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# Flames and Frogs – The Impact of Environmental Disturbances on Host-Parasite Dynamics

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Flames and Frogs – The Impact of Environmental Disturbances on Host-Parasite Dynamics

by

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A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
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College of Arts and Sciences  
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## DEDICATION

The successful completion of this work is dedicated first to my grandparents for having always shown their unwavering love and encouragement in my journeys (most of which they kindly and politely only pretended to understand) and for having also served as life-long role models who upheld an unparalleled work ethic. To many whom I consider to be my chosen family, especially Ann Williams and Brittany Sears, who kept me laughing, but more importantly, kept my crazy train from derailing during these tumultuous years. To Wayne Price and Tom Jackman, who not only fostered the success of my career, but have also been the epitome of patience and kindness. To DeAngelis, for the many hours of laughter, conversations, and adventuresome treks that further kindled my knowledge, love, and respect for Florida's ecology. To family in Alabama who have either helped shape my brazen character or made this education possible. To Taego, the one to whom I am bound through so many of the stories that begin with, "Remember when...?" and who is often so kind and thoughtful though he still holds tightly to the stereotype of the youngest sibling. Finally, to Fen for being my smiling, bright blue-eyed, spunky kid who has been on this journey with me from the get-go; for keeping me from getting too big for my britches; for his intrinsic fire that burns brightly for equality, fairness, and friendship; and for inspiring me to be the best example of a mother that I can possibly be.

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## ABSTRACT

Wildlife diseases impact the success and sustainability of native populations; therefore, it is crucial to understand the disturbances that are capable of shifting the dynamics between hosts and parasites. In terms of elucidating the effects of introduced parasites or determining how alterations occur in the abundance of parasites already present in the environment, ecological disturbances are understudied. Here, I use the Cuban treefrog (CTF), *Osteopilus septentrionalis*, to examine both of these facets of disturbance because 1) the CTF is an introduced species for which little work has been conducted on the parasites it is either introducing and/or acquiring, and 2) the CTF is also common to habitats that undergo frequent prescribed burning. Thus, the CTF can be used to address parasite spillover, spillback, and dilution while also addressing the direct and indirect effects that prescribed burning may have on parasites.

Even though there is strong evidence that introduced parasites (novel weapons) can greatly impact native host populations, there is also a growing body of literature that suggests that invasive species often lose many of their parasites after invading and colonizing new areas (enemy release hypothesis). I first show that the CTF has undergone enemy release because it is nearly void of parasites that are common to it in its native range. Next, I show that the CTF may be simultaneously changing parasite burdens of native treefrogs by either introducing new parasites (spillover effect) to these frogs or by the CTF acquiring new species parasites in the invaded area that can then be subsequently transmitted back to the native wildlife. For example, I show that one of these introduced parasites, *Aplectana* sp., can successfully infect a native

treefrog, *Hyla femoralis*, and that *H. femoralis* is less tolerant of these infections than *O. septentrionalis* because this nematode caused a greater loss in body mass for *H. femoralis* than *O. septentrionalis*. Despite *H. femoralis* shedding fewer total worms in their feces than *O. septentrionalis*.

To determine how host age and behaviors might influence the spread or acquisition of the three most prevalent parasites (*Aplectana* sp., acuariids, metacercariae) in CTFs, I investigated the relationship between parasite intensity and CTF age (host age-intensity relationship). These data show that *Aplectana* sp. intensity tends to increase with host age for both male and female CTFs. As such, these older frogs are likely to be the individuals spreading the greatest number of *Aplectana* sp. in the wild. I also show that as female and male CTFs age, they continue to acquire native acuariid nematodes. Because the CTF is likely a paratenic host to the acuariids, at this time it is unclear as to whether these infections may result in an increase in acuariids in native frogs (spillback effect) or whether the CTF is helping to remove the parasites from the environment (dilution effect) because we do not know how often these infected CTFs are being consumed by final hosts. In contrast to the acuariids and *Aplectana* sp. nematodes, there does not appear to be a change in trematode metacercarial intensity as CTFs age. The CTF serves as an intermediate host for the metacercariae and could be contributing to spillback to native frogs because it is not uncommon for intermediate hosts to be preferentially preyed upon by larger predators, many of which are final hosts for the trematodes.

In addition to using the CTF to determine how an invasive host may change the host-parasite relationships of native species, I also used the CTF to determine how parasites may change in burned habitats. Here, I show that fire can interact with disease dynamics in predictable manners, but these results may also depend on the sex of the host. In summary,

where fire recruits hosts, we can expect parasite abundance to increase, and where parasites are in direct contact with the intense heat of fire, we can expect a decrease in parasites. For example, CTFs that harbor parasites (e.g. acuariids and metacercariae) that are transmitted by invertebrate intermediate hosts that tend to be 1) recruited to recently burned areas due to increased food availability or 2) experience increased productivity/fitness due to increased nutrient availability show an increase in abundance after burns. In contrast, juveniles of *Aplectana* sp. nematodes are free-living and are subjected to extreme heat during burns. As expected, the intensity of *Aplectana* sp. in CTFs tends to decrease through time after burns. This correlation between increased temperature and nematode (both free living and parasitic) death was confirmed during controlled burns in the field and in mesocosms. Furthermore, the significant reduction in nematodes was observed despite variation in burn duration and intensity. Surprisingly, there were no discernible changes in parasite trajectories through time, indicating that there was no evidence of parasite recovery in CTFs that had been captured from wetlands that had experienced fire nearly seven years prior to collection. These results suggest that disturbance by fire might create long-term effects on host-parasite dynamics.

My collection of work establishes the need to further consider the factors that can shift host-parasite dynamics and how host populations may be influenced by these altered dynamics. Given that invasive species continue to threaten native populations on a global scale, it is important to not only understand how they outcompete or replace native species, but to also appreciate that they can cause shifts in host-parasite relationships with potential positive and negative effects on native hosts (e.g. spillover, spillback, and dilution effect). In addition to invasive species altering parasite abundance, there are other ecological disturbances, such as fire,

that can alter parasite abundance. Consequently, managing diseases of wildlife stands to benefit from appreciating these impacts that invasive species and fire can have on infectious diseases.

## INTRODUCTION

Infectious diseases of wildlife are associated with considerable losses to human lives, biodiversity, and ecosystem services [1-5], and the emergence of such diseases typically follows a change in the relationship or the interaction between hosts and parasites [5-7]. Thus, identifying the disturbances that alter host-parasite relationships is important to understanding emerging diseases. For example, several biotic and abiotic disturbances, such as climate change [8, 9], urban development [10, 11], deforestation [12-15], wildfire [16, 17], and introduced species [18, 19], are capable of structuring communities of hosts. However, how such disturbances influence the spread and emergence of diseases is widely debated (e.g. climate change: [20-25]; urbanization: [23, 26]; habitat fragmentation and deforestation: [27]). Though some disturbances, such as introduced hosts and parasites, disrupt native host-parasite relationships [18, 28-32], less is known about how introduced species contribute to declines in native host populations. Furthermore, fire, which is a relatively common disturbance, likely affects host-parasite relationships, but little is known about these interactions. By improving our understanding of what catalyzes changes in host-parasite dynamics, we can better predict disease patterns for both humans and wildlife.

### **Shifting of host-parasite relationships via introduced host species**

The spread of introduced species is a global problem that has gained widespread attention because it can cause long-lasting ecological changes [33]. Such deleterious impacts include

economic damage [34], disease transmission [2], displacement and consumption of native species [29, 33, 35], and hybridization with native species [19, 36]. The role that introduced species play in disrupting ecosystem balance [37] and extirpating native organisms [29] may be mediated by changes to parasite burdens of introduced and/or native hosts [30-32, 38], whereby parasites may be lost (enemy release), transmitted (spillover or spillback effect), or diluted.

The enemy release hypothesis proposes that introduced species readily establish in nonindigenous areas because they leave behind many natural enemies, such as parasites, in their native ranges [39-41]. For example, Burke *et. al.* (2007) report that two introduced species of lizards in several different populations across the United States have lost most of their gut and blood parasites. In Hawaii, the introduced Coqui frog, *Eleutherodactylus coqui*, does not have any parasites that commonly infect it in its native Puerto Rican habitats [43, 44]. Although there is growing evidence in support of this loss of parasites from the native areas into the invaded areas, studies suggest that evaluation of enemy loss should 1) be considered relative to parasite infection intensities of native hosts and 2) with respect to the expanding ranges of the introduced hosts. For example, Colautti, Ricciardi [45] caution against accepting that enemy release holds true at the community level where introduced hosts might not have fewer enemies (parasites) than the native hosts. In this case, in order for the enemy release hypothesis to hold at both the biogeographical and at community levels for the introduced host, both the introduced and native hosts' parasites must be considered. In the Panama Canal watershed, the introduced Nile tilapia, *Oreochromis niloticus*, harbored fewer parasite species than in its native range, and it also had lower parasite abundance compared to the native Blackbelt cichlid, *Vieja maculicauda* [46]. Furthermore, even though introduced hosts do lose parasites, studies suggest that a significant loss of native parasites in introduced hosts is most noticeable along the invasion front; introduced

hosts at the original invasion sites might not be entirely free of parasites harbored in its native ranges (reviewed in Poulin [47]).

In addition to undergoing a shift in their own parasite loads, introduced host species can also impact parasite abundance of native host species [30-32]. The parasite spillover effect suggests that introduced host species can introduce new parasites to native host species (Figure 1). If these introduced parasites happen to be more virulent in the native hosts than in the introduced hosts, then the parasites might become a source of “novel weapons” that allow the introduced hosts to obtain a greater competitive advantage over the natives [48-53]. A review by Lymbery, Morine [53] found that 85% of introduced parasites were more virulent in native hosts than in the introduced hosts. For example, in Hawaii, malaria transmitted via a non-native mosquito is causing significant declines in many native species of birds [54]. Even if some spillover parasites are not as virulent in native hosts, they can still negatively impact native hosts. For example, the introduced castrating barnacle, *Loxothylacus panopaei*, causes the mud crab, *Eurypanopeus depressus*, to be more susceptible to the predatory blue crab, *Callinectes sapidus* [55]. Additionally, several different native species of aquatic invertebrates and vertebrates in Europe have declining populations as a result of parasite spillover [31].

Introduced hosts might intensify the abundances of native parasites if the introduced species are suitable hosts or reservoirs for the native parasites (“spillback effect”; Figure 1) [32, 56, 57]. For example, an introduced species of the American mink, *Neovison vison*, can release nearly three times as many trematodes that normally infect the Eurasian otter, *Lutra lutra* [58]. In another example, an introduced snail, *Pseudosuccinea columella*, can successfully be infected with the liver fluke, *Fasciola gigantica*, which can then infect humans. The introduced snail is hypothesized to increase fascioliasis (disease caused by *F. gigantica*) in humans in Egypt by

increasing the abundance of *F. gigantica* in the environment [59]. By amplifying parasite abundance or serving as reservoir hosts, introduced hosts could be negatively impacting the success of native host populations. For example, Faillace et al. [60] discuss how parasites (e.g. viruses) transmitted between introduced and native hosts led to population declines of several native host species.

Introduced hosts may remove native parasites from the environment if: 1) they become infected with parasites but do not then directly transmit them back to the native hosts, or 2) they reduce the probability that the parasite can effectively be transmitted to the proper final host; this process is termed the dilution effect (Figure 1) [53, 57, 61, 62]. Gendron and Marcogliese [63] show that an introduced round goby, *Neogobius melanostomus*, can successfully be infected by the native acanthocephalan, *Neoechinorhynchus tenellus*, but the parasite then dies. Additionally, when *N. melanostomus* was absent from waters that harbored a native darter, *Etheostoma nigrum*, this native fish species had a higher parasite infection intensity and abundance, which suggests that the presence of *N. melanostomus* can cause a diversion for parasites to infect it rather than the native darter. Similarly, Gagne, Heins [64] found that both native and introduced fishes in several Hawaiian watersheds have an overall lower parasite abundance when they live in the same watersheds, suggesting that a mutual dilution effect may be occurring between the different species of fishes. Though introduced species can impact host-parasite relationships of natives, research of these shifting dynamics pales in comparison to general research conducted on the success of biological invasions, and we must continue to



investigate how introduced hosts are altering parasitism of natives [47].

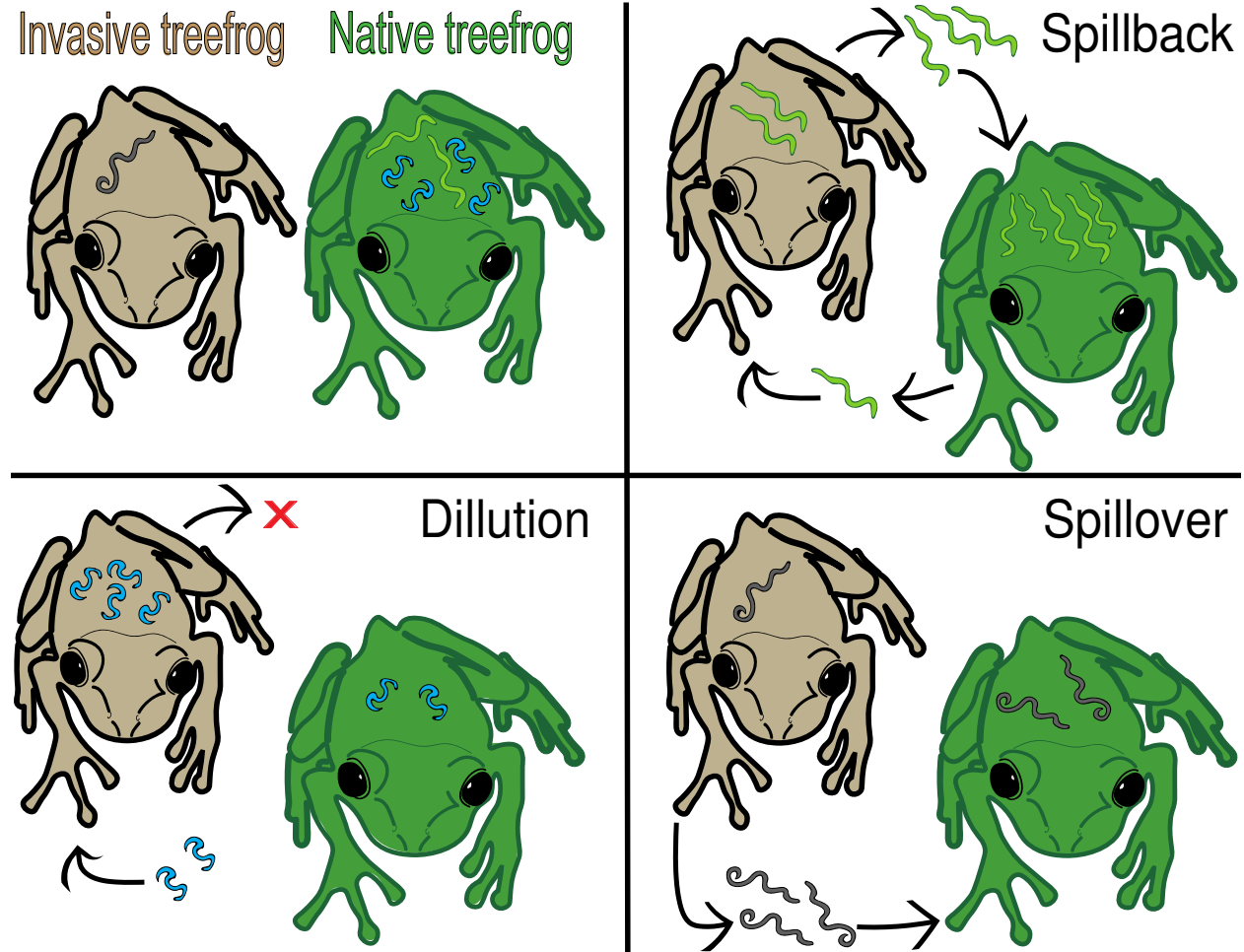


Figure 1. Shifting parasite relationships via an introduced species. Each panel is respective to the top left diagram, which shows the parasites harbored by an introduced and native host prior to parasite exchange. Each color worm represents a different species of nematode. The top right panel represents an introduced species becoming successfully infected by a native parasite and then increasing the abundance of that parasite species. The bottom left panel is an example of the introduced host removing parasites from the environment that would normally infect native hosts. Here, the parasite may not be able to further complete its life cycle to then go on to infect natives; therefore, it is diluted in the environment. As a result, the native host may now harbor fewer of these parasites. In the bottom right panel, an introduced host is introducing a parasite

that can successfully infect the native hosts that may then be more adversely affected by the parasite compared to the infected introduced hosts.

### **Altering parasite abundance with fire**

Fire can impart drastic changes across both aquatic and terrestrial habitats [1, 65], and such changes include cycling of nutrients, supplying new food and habitats to animals, causing seedling recruitment and sprouting of vegetation, preserving indigenous animal and vegetation species, and also reducing the occurrence of destructive fires that are fueled by unnaturally accumulated biomass [16, 66-69]. We also know that humans have greatly impacted the occurrence of natural wildfires and could thus be interfering with an ecological process that might have profound effects on parasite burdens of wildlife. For example, in much of the present day developed world, fire is suppressed to minimize property and human losses, but this suppression dangerously facilitates fuel buildup, which can lead to intense and unmanageable wildfires [16, 66, 67, 70, 71]. Also, where anthropogenic-induced climate change facilitates drought and increases in temperatures, there will likely be an increase in the occurrence of wildfires [72-74]. However, a strong, predictive framework is required for establishing how the presence or absence of fire will influence diseases.

Community changes induced by fire, such as the clearing of overgrown vegetation followed by new growth that is more palatable to herbivorous invertebrates and vertebrates [75-77], likely impacts recruitment or movement of hosts to recently burned areas [78]. As a result, these preferred areas might subsequently either concentrate infected hosts or change how frequently hosts are encountering parasites [79, 80]. For example, studies present conflicting evidence for how tick abundance is expected to change with respect to fire. Cully (1999) found that tick abundance decreases with increased burning. In contrast, other studies have found that

tick abundance is higher in burned sites compared with control areas [81, 82], which is likely due to the presence of herbivores, such as deer (harboring ticks), that prefer burned habitats [80]. In a similar example, McCoy, Styga [79] show that there is a tendency for ectoparasitic mites, *Eutrombicula* sp., to have a higher infection prevalence on the six-lined racerunner, *Aspidoscelis sexlineata*, and the Florida scrub lizard, *Sceloporus woodi*, captured from recently burned Florida scrub communities.

In addition to attracting vertebrates to the burned areas, new, poorly defended vegetation may also serve as food for several arthropod species [83, 85-88]. An influx of these invertebrates may then attract birds that consume these arthropods [89-92]. As such, we might expect that bird-transmitted and/or arthropod-transmitted parasites to increase in burned habitats.

Another mechanism by which fire may alter parasite abundance is by changing the availability of nutrients. Through combustion of the vegetation, fire quickly converts compounds in the vegetation into char and ash. These carbon, nitrogen, and phosphorous compounds can then be readily taken up by terrestrial plants, but they may also be washed into nearby waterbodies. As nutrients become available in the water, they can enhance freshwater productivity via a bottom-up effect where more nutrients lead to more primary producers that can subsequently support a larger abundance of hosts. For example, nutrient-enhanced freshwater systems can increase snail abundance, which also likely increases metacercarial (trematode) infections due to an increase in cercariae shed from snails [93, 94]. Similarly, Hossack et al. (2013a) found that there was a positive correlation between burn severity and infection intensity of an aquatically-transmitted nematode, *Gyrinicola batrachiensis*, which is likely an artifact of increased tadpole densities in these more productive freshwater systems.

In contrast to indirectly increasing parasitic infections, we might expect fire to reduce parasites that are temperature-sensitive because they could be killed by the intense heat of fire or may become desiccated in the arid habitats that are created when most of the shade and soil moisture is reduced by the fire. For example, Derek Scasta, Engle [95] show that heat from fire in pastures can directly reduce the eggs and pupae of ectoparasitic horn flies, *Haematobia irritans*, that infect livestock. Furthermore, Hossack et al. [96] found that when toads were collected from recently burned areas (i.e. more arid), they were less likely to be infected by the chytrid fungus, a disease that is implicated in the global decline of amphibians. In a different study, Hossack, Lowe [84] found that burn severity negatively impacted the mean intensity of *Cosmocercoides variabilis*, a skin-penetrating nematode of amphibians. As previously discussed, there is evidence that fire can impact host-parasite relationships by either increasing or decreasing wildlife diseases, but a straightforward framework does not exist for predicting the occurrence of such differing effects.

### **Dissertation study system and objectives**

Florida harbors an abundance of introduced reptiles and amphibians (137 taxa) [35, 97], and it also holds statewide prescribed burning programs that aggressively monitor and manage lands with fire. Thus, the effect of ecological disturbances on host-parasite interactions can be thoroughly studied in this area. Specifically, I studied Cuban treefrog, *Osteopilus septentrionalis*, an introduced amphibian with a population range that overlaps lands that are managed with fire. *Osteopilus septentrionalis* outcompetes and displaces native treefrogs because it has a much larger body size, broad dietary niche [98], and year-round reproduction with average clutch sizes of 2000-4000 eggs [99], but no studies have addressed the possible success of *O. septentrionalis*

being mediated through parasites or a lack thereof. Furthermore, no studies in Florida have determined what effect fire has on parasites that infect *O. septentrionalis*.

My dissertation addressed how an introduced host species can disturb native host-parasite relationships and quantified the direct and indirect effects of fire on parasite transmission in aquatic and terrestrial environments. First, I determined if *O. septentrionalis* has indeed experienced enemy release from parasite species that commonly infected it in its native range (Chapter 1). Next, I showed how *O. septentrionalis* contributes to parasite spillover, spillback, and dilution, and I then quantified the effects of an introduced parasite on a native treefrog, *Hyla femoralis* (Chapter 2). Finally, I constructed and tested a framework for predicting the effects of fire on parasite transmission by first quantifying the effect of time-since-burn on parasites found in *O. septentrionalis*, and then I verified that fire directly induced mortality of soil-dwelling, parasitic nematodes (Chapter 3).

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CHAPTER 1: ACQUIRED AND INTRODUCED MACROPARASITES OF THE INVASIVE  
CUBAN TREEFROG, *OSTEOPILUS SEPTENTRIONALIS*

Note to Reader:

This chapter has been previously published and is attached as Appendix A. See Appendix B for permission from the publisher.

## CHAPTER 2: INTRODUCED SPECIES AND HOST-PARASITE DYNAMICS

### **Abstract**

Host-parasite dynamics can be disrupted by introduced species. Introduced hosts are capable of introducing new parasite species and altering the abundance of parasites that are already present in native hosts. The traits of introduced hosts might shed light on what type of an effect they will have on overall parasite abundance in the environment. For the first part of this study, we evaluated the effects of a likely introduced nematode (*Aplectana* sp.) on native treefrogs (*Hyla femoralis*) compared to the invasive Cuban treefrogs (*Osteopilus septentrionalis*). There was a marginally significant effect of worms on the proportional mass loss for both species of treefrogs. *H. femoralis* was less tolerant of these infections than *O. septentrionalis* because this nematode caused a greater loss in body mass for *H. femoralis* than *O. septentrionalis* despite *H. femoralis* shedding fewer total worms in their feces than *O. septentrionalis*. In the second part of the study, we tested for monotonic and nonmonotonic relationships between host age and the intensity of infection for the three most prevalent parasites (*Aplectana* sp., acuariid nematodes, and trematode metacercariae) in *O. septentrionalis* to determine what effect age may have in spreading or acquiring parasites. We found that, for both males and females, there is a significant linear increase in *Aplectana* sp. and encysted acuariids with host age. There was no detectable significant effect of host age on the intensity of metacercariae. These results suggest that an introduced host along with one of its introduced parasites could have effects on native treefrog populations.

## Introduction

Introduced hosts can disrupt host-parasite relationships of native species in several ways [30-32]. For example, introduced hosts can introduce new parasites to native host species, a phenomenon described as “parasite spillover”. This can be particularly problematic to native hosts if they lack sufficient defenses against these new infections [49-52]. For instance, in Europe native aquatic wildlife species, ranging from invertebrates like crayfish and mollusk species to aquatic vertebrates like fishes and amphibians, have experienced population declines because of parasite spillover from introduced hosts [31]. In addition to introducing parasites, introduced hosts that become suitable hosts or reservoirs for parasites already common to native host species can then magnify the abundances of native parasites, a phenomenon termed “parasite spillback” [32, 56, 57]. For example, Sherrard-Smith, Chadwick [58] showed that the introduced American mink, (*Neovison vison*) can be infected with a trematode species that is native to otters (*Lutra lutra*), and it potentially sheds nearly three times as many of these parasites as the native otter. In another possible situation, the introduced host may acquire but not transmit native parasites, thereby effectively removing them from the environment and decreasing the risk to natives, which is often referred to as the dilution effect [53, 57, 61]. Gendron and Marcogliese [63] found that an introduced goby, *Neogobius melanostomus*, could potentially decrease an acanthocephalan, *Neoechinorhynchus tenellus*, because the parasite infects the goby but then dies prematurely in contrast to the native species of fishes that successfully serve as paratenic hosts. Although in the short-term introduced hosts are often thought to leave most of their parasites behind in their native range (enemy release hypothesis) and only acquire a few new parasite species in their introduced range [39, 100], it is imperative to understand how any potential parasite spillback, spillover, or dilution effects can impact native populations. Given that many of the parasite-mediated effects of

introduced species on native species are adverse to native hosts, there is extensive interest in understanding the role parasites play in facilitating these invasions and how to best-manage their impacts on the natives [53].

For populations of introduced hosts that either become suitable hosts for native parasites or serve to introduce new parasites, employing the relationship between the ages of hosts and their parasite infection intensities (age-intensity relationship) could help to identify which introduced hosts pose the greatest risk to native hosts. For example, many age-intensity relationships increase monotonically, either increasing continuously with age or reaching some asymptote because parasite death balances parasite colonization [101](Fig. 1). In these cases, the oldest introduced hosts would have the most parasites and thus would represent the greatest risk of transmitting or amplifying native or introduced parasites in native hosts. In contrast, if hosts acquire the majority of a parasite species as juveniles but then are minimally re-infected as adults, we may expect to see a monotonically negative age-intensity relationship as parasites either die naturally or are killed by the immune system of the host (Fig. 1). In this case, the youngest introduced hosts would have the most parasites and thus would represent the greatest risk of transmitting or amplifying native or introduced parasites in native hosts. In some cases, age-intensity relationships are convex, peaking at intermediate ages [102-104] because of acquired immunity or parasite-induced mortality in older hosts [102, 105, 106] (Fig. 1). In this case, intermediate-aged introduced hosts might pose the greatest threat to native hosts.

The Cuban treefrog (CTF), *Osteopilus septentrionalis*, is an ideal host for evaluating parasites that are either associated with spillover, spillback, or dilution, and also for addressing age-relationships. First, it is a highly invasive amphibian species in Florida that is extremely abundant and easily captured [107, 108], and is already negatively impacting native treefrogs

because of its large body size, broad dietary niche, and extended reproduction period [98, 99]. Additionally, CTFs have acquired parasites from native frogs and have also introduced parasites to native frogs [109]. Additionally, CTFs have an aquatic and terrestrial life stage, which allows us to address how their parasite loads change as a function of age for infections primarily acquired early in life as aquatic tadpoles or later in life as predominantly terrestrial juveniles and adults.

Our first objective was to determine if an introduced parasite of the CTF could effectively spillover to native Pinewoods treefrogs, *Hyla femoralis*. [109]. To address this objective, we exposed both CTFs and *H. femoralis* to *Aplectana* sp., a nematode that has likely been introduced by CTFs, and determined infection success and growth rates of each frog species. We hypothesized that *Aplectana* sp. would not be as infective to *H. femoralis*, a novel host, as it is to the CTF, a host with which it has coevolved (naïve host syndrome) [110]. Furthermore, we hypothesized that any *H. femoralis* that do become infected might experience greater pathology and lower growth rates because of this lack of coevolution with *Aplectana* sp. [53, 110, 111].

Our second objective was to offer insights into the traits of CTFs that might predict their potential for spillover, spillback, or dilution by quantifying the relationship between the abundance of their parasitic infections (native and introduced) and the age/size of both male and female CTFs. To address this second objective, we determined the best-fitting model of parasite burdens as a function of age for both sexes of CTFs. These analyses were conducted on the most common parasites recovered from CTFs: *Aplectana* sp. (gut nematode; likely introduced from Cuba), acuariids (encysted nematodes in gut wall; native to FL), and trematode metacercariae (encysted in mesenteries and body cavity; native to FL). Below we detail the life cycles of each parasite and describe and justify our hypotheses. By examining the age-intensity relationships for *Aplectana*

sp., acuariids, and metacercariae, we can begin to understand the impact that the CTF may be having on native treefrogs through either the introduction and/or the acquisition of parasites.

### *Life Cycles*

For *Aplectana*, the CTF serves as the only host in the nematode's direct life cycle. Gravid, adult female worms live in the intestinal tract of the CTF and release juveniles or eggs (viviparous or ovoviviparous) in the feces of infected frogs. These juveniles penetrate the skin of another frog to complete the life cycle. For acuariids, the CTF is a paratenic host. Acuariids are nematodes that require a bird as a final host with arthropods serving as intermediate hosts, but sometimes they will infect paratenic hosts (i.e. a host that can facilitate life-cycle completion but one in which the parasite does not continue to develop), such as fishes or amphibians that have ingested the infected arthropod [112]. Thus, CTFs acquire larval acuariids on the gastrointestinal tract when consuming another infected host. Finally, the CTF is an intermediate host for trematode metacercariae. Metacercariae are primarily acquired during the aquatic stage, where the tadpoles are exposed to cercariae, a larval swimming stage of the trematode life cycle. Cercariae typically infect amphibians by penetrating their skin or crawling up their cloaca.

### *Hypotheses*

Based on host traits, we can make general predictions about how age and sex of CTFs should affect the parasite burden of each of the three focal parasite species. First, we hypothesized that older, larger frogs would have the largest burdens of both *Aplectana* and acuariids because the predominantly terrestrial CTFs have accumulated exposure to these predominantly terrestrial parasites throughout their lifetime. Furthermore, CTFs are sexually dimorphic, with females often being much larger than males [99]. Thus, females have more surface area to contact larval *Aplectana* and need to eat more than males to maintain their larger body size increasing their



likelihood of consuming acuariids. Consequently, we expect older females to have the highest burdens and pose the biggest risk of transmitting *Aplectana* sp. and acuariids to natives.

Unlike *Aplectana* and acuariids, trematode metacercariae are mostly obtained when hosts are young and exclusively aquatic. Thus, CTFs should have predominantly decreasing exposure to metacercariae with age post-metamorphosis. For female CTFs, we hypothesize that the encysted metacercariae would either die naturally or be cleared as the host ages [113], producing a negative linear age-intensity relationship. Relative to females, males spend more time in the water during the breeding season in an effort to maximize mating opportunities [114]. Hence, males should acquire more of these aquatic infections as adults than females and should therefore have a less negative age-intensity relationship for this parasite.

## **Methods**

### *Aplectana* Spillover into *Hyla femoralis*

#### Host and Parasite Collections

Cuban treefrogs, *Osteopilus septentrionalis*, and Pinewoods treefrogs, *Hyla femoralis*, were collected as tadpoles in July of 2015 from Flatwoods Wilderness Park in Tampa, FL (28°07'01.08"N 82°18'11.15"W). Tadpoles were separated by species and reared outdoors in the shade in plastic boxes (33 x 20 x 15 cm) containing roughly 8 L of artificial spring water with approximately 10 tadpoles per box. Tadpoles were fed *ad libitum* on a spirulina-agar diet. After metamorphosis, the juvenile frogs were housed in the laboratory (22°C, 12 hr light-dark photoperiod) in individual plastic containers along with a wet, unbleached paper towel for bedding. All frogs received a new, clean container with bedding on a weekly basis. These juvenile frogs were fed *ad libitum* with vitamin-dusted crickets until all frogs reached a minimum snout-vent-length (SVL) of 20 mm.

Two weeks before nematode exposures, adult CTFs were collected from Flatwoods Wilderness Park, euthanized with tricaine methanesulfonate (MS-222), and necropsied to obtain adult *Aplectana*. Once the gravid, adult female *Aplectana* sp. nematodes were isolated from the CTF intestinal tract, the worms were then individually transferred to petri dishes (3.5 x 1 cm) along with approximately 2 mL 0.7 % saline water where they shed juvenile worms. Juvenile worms were allowed to grow for approximately two weeks to ensure that they had reached their infective, skin-penetrating stage before exposing them to the juvenile frogs. To also ensure that none of the juvenile frogs had been previously infected as tadpoles with nematodes in the wild, two weekly fecal checks for juvenile nematodes were conducted prior to experimental exposure. Fecal checks were performed by rinsing individual frog containers with approximately 2 mL of 0.7 % saline water and then observing this rinse water and any feces under a dissecting microscope to check for the presence of juvenile nematodes.

### *Experimental Design*

For the nematode exposures, each frog was first placed in a single petri dish (3.5 x 1 cm) that was capped and then sealed with parafilm (n=20 worm-exposed frogs and n=20 sham-exposed frogs per species). Next, through one large, premade hole in the petri dish lid, 20 juvenile *Aplectana* were counted with a dissecting microscope and then transferred to each worm-exposed frog along with approximately 1 mL of 0.7 % saline water. For sham-exposed frogs, approximately 1 mL of the same nematode water solution (but without nematodes) was pipetted through the hole into the petri dish. Each large hole was then sealed with tape to reduce evaporation of the water, but a smaller, premade hole in the lid was left uncovered to allow for airflow. These petri dishes containing the frogs were then randomly placed in a dark cabinet to minimize stress to the frog and left for 24 hr. After the 24hr exposure time, frogs were removed

from the petri dishes and placed back into their original individual plastic housing containers. The remaining juvenile *Aplectana* nematodes in each petri dish that were unable to successfully penetrate the frog were counted with a dissecting microscope. All frogs were kept at the same 22°C, 12 hr light-dark photoperiod throughout the remainder of the experiment.

Before exposure to *Aplectana* and each week thereafter, frog snout-vent-length (SVL) (to the nearest 0.1 mm) and mass (to the nearest 0.001 g) were recorded once per week for a total period of six weeks. Additionally, successful infections of established adult *Aplectana* nematodes in the gut were confirmed by performing fecal checks as described above. To isolate the effect of *Aplectana* sp. on frog growth, we fed each frog a restricted diet of three vitamin-dusted crickets two days per week to ensure that frogs could not compensate for resources lost to parasitism by consuming crickets *ad libitum*.

*Age-Intensity Relationships for Ctf's And Aplectana sp., Acuariid, and Metacercariae*

#### Host and Parasite Collections

During the summers of 2005-2008, 330 CTFs were collected from polyvinyl chloride (PVC) pipes encircling 18 wetlands within the Flatwoods Wilderness Park in northeastern Hillsborough County, Florida (28°07'01.08"N, 82°18'11.15"W). Within this park, the plant community is mainly a second-growth pine flatwoods forest matrix with numerous borrow pits, hardwood swamps, freshwater marshes, and cypress domes [108, 115]. After removing frogs from the PVC pipes, each frog was placed in a plastic bag, euthanized, and kept frozen until necropsied for parasite quantification. Frog SVL and wet weight were recorded. For frogs  $\geq 42$  mm, sex was determined by the presence/absence of nuptial pads and evidence of mature reproductive organs. All frogs  $\leq 41$  mm were considered juveniles due to a lack of discernable reproductive organs. The body cavity was opened by a longitudinal incision from vent to throat

and all internal body organs and mesenteries were examined for *Aplectana* sp., acuariids, and metacercariae. Parasites were counted and preserved in 70% ethanol. Identification and confirmation of parasites were provided by Charles Bursey at Pennsylvania State University's Shenango Campus and by Dr. Omar M. Amin at Parasitology Center, Inc. (PCI) in Scottsdale, Arizona.

## **Statistical analyses**

### *Aplectana Spillover into Hyla femoralis*

We used a linear model to determine the effect of treatment (control vs infected) on proportional mass change while controlling for the initial mass of each individual. For infected individuals, we also used a linear model to quantify the effect of total worms shed (a proxy for intensity of infection), initial mass, and species on the proportional mass change between week 1 and week 6. Initial mass of the frogs was included in the models because initial host mass may influence the number of worms that were successfully able to penetrate the host (resistance), or it may also be correlated with bigger frogs being able to support larger worms that could then shed more larvae. To control for the overdispersion of nematode counts in the feces, the counts were log transformed. We used the Anova function in the car\_package to generate probability values via log-likelihood ratio tests.

### *Age-Intensity Relationships for CTFs and Aplectana sp., Acuariids, and Metacercariae*

To address our second objective of quantifying the relationship between the intensity of parasitic infections (response variable) and the age/size (SVL) and year of collection of both male and female CTFs (explanatory variables treated as fixed effects), we used model selection to compare among several plausible models (Table 1). To best-account for the overdispersion of parasite counts in the hosts, our models included the comparison of negative binomial and Poisson error

distributions. To facilitate identifying nonlinear relationships that might exist between predictors and parasite intensity, we considered generalized additive models (GAMs) using the `gam` function in the `mgcv` package (with the default smoothing spline) [116]. With the `mgcv` package, a term called the *effect degrees of freedom* (edf) was used to assess how linear or non-linear the models were. These values range between 0 and infinity, and lower values indicate that the relationship is more linear. Finally, since CTFs were captured across multiple wetlands, we also considered models that treated wetland as a random effect (generalized additive mixed models, GAMMs). CTF infection was modeled independently for each parasite (*Aplectana*, metacercariae, and acuariids) and separately for each sex of the CTF. Juvenile frogs were used to model both the male and female populations.

To determine the best fitting age-intensity model for each parasite (*Aplectana*, metacercariae, and acuariid), we compared among models by comparing their AIC values as recommended by [116]. AIC considers a trade-off between the goodness of fit and complexity of a statistical model. Required normality and homoscedasticity assumptions were confirmed by visually inspecting plots of the fitted values versus the residuals [116]. All statistical analyses were conducted with R statistical software [117].

## Results

### *Aplectana* Spillover into *Hyla femoralis*

A total of 13 CTFs and 9 *H. femoralis* became infected, and the average ( $\pm$  SE) initial starting mass for each species was  $0.709 \pm 0.040$  g and  $0.547 \pm 0.038$  g, respectively. Hence, *Aplectana* can indeed spillover from CTF to the native *H. femoralis*. There was a marginally significant effect of treatment on change in proportional mass overall, both the control and infected CTFs and the control *H. femoralis* tended to gain mass throughout the experiment (Fig.

1). However, the infected *H. femoralis* lost mass (Fig. 1). Infected *H. femoralis* lost mass despite shedding significantly fewer worms (mean  $\pm$  SE:  $77.12 \pm 31.21$ ) than CTFs (mean  $\pm$  SE:  $171.5 \pm 19.74$ ;  $df = 20$ ,  $t = 2.686$ ,  $P = 0.01$ ) throughout the experiment (Fig. 2). Additionally, *H. femoralis* were less tolerant of infections than CTFs (Fig. 3). When controlling for initial mass, *H. femoralis* showed a greater decline in mass with each additional worm shed than CTFs (Species  $\times$  worms shed  $\times$  initial mass:  $F_{1,1} = 11.97$ ,  $P = < 0.01$ ; Fig. 3).

#### *Age-Intensity Relationships for CTFs and Aplectana sp., Acuariids, and Metacercariae*

The best-fitting models for all parasite species and each host sex were the GAMS with negative binomial distributions (Table 2). For both males (Fig. 4) and females (Fig. 5), all plots of the best fitting models against the partial residuals showed that the relationships for all parasite intensities and host age (using SVL as a proxy) were linear. These linear results are corroborated by the GAM edf values all having a value of one (Table 3). Host age was a significant positive predictor for both *Aplectana* sp. and acuariid intensities in both males (Fig. 4A and D) and females (Fig. 5A, B, and D), but there was no significant relationship between metacercariae intensity and age for males (Fig. 4B) or females (Fig. 5C; Table 3).

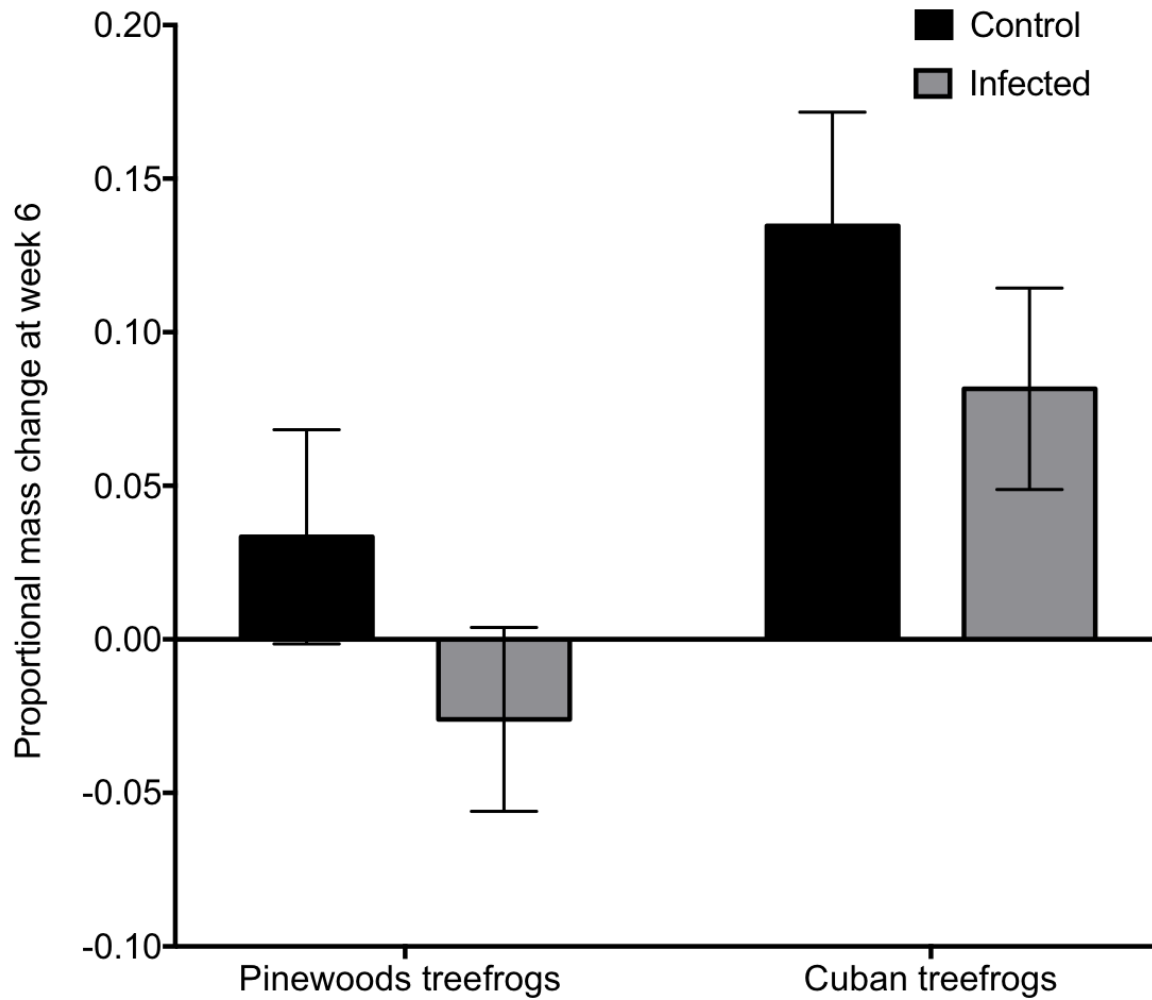


Figure 1. Proportional mass change between week 1 and week 6 for *Aplectana*-infected and control individuals. All treatment groups gained mass except for infected Pinewoods treefrog, *H. femoralis*, and there was a marginally significant effect of treatment on proportional mass change.

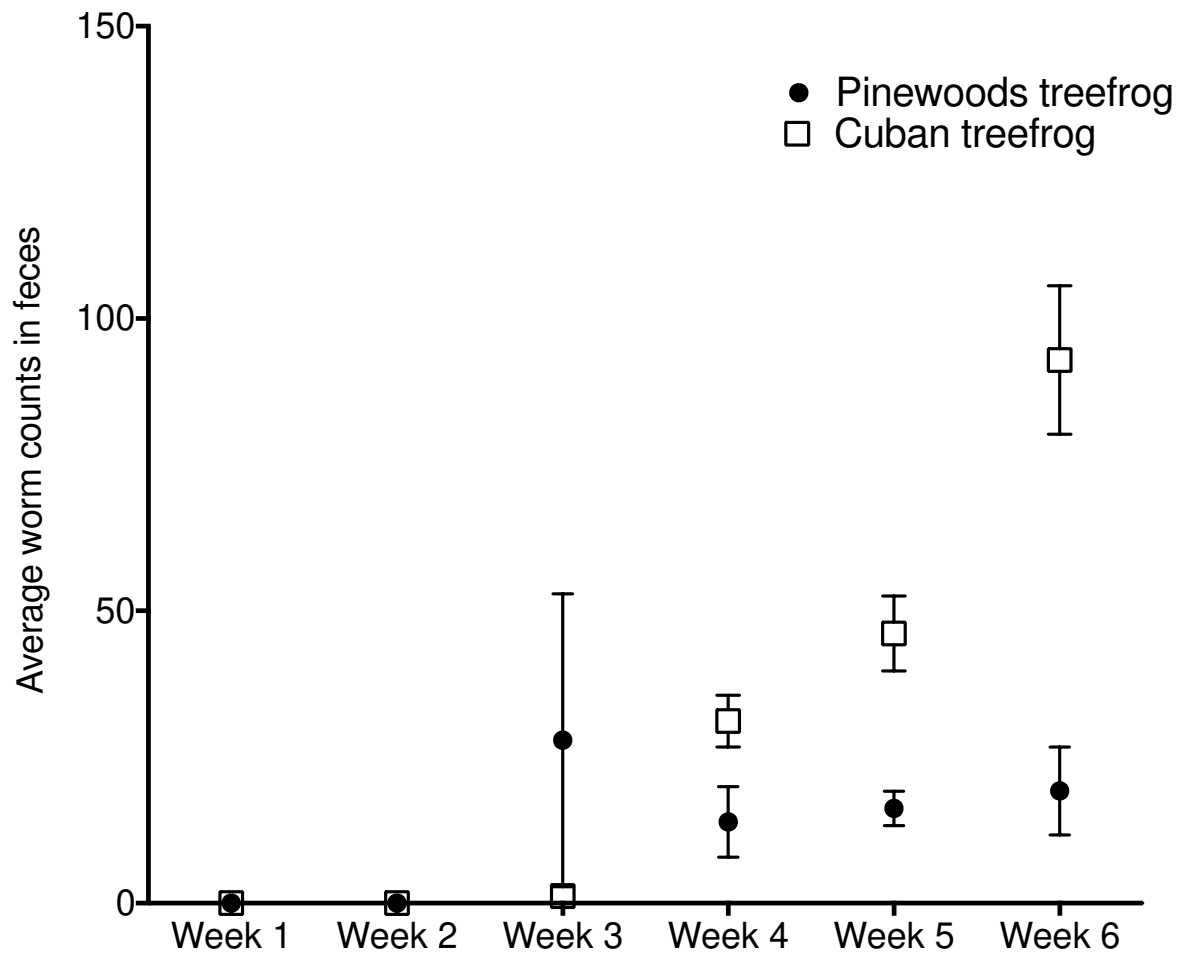


Figure 2. Average weekly fecal *Aplectana* worm counts for infected Cuban treefrogs and Pinewoods treefrogs. With the exception of the week 3 outlier, Cuban treefrogs consistently shed more worms each week.



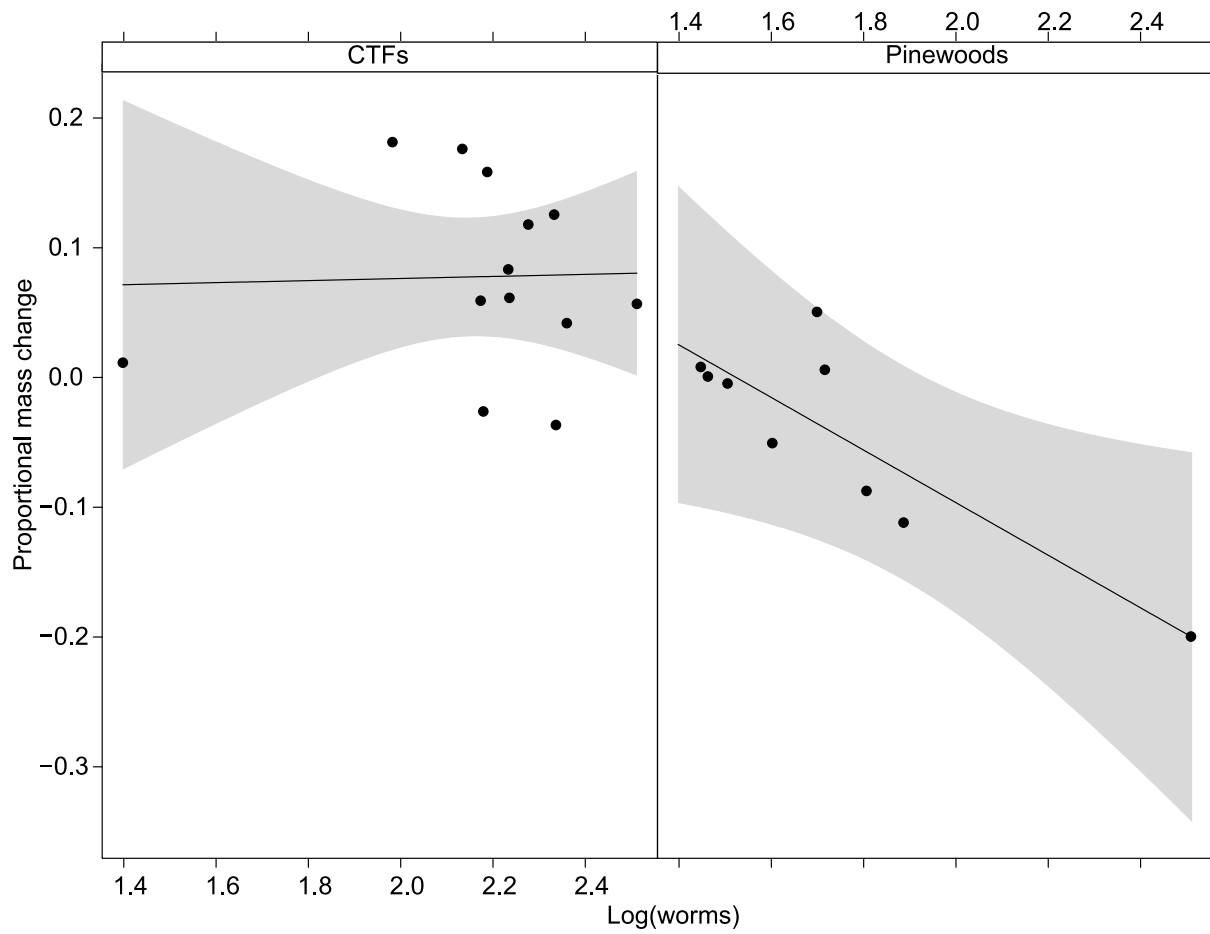


Figure 3. The proportional mass change for Cuban treefrogs and Pinewood treefrogs as a function of the number of *Aplectana* worms shed, controlling for initial mass (Species x worms shed x initial mass:  $F_{1,1} = 11.97$ ,  $P = < 0.01$ ). These results reveal that Pinewood treefrogs are less tolerant of infections than Cuban treefrogs

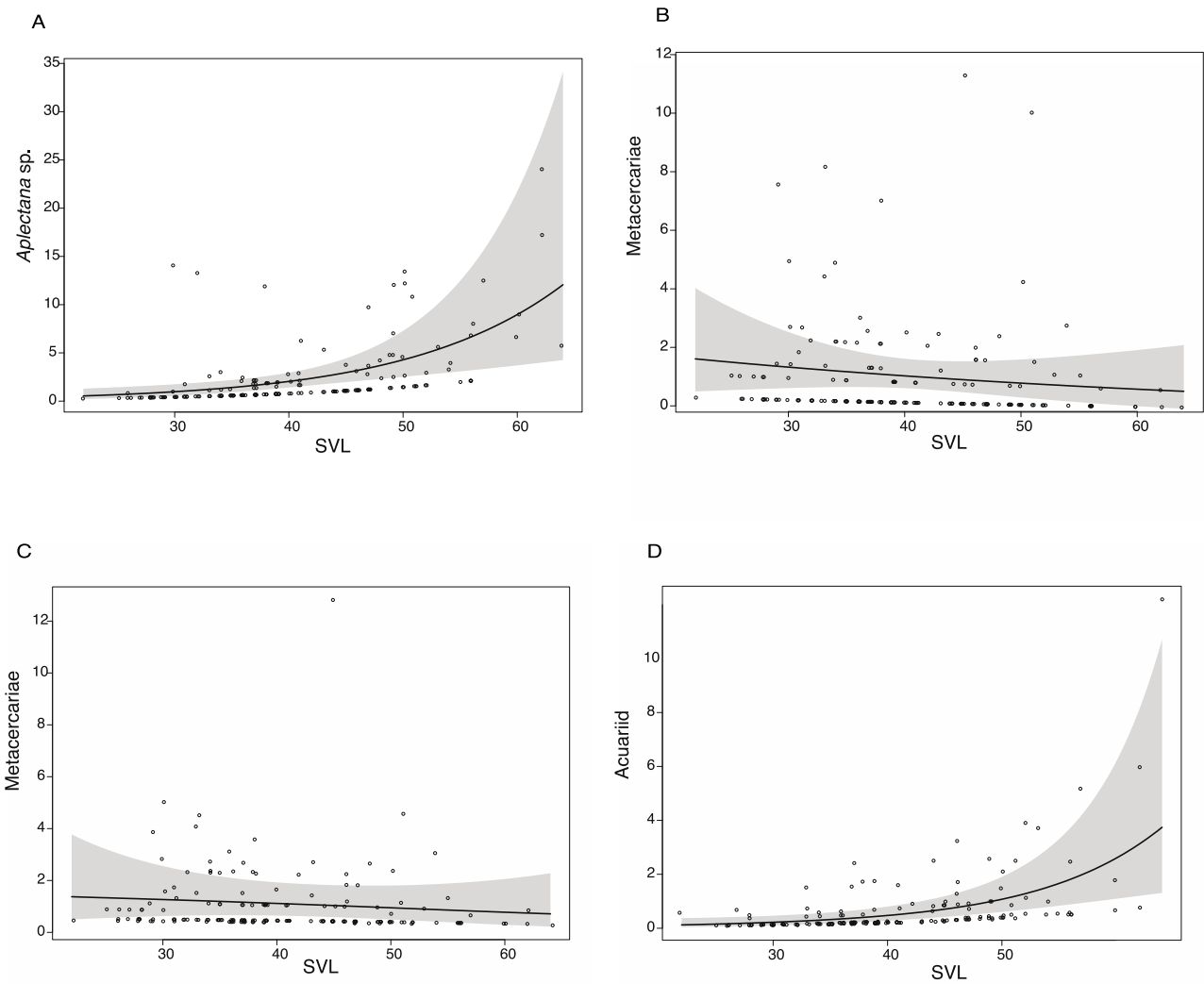


Figure 4. Plots of the partial residuals for the GAM best-fit models of parasite intensity against host snout-vent length (proxy for age) for male Cuban treefrogs. Each panel represents the following: A) *Aplectana* sp. (model I), B) metacercariae (model I) C) metarcercariae (model II), and D) acuariids (model II). Snout-vent length is a significant predictor of parasite intensity for *Aplectana* sp. ( $X^2 = 43.38$ ,  $P < 0.001$ ) and acuariids ( $X^2 = 18.16$ ,  $P < 0.001$ )

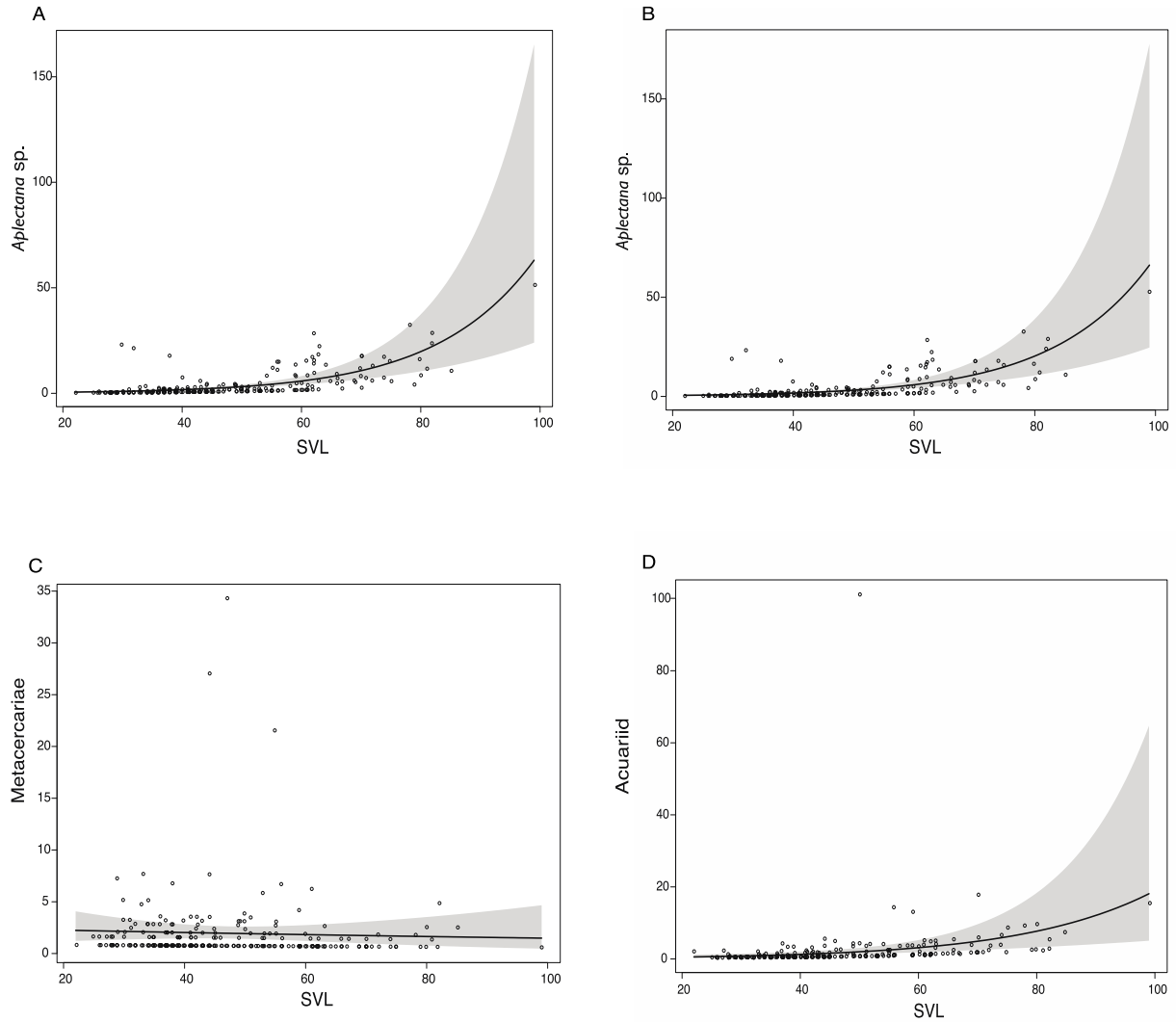


Figure 5. Plots of the partial residuals for the GAM best-fit models of parasite intensity against host snout-vent length (proxy for age) for female Cuban treefrogs. Each panel represents the following: A) *Aplectana* sp. (model I), B) *Aplectana* sp. (model II), C) metacercariae (model I), and D) acuariids (model II). Snout-vent length is a significant predictor of parasite intensity for *Aplectana* sp. (model 1;  $X^2 = 44.01$ ,  $P < 0.001$ ) and (model II;  $X^2 = 42.33$ ,  $P < 0.001$ ), and acuariids ( $X^2 = 13.22$ ,  $P < 0.001$ ).

Table 1. Comparison of plausible models for quantifying the relationship between host age and parasite intensity. Each model was run separately for male and female frogs.

Response	Model type	Predictors	Random effect	Error distribution*	Model ID
Parasite abundance	GAM	SVL		Nb	I
	GAM	SVL + Collection year		Nb	II
	GAM	SVL		Poisson	III
	GAM	SVL + Collection year		Poisson	IV
	GAMM	SVL	Wetland	Nb	V
	GAMM	SVL + Collection year	Wetland	Nb	VI
	GAMM	SVL	Wetland	Poisson	VII
	GAMM	SVL + Collection year	Wetland	Poisson	VIII

\*Nb = negative binomial

Table 2. Results of best fit models according to Akaike information criterion (AIC). All models with  $\Delta AIC \leq 2$  are listed for each parasite for male and female frogs. See model ID and Table 1 for corresponding predictors, error distribution, and model type.

Parasite	Host Sex	AIC	df	Model weight	Model ID
<i>Aplectana</i> sp.	Males	0.0	3	0.81	I
Metacercariae		0.0	3	0.72	I
		1.9	4.0	0.48	II
Acuariids		0.0	4.0	0.91	II
<i>Aplectana</i> sp.	Females	0.0	3.0	0.71	I
		1.8	4.0	0.29	II
Metacercariae		0.0	3.0	0.72	I
Acuariids		0.0	4.0	0.91	II

## Discussion

### *Aplectana* Spillover into *Hyla femoralis*

Several studies show that introduced parasites (spillover) can have negative impacts on native populations [49-52]. Although the CTF is a well-established invasive species throughout nearly the entire state of FL, and native treefrog populations have been shown to decline in areas where they are sympatric with CTFs [99], no studies have measured the effects of any introduced parasites of the CTF on native treefrogs. Here we show that infected CTFs do not experience a significant loss in body mass when infected with *Aplectana* nematodes, yet they shed more juvenile worms than the native *H. femoralis*, which is significantly less tolerant of these infections than CTFs.

*Aplectana* sp. is likely an introduced parasite of the CTF, and their shared evolutionary history might explain why infected CTFs shed more worms and experience less pathology (weight loss) than native treefrogs. When hosts are investing in costly immune functions (e.g. inflammation) to

resist pathogens, the host may have fewer resources to devote towards growth and/or reproduction [118-120]. Consequently, it could be beneficial for hosts to mount less of this type of resistance response if, for example, increased tolerance (i.e., less demand of energetic resources) towards the infection improves host fitness in the presence of this parasite [119]. Hosts that do not share an evolutionary history with a pathogen, such as native treefrogs and *Aplectana* sp., might suffer increased pathology due to this lack of evolved tolerance [53, 110, 111]. In this case, it is likely that *H. femoralis* may have invested in resistance to *Aplectana* sp., and the use of resources to fuel such an investment may explain why infected *H. femoralis* were unable to invest in growth [121, 122].

In addition to not observing a significant decline in mass of the CTF, our data also show that the CTFs are capable of shedding significantly more worms, and thus likely harbor a greater number of adult nematodes in the intestinal tract. This immune response is not uncommon in hosts that become infected with a foreign parasite with which they have not coevolved [53, 110, 111]. Given that *Aplectana* sp. is one of the most abundant nematodes in the CTF at our study site (Flatwoods Wilderness Park) [109], it is possible that the negative effects of *Aplectana* sp. are being experienced by several species of native treefrogs, in which case the invasive CTF and *Aplectana* sp. would contribute to the growing body of literature that shows that populations of native hosts can be altered by introduced parasites [31, 49-52]

#### *Age-Intensity Relationships for CTFs and Aplectana sp., Acuariids, and Metacercariae*

In addition to quantifying the effects that introduced parasites have on native populations, it is also beneficial to understanding how host traits and behaviors influence the spread or acquisition of parasites [123-125]. Deciphering how host traits influence parasite dynamics can be particularly useful for exploring how an invasive species may be contributing to parasite

spillover, spillback, and dilution effect. By understanding each of those three general effects, we can better predict how CTFs directly or indirectly change parasite burdens within native treefrogs. Here, we show that older CTFs continue to gain *Aplectana* sp. and acuariids, but that there is no effect on metacercariae loads as CTFs age.

As CTFs get older (larger), they also harbor more *Aplectana* sp... This larger size in hosts increases surface area for penetrating the host as well as larger hosts providing more gut resources to support a larger nematode burden within the intestinal tract because the nematodes are feeding on digested food. Older hosts tend to harbor more parasites due to accumulated exposures (cumulative hypothesis) [126]; therefore, older CTFs should have a greater number of *Aplectana* sp. than younger CTFs, which is exactly what our data show. Because juvenile *Aplectana* sp. exit the CTF in the feces, host age leads to an increase in *Aplectana* sp. in the wild; therefore, older individuals likely contribute the most to con- and heterospecific transmission, the latter of which is spillover.

The increasing amounts of acuariids in older hosts is likely a result of these hosts consuming a larger quantity of arthropods that are also infected with acuariids. Because the acuariids are encysted in the CTF (a paratenic host), there is no chance of the parasite being transmitted back to the native treefrogs unless the CTF is consumed by a final host (bird), whereby the acuariid can complete its lifecycle and then exit in the feces of the birds [112]. If birds preferentially consume larger CTFs, this invasive treefrog may then contribute to the spillback effect because birds may become infected with a larger quantity of acuariids, thus eventually releasing more eggs into the environment. On the other hand, if birds selectively consume smaller CTFs, then the larger CTFs may be contributing to the dilution effect because they essentially become a sink for the acuariids, which removes them from the environment.

The metacercarial loads within the CTFs do not significantly change as the frogs get older. Although the frogs are likely to be predominantly infected as tadpoles, it is possible that these infections can be cleared as the frogs mature. For example, bluegill fish infected with *Ribeiroia ondatrae* metacercariae showed a decline in their infection over the course of roughly two months, and these declines were correlated with circulating leukocytes and neutrophils (immune system cells) [127]. Because the metacercariae can be cleared, we should expect a decline with age of the CTF, but instead, our results show that there is no difference between the adult and juvenile metacercariae infections in CTFs. These infections in the adult frogs can be explained by the adult frogs returning to these water bodies to breed, at which time they become re-infected by cercariae. Studies have also shown that fishes infected with trematodes tend to be easier prey for fish-eating birds (the final host) [128, 129]. Additionally, Goodman and Johnson (2011), show that metacercarial-induced morphological changes result in decreased survivorship for frogs. In our case, if the infected CTFs are preferentially consumed by prey, then more eggs may be passed from the final host into the environment whereby native hosts may eventually encounter more cercariae in the water. In such case, the CTF will amplify parasites of the native treefrogs. This increase in cercariae by CTF would lead to a spillback effect of native trematodes.

In summary, our data show that *H. femoralis* can be infected by a parasitic nematode that is spread by an invasive host with which it is sympatric. More studies need to be conducted to confirm that this nematode is negatively impacting native amphibian populations in the wild. It also stands to reason that older CTFs are the individuals that are most responsible for spreading juvenile nematodes in the environment because there is a positive relationship between *Aplectana* sp. intensity and host age. Although this same positive relationship was also shown for



acuariids, additional studies would be needed to examine the feeding habits of predators of infected CTFs to elucidate whether or not the CTFs are a sink or a source of amplification for these nematodes. In a similar manner, even though there is no discernable change in metacercariae intensity as frogs age, it is likely that the CTF is resulting in a spillback effect for trematodes that utilize birds as a final host. By investigating the mechanisms by which invasive species can lead to declines in native populations while also discerning which host traits result in alterations to relationships between native hosts and their parasites, biologists can better predict how new invasions may impact native populations as well as develop better management plans for native populations that are already experiencing declines.

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## CHAPTER 3: EFFECT OF FIRE ON PARASITE TRANSMISSION

### **Abstract**

Disease emergences are often associated with a change to host-pathogen ecology, and wildfires are known to profoundly modify the ecology of terrestrial and freshwater ecosystems. Nevertheless, a proposed and validated predictive framework for the effects of fire on parasite transmission has remained elusive. Here we provide that framework by proposing that fire should directly kill parasites with terrestrial free-living stages but should indirectly increase parasites with hosts whose population densities increase after fire. Using field and mesocosm before-after-control-impact experiments, we provide support for these hypotheses for two nematodes and several trematode species of Cuban treefrogs (*Osteopilus septentrionalis*). Moreover, we found no change in the fire-driven trajectories of these parasites seven years after burns. These results suggest that fire has predictable direct and indirect positive and negative effects on parasite transmission and that these effects on host-parasite dynamics can be highly persistent.

### **Introduction**

Fire and disease are ecological factors that occur naturally, but the occurrence of both are also highly modified by humans [131, 132]. In the developing world, fire is being used intentionally to clear tropical forests for agriculture. In contrast, in the developed world, fire has often been intentionally suppressed to reduce property and human losses, which in turn facilitates fuel accumulation, making wildfires more intense and less manageable when they do occur [16,

66, 67, 70, 71]. Indeed, the incidence of large, uncontrolled fires has increased on all vegetated continents, with resulting annual economic costs exceeding \$US 15 billion in some years [1]. To prevent such catastrophic damage from over-fueled wildfires and to restore and maintain naturally fire-adapted communities, prescribed burns are now widely used as a land management tool [16, 133, 134].

Like fire, infectious diseases of both humans and wildlife have increased in incidence, geographic range, and intensity in the 21st century [1, 16, 133, 134]. Also similar to fire, these increases have had enormous costs to human societies, resulting in declines in biodiversity and ecosystem services and losses of human lives [1-5]. Owing to these costs, there is considerable interest in understanding factors that drive wildlife disease emergence, as well as considerable management efforts to control these diseases [132].

The emergence of most diseases is generally associated with a change to host-pathogen ecology [5, 6], and we know that fire often profoundly modifies the ecology of terrestrial and freshwater systems [1, 65]. This suggests that fire might be an important but overlooked driver of infectious disease dynamics [135]. For example, Hossack, Lowe [84] showed that fire can either increase or decrease parasite densities depending on how host densities change in response to fire severity. Additionally, Derek Scasta, Engle [95] found that ectoparasitic horn flies (*Haematobia irritans*) of cattle can be directly reduced by burning pastures because the heat kills both the eggs and pupae living in the cow fecal pats. However, with the exception of these few studies, the effects of fire on wildlife diseases have not been well-studied. Additionally, these few studies suggest that fire can increase and decrease wildlife diseases, but no clear hypotheses have emerged for when and why fire should have these opposing effects. To fill this gap, we develop and test a framework for predicting the effects of fire on parasite transmission.

The first hypothesis of our framework is that fire will directly reduce soil-dwelling, skin-penetrating nematodes of vertebrates because fire should have more detrimental direct effects on these parasites than the hosts. The basis for this hypothesis is that parasitic nematodes that infect vertebrate hosts via skin penetration generally spend a substantial portion of their time at the soil surface waiting for a host, and thus might not be buffered from extreme temperatures by being deep in the soil. Moreover, nematodes cannot move as quickly as their vertebrate hosts, so it seems more likely that hosts could avoid the adverse effects of fire than soil dwelling nematodes. Indeed, numerous studies have shown that many species of herpetofauna, mammals, and birds can escape or withstand fires by fleeing or seeking refuge in bodies of water or burrows [136-138].

The second hypothesis of our framework is that fire should increase terrestrial parasites that use arthropods as intermediate hosts. Arthropod diversity often increases after fire, likely because of increased habitat heterogeneity that results from patches of burned and unburned areas [91]. For example, the diversity of 20 species of ground beetles increased within forests managed with prescribed burns [92]. Furthermore, Moretti, Obrist [89] showed that at least nine arthropod families either significantly increased in abundance or showed positive increasing trends in areas that had experienced fire. Often times, herbivorous arthropods are attracted to poorly defended new plant growth that appears soon after fires [139]. This increase in arthropod biodiversity and abundance after fires should promote parasites that require these species to complete their life cycles.

Finally, we hypothesize that fire will increase aquatic parasites. It is well-documented that fire can cause an influx of nutrients from terrestrial systems into freshwater ecosystems, enhancing aquatic algal and invertebrate productivity [140, 141]. It is also well-established that

nutrient additions to freshwater ecosystems can lead to increased aquatic parasites, especially trematodes [93, 94]. Hence, through bottom-up effects, fire might indirectly increase aquatic parasites.

To test this framework for how fire might affect parasite abundance in wildlife, we quantified helminth parasites in the Cuban tree frog (CTF), *Osteopilus septentrionalis*, as a function of time since prescribed burns. Cuban tree frogs are an optimal host for testing our hypotheses because they can acquire parasites from freshwater and terrestrial ecosystems. Next, because no previous experiments have demonstrated that natural or prescribed fires can kill soil-dwelling nematode parasites of vertebrates, we tested this hypothesis using a before-after-impact experiment in the field. Finally, to isolate the effects of fire specifically on parasitic soil-dwelling nematodes, we conducted a before-after-control-impact experiment in mesocosms. Here, we provide support for our proposed framework suggesting that, through fire suppression, the well-known “Smokey the Bear” campaign in the U.S. altered host-parasite dynamics of wildlife.

## **Methods**

### *A Natural Experiment on the Effects of Fire on Parasites in Frogs*

From 2006-2009, 239 CTFs (Table S1 in Supporting Information) were collected from polyvinyl chloride (PVC) pipes encircling five wetlands within Flatwoods Park in northeastern Hillsborough County, Florida (28°07'01.08"N 82°18'11.15"W). The park is primarily a matrix of second-growth pine flatwoods forest, borrow pits, hardwood swamps, freshwater marshes, and cypress domes [108]. This 4,000 acre property has been compartmentally burned by Southwest Florida Management District (SWFWMD) for approximately the last 33 years. Sections of the park are preferentially burned at times when water levels can prevent fire from moving directly



through the wetlands, which mostly results in the burning of wetland edges and uplands. Burn histories for each wetland were obtained from SWFWMD.

Upon capture, each of the CTFs was euthanized, placed in a plastic bag, and kept frozen until they were later necropsied. Date of capture, host snout-vent length (SVL), and wet weight were recorded. Sex was determined by evidence of mature reproductive organs upon necropsy. For most hosts, sex could be determined if they were > 41 mm. Hosts that lacked discernible reproductive organs are referred to as juveniles. After thawing, the body cavity of each CTF was opened by a longitudinal incision from vent to throat and all internal body organs were examined for macroparasites. Parasites were identified, counted, and preserved in 70% ethanol. Parasite identification was confirmed by Charles Bursey at Pennsylvania State University's Shenango Campus and by Dr. Omar M. Amin at Parasitology Center, Inc. in Scottsdale, Arizona, and voucher specimens of each species have been placed in the United States National Parasite Collection Beltsville, Maryland.

*Before-After-Control-Impact Experiments on the Effects of Fire on Soil-dwelling Nematodes*

Fires often result in influxes of terrestrial nutrients via runoff into local waterbodies, and nutrient additions have been shown to increase aquatic parasites, such as trematode metacercariae [93, 94]. Also, the diversity of arthropods emerging from waterbodies can increase after fires [89] along with terrestrial insects that arrive to feed on new plant growth in burned areas [90]. Hence, the hypotheses that fire should increase both aquatic parasites and parasites that use insects seemed reasonably well-founded. However, many nematodes have hardy resting stages [142] that might be resistant to fire. Moreover, temperature attenuates rapidly as soil depth increases, and thus it is seemingly possible that soil-dwelling parasites could seek refugia at

deeper soil depths to survive fire. Hence, we set out to empirically test how fire affects the survival of soil-dwelling nematodes.

#### *A Field Experiment on the Effects of Fire on Soil-dwelling Nematodes*

The field experiment offered us information on the degree of variability in the duration and intensity of prescribed burns in Florida and their impacts on both free-living and parasitic nematodes. During two prescribed burns at Flatwoods Park in April and May of 2015, we collected temperature profiles and soil samples before and after fire in the uplands of Flatwoods Park. The dominant vegetation was *Aristida stricta* (wiregrass), *Ilex glabra* (gallberry), *Pinus* spp. (pine straw), *Serenoa repens* (saw palmetto), and *Myrica cerifera* (wax myrtle).

Temperature loggers (Onset™ # U12-014 HOBO data loggers with additional HOBO TC6-K Type K beaded thermocouple attached to insulated 30-AWG wire) were used to capture the intensity and duration of burns. Each logger was encased in a Pelican™ 1010 micro case with a small hole to allow the wire to extend beyond the case. For each burn, seven loggers were deployed.

Each sample plot was haphazardly chosen from an area that was most likely to be burned soon after the initial fire line was strung. At each sample plot, two neon-painted rebar (for visibly locating plots) were placed in the ground ~0.5m apart, and between them, a temperature logger was buried ~15cm below the surface with only the tip of the wire being exposed at the surface with care given to not disturb the area from which the soil was being collected. A 50 mL centrifuge tube was pressed approximately 2.5 cm into the soil to remove a plug of the before and after burn soil, which was then sealed inside the tube, placed in a cooler, and analyzed within 24 hrs. Preburn soil was collected ~1hr before the burn, and the postburn soil was collected ~4hrs after the burn. For each of the two burns, soil was collected and temperatures were recorded for

seven plots. At each plot, 1 ‘before’ and 1 ‘after’ fire soil cores were collected (14 total), but only 12 soil cores (6 ‘before’ and 6 ‘after’ soil cores) were used for each burn in this study because they were confirmed to be from plots that experienced fire. Seven temperature loggers were deployed per burn at each plot for a total of 14 temperature profiles; however, two profiles were excluded (one for each burn) because they did not appear to burn, leaving a total of 12 temperature profiles used in the statistical analyses. Due to time constraints during sampling, control samples from adjacent unburned forests were not obtained, but given that the preburn samples were taken  $\leq 1$  hr prior to ignition, we have no reason to think that any change in nematode counts could be attributed to any other factor than fire, and to verify, we included controls in the mesocosm experiment. All plots were at least 10m apart

Nematodes were extracted from the soil using modified Baermann’s funnels. Ten grams of soil was added to each Baermann funnel and allowed to stand for roughly 24 hrs, after which the pinchcock at the bottom of the funnel was opened to allow for the collection of 5 mL of the bottom-most portion of the extraction solution to where the live nematodes had fallen. The 5 mL extraction solution was transferred into a petri dish (35 x 10 mm) that had a transparent grid affixed to the bottom, and the nematodes were counted under 40x magnification and then preserved in a solution of 1 part glycerine, 79 parts dionized water, 20 parts 96% ethanol.

#### *A Mesocosm Experiment on the Effects of Fire on Parasitic Nematodes*

The previous field experiment did not discriminate between the effects of fire on free-living and parasitic worms and thus could not definitively test the sensitivity of parasitic nematodes to fire. To address this specifically, we intended to repeat the field experiment with and without inoculations of parasitic nematodes to our field plots, however, one of the wettest summers and winters followed the field experiment and burns were unsuccessful. Hence, we

chose to isolate the effects of fire on parasitic nematodes using a BACI mesocosm experiment with fuel and burn durations and intensities that matched the field (see Results below). For this experiment, our mesocosms consisted of four steel tubs (57 L, 56 cm long x 33 cm wide by 19 cm high, respectively) containing a rectangular section of soil (~ 30 cm x 19 cm deep) obtained from Flatwoods Park, and the soil was kept intact with aluminum foil. Next, vegetation was added to each tub so that it mimicked the plant species and average duration and intensity of fires observed during our field experiments at Flatwoods Park. The same previously described temperature loggers were placed on the ground, away from the bin, and the thermocouple wire was run alongside the bin and buried approximately 2.5 cm under the soil except for the tip of the wire, which remained exposed at the soil surface (Figure S2A).

One of the following four treatments was applied to a steel tub: 1) fire + parasitic nematode inoculation, 2) fire with no parasitic nematode inoculation, 3) no fire + parasitic nematode inoculation, or 4) no fire and no parasitic nematode inoculation. Using a 50 mL centrifuge tube, two circular indentations were made on each side of the exposed thermocouple wire to mark the area where either the nematodes or control water inoculations were to be pipetted onto the soil (Figure S1B). The circular indentation created a mark in the soil that allowed us to see the exact location of the added nematode/control water treatment after the fires occurred. For tubs receiving parasitic nematode inoculations, we added 200 juveniles (~1 mL total water volume) of a known skin-penetrating parasitic nematode, *Aplectana* sp., attained from freshly necropsied CTFs. This addition of *Aplectana* allowed us to isolate the effect of fire on parasitic nematodes. For the non-inoculated tubs, ~1mL of control water was added to the center of each circular indentation. The non-inoculated tubs provided a background level of the naturally occurring densities of both the free-living and parasitic worms. Next, the “preburn” soil

core samples were taken from each of the four tubs by aligning a clean 50 mL centrifuge tube with the aforementioned indentation and then pressing the tube approximately 2.5 cm into the soil to remove a plug of the soil, which was then sealed inside the tube until further analysis. In this same fashion, a “postburn” soil core was taken from each of the four tubs after soil in the burned bins had reached ambient temperature. Nematode extractions were carried out as described above. This procedure was repeated in five additional temporal blocks between May and June 2015 for a total of six replicates per treatment.

### **Statistical analyses**

All statistical analyses were conducted with R statistical software. For all models, we used a negative binomial distribution, instead of Poisson distribution, to control for the overdispersion that is associated with parasite count data. For the natural experiment on the effects of fire on parasites in CTFs, we used the `glm.nb` function in the MASS package [143] to conduct a generalized linear model with a negative binomial error distribution to quantify the effects of spatial block (individual wetland), time since burn, frog snout-vent length, and frog sex (male, female, or unknown juvenile) on the number of parasites per CTF. For the before-after field burns, we used the `glmmadmb` function in the `glmmADMB_package` [144, 145] to conduct a mixed effect generalized linear mixed model with a negative binomial error distribution to quantify the effects of burn (before and after), maximum temperature, and burn duration on number of nematodes per gram of soil treating plot as a random effect. For the BACI experiment in the mesocosms, we also used the `glmmadmb` function in the `glmmADMB` package with a negative binomial error distribution to quantify the crossed effects of parasitic nematode additions, timing of sample collection (before or after burns) and burn treatment (burned or not burned) on number of nematodes in soil samples controlling for temporal block (day) and

treating the replicate as a random effect to account for the lack of independence between soil samples collected from the same mesocosm. Probability values were calculated using log-likelihood ratio tests using the Anova function in the car\_package [146], and coefficients were generated using the summary function. The Anova function generates an ANOVA table, and the associated probability values control for the other factors in the model. The summary function tests whether the coefficient for a given level of a predictor is significantly different than zero.

## Results

### *A Natural Experiment on the Effect of Fire on Parasites in Frogs*

Three helminths were found commonly enough in the CTFs to justify statistical analyses - a soil dwelling, skin-penetrating nematode, *Aplectana* sp., an encysted larval acuariid nematode transmitted by arthropod intermediate hosts, and larval trematode metacercariae that are transmitted from aquatic snails to frogs. As predicted, there was a significant overall decrease (Fig. 1A) in the abundance of *Aplectana* sp. per host with time-since-burns ( $\chi^2=6.0$ ,  $P=0.014$ ,  $df=1$ ; Tables S2 and S3). However, this decrease was greater for adult female CTFs than adult males (Sex x days-since-burn:  $\chi^2 = 7.6$ ,  $P = 0.023$ ,  $df = 2$ ). Also as predicted, for all classes of frogs, both acuariid nematodes and metacercariae per host increased with time-since-burn (Fig. 1). However, for acuariids, adult female CTFs showed a significantly greater increase as a function of time-since-burn than did males (Sex x days since burn:  $\chi^2 = 6.8$ ,  $P = 0.033$ ,  $df = 2$ ; Fig. 1B; Tables S2 and S3). In contrast, for metacercariae, adult male CTFs showed a significantly greater increase as a function of time-since-burn than did females (Sex x days since burn:  $\chi^2 = 6.2$ ,  $P = 0.04$ ,  $df = 2$ ; Fig. 1C; Tables S2 and S3).

### *Before-After-Impact Field Experiment*

The average number of nematodes recovered per gram of soil was significantly lower with than without fire ( $X^2 = 9.33$ ,  $P < 0.01$ ,  $n=12$ ,  $df=1$ ; Fig. 2A; Tables S4 and S5), suggesting that fire directly decreased densities of nematodes in soil. Additionally, there was no effect of maximum temperature ( $X^2 = 1.1$ ,  $P = 0.29$ ,  $df = 1$ ) or burn duration ( $\chi^2 = 0.68$ ,  $P = 0.41$ ,  $df = 1$ ) on the number of nematodes recovered. The prescribed burns at Flatwoods Park had a median burn duration (defined as time recorded for temperatures  $\geq 100$  °C) of 3.7 min (1-117min) and reached a median maximum soil surface temperature of 771°C (197-1304°C,  $n=12$ ). The maximum temperature of 1,304°C was likely an underestimate because the temperature logger is not equipped to record greater temperatures.

#### *Before-After-Control-Impact Mesocosm Experiment*

In this experiment, we tested the effects of fire on parasitic versus free living nematodes. When juvenile parasitic *Aplectana* nematodes were added to the soil before the burns, we recovered two and half times the number of nematodes compared to tubs without juvenile *Aplectana* added (Fig. 2B), which demonstrates that the inoculations effectively increased the density of parasitic worms ( $\chi^2 = 14.18$ ,  $P < 0.001$ ,  $df = 1$ ; Tables S6 and S7). Abundance of worms recovered from mesocosms that were not burned was similar before and after the burn treatments (Fig. 2C). However, there was a significant reduction in live worms recovered after the burns ( $\chi^2 = 21.9$ ,  $P < 0.001$ ,  $df = 1$ ; Fig. 2C), and like the field study, there was no effect of maximum temperature ( $\chi^2 = 0.36$ ,  $P = 0.55$ ; Figure S2; Tables S8 and S9) or burn duration ( $\chi^2 = 1.74$ ,  $P = 0.18$ ; Figure S3; Tables S8 and S9). Given that the proportional decline in nematodes was the same when tubs were inoculated with parasitic worms or not, the parasitic and free-living worms exhibited similar sensitivities to fire (three-way interaction:  $\chi^2 = 0.21$ ,  $P=0.65$ ; Tables S6 and S7). The median burn duration was 4.9 min (0.27 – 11min), and the median maximum soil surface

temperature was 774°C (150-1304°C,  $n = 12$ ). This burn regime was very similar to the aforementioned burns at Flatwoods Park where the experiment on the effects of fire on soil-dwelling nematodes in the field was conducted.



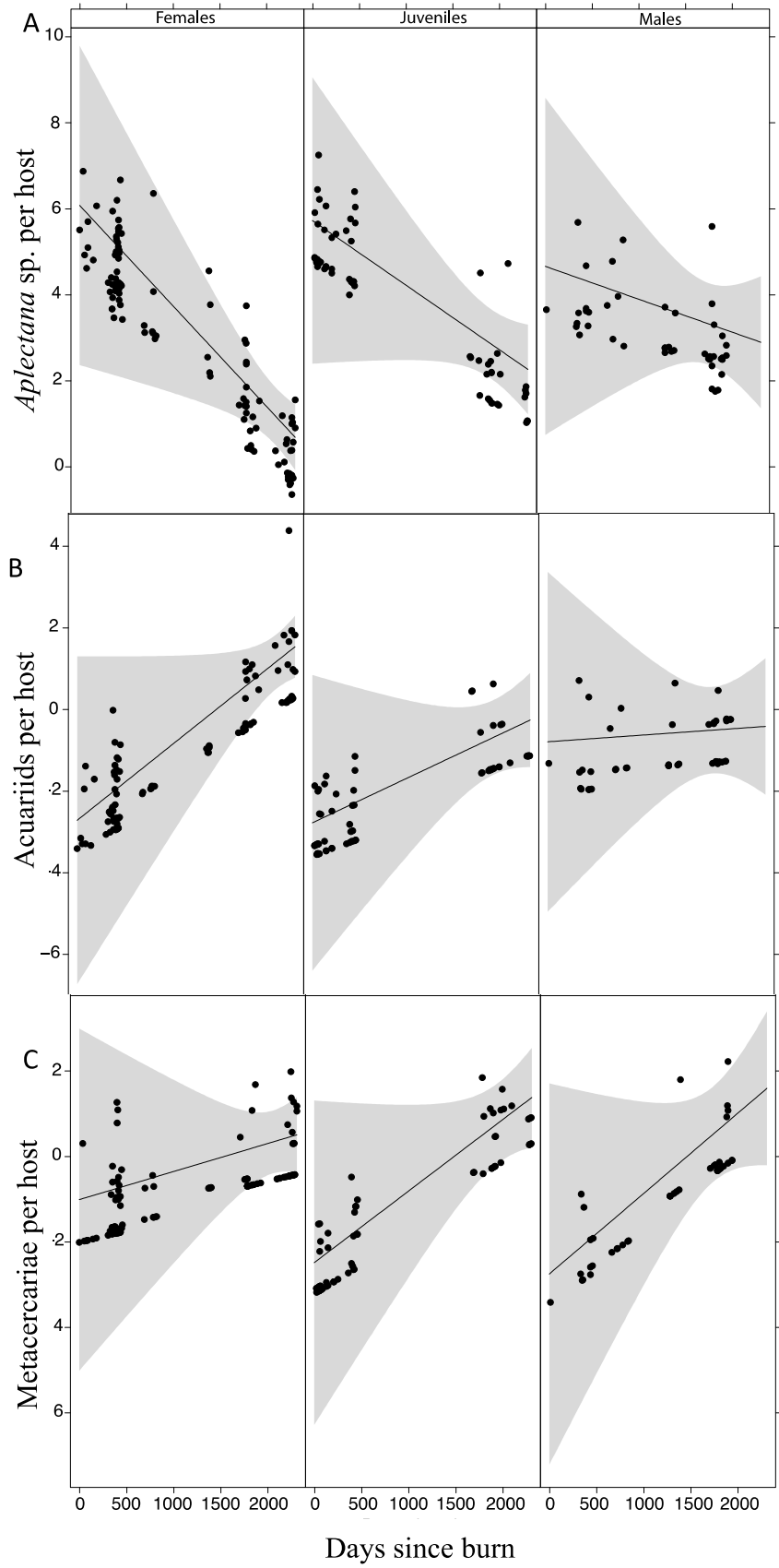


Figure 1. Sex-dependent effects of fire on the three most prevalent parasites recovered from *O. septentrionalis*. A) The soil-dwelling *Aplectana* declines with time since burn, whereas the B) acuariid, which has an arthropod intermediate host, and C) metacercariae, acquired in freshwater, both increase with time since burn. See text for statistics and sample sizes. Bands represent 95% confidence bands. The y-axis represents the natural logarithm of parasites per host.

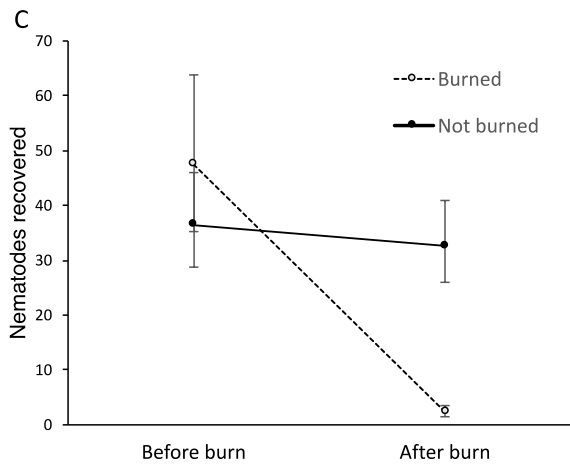
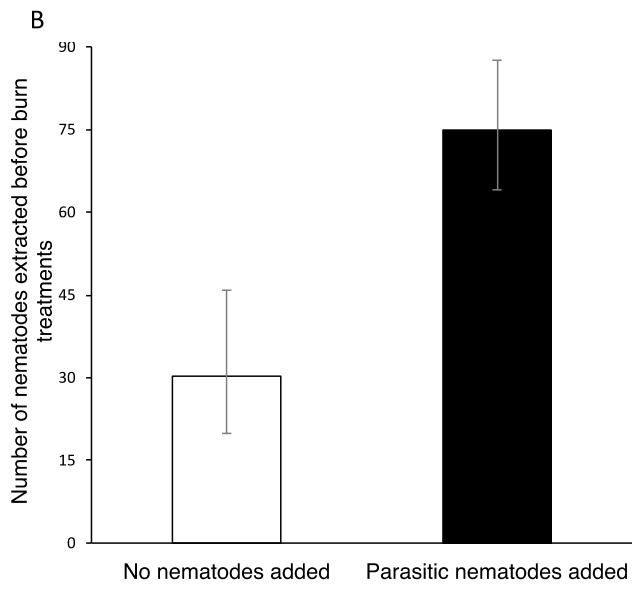
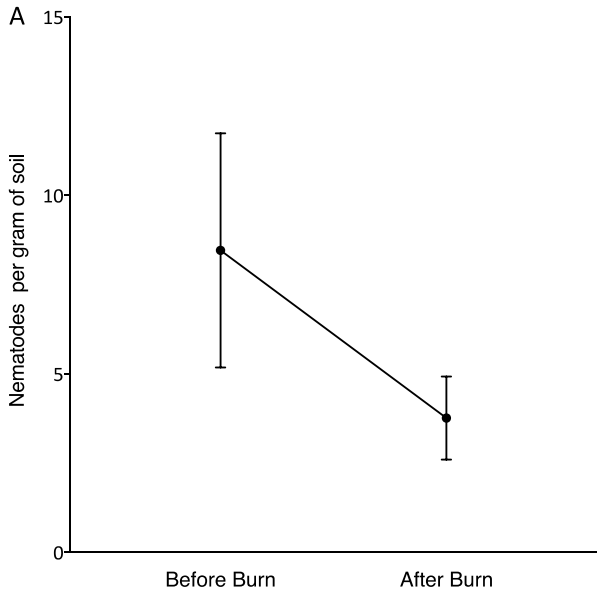


Figure 2. Mean ( $\pm 1$  SE) nematode recovery from the before-after-impact field experiment and the before-after-control-impact mesocosm experiment. A) Fire reduced the total nematodes recovered per gram of soil ( $X^2=9.33$ ,  $P<0.01$ ,  $n=12$ ) in the field. B) Soil inoculated with *Aplectana* (solid bar) increased nematode recovery from Baermann funnels at least two-fold before fire was applied ( $n=6$ ) in mesocosms. C) Fire reduced the nematodes recovered from mesocosms ( $X^2=21.9$ ,  $P<0.001$ ,  $df=1$ ,  $n=6$ ). Means and standard errors are back-transformed from log values to more accurately reflect the underlying error distribution.

## Discussion

As hypothesized, fire was associated with decreases in soil-dwelling, skin-penetrating nematodes and increases in aquatic metacercariae and the acuariid nematodes that require arthropod intermediate hosts. Additionally, the effects of fire on changes in parasite abundances in the CTFs were sex-dependent, and none of the parasite abundance trends showed any discernible sign of recovery after the burns. We also found clear adverse effects of fire on both free-living and parasitic nematodes in our field and mesocosm experiments. Moreover, these direct effects of fire on soil-dwelling nematodes occurred despite considerable heterogeneity in the field and variation in the duration and intensity of burns. Below we discuss each of the results in more detail.

Although heat has been shown to be an effective tool for reducing parasitic nematodes of plants [83, 85-88], the direct effects of increased soil temperatures (i.e. fire) on soil-dwelling parasites of animals has remained understudied before our work (but see Hossack *et al.* 2013a,b). To kill parasitic nematodes in crops, farmers in tropical and subtropical regions use soil solarization, a process by which a clear plastic sheet is placed atop the soil to create heat through the “greenhouse effect”. The difference between soil solarization and fire is that temperatures are

higher but the duration of heat is shorter with fire than soil solarization. In fact, the median duration during which soil temperatures exceeded 100 degrees Celsius during our prescribed burns was <5 minutes. Despite these short burns, the intensity of the heat produced was sufficient to consistently reduce both free-living and parasitic, larval *Aplectana* nematodes. Given that larval *Aplectana* infect hosts and replace adult worms that have either died naturally in or have been cleared by hosts, the overall decline in *Aplectana* infections in CTF hosts after prescribed burns was likely a direct product of fire reducing *Aplectana* recruitment to the adult subpopulation.

In addition to elucidating the direct effects of fire on parasite transmission, it is also important to understand its indirect effects. We hypothesized that fire would indirectly increase parasites whose hosts benefited the most from fire. We found support for this hypothesis in two parasite species of CTFs, an acuariid nematode and trematode metacercariae. Occasionally, acuariids will infect paratenic hosts (i.e. a host that can facilitate life-cycle completion but one in which no further parasite development occurs), such as fish or amphibians, but typically the acuariid lifecycle requires a bird definitive host to eat an infected arthropod intermediate host [112]. Given that arthropod diversity and abundance commonly increases after burns because of a rise in habitat heterogeneity (patchy burned and unburned areas) and a proliferation of the preferred food of many herbivorous arthropods (poorly defended new plant growth) [83, 85-88], and that this, in turn, often recruits predatory birds that forage on these arthropods [89-92], it was not surprising to discover that the abundance of acuariids in the CTFs also increased after burns.

Similar to the acuariids, metacercariae in CTFs likely increased after burns because fire was beneficial to their intermediate hosts, freshwater snails. It is well documented that freshwater ecosystems receive an influx of nutrient-rich run-off from burned sites nearby. These

nutrients can enhance freshwater productivity and the abundance of snails, which likely increased metacercarial infections [93, 94]. The increase in metacercariae and acuariids parasites after burns is consistent with the hypotheses that fire indirectly 1) increases aquatic parasites and terrestrial parasites associated with arthropods and, 2) more generally, increases parasites whose hosts' densities increase as a consequence of fire.

Just as fire had discrepant effects on parasite species, it also had varying effects on the parasitism of frogs of different sexes. Although the exact causes of these differences are not entirely clear, they are likely a product of differences in the behavior, diet, and immunity of male and female CTFs. For example, after fire, metacercariae increased more for male than female frogs, which is unsurprising given that male frogs tend to spend more time in the water during the breeding season searching for extra mating opportunities [114], a behavior that would increase exposure to aquatic metacercariae. In contrast, after fire, acuariids, which are acquired via diet, increased more for female than male frogs. This is consistent with the larger females (i.e., sexual dimorphism), whose fitness is determined by the size of their egg masses [147, 148], consuming a greater quantity of infected arthropods than males. The greater decline in *Aplectana* infections of females than males after burns is more challenging to explain, but it is consistent with the hypothesis that testosterone is immunosuppressive [149]. The response of infections in juvenile frogs to fire was intermediate to that of the adult males and females, which probably just reflects that the juvenile subpopulation was likely half males and half females.

We tracked parasite infections in CTFs for nearly seven years after prescribed burns and did not see any discernible changes in trajectories or signs of parasite recovery after the burns. In short, the trajectories for all three parasites were linear through time suggesting that the metapopulation structure of the hosts and parasites was not sufficient to allow recovery from this

disturbance within the time course of our study. This suggests that the disturbance created by prescribed or natural burns might have long-term effects on host-parasite dynamics.

Here we provided a tested framework for how fire can directly and indirectly affect macroparasites (nematodes and trematodes), but there is some, albeit limited, data from the literature that provides insights on how fire might affect other types of parasites as well. For example, fungi thrive in cool, moist environments, but burned areas have less ground cover and are therefore hotter and less humid than more vegetated areas, a condition that is likely less favorable for pathogenic fungi. Indeed, Hossack et al. (2013) found that toads from recently burned areas in a boreal forest were less likely to be infected with chytrid fungus, an infection implicated in the global decline of amphibians. For Lyme disease, a bacterial infection transmitted to humans by ticks, the effects of fire are conflicting. Tick abundance can be reduced by burns [150], but the populations may sometimes be even greater in burned sites than control sites [81, 82], perhaps because tick populations quickly rebound after fires [151]. For other microparasites, like viruses transmitted by “container-breeding” mosquito vectors, there is likely a negative impact on the parasite because the natural, shallow water reservoirs that serve as breeding sites tend to be reduced by fire, and thus, so too are the mosquitoes [152]. Continued research is needed on a greater variety of parasites and their hosts to better understand how fire ecology more generally impacts disease dynamics.

This study shows that ecological disturbances created by fire can have long-lasting alterations to host-parasite dynamics, but that these changes are not the same for all types of parasites.

Parasites exposed to the intense heat of fires are expected to experience direct reduction in abundance; whereas the abundance of parasite species whose host populations increase after fires are also expected to increase. Based on these findings and the current review by Scasta [135], it

is imperative to recognize that fire, or lack thereof, may be a driver of infectious disease dynamics. These results suggest that the Smokey the Bear campaign that promoted the suppression of wild fires could have caused shifts in disease dynamics in the U.S., increasing some infectious disease and decreasing others. Consequently, when forming management plans to monitor or control diseases of humans and wildlife, scientists and managers might benefit from more explicitly considering the impact of fire ecology on infectious diseases.

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## CONCLUSION

### *Introduced species disturbance on host-parasite dynamics*

On a global scale, introduced species are responsible for the decline of many native species [29] and elicit long-lasting changes to ecosystems [33]. Some change in native populations reflects the shift in parasite burdens [30-32, 38]. For instance, introduced species often lose many of their parasites during the process of invading and colonizing novel areas (i.e., enemy release hypothesis) [39-41]. Therefore, introduced species might have a competitive advantage over native species as a result of having to invest fewer resources in immune function. Furthermore, by releasing parasites (i.e., novel weapons) in the introduced range, introduced species can often cause detrimental effects on native species populations [48-53]. Alternatively, the introduction of non-native species might change host-pathogen dynamics in the introduced range if the introduced species can serve as suitable hosts for native parasites (e.g., dilution effect). [30-32]. Using the Cuban treefrog (CTF), *Osteopilus septentrionalis*, I investigated the effects that an introduced species can have on host-parasite dynamics in native populations.

I first showed that the CTF has undergone enemy release in introduced habitats and that it is also acquiring parasites that are common to native amphibians. In summary, at least eight parasite species were recovered, six of which had never been reported in the CTF. Two nematodes, *Oswaldocruzia lenteixeirai* and *Aplectana* sp., were the only species that exist in both the CTF's introduced and native ranges. The acquired parasites were trematodes (encysted metacercariae), nematodes (encysted acuariid larvae, *Physaloptera* sp., and *Rhabdias* sp.), a

cestode (*Cylindrotaenia americana*), and one pentastomid. I then demonstrated that the nematode, *Aplectana* sp., (likely introduced by the CTF) can successfully infect a native species (Pinewoods treefrog, *Hyla femoralis*), and that the native treefrog is less tolerant to the nematode than the CTF, which supports the idea of introduced species being successful as a result of the introduction of parasites (novel weapons). Thus, the CTF alters parasite burdens of native treefrogs through the introduction of *Aplectana* sp. and by the CTF acquiring new parasites in the invaded area.

I also determined that for three of the most prevalent parasites (two nematodes and one trematode) in the CTF, the age and sex of the CTF can predicted the intensity of infection for the two nematodes (*Aplectana* sp. and encysted acuariid larvae). *Aplectana* sp. was used to address spillover due to its introduction by the CTF, and encysted metacercariae and acuariid larvae were used to determine the CTF's effect on spillback and dilution because these parasites are most likely being acquired from native amphibians. By paring the life cycle of each parasite with these age-intensity relationships, we can predict which hosts are likely to be contributing to parasite spillover, spillback, and dilution in the wild.

#### *Fire disturbance on host-parasite dynamics*

Disease emergence is often associated with changes in host and/or parasite ecology [5, 6]; therefore, where disturbance results in major ecological changes, we might also expect to see a shift in disease dynamics [135]. For example, fire disturbance can drastically alter ecosystems [1, 65], but there is a gap in the literature for explaining how fire may be driving disease emergence [135]. Consequently, there are no clear predictions for how changes to fire regimes, whether it be through 1) fire suppression, 2) increase in incidence of wildfires, or 3) prescribed burning wildlife will affect wildlife diseases. In this research, I used the CTF as a model to determine

how its parasites change relative to time-since-burn, and thus elucidate how fire can directly and indirectly influence parasite transmission.

Using parasites from necropsied CTFs paired with mesocosm and field data, I show that fire can have predictable direct and indirect effects on disease dynamics, which are dependent upon host sex. When parasites are in direct contact with heat, there is a decline in parasite abundance. Regardless of habitat heterogeneity and burn variation, field and mesocosm data show a significant reduction in both free-living and parasitic nematodes that are exposed to the heat generated by fire. For parasites that have hosts which benefit from recently burned areas (i.e., nutrient pulses, new vegetative growth, invertebrate recruitment), there is an increase in parasites relative to time-since-burn. Even more surprising is that for CTFs captured from wetlands that had not experienced fire for nearly seven, there was no turnover in the relationship between the parasite intensity relative to time-since-burn (i.e., parasites continued to either increase or decrease, depending on the parasite species). These results suggest that direct and/or indirect disturbance by fire might create long-term effects on host-parasite dynamics as parasites are either removed or recruited into an area.

*Concluding remarks and future work for host-parasite dynamics and disturbance ecology*

As global climate change continues, it is expected that occurrence of diseases [20-25] and fire [153-156] will also shift. The need to identify mechanisms responsible for the decrease in native populations and biodiversity is of utmost importance as humans continue to affect ecosystems through the spread of non-native species and alteration of natural fire regimes. Moreover, we also need to be able to better-predict and manage disease emergence for humans and wildlife alike. As such, it is imperative that future work continues to investigate how ecological disturbances modify relationships between hosts and their parasites.

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## Appendices

# APPENDIX A: Acquired and introduced macroparasites of the invasive Cuban treefrog, *Osteopilus septentrionalis*

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## Acquired and introduced macroparasites of the invasive Cuban treefrog, *Osteopilus septentrionalis*



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### ABSTRACT

Because shifts in host–parasite relationships can alter host populations, attention should be given to the parasites that introduced species take with them or acquire in their introduced range. The Cuban treefrog, *Osteopilus septentrionalis*, is a successful invasive species in Florida with its parasites in the native range being well-documented, but there is a void in the literature regarding what parasites were lost or introduced in its expansion. We necropsied 330 *O. septentrionalis* from Tampa, FL and compared their macroparasites to those of *O. septentrionalis* in their native range and to the parasites of anurans native to the Tampa, FL area to determine the species *O. septentrionalis* likely introduced or acquired in Florida. At least nine parasite species (*Aplectana* sp., *Oswaldocruzia lenteixeirai*, *Cylindrotaenia americana*, *Physaloptera* sp., *Rhabdias* sp., *Centrorhynchus* sp., unidentified trematode metacercariae, unidentified larval acuariids, and unidentified pentastomids) were isolated. We found no differences in parasite communities of adult male and female frogs, which averaged 19.36 parasite individuals and 1.39 parasite species per adult frog, and had an overall prevalence of 77.52%. Acuariid larvae were likely acquired by *O. septentrionalis* in FL because they are not found in their native range. *O. lenteixeirai* was likely introduced because it is commonly reported in *O. septentrionalis*' native range but has never been reported in FL-native anurans. *Aplectana* sp. is also likely introduced because it has been reported in several anurans in Cuba but only reported once in Florida. *O. septentrionalis* tended to harbor fewer of its native parasites in the introduced range, which is consistent with the enemy release hypothesis and potentially creates an immunological advantage for this invasive host. Because native populations can be threatened by introduced parasites, there is a need to further explore the frequency and rate at which non-native hosts introduce parasites.

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### 1. Introduction

Invasive species can be expensive pests, causing changes to local ecosystems that can interfere with ecosystem services and extirpate native organisms (Mack et al., 2000; Sakai et al., 2001). Invasive hosts can also disrupt native host–parasite relationships that are important to ecosystem productivity, stability, and biodiversity (Anderson and May, 1978). Host–parasite dynamics within the native populations can be altered by the introduction of non-indigenous parasites because native host populations might lack the defenses necessary to combat the introduced pathogens (Bar-David et al., 2006). Likewise, increases in the abundance of native

parasites can occur if populations of the introduced host are capable of being suitable hosts and/or reservoirs for parasites of native species (Hudson et al., 2006; Kelly et al., 2009). Given that invasives can alter native species diversity via their introduced parasites (Strauss et al., 2006), it is important to investigate the parasite–host interactions when areas become invaded (Peeler et al., 2011; Frankel et al., 2015; Scholz et al., 2015). The Cuban treefrog (CTF), *Osteopilus septentrionalis*, is an invasive amphibian species that is ideally suited to address any such loss or new acquisition of parasites because they are extremely abundant and easily captured.

The CTF is an invasive anuran native to Cuba, the Cayman Islands, Island de Pinos, and the Bahamas. It was introduced to Puerto Rico, various islands in the Lesser Antilles, Alabama, Georgia, Maryland, Minnesota, Hawaii, and Florida (Schwartz and Henderson, 1991; Meshaka, 2001; Johnson, 2004). The original

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introduction of CTFs into Florida is believed to have occurred in the Keys and spread throughout Florida as a result of highway construction (Barbour, 1931; Meshaka, 2001; Krysko et al., 2011). Because *O. septentrionalis* is often larger than the Florida-native treefrogs, has a broad dietary niche (Meshaka, 1996), and can reproduce throughout the year with average clutch sizes of 2000–4000 eggs, it is displacing native treefrogs (Meshaka, 2001).

Although CTFs have inhabited Florida for nearly 100 years, we are unaware of any published surveys of its parasites in this introduced range. Thus, we quantified macroparasites in CTFs from a natural area near Tampa, Florida, USA where the CTFs have been established since 1992 (Campbell et al., 2010) and compared these parasites to those previously reported in the CTF from its native range, as well as the parasites of native anurans. We hypothesized that CTFs from Tampa harbor parasite species from their native range and that an exchange of parasites between native hosts and CTFs has resulted in CTFs acquiring new parasite species.

## 2. Materials and methods

### 2.1. Experimental details

During the summers of 2005–2008, 330 CTFs were collected from polyvinyl chloride (PVC) pipes encircling 37 wetlands spanning 4000 acres within or near the Morris Bridge Wellfield (MBWF) within the Flatwoods Wilderness Park in northeastern Hillsborough County, Florida (28°07'01.08"N 82°18'11.15"W). The MBWF is primarily second-growth pine flatwoods forest matrix with numerous borrow pits, hardwood swamps, freshwater marshes, and cypress domes (Guzy et al., 2006; Campbell et al., 2010).

Each host was placed in a plastic bag and frozen until necropsied. Host snout-vent length (SVL) and wet weight were recorded. For hosts >41 mm, sex was determined by the presence/absence of nuptial pads and evidence of mature reproductive organs. All hosts <42 mm were considered juveniles due to a lack of discernable reproductive organs. The body cavity was opened by a longitudinal incision from vent to throat and all internal body organs were examined for macroparasites. Species of parasites were counted and preserved in 70% ethanol. Identification and confirmation of parasites were provided by Charles Bursey at Pennsylvania State University's Shenango Campus and by Dr. Omar M. Amin at Parasitology Center, Inc. (PCI) in Scottsdale, Arizona. The following voucher specimens were deposited in the United States National Parasite Collection Beltsville, Maryland: *Cylindrotaenia americana* (No. 105165), *Oswaldocruzia lenteixeirai* (No. 105160, 105161), *Centronychus* sp. (No. 105166), *Physaloptera* sp. (No. 105162), acuariid larvae (No. 105163), and trematode metacercaria (No. 105164).

### 2.2. Statistical analyses

Statistical analyses were conducted using R statistical software (R Development Core Team, 2014). Prevalence, mean intensity, and mean abundance were calculated in accordance with definitions provided by Bush et al. (1997). We tested for differences among males, females, and juveniles in their parasite mean abundance and mean intensity based on a negative binomial error distribution using the "glm.nb" function ("MASS" package). Differences among males, females, and juveniles in their parasite prevalence and richness were determined using binomial and Poisson error distributions, respectively, by using the "glm" function ("MASS" package). Parasite evenness was calculated using Simpson's Diversity Index, and differences in parasite evenness among males, females, and juveniles were tested using a normal distribution and the "lm" function. A Bonferroni's alpha adjustment was used to

keep the experiment-wise error rate at 0.05, which means that a  $p$  value  $\leq 0.017$  was considered statistically significant when we tested for differences among males, females, and juveniles.

## 3. Results

Three hundred thirty *O. septentrionalis* were collected and necropsied (68 males, 150 females, and 112 juveniles; mean mass [g]  $\pm$  SE: males  $6.28 \pm 0.32$ , females  $10.79 \pm 0.66$ , and juveniles  $2.14 \pm 0.08$ , respectively). The overall parasite prevalence was 74.2%, and the overall mean abundance was  $14.7 \pm 2.5$ . At least nine species of parasites were isolated from the CTFs, which included six host records (Table 1). *Aplectana* sp., metacercariae, and acuariid larvae had the highest prevalences and mean abundances; the remaining helminths were relatively rare (Table 2). *Oswaldocruzia lenteixeirai* and *Aplectana* sp. were the only species that have been reported in the CTF's native and introduced ranges (Table 3).

Host type (male, female, or juvenile) significantly affected parasite mean abundance ( $X^2 = 29.44$ ,  $P < 0.001$ ) and mean intensity ( $X^2 = 29.55$ ,  $P < 0.001$ ) (Fig. 1) but did not affect prevalence ( $X^2 = 3.55$ ,  $P = 0.170$ ), evenness ( $F_{2,201} = 0.632$ ,  $P = 0.532$ ), or richness ( $X^2 = 6.96$ ,  $P = 0.031$ ) (Fig. 2). Juveniles had lower mean abundance and mean intensity, but the male and female adults did not significantly differ in these two categories ( $P > 0.58$ ; Fig. 1). On the other hand, juveniles did not significantly differ from adults, in prevalence and evenness, but they did significantly differ from adult females for parasite richness ( $X^2 = 6.71$ ,  $P = 0.01$ ; Fig. 2). There was no significant difference between adult males and females in parasite evenness, richness, or prevalence ( $P > 0.42$ , Fig. 2). Adults averaged ( $\pm$ SE) 19.36 ( $\pm 3.67$ ) parasite individuals, 1.39 ( $\pm 0.07$ ) parasite species, and 77.52% ( $\pm 2.83$ ) prevalence, whereas juveniles averaged 5.71 ( $\pm 1.57$ ) individuals, 1.05 ( $\pm 0.09$ ) species, and 67.86% ( $\pm 4.41$ ) prevalence, respectively.

## 4. Discussion

### 4.1. Variation in parasites among juveniles and adult males and females

Our results indicate that there was no significant difference in abundance, intensity, evenness, richness, or prevalence between adult male and female CTFs despite males having greater home ranges because of extensive mate searching (Vargas-Salinas, 2006). However, adult CTFs had many more parasites than juveniles. For many host species, infections can accumulate with age (Raffel et al., 2009, 2010, 2011). Our results suggest that CTFs acquire parasites through time such that older hosts have more parasites than younger hosts.

**Table 1**

Parasites found in *Osteopilus septentrionalis* from Tampa, FL and whether they were introduced, acquired, and represent new host records.

Parasite	Status in <i>O. septentrionalis</i>
Acuariid larvae (nematode)	Acquired; new host record
<i>Oswaldocruzia lenteixeirai</i> (nematode)	Introduced; previously reported
<i>Aplectana</i> sp. (nematode)	Likely introduced; previously reported
<i>Physaloptera</i> sp. (nematode)	Undetermined; new host record
<i>Rhabdias</i> sp. (nematode)	Undetermined; new host record
<i>Physaloptera</i> sp. (nematode)	Undetermined; new host record
Digenean metacercaria (trematode)	Undetermined
<i>Cylindrotaenia americana</i> (cestode)	Undetermined; new host record
Pentastomid	Undetermined; new host record

**Table 2**  
Prevalence, mean intensity (range), mean abundance, and location of parasites found in 330 *Osteopilus septentrionalis* from Tampa, Florida.

Parasite	Prevalence (# infected/# examined, %)	Mean intensity $\pm$ SE (range)	Mean abundance $\pm$ SE	Habitat <sup>a</sup>
<b>Nematoda</b>				
<i>Aplectana</i> sp.	50	24.82 $\pm$ 5.2 (1–150)	11.3 $\pm$ 2.5	a–c
<i>Oswaldocruzia lenteixeirai</i>	4	1.4 $\pm$ 0.2 (1–14)	0.06 $\pm$ 0.02	b
<i>Physaloptera</i> sp. larvae	1	11.3 $\pm$ 9.4 (1–3)	0.10 $\pm$ 0.09	a–c
<i>Rhabdias</i> sp.	1	1.0 $\pm$ 0 (1–3)	0.01 $\pm$ 0.005	e
Unidentified larval acuariid (cysts)	32	2.49 $\pm$ 0.07 (1–105)	1.2 $\pm$ 0.3	a–d, i
<b>Trematoda</b>				
Unidentified metacercaria	36	5.2 $\pm$ 0.71 (1–52)	1.9 $\pm$ 0.3	a–g
<b>Cestoda</b>				
<i>Cylindrotaenia americana</i>	0.3	3.0 $\pm$ 0 (1)	0.03 $\pm$ 0.01	b
<b>Acanthocephala</b>				
<i>Centrorhynchus</i> sp. (cystacanth)	7	2.1 $\pm$ 0.47 (1–11)	0.15 $\pm$ 0.04	a–d, f, h, j
<b>Pentastomid</b>				
Unidentified pentastomid	1	1.0 $\pm$ 0 (1–4)	0.01 $\pm$ 0.006	e

<sup>a</sup> a = stomach, b = small intestine, c = large intestine, d = body cavity, e = lungs, f = gallbladder mesenteries, g = kidney mesenteries, h = testes mesenteries, i = liver mesenteries, j = urinary bladder.

**Table 3**  
Parasite species previously reported in *Osteopilus septentrionalis* in its native range.

Parasite	Habitat	Locality	Reference(s)
<b>Nematoda:</b>			
<b>Rhabditiodea</b>			
<i>Strongyloides</i> sp.	c	Bahamas	Goldberg et al., 1994
<b>Diocotylphymatoidea</b>			
<i>Eustrongylides</i> sp. (larvae)	d	Cuba	Walton, 1940
<b>Oxyuroidea</b>			
<i>Parapharyngodon bassi</i>	d	Cuba	Walton, 1940; Baruš and Moravec, 1967; Coy Otero and Ventosa, 1984
<i>Parapharyngodon osteopilli</i>	c	Cuba	Adamson, 1981
	c,d	Bahamas	Goldberg et al., 1994
Larval oxyurids	b	Cuba	Coy Otero and Ventosa, 1984; Goldberg et al., 1994
Unidentified oxyurid	d	Cuba	Coy Otero and Ventosa, 1984
<b>Cosmocercidae</b>			
<i>Aplectana</i> sp.	d	Cuba	Coy Otero and Ventosa, 1984
<i>Aplectana hamatospicula</i>	d	Cuba	Coy Otero and Ventosa, 1984
<b>Ascaridoidea</b>			
<i>Contracecum</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
<i>Porrocaecum</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
<b>Physalopteroidea</b>			
<i>Abbreviata</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
<i>Physalopteroides bahamiensis</i>	a–d	Bahamas	Goldberg et al., 1994
<i>Physalopteroides valdesi</i>	b	Cuba	Coy Otero and Ventosa, 1984
<b>Filarioidea</b>			
<i>Foleyellides brachyoptera</i>	e	Cuba	Coy Otero and Ventosa, 1984
<b>Trichostrongyloidea</b>			
<i>Oswaldocruzia lenteixeirai</i>	b,c	Cuba	Baruš and Moravec, 1967
	c	Cuba	Coy Otero and Ventosa, 1984
	b,c	Bahamas	Goldberg et al., 1994
<b>Thelastomatidae</b>			
<i>Hammerschmidtella</i>	?	Cuba	Coy Otero et al., 1980
<b>Tematoda:</b>			
<i>Mesocoelium crossophorum</i>	b,c	Cuba	Baruš and Moravec, 1967

a = esophagus, b = stomach, c = small intestine, d = large intestine, e = body cavity.

#### 4.2. Ecology of the observed parasites

*Aplectana* spp. are cosmopolitan cosmocercid nematodes that usually reside in the gastrointestinal tract of amphibians and reptiles (Baker, 1987; Anderson, 2000). Identity to species is based on males; since only juveniles and females were found in the present study, specific identity was not possible. Gravid females can be viviparous or ovoviviparous and shed eggs and larvae in feces. At this time, the complete life cycle of *Aplectana* spp. is unknown (Anderson, 2000), but a single study suggests that tadpoles can ingest juvenile nematodes, but that direct penetration of the skin of frogs is unsuccessful (Vhora and Bolek, 2013). In contrast, preliminary data from our lab show that post-metamorphic CTFs can be experimentally infected via skin penetration. Although there

have been three reported *Aplectana* spp. in North America, only one, *Aplectana hamatospicula*, has been found in both Cuba and the U.S.A. In Cuba, this species has been found in 14 species of anurans, but it only occurs in one species native to Florida (Baker, 1987; Vhora and Bolek, 2013).

Acuariids, nematodes belonging to the order Spirurida, have a heteroxenous life cycle, with birds and arthropods as definitive and intermediate hosts, respectively (Anderson, 2000). CTFs may only serve as a paratenic hosts. Meshaka (1996) showed that the CTFs diet consists of an array of arthropods, thus the most likely mode of infection is by consumption of an infected arthropod intermediate host.

CTFs most likely acquired metacercaria through infection by cercariae during the tadpole stage, when they are often found in



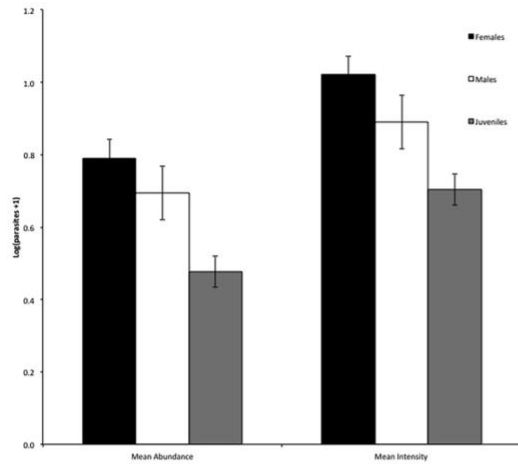


Fig. 1. Mean ( $\pm$ SE) abundance (log transformed) and mean intensity (log transformed) of parasites in male, female, and juvenile Cuban treefrogs, *Osteopilus septentrionalis*, collected from Tampa, FL.

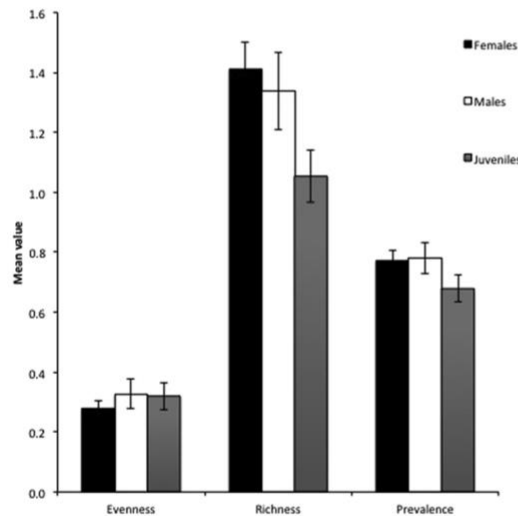


Fig. 2. Prevalence, richness, and evenness (Simpson's Diversity Index) of parasites in male, female, and juvenile Cuban treefrogs, *Osteopilus septentrionalis*, collected from Tampa, FL.

sympatry with trematode-infected snails. However, we cannot rule out the adults being infected with cercariae when they returned to the water to reproduce or by postmetamorphic frogs consuming trematode-infected terrestrial snails.

*C. americana* is a tapeworm that is found in at least 30 species of amphibians and reptiles in North America, three of which are native species in our study site. Because *C. americana* is also found in South America, Europe, Asia, and the Caribbean, it is impossible to determine whether or not this species was introduced to or acquired in Florida (McAllister, 1991; Goldberg et al., 2002). The life cycle of *C. americana* is not yet entirely understood, but transmission can occur in two ways: 1) directly, through coprophagy, or

2) indirectly, in which an infected invertebrate intermediate host (probably an insect) is ingested by the definitive host (Prudhoe and Bray, 1982).

*O. lenteixeira* is a common species of strongylid nematode reported from about forty species of amphibian and reptile final hosts, including CTFs, in the Caribbean (Coy Otero and Baruš, 1979; Baker, 1987; Moravec and Kaiser, 1995). The third stage larva penetrates the skin or is ingested by the final host where it matures into an adult in the intestines (Pérez Viguera, 1938). This species is also known to parasitize brown anoles, *Anolis sagrei*, in the Caribbean, but has never been reported from this common nonindigenous lizard in Florida (Goldberg and Bursey, 2000).

*Rhabdias* is a cosmopolitan nematode genus found as adults in lungs of amphibians and reptiles. These protandrous hermaphrodites typically infect anuran hosts via skin penetration. (Runey et al., 1978; Anderson, 2000; Langford and Janovy, 2009). At present, eight species of *Rhabdias* are known from North America, and at least two are from the insular Caribbean (Bursey et al., 2003; Martínez-Salazar and León-Règagnon, 2007). Since the few specimens found in our study could not be identified to species, it is impossible to determine whether this nematode was acquired or introduced.

*Physaloptera* spp. are known to occur in North America and in the Caribbean (Morgan, 1943; McAllister and Bursey, 2007). Generally, adult nematodes are found in the stomachs of mammals, reptiles, and birds that have ingested insect intermediate hosts. Although amphibians have not been reported as definitive hosts for *Physaloptera*, this group, as well as lizards, may harbor larvae that attach to the gastric mucosa, but do not encyst (Goldberg et al., 1993; Anderson, 2000). Some studies suggest that these hosts should not be considered paratenic since larvae do not encyst and often do not persist long in the host (Goldberg et al., 1993; Bursey and Goldberg, 1994). Therefore, CTFs may not serve as functional paratenic hosts for the unencysted *Physaloptera* larvae found in the present study as well as for another larval physalopterid, *Abbreviata* sp., reported from CTFs in Cuba (Coy Otero and Ventosa, 1984).

The developing larval stages of the acanthocephalan *Centrorhynchus* sp. are commonly found in terrestrial insects or isopods, but adults are found in the small intestines of carnivorous birds. Furthermore, amphibians and reptiles may serve as paratenic hosts by consuming arthropods infected with cystacanths (Nickol, 1985).

Pentastomids (worm-like arthropods bearing two pairs of retractable hooks in the mouth region) have a heteroxenous life cycle, and adults are commonly found in the respiratory tract of vertebrates (Paré, 2008). Although all classes of vertebrates and some invertebrates can serve as intermediate hosts, only fishes are reported to be unsuitable as final hosts (Riley, 1986; Barton and Riley, 2004). It is likely that intermediate hosts are infected by ingesting eggs that are shed in the feces of an infected final host. After the larva penetrates the gut, it continues to migrate in the abdomen/coelem, and often ends up in the visceral tissue where it undergoes several molts until it becomes an infective nymph (Paré, 2008). Although a few anurans can harbor adult pentastomids, most anurans are considered to be intermediate hosts with the final host being reptiles, a group that makes up approximately 90% of pentastomid species (Riley, 1986; Barton and Riley, 2004; Paré, 2008). Despite the fact that we found pentastomid nymphs in the lungs of the CTFs, we were unable to further identify this group because the pentastomid maturation was either incomplete or stunted in the CTF host.

#### 4.3. Implications for enemy release

Perhaps the best-described effect of invasive species on host–parasite interactions is the enemy release hypothesis (ERH)

(Torchin et al., 2001; Mitchell and Power, 2003). ERH suggests that invasive species have a competitive advantage in their introduced range because they leave behind many of their natural enemies in their native range. This is a product of several phenomena. First, most of the parasites in a population are in a small proportion of the hosts (Shaw and Dobson, 1995; Shaw et al., 1998). Thus, by chance alone, it is unlikely that a heavily infected host will be introduced and successfully become established in a new environment; it is also unlikely that a heavily parasitized host would survive translocation. Second, most infected hosts are not infected with all the parasite species in their native range (Poulin, 2013). Therefore, even if parasites are introduced with the host and successfully establish, the parasites typically only represent a small portion of the host's parasites from its native range. Third, many parasites have complex life cycles requiring multiple host species. Hence, even if a parasite with a complex life cycle is introduced with the host, it is unlikely to establish because other required hosts are typically missing. In addition to the loss of natural enemies, introduced hosts are also thought to acquire few parasites in their introduced range because these parasites lack an evolutionary history with the introduced host; therefore, the parasites might not recognize or be capable of infecting a novel host (Mitchell and Power, 2003; Torchin et al., 2003).

In the present study, we found that CTFs harbored at least one parasite species from its native range but have also likely acquired parasites from herpetofauna native to the Tampa, FL area. Of the three types of parasites reported with the highest prevalences and mean abundances, the larval acuariids appear to have been acquired post invasion because they have never been reported in the CTF native range (Table 3) (Baker, 1987; Anderson, 2000). Only one parasite species, *O. lenteixeira*, appears to be a confirmed introduced species because it is found commonly in the native range of CTFs (Table 3), but there are no records of this species from frogs native to Florida. Because the *Aplectana* specimens were not identifiable to species, we cannot be certain whether it was introduced or acquired by the CTF because this genus has been reported in Cuba as well as in Florida. However, it is worth noting that only one species, *A. hamatospicula*, has ever been reported from native anurans of Florida, and it was found only in one species, *Gastrophryne carolinensis*, in 1939, which means that it has most likely been introduced (Walton, 1940).

CTFs at our study site were infected with fewer species of parasites than in their native range (Table 2). With the exception of *O. lenteixeira* and possibly *Aplectana* sp., CTFs at our study site were devoid of the parasite species found in their native range (Table 3). Moreover, the prevalence of *O. lenteixeira* is eight to fifteen times higher in its native than introduced range (Table 3); however, caution must be taken when making these comparisons between the parasites being reported in the native and introduced ranges because it is likely that several populations were surveyed across extensive areas ranging from the Bahamas to the Cuban Archipelago. As a result, we may be comparing fewer populations of CTFs or populations over a smaller spatial extent in Florida to more populations or at least populations distributed over a greater spatial extent in its native range. Because several of the previous studies do not list their sites of collection, we can not be sure as to how many populations of Cuban treefrogs were surveyed in their native range. However, we know that we collected frogs from thirty-seven wetlands over a 4000 acre park.

According to the enemy release hypothesis, a loss of enemies (in this case, parasite species) could, to some extent, facilitate the establishment and subsequent invasiveness of introduced species (Torchin et al., 2001; Mitchell and Power, 2003; Torchin et al., 2003). Nevertheless, Colautti et al. (2004) advocate further investigation of the ERH because very few introduced species exhibit

evidence of an enemy release; rather, they undergo an exchange or reduction of enemies. For example, in comparison to an overall mean parasite abundance of 4.6 calculated from Coy Otero and Ventosa (1984) and 12.7 calculated from Goldberg et al. (1994) in the CTF native range, this study indicates that the CTF has a higher mean abundance of 14.7 in the introduced range. Thus, careful consideration should be given to the vulnerability of introduced species to all of its enemies in native and invaded locations before attributing the colonization success to ERH. Though ERH is a plausible explanation for the colonization success of introduced species, research is still needed address whether or not shifts in host–parasite dynamics of introduced species aid in their colonization or in the decline of natives.

Although comparison to natives is not imperative to substantiate the ERH because the hypothesis is strictly based on the abundance and richness of parasites in an introduced species in its native versus introduced range, we acknowledge that this study would have been much stronger with the inclusion of a comparison of the parasites of the native treefrogs in Florida to parasites found in the invasive Cuban treefrogs in Florida. This might have suggested that exposure to parasites is higher in the introduced than native range of CTFs, which might help to explain their higher parasite abundance in their introduced range. Unfortunately, at the time of these collections, the Southwest Florida Water Management District prohibited collection of native frogs from this county park. Understanding how diseases shift between introduced and native populations can be imperative to prioritizing efforts in the conservation of species, and future work should focus on collecting CTFs as well as native treefrogs from various locations throughout the introduced range of CTFs to determine if their success is at least partially attributable to a loss or introduction of parasites.

#### Acknowledgments

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APPENDIX C: Supplemental materials for Chapter 3.

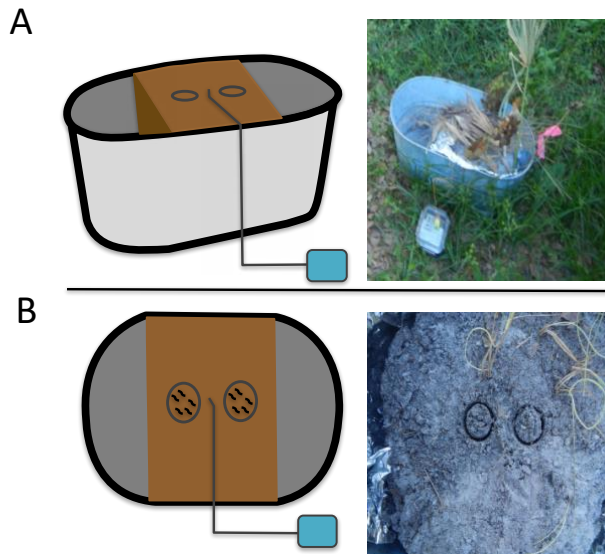
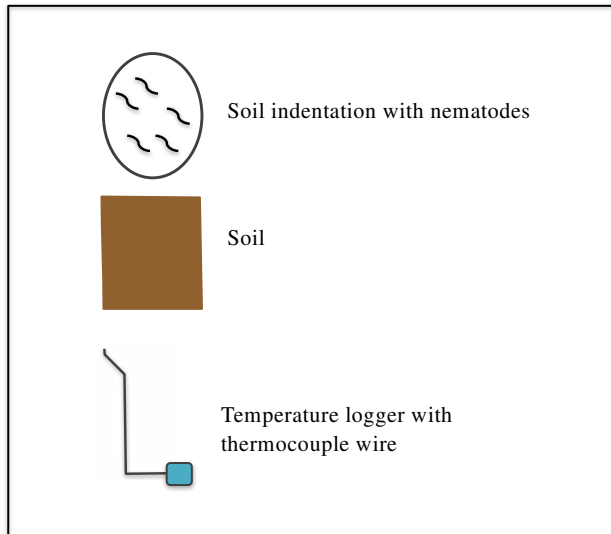


Figure S1. Schematics of experimental mesocosm burns. A) Side view of an experimental mesocosm with temperature logger alongside. Vegetation and soil are held together with aluminum foil. B) Top-down view of mesocosm and soil, with indentations made in the soil where either nematodes or control solutions were inoculated before vegetation was added. Soil samples were taken from each circular indented area before or after a burn.

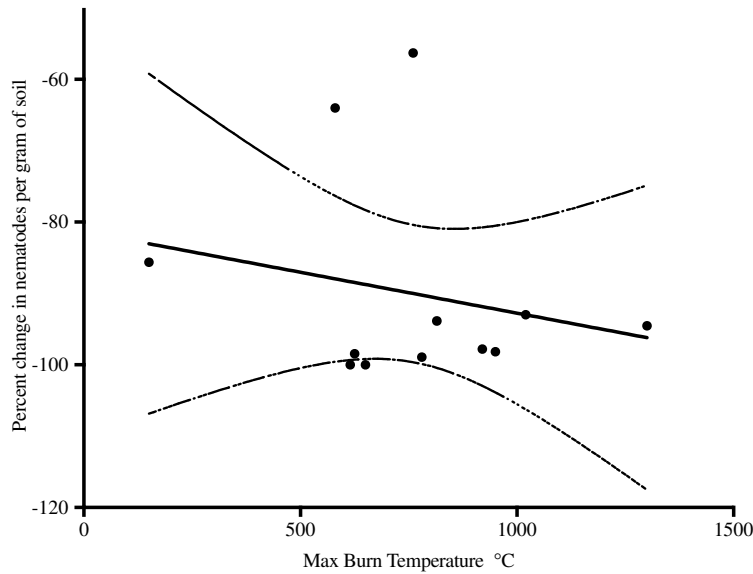


Figure S2. Effect of maximum temperature on the percent of nematodes recovered from mesocosms. Only burned mesocosms are included in the figure. Temperature ( $^{\circ}\text{C}$ ) did not have a significant effect on nematodes recovered from mesocosms ( $\chi^2=0.36$ ,  $P=0.55$ ,  $n=6$ ). Bands represent 95% confidence bands.

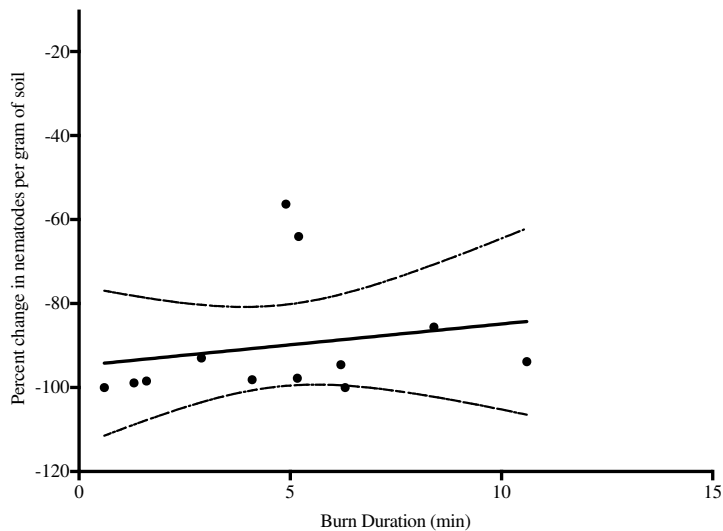


Figure S3. Effect of burn duration on the percent of nematodes recovered from mesocosms. Only burned mesocosms are included in the figure. Burn duration (minutes) did not have a significant effect on nematodes recovered from mesocosms ( $\chi^2=1.74$ ,  $P=0.18$ ,  $n=6$ ). Bands represent 95% confidence bands.

Table S1. Total *O. septentrionalis* collected from Flatwoods Park, Tampa, FL for each of the five burned wetlands.

Wetland ID	Female	Juvenile	Male	Unidentified	Total
A	40	23	14	2	79



Table S1 (Continued)

Wetland ID	Female	Juvenile	Male	Unidentified	Total
B	33	31	6	2	72
C	12	3	13	2	30
D	12	14	0	0	26
E	18	1	11	0	32

Table S2. ANOVA table for the effect of time-since-burn on *Aplectana* sp., acuariids, and metacercariae quantified from *O. septentrionalis*.

Response	Predictors	$\chi^2$	df	P
<i>Aplectana</i>	Days since burn	5.99	1	0.01
	Sex (Female, Juvenile, Male)	0.97	2	0.62
	Wetland	25.83	4	<0.001
	Frog mass	32.12	1	<0.001
	Days since burn x Sex	7.578	2	0.02
Acuariid	Days since burn	2.78	1	0.10
	Sex	2.57	2	0.28
	Wetland	8.33	4	0.08
	Frog mass	0.78	1	0.38
	Days since burn x Sex	6.79	2	0.03
Metacercariae	Days since burn	0.14	1	0.71
	Sex	1.28	2	0.53
	Wetland	5.33	4	0.25
	Frog mass	0.27	1	0.60
	Days since burn x Sex	6.53	2	0.04

Table S3. Coefficients table for the effect of time-since-burn on *Aplectana* sp., acuariids, and metacercariae quantified from *O. septentrionalis*.

Response	Predictors	Coefficient (SE)	Z	P
<i>Aplectana</i>	Intercept	5.017 (1.96)	2.554	0.01
	Days since burn	-0.002 (0.001)	-2.628	0.01
	Sex (Female, Juvenile, Male)			
	Juvenile	-0.349 (0.62)	-0.57	0.57
	Male	-1.410 (0.86)	-1.63	0.10
	Wetland			
	B	-5.013 (1.54)	-3.251	<0.01
	C	-3.800 (0.67)	-5.664	<0.001
	D	-5.140 (1.63)	-3.148	<0.01

Table S3 (Continued)

Response	Predictors	Coefficient (SE)	Z	P
	E	-3.626 (1.35)	-2.676	<0.01
	Frog weight	0.229 (0.04)	6.369	<0.001
	Days since burn x Sex			
	Days since burn x Juveniles	0.001 (0.0004)	2.162	0.03
	Days since burn x Males	0.0016 (0.001)	2.576	0.01
Acuariid	Intercept	2.5283 (2.12)	-1.190	0.23
	Days since burn	0.002 (0.001)	1.926	0.05
	Sex (Female, Juvenile, Male)			
	Juvenile	-0.065 (0.65)	-0.100	0.920
	Male	1.916 (0.90)	2.133	0.03
	Wetland			
	B	2.293 (1.66)	1.380	0.17
	C	0.143 (0.66)	0.216	0.83
	D	1.199 (1.78)	0.676	0.50
	E	0.300 (1.47)	0.204	0.84
	Frog weight	-0.0386 (0.04)	-0.944	0.34
	Days since burn x Sex			
	Days since burn x Juveniles	-0.001 (0.0004)	-1.810	0.07
	Days since burn x Males	-0.002 (0.001)	-2.611	0.01
Metacercariae	Intercept	-0.464 (2.30)	-0.201	0.84
	Days since burn	0.001 (0.001)	0.443	0.66
	Sex			
	Juvenile	-1.603 (0.70)	-2.303	0.02
	Male	-2.017 (1.03)	-1.960	0.05
	Wetland			
	B	1.660 (1.81)	0.916	0.36
	C	-0.909 (0.75)	-1.214	0.22
	D	1.192 (1.91)	0.623	0.53
	E	1.057 (1.60)	0.660	0.51
	Frog weight	-0.024 (0.04)	-0.552	0.58
	Days since burn x Sex			
	Days since burn x Juveniles	0.001 (0.0004)	2.297	0.02
	Days since burn x Males	0.001 (0.001)	1.861	0.06

Table S5. Coefficients table for the effect of fire on nematodes recovered before and after field burns.

Predictors*	Coefficient (SE)	Z	P
Intercept	10.023 (2.41)	4.16	<0.001
Sample event (before or after)			
Before	0.540 (0.18)	3.05	<0.01
Soil mass	-0.481 (0.11)	-4.24	<0.001

Table S5 (Continued)

Predictors*	Coefficient (SE)	Z	P
†Maximum temperature	-0.739 (0.70)	-1.05	0.29

\*Random effect = Sample location; Variance = 0.156, StdDev = 0.395

Negative binomial dispersion parameter = 7.37

†log transformed

Table S6. ANOVA table for the effect of fire on nematodes recovered before and after mesocosm burns.

Predictors	$\chi^2$	P	df
Sample event (before or after)	29.344	<0.001	1
Inoculation (nematodes or sham added)	1.536	0.215	1
Fire (burned or unburned)	21.873	<0.001	1
Day	17.004	<0.01	5
Fire x Inoculation	0.004	0.95	1
Fire x Inoculation x Sample event	2.207	0.649	1

Table S7. Coefficients table for the effect of fire on nematodes recovered before and after mesocosm burns.

Predictors*,†	Coefficients (SE)	Z	P
Intercept	0.543 (0.43)	1.26	0.21
Sample event (before or after)			
Before	2.39 (0.44)	5.44	<0.001
Inoculation (nematodes or sham added)			
Nematodes added	0.482 (0.47)	1.02	0.31
Fire (burned or unburned)			
Unburned	2.39 (0.44)	5.44	<0.001
Day			
Day (2)	1.078 (0.37)	2.89	<0.01
Day (3)	0.387 (0.38)	1.03	0.31
Day (4)	-0.298 (0.38)	-0.79	0.43
Day (5)	0.776 (0.36)	2.14	0.03
Day (6)	0.616 (0.36)	1.69	0.09
Fire x Inoculation			
Unburned x Nematodes added	0.0002 (0.59)	0	1.00
Inoculation x Sampling event			
Nematodes added x Before	0.655 (0.53)	1.22	0.22
Fire x Inoculation x Sample event			
Unburned x Nematodes added x Before burn	-0.314 (0.69)	-0.45	0.65

\*Random effect = Day; Variance < 0.0001, StdDev = 0.0003

†Random effect = Tub within Day; Variance = 0.10, StdDev = 0.3176

Negative binomial dispersion parameter: 4.04

Table S8. ANOVA table for the effect of temperature and duration of burn on nematodes recovered only from burned mesocosms.

Predictors	$\chi^2$	<i>P</i>	df
Sample event (before or after)	9,407	<0.01	1
Inoculation (nematodes or sham added)	2.463	0.12	1
Day	19.541	<0.01	5
Burn time above 100°C	1.738	0.19	1
Maximum Temperature	0.364	0.55	1
Inoculation x Sample event	2.448	0.12	1

Table S9. Coefficients table for the effect of temperature and duration of burn on nematodes recovered only from burned mesocosms.

Predictors <sup>*†</sup>	Coefficients (SE)	Z	<i>P</i>
Intercept	0.181 (1.03)	0.18	0.86
Sample event (before or after)			
Before	2.889 (0.99)	2.92	<0.01
Inoculation (nematodes or sham added)			
Nematodes added	0.426 (0.43)	0.99	0.32
Day			
Day (2)	1.759 (0.44)	0.18	0.86
Day (3)	-0.221 (0.451)	-0.49	0.624
Day (4)	-0.514(0.45)	-1.15	0.25
Day (5)	0.819 (0.42)	1.96	0.05
Day (6)	0.04 (0.43)	0.08	0.93
Burn time above 100°C	0.129 (0.10)	1.32	0.19
Maximum Temperature	-0.0004 (0.001)	-0.60	0.55
Inoculation x Sample event			
Nematodes added x Before burn	0.824 (0.53)	1.56	0.12

\*Random effect = Day; Variance < 0.0001, StdDev = 0.0003

†Random effect= Tub within Day; Variance = 0.10, StdDev = 0.3176

Negative binomial dispersion parameter: 4.04


APPENDIX D: IACUC letter of approval for amphibians used.



RESEARCH INTEGRITY AND COMPLIANCE  
INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

MEMORANDUM

TO: Jason Rohr,

FROM:   
Farah Moulvi, MSPH, IACUC Coordinator  
Institutional Animal Care & Use Committee  
Research Integrity & Compliance

DATE: 6/30/2014

PROJECT TITLE: Human modified landscapes: Effects on wildlife disease risk and host population dynamics

FUNDING SOURCE: USF department, institute, center, etc.

IACUC PROTOCOL #: W IS00000587

PROTOCOL STATUS: **APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC **APPROVED** your request to use the following animals in your **protocol for a one-year period beginning 6/27/2014:**

Hyla cinerea (green treefrog) (Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	10000
Hyla femoralis (pinewoods treefrog) (Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	10000
Hyla gratiosa (barking treefrog) (Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	10000
Hyla squirella (squirrel treefrog) (Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and	10000

adults). We expect to capture <10,000.)	
Osteopilus septentrionalis (cuban treefrog)	10000
(Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	
Bufo terrestris (Southern toad)	10000
(Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	
Bufo marinus (Cane toad)	10000
(Unknown because we are proposing a capture-mark-recapture field study. Most are expected to be postmetamorphic (i.e. juveniles and adults). We expect to capture <10,000.)	

Please take note of the following:

- **IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol through the eIACUC system.** After three years, all continuing studies must be completely re-described in a new electronic application and submitted to IACUC for review.
- **All Comparative Medicine pre-performance safety and logistic meetings must occur prior to implementation of this protocol.** Please contact the program coordinator at [compmed@research.usf.edu](mailto:compmed@research.usf.edu) to schedule a pre-performance meeting.
- **All modifications to the IACUC-Approved Protocol must be approved by the IACUC prior to initiating the modification.** Modifications can be submitted to the IACUC for review and approval as an Amendment or Procedural Change through the eIACUC system. These changes must be within the scope of the original research hypothesis, involve the original species and justified in writing. Any change in the IACUC-approved protocol that does not meet the latter definition is considered a major protocol change and requires the submission of a new application.
- **All costs invoiced to a grant account must be allocable to the purpose of the grant.** Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.
- **The PI must assist the IACUC with tracking wild animal field research activities.** The PI must report episodes of wild animal use, the approximate range of taxa, and the approximate numbers of animals encountered or used at intervals appropriate to the study but at least once a year.