Movement and Physiology of Invasion Front Cane Toads (*Rhinella marina*) in Central Florida

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Abstract

In Australia and Florida, cane toads (*Rhinella marina*) are an invasive species that have wreaked havoc on local ecosystems. Native to South and Central America, these toads secrete a toxin harmful to both fauna and humans, strongly impacting Florida wildlife. Previous studies in Australia have established that actively invading toads change morphologically to have longer limbs, greater speed, and greater endurance compared to those that remain established in a single population. However, our research in central Florida suggests that the opposite may be occurring at this invasion site. Our experimental studies on toads suggest that those at Florida invasion fronts have shorter limbs, lower muscle mass, and less endurance. In addition, surveys evaluating the movement of the invasion itself were taken over 6 years to follow the path of travel and establish mean migration across the state. Contrary to the rapid expansion in all directions seen in Australia, our invasion appears to follow human development such as roadways eastward from Tampa, Florida at a slow and steady pace. Additional research is required to establish a mechanism creating the discrepancies between the two populations of invasion and established toads in both Florida and Australia. Establishing the differences between the two groups can give key insight into the management strategy of this invasive species and help preserve the health of Florida's ecosystem.

Introduction

Non-native species are organisms that are introduced by human activity into areas in which they are not native. Many non-native species are invasive, meaning the population is actively expanding its range and often posing a threat to native wildlife.. In many cases, invasive organisms have adaptations that native flora or fauna do not, forcing greater competition and often outcompeting them for resources (Duckworth and Badyaev 2007). If an invading species has adaptations that increase their ability to expand their range, they can have a greater dispersal and wider impacts on the ecosystem (Phillips *et al.* 2006). The outskirts of these dispersals are known as invasion fronts. Individuals from these areas are of particular interest, as they are directly responsible for the movement of the population and may have unique traits (Cote *et al.* 2010; Duckworth and Badyaev 2007; Llewelyn *et al*. 2010; Phillips *et al*. 2006; Pintor *et al*. 2009).

Spatial data is useful when observing invasive species as it can indicate the rate of invasion, range the species covers, as well as the habitats that are preferred by the population. Understanding where a species spreads can give insight on how to better estimate where they will be seen next (Joshi 2004). When looking at spatially separated populations, behavioral characteristics are often of interest, especially within an invasive species. Traits such as aggression, sociability, and the tendency to travel long distances in a straight line have been previously examined in invasive species (Duckworth and Badyaev 2007; Cote et al. 2010; Pintor et al. 2009). Studies on morphological differences specific to the invasion front have occurred less frequently. However, invasion vanguard-specific morphological adaptations have been recorded in invasive cane toads in Australia (Phillips et al. 2006; Llewelyn et al. 2010; Brown et al. 2013).

Since its introduction to Australia in 1935 to control cane beetle larvae, *R. marina* has spread over more than a million square kilometers (Phillips et al. 2006; Shine 2020), and its rate of dispersal has increased fivefold (Llewelyn et al. 2010). Cane toads have been highly effective invaders due, in part, to their extreme toxicity to any Australian predator that attempts to prey upon them (Phillips et al. 2007). Bufonid toads have a cosmopolitan distribution, except for Australia, which means Australian mesopredators are not adapted to encountering and preying upon toads (Shanmuganathan 2010). Some Australian studies have focused specifically on the morphology of cane toads at the invasion front. Phillips et al. (2006) and Hudson et al. (2016) found that newly colonizing toads have longer limbs and structurally different skeletal shapes than conspecifics from long-established populations. Longer-legged toads have the ability to travel farther over a 24 hour period. In addition, the elongation of the forearms in invading toads allow for a difference in gait with more successive leaps at shorter distances to conserve energy. Given that selection at the invasion front has continued to favor longer legged toads, researchers predicted correctly that the speed of dispersal would continue to increase (Brown et al. 2011). Llewelyn et al. (2010) found that toads at the invasion front have a significantly higher level of endurance than those of long-standing populations. However, in contrast to the findings of Brown et al. (2011) and Phillips et al. (2006), Llewelyn et al. (2010) failed to show a significant difference in speed or in rear leg length between established and invasion front sites, although the latter result was complicated by greater sexual dimorphism in limb length at the invasion front. It was unclear whether increased endurance at the invasion front was a cause of greater dispersal or a consequence of a behavioral shift toward repeated prolonged exertion. Llewelyn et al. (2010) argue that the increased rate of dispersal has been a consequence of traveling in straighter paths and greater endurance, rather than greater speed.

A study by Brown et al. (2013) found that cane toads at the invasion vanguard have greater fat deposits, higher growth rates in both mass and length, and fewer parasites. Brown et al. (2013) argue that, because parasites such as lungworms can reduce growth rates of adult toads, cane toads having fewer parasites could explain the increased growth rates. The toads at the invasion front have better prey availability due to less conspecific competition, which also could contribute to their growth rates and large fat deposits.

Rhinella marina was introduced to Florida in the 1930s and has resided in Tampa uninterrupted since the 1970s (Dodd, 2013). The invasion front has recently spread east from Tampa to cover Polk county. Thus there is the opportunity to study the morphological differences between the long-standing population in Tampa and the newly-colonized Lakeland population. In addition, novel sightings of the species established a baseline location for the invasion front and have given the opportunity to track the direction and rate of invasion. In previous research on preserved specimens of cane toads in Florida, there have been unexpected trends of reduced leg length over historical time, however, this has not been corroborated with invasion front vanguard toads compared to established populations (Rubio et al. 2020). This study aims to assess the invasion front toads in central Florida by evaluating the spatial data of 6 years of movement as well as comparing the physiological differences between actively invading toads and resident populations.

Methods

Spatial data of the invading cane toads was recorded from direct observations. In the fall of 2010 and spring of 2011, an extensive search, road-cruising for toads and dip-netting roadside ditches for tadpoles, was conducted in western Polk Co. and eastern Hillsborough Co. In addition, veterinary offices in Plant City and Lakeland were contacted to inquire if clients had reported cane toad interactions with pets. During this initial search, the invasive toads were located in Plant City, Florida, but not east of this location. Spatial data collection began over a period of six years between 2010 and 2016. Sampling occurred along major and minor roadways beginning at the last known extent of their range. Every 2-3 months, during favorable conditions, e.g., during rainfall , roadway surveys were performed during warm seasons. Each survey period lasted approximately 2-3 hours beginning at dusk. Routes were mapped prior to departure to systematically search along the leading edge of the invasion with a focus on the area within 2 km in front of the invasion edge. However, additional routes were driven further from the invasion front out to5 km from the outermost sighting, with the intent of catching any rapidly advancing toads. In addition to visual observations, calling males were recorded as a single spatial data point. Citizen scientists supplemented data between surveys when accompanied by identifiable photographs with GPS data. ArcGIS Pro software was used to generate and analyze statistical and spatial data.

Cane toads used in physiological experiments were collected by hand from 2 locations: the University of South Florida campus (in Tampa) and Florida Southern College campus (in Lakeland). Following capture, the toads were transported (N 1 = 50, N 2 = 50) to Florida Southern College. Specimens were housed individually in plastic cages designed for small pets (Petco Pet Keeper: 27 cm L · 17 cm W · 16 cm H) in a temperature-controlled room designed for housing amphibians and reptiles for no more than 48 hours prior to endurance testing. Endurance was measured by placing toads individually on an enclosed square track (1.98 m · 1.98 m) and encouraging them to round the edges by gentle prods to the urostyle with a wooden pole. When a toad failed to respond, despite 10 taps to the urostyle, it was presumed exhausted and its trial ended (its total distance run and time to exhaustion were recorded). All endurance trials were conducted between 20:00 and 24:00 h, when cane toads are most active.

After the endurance trials, and no more than 48 hours following capture, subjects were euthanized by double pithing for dissection.

The following external morphological traits were measured: total mass (g), snout-vent length (mm), leg length (acetabulum to tip of longest phalanx, mm), and thigh width (mm) at its widest point. The rear legs were flayed and detached from the body by separating the femur head from the joint capsule of the acetabulum. Separated from the bone were all of the leg muscles inserting at the femur from their origins on the pelvis, except the gluteus, and all muscles inserting and originating at the tibiofibula. The feet were separated from the tibiofibula by severing the Achilles tendon. All of the leg muscles, except the gluteus, were weighed together for each leg, and then the two legs' masses were averaged together for each toad. Next, the tibiofibula and femur were separated and their lengths were measured at the longest point (mm). The lengths of the bones of the same type from each leg were averaged. Concluding the dissection, the masses of the paratoid glands and the heart (cut at the top of the atrium) were obtained. The morphological measurements obtained were normalized by SVL and by total mass. Mann Whitney U tests were used to analyze and compare the resulting data.

Results

Endurance trials were run on 50 cane toads from the invasion front population in Lakeland and 50 from the long-standing population in Tampa and dissected following the trials. Cane toads from Tampa outperformed toads from Lakeland in endurance, measured both by average time to exhaustion ($W = 1933$, $p = 0.0005$) and average distance traveled (Figure 3; $W = 1907$, $p = 0.0002$). Toads from the long-standing population also had longer legs, when normalized by SVL (Figure 2; $W = 1940$, $p = 0.0006$), and more muscular legs, as a proportion of total body mass (Figure 1; $W = 1779$, $p = 0.0000$).

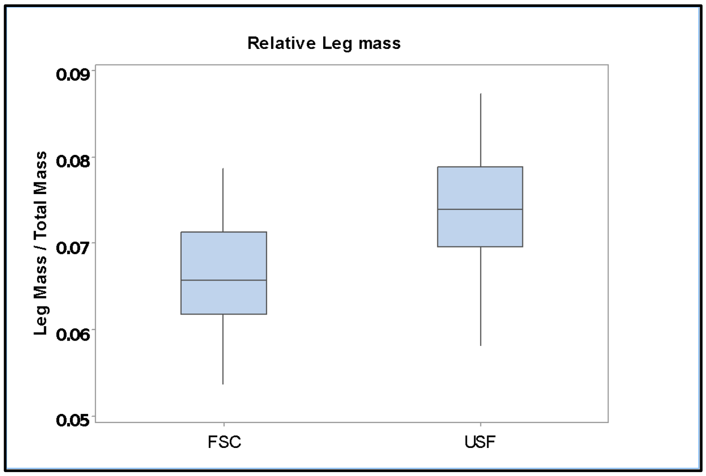

Figure 1. Comparison of mean leg mass, as a proportion of total mass, between populations. Relative leg mass was significantly different between populations at $p < 0.001$.

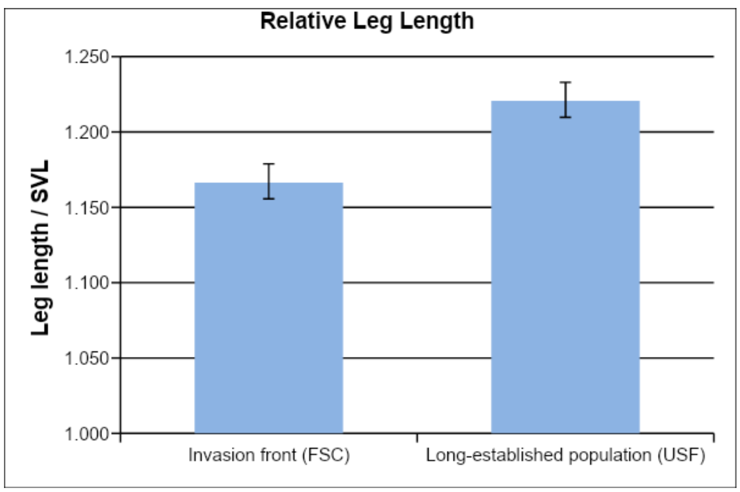

Figure 2. Comparison of mean leg length, normalized by SVL, between populations.

Relative leg length was significantly different between populations at $p < 0.001$.

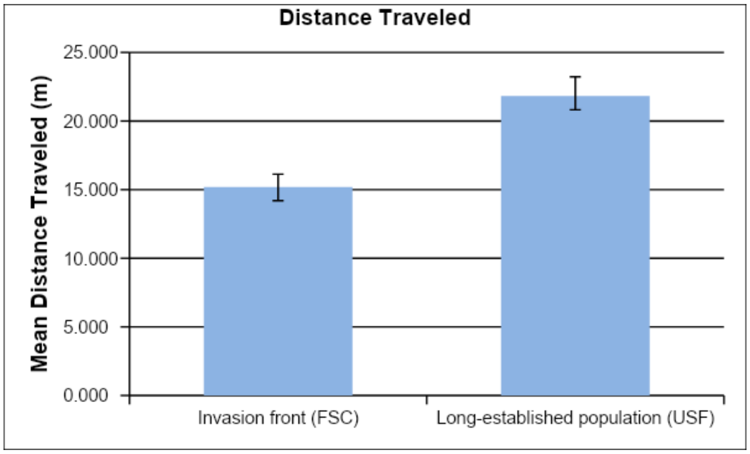

Figure 3. Comparison of mean distance traveled between populations. Distance traveled was significantly different between populations at $p < 0.001$.

The individual observations of toads when plotted resulted in a dispersal radiating eastward along major roadways. The approximate distance between the two farthest plotted observations was 38.62 kilometers. The mean center per year follows a linear pattern, and had a total distance of 29.05 kilometers between 2010 and 2016 points. Table 1 contains values of the averaged rate of invasion per year.

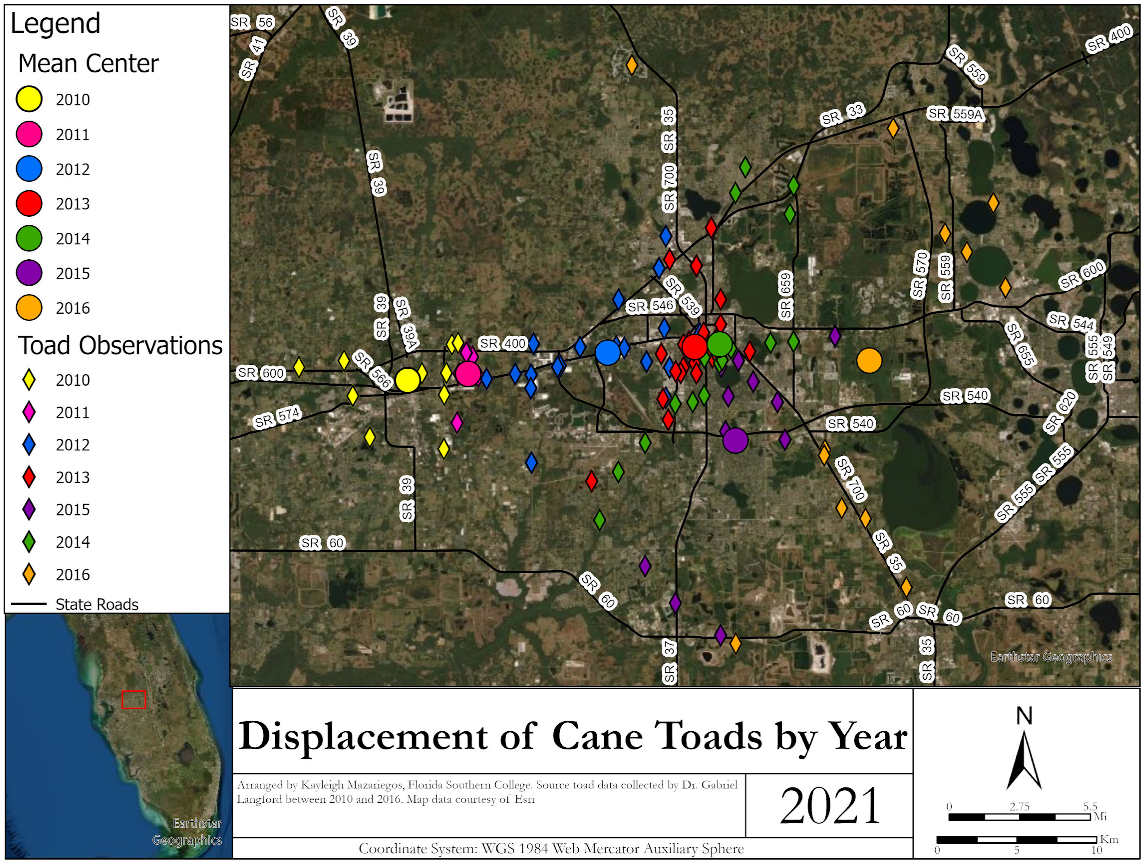

Figure 4. Displacement of Cane Toads by Year, plotted individual toad sightings and calculated mean centers per year.

Year	Rate of invasion per year
2011	3.96 km/yr
2012	8.67 km/yr
2013	5.15 km/yr

2014	1.77 km/yr
2015	6.04 km/yr
2016	9.78 km/yr

Table 1. Values of Average Rate of Invasion by Year. Calculated using distance between mean center values per year.

Discussion

Cane toads in central Florida disperse at rates ranging from only 1.77 to 9.78 kilometers per year, well below a conservative Australian average of 15 kilometers per year and record single day travel of 1.8 kilometers per night (Phillips et al. 2006; Urban 2007). In addition, the physiological traits of invasion front and long standing populations appear to be reversed. Toads from the Lakeland, Florida invasion front exhibited significantly shorter legs and reduced leg mass when compared to the established Tampa population. Similarly, endurance testing resulted in invasion front toads performing more poorly and tiring quickly compared to their established counterparts. There was not an observed difference between the locomotive speeds of the two populations, which was consistent with Australian studies (Llewelyn et al. 2010).

Cane toads from the invasion front were noted to have shorter limbs than established populations as opposed to their Australian counterparts. Hudson (2016) acknowledges shortening of the forearms as physiological adaptation in order to migrate at a more consistent gait; however, this was in conjunction with elongated hind limbs. Our discovery of a lower muscle mass and length may indicate that Florida cane toads actively invading may experience a reduction in length as a stress response, as resources may be less available to those at the forefront of new areas with higher competition. Geographically, the invasion front appears to move linearly west to east with preference for intermediate human development. There is a notable lack of toads found in undisturbed areas. We hypothesize that invasion into more natural areas may be hindered by existing competition by other ground-dwelling anurans.

These results are notably different from those in Australia, and may point to many differences in their respective ecosystems. Specialized traits seen in Australian toads may not be specific to increasing invasion but instead arisen from differences in environmental stimuli. Australian ecosystems are generally more arid, requiring greater endurance when traveling between suitable habitats to avoid dessication. Central Florida has an abundance of wetland habitat, and it would be a much smaller distance between suitable breeding areas. In addition, differences in the native fauna of both areas may lead to differences in fitness between the populations. Australia was devoid of true ground-dwelling toads, and as a result had few barriers to invasion in regards to occupying a new niche (Shanmuganathan 2010). In Florida, there are many other species of toads and greater competition for the same prey items, which may be a cause for the slower spread in the area.

One aspect that is similar between the Australian and Central Florida populations relates to spread and human settlement. In Urban's (2007) analysis of rate of invasion in Australia, the heavily populated northeastern coast of Australia experienced a slower rate of spread than the less inhabited central and western coasts. In central Florida, the rate of invasion also significantly reduces when approaching urban areas. Urban areas are prone to greater traffic, leading to mortality in roadways, and limiting paths of travel. It is likely that this explains the reduction in invasion rate when encountering these areas. Developed areas are also artificially lit, creating prime hunting grounds for toads seeking an easy meal in the attracted insects. It is possible that when abundant resources are located, the toads are less likely to migrate.

The physiological studies did not control for sex, which may account for some variation in results. Cane toads are sexually dimorphic in size, and additionally many of the females dissected carried a large proportion of their body mass in eggs. A female carrying a brood of eggs would presumably be at a disadvantage in speed and endurance from the extra mass alone, without taking into account possible energy expenditures associated with reproduction. It is possible that the proportion of females in the populations differed or, as in the Llewelyn et al. (2010) study, sexual dimorphism might differ between populations; both of these scenarios could have influenced the results. Future research should control for the sex of each cane toad to clarify this issue. Toads from the invasion front in Australia had fewer parasites (Brown et al. 2013) than toads from a long-standing population; if the two populations studied in Florida have different amounts of parasites, the poor health of the parasite-laden toads could have resulted in low growth rates and poor endurance.

There is limited published data on the spatial ecology of Florida cane toads and their dispersal within the state. Given the direction of migration, it can be assumed that the Lakeland population originated near Tampa or the surrounding area and travelled eastward. However, because the spatial sampling began in Plant City, it cannot be definitively concluded that these invading toads are directly descended from the Tampa resident population. Given that Tampa has been invaded at multiple points in time, it is possible that the populations between Tampa and Lakeland used in the physiology studies are not directly related or linearly migrated from one area to the other. Limitations in sampling resulted in more observations along roadways and human development. Due to the nature of the study, there was limited access to private property, and thus there are clear patterns in the projected path of invasion. This likely accounts for the slight southward dip in the mean center point of 2015 as there are limited roads in the area from which to sample.

If the observed trends from this study in Florida are truly representative of a less fit invasion vanguard, then the mechanisms driving invasion in the Australian and Florida populations appear significantly different. The slower and less effective invasion of cane toads in Florida may be indicative of a more controlled spread. While this study effectively shows direction, rate, and fitness of invading toads in central Florida, environmental stimuli and their correlation is needed to soundly determine what factors determine physiological changes between populations. In the future, more information is needed to effectively help understand and eradicate this aggressive invasive.

Acknowledgments:

I would like to thank Dr. Gabriel Langford for his support and advice especially given the accelerated project timeline due to the COVID-19 pandemic. Former students Aaron Bias, Nathan Roddenberry, and Bill DeMott were critical in data collection and analysis, and I am grateful for inheriting their hard work. Thank you to the citizen scientists whose supplementary data helped complete the picture of cane toad distribution. I would like to thank the Florida Southern College Department of Biology and Honors Program for supplying technology and supporting the project. This research was permitted by Florida Southern College Institutional Animal Care and Use Committee protocol #1014.

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