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The Behavior of Humans and Wildlife with Respect to Roads: Insights for Mitigation and Management

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THE BEHAVIOR OF HUMANS AND WILDLIFE WITH RESPECT TO ROADS: INSIGHTS
FOR MITIGATION AND MANAGEMENT

by

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B.S. Duke University, 2012

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Biology
in the College of Sciences
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ABSTRACT

Road ecology is the study of how roads and wildlife interact. Traditionally, road ecologists have primarily focused on one effect of roads: roadkill. Though roadkill can have devastating effects on wildlife populations, roads have sub-lethal impacts that are gaining more and more attention from the scientific community. These sub-lethal impacts include noise, light, and chemical pollution as well as altered habitat structure, which can all influence animal behavior. In this dissertation, I applied a behavioral ecology framework to study specific lethal and sub-lethal road effects with the goal of improving mitigation efforts. In Chapters 1 and 2, I evaluated how human behavior may be modified to reduce wildlife-vehicle collisions; traditionally efforts have been made to modify wildlife road crossing behavior. I found that Roadside Animal Detection Systems, which warn drivers when animals are near the road, are successful in reducing crash risk; however, care must be taken to ensure that drivers do not become acclimated to the warning system. In Chapters 3 and 4, I evaluated how traffic noise affects subadult growth and adult abundance and communication of anuran amphibians (frogs and toads), a taxon widely recognized as one of the most negatively impacted by roads. I found that through traffic noise alters tadpole behavior, it does not appear to have a negative effect on their growth. Traffic noise does, however, negatively affect adult anuran abundance. My results indicate that this reduction in abundance is caused by the interference of traffic noise with anuran communication.

“The last word in ignorance is the man who says of an animal or plant, "What good is it?" If the land mechanism as a whole is good, then every part is good, whether we understand it or not. If the biota, in the course of aeons, has built something we like but do not understand, then who but a fool would discard seemingly useless parts? To keep every cog and wheel is the first precaution of intelligent tinkering.”

-Aldo Leopold

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PREFACE: THE NEED FOR BEHAVIORAL RESEARCH IN ROAD ECOLOGY

Road ecology is the study of how transportation infrastructure affects our environment. To the layperson, this description probably makes them think immediately of roadkill, due to its graphic visibility and the fact that collisions hurt humans as well as animals. However, we have come to realize that there are myriad interactions between roads and wildlife that are more difficult to study, but are equally important to conservation. It is becoming clear that even if roads do not kill an animal, they can influence its behavior in complex ways, and there is a need to devote study to the behavior of animals near roads (Rytwinski and Fahrig 2012). It may also be possible to alter human behavior to make roads safer for wildlife. Yet, our understanding of behavioral responses to roads has advanced slowly because such studies require large amounts of data to draw meaningful conclusions. Historically, road ecology as a field has focused on roadkill and landscape fragmentation, as these questions are “low-hanging fruit” in terms of study design. In fact, in the recently published *Handbook of Road Ecology* (van der Ree et al. 2015a), 47 out of 62 contributed chapters discuss roadkill, roadkill prevention, and fragmentation, whereas only 4 chapters address how roads alter animal behavior. Therefore, behavior is the focus of my dissertation research.

The first two chapters of my dissertation apply a human behavioral approach to roadkill prevention. Traditionally, we have installed fences to prevent animals from becoming casualties, but this can divide wildlife populations into fragments (reviewed in Laurance 2015; van der Ree et al. 2015b). So, instead of keeping animals off the road, why not warn drivers when animals are near? Roadside Animal Detection Systems (RADS) attempt to do just that, but so far studies have focused on whether or not they successfully detect animals (Huijser et al. 2003, 2009a,b),

not on whether they cause humans to drive more carefully. In the 1990s and early 2000s, when RADS were relatively new, there were some studies that measured their effect on driver speed (Muurinen 1998; Kinley et al. 2003), but the technology of these systems has progressed rapidly since then. Past studies have also not determined whether RADS actually reduce wildlife-vehicle collision rates. In **Chapter One**, I used a driving simulator to test the potential of RADS to change human behavior and reduce crash rates in a controlled, safe environment. I found that the use of a RADS decreased driver speed, increased their reaction time to an animal entering the road, and reduced crash probability by over six-fold. In **Chapter Two**, I studied driver responses to a real-world RADS in southern Florida, and the results of that study corroborated my driving simulator findings.

The last two chapters dive into the much less-studied realm of wildlife behavior near roads. Specifically, I looked at how animal behavior is affected by a non-lethal effect of roads: traffic noise. Traffic creates pervasive noise pollution in our environment, and previous work shows correlations between traffic noise intensity and reduced animal abundance and reproductive success (see Fahrig and Rytwinski 2009; Francis and Barber 2013; Potvin 2016). However, because many past studies were correlative and performed next to actual roads, it is unclear if traffic noise itself was the culprit, or if another unaccounted-for effect of roads (e.g., mortality, chemical pollution) was to blame. In **Chapter Three**, I conducted a series of laboratory experiments to identify the effects of traffic noise exposure on tadpoles (frogs often deposit their eggs in roadside ditches). I found that traffic noise caused tadpoles to eat less and alter their activity patterns, although this did not result in changes in growth rate or time to metamorphosis. Finally, In **Chapter Four**, I conducted a large-scale field experiment wherein I

played pre-recorded traffic noise in a roadless area to study how the noise affected amphibian abundance. I found that the noise alone caused a reduction in amphibian abundance, providing empirical evidence to support the correlative studies.

CHAPTER ONE ~ ROADSIDE ANIMAL DETECTION SYSTEMS: DRIVING SIMULATOR PROOF OF CONCEPT

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Introduction

Collisions between large mammals and vehicles are costly for wildlife and humans alike. In Sweden, road-kills are responsible for an average loss of between 1 and 12 percent of the population size of medium- and large-sized mammal species (Seiler et al. 2004). Road-kill disproportionately affects small reptiles, amphibians, and mammals, but collisions with large mammals are most frequently reported (Huijser and McGowen 2003). This is because collisions with large mammals come at a greater cost to humans; in the United States, they cause 211 deaths, 29,000 injuries, and more than \$1 billion USD in property damage every year (Huijser et al. 2007a). Likewise, large animal-vehicle collisions (hereafter LAVCs) take a heavy toll on wildlife populations, through increased mortality and reduced landscape connectivity. In fact, roads are thought to be one of the greatest threats to wildlife worldwide (Noss and Cooperider 1994; Trombulak and Frissel 2000; Forman et al. 2003; Smith 2003; Laurance et al. 2014).

The most common measures used to reduce the incidence of LAVCs are static warning signage, wildlife fencing and ecopassages (e.g., overpasses, underpasses, and tunnels/culverts). The first, static warning signage, has been shown to be largely ineffective; drivers easily habituate to it and fail to make adequate reductions in speed (Huijser et al. 2007a). The other two

measures are largely successful, but they come with limitations. On its own, wildlife fencing merely creates another barrier to animal movement throughout the landscape (Smith 2003), so it is rarely a standalone mitigation method for large, wide-ranging animals, though it can be a useful interim measure (Jaeger and Fahrig 2004). Using fences in combination with ecopassages is a common strategy, but the effectiveness of ecopassages is almost never evaluated in a BACI design (van der Ree et al. 2007; Lesbarrères and Fahrig 2012) and they can be very expensive (several million USD; Huijser et al. 2007a). In addition, their installation is disruptive to traffic, meaning that they are rarely installed unless a road is being widened or a new road built (Smith 2003; Huijser et al. 2007a). Because of the cost and difficulty of installation, ecopassages cannot be installed at every location that could benefit from one.

Roadside Animal Detection Systems (RADS) are a relatively new alternative to the previously listed measures. RADS use sensors (e.g. motion-sensing, thermal, infrared) to detect when large animals are near the road; when an animal is detected, the sensors send a signal to lights on warning signs, which begin to flash. Unlike fencing and ecopassages, RADS are not intended to keep wildlife off of the road, but rather to alert drivers when there is an increased risk of a collision. Because the lights only activate when a large animal is detected near the road, it should decrease the likelihood of drivers habituating to the warning signs. In addition, RADS are easier to install than ecopassages and are less expensive, ranging from \$11,500–\$60,000 USD plus installation and maintenance costs (Huijser and McGowen 2003), so they could potentially be deployed on a larger scale. RADS were first installed and tested in 1993 in Switzerland (Kistler 1998, Tschuden 1998; cited in Huijser and McGowen 2003). Since then, many more

systems have been implemented in North America and in Europe: in 2006, there were 34 separate locations with RADS installed (14 inactive), with 27 more planned (Huijser et al. 2006).

Despite the promising nature of the technology, the success of a particular RADS depends largely on its ability to influence human behavior. However, studies to evaluate driver response to RADS in the field have encountered significant difficulties (reviewed in Huijser and McGowen 2003). For example, false triggers—instances where the system activates when no large animal is present, often caused by vegetation or deep snow—have been a common problem. Over time, this could cause driver habituation and skew the results of a field evaluation. Broken sensors and loss of power have also been an issue in certain locations, especially where weather conditions are severe, e.g., excess rain or snow. As a result of these technical challenges, it is hard to draw conclusions from previously collected data; however, one study of a RADS at a deer crossing that did not experience malfunctions until after the study period found that the system reduced driver speed by 6.5 km/h at night and 3 km/h during the day (Gordon et al. 2004).

We circumvented these difficulties by using a driving simulator to evaluate how a RADS affects driver behavior. Our study is the first to use a driving simulator to assess a RADS; however, simulator studies have been used to assess driver reaction to variations in road signs, e.g. Hammond and Wade (2004). The use of a driving simulator is ideal for evaluating RADS in many ways: first, it provides a controlled setting in which to observe driver behavior, with no confounding effects of weather, time of day, or equipment malfunction. Second, it allows us to safely assess the risk of an animal-vehicle collision; in a simulated setting, this can be done with

no threat to humans or wildlife, as well as generating a much larger sample of potential collisions than could be observed in the field.

Another key feature of the simulator is that the controlled setting allows us to compare alternative designs for the RADS; in this case, designs for warning signage. Bond and Jones (2013) show that drivers rate warning signs with silhouettes of animals (picture-based) higher than text-only signs (word-based) in their ability to reduce speed and increase alertness. Both picture- and word-based signs have been used in RADS systems (reviewed in Huijser and McGowen 2003); however, the two alternatives have never been compared in a controlled setting. It is possible that one design could produce the desired reduction in speed and/or increase in alertness more often or with a greater magnitude. For identifying best practices in animal-vehicle collision mitigation, we sought to determine which design yields greater potential benefits.

In this study, we asked (1) does the presence of a RADS have an effect on driver speed, latency to brake when an animal enters the road unexpectedly (treated here as a proxy for alertness), and/or the probability of animal-vehicle collisions? and (2) do word-based and picture-based RADS signs perform differently? Because the use of a driving simulator removes the technical challenges often experienced in the field, we can evaluate whether RADS have the potential to significantly affect driver behavior and reduce crashes with wildlife. If so, it will be worthwhile to invest in research and development to overcome these challenges.

Methods

Focal RADS

In January 2012, an experimental RADS (manufactured by Simrex Corporation) was installed in Big Cypress National Preserve (BCNP) on Highway 41 near Turner River in Collier County, Florida, USA. Daisy-chained infrared sensors, spaced approximately 153 m apart and placed just beyond the road shoulder on both sides of Highway 41, create a detection beam parallel to the road surface spanning 2.1 km. The system is designed specifically to detect large wildlife, so the infrared beam is 46 cm above the ground and will not detect shorter animals. The standard yellow diamond warning signage is word-based, not picture-based, and reads, “WARNING WILDLIFE ON ROADWAY REDUCE SPEED.” These warning signs are placed every 610 m. In addition, an informational sign informs drivers of the RADS system 0.8 km before entering the animal detection area (Fig. 1).

The 2.1-kilometer-long road segment was identified by federal and state wildlife agencies as a critical hotspot for road-kills of the federally endangered Florida panther (*Puma concolor coryi*), whose population is currently estimated at 100–180 individuals (FFWCC 2014). Five panther road-kills occurred at this location between 2004 and 2009, of which four were breeding-age females. Although collisions with Florida panthers were the catalyst for this experimental installation, a successful RADS would also benefit other large wildlife, such as Florida black bear (*Ursus americanus floridanus*), and white-tailed deer (*Odocoileus virginianus*). Since its installation, the RADS has experienced a large number of false triggers during daylight hours, which could potentially reduce its effectiveness as a warning system.



Figure 1. Images of the RADS signs used in the driving simulator. Left: simulated images of the Highway 41 RADS signs, including a word-based informational sign posted 0.8 km before entering the animal detection area and four word-based warning signs with 8 LED lights that flash when animals break the infrared beam parallel to the road. Right: simulated images of the modified RADS signs, which feature a picture-based informational sign and four picture-based flashing warning signs.

Driving Simulator

The driving simulator was provided and programmed by the Research in Advanced Performance Technology and Educational Readiness Lab at the University of Central Florida's Institute for Simulation and Training. They programmed the simulator to create a digital version of the RADS installation site on Highway 41. Using this digital version of the roadway and surroundings as a base, three alternatives were created: one had no RADS warning signs (control), another included word-based RADS signs (which reflects the Highway 41 RADS), and the third included redesigned, picture-based RADS warning signs (Fig. 1).

Participants

The use of human subjects in this research was approved by the UCF Institutional Review Board, IRB number SBE-13-09322. Ninety people participated in the simulator experiment between 15 July and 18 August 2013. The participants were either students or employees of the University of Central Florida or members of the surrounding community. Undergraduate students were offered course credit as incentive to participate, while all other participants received a small monetary compensation. All were at least 18 years old, and all had been licensed to drive for at least one year.

Participants were recruited systematically so that there were 30 participants in each age group (age group 1= 18–24 years, age group 2= 25–44 years, age group 3= 45+ years). We recruited into these age groups to obtain a more balanced participant pool, and because age is known to affect driver behavior: young or inexperienced drivers make up a disproportionate amount of accidents on the road because they have an underdeveloped ability to recognize hazards, yet tend to overestimate their own driving skills (reviewed in Deery 2000).

Participants in each age group were assigned to a treatment (control, word-based RADS, or picture-based RADS) using a systematic random design: i.e., the first member of each age group tested was assigned to the control treatment; the second, word-based; third, picture-based; fourth, control, *etc.* Thus, exactly one-third of each age group was assigned to each treatment.

Participants were told that they were participating in a study aiming to evaluate driver response to various hazards on the road. The full nature of the study, i.e., the intent to evaluate the RADS, was not disclosed to the participants until debriefing after the testing session. This

was done so that participants did not anticipate seeing animals on the road, which could affect their responses.

Simulator Experiment

Each participant completed six runs in the driving simulator, three “twilight” scenarios and three “night” scenarios. We chose these times of day because these are the times that collisions with large animals are more likely to occur (Danks and Porter 2010; Neumann et al. 2012), and also because in Big Cypress National Preserve, there is a nighttime speed limit of 45 mph (72 km/h), while during the day (and twilight) the speed limit is 60 mph (97 km/h). This was reflected in the speed limit signs programmed in the simulation. Before participants began the six runs, there was an acclimation period during which the participant was able to familiarize themselves with and become accustomed to the driving simulator. A five-minute break was offered between each run, during which the participant could walk around, get water, or eat.

In each set of three runs, there was one target run featuring an animal hazard in which a deer entered the road directly in front of the car at a certain point in the run. The other two were non-target runs, meaning that they did not include an animal hazard, but instead a different type of hazard (either a car crashed on the roadside or a driver entering the road in front of the participant suddenly) to prevent the participant from realizing the true purpose of the study. Each run featured only one hazardous situation. Participants completed the six runs in a random order.

Each run featured a 0.8 km baseline period at the beginning, a 0.8-km zone after the RADS informational sign, and a 2.1 km RADS zone. All hazards, animal or not, occurred within the RADS zone. Driver speed and brake pressure were automatically recorded every 0.014

seconds. We also recorded whether or not a participant crashed into the animal hazard during target runs.

Analyses

We only analyzed the target runs, in which participants were presented with an animal hazard. Each participant completed one target run during the twilight scenario and one during the night scenario. Because each participant was tested both at night and twilight, which had different speed limits, the effects of these factors were tested using paired methods. All statistical tests were performed in JMP[®] (version 10, SAS Institute Inc., Cary, NC, USA) and figures generated using JMP[®] or R Statistical Computing software (version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria, 2013).

Pairwise Comparisons between Twilight and Night

To assess whether there was a significant difference in crashes between runs that occurred at twilight vs. those at night, we performed McNemar's chi-squared test for paired samples. To test whether driver speed was greater at twilight than at night, we used Wilcoxon's signed rank test (1-tailed). The speed considered was the average speed between the point where the participant entered the RADS sensor array and the point just before the deer appeared in the road. We used a nonparametric test because the differences between the pairs were not normally distributed (Shapiro-Wilk test, $W = 0.953$, $p = 0.00260$). To test if there was a difference in latency to brake (measured as the distance between the location where the participant started braking and the location of the deer on the road) between night and twilight, we again used Wilcoxon's signed rank test (1-tailed) (Shapiro-Wilk test, $W = 0.945$, $p = 0.00131$). We excluded data from six

participants who did not brake in response to the animal hazard in one or both of their target runs: four participants from control treatment, one participant each from word- and picture-based treatments; three participants from age group 1 and three participants from age group 2.

Effect of RADS

To assess whether treatment or age had an effect on the probability of crashing, we fit a multiple logistic regression model using “crash” (yes/no) as the dependent binomial outcome and treatment (control, word-based RADS, and picture-based RADS) and age group as fixed factors. We intended to do this analysis for both twilight and night, but so few crashes occurred at night ($n = 2$) that the logistic model becomes unstable and uninformative. Therefore, this analysis was done only for twilight.

We used One-Way Analysis of Variance (ANOVA) to assess whether treatment or age had an effect on speed or latency to brake (measured as the distance between the location where the participant started braking and the location of the deer on the road). Separate ANOVAs were done for twilight and night datasets.

Results

Descriptive Statistics

We tested 43 females and 47 males. Mean age (yrs.) and standard error in age group 1 was 20.4 ± 1.98 (range 18–24); age group 2, 30.3 ± 4.45 (range 25–41); age group 3, 52.2 ± 5.62 (range 45–65). Of the 90 participants, 30 crashed (Table 1).

Table 1. Number of crashes per factor

Factor	Number of crashes			
	Control	Word-based	Picture-based	Total
Treatment	18	8	4	30
Age group	Age group 1	Age group 2	Age group 3	Total
	18	6	6	30
Time of day	Twilight	Night		Total
	28	2		30
Gender	Males	Females		Total
	13	17		30

Pairwise Comparison of Crashes Occurring in Twilight vs. Night Runs

There were 30 total crashes observed in our experiment. During twilight runs, participants crashed 31% of the time ($n = 28$ crashes), while in night runs, participants crashed 2% of the time ($n = 2$ crashes). This difference is significant (McNemar's $\chi^2 = 22.3214$, $DF = 1$, $p < 0.00001$; Table 1). Thus, we analyzed the effect of experimental factors separately for twilight and night. However, since only 2 crashes occurred at night, we were only able to further analyze crashes that occurred in twilight simulations.

Effect of RADS on Crash Rate

The overall model for crash probability was highly significant ($\chi^2 = 24.9$, $DF = 5$, $p < 0.0001$).

The independent variables treatment and age group had a significant influence on crash

likelihood (respectively, $\chi^2 = 17.5$, $DF = 2$, $p = 0.0002$; $\chi^2 = 7.08$, $DF = 2$, $p = 0.0290$; effect likelihood ratio tests).

Between levels of experimental factors, participants in the youngest age group (age group 1) were significantly more likely to crash than those in the oldest age group (age group 3; Table 2). There was no significant difference in crash rate between age groups 1 and 2 or 2 and 3. Participants in the control treatment were significantly more likely to crash than those in the word-based treatment or the picture-based treatment (Table 2). There was no significant difference in crash rate between the word-based treatment and the picture-based treatment.

Pairwise Comparison of Speed in Twilight vs. Night Runs

Mean speed of participants during twilight runs was $93.7 \text{ km/h} \pm 1.21 \text{ SE}$ and ranged from 70–160 km/h, compared to a nighttime average of $78.2 \text{ km/h} \pm 1.24 \text{ SE}$ with a range of 23.0–160 km/h. Extreme outliers (>3 standard deviations from the mean) were seen in both twilight and night and were from the same participant; these were removed from further analyses. The average difference in speed between twilight and night runs for a participant was $15.4 \text{ km/h} \pm 1.09 \text{ SE}$ faster at twilight than night, with a range of -20.4–34.1 km/h. This difference is significant (Wilcoxon signed rank test with continuity correction, $W = 120$, $p < 1.0e^{-13}$).

Table 2. Crash probabilities between treatments and age groups. Odds ratios showing likelihood of a member of the first group crashing compared to the likelihood of a member of the second group crashing. A dagger (†) indicates significant odds ratios at $\alpha = 0.10$ while an asterisk (*) indicates effects significant at $\alpha = 0.05$.

Comparison	Odds Ratio	$p > \chi^2$	Lower 95%	Upper 95%
control vs. word-based	6.29	0.0026*	1.85	25.4
control vs. picture-based	14.0	<0.0001*	3.59	68.9
word-based vs. picture-based	2.22	0.267	0.546	10.1
age group 1 vs. age group 2	3.54	0.0514†	0.993	14.1
age group 1 vs. age group 3	5.15	0.0114*	1.43	21.5
age group 2 vs. age group 3	1.46	0.591	0.36	6.06

Effect of RADS on Speed

The overall twilight model for speed was significant ($F = 2.55$, $DF = 8$ and 80 , $p = 0.0159$). Both treatment and age group had a significant influence on speed, and there was no interaction between treatment and age group (Table 3). Between the different treatments, participants in the control treatment (mean speed 97.0 km/h) drove faster than those in the picture-based treatment (89.5 km/h), but not the word-based treatment (92.2 km/h) (Tukey-Kramer HSD, $p = 0.0098$ and 0.137 , respectively; Fig. 2). Participants in age group 3 drove significantly slower than those in age group 2 at $\alpha = 0.1$ (Tukey-Kramer HSD, $p = 0.0560$) but there were no other significant differences between the age groups (Fig. 2).

Nighttime speed data were transformed using the Box-Cox transformation to meet the assumption of normal residuals. The overall nighttime model was not significant ($F = 1.75$, $DF = 8$ and 80 , $p = 0.0991$), and no factor significantly affected speed.

Table 3. ANOVA table for average speed of participants within the RADS zone at twilight. An asterisk (*) indicates effects significant at $\alpha = 0.05$.

factor	DF	SS	F ratio	p > F
age group	2	211.4	3.19	0.0464*
treatment	2	327.0	4.94	0.00951*
age group*treatment	4	136.8	1.03	0.396

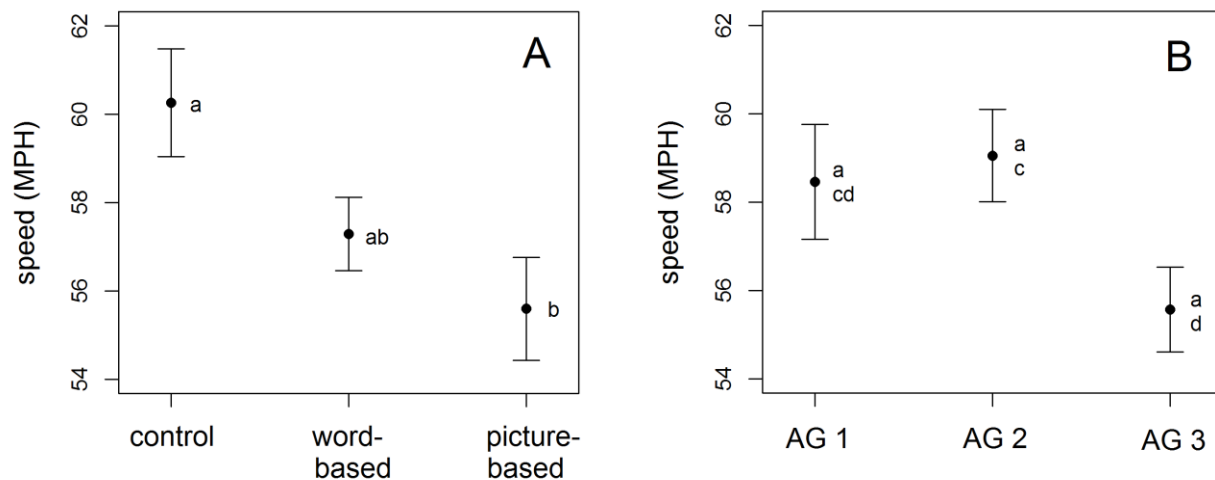


Figure 2. Participant speed at twilight by treatment and age group. Means and standard errors of speeds (mph) of participants at twilight in: **A)** the three treatments; **B)** the three age groups. Significantly different treatments have different letters next to them (a's and b's indicate significant differences at $\alpha = 0.05$; c's and d's indicate significant differences at $\alpha = 0.10$). $n=30$ for word- and picture-based treatments; $n=29$ in control because we removed an outlier (>3 standard deviations from the grand mean).

Pairwise Comparison of Braking Distance in Twilight vs. Night Runs

Mean braking distance during twilight runs was 45.7 m before the deer's location on the road (± 1.99 SE; range 14.4–79.8), compared to 51.7 m during night runs (± 1.81 SE; range 22.2–79.8). The average paired difference between night braking start distance and twilight braking start distance was that participants braked 5.93 m earlier at night than twilight (± 2.80 SE), though there was a very wide range (-57.4 m–65.1 m). This difference is significant (Wilcoxon signed rank test with continuity correction, $W = 2350$, $p = 0.00274$).

Effect of RADS on Braking Distance

Both the twilight and nighttime overall models were significant ($F = 3.77$, $DF = 8$ and 75 , $p = 0.0009$ and $F = 3.81$, $DF = 8$ and 78 , $p = 0.0008$, respectively; nighttime braking distance data were transformed using the Box-Cox transformation to meet the assumption of normal residuals). At twilight, braking distance was influenced by treatment, though the effect of age group was also significant at $\alpha = 0.1$ (Table 4). There was no significant interaction between treatment and age. Participants in both the picture-based and word-based treatments began to brake earlier than participants in the control treatment (on average, 8.09 m and 7.59 m earlier, respectively; Tukey-Kramer HSD, $p = 0.0007$ and $p = 0.0017$, respectively; Fig. 3). There was no significant difference in braking distance between participants in the picture-based and word-based groups (Tukey-Kramer HSD, $p = 0.968$; Fig. 3).

At night, braking distance was influenced by treatment, but not age, although the interaction between treatment and age was significant at $\alpha = 0.10$ (Table 4). Participants in the picture-based treatment started to brake on average 5.53 m before participants in the control

treatment (significant difference; Tukey-Kramer HSD, $p = 0.0006$; Fig. 3) and 3.04 m before participants in the word-based treatment (significant at $\alpha = 0.1$; Tukey-Kramer HSD, $p = 0.0725$; Fig. 3). Participants in the word-based treatment started to brake 2.49 m before participants in the control treatment, but this difference was not significant (Tukey-Kramer HSD, $p = 0.189$).

Because the interaction between age and treatment at night was close to being significant at $\alpha = 0.05$, and because we tested a relatively small sample of participants, we investigated the interaction for any trends indicating that the different RADS designs affected participants differently within an age group. Within age group 1, the youngest age group, those in the picture-based group braked on average 7.76 m before those in the control group (Tukey-Kramer HSD, $p = 0.0749$). Within age group 3, the oldest age group, those in the picture based group braked on average 7.81 m before those in the word-based group (Tukey-Kramer HSD, $p = 0.0714$).

Table 4. ANOVA tables for braking distance. The distance considered is the distance at which participants began to brake when the deer ran out in front of them during the simulation. Separate ANOVAS were calculated for twilight and night data. A dagger (†) indicates effects significant at $\alpha = 0.10$ while an asterisk (*) indicates effects significant at $\alpha = 0.05$.

time of day	factor	DF	SS	F ratio	p > F
twilight	age group	2	331.9	2.91	0.0605†
	treatment	2	1121.8	9.85	0.0002*
	age group*treatment	4	252.9	1.11	0.358
night	age group	2	110.4	2.16	0.122
	treatment	2	426.5	8.36	0.0005*
	age group*treatment	4	236.9	2.32	0.0643†

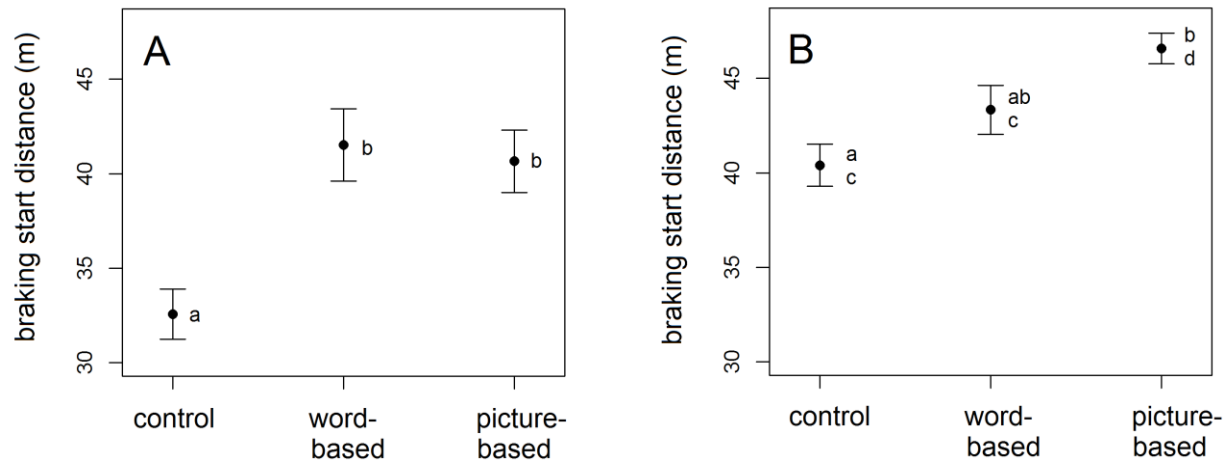


Figure 3. Braking distances by treatment. Means and standard errors of braking distances (m). Significantly different treatments have different letters next to them (a's and b's indicate significant differences at $\alpha = 0.05$; c's and d's indicate significant differences at $\alpha = 0.10$). **A)** The three treatments at twilight. Control $n=26$, word- and picture-based $n= 29$ because we removed outliers (>3 standard deviations from the grand mean); **B)** The three treatments at night. Control $n=27$, word- and picture-based $n=30$. Nighttime data were transformed using the Box-Cox transformation for analysis, but values in this figure are untransformed.

Discussion

In our simulator study, we found that the RADS produced significant positive outcomes: participants in RADS treatments reduced their speed, braked earlier in response to an animal in the road, and were involved in animal-vehicle collisions less often. In addition, the picture-based RADS signs were more effective than the word-based RADS signs at reducing driver speed at twilight, and the same was true of differences in reaction time (braking distance) at night. These results lend empirical support to Bond and Jones' (2013) survey results reporting that drivers rate picture-based signs higher for increasing alertness. Although fewer crashes occurred in the picture-based treatment than in the word-based treatment, this difference was not significant.

However, this could be the result of our relatively low sample of crashes; in a larger study with more participants, we suspect that these trends would become significant.

The reduction in mean speed at twilight from 97 km/h in the control group to 89.5 km/h in the picture-based group (7.5 km/h difference) may seem small, but previous work shows that a change of this size could greatly reduce crash probability. In a meta-analysis of crash rates before and after speed limit changes on rural roads in Europe and the United States, Finch et al. (1994) fit a model predicting that with a 1 mph (1.6 km/h) decrease in speed, there is a corresponding 5% decrease in crash rate. Taylor *et al.* (2002) also found that as speed increases, crash rate increases, and particularly concerning severe crashes: in their model, a 10% increase in the mean speed on a roadway predicts a 30% increase in fatal and serious crashes. Therefore, our observed differences in speed would result in tangible safety benefits for humans and wildlife.

We also found significant differences in driver behavior at twilight and at night. Average speed was lower at night, which is almost certainly due to the different speed limits at twilight and night (60 mph vs. 45 mph). This reduction in speed was accompanied by earlier braking distances and a greatly reduced number of crashes at night. The lower nighttime speed limit in Big Cypress National Preserve was put in place to reduce animal-vehicle collisions, especially collisions with Florida panthers, and our simulator data support the potential effectiveness of this measure.

Our study also re-confirmed that age plays a significant role in driver safety, with participants in age group 1 being much more likely to crash than participants in age group 3. The difference between age groups 1 and 2 was very close to being significant as well, and we believe that with a larger sample size of crashes, the difference would probably be significant.

This trend highlights the need to educate younger drivers about the danger of animal-vehicle collisions.

The trends within the interaction of RADS treatment and age group on braking start distance are also worth considering. Drivers in both the youngest and oldest age groups started braking earlier in response to picture-based RADS signs. Because the animal entered the road at the same point for all participants, the distance at which participants started braking is a proxy for brake reaction time. Brake reaction time (BRT) is the amount of time that passes between the moment the stimulus appears and when the driver's foot actually reaches the pedal (Shinar 2007). Contained within BRT is perception reaction time (PRT), the amount of time that passes between when a stimulus reaches a driver and the driver initiates a response. The BRT is therefore affected by the PRT; if people are primed to expect a stimulus, their PRT (and therefore, BRT) should be reduced. The slower the PRT, the longer the stopping distance, increasing the chance of collision (Shinar 2007). Thus, it appears that for two of three age groups tested, the picture-based RADS signs did a better job of priming participants to expect an animal, and therefore may be more effective in preventing collisions.

Simulator validity

Behaviors observed in a driving simulator may not accurately reflect real-life behaviors. The validity of using driving simulators to predict real-world crash rate was reviewed by Rudin-Brown et al. (2009). Although simulators cannot perfectly recreate real-world driving conditions, Rudin-Brown et al. conclude that the use of a simulator is acceptable if it recreates conditions with enough validity to measure the behavior being investigated. Behavioral validity can be

absolute—do the simulator observations exactly match those in the real world?—or relative—do simulator observations have the same direction and similar magnitude to those in the real world? (Blaauw 1982). If the goal of a simulator experiment is to measure the effect of one treatment vs. another, as is often the case in human factors research, ensuring that simulators have adequate relative validity is more important than ensuring absolute validity (Törnros 1998).

In many studies evaluating driver speed, simulations achieved relative validity but not absolute validity (Törnros 1998; Klee et al. 1999; Godley et al. 2002; Bella 2008), though absolute validity has been documented (Yan et al. 2008). It is more common for simulations to achieve absolute validity in addition to relative validity with regard to certain behaviors (reviewed in Kaptein et al. 1996), such as lateral position of the vehicle in the lane (Törnros 1998) and route choice.

Future directions

To determine whether the results of our simulator study reflect responses to the RADS in the real world, Chapter Two will validate the simulator data using field data. We will test the relative and absolute validity of our speed results by comparing speeds recorded during the simulation experiment to field recordings of speed through the RADS zone in Big Cypress National Preserve (as in Godley et al. 2002). We predict that speeds of participants in the simulator may be higher than those observed on the road, since previous studies have shown that when the road is relatively straight, participants drive faster in simulators (Boer et al. 2000). By comparing the simulator data to real-world data, we will gain a solid foundation from which to make management recommendations about the design and effectiveness of RADS.

Conclusions

Roadside animal detection systems are a promising technology to reduce the frequency of animal-vehicle collisions, but empirical testing has been difficult because of system malfunctions in the field. We overcame these difficulties by studying the effects of a particular RADS using a driving simulator, and our data suggest that RADS can indeed produce the intended results on crash probability, driver speed, and latency to brake. With improved RADS technology, these systems could be deployed on a larger scale as a cost-effective way to improve safety for humans and wildlife.

CHAPTER TWO ~ ROADSIDE ANIMAL DETECTION SYSTEMS: EVALUATION OF HUMAN RESPONSES IN THE FIELD

Introduction

Roads are a ubiquitous feature in today's landscape; as of the year 2003, only an estimated 3% of land in the United States was more than 5 kilometers from a road (Riitters & Wickham 2003), a figure that has almost certainly decreased since then. The effects of these pervasive anthropogenic features on wildlife have been studied for decades (reviewed in Forman et al. 2003; Coffin 2007; van der Ree et al. 2015a). We now know that the impacts of roads on wildlife are myriad: from altering patterns of animal abundance (reviewed in Fahrig & Rytwinski 2009); producing a novel source of environmental noise, causing animals to change spectral and temporal features of their vocalizations (reviewed in Slabbekoorn & Ripmeester 2008; Lowry et al. 2013); and even releasing certain species from predation pressure (Rytwinski & Fahrig 2007; Munro et al. 2011). Despite the innumerable and complex ecological effects of roads, their best-known effect on wildlife is the simplest: roadkill. In addition to being graphic and easily observed, roadkill is in the forefront because of its negative effects on humans: In the United States alone, collisions with large animals cost over \$1 billion per year in property damage, in addition to hundreds of human deaths and tens of thousands of injuries (Huijser et al. 2007a). Probably due to these factors, most efforts to mitigate road effects on wildlife have focused on preventing roadkill (van der Ree et al. 2015a).

A traditional solution to reducing roadkill, especially for mammals, is to install a combination of fences and crossing structures, keeping animals off of the road and allowing them to cross only at certain points (van der Ree et al. 2015b; Smith et al. 2015). Although this

type of mitigation works well for some species, it has disadvantages. Unless fences are well-maintained, animals will breach them soon after installation (van der Ree et al. 2015b). In addition, to be effective in excluding animals that can climb, fences must incorporate smooth surfaces that make it difficult for animals to get purchase or have an overhanging lip at the top of the fence (Klar et al. 2009), features that can detract from the aesthetic of the roadside area. Moreover, crossing structures are expensive, with single structures often costing millions of dollars (Huijser et al. 2007b).

In response to the costs and limitations of fence-crossing systems, in the 1990s an alternative mitigation strategy was introduced: Roadside Animal Detection Systems (RADS). First tested in Switzerland (Huijser & McGowen 2003), RADS differ from fencing in that they do not attempt to keep wildlife off of the road; rather, they attempt to warn drivers when wildlife are near the road. Animals are detected by sensors in the roadside area (often infrared, but sometimes thermal or motion-activated); when the sensors are tripped, a signal is sent to road signs, which begin to flash in warning. Since the 1990s, at least 34 systems have been installed across North America and Europe (Huijser et al. 2006).

Mitigation efforts in road ecology are consistently plagued by a lack of empirical evaluation, a problem found throughout conservation biology due to low statistical power and limited budgets (Legg & Nagy 2006; Field et al. 2007; Lindenmayer & Likens 2010). As such, there have been calls for increased rigor when testing mitigation strategies (Roedenbeck et al. 2007; van der Ree et al. 2007). This issue extends to RADS as well: despite the proliferation of animal detection systems around the world, only one peer-reviewed study evaluating its effect on driver behavior has been published (Gordon et al. 2004). Gordon et al. studied a RADS installed

to reduce wildlife-vehicle collisions during mule deer migration in Wyoming and found that, on average, drivers reduced speed by 6-7 km/h in response to the warning lights at night; when drivers were tested at night with the addition of a roadside deer decoy, they reduced speed by 20 km/h. These results are promising, but some questions remain. For example, Gordon et al. reported that the road along which the system was placed was traveled primarily by non-local motorists; it remains to be seen if local drivers may adapt to the RADS and begin to ignore the system.

In addition, and perhaps more importantly, a short-term field study is not able to assess whether a RADS actually reduces wildlife-vehicle collisions, an assessment that would require years of pre- and post-mitigation monitoring. To address this issue, we performed a study with a driving simulator that not only assessed the effect of RADS on driver speed, but required subjects to react to a deer coming out in the road in front of them (Grace et al. 2015). We found that a RADS reduced the likelihood of the subject colliding with the deer by either 6.29 or 14 times, depending on the design of the warning signage. In addition, drivers in the simulation reduced their speed by an average of 4.8 km/h in response to a RADS with words-only warning signs and by 7.5 km/h to signs with a picture of an animal, a reduction comparable to what was observed in the field study by Gordon et al. (2004).

Here, we studied driver response to a RADS installed in southern Florida as a way to mitigate collisions with the endangered Florida panther (*Puma concolor coryi*). The driving simulator study (Grace et al. 2015) was based on this real-world RADS, and if we observe similar reductions in speed in the field, it would support the potential for RADS to reduce crashes as well. By sampling throughout an entire year, we aimed to assess not only whether the

RADS was successful at reducing driver speed, but whether or not its effect varied with a seasonal influx of tourists. In addition, we began the study more than a year after the system was installed, so we hoped to shed light on whether drivers acclimate to and become desensitized to these kind of warning systems.

Methods

Study Area

We studied a Roadside Animal Detection System installed in Big Cypress National Preserve (BCNP), an area which encompasses a large portion of occupied Florida panther habitat (FFWCC 2016). Dedicated conservation efforts have brought this subspecies back from the brink of extinction, yet the single remaining population currently numbers 100-180 adult and subadult individuals (McClintock et al. 2015; FFWCC 2016), a far cry from the three populations of 240 individuals that are required to delist the Florida panther (USFWS 2008). Roadkill has been identified as a leading cause of death for the Florida panther, accounting for 55% of observed mortalities as of the year 2012 (FFWCC 2012). In an attempt to mitigate this, a RADS was installed in 2012 on the most traveled road in BCNP as an experimental alternative to a wildlife crossing structure and fencing, which were opposed by key stakeholder groups because of potential restriction of access.

The RADS covers a 1.3-mile section of U.S. Highway 41 (U.S. 41), which runs east/west through BCNP (Fig. 4) and is a main thoroughfare between Naples and Miami. The preserve is a popular tourist attraction during the cooler months of the year. Annual average daily traffic

volume on U.S. 41 during our period of study was 3100 cars/day in 2014 and 4400 cars/day in 2015, though traffic volume varied throughout the year: the difference in traffic volume between the peak of the tourist season (November-March) and the low point of the off-season (April-October) is about 1200 cars per day (FDOT Florida Traffic Online Database). Posted speed limits on U.S. 41 are 60 mph (96.6 km/h) during the day and 45 mph (72.4 km/h) after dark.

Roadside Animal Detection System Configuration

The RADS on U.S. 41 consists of two arrays of infrared sensors, one on each side of the road and running parallel to the pavement. The sensor arrays are made up of daisy-chained sensors and receivers, with approximately 150 m between each sensor and its receiver, altogether covering 2.1 km of road. The infrared beams are located 18 inches (45.7 cm) above the ground, a height that was chosen to maximize detections of Florida panther and other local species large enough to cause severe damage to vehicles and their inhabitants in a crash (e.g. Florida black bear (*Ursus americanus floridanus*), white-tailed deer (*Odocoileus virginianus*), coyote (*Canis latrans*), and bobcat (*Lynx rufus*)). The selected height was also meant to avoid detections of smaller animals, which cross the road more frequently and could desensitize drivers to the warning. To increase driver awareness of the RADS, informational signs were placed 0.5 miles before the sensor array (i.e. animal detection zone) that read, “ENTERING WILDLIFE SENSOR DETECTION AREA.”

Whenever the infrared beam is broken, a signal is sent to lights on solar-powered warning signs, which begin to flash (Fig. 4). The warning signs are spaced every 2000 feet (610 m) both eastbound and westbound, so that after a driver passes a sign, the next sign is visible. No matter

where in the 1.3-mile (2.09-km) array the sensor is tripped, all of the warning signs will flash. The lights remain flashing for 5 minutes. A central computer records the date and time of all instances in which the beam is broken. We placed trail cameras parallel to the sensor arrays to allow cross-referencing of animals captured in time-stamped images to the beam breaks.

Driver Speed

To assess driver response to RADS, we measured the speed of individual cars on U.S. 41 when the RADS was active (flashing) and inactive (not flashing). Vehicles traveling east and west were both included. We made two separate speed measurements for each vehicle: vehicle speed outside of the RADS sensor array (control measurements) and speed of the same vehicles after traveling halfway through the RADS array (experimental measurements) (Fig. 4). Control vehicle speed and type (number of axles) was recorded using twin-tube traffic counters (Mini TRS, International Road Dynamics; accurately measures speeds >20 mph) placed on the eastbound and westbound lanes of U.S. 41, 0.3 mi before drivers encountered the informational RADS signs. Drivers were not able to see the RADS signage or warning lights from this distance, even when active (Fig. 4). For the experimental measurements, we recorded the speed of drivers in the center of the array, after they had seen the RADS signs (flashing or not flashing) using Bushnell Velocity DSP Speed Guns (accuracy to +/- 1 mph (1.6 km/h)). We recorded speeds in the center of the RADS zone because by that point drivers would have been exposed to two RADS warning signs (flashing or not).

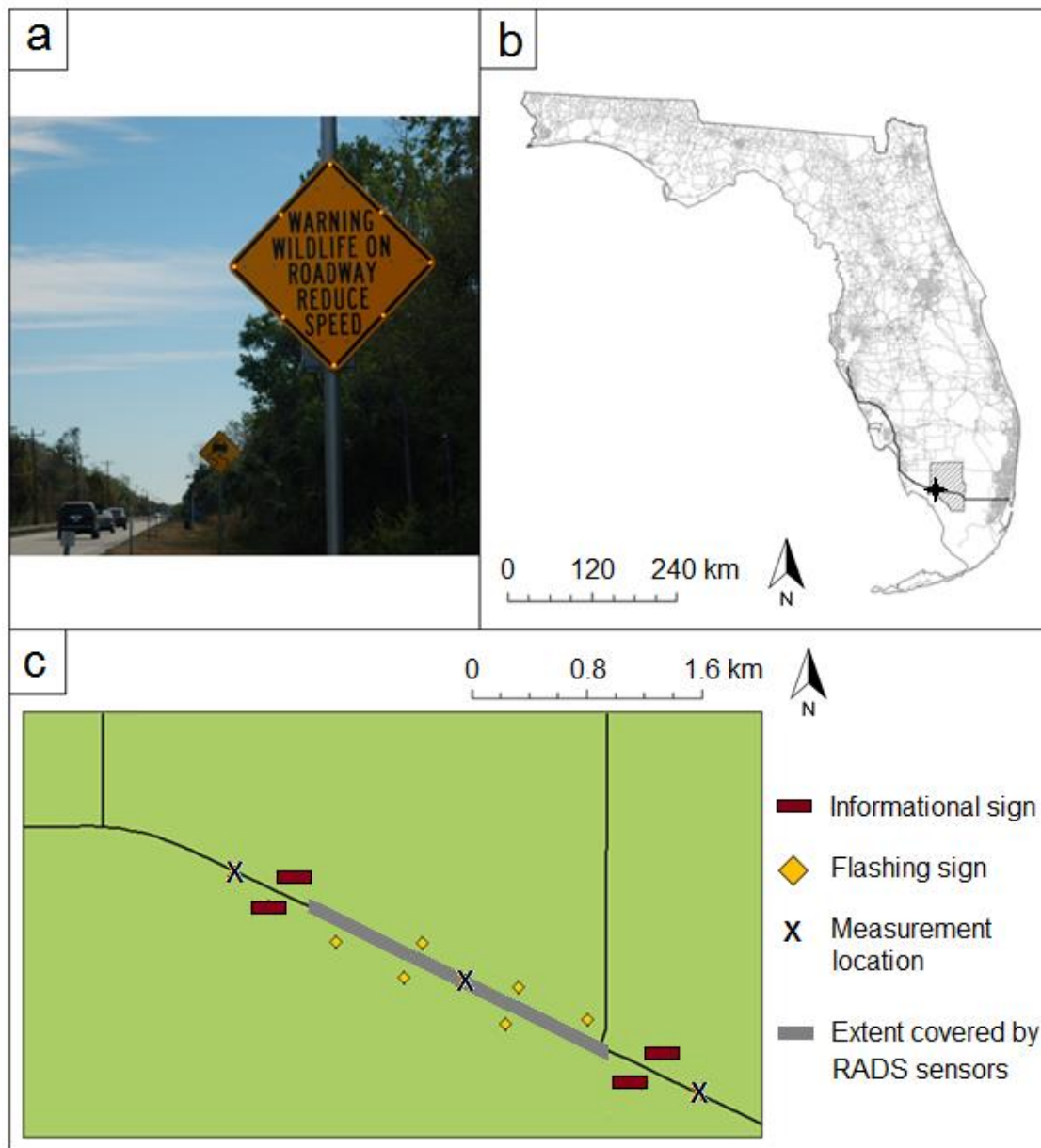


Figure 4. RADS signage, landscape context, sensor array and speed test locations. a) Image of the RADS warning signs, with the 8 LED lights visible around the edge of the sign that flash when the infrared beam is broken. b) Big Cypress National Park (striped polygon) in southern Florida, with U.S. Highway 41 highlighted with a bold black line. The star indicates the location of the RADS, in the west of Big Cypress. c) The area covered by RADS infrared sensors on U.S. Highway 41 (RADS array).

We recorded speeds from 1609 cars over four time blocks: 14-17 May 2014 and 24-27 September 2014 (off-season) and 3-6 December 2014 and 11-14 February 2015 (tourist season). We recorded driver speeds from approximately 1 hour before sunset to approximately 3 hours after sunset on each day within a block. This time period was chosen because the animal species of interest are crepuscular or nocturnal, and we sampled for a longer period after sunset because traffic volume was lower after dark. We manipulated the RADS to rotate through ~10 min. periods of on (flashing) and off (not flashing) while we recorded driver speeds, to achieve roughly equal numbers of vehicles in both treatments.

Driver Demographics

Traffic volume on U.S. 41 in BCNP increases during the tourist season (see “Study Area” above), and we assumed that many of these additional drivers would be naïve to the RADS and that their responses would differ from the responses of drivers during the off-season, who use the road mainly as a commute to and from work and have seen the RADS many times. We were not able to conduct a formal survey to test the assumption of increased driver naiveté in the tourist season, but during January 24-25 and February 13-14, 2015 (during the tourist season) we asked visitors to the BCNP welcome center (about 2.5 miles west of the RADS sensor array) and H.P. Williams Roadside Park (located just within the eastern end of the array) where they were from. 89.8% of respondents (n=166) were visiting from a location more than an hour’s drive from the study area.

Peaks in Animal Activity

As part of our assessment of the RADS, we maintained track beds and used images from our trail cameras to monitor animal activity near the road in 2013 and 2014 (methods described in Smith et al. 2016). To identify peaks in animal activity, during which increased driver awareness would be more important, we counted the number of days per month that a species' tracks were observed in at least one of our roadside track beds and the number of days a species was observed in at least one of our trail cameras to examine how it varied from month to month. We only included camera data from the year 2014 because cameras were not deployed for the full year in 2013. This was done for all species large enough to trip the RADS sensors whose tracks we observed: coyote, bobcat, white-tailed deer, Florida panther, and Florida black bear.

Analysis

Sample Independence

We excluded buses, motorcycles, RVs, semi-trucks, and cars with trailers from the analysis because there were too few of them. Vehicles analyzed were passenger cars, pickup trucks, and vans. To ensure that each vehicle's speed was independent, the raw data were sorted and groups of 2 or more consecutive vehicles going the same speed (± 2 mph (3.2 km/h)) in the same minute were grouped together. All but the first vehicle recorded in that group were excluded from analysis. The person doing the data censoring (M.G.) did so blind to the status of the RADS system (on or off). This resulted in a reduced data set of 1309 vehicles (Table 5).

Table 5. Number of vehicles per treatment combination

RADS				RADS			
on				off			
twilight		night		twilight		night	
tourist	off-season	tourist	off-season	tourist	off-season	tourist	off-season
205	138	195	196	206	73	165	131

Effect of RADS on Driver Speed

The control data and experimental data were analyzed separately because different methods were used to obtain each (traffic counters vs. speed guns); therefore, they are not directly comparable and a paired test cannot be used, even though the same vehicles were measured in both groups. Nevertheless, it was necessary to ensure that any patterns observed in the experimental data were not artefacts of chance associations. We assessed this by performing the same analysis on the control data as on the experimental data. We expected that the RADS would explain differences in driver speed in the experimental data, but we would expect that the RADS being on or off would have no effect in the control data, since drivers could not see the RADS at the control measurement point.

To assess the effect of the RADS on driver speed, we performed Analysis of Variance (ANOVA) on our experimental speed data and our control speed data using the following factors: whether or not the RADS was active (flashing) when speed was recorded, season (tourist season or off season), time of day (twilight vs. night) and their interactions. To meet the

assumptions of ANOVA, the speeds of all groups (Table 5) were checked for homogeneity of variances (F tests; JMP 11) and visually inspected for normality.

In the control data, F tests for homogeneity of variances (JMP 11) found that variances of speeds between seasons were unequal. Because there are twice as many data points in tourist season than the off season, and the variance is 1.8 times greater in the off season, this inflates the Type I error rate for comparison of these means to 0.12 instead of 0.05 (Glass et al. 1972), so if a season is found to be significant in the control data, interpretation must be conservative.

Results

Control Speed

As we expected, there was no significant effect of RADS status (on vs. off) in the control speeds measured before drivers entered the RADS sensor array, though the overall model including all factors was significant (ANOVA, $F_{7, 1020} = 10.5$; $p = 8.62 \times 10^{-13}$). The only factor that significantly affected speeds in the control data was time of day (twilight vs. night; Table 6), though since the speed limit changes from 60 mph to 45 mph after dark, this was not unexpected.

Experimental Speed

The overall model including the effects of RADS, season, and time of day was significant (ANOVA, $F_{7, 1287} = 13.0$; $p = 3.58 \times 10^{-16}$). Interaction effects were found at the $\alpha = 0.1$ significance level between RADS status and season (tourist vs. off-season) and RADS status time of day (twilight vs. night; Table 7).

Because we were interested in knowing whether driver response to the RADS varies between the tourist and off-seasons, we investigated the interaction between RADS status and season ($p = 0.0896$, Table 7) with Tukey's post-hoc test for Honestly Significant Difference (Fig. 5). In the tourist season, the activation of the RADS caused a significant difference in driver speed (3.81 km/h slower with the RADS on; Tukey's HSD, $p = 1.14 \times 10^{-4}$); however, this effect was not seen during the off-season (1.47 km/h slower with RADS on; Tukey's HSD, $p = 0.540$). This is an interesting result, considering that when the RADS was turned off, drivers in the tourist season drove faster than drivers in the off-season (3.49 km/h; Tukey's HSD, $p = 0.00697$).

Table 6. ANOVA table for driver speed at the control point (drivers had not seen any informational signs about the RADS or any warning signs). "RADS" = whether the warning signs were flashing or not flashing at the time; "TOD" = time of day (twilight or night); "season" = tourist season or off-season.

	d.f.	SS	MS	F	p > F
RADS	1	49	49	0.643	0.423
TOD	1	5214	5214	67.974	5.06×10^{-16} *
season	1	85	85	1.107	0.293
RADS*TOD	1	25	25	0.325	0.569
RADS*season	1	44	44	0.577	0.447
TOD*season	1	10	10	0.127	0.722
RADS*TOD*season	1	212	212	2.760	0.097

*significant at $\alpha=0.05$

Table 7. ANOVA table for driver speed in the center of the RADS zone (after passing 2 active or non-active warning signs); see Methods for explanation of variables.

	d.f.	SS	MS	F	p > F
RADS ^a	1	1190	1190	20.2	6.88 x 10 ⁻⁶ *
TOD ^b	1	2964	2964	50.8	1.69 x 10 ⁻¹² *
season	1	549	549	9.41	0.0022*
RADS*TOD	1	46	46	.791	0.374
RADS*season	1	168	168	2.89	0.0896 [†]
TOD*season	1	172	172	2.94	0.0867 [†]
RADS*TOD*season	1	155	155	2.65	0.104

a= Roadside Animal Detection System; b= time of day; *significant at $\alpha = 0.05$; [†]significant at $\alpha = 0.10$

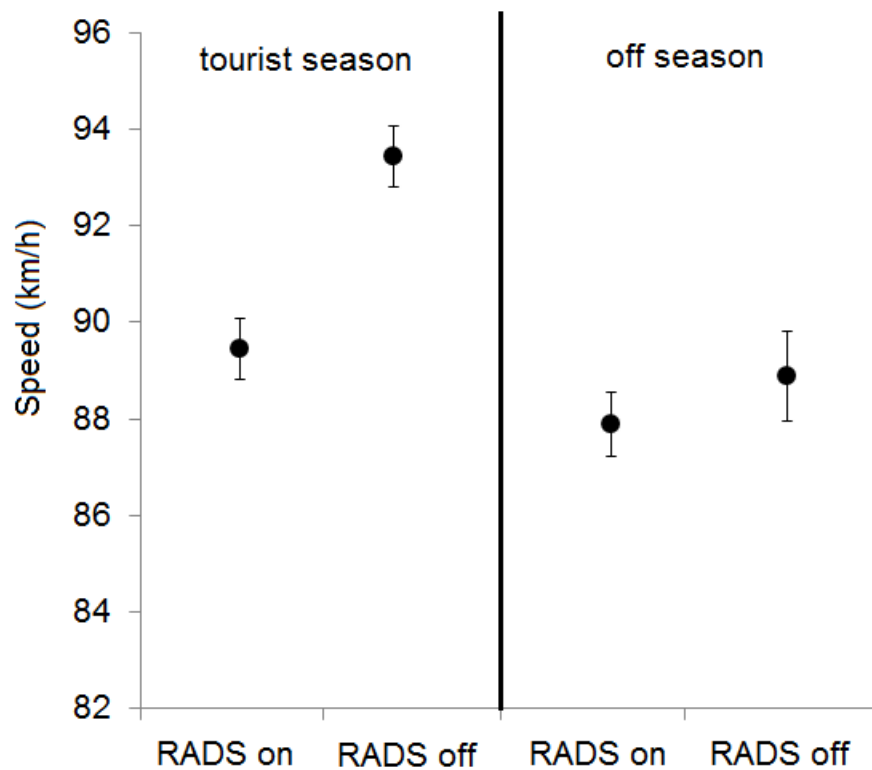


Figure 5. Interaction between RADS and season on driver speed. Markers indicate group means, while error bars indicate standard error.

Peaks in Animal Activity

Of the five large animal species whose tracks and camera appearances were measured, four showed a pattern of increased roadside activity during certain months of the year: Florida panther, Florida black bear, coyote, and white-tailed deer. The increase in activity coincided with the tourist season for the first three species (Fig. 6), whereas deer activity peaked prior to the tourist season.

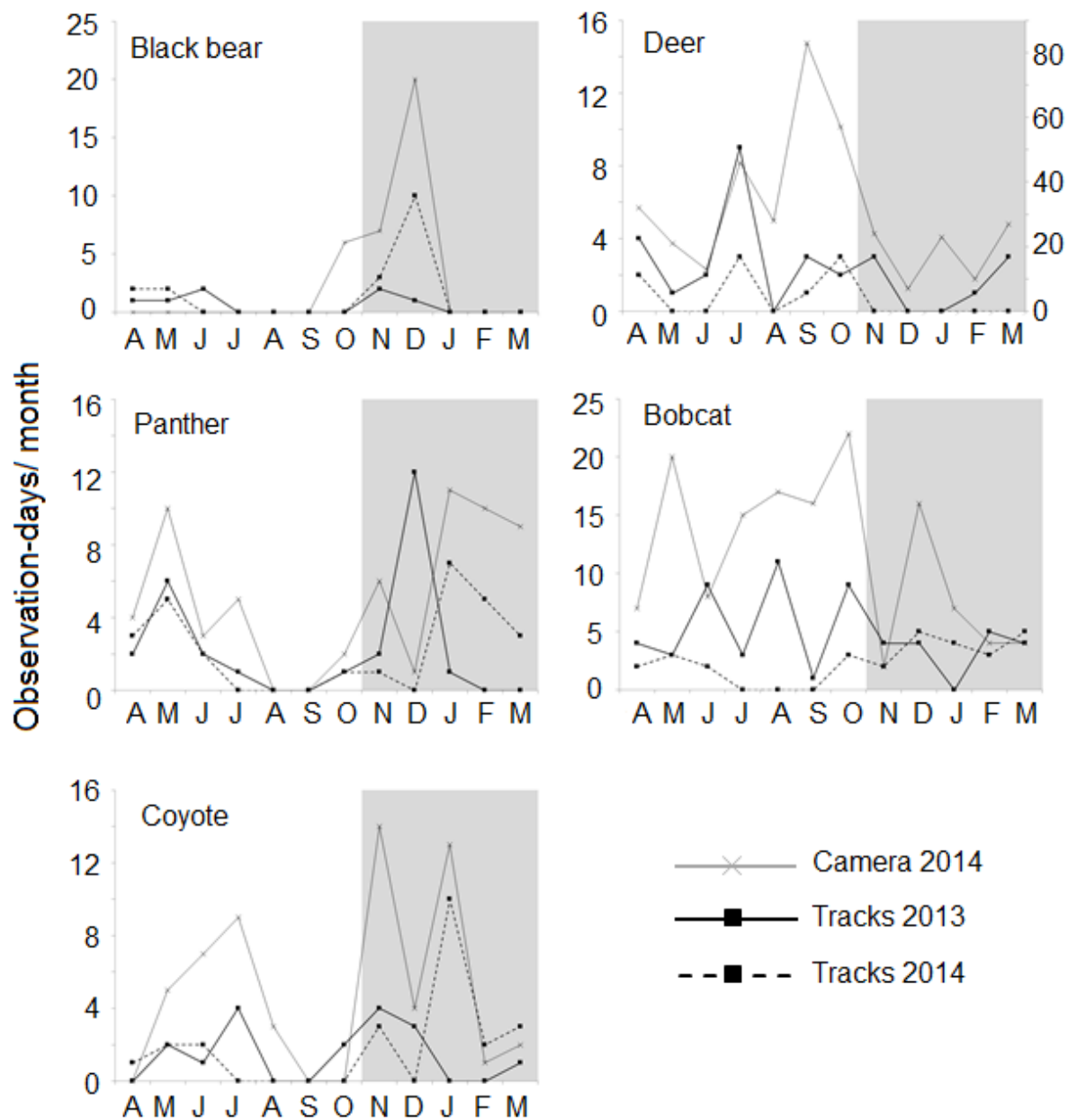


Figure 6. Increase in animal roadside activity during the tourist season (gray shading). For white-tailed deer, camera observations far outnumbered track observations, so camera observations are plotted on the secondary axis.

Discussion

Our field study found that the Roadside Animal Detection System did cause some drivers to reduce their speed, on average by 3.82 km/h. This value corresponds to the results of a RADS study in California (Mohammad Ashkan Sharafsaleh et al. 2012) and is very similar to what we observed in our driving simulator study (Grace et al. 2015) where drivers reduced their average speed by 4.80 km/h in response to the RADS. Although this may seem like a small decrease, incremental changes in speed can have large effects on crash probability. For example, a 10% increase in mean speed predicts a 30% increase in fatal and serious car accidents on rural roads (Taylor et al. 2002), while a 1 mph (1.6 km/h) decrease in speed corresponds with a 5% decrease in crash rate (Finch et al. 1994). Case (1978) found that decreases in average vehicle speed were linearly correlated with decreases in roadkills of pheasants, roadkills, opossums, and coyotes. In our simulator study, the participants that decreased their speed by an average of 4.80 km/h were also 6.29 times less likely to crash into an animal in the road (Grace et al. 2015).

Nevertheless, not all drivers in the current study reduced their speed in response to the RADS. Our most interesting finding was the interaction between the activation status of the RADS and the season (tourist vs. off-season), where the only drivers who reduced their speed were drivers in the tourist season. Traffic during the off-season is assumed to be primarily made up of locals, who had presumably observed the RADS multiple times before, if not daily, considering that U.S. Highway 41 is a main commuter route. Traffic observed during the tourist season would have still contained much local traffic, but would also contain a higher percentage of drivers who were unfamiliar with U.S. Highway 41, as indicated by our survey of park visitors (see Methods).

Despite our finding that drivers in the tourist season generally drove faster than those in the off-season, tourist-season drivers responded to the RADS by driving more slowly, while off-season drivers did not. We hypothesize that this result is due to acclimation to the RADS by local drivers. Throughout the experimental period, the RADS experienced a number of malfunctions causing it to flash almost continuously during the day, though it appears to have functioned correctly at night. Local drivers, who were accustomed to seeing the RADS lights flash non-stop, probably had begun to disregard the warning system. Non-local traffic, however, would not have experienced the same acclimation.

Though the RADS on U.S. 41 malfunctioned during our study period, it still served to reduce the speed of drivers during the tourist season, which may mean that a malfunctioning RADS is better than none. Tourists, perhaps not as aware of the potential for collisions with wildlife in the area, drove consistently faster than locals. The constantly activated RADS, though possibly ignored by locals, caused tourists to slow to the same speed that locals drive (Fig. 2). This is important because there is an increase in collisions with Florida panthers during the tourist season, November- March (Maehr et al. 1991; FFWCC 2015). This is likely due to three factors: (1) traffic volume increases (the addition of ~1200 cars per day; FDOT Florida Traffic Online Database), (2) drivers travel at higher speeds on average during the tourist season, as our data show, and (3) the tourist season coincides with panther breeding season, when males are more active as they search for mates (Maehr 1992). Our track data support this, showing a spike in panther activity during the tourist season in both 2013 and 2014 (Fig. 3). Black bears and coyotes showed the same seasonal spike in activity. Therefore, a system that produces awareness

and reduction in speed during the tourist season may be effective in reducing wildlife mortality because that is the period during which collisions are more likely to occur.

In conclusion, given that driver speed was affected similarly in the field as in the simulator study, we believe that Roadside Animal Detection Systems can be a useful tool in helping to reduce the number of collisions with Florida panthers and other large-bodied wildlife species. However, based on our results we suggest that, if the detection capabilities of the RADS remain as they are (many false positives; Huijser & McGowen 2003), these systems will probably be most effective if used during periods of greatest risk for wildlife-vehicle collisions (e.g. times with increased traffic volume or during spikes in animal movement; Huijser et al. 2015), in order to reduce acclimation by local drivers. If drivers ignore the RADS, then there is no benefit to wildlife, since it does not keep animals off of the road. However, we strongly suggest that improvements in the reliability of the RADS should be prioritized (Huijser et al. 2015; Smith et al. 2016). Once the technology of RADS has been refined to reliably detect target species, we also suggest that the effectiveness could be additionally improved by reducing the spatial extent of the system to focus more narrowly on areas with high crossing rates by target species. In our experiment, signs along all 2.1 km covered by the RADS sensors flashed no matter where an animal crossed, which drivers may react to as a false positive. In the Netherlands, RADS combined with fences guiding animals to a crossing point have produced a 94% success rate at preventing collisions across seven locations (Hamburg 2016).

Preventing collisions with wildlife, in particular the endangered Florida panther, is critical: of all mammalian orders studied, carnivores are the most negatively affected by roads (Rytwinski & Fahrig 2015). Across all animals, large-bodied species with low reproductive rates,

traits that describe many endangered species, suffer the greatest toll from roads (Rytwinski & Fahrig 2015). Indeed, habitat loss and mortality caused by roads may be currently among the greatest threats to carnivore population persistence (Burkey & Reed 2006).

CHAPTER THREE ~ ARE ROADSIDE DITCHES AN ECOLOGICAL TRAP? THE EFFECTS OF TRAFFIC NOISE ON TADPOLE DEVELOPMENT AND BEHAVIOR

Introduction

We have known for decades that chronic exposure to traffic noise has negative effects on human health and quality of life (Broner 1978), ranging from annoyance (Ouis 2001), to disrupted sleeping patterns (Öhrström et al. 2006), to increased risk for heart disease (Babisch 2000). Roads and their associated traffic are one of the most pervasive and obvious signatures of humans on the landscape; Riitters and Wickham (2003) estimated that less than 3% of the contiguous United States is located more than 5 km from a paved road, and that percentage has almost certainly shrunk since then. In fact, there are plans for a 60% increase in paved miles worldwide by 2050 (Laurance et al. 2014). It is therefore not surprising that ecologists have also been curious about how traffic noise affects wildlife.

As one might expect, traffic noise has been found to produce mainly negative outcomes for wildlife (reviewed in Francis and Barber 2013; Laurance 2015). In a study of woodland birds, traffic noise was the best predictor of the reduced abundance observed near roads (Reijnen et al. 1995), an effect that was reproduced empirically when traffic noise was broadcast in a roadless area (McClure et al. 2013). For individuals and species that do remain in noisy roadside areas, there can be detrimental effects. Traffic noise elevates stress levels in wood frogs (*Rana sylvatica*; Tennessen et al. 2014) and northern spotted owls (*Strix occidentalis caurina*; Hayward et al. 2011), and chronically elevated stress levels have been shown across taxa to negatively impact immune function (Martin et al. 2005), growth (Belden et al. 2005), reproduction (Ouyang et al. 2011), and survival (Pride 2005). Chronic environmental noise is associated with decreases

in the quality of avian parental care (Leonard and Horn 2012; Schroeder et al 2012). White-crowned sparrows (*Zonotrichia leucophrys*) exposed to traffic noise spent less time foraging and more time exhibiting vigilance behaviors, presumably because the noise interferes with their ability to detect predators (Ware et al. 2015). Species that use vocalizations as their primary form of communication (e.g. birds, frogs, many orthopterans) may find their signals masked by traffic noise, necessitating adjustments in amplitude, frequency, and/or timing (work on birds reviewed in Gil and Brumm 2014; frogs: Sun and Nairns 2005, Bee and Swanson 2007, Cunningham and Fahrig 2010; monkeys: Brumm et al. 2004). Interference with vocal communication can reduce mating success (frogs: Cunningham and Fahrig 2013; Tennessen et al. 2014).

While most work has focused on terrestrial animals, aquatic species are not necessarily immune to the negative effects of traffic noise. Roads often pass by or over bodies of water, and the sound can be transmitted to the aquatic environment. Holt and Johnston (2015) found that low-frequency noise from truck traffic on a bridge, which potentially masks the low-frequency signals of the Blacktail Shiner (*Cyprinella venusta*), propagated throughout a shallow stream further than signals of the fish, essentially creating “dead zones” for fish communication. While Holt and Johnston’s study is the first to show that traffic noise can infiltrate the aquatic environment, other anthropogenic sources of aquatic noise pollution have long been identified, and the effects on aquatic fauna are similar to what has been observed in terrestrial species (Slabbekoorn et al. 2010). Noise from passing ships induces a stress response in various fish species (Wysocki et al. 2006; Purser et al. 2016) and shore crabs (*Carcinus maenas*, Wale et al. 2013a). It is also known to interfere with schooling behavior in bluefin tuna (*Thunnus thynnus*, Sarà et al. 2007) and foraging behavior in shore crabs (Wale et al. 2013b). Aquarium noise from

filters and pumps increases stress and decreases body condition of lined seahorses (*Hippocampus erectus*; Anderson et al. 2011).

In this study, we focus on the effects of traffic noise on activity level, feeding behavior, and growth of tadpoles. Though previous studies have examined how traffic noise affects adult anurans, i.e. frogs and toads (Sun and Nairns 2005, Bee and Swanson 2007, Cunningham and Fahrig 2010; Cunningham and Fahrig 2013; Tennessen et al. 2014), there have been no studies on tadpoles. The effect of traffic noise on tadpoles is relevant because some anuran species lay their eggs in roadside ditches, presumably because these ephemeral water bodies are generally free from fish predators (Mobberly and Pfrimmer 1967; Bridges and Semlitsch 2001; Eric Hoffman, pers. comm.). If traffic noise negatively affects tadpole development, however, then roadside ditches could actually be functioning as an ecological trap (Gates and Gysel 1978; Schlaepfer et al. 2002). In addition to noise, anurans have been identified as one of the taxa most vulnerable to direct mortality on roads (reviewed in Andrews et al. 2008; Fahrig and Rytwinski 2009), so gaining a more complete picture of anuran road ecology will help conservation efforts. Given the vulnerability and extinction of many amphibian species worldwide (Houlahan et al. 2000; Stuart et al. 2004), it is critical to understand which factors could contribute to their decline.

We hypothesized that, similar to what was observed in Ware et al.'s (2015) study, traffic noise would cause tadpoles to become more vigilant, which would translate into changes in feeding and behavior, in turn affecting growth. A similar reduction in foraging in response to noise has been observed in Mediterranean damselfish (*Chromis chromis*; Bracciali et al. 2012). In the presence of a predator, tadpoles from the majority of anuran species tested reduce their activity level (Lawler 1989; Richardson 2001), so we expected to observe a similar reaction to

traffic noise. For this study, we had three specific predictions; if traffic noise has a negative effect on tadpole fitness, then:

- 1) Traffic noise exposure will decrease the amount of food consumed by tadpoles, as it has been shown to decrease foraging efficiency in other species
- 2) The decrease in food consumption will be accompanied by a decrease in activity level
- 3) The combination of these factors should result in significant reduction of mass at the time of metamorphosis and/or earlier metamorphosis, which is a signal of stress (Denver 1997; Warne et al. 2011).

Methods

Tadpole Collection and Housing

Southern Toad Tadpoles

We collected southern toad (*Anaxyrus terrestris*) tadpoles (n=16) on June 20, 2016 at Ralph V. Chisholm Regional Park on East Lake Tohopekaliga (St. Cloud, Osceola County, FL). Tadpole age was unknown, but they were less than 1cm long and had not yet developed hind limb buds (Gosner stage 25). All testing of southern toad tadpoles was completed before Gosner stage 25. Southern toad tadpoles were housed individually in 64-oz food-grade plastic containers filled with 1.5 inches of dechlorinated water and fed ground TetraMin Tropical Flakes ad libitum daily. Water was changed every 2-3 days to prevent fouling. Due to the small number of individuals available, southern toad tadpoles were only used in one experiment.

Cuban Treefrog Tadpoles

Cuban treefrog (*Osteopilus septentrionalis*) tadpoles (n=300) were collected on July 7, 2016 one day after hatching in a residential area near the University of Central Florida (Orlando, Orange County, FL). They were housed in groups of approximately 30 tadpoles in 64-oz containers filled with 4 inches of dechlorinated water and fed TetraMin flakes ad libitum. Water was changed every 2-3 days to prevent fouling. Because we had such a large sample of Cuban treefrog tadpoles, individual tadpoles were not tested in more than one experiment. With the exception of the metamorphosis experiment, all testing of Cuban treefrog tadpoles was completed before Gosner stage 25.

Tadpole Hearing Ability

Early in development, before the development of the tympanum, tadpoles hear via vibrations passing through the side of their head and react to noise (Horowitz 2012). However, they undergo a 48-hour deaf period just before their forelimbs emerge in Gosner stage 41, when the developing muscle and cartilage associated with the shoulder girdle block the opening of the inner ear. Because all tadpoles in this study were tested before Gosner stage 25, this deaf period does not affect the results.

Traffic Noise Stimulus

To test tadpoles' reaction to traffic noise, we created a digital compilation of vehicle pass-by events (as in McClure et al. 2013). The road on which the traffic noise stimulus was based is State Road 528, a highway with a speed limit of 70 mph (113 km/h) that runs east to west

through Tosohatchee Wildlife Management Area, a site rich in anuran diversity (16 documented species, including southern toad and Cuban treefrog; pers. obs.). S.R. 528 maintains a relatively high traffic volume even at night, when anurans are most likely to be calling (Fig. 7). This traffic volume remains consistent throughout the summer breeding season for anurans in Florida (March-October; Fig. 7). During the sampling period for the North American Amphibian Monitoring Program (19:00–0:00 hours), average traffic volume on S.R. 528 is 1126 vehicles/hour (standard deviation 476.3 vehicles/h). A study measuring the effects of road noise on the abundance of migratory birds used a playback file with 720 vehicles/h traveling at 45 mph (72 km/h), and even at this lower traffic volume found significant effects of noise (McClure et al. 2013).

We recorded individual vehicle pass-by events on April 30, 2015 on State Road 50, which lies just north of Tosohatchee WMA. We did not record on S.R. 528 because it was easier to pull off safely on the side of the road on S.R. 50, and also because it has a lower traffic volume, making it possible to obtain clean recordings of individual vehicles. The vehicles from which we recorded traffic noise were traveling between 60 and 70 mph (97-113 km/h), and we recorded them at a microphone height of 1.1 m and at a distance of 7 m from the edge of the pavement. The recordings were made in WAV format using a Sennheiser MKH 50 P48 microphone with wind guard and Marantz PMD670 solid state recorder. From our recordings, we chose nine individual vehicle pass-by events based on the recording clarity. These pass-by events were individually normalized to a common peak amplitude in the program Audacity v. 2.1.0 and combined in a repeating sound file which played back 1120 pass-by events per hour.

This sound file was exported in WMA format because our playback equipment did not recognize files in WAV format (as in Cunningham and Fahrig 2013).

During all experiments, to create the traffic noise treatment the traffic noise file was played to create an average ambient noise level of 70 dB, which is the noise level ~30m from a real road (Reijnen et al. 1995). This noise level is conservative, because some anuran species lay their eggs directly alongside the road in ditches (Mobberly and Pfrimmer 1967; Bridges and Semlitsch 2001; Eric Hoffman, pers. comm.).

To determine how much of this noise would actually be transmitted across the air-water interface and be audible to the tadpoles, we played the traffic noise stimulus at 80 dB SPL (5m) and recorded the noise with a microphone both in the air and submerged 2 inches under water. As expected, some of the higher frequencies were lost at the water's surface, but much of the signal was transmitted and would be audible to tadpoles (Fig. 8).

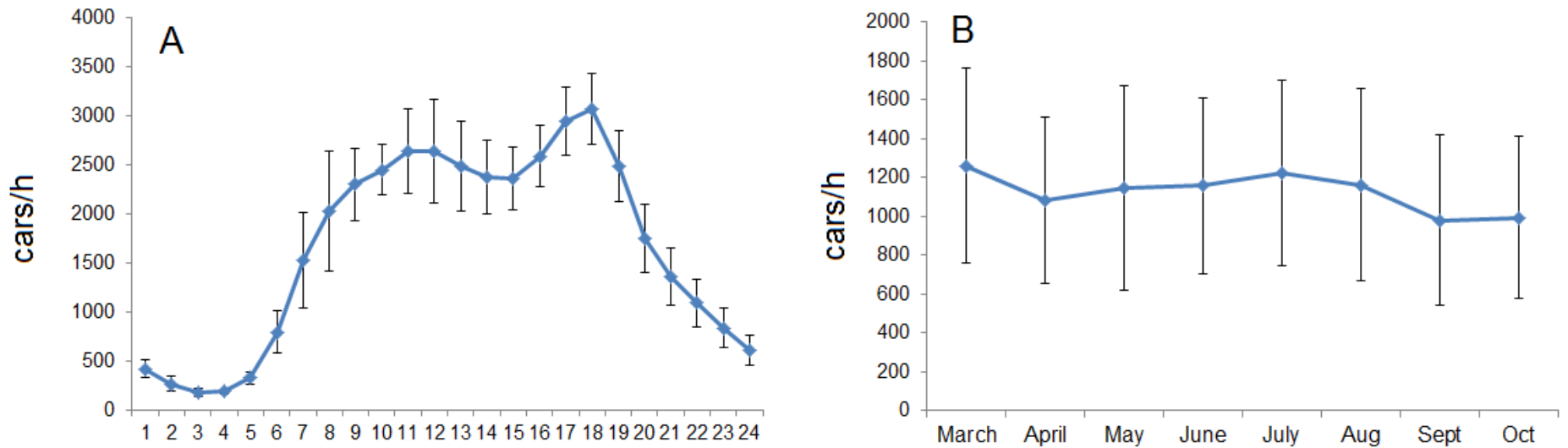


Figure 7. Variation in traffic volume on SR 528 by hour and month. **A)** Average number of vehicles observed per hour on State Road 528 in March-October 2013 (Florida Department of Transportation statistics). Though traffic volume peaks in the middle of the day, SR 528 experiences significant traffic throughout the evening. Error bars represent standard deviation. **B)** Average number of vehicles observed per hour on State Road 528 during time relevant to anuran calling behavior (19:00-24:00) in March-October 2013 (Florida Department of Transportation statistics). Error bars represent standard deviation. Traffic levels remain basically unchanged throughout the breeding season for Florida anurans (March-October).

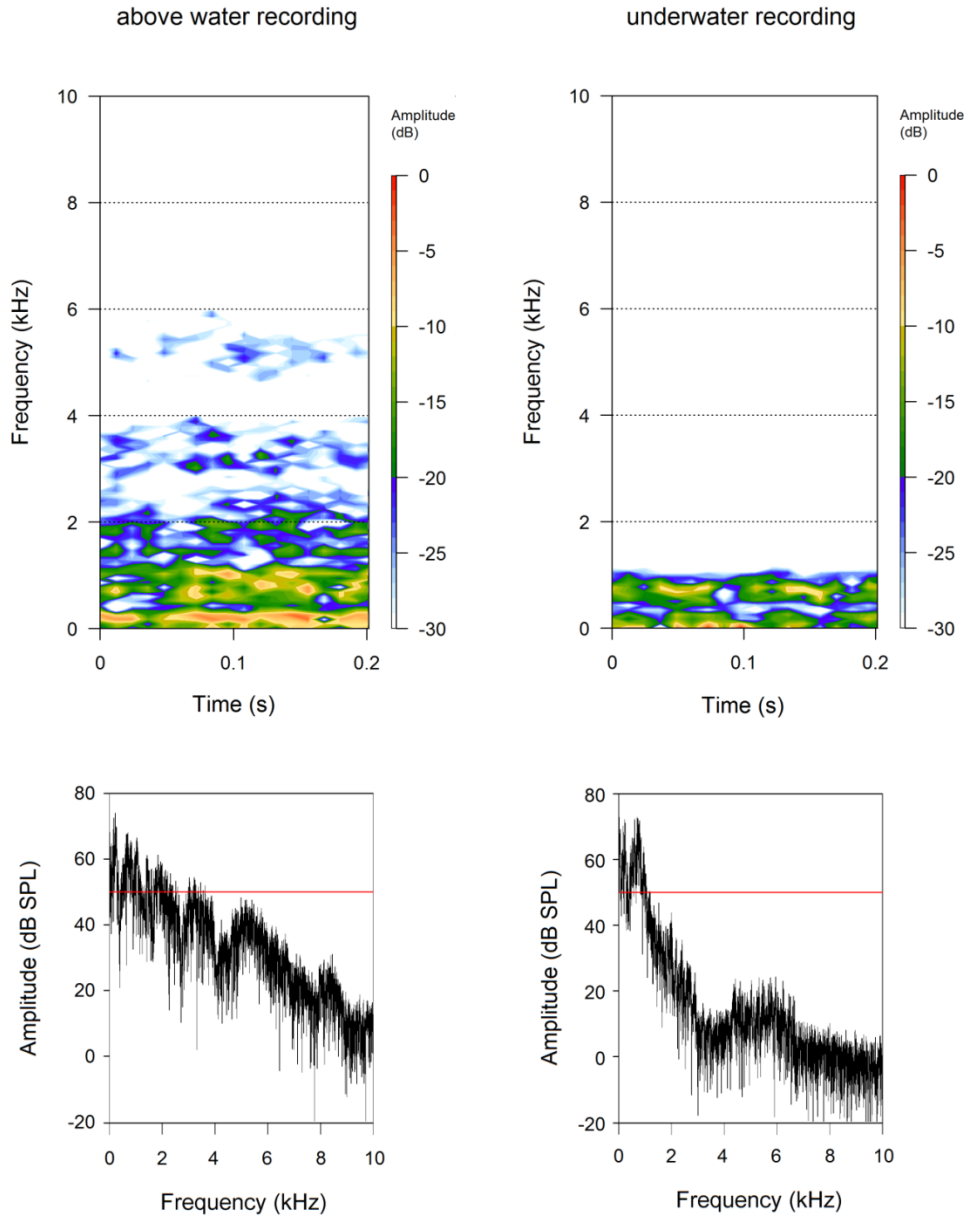


Figure 8. Spectrograms (top) and power spectra (bottom) of the traffic noise stimulus broadcast at 80 dB SPL at 5m. The spectrograms show that the transition from air to water caused the loss of the stimulus' higher frequencies. However, the power spectra show that the frequencies that were transmitted across the air-water interface were transmitted at comparable amplitude. The red lines on the spectra show the cutoff point for frequencies shown on the spectrograms (30 dB SPL below the loudest frequency in the signal).

Effect of Traffic Noise on Feeding Behavior

We randomly assigned 60 Cuban treefrog tadpoles to either the traffic noise treatment (n=30) or the control treatment (n=30) and placed them in individual containers. The tadpoles assigned to the traffic noise treatment were moved to the experimental room, while those in the control treatment remained in the housing room. The two rooms were light- and temperature-controlled to minimize differences in environmental conditions. Because we wished to measure changes in food consumption, we needed to be able to measure the mass of the food, and the TetraMin flakes dissolved too quickly for this to be feasible. Instead, we switched the tadpoles to a diet of boiled lettuce. Tadpoles were allowed to acclimate to their individual containers and new diet for two weeks.

For four days prior to the experiment, the tadpoles were food-starved to ensure that they would eat during the experimental period. 48 hours before the experiment, we measured each tadpole's body mass (gently blotted dry) so that we could correct the amount of food eaten for body size. The scale was accurate to three decimal places, so all mass measurements are rounded to three decimal places and final calculations are reported at three significant figures. On the day of the experiment, the tadpoles were given a pre-weighed amount of boiled lettuce (blotted dry) and allowed to eat *ad libitum*, undisturbed for 2 hours. At the end of the experiment, the lettuce was weighed again and we subtracted the final mass from the original mass to determine how much lettuce had been consumed. The lettuce mostly remained in large pieces that were removed and weighed, although it is possible that we missed some small particles.

Intriguingly, we found that the lettuce samples weighed *more* at the end of the experiment, even though the tadpoles had clearly eaten. We discovered that the cause of this was

that the lettuce absorbed water during the two hour experiment. We realized that we needed to know the dry mass of the lettuce, but it was not feasible to dry the lettuce samples before feeding them to the tadpoles. To account for this, we devised a method to convert the wet mass of the lettuce at both the beginning and end of the experiment to dry mass. We took freshly prepared boiled lettuce and created samples of different masses that covered the range of masses given to the tadpoles at the beginning of the experiment. We measured the wet mass, then desiccated the samples in a drying oven and measured their dry mass. We repeated this procedure with boiled lettuce samples that had soaked in water for two hours, mimicking the conditions at the end of the experiment.

We then plotted the wet mass against the dry mass for both data sets and fit linear, exponential, logarithmic, and power curves to determine the best fit based on R^2 values. For the freshly prepared lettuce, a linear function was the best fit, while the best fit for the samples that had soaked for two hours was a power function (Table 8). We used these fits to calculate an estimate of the dry mass of the lettuce used in our experiment from the wet mass at the beginning and end of the experiment (Fig. 9).

Using the fitted values, we used a one-tailed two-sample t-test to determine if traffic noise reduced food consumption by the tadpoles. Prior to performing the t-test, we checked for homogeneity of variances (F-test for equal variances, $F = 0.970$, $df = 29$ and 29 , $p = 0.468$).

Table 8. R^2 values obtained by fitting different curves to the regression of wet lettuce mass against dry lettuce mass.

Function type	R^2 freshly boiled	R^2 two hours
linear	.585	.727
exponential	.553	.707
logarithmic	.545	.667
power	.567	.732

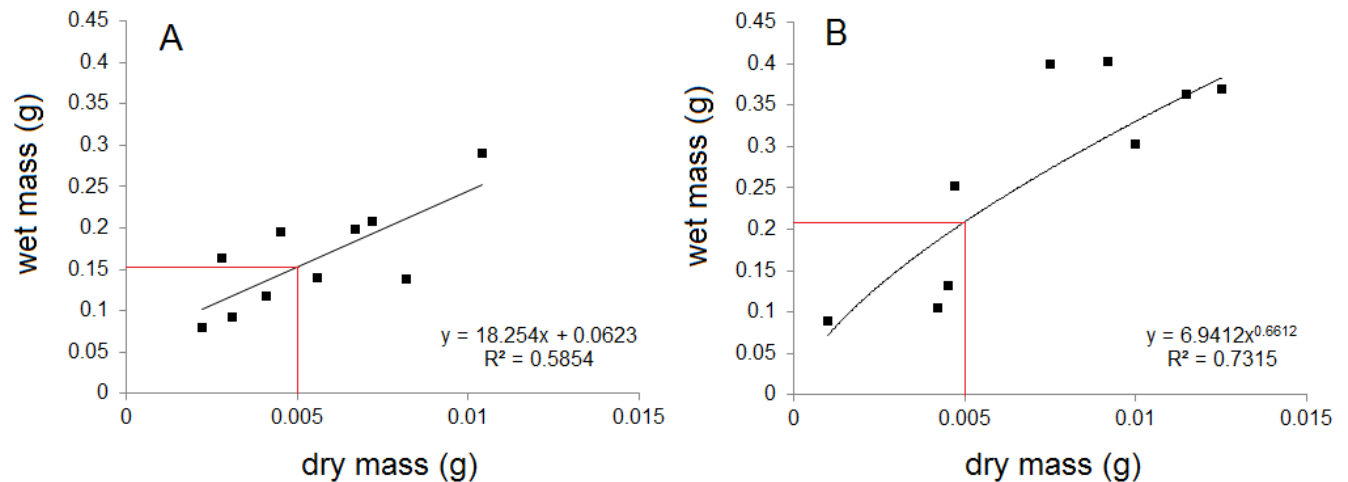


Figure 9. Fitted curves used to convert the wet mass of the lettuce at the beginning (A) and end (B) of the experiment into dry mass. The fitted equation was solved for x (dry mass) and the wet mass (y) was plugged in to estimate the dry mass of experimental samples. Notice that the wet mass at the end of the experiment for a given dry mass is higher than for that same dry mass at the beginning of the experiment. This is due to water absorption during the experiment and is the reason that two separate curves are necessary.

Effect of Traffic Noise on Tadpole Activity Level

Southern Toad

Each of the 16 tadpoles was randomly assigned to either the traffic noise treatment or control treatment (no noise) so that there were 8 tadpoles per treatment. We tested tadpoles one by one, but all tadpoles were tested on the same day. Each tadpole's container was moved from the housing room to the experimental room and the tadpole was allowed to acclimate for one minute. The experimental room was maintained at the same temperature as the housing room (~27°C). At the end of the minute, the traffic noise treatment was turned on when testing a tadpole in the treatment group. In both treatment groups, we recorded the duration of all tadpole movements using a stopwatch for 5 minutes. The total summed duration of all movements was used as a measure of tadpole activity level (Richardson 2001).

Because we had such a small sample of southern toad tadpoles, we repeated the experiment two days later but switched the treatment assignments of each tadpole, allowing us to measure changes in individual behavior using a paired one-tailed t-test. Prior to performing the test, we checked that the individual differences in activity between treatments (delta) were normally distributed (Shapiro-Wilk test, $W = 0.918$, $p\text{-value} = 0.156$).

Cuban Treefrog

We randomly assigned 30 tadpoles each to the traffic noise and control treatment. Since the Cuban treefrog tadpoles were housed in groups, each experimental tadpole was moved to its own container and allowed to acclimate to the container for five minutes before being moved to the experimental room. To accommodate the larger number of tadpoles being tested, tadpoles were

tested six at a time (block) and videotaped, and their activity level was measured from these videos. Because we had a larger sample of Cuban treefrog tadpoles, we did not test the same tadpoles twice. Differences in activity level were assessed using analysis of variance (ANOVA) with treatment, block, and their interaction as factors. Groups were tested for homogeneity of variances using the F test (control:treatment, $F_{(29,29)} = 1.51$, $p = 0.138$; most variable block:least variable block, $F_{(11,11)} = 1.73$, $p = 0.188$; most variable interaction:least variable interaction, $F_{(5,5)} = 2.47$, $p = 0.172$).

Effects of Traffic Noise on Growth and Time to Metamorphosis

To examine if traffic noise exposure decreased growth rate and time to metamorphosis, we assigned tadpoles to one of four treatments: all combinations of either the traffic noise or control treatment and either a high competition or low competition treatment. We added competition as a factor because the effect of another environmental stressor, pesticides, on tadpole development is known to vary depending on the level of competition (Relyea and Diecks 2008). The low-competition and high-competition treatments were kept in the same sized containers (64-oz) with the same amount of water, but the number of tadpoles varied: 5 tadpoles per container in the low-competition treatment and 12 tadpoles per container in the high-competition treatment. Each treatment combination was replicated 4 times for a total of 16 groups.

Each day, we fed each group 0.05g of fish flakes. While this seems miniscule, based on average tadpole weight, this amounts to each tadpole receiving ~5% of its weight in food each day in the low-competition treatment and ~2% in the high-competition treatment. At the beginning of the experiment, all tadpoles were prior to Gosner stage 25 and were all the same

age (same clutch). Every other day for 3 weeks, tadpoles were weighed to track their growth rate, and every day the groups were checked for metamorphs. We defined metamorphs as individuals that had developed all 4 limbs and were able to climb. When we removed metamorphs, we adjusted the amount of food given to each group in order to maintain the relative amount of food received by each tadpole (5% or 2%).

We assessed the effects of noise treatment and competition on mass at metamorphosis and day of metamorphosis using Analyses of Variance (ANOVA) with replicate as a covariate. We checked that the variances of all treatment combinations were normally distributed (multiple F-tests). Some of the groups were not normally distributed, but ANOVA remains a robust test even if this assumption is violated (Schmider et al. 2010).

Results

Effect of Traffic Noise on Feeding Behavior

Tadpoles ate on average 0.00334 grams of food during the two-hour experiment ($SE \pm 0.000364$ g). Average tadpole body mass was 0.154 grams ($SE \pm 0.00521$ g), so on average they consumed an amount equivalent to 2.18% of their body mass. When adjusted for tadpole mass, tadpoles in the traffic noise treatment consumed less lettuce during the experiment than tadpoles in the control treatment: 0.0177 g lettuce/ g tadpole mass on average for traffic and 0.0315 g lettuce/ g tadpole mass for control (one-tailed two-sample t-test, $t = 3.23$, d.f. = 58, $p = 0.00103$; Fig. 10).

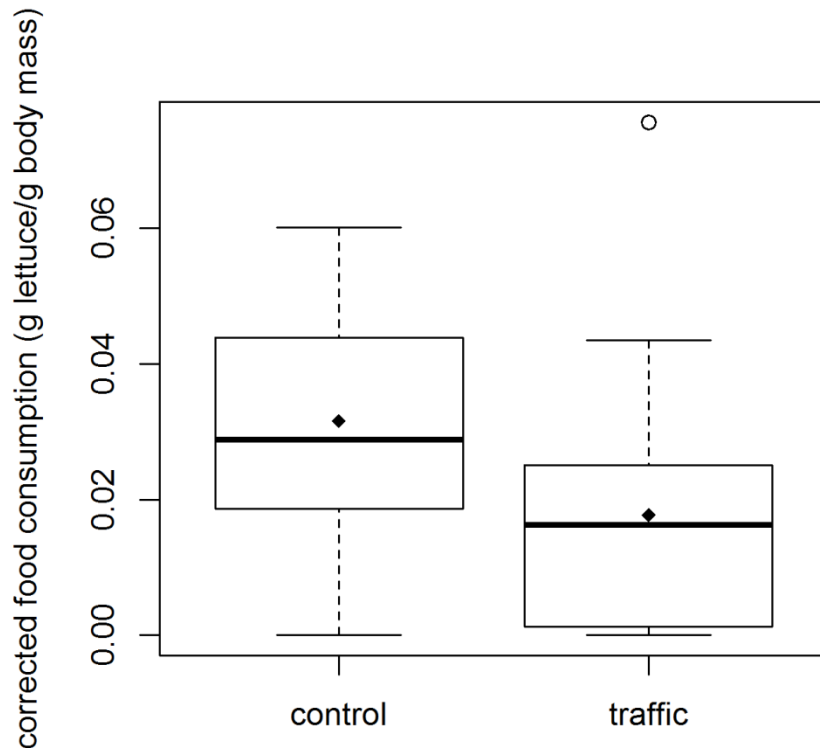


Figure 10. Tadpoles in the control group ate significantly more than those in the traffic noise treatment. n=30 per treatment. The values in this figure are dry weights. Diamonds indicate group means.

Effect of Traffic Noise on Tadpole Activity Level

Both southern toad and Cuban treefrog tadpoles were more active in the traffic noise treatment compared to the no noise control (Fig. 11, 12, Table 9). On average, southern toad tadpoles increased their activity by 40.2 seconds (170% increase in activity; control average= 27.8s, traffic average 75.4s), while Cuban treefrog tadpoles increased activity to a lesser extent by an average of 44.7 seconds (44.1% increase in activity; control average= 101.3s, traffic average 146.0s).

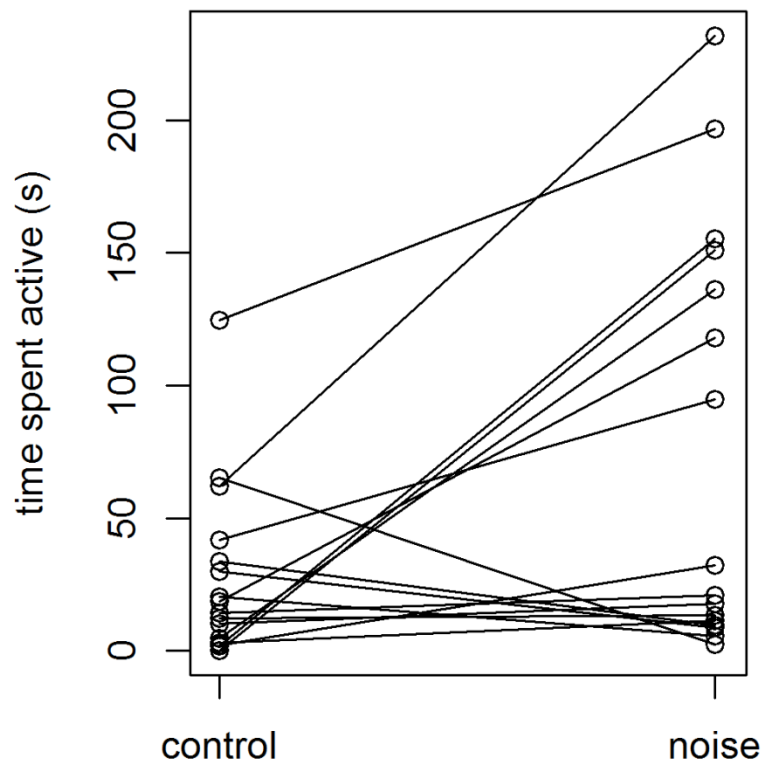


Figure 11. Individual southern toad tadpoles increased activity level in response to traffic noise. Each line represents one tadpole's change in activity level from the control treatment to the noise treatment. This difference is significant (paired t-test, $t = 2.5984$, $df = 15$, $n = 16$, $p = 0.0101$).

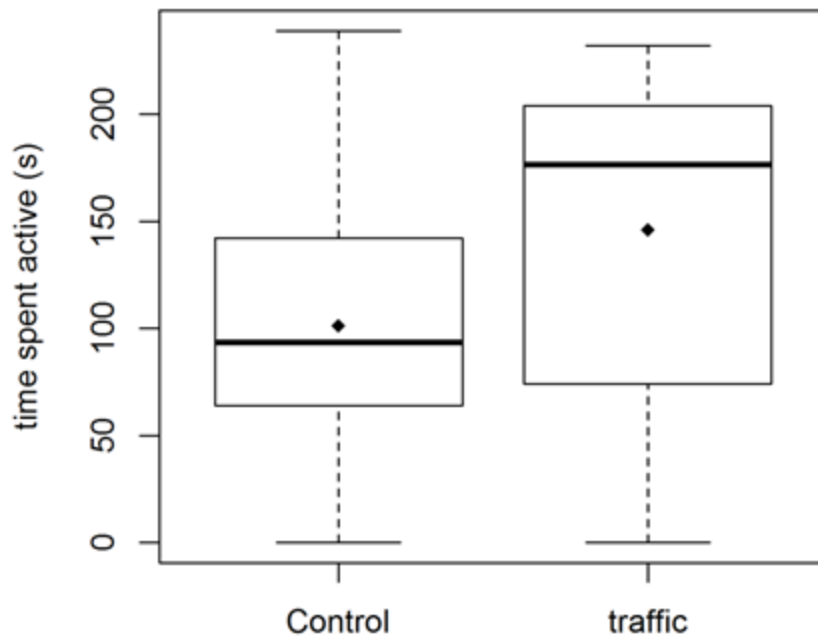


Figure 12. Cuban treefrog tadpoles spent more time active in the traffic noise treatment than in the control treatment (ANOVA; $F_{(1,82)}=6.09$; $p=0.0170$). Diamonds show group means.

Table 9. Noise treatment significantly affected Cuban treefrog activity level.

	d.f.	Sum Sq	Mean Sq	F	p
treatment	1	29987	29987	6.09	0.0170*
block	1	30463	7616	1.55	0.203
treatment:block	1	45950	11488	2.33	0.0683
residuals	82	246048	4921		

Effects of Traffic Noise on Growth and Time to Metamorphosis

Although traffic noise reduced food consumption in the previous experiment, this did not translate into a decreased body mass at metamorphosis (Fig. 13, Tables 10 and 11). As expected, increased competition did have a negative effect on mass at metamorphosis. However, there was no significant effect of either traffic noise or competition on day of metamorphosis (Fig. 14, Table 12).

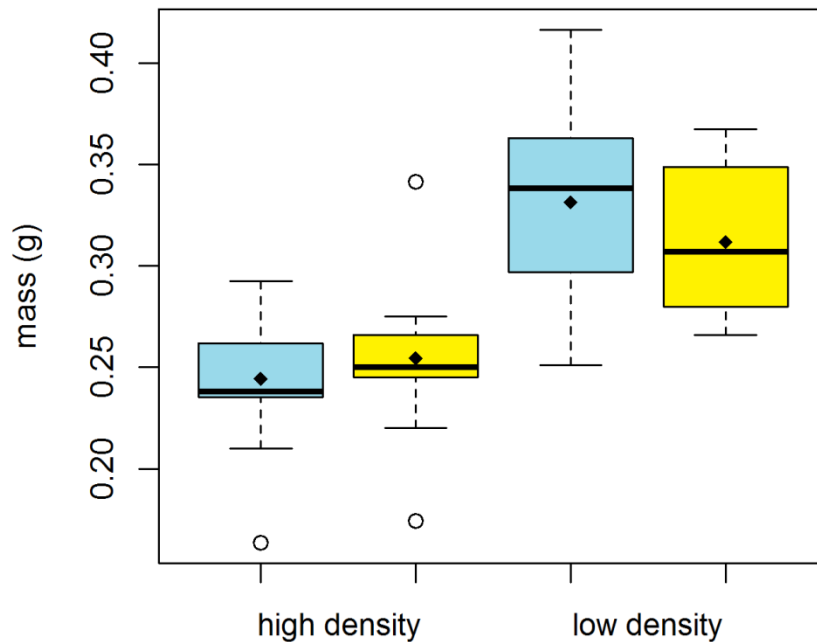


Figure 13. Tadpole competition, but not traffic noise, has a significant effect on tadpole size at metamorphosis. Blue indicates the control group while yellow indicates the group that was exposed to traffic noise. Black diamonds show means.

Table 10. ANOVA table for the effects of traffic noise and tadpole competition on tadpole size at metamorphosis. Though the interaction between treatment and density appears significant, it is actually not (see Fig. 7, Table 3).

	d.f.	Sum Sq	Mean Sq	F	p
treatment	1	0.00012	0.000120	0.111	0.7399
competition	1	0.106	0.106	100	$7.31e^{-16}$ *
rep	3	0.0104	0.00346	3.27	0.0253*
treatment:comp	1	0.00465	0.00465	4.39	0.0392*
residuals	82	0.0869	0.00106		

Table 11. Results of Tukey's Honestly Significant Differences test that relate to the interaction between traffic noise treatment and competition. The contrasts compare means across every combination of variables. H= high competition treatment, L= low competition treatment. The only significant contrasts are those that compare a low competition group to a high competition group.

contrast	mean difference	lower bound	upper bound	p
Traffic:H-None:H	0.0102	-0.0124	0.0329	0.637
None:L-None:H	0.0872	0.0603	0.114	$< 1.0e^{-10}$ *
Traffic:L-None:H	0.0675	0.0406	0.0944	$< 1.0e^{-10}$ *
None:L-Traffic:H	0.0770	0.0506	0.103	$< 1.0e^{-10}$ *
Traffic:L-Traffic:H	0.0573	0.0308	0.0837	$1.2e^{-6}$ *
Traffic:L-None:L	-0.0197	-0.0499	0.0104	0.323

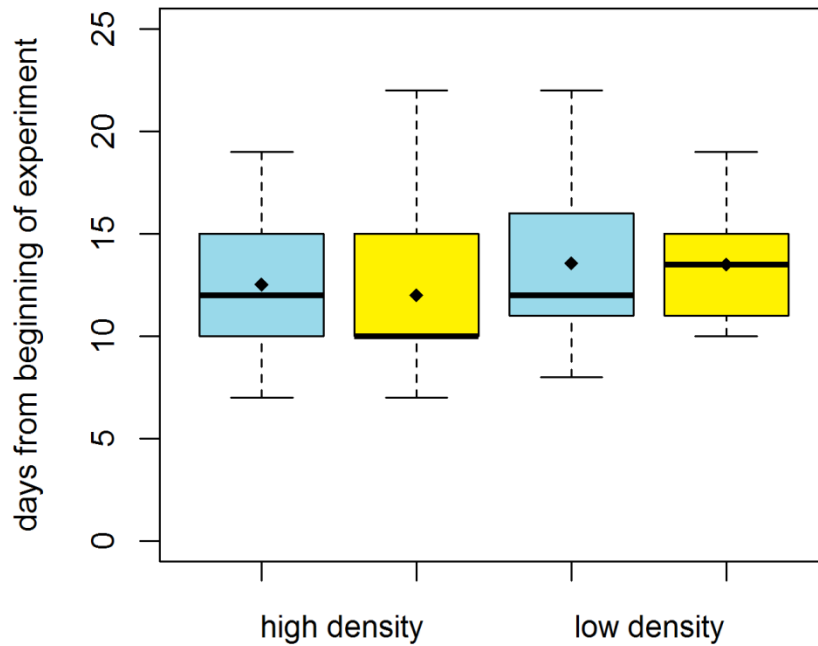


Figure 14. No effect of competition or traffic noise treatment on the timing of metamorphosis. Blue indicates the control group while yellow indicates the group that was exposed to traffic noise. Black diamonds show means.

Table 12. ANOVA table for the effects of traffic noise and tadpole competition on day of metamorphosis.

	d.f.	Sum Sq	Mean Sq	F	p
treatment	1	3.3	3.3	0.275	0.601
competition	1	33.4	33.4	2.78	0.0991
rep	3	58.2	19.4	1.62	0.192
treatment:comp	1	0.5	0.54	0.045	0.833
residuals	82	983	12.0		

Discussion

Although we observed that exposure to traffic noise increased tadpole activity level and decreased the amount of food consumed during an experimental trial, we did not observe any corresponding effects on timing or size at metamorphosis. This aligns with studies in cultured fish; a study of how rainbow trout (*Oncorhynchus mykiss*) respond to aquarium noise found no significant effects on growth rate or mortality (Wysocki et al. 2007), and a study of the effects of white noise on goldfish (*Carassius auratus*) found that the noise produced temporary, but not permanent, spikes in plasma cortisol (Smith et al. 2004). A possible explanation for our findings is that the brief exposure to noise in the activity and feeding experiments produced an acute spike in stress that affects behavior, but that tadpoles are able to acclimate and resume normal patterns of behavior when facing chronic exposure to noise. This could be a positive finding in terms of anuran conservation, but several caveats must be addressed.

Most anuran species decrease their activity level in the presence of a predator or predator cues (Lawler 1989; Richardson 2001), but some species (across the anuran families Bufonidae, Ranidae, and Hylidae) show increased activity, so our finding that both Cuban treefrogs and southern toads increased their time spent active in response to traffic noise is not unprecedented. However, though we hypothesized that traffic noise would induce similar responses in the tadpoles as a predator would, our experiment did not include any actual predators. Previous work has shown that tadpoles with higher activity levels were less likely to survive in the presence of a predator (Lawler 1989), so it is possible that increased activity in response to traffic noise could make tadpoles more vulnerable to predation. Chan et al. (2010) found that Caribbean hermit

crabs (*Coenobita clypeatus*) allowed a simulated predator to approach closer under conditions of anthropogenic noise. However, our experiment did not test for this.

Another caveat that limits extrapolation from our results is that, due to the difficulty in obtaining tadpoles, we only tested one clutch of tadpoles for each species. While a clutch of Cuban treefrog tadpoles provides more than enough individuals to achieve statistical power in multiple experiments, an experiment where all individuals come from the same clutch is pseudoreplicated. That is, it is possible that the behavioral responses were a quirk of this particular clutch and not a general pattern for the species as a whole. The fact that similar results were found in both species helps alleviate this concern, but it is a definite limitation to this set of experiments.

In summary, we did not find that traffic noise had a negative impact on tadpole size at metamorphosis, but it is crucial to empirically investigate the interaction between traffic noise and predation before concluding that traffic noise does not produce negative outcomes for tadpoles. If roadside ditches are predator-free, however, as many are, our preliminary findings suggest their use as egg deposition sites by anurans is not an ecological trap in terms of tadpole growth.

CHAPTER FOUR ~ THE EFFECT OF TRAFFIC NOISE ON ANURAN ABUNDANCE AND COMMUNICATION

Introduction

Roads are associated with reduced abundances of many animal species, from arthropods to mammals (reviewed in Fahrig and Rytwinski 2009), and it is hypothesized that traffic noise is a contributing factor (Francis and Barber 2013). This would be relevant to the design of ecopassages (a.k.a. wildlife overpasses and underpasses); their aim is solely to reduce roadkill and fragmentation, and they do not reduce traffic noise. Due to the logistical challenges, there are very few studies which have experimentally tested the effects of noise on wildlife abundance. In contrast, there are innumerable observational studies which suggest that noise affects abundance (reviewed in Potvin 2016), but these studies are confounded by other road effects, such as direct mortality via roadkill or chemical and light pollution (e.g., Reijnen et al. 1995; Eigenbrod et al. 2009).

One way to circumvent these confounding factors is to use a “phantom road” approach (McClure et al. 2013), in which pre-recorded traffic noise is broadcast in an otherwise roadless area. Use of such an approach has shown that traffic noise causes certain frog species to modify their vocalizations (Cunnington and Fahrig 2010); that traffic noise may impede detection of calling male frogs by females (Bee and Swanson 2007; Tennessen et al. 2014) and increases stress levels (Tennessen et al. 2014). Most relevantly to the question at hand, the phantom road approach has been used to show that migratory birds spend less stop-over time in areas with low to moderate levels of road noise (McClure et al. 2013). However, to date, this approach has not been used to study the effect of traffic noise on site occupancy by non-migratory species.

In this project, I broadcast traffic noise in roadless areas to observe the effect on anuran (frogs and toads) abundance as measured by recordings of vocalizations. Using vocalizations as a proxy for abundance (rather than trapping individuals) was chosen to increase sampling effort, and number of vocalizations has been found to correlate with number of anuran individuals (Nelson and Graves 2004). This method also produced a library of vocalization recordings, which I used to assess whether traffic noise induces changes in anuran vocalizations. It has long been known that many songbird species alter properties of their vocalizations, such as frequency, amplitude, duration, and song type, in response to traffic noise (reviewed in Slabbekoorn 2013 and Potvin 2016). While several studies have experimentally tested for this in anurans (Sun and Narins 2005; Lengagne 2008; Cunningham and Fahrig 2010, 2013; Hanna et al. 2014), they have produced conflicting results. Given the wide diversity in vocalization properties displayed by anuran species around the world, it is unsurprising that there is a diversity of responses to masking noise, and my study is another piece of information that may help uncover a broad pattern.

Methods

Study Sites

To determine whether traffic noise alone truly has a negative impact on wildlife abundance, I broadcast traffic noise at roadless pine flatwoods sites in Tosohatchee Wildlife Management Area (Christmas, FL, USA), a habitat that supports at least 16 anuran species (pers. obs.), one of

which is federally threatened (Gopher frog, *Rana capito*). Due to Florida's sub-tropical climate, anurans can be found calling almost year-round. I selected three sites within Tosohatchee WMA that are at least 1000 m from each other and 1000 m from any paved roads (Figs. 15, 16). One thousand meters is the maximum road-effect zone recorded for anurans, i.e., the distance that the effects of a paved road extend into the landscape (Eigenbrod et al. 2009). By selecting sites beyond this range, I attempted to eliminate any confounding factors from paved roads or from our other study sites. Though there are dirt roads in Tosohatchee near the study sites, they are not frequently traveled and experience a traffic volume of fewer than 50 cars/day, and the park is closed after sunset (which is when sampling occurred), so I consider any noise effects from these unpaved roads to be negligible, if present.

At each of the three sites, we buried an open-top cattle tank (8 ft. circular diameter, 2 ft. deep) filled with dechlorinated water to ensure that there would always be a water source for breeding anurans, many of which seek out fishless water in which to lay their eggs. Although all three sites flooded seasonally, the ponds were installed in case of dry-down events. The ponds were installed in early June 2015 and were stocked with native aquatic vegetation. Though we expected that anurans may have been slow to recognize these ponds as viable breeding spots, anurans and tadpoles had been observed in some of the ponds as early as July 2015.

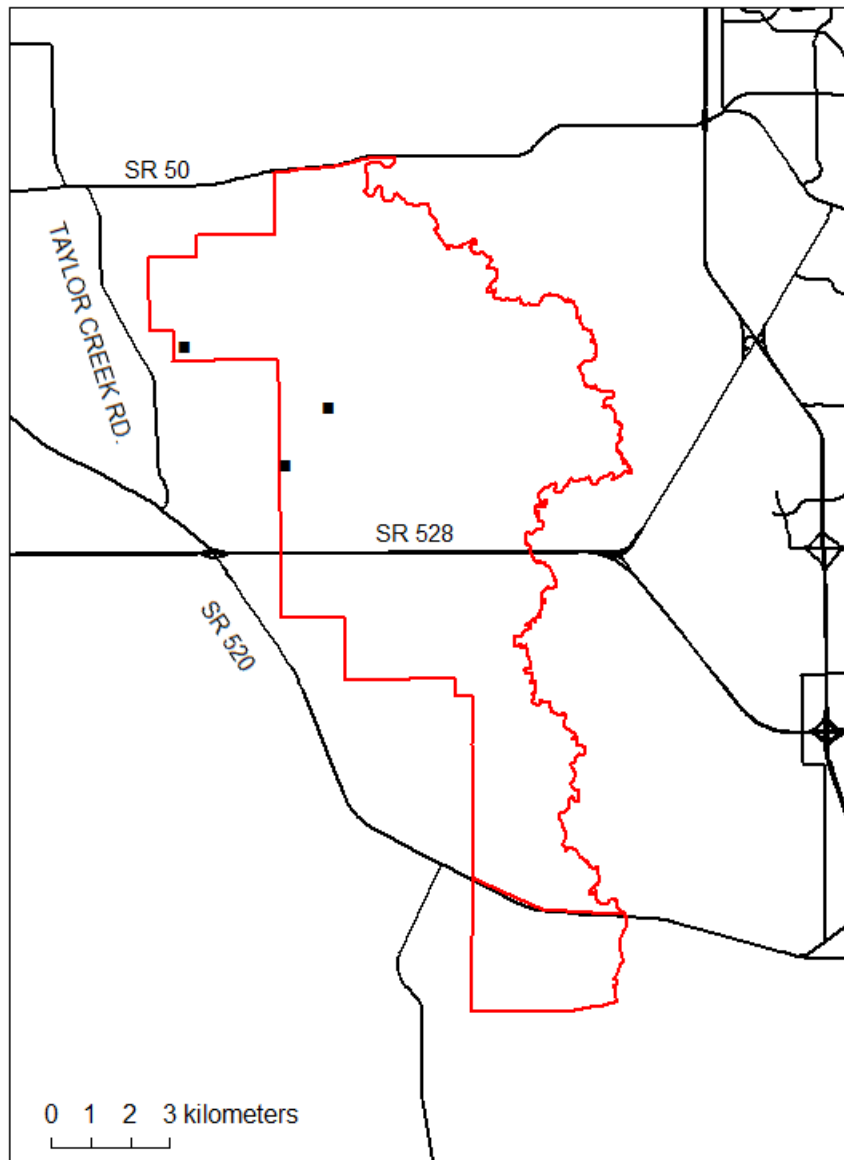


Figure 15. Tosohatchee Wildlife Management Area's location (red outline) relative to paved roads. Black squares show sampling locations.

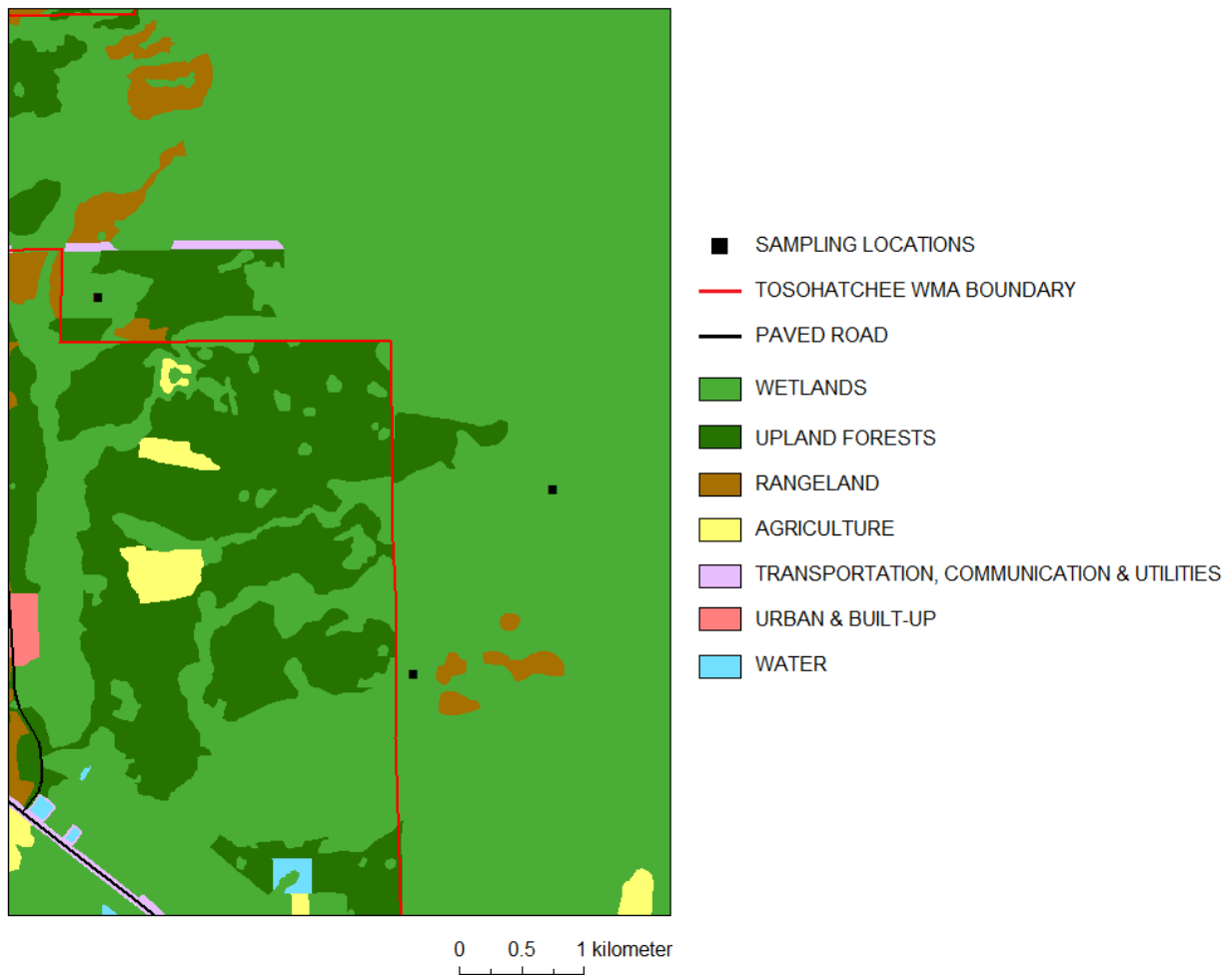


Figure 16. Land use at sampling locations and the surrounding area. Tosohatchee WMA is primarily wetlands, and the sampling locations are in wet flatwoods.

Traffic Noise Playback

Beginning in May 2016, I began broadcasting traffic noise treatments at each of the three pond sites and surveying for adult anuran abundance. The digital traffic noise file was the same as the one used in Chapter 3. Treatments were played using car stereos (XO Vision) connected to 40-

watt coaxial marine speakers (SeaWorthy). The stereo and speakers at each site were powered by a group 24 marine battery connected to a 5-watt solar battery maintainer.

The traffic noise treatments played at the three sites were: (1) control (no noise), (2) traffic, and (3) filtered. Traffic noise was played at 65-70 dB(A) measured at 5m from the speaker, which is comparable to standing 30m from a busy highway (Reijnen et al. 1995). Filtered noise was also played at 65-70 dB(A), but I digitally filtered the noise with the result that signal's amplitude was concentrated at frequencies below 2 kHz; this removes the frequencies of the noise that overlap with many anuran vocalizations (Fig. 17). It was not possible to filter out additional frequencies without sacrificing broadcast amplitude. Study site frogs all vocalize above 400 Hz (Cunnington and Fahrig 2010). Including filtered noise allowed me to test whether anurans avoid all noise, or just noise that interferes with breeding communication. If they only avoid noise that masks their vocalizations, I predicted that abundance should be lower in the traffic treatment than in filtered.

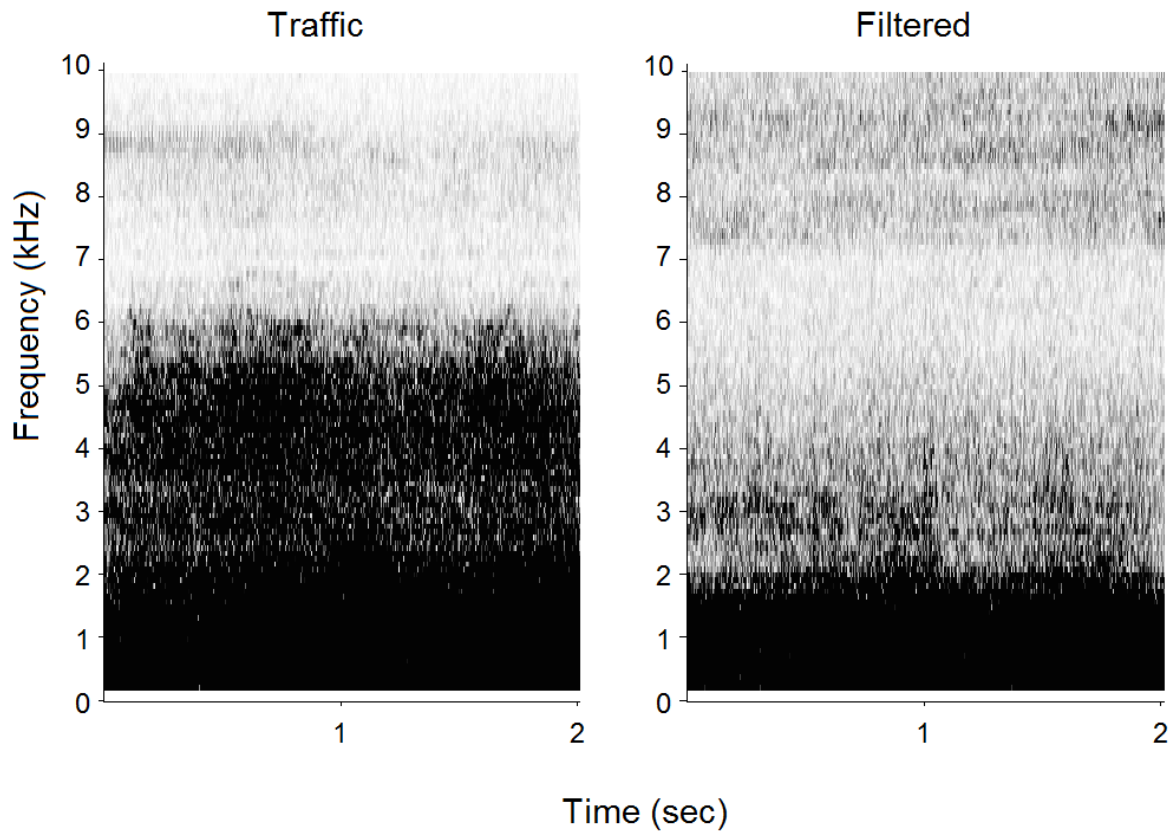


Figure 17. Spectrograms showing the difference between the traffic noise and filtered noise treatments. Anuran species whose vocalizations are above 2 kHz were not masked by the traffic noise.

I played one treatment at each site for 24 hours, turning the noise on in the morning (between 08:00 and 09:00) and off the next morning. Playing the noise for longer periods of time was impossible due to battery limitations; however, bird species have shown immediate decreases in abundance in response to experimental traffic noise (McClure et al. 2013). I sampled one night per week, swapping treatments among sites using a Latin square design that accounts for carry over effects (Wang 2009). With this design, each site experienced each treatment the same number of times and treatment effects can be directly compared while

accounting for day and site. I sampled over 13 weeks from May 2016- August 2016. Due to technical difficulties, the traffic noise broadcast was sometimes cut short (the noise was present in daytime recordings but absent from evening recordings at that site), so those dates could not be included in analysis and resulted in an unbalanced dataset (Table 13).

Table 13. Number of sampling events by site and treatment.

treatment	site 1	site 2	site 3
control	3	3	5
filtered	1	4	3
traffic	3	3	2

Measuring Anuran Abundance

Field recorders (Raspberry Pi computers connected to a microphone) were set out at each site to passively log anuran calls. The devices recorded continuously, but I subsampled by restricting the sampling hours to between 20:00 and 02:00 (when anurans are most likely to be calling; Bridges and Dorcas 2000; North American Amphibian Monitoring Program protocol). To prevent undercounting of species whose vocalizations are masked by traffic noise, I counted the number of calling individuals during 15-minute silent periods built into the traffic noise stimuli that occurred once per hour.

I counted calling individuals during six different hours per night because anuran activity varies by species throughout the night (Bridges and Dorcas 2000). However, it was impossible to

know whether or not calls heard in a different hour represent different individuals. To prevent spurious inflation of my dataset, I used the greatest number of individuals heard in one of the sampled hours as the number of individuals calling per site per night. For example, if I recorded three individual oak toads at 20:00, one individual at 22:00, and two individuals at 1:00, the total number of oak toads heard at that site at that night would be recorded as 3 individuals, not 6.

It is important to note that I indirectly measured anuran abundance using calls as a proxy. However, green frog (*Rana clamitans*) abundances measured using mark-recapture are positively correlated with calling index and calls per minute (Nelson and Graves 2004). Though only males produce calls, male and female frog abundances are positively correlated (Nelson and Graves 2004). Therefore, I expected that any differences in number of calling individuals reflect a true difference in abundance.

Modeling the Effects of Traffic Noise

I tested whether species richness and total abundance (number of individuals encountered, regardless of species) varied with noise treatment in R. Because 2016 was a relatively dry year, I recorded too few individuals of each species (no more than 3 individuals/site/night) to perform analyses of abundance of individual species. I modeled the effects of noise treatment on species richness and abundance using generalized linear models because the amount of samples from each treatment combination were unbalanced (Table 13) and using a Poisson distribution because the dependent variables (species richness and number of individuals) were counts.

I created candidate model sets to explain richness and total abundance using all combinations of treatment, site, humidity and temperature as explanatory variables, as well as a null model. Humidity and temperature were measured at the Tosohatchee weather station and found to be somewhat collinear (Pearson correlation = 0.655; Fig. 18) so the candidate sets did not include models with both factors. The candidate models were tested for overdispersion (R package AER, function “dispersiontest”) and were found to meet the assumptions of the Poisson distribution (they were not overdispersed). I used model selection (AICc; R package MuMIn) to determine the models that explained most of the variance in species richness and total abundance.

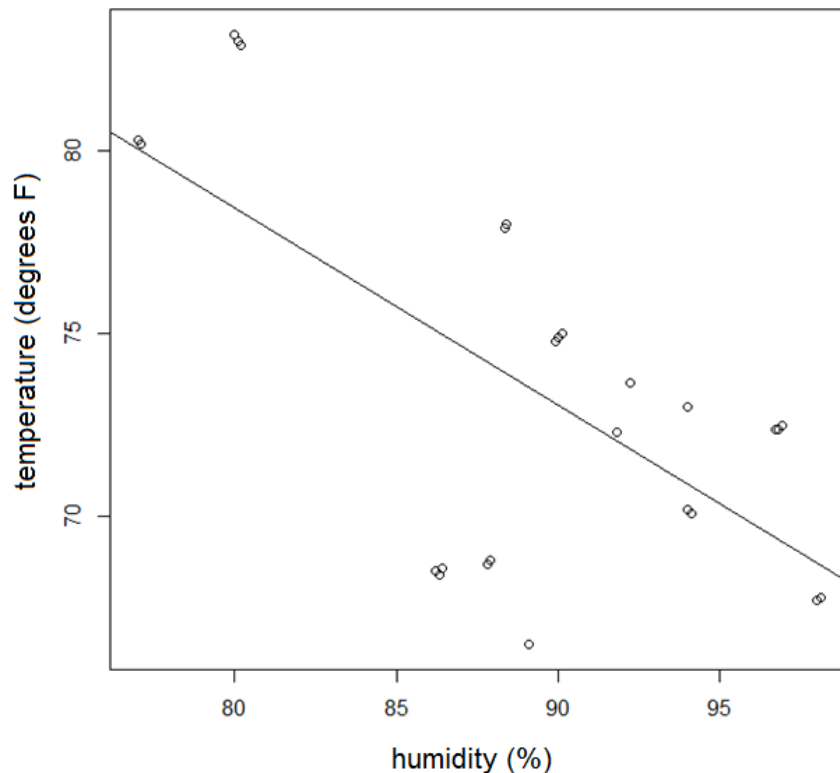


Figure 18. Humidity and temperature were collinear and so were never included in the same candidate model.

Effects of Traffic Noise Treatments on Vocalization Properties

Because I had recordings of anuran vocalizations, I decided to test whether any properties of the vocalizations change when individuals are exposed to traffic noise. Changes in various properties of bird and anuran vocalizations have been observed in response to traffic noise, though response varies greatly between species (reviewed in Slabbekoorn 2013 and Potvin 2016). The most well-documented change is an increase in the minimum frequencies of notes, possibly to reduce the amount of the vocalization that overlaps with (and is masked by) low-frequency traffic noise or as a side effect of singing louder to compensate for the noisy environment (Halfwerk and Slabbekoorn 2009). However, proper assessment of this phenomenon requires recordings made from individuals whose vertical position is known and that are relatively close to the recording equipment, because frequencies of a signal attenuate (are lost) depending on the distance between the signal and receiver and the height at which the signal is produced (Marten and Marler 1977).

Because my recordings were made passively, without knowledge of the distance from the microphone and position of each individual frog, it was not possible to accurately measure the frequencies of calls. A similar problem occurs when trying to measure the amplitude (loudness) of calls; the distance between the calling individual and microphone must be known. Therefore, I measured properties that could be easily identified from a spectrogram, a visual representation of a sound which plots frequency in kHz on the y-axis vs. time on the x-axis (Fig. 19): the duration of the call, number of notes per call, and number of notes per second (rate).

This analysis was limited to one species, the oak toad (*Anaxyrus quercicus*). This was due to spectral overlap between traffic noise and the vocalizations of many species; for these species,

if a call began before a car pass-by, it was impossible to tell if it continued during the car pass-by (illustrated with squirrel treefrog *Hyla squirella* notes in Fig. 19). Therefore, it would be impossible to accurately measure the duration of calls from these species, and would probably result in an observation of spuriously shorter calls in the traffic noise treatment. However, the extremely high-amplitude call of the oak toad (100 dB at 100 cm, Gerhardt 1975) remains both audible and visible on a spectrogram even if there is interference from traffic noise (Fig. 19).

Because there was no way to identify whether one individual called throughout the night, or if different individuals called during different hours, I again only measured calls during one hour per site per night to prevent pseudoreplication. If oak toads called during more than one hour of the night, I chose the hour closest to the peak in oak toad calling activity (Fig. 20), which I calculated by summing the number of times oak toads were found to be calling during each hour over every night that I surveyed for the abundance/species richness analysis. During this hour, I measured the duration, number of notes, and notes/s for all calls during two periods: 5 minutes during the noise treatment (traffic, filtered, or control) and 5 minutes immediately following during the silent period of the recording (Cunnington and Fahrig 2010). These calls were considered subsamples and all values within these two time periods were averaged to determine the average value for the sampling occasion

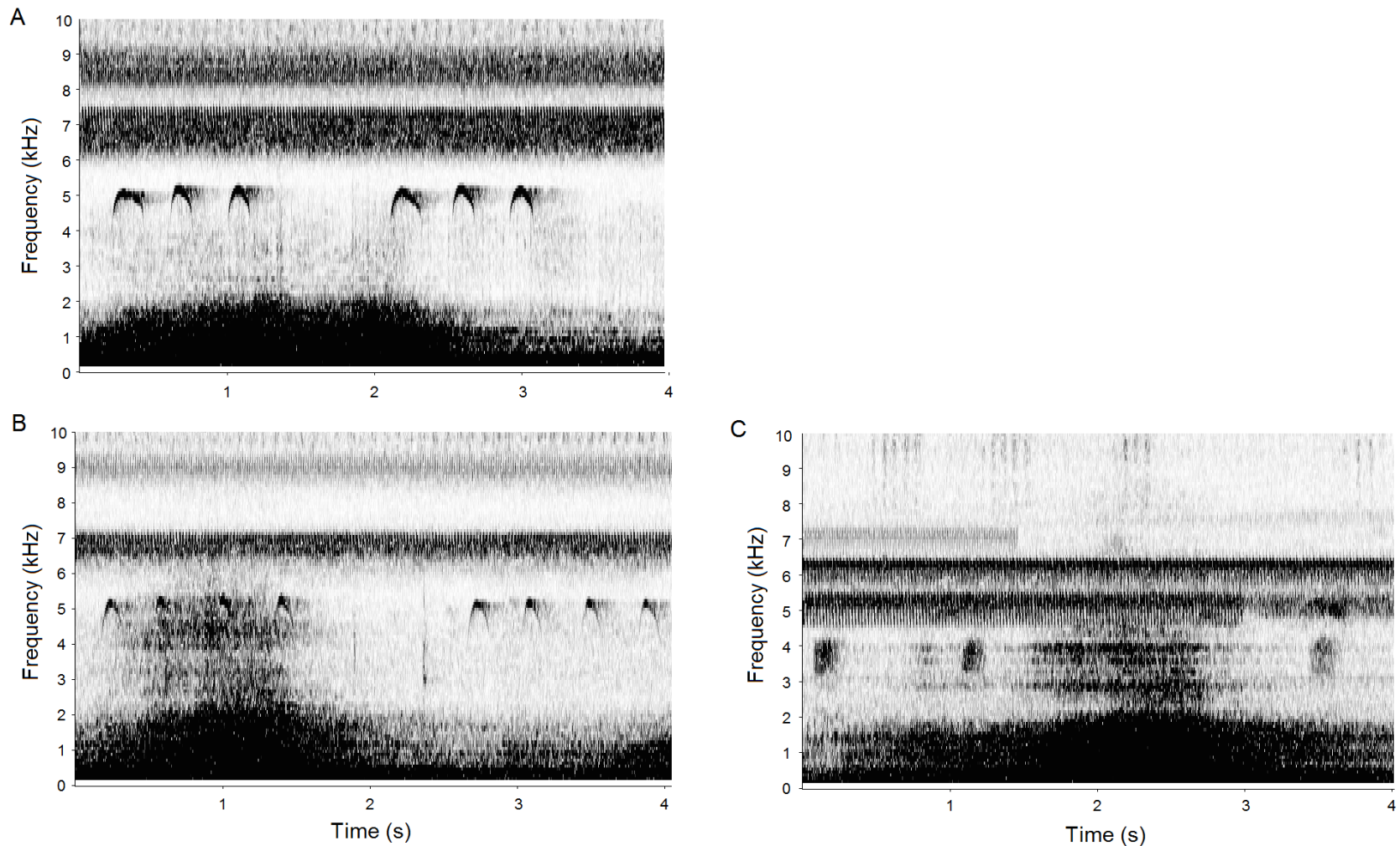


Figure 19. Spectrograms showing oak toad vocalizations in filtered noise (A) and traffic noise (B). Each chevron represents a single oak toad note, while the dark signal along the bottom is the noise treatment; the rise and fall in the noise signal is caused by the sound of a car moving past (car pass-by). Darker colors on the spectrogram represent relatively higher amplitudes. The filtered noise does not contain high-amplitude frequencies that overlap with the oak toad notes, but the traffic noise does. (C) shows squirrel treefrog vocalizations (buzzes between 3-4 kHz) in traffic noise. Because they are not as loud as oak toads, it is impossible to know if the vocalization continues during the car pass-by or if it stops.

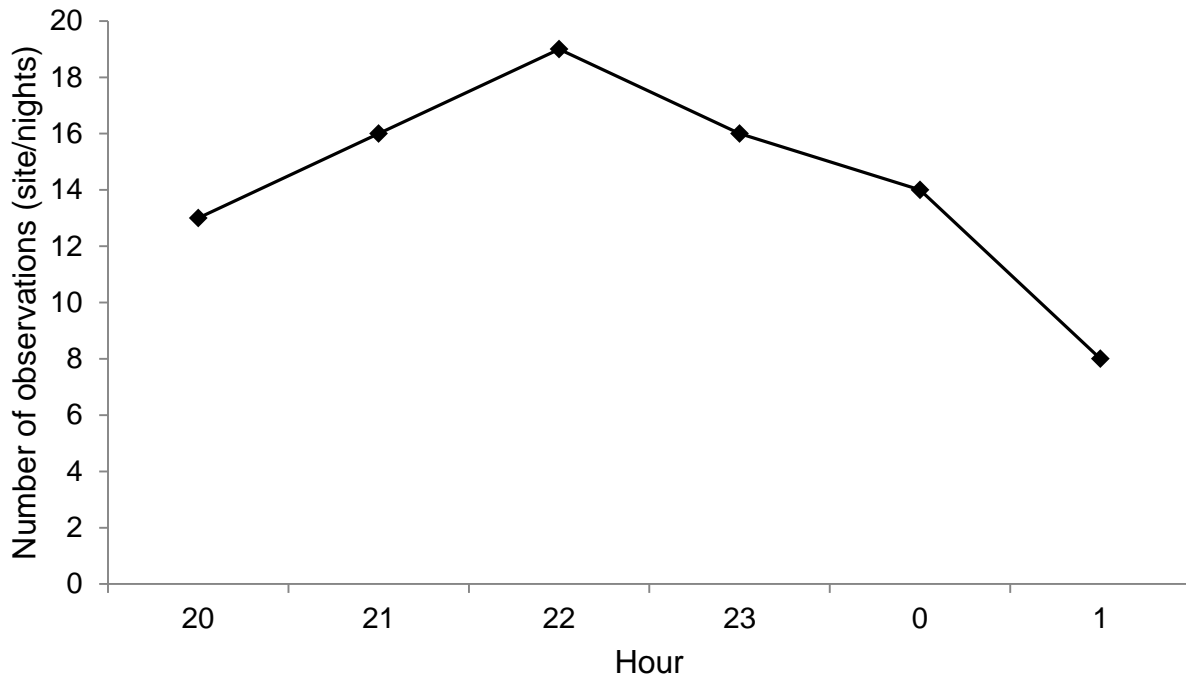


Figure 20. Chart showing the number of times that oak toads were recorded calling during each hour of the night. Calling activity peaks between 22:00 and 23:00. There were 27 possible combinations of date and site during which they could have been detected (Table 13) and oak toads were observed between 22:00 and 23:00 on 19 of those occasions.

I compared the average values for call duration, number of notes per call, and notes per second from the different noise treatments using ANOVA. Visual inspection indicated that variances between treatments were not equal for call duration and number of notes per call, and this was confirmed by F-tests for equal variance (Fig. 21). Therefore, call duration and notes per call were natural log-transformed to meet this assumption (duration: $F_{(6,6)} = 1.71$, $p = 0.265$; notes per call: $F_{(6,6)} = 2.70$, $p = 0.0975$). The assumption of normality was met for all treatment

groups for the natural log-transformed duration and notes/call, as well as for the untransformed call rate (notes/s).

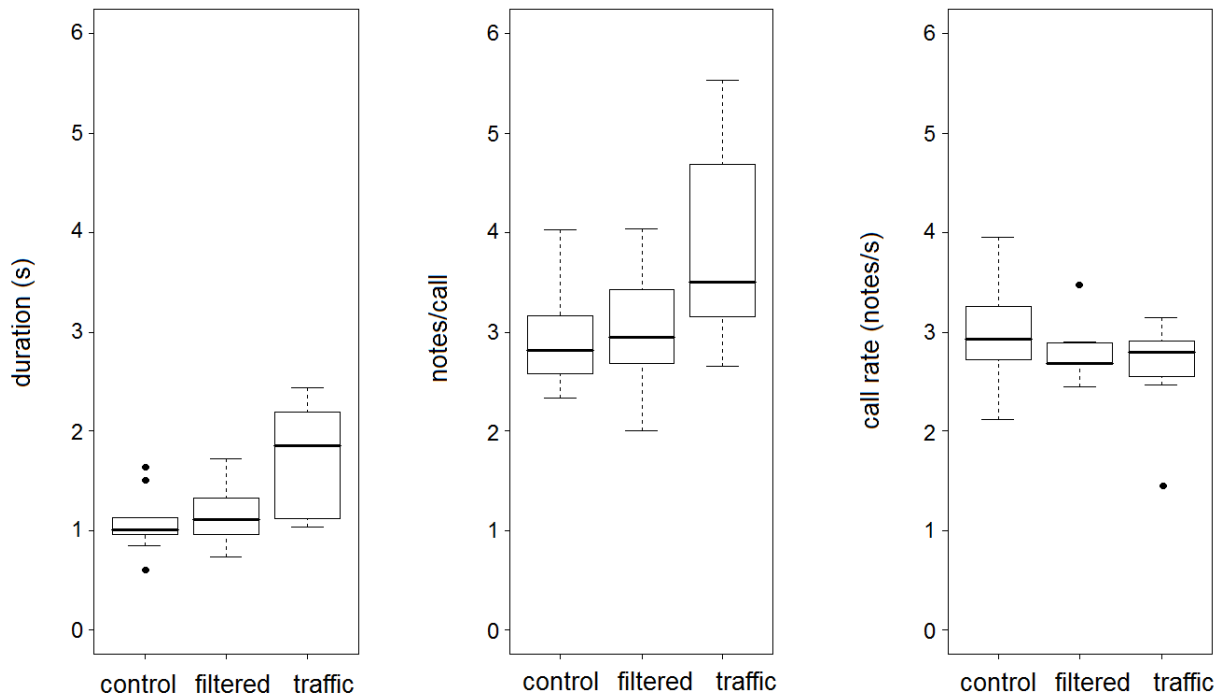


Figure 21. Boxplots of the raw measurements of call parameters (control n= 9, filtered n=7, traffic n=7). Values for duration and notes/call did not meet the assumption of equal variance between groups, so they were natural log-transformed before performing ANOVA.

I also compared the paired values between the noise treatment (filtered or traffic) and the five minutes of silence immediately following to test for plasticity in vocalization properties. This was done using Wilcoxon's signed rank tests due to small sample size.

Results

Anuran species observed

From the recordings, 16 anuran species from 5 different families were identified; however, some species were much more common than others (Table 14).

Table 14. Species identified by their calls from the recordings, reported with the number of independent sampling occasions during which the species was observed.

Species binomial	Common name	Family	# of sites/night species observed (out of 27)
<i>Anaxyrus quercicus</i>	Oak toad	Bufonidae	21
<i>Anaxyrus terrestris</i>	Southern toad	Bufonidae	4
<i>Eleutherodactylus planirostris</i>	Greenhouse frog	Eleutherodactylidae	22
<i>Acris gryllus</i>	Cricket frog	Hylidae	4
<i>Hyla cinerea</i>	Green treefrog	Hylidae	4
<i>Hyla femoralis</i>	Pine woods treefrog	Hylidae	4
<i>Hyla gratiosa</i>	Barking treefrog	Hylidae	3
<i>Hyla squirella</i>	Squirrel treefrog	Hylidae	22
<i>Osteopilus septentrionalis</i>	Cuban treefrog	Hylidae	5
<i>Pseudacris crucifer</i>	Spring peeper	Hylidae	1
<i>Pseudacris nigrata verrucosa</i>	Florida chorus frog	Hylidae	3
<i>Pseudacris ocularis</i>	Little grass frog	Hylidae	19
<i>Gastrophryne carolinensis</i>	Eastern narrow-mouthed toad	Microhylidae	3
<i>Rana catesbeianus</i>	American bullfrog	Ranidae	2
<i>Rana grylio</i>	Pig frog	Ranidae	2
<i>Rana sphenoccephala</i>	Southern leopard frog	Ranidae	15

Species richness

Overall, I recorded a mean of 3.85 species/site/night (median 4 species). Model selection found that none of the proposed models explained species richness better than the null model (Table 15). The second model, within 2 $\Delta AICc$ of the null model and weighted similarly, included treatment, site, and humidity as explanatory factors. However, looking at the output of this model (Table 16), the species richness at neither the filtered or traffic sites differed significantly from control sites at $\alpha=0.05$.

Table 15. Candidate model set for species richness.

model	df	AICc	$\Delta AICc$	Akaike weight
glm(richness ~ 1, family=poisson)	1	109	0	0.408
glm(richness ~ treatment + site + humidity, family=poisson)	6	109	0.27	0.357
glm(richness ~ treatment, family=poisson)	3	111	2.39	0.124
glm(richness ~ site, family=poisson)	3	112	3.09	0.0869
glm(richness ~ treatment + site, family=poisson)	5	115	6.65	0.0147
glm(richness ~ treatment + site + temp, family=poisson)	6	116	7.71	8.66E-03
glm(richness ~ treatment*site + humidity, family=poisson)	10	125	16.4	1.13E-04
glm(richness ~ treatment*site, family=poisson)	9	128	19.5	2.38E-05
glm(richness ~ treatment*site + temp, family=poisson)	10	132	23.3	3.62E-06
glm(richness ~ treatment*site*humidity, family=poisson)	17	193	84.6	1.75E-19

Table 16. Model output from the “second-best” model in the candidate set explaining species richness. Estimates of effect sizes comparing the listed treatments to the control and listed sites to site 1 are given.

	Estimate	SE	z	Pr(> z)
Intercept	-3.10	1.62	-1.91	0.0561 [†]
filtered treatment	-0.346	0.256	-1.35	0.177
traffic treatment	-0.506	0.262	-1.93	0.0533 [†]
site 2	-0.505	0.263	-1.92	0.0547 [†]
site 3	-0.510	0.270	-1.89	0.0591 [†]
humidity	0.056	0.019	3.01	0.00261*

* significant at $\alpha=0.05$

[†] significant at $\alpha=0.10$

Abundance

Overall, I recorded on average 5.54 individuals/site/night (this is considering individuals of all species; median 5 individuals). Unlike with species richness, anuran abundance was best explained by a model that included treatment, site, and humidity as factors, a model which was weighted extremely highly (Table 17). The model output (Table 18) shows that overall abundance was significantly lower in the traffic treatment than in the control treatment (about 1 individual fewer), but the filtered treatment was not significantly different from the control.

Table 17. Candidate model set for overall abundance of anurans.

model	df	AICc	Δ AICc	Akaike weight
glm(abundance ~ treatment + site + humidity, family=poisson)	6	131	0	0.999
glm(abundance ~ treatment*site + humidity, family=poisson)	10	145	14.2	0.000826
glm(abundance ~1, family=poisson)	1	154	23.3	8.93E-06
glm(abundance ~ treatment, family=poisson)	3	155	24.0	6.19E-06
glm(abundance ~ site, family=poisson)	3	159	28.1	8.06E-07
glm(abundance ~ treatment + site + temp, family=poisson)	6	160	29.3	4.42E-07
glm(abundance ~ treatment + site, family=poisson)	5	160	29.7	3.48E-07
glm(abundance ~ treatment*site, family=poisson)	9	166	35.4	2.06E-08
glm(abundance ~ treatment*site + temp, family=poisson)	10	170	38.9	3.49E-09
glm(abundance ~ treatment*site*humidity, family=poisson)	17	204	73.3	1.22E-16

Table 18. Output from the top model in the candidate set explaining anuran abundance.

Estimates of effect sizes comparing the listed treatments to the control and listed sites to site 1 are given.

	Estimate	SE	z	Pr(> z)
Intercept	-6.04	1.50	-4.02	5.73E-05*
filtered treatment	-0.119	0.200	-0.594	0.553
traffic treatment	-0.730	0.235	-3.11	0.00189*
site 2	-0.282	0.228	-1.24	0.215
site 3	-0.411	0.245	-1.68	0.0931 [†]
humidity	0.0911	0.0170	5.37	7.97E-08*

Vocalization properties- between treatments

Call duration was on average 1.53 seconds longer in the traffic treatment than in the control treatment (Tukey's post-hoc test, log-transformed average difference = 0.426, $p = 0.0388$; Table 19) but there was no significant difference between the control and filtered treatment. There was a trend for number of notes per call to be higher (average 1.30 notes more) in the traffic treatment than the control treatment (Tukey's post-hoc test, log-transformed average difference = 0.264, $p = 0.0745$; Table 19), but there was no significant difference in call rate (notes/s) between treatments (Table 19).

Table 19. ANOVA outputs comparing vocalization properties across treatments.

		d.f.	Sum Sq.	Mean Sq.	F	Pr(>F)
ln(duration)	treatment	2	0.7879	0.3939	3.877	0.0378*
	residuals	20	2.0324	0.1016		
ln(notes/call)	treatment	2	0.3203	0.16016	3.158	0.0643 [†]
	residuals	20	1.0143	0.05071		
call rate (notes/s)	treatment	2	0.5	0.25	0.99	0.389
	residuals	20	5.051	0.2525		

Plasticity in vocalization properties

Wilcoxon's signed rank tests found no significant differences in call duration, notes/call, or call rate between exposure to traffic noise and the silent period immediately following (Fig. 22).

There was a statistically significant increase in call duration and notes/call in the filtered treatment, but these results appear to be strongly influenced by one data point, and so I reject the idea that there is a biologically significant trend indicating immediate plasticity (Fig. 22).

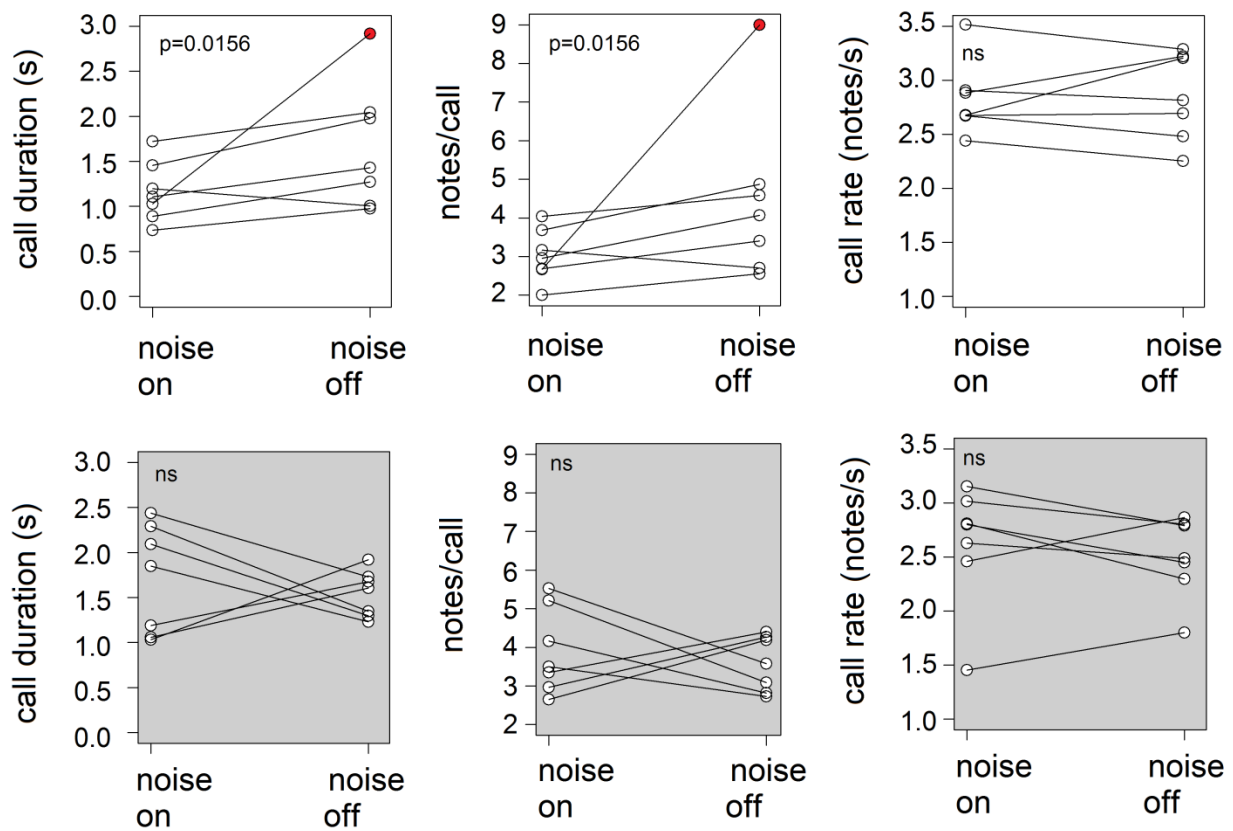


Figure 22. Wilcoxon's signed rank tests reveal little evidence for plasticity in oak toad vocalizations between noise treatments and the silent period immediately following (n=7 pairs in all cases). Plots with unshaded backgrounds show comparisons between filtered noise and silence while shaded plots show comparisons between traffic noise and silence.

Discussion

Traffic noise significantly reduced the total number of anurans observed compared to the control treatment, an effect previously observed in migrating birds (McClure et al. 2013). Due to the low overall abundance of anurans during the 2016 field season, the observed difference was small- an effect size of roughly one individual fewer- but the fact that this pattern was detected despite low sample sizes indicates that it is probably an ecologically significant pattern. The low sample size is likely due to a relatively dry field season in 2016 during which the wet flatwoods did not flood, compared to pilot site selection in 2015. There was not a significant difference in number of individuals observed between the control and filtered treatments, but the effect size for the filtered treatment was also negative, so it is possible that my study simply lacked the power to detect a difference. Therefore, I am not willing to say that anurans are unaffected by noise that does not overlap with their vocalizations; however, this study did not provide evidence to the contrary. Low power may also explain the lack of significant results regarding species richness, though both the filtered and traffic treatments had negative effect sizes. In the future, I would like to expand upon this study to include more sites and multiple field seasons to increase sample size and power.

Oak toads produced longer calls at traffic noise sites than at control sites, an effect that appears to be due to an increase in number of notes per call rather than an increase in call rate (notes/s). The fact that increased call duration did not occur at filtered noise sites indicates that it is a response to the decreased signal-to-noise ratio of oak toad calls experienced during the traffic, but not the filtered, treatment. This was a curious result, because previous work on anurans has found the exact opposite pattern: green frogs (*Rana clamitans*) and northern leopard

frogs (*Rana pipiens*) reduce call rate (calls/min) and amplitude but increase dominant frequency in response to traffic noise; gray treefrogs (*Hyla versicolor*) also reduce call rate (Cunnington and Fahrig 2010). European tree frogs (*Hyla arborea*) reduce the length of their calling bouts when exposed to traffic noise (Lengagne 2008), as do spring peepers (*Pseudacris crucifer*) exposed to masking noise (Hanna et al. 2014). These responses suggest that these species expend less energy calling when there is interference from traffic noise, which makes sense from a theoretical standpoint; calling is one of the most energetically expensive activities frogs perform (Pough et al. 1992), and therefore males should spend more time calling at times when the effective transmission distance of those calls will be greater (Wells and Schwartz 2007).

However, not all anuran species decrease call duration in response to anthropogenic noise. Sun and Narins (2005) found that while three species of co-occurring frogs in Thailand (*Microhyla butleri*, *Rana nigrovittata* and *Kaloula pulchra*) vocalized less during airplane flyovers, another species found in the pond assemblage, *Rana taipehensis*, consistently increased calling effort during flyovers. Sun and Narins hypothesize that *R. taipehensis* was exploiting the reduction from interspecific calls during the airplane flyovers, and it is possible that the same thing is happening here; however, my data were not collected for the purpose of answering that question. It is also possible that oak toads are exhibiting another adaptive strategy: increasing signal redundancy to increase the probability that the signal will be communicated (Shannon and Weaver, 1949). This adaptation is well documented in birds; Japanese Quail (Potash 1972) and King Penguins (Lengagne et al. 1999) increase the number of syllables per call as background noise increases, and Silvereyes (*Zosterops lateralis*) and Chaffinches (*Fringilla coelebs*) increase call duration in response to noise playback (Brumm and Slater 2006; Potvin and Mulder 2013).

Though my study was the first to observe increased redundancy of anuran calls in response to traffic noise, redundancy has been observed in many (but not all) species as a response to anuran chorusing (reviewed in Schwartz and Bee 2013), which has been studied far more extensively than anurans' response to traffic noise. Anurans often increase the duration and/or number of notes in noisy chorus situations, so it makes sense that the behavior could be a response to other noisy situations like traffic noise.

I found no evidence for immediate plasticity in oak toad call characteristics, but the fact that I did find significant differences in call duration between sites receiving different treatments suggest that this may be due to a lack of power. Plasticity in call characteristics in response to experimental traffic noise has been observed in some anurans (Sun and Narins 2005; Lengagne 2008; Cunningham and Fahrig 2010; 2013) and in a long list of birds (reviewed in Slabbekoorn 2013 and Potvin 2016).

Great strides have been made in designing and installing safe routes for wildlife to cross roads without being hit by cars, including "ecopassages" designed specifically for reptiles and amphibians. However, we are usually not able to assess if installing such structures actually increases the rate of successful crossings, since monitoring often does not begin until after the mitigation is in place. If noise has a deterrent effect on wildlife and causes habitat fragmentation because animals avoid roadside areas, then these crossings, which are expensive and difficult to maintain, may not be the best option for conservation of certain species. In addition, if traffic noise itself is found to have harmful effects, then research can be done into creating species- or site-specific noise mitigation along with road-kill prevention.

RECOMMENDATIONS BASED ON THIS RESEARCH

This work presented within this dissertation provides new insights in the field of road ecology and has implications for improving mitigation of roads' lethal and sub-lethal effects. As our understanding of road-related behaviors improves, these insights should be incorporated into strategies to minimize road effects.

Recommendations for improving the effectiveness of Roadside Animal Detection Systems

My simulator study, verified by our field study, shows that RADS have great potential to reduce crash probability, making roads safer for large animals and for humans. They are also much less expensive than wildlife crossings such as overpasses and underpasses. However, currently these systems are plagued with false positives, contributing to driver acclimation. I recommend the following strategies based on our observations:

- Now that the potential for RADS to greatly reduce crashes has been empirically demonstrated, efforts to improve system reliability are crucial and warranted. To reduce false positives, options such as sensor shades to prevent solar interference should be explored. In regions with a low water table, buried sensors, which are less sensitive to environmental conditions, could be a good option.
- To reduce driver acclimation to the warning system, I recommend that use of these systems should be limited in time and space. Specifically, these systems will have greatest effect if they are used only during times of peak animal movement (e.g., migrations). In addition, I recommend reducing the spatial extent of the system (the system we studied incorporated 1.3 miles of sensors, and if an animal was detected at any

point, all signs flashed). Using sensors only at known crossing points (or funneling animals toward those crossing points using fences) will improve driver confidence in the system.

- Because picture-based signs performed better at reducing crashes than word-based signs in the driving simulator, I recommend using picture-based signs in all field installations.

Recommendations for reducing negative effects of traffic noise

My research found that traffic noise reduces adult anuran abundance, evidently by interfering with their ability to communicate. It is likely that other vocal species (e.g., birds), are similarly affected. I also found that traffic noise alters tadpole behavior, though I did not find evidence that tadpole size at metamorphosis is negatively affected.

- Efforts should be made to minimize the number of new roads built whenever possible to keep large patches of habitat intact and unaffected by traffic noise.
- Reducing traffic volume (cars/min) and speed reduces the overall level of traffic noise. I recommend introducing speed limits, especially in state and national parks, which can be reinforced with speed bumps/ rumble strips.
- Future research should explicitly test for species-specific differences in reactions to traffic noise within a single taxon.

APPENDIX A: IRB APPROVAL LETTER



University of Central Florida Institutional Review Board
Office of Research & Commercialization
12201 Research Parkway, Suite 501
Orlando, Florida 32826-3246
Telephone: 407-823-2901 or 407-882-2276
www.research.ucf.edu/compliance/irb.html

Approval of Human Research

From: **UCF Institutional Review Board #1**
FWA00000351, IRB00001138

To: **Daniel J. Smith and Co-PI: Reed F. Noss**

Date: **May 06, 2013**

Dear Researcher:

On 5/6/2013, the IRB approved the following human participant research until 5/5/2014 inclusive:

Type of Review: UCF Initial Review Submission Form
Project Title: Assessing the Effectiveness and Reliability of the Roadside
Animal Detection System on US Highway 41 near the Turner
River in Collier County
Investigator: Daniel J. Smith
IRB Number: SBE-13-09322
Funding Agency: FL Department of Transportation
Grant Title: 1cAssessing the Effectiveness and Reliability of the Roadside
Animal Detection System on US Hwy 41 near the Turner River
in Collier County. Contract No. BVD 37, TWO No. 1
Research ID: N/A

The scientific merit of the research was considered during the IRB review. The Continuing Review Application must be submitted 30 days prior to the expiration date for studies that were previously expedited, and 60 days prior to the expiration date for research that was previously reviewed at a convened meeting. Do not make changes to the study (i.e., protocol, methodology, consent form, personnel, site, etc.) before obtaining IRB approval. A Modification Form **cannot** be used to extend the approval period of a study. All forms may be completed and submitted online at <https://iris.research.ucf.edu>.

If continuing review approval is not granted before the expiration date of 5/5/2014, approval of this research expires on that date. When you have completed your research, please submit a Study Closure request in iRIS so that IRB records will be accurate.

Use of the approved, stamped consent document(s) is required. The new form supersedes all previous versions, which are now invalid for further use. Only approved investigators (or other approved key study personnel) may solicit consent for research participation. Participants or their representatives must receive a copy of the consent form(s).

In the conduct of this research, you are responsible to follow the requirements of the Investigator Manual.

On behalf of Sophia Dziegielewski, Ph.D., L.C.S.W., UCF IRB Chair, this letter is signed by:

Signature applied by Joanne Muratori on 05/06/2013 11:12:48 AM EDT

IRB Coordinator

APPENDIX B: IACUC APPROVAL LETTERS



3/23/2015

Dr. Reed Noss
Biology
BL210
4000 Central Florida Blvd.
Orlando, FL 32816

Subject: Institutional Animal Care and Use Committee (IACUC) Protocol Submission

Dear Dr. Reed Noss:

This letter is to inform you that your following animal protocol was approved by the IACUC. The IACUC Use Approval Form is attached for your records.

Animal Project #: 15-11W
Title: How does traffic noise influence abundances of animal populations? An investigation using anurans as a model.

First Approval Date: 3/20/2015

Please be advised that IACUC approvals are limited to one year maximum. Should there be any technical or administrative changes to the approved protocol, they must be submitted in writing to the IACUC for approval. Changes should not be initiated until written IACUC approval is received. Adverse events should be reported to the IACUC as they occur. Furthermore, should there be a need to extend this protocol, a renewal must be submitted for approval at least three months prior to the anniversary date of the most recent approval. If the protocol is over three years old, it must be rewritten and submitted for IACUC review.

Should you have any questions, please do not hesitate to call the office of Animal Welfare at (407) 882-1164

Please accept our best wishes for the success of your endeavors.

Best Regards,

A handwritten signature in black ink that reads "Cristina Caamaño".

Cristina Caamaño
Associate Director, Research Program Services
Office of Research and Commercialization

Copies: Appropriate Facility Manager (when applicable)



THE UNIVERSITY OF CENTRAL FLORIDA
INSTITUTIONAL ANIMAL CARE and USE COMMITTEE (IACUC)
Approval to Use Animals

Dear Dr. Reed Noss,

Your application for IACUC Approval has been reviewed and approved by the UCF IACUC Reviewers.

Approval Date: 3/20/2015

Title: How does traffic noise influence abundances of animal populations? An investigation using anurans as a model.

Department: Biology

Animal Project #: 15-11W

Expiration: 3/19/2016

You may purchase and use animals according to the provisions outlined in the above referenced animal project.

Christopher Parkinson, Ph.D.
IACUC Chair



1/20/2016

Dr. Reed Noss
Biology
BL210
4000 Central Florida Blvd.
Orlando, FL 32816

Subject: Institutional Animal Care and Use Committee (IACUC) Protocol Submission

Dear Dr. Reed Noss:

This letter is to inform you that your following animal protocol was re-approved by the IACUC. The IACUC Animal Use Renewal Form is attached for your records.

Animal Project #: 15-11W
Title: How does traffic noise influence abundances of animal populations? An investigation using anurans as a model.

First Approval Date: 3/20/2015

Please be advised that IACUC approvals are limited to one year maximum. Should there be any technical or administrative changes to the approved protocol, they must be submitted in writing to the IACUC for approval. Changes should not be initiated until written IACUC approval is received. Adverse events should be reported to the IACUC as they occur. Furthermore, should there be a need to extend this protocol, a renewal must be submitted for approval at least three months prior to the anniversary date of the most recent approval. If the protocol is over three years old, it must be rewritten and submitted for IACUC review.

Should you have any questions, please do not hesitate to call the office of Animal Welfare at (407) 882-1164

Please accept our best wishes for the success of your endeavors.

Best Regards,

A handwritten signature in black ink that reads "Cristina Caamaño".

Cristina Caamaño
Associate Director, Research Program
Services

Copies: Facility Manager (when applicable.)



THE UNIVERSITY OF CENTRAL FLORIDA
INSTITUTIONAL ANIMAL CARE and USE COMMITTEE (IACUC)
Re-Approval to Use Animals

Dear Dr. Reed Noss,

Your application for IACUC Re-Approval has been reviewed and approved by the UCF IACUC Reviewers.

Approval Date: 1/15/2016

Title: How does traffic noise influence abundances of animal populations? An investigation using anurans as a model.

Department: Biology

Animal Project #: 15-11W

Expiration: 3/19/2017

You may purchase and use animals according to the provisions outlined in the above referenced animal project. This project will expire as indicated above. You will be notified 2-3 months prior to your expiration date regarding your need to file another renewal.

Christopher Parkinson, Ph.D.
IACUC Chair

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