on weather data for the area (mm per year for the last 15 years), each year is pre-determined to be a wet or dry year based on the observed values.

- Wetlands each have a predetermined value for how likely they are to produce metamorphs, this value is added to the weather number and divided by 2 to determine how many clutches the pond can produce in a given year.

Literature Cited

ESRI. 2011. ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, CA.

Garton, J. S., and R. A. Brandon. 1975. Reproductive ecology of the Green Treefrog, *Hyla cinerea*, in southern Illinois (Anura: Hylidae). Herpetologica **31**:150-161.

Pellet, J., L. Rechsteiner, A. K. Skrivervik, J. F. Zurcher, and N. Perrin. 2006. Use of the Harmonic Direction Finder to study the terrestrial habitats of the European tree frog (*Hyla arborea*). Amphibia-Reptilia **27**:138-142.

Roble, S. M. 1979. Dispersal movements and plant associations of juvenile Gray Treefrogs, *Hyla versicolor* Le Conte. Transactions of the Kansas Academy of Science (1903-) **82**:235-245.

Semlitsch, R. D., and J. R. Bodie. 2003. Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology **17**:1219-1228.

Figures

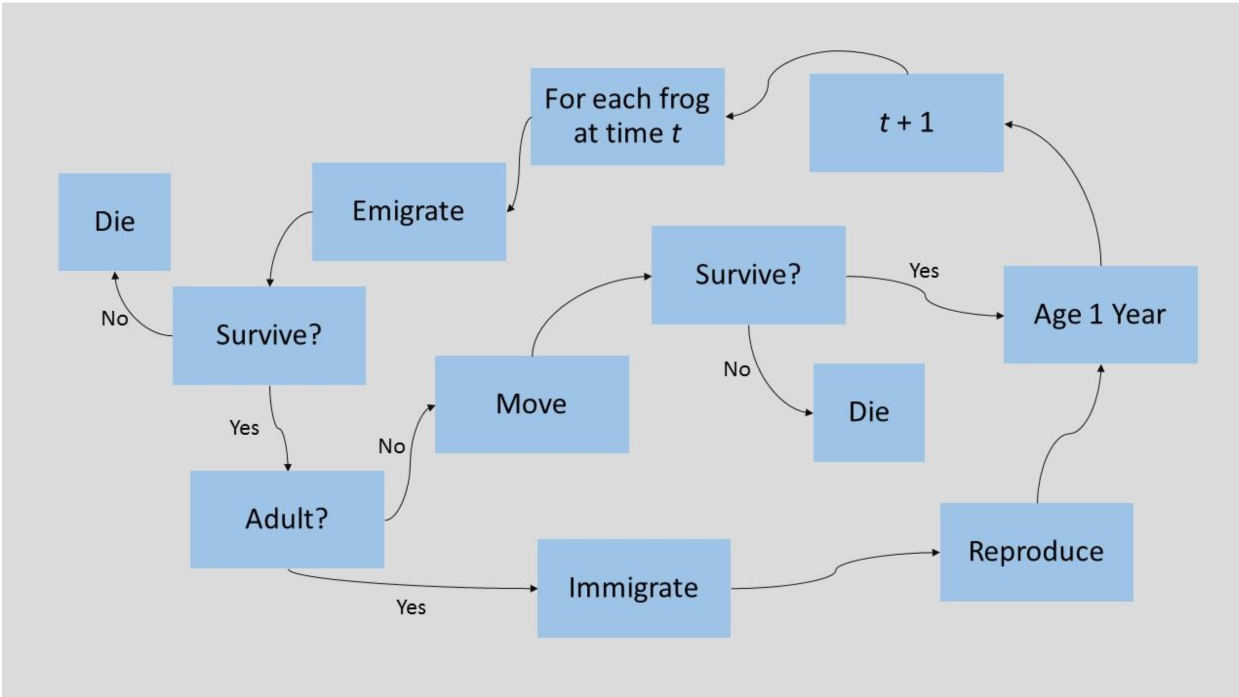

Figure A.1. For each frog at time t, the above schedule is observed.

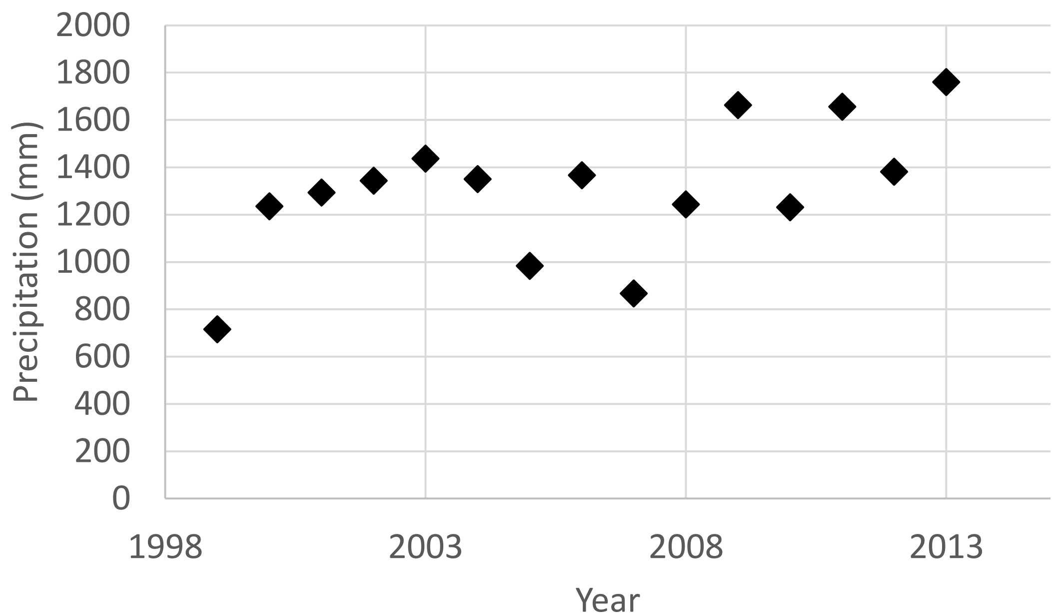

Figure A.2. Precipitation values each year in millimeters per year.

Tables

Table A.1.1. Classification scheme for converting the vegetation file into a binary forest/non-forest layer

Original Category	Reclassified Category
Chestnut oak forest	forest
Cove forest (typic type)	forest
Chestnut oak forest (heath type)	forest
White pine-xeric oak forest	forest
Yellow pine forest	forest
Acid cove forest (typic type)	forest
Hemlock forest (typic type)	forest
Water	non-forest
Oak-hickory forest (red oak type)	forest
Successional hardwood forest	forest
Cove forest (rich type)	forest
Oak-hickory forest (typic acidic type)	forest
Rock	non-forest
Floodplain forests	forest
Cultivated/pasture/old-field	non-forest
White pine forest	forest
Grape opening	non-forest
Human influence	non-forest
Alluvial vegetation (non-forested)	non-forest
Sparse vegetation	non-forest
Northern hardwood/acid hardwood forest	forest
Northern hardwood/boulderfield forest	forest

Table A.1.2. Values used for reproduction rates at different wetlands. Locations that were not included in the original study but had permanent water were given a value of 1 for survival (to intentionally make the model more generous).

	Percent Survival	Scaling Value	Value Used
Abrams Creek Oxbow	0.811	0.05	0.040556
Gourley Pond	0.644	0.05	0.032222
Shield Pond	0.856	0.05	0.042778
Abrams Creek Spring	1.000	0.05	0.05
Beaver Pond	1.000	0.05	0.05
Methodist Pond	0.467	0.05	0.023333
Gum Swamp	0.533	0.05	0.026667
Stupkas Sinkhole	0.722	0.05	0.036111
Settling Lagoons	1.000	0.05	0.05