TRACKING TOADS:

MOVEMENT PATTERNS OF GULF COAST TOADS

(*INCILIUS NEBULIFER*) AND THE URBAN ENVIRONMENT

by

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ABSTRACT

Gulf Coast Toads (*Incilius nebulifer*) are a familiar urban resident in the southeast United States and Mexico. As urbanization increases, the Gulf Coast Toad may be expanding its range. Studying movement behavior can reveal how invasive a species may be and is a key factor in understanding the ecology of a species. This study used standardized movement trials in the field to gather behavioral data from toads in two different urban environments within Houston, Texas. Two hypotheses were tested: (1) differences among urban environments will affect the movement behavior of Gulf Coast Toads, and (2) leg length will affect hopping distance. The results support both hypotheses. I also observed notable qualitative differences in toad coloration between the two sites. Gulf Coast Toads may be an excellent model for observing how different types of urbanization exert different environmental pressures upon a species.

INTRODUCTION

The plight of amphibians is a global concern, as many species are currently facing extinction from threats such as disease and habitat destruction (Bolochio et al., 2020; Fisher & Garner, 2020). As humans continue to alter landscapes around the world (United Nations & Department of Economic and Social Affairs, 2018), some species struggle to adapt while others have no trouble thriving in the urban environment.

The Gulf Coast Toad (*Incilius nebulifer*) is one of the amphibian species that appears to thrive in urban settings. They range from Mississippi to Texas and eastern Mexico (Mendelson et al., 2015), making themselves at home in a variety of ecosystems, including apartment complexes, city streets, and residential lawns. They grow rapidly in their first year of hatching, attaining snout-vent lengths of up to 55 mm, and they can attain sexual maturity and snout-vent lengths of up to 100 mm within 10 months (Blair, 1953). Growth rate slows in the cold season and as the toad ages, and also varies between individuals and with environmental conditions (Blair, 1953). Individuals have been recorded living up to 8 years of age (Blair, 1960). Toads require a refuge in which they can shelter during daylight hours and cold temperatures, and their variety of shelter selection showcases their adaptability. Their refuges can include simple holes in the ground, in hollow logs, underneath rocks, sidewalks, water valve covers, and even in tree hollows several meters above the ground (Neill & Grubb, 1971).

Gulf Coast Toads have been documented as surviving and maintaining large numbers in the face of great disturbance, such as in the conversion of savanna habitat to suburban homes, in which 55% to 75% of other amphibian species may be extirpated or experience population declines (Gehlbach, 2010). In a rapidly urbanizing world, it is important to understand how species adapt to living within urban environments, and how these adaptations could impact entire ecosystems (Olivier et al., 2020). This species has been so tolerant of human habitation that there is now concern the Gulf Coast Toad is expanding its range east and may out-compete other native amphibian species (Milko, 2012). Bocxlaer et al. (2010) identified 7 life history traits that are correlated with range-expansion ability in *Bufonidae* species, and many are present in the Gulf Coast Toad: large body size, terrestrial adult niche, parotoid glands, large clutch sizes, and exotrophus larvae. Studying movement patterns can reveal how invasive a species may be and predict where an invasive species may cause major impacts to an ecosystem (Feit et al., 2019; Kim & Mandrak, 2016). Movement behavior is a key factor in understanding the ecology of a species, as animals move to perform crucial life history functions such as foraging for food, seeking shelter, and finding mates. Movement behavior is critically important for the survival of amphibians especially, as they must navigate connections between land and water (Bredeweg et al., 2019). Habitat fragmentation and inhospitability due to anthropogenic land use changes can force changes to movement behavior and can result in impacts such as reduced gene flow between populations (Trochet et al., 2019; Cushman, 2006).

The goal of this study is to learn more about the movement behavior of Gulf Coast Toads and how toad behavior and ecology may be affected by urbanization. I use standardized movement trials to gather data on movement behavior from toads in two different urban environments. Urbanization likely impacts such variables as prey availability, shelter, and access to water, and these impacts will likely differ between different types of urban settings. I test the hypothesis that environmental differences between urban environments will affect the movement behavior of Gulf Coast Toads, and predict that there will be a difference in movement behavior between toads in different environments. I also examine the effect of morphology on behavior by testing the hypothesis that leg length will affect hopping distance, which affects dispersal ability, and I predict that longer legs will correlate with longer hops.

METHODS

Two study sites were chosen based on ease of access to large toad populations and to compare two different types of urban environment. Both sites are within the greater Houston metropolitan area (Texas, USA) and are separated from each other by 25 kilometers. Site DA (Figure 1) is a residential area that includes apartment complexes, suburban homes, and associated detention ponds. Site CP (Figure 2) is a 43-hectare city park surrounded by suburban homes and featuring sports facilities, walking trails, and gazebos. Both sites featured access to large bodies of water in which toads were breeding. Data were collected in April 2021.

Eighteen toads were collected at each study site through random searches. I noted the GPS location of each toad as it was found. Every toad was put through two trials: a runway movement trial and a round arena movement trial (Figure 3). The order of the two trial types was randomized for each individual. I conducted trials in the field at night under red light, and periodically moistened the substrates with dechlorinated water. Ambient temperature was noted for each night of the experiment. In both trial types, I acclimated toads for 5 mins underneath a clay pot. After acclimation, toads could move around the experimental chamber freely. If there were no hops after 10 mins, the trial would end. After testing, I weighed and photographed each toad, then released each toad back at their original point of capture.

Runway movement trials were based partly on methods in Bredeweg et al., 2019 and Trochet et al., 2019. I constructed a 182 x 20 (cm) portable runway using landscape edging, corrugated plastic board, shelf liner, wooden dowels, and plastic mesh (Figure 3). When a toad was released into the runway, I recorded number of hops, time until first hop, and time from first hop until the toad reached the end of the runway. The length of 5 hops was measured using a ruler adjacent to the runway. The trial ended when the toad reached the end of the runway, or when the toad failed to reach the end after 10 mins from the first hop. Arena movement trials utilized a round plastic pool (Yaheetech, 120 x 30 cm; Figure 3). I recorded time until first hop, and then the number of hops for 5 mins. For both trials, walking, climbing, or reorientation were not considered to be hops and excluded from counts.

Photos of each toad were processed using ImageJ to determine snout-vent length and hind leg length. To control for allometry in leg length and body size, I used a standard least squares regression on hind leg length (mm) with snout-vent length (mm) and saved the hind leg length residuals (HLLresids) to use in subsequent analysis. To determine if toads with relatively longer legs can hop longer distances, and if this varies between the two sites, I used ANOVA on the longest hop length (cm) in the runway trials by site, with the hind leg length residuals (HLLresids) as a covariate. JMP Pro (v15) was used for all statistical analyses. To evaluate if toads from the two sites differed in overall hopping activity, we used a repeated measures ANOVA to take into account the different trial designs (runway and arena). Site and the trial type that was given first were the between subject effects, and the individual toad tested by the trial type given first and the individual toad tested by site interactions were the within subject effects.

Figure 1: Satellite image of residential site DA, with points marking where each toad was found. Major features of the site include detention ponds, suburban homes, apartment complexes, green space along walking paths, a powerline easement, and undeveloped fields. Toads were chorusing only at the bayou to the east side of Long Prairie Trace road.

Figure 2: Satellite image of park site CP, with points marking where each toad was found. The construction area pictured on the east side was developed into suburban homes since the satellite image was taken and prior to data collection. Major features of the site include a central pond, a bayou along the eastern park border, sports facilities, manicured fields, and suburban homes. Toads were chorusing only at the bayou.

Figure 3: The two trial types, arena (left) and runway (right).

RESULTS

There was no significant effect of site (ANOVA, $F_{(1,30)} = 0.053$, $P = 0.820$) nor interaction with HLLresids (ANOVA, $F_{(1,30)} = 0.001$, $P = 0.981$), but there was a significant positive relationship (Figure 4) between HLLresids (relative hind leg length) and longest hop distance in the runway (ANOVA, , $F_{(1,30)} = 5.044$, $P = 0.033$; Figure 4).

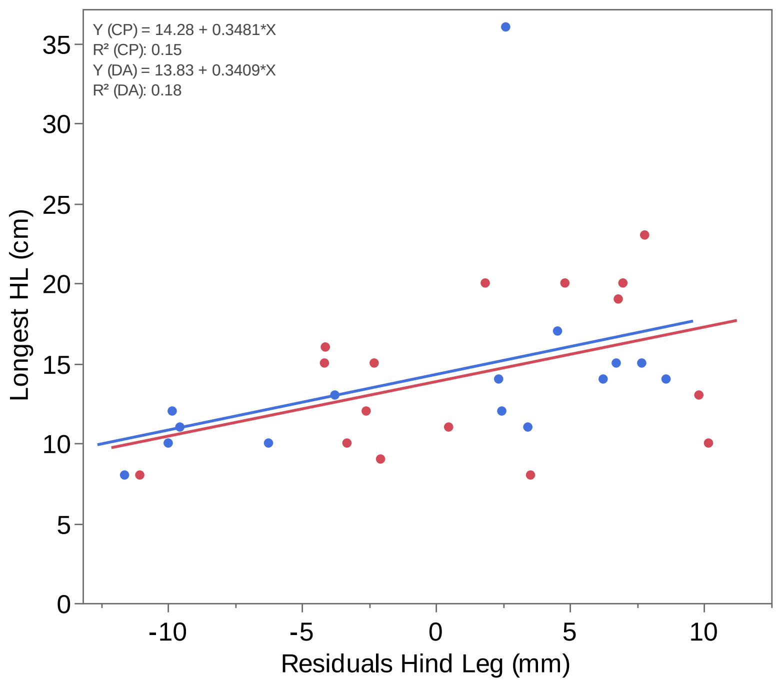

Figure 4: Variation in longest hop length (cm) by hind leg length residuals (mm) at both sites CP (blue) and DA (red). There is a significant positive relationship between HLLresids and longest hop distance.

There was a significant difference in mean number of hops in the runway trial between sites (Table 1; Figure 5), with toads hopping less at the CP site. There was no significant difference in mean number of hops in the arena trial between sites (Figure 5), although it exhibits the same trend of fewer hops at site CP. Which trial the toad experienced first did have a significant effect on number of hops (Table 2), with the toads that did the runway trial first hopping the least. Mass (g) did not have a significant effect on hop activity, nor did temperature (18 – 22 °C) (All $p > 0.05$).

Measurement photographs of each toad revealed qualitative differences between individuals in coloration and patterning, and general differences in coloration between the populations at each site. At site DA the majority of toads were pale brown, whereas the majority of toads at site CP were dark brown (Figure 6).

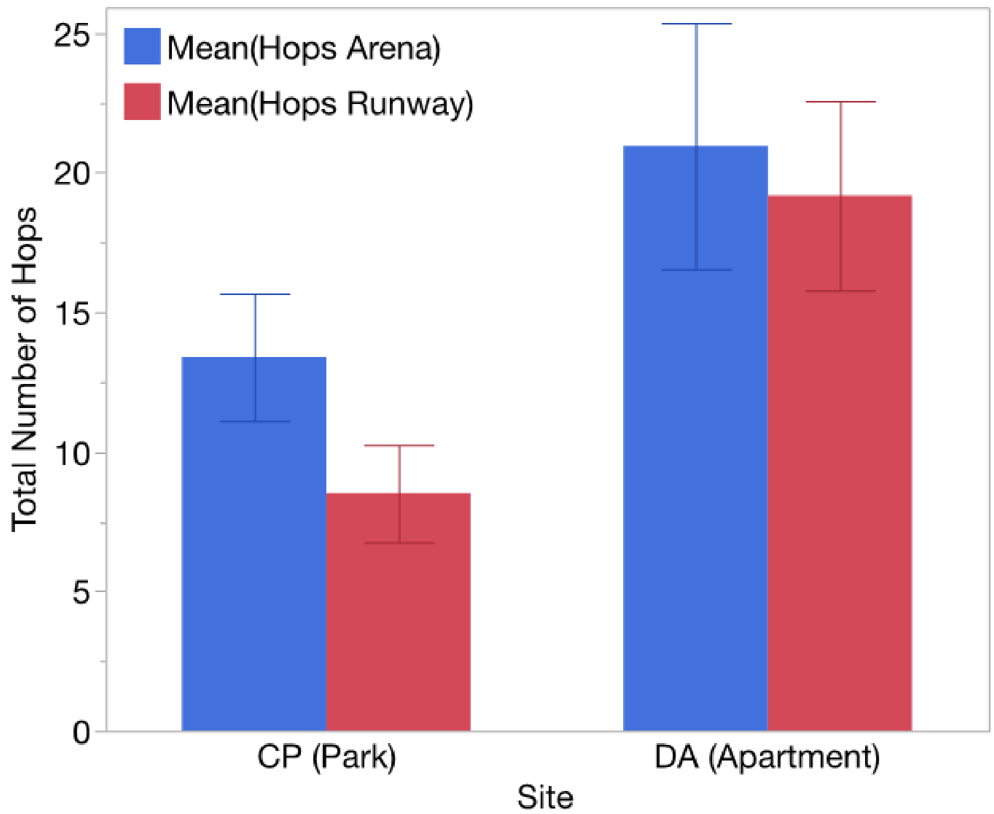

Figure 5: Mean number of hops in the arena trial (blue) vs mean hops in the runway trial (red) at each site. There was a significant difference in mean number of hops in the runway trials. Mean number of hops in the arena trials were not significant but exhibited the same trend.

Table 1: Results of rmANOVA with site and which trial type first as between subject effects and individual toad tested by which trial type first and by site interactions as within subject effects.

Site					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.1971337	6.3083	1	32	0.0173
Which trial first?					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.0035816	0.1146	1	32	0.7372
Which trial first*Site					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.0004593	0.0147	1	32	0.9043
Time					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.1001922	3.2062	1	32	0.0828
Time*Site					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.0038841	0.1243	1	32	0.7267
Time*Which trial first?					
Test	**Value**	**Exact F**	**NumDF**	**DenDF**	**Prob>F**
F Test	0.2189901	7.0077	1	32	0.0125

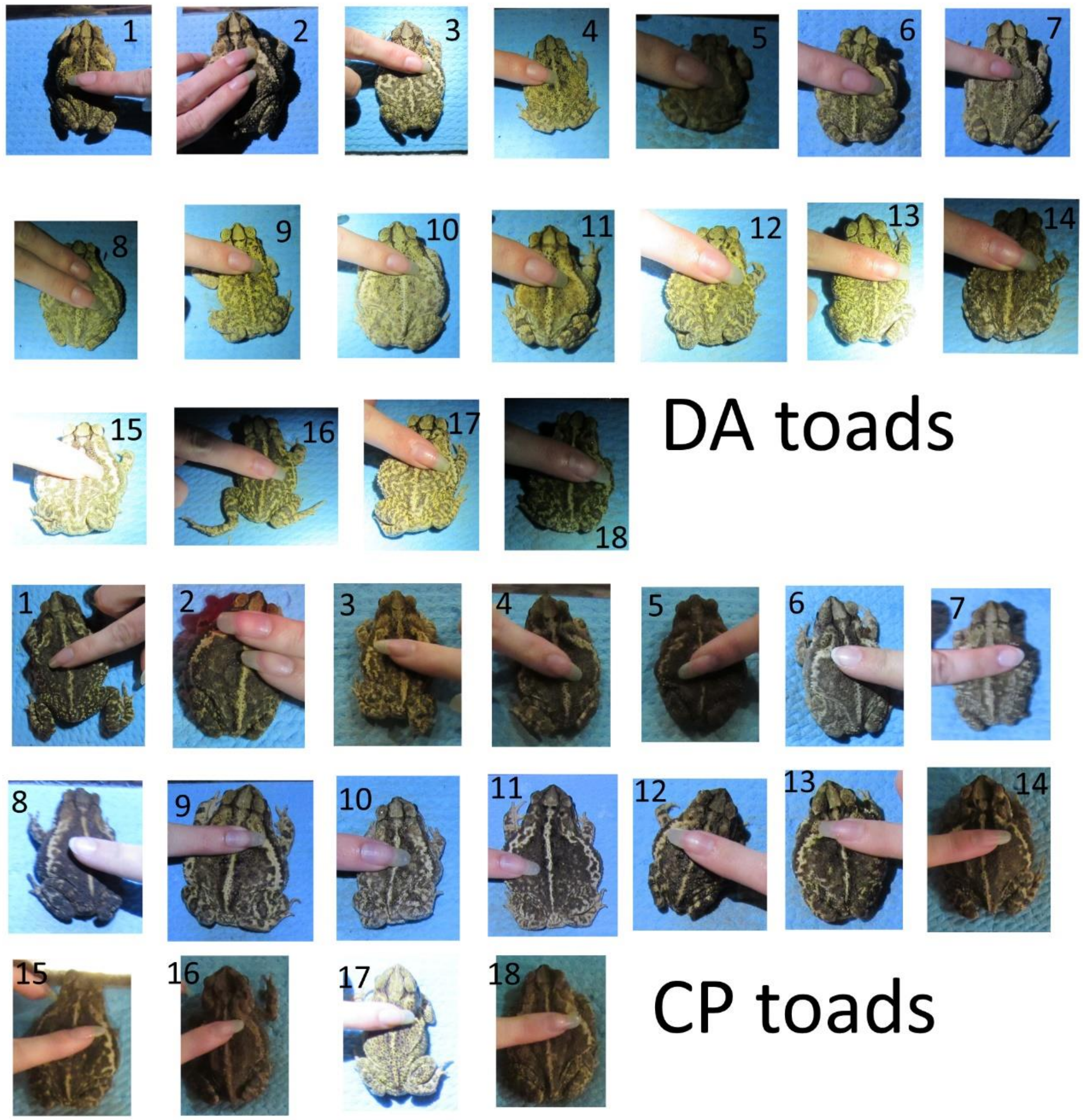

Figure 6: Differences in coloration and patterning among individuals, and differences in general coloration between the two toad populations.

DISCUSSION

My results indicate that toads with relatively longer legs have the ability to make longer hops. This result is consistent with findings from Trochet et al. (2019) where individual amphibians with longer legs were able to cross difficult human-made substrates such as concrete faster than shorter legged individuals. An analysis by Gomes et al. (2009) also found that hind leg length in amphibians is correlated with jumping performance, as well as habitat use of the species. The effect of hind leg length on hop length was the same at both sites, indicating that this trait was unaffected by environmental differences between the two sites. As shown by Trochet et al. (2019), longer legged toads could have an advantage in the urban environment, as they may be more successful at evading predators and crossing obstacles. Phillips et al. (2006) demonstrated that invasive Cane Toads (*Bufo marinus*) with longer legs were able to move faster and were the first to colonize new areas, implying that there may be a link between dispersal ability and hind leg length in other toad species as well. My result was consistent with this prediction and supports the hypothesis that toads with longer legs will have longer hops.

The results also show that there was a significant difference in average number of hops between the two sites. In the runway trials, toads hopped more at residential site DA than at park site CP. In the arena trials, the difference in hop number was not significant between the two sites, but it did exhibit the same trend of fewer hops at site CP. Bredeweg et al. (2019) also conducted movement experiments using runways and found that environmental variation and stressors affected frog movement behavior. Various environmental factors could have contributed to toads being less likely to move at CP, most notably the amount of human disturbance and the presence of predators. Residential site DA experienced little to no human activity after dark, particularly in green spaces adjacent to roads in which most of the toads for the study were located. In addition, few potential predators were encountered during searches at DA. In contrast, park site CP was typically very busy during daylight hours as human visitors and their dog companions utilized the park spaces for sports, parties, exercise, play, and fishing. Often the human activity would continue, albeit at lower levels, through sunset and into the night. Potential predators were also frequently encountered throughout the night: large American Bullfrogs (*Lithobates catesbeianus*) were common along the shoreline of the park's central pond, as were Great Blue Herons (*Ardea herodias*) and Yellow-crowned Night-Herons (*Nyctanassa violacea*). Although Gulf Coast Toads are likely unpalatable to these predators due to the toxins they possess, observations such as those by Brown (1974) indicate that predators such as the Bullfrog may still attempt to eat toads, and that consumption of toads can occur on very rare occasions. It is possible that toads at site CP are more reliant on crypsis or more wary of potential harassment. The difference in hop number between the two sites is consistent with this prediction and supports the hypothesis that environmental differences among urban environments will affect the movement behavior of Gulf Coast Toads.

There was a significant effect of the experimental design, specifically which trial type (runway vs. arena) the toads completed first. Toads that did the runway trial first completed fewer of hops than those that did the arena trial first. This negative effect on hop number was observed at both locations. This could have been due to a variety of factors associated with runway design in contrast to arena design. The meshed roof of the runway required the observer to be closer to the toad to see its behavior clearly. Close observation was also necessary to accurately measure hop length. The initial closeness of a human observer could have caused the toads to be more hesitant. Because light was provided through a headlamp attached to the observer, intensity of light was greater on the runway than in the arena, and this intensity of artificial light could have caused hesitancy as well (Buchanan, 1993). Additionally or alternatively, the smaller, more enclosed space of the runway could also have affected toad behavior.

An unexpected qualitative result was the difference in toad coloration between the two locations. Toads at the DA site were in general much paler than toads at the CP site. The coloration difference may be due to environmental differences, different selection regimes, or random genetic differences between the two populations. The two sites do have different soil compositions, with site CP being primarily Texana-Cieno, and site DA being primarily Bernard Clay Loam (SoilWeb, 2021). However, these two soils do not differ significantly in color and it is unclear how soil type could affect coloration. When searching for toads, we did notice a difference in surface topography and where toads were found. At CP, toads were frequently caught on patches of dirt or dark mulch, on which they blended in very well. At DA, toads were frequently caught in patches of grass, on which they blended in well. The difference in coloration might be explained by differences in vegetation composition and ratio of bare ground or mulch to grass between the sites, and the effectiveness of different colorations as crypsis on these different substrates.

The nature of a field study invites a certain level of error: many variables were present that I did not or could not control for. Because toads were tested immediately in the field, they varied in level of hunger, hydration, and other body condition factors that could have affected their behavior. In addition, there was variation in how long a toad stayed within its holding container before being tested. Environmental conditions of temperature, humidity, time of night, and rainfall were not the same for every trial, although I do know that temperature did not significantly affect any of the hopping metrics that I measured. More accurate methods of hop length measurement, such as video recording, were prevented by the mesh screen roof design of the runway. Observation techniques and lighting were not standardized to be the same across both trial types. Any number of these factors could have unduly influenced the study results. But, the benefit of the field study is that toads were tested soon after capture without any additional stressors, and they could be returned to their original locations. In the future, a combination of lab and field studies could provide a better understanding of these questions.

Future studies could attempt to narrow down exactly which, if any specific one, urban environmental variables affect Gulf Coast Toad behavior, and if such variation in behavior is a consistent result across more sites and with larger samples of toads. Examining morphological differences such as coloration across more toad populations would also be an interesting route to explore. Future work could also optimize on runway trial design to minimize or eliminate its potential negative effect on hopping behavior. This study found evidence that some aspects of behavior, such as hop length, are likely to be consistent across environments, while others, such as number of hops, likely vary with environmental differences. Because Gulf Coast Toads are so adaptable and inhabit such a wide array of habitat types, they could be an excellent model for observing how different types of urbanization exert different environmental pressures upon a species.

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