The complexities of living with the nine-banded armadillo (*Dasypus novemcinctus*): leprosy, management, and homeowner perceptions

by

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Abstract

The management of nuisance wildlife damage is complex and requires the understanding of human perceptions, practical management methods, and species-specific factors. For the nine-banded armadillo (*Dasypus novemcinctus*), management is complicated by a lack of research on effective methods and the species' tendency to occupy urbanized areas. Although extension agents in Alabama and the remainder of the southeast United States recommend several management methods, residents' acceptance of these methods and their tolerance for the species is not well understood. Armadillo management is also further complicated by the presence of leprosy-causing bacteria which can negatively impact armadillo populations. Leprosy may also be a public health concern, however, prevalence studies in the United States have been limited to the lower coastal plain and few studies have been conducted in Alabama. In this thesis, I address these research gaps in three studies. First, I conducted a systematic review of the existing literature related to leprosy's impact on wild armadillo populations and the spatial and temporal patterns of infection. Next, I evaluated the prevalence of leprosy in wild armadillo populations in two Alabama counties, Mobile and Lee County, AL, and found a lower disease prevalence in central Alabama compared to the south; these findings may follow the ecologic-constraints hypothesis and be a result of different ecoregions. Finally, I evaluated Alabama residents' tolerance for armadillos using a wildlife acceptance capacity (WAC) model and based on the results, refined this model to include a factor of disease risk perception. Residents in Alabama generally have a low tolerance for armadillos and expressed difficulties managing them on their property. The results of this thesis provide further insights into armadillo management in the

southeast and the current prevalence of leprosy infection in populations further north of the Gulf Coast.

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Abstract

Leprosy is a chronically infectious disease in humans that is caused by infection with *Mycobacterium leprae,* or the more recently discovered *Mycobacterium lepromatosis.* Wild nine–banded armadillos (*Dasypus novemcinctus)* of North and South America are known to be natural hosts of *M. leprae* and can develop a full range of the disease, symptomatically similar to that in humans. The current global knowledge of the epidemiology and patterns of *M. leprae* infection in wild armadillo populations were reviewed in this paper. I recovered 158 records, with only six relevant papers that evaluated leprosy's impacts on wild armadillo demographics and patterns of infection. A common finding of the included papers is that leprosy does not appear to significantly impact wild armadillo populations in terms of reproduction; however, differences in infection between the sexes was inconclusive. Additionally, leprosy appears to significantly impact mortality and age structure of armadillo populations, due to older adults being increasingly infected. Spatially, leprosy does not appear to cluster within wild armadillo populations. Temporal patterns of infections were inconclusive and require further study. Major limitations of the reviewed studies include a small geographic scale limited to the southeast United States, an inclusion of populations only in natural or rural areas, and limited diagnostic techniques for detecting infection. Future research should evaluate the spatiotemporal and epidemiological impacts on populations further east of the Mississippi River and in South and Central America, where populations may be genetically distinct. The findings documented should also attempt to be replicated using more sensitive diagnostic testing such as polymerase

chain reaction (PCR). Additionally, more research needs to be done on other strains of leprosy–causing bacterium, such as *M. lepromatosis*, to evaluate its presence and potential impacts on wild nine–banded armadillo populations. A better understanding of the impacts and distribution of leprosy in wild populations would allow for improved management recommendations for nuisance armadillos throughout their range. Additionally, the cultural consumption of armadillo meat and management of nuisance armadillos in human–dominated environments allows for potential exposure to leprosy–causing bacteria that may present a public health risk.

Introduction

The nine–banded armadillo (*Dasypus novemcinctus*), hereafter referred to as 'armadillo,' is native to South and Central America, expanding to North America in the nineteenth–century (Taulman and Robbins 1996). The armadillo has a low tolerance for colder weather, limiting northward expansion in the United States. As a burrowing insectivore, it is also believed that suitable soils and sufficient precipitation are factors limiting the species' westward expansion (Taulman and Robbins 2014). As a result, armadillos are common in South America, Mexico, and the southeast United States, although, in recent years they have been found as far north as southern Illinois (Taulman and Robbins 2014; Haywood *et al.* 2022).

Armadillos are mainly nocturnal but can be seen during periods of daylight; individuals are also solitary with the exception of females with young offspring (Clark 1951). In the late spring and early summer, males disperse further distances than they would typically travel the remainder of the year in order to find a mate (McDonough 2000). Once a mate is found, males and females breed annually during the summer in non–monogamous pairs (McDonough 1997). If fertilization is successful, female armadillos delay implantation of the fertilized egg until the fall, giving birth to identical quadruplets around 18 weeks later in early spring (Prodöhl *et al.* 1996). Among the four littermates, kin selection does not appear to occur, and juveniles typically disperse out of the population, away from siblings, by the following fall (McDonough and Loughry 1997; McDonough *et al.* 1998). According to McDonough and Loughry (1997), the age structure of a wild armadillo population is mainly adults as most juveniles are depredated before reaching adulthood. If juveniles do reach adulthood, the lifespan for an adult armadillo in the wild is estimated to be between eight and twelve years (Loughry *et al.* 2013).

Mortality in wild armadillo populations may be a result of extreme environmental conditions such as drought, depredation of juveniles, and vehicular collisions (McDonough and Loughry 1997). An additional source of mortality may be disease, specifically leprosy caused by *Mycobacterium* spp. Leprosy, often known as "Hansen's Disease" in human health, is a neglected tropical disease (NTD), or one that causes potentially deadly impacts and persists in developing nations; leprosy, in particular, mainly affects the peripheral nerves and mucus membranes of its host (Joshi *et al.* 2021). The disease–causing agents of leprosy are acid–fast rod–shaped bacteria, *Mycobacterium leprae,* and the more recently discovered *M. lepromatosis*, which are obligate, intracellular pathogens (Scollard *et al.* 2006; Han *et al.* 2008; Scollard 2016). In North America, nine–banded armadillos are the only known natural reservoirs of the bacteria, outside of humans (Truman 2005).

Armadillos were discovered to be efficient animal models of leprosy in the 1960s, contributing to the use of the species in clinical research (Balamayooran *et al.* 2015). In 1974 and 1975, wild armadillos in Louisiana were discovered to have leprosy (Center for Disease Control and Prevention 1976). Since the 1970s, leprosy has been detected in armadillo populations throughout parts of their range in the United States and in South America (Loughry *et al.* 2009; Truman *et al.* 2011; Sharma *et al.* 2015; da Silva *et al.* 2018; Stefani *et al.* 2019). In the southeast United States, where most of the research on leprosy in wild armadillos has occurred, prevalence has ranged from 0% to 25%, using a combination of serologic, molecular, and histopathologic detection (Howerth *et al.* 1990; Loughry *et al.* 2009; Truman *et al.* 2011; Sharma *et al.* 2015). Leprosy prevalence has also been studied in South America, particularly in Brazil where nine–banded armadillos are native, and prevalence has ranged from 0% to 63% (Antunes and Deps 2003; Deps *et al.* 2008b; Pedrini *et al.* 2010; Frota *et al.* 2012; de Souza 2016; da Silva *et al.* 2018; Stefani *et al.* 2019). However, sample sizes in South American countries have been smaller than in the United States due to legal protections that do not permit the use of many specimens, particularly the limitations of Brazilians laws where most of the research has occurred (as reviewed in: Deps *et al.* 2020).

Inoculation of armadillos with *M. leprae* in the laboratory has demonstrated that detectable signs of infection can occur between six months to four years later, depending on the route of infection (Storrs *et al.* 1974; Duthie *et al.* 2011). Once infected, the bacteria become localized mainly in the reticuloendothelial system and by 18 to 24 months, an individual has typically developed a severe infection (Balamayooran *et al.* 2015). Balamayooran *et al.* (2015) also notes that, although armadillos display the full spectrum of disseminated disease, most infections in armadillos are a lepromatous–type response, which is indicative of a poor immune response to the bacteria. Once infected, armadillos with leprosy succumb to the disease within a year or two of infection as a result of bacillated macrophages in the skin, liver, spleen, lungs, and lymph nodes; infection with the bacteria can result in lesions in the lungs and other organs, leading to organ failure, or make an individual more susceptible to concurrent infections, resulting in death (Job *et al.* 1985; Truman 2005; Vijayaraghavan 2009).

Armadillos are believed to be highly susceptible to infection with *M. leprae*, however, one study found that there were different individual–level immune responses with 15% to 20% of armadillos resisting infection; it is currently believed that resistance to infection may have a genetic basis in armadillos (Truman *et al.* 2014). Genetic resistance is similar in humans with the discovery of genes such as NRAMP1 that control immune response and thus susceptibility (Scollard *et al.* 2006). In armadillos, those that are susceptible will likely become infected through the inhalation of infectious aerosols of an infected individual; however direct and indirect leprosy transmission routes require further investigation (as reviewed in: Ploemacher *et al.* 2020).

Armadillos' susceptibility to infection with leprosy may be due to a shorter period of co–evolutionary history with the pathogen as, according to genetic studies, the species was first exposed within the past 500 years (Monot *et al.* 2005). A shorter co–evolutionary period may cause leprosy to significantly impact the physiology of armadillos. Steuber (2007) demonstrated that infected armadillos had a metabolic rate that was 23.9% higher than non–infected armadillos. Steuber (2007) also suggested that since metabolism is required for reproduction, migration, and foraging, a substantial decrease in metabolic rate of infected individuals may impact an individual's ability to carry out normal behaviors. As a result, physiological changes due to infection may lead to significant impacts on population dynamics (Tompkins *et al.* 2011).

Much of the previously discussed literature about leprosy in armadillos has involved laboratory populations. Research that has been done with wild populations has mainly been limited to leprosy prevalence studies in the southeast United States and Brazil (Truman *et al.* 2011; Frota *et al.* 2012; Sharma *et al.* 2015; Stefani *et al.* 2019). Studies that have evaluated leprosy's spatiotemporal patterns of infection and impact on wild armadillo population dynamics have not yet been reviewed.

Armadillos are also often considered nuisance wildlife, causing damage to property that requires management. Management may take the form of trapping and relocating, shooting, or forms of habitat modification (Gammons *et al.* 2009). Additionally, the cultural consumption of armadillo meat is common in parts of the specie's range and may be a source of potential human exposure to leprosy–causing bacteria (Deps *et al.* 2008a). Recent genetic and case studies in the southeast United States and South America have also demonstrated that leprosy has likely been transferred between humans and armadillos (Truman *et al.* 2011; da Silva *et al.* 2018). Therefore, because of nuisance armadillo management and cultural consumption of armadillos, exposure to potentially infected individuals may present a public health concern.

Evaluating leprosy's impact on wild armadillo population dynamics as well as spatial and temporal patterns of infection is important in understanding the epidemiology of leprosy in wild populations. Additionally, an understanding of the disease in armadillo populations can inform how people interact with the species and decrease the potential for zoonotic transmission. The aim of this systematic review was to characterize studies which have investigated the effects of leprosy on population dynamics as well as the spatiotemporal patterns of infection in wild armadillo populations. This review also identifies research gaps and potential areas for further study.

Materials and methods

Documents, including peer–reviewed research articles and gray literature such as conference proceedings and theses dissertations, were queried. The inclusion criteria included papers that analyzed population effects or spatiotemporal patterns of leprosy infection in wild nine–banded armadillos. The queried results included all past documents with no defined time period as the goal was to perform a complete review of all research related to my question. We were also limited to reports in English or that have been translated to English. Records discussing armadillos in any part of their geographic range or in a laboratory setting were included if an objective was also to investigate the spatiotemporal pattern of leprosy infection or the impacts on population dynamics. Population dynamics for the purposes of this review include sex ratio, age structure, fecundity or reproduction, and mortality or morbidity.

A structured literature search following PRISMA guidelines was conducted to retrieve records (Page *et al.* 2021). The search string ('nine–banded armadillo*' OR 'Dasypus novemcinctus' OR 'D novemcinctus') AND (lepro* OR 'M leprae' OR 'M lepromatosis' OR 'Mycobacterium leprae' OR 'Mycobacterium lepromatosis' OR 'Hansen's Disease') AND (surviv* OR demograph* OR migrat* OR dispers* OR reproduc* OR breed* OR fecund* OR fertil* OR mortal* OR death OR predat* OR fatal* OR lethal OR 'population dynamics' OR 'life history'

OR size OR density OR ‘sex ratio’ OR age OR spatial* OR temporal*) was used to search the databases of Web of Science Core Collection, Biosis Citation Index, Dissertations and Theses, and PubMed. Google Scholar was also searched to recover any missing reports. Due to character limits, the search string used to query the Google Scholar database was ‘allintitle: leprosy OR leprae OR lepromatosis OR Hansens AND ‘nine banded armadillo’’. All search queries were conducted on April 16th, 2022.

Exclusion criteria included (1) clinical trials on leprosy pathogenesis or susceptibility (2) a focus on a disease other than leprosy (3) a study focused on a different armadillo species (4) clinical trials focused on developing detection methods and (5) disease prevalence studies that did not include an analysis of population demographics or spatiotemporal patterns of infection. Commentaries, reviews, and other secondary records were excluded prior to the first screening. Gray literature including theses, conference proceedings, and technical reports were included in the first screening. In the first screening, I reviewed the titles and abstracts of each paper. Papers that were not relevant based on the first screening were removed before the next screening phase. The second screening was a full–text review of each paper. Records were excluded if the full review revealed that an article did not meet the selection criteria previously described. Search strategies are listed in the PRISMA flowchart (Figure 1.1).

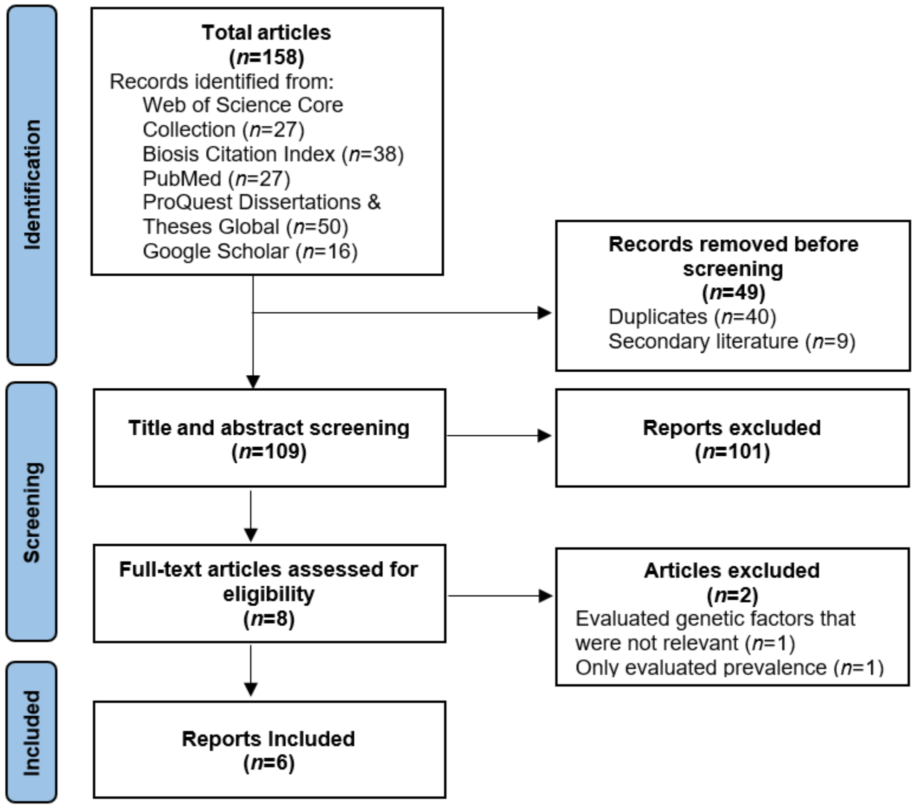

Figure 1.1. The PRISMA flowchart describing the search strategies for this study.

The selected records were summarized based on the leprosy prevalence recorded in each, the detection method used for leprosy, the study years, and location. From the selected records, findings from one or more of the papers were also summarized into main findings. All relevant and distinct findings from each record were included.

The impacts of leprosy infection on population demographics were grouped into four main categories: reproduction, age structure, sex ratio, and mortality. Reports that included findings of leprosy infection patterns were grouped into two broad categories: spatial and temporal. Some records discussed both population demographics and spatiotemporal patterns; in these instances, the same report's findings were separated into the applicable categories during the review process.

Results

The literature search for leprosy's effect on wild armadillo population demographics and spatiotemporal patterns of infection revealed a total of 158 articles. Before the first screening, 49 records were removed, including 40 duplicates and nine secondary literature sources. During the title and abstract screening, 101 records were removed according to the inclusion and exclusion criteria (Figure 1.1). Common reasons for exclusion include the clinical study of laboratory armadillos, the study of a disease other than leprosy, or the study of an individual armadillo rather than a population. The final screening of the full text was done for the remaining records. Two records were removed due to their evaluation of *D. novemcinctus* genetic factors and recording leprosy prevalence without at least one of the topics related to demographic effects or spatiotemporal patterns. The result was therefore six relevant records that were included in the review (Truman *et al.* 1991; Paige *et al.* 2002; Morgan and Loughry 2009; Andrew and Loughry 2012; Perez–Heydrich *et al.* 2016; Oli *et al.* 2017).

All six of the included studies analysed wild populations in the southeast United States and were limited to three states: Louisiana, Mississippi, and Texas. The average sample size of armadillos across the six studies was 449 (± 34 SE). The studies detected leprosy infection by either enzyme–linked immunosorbent assay (ELISA) for the *M. leprae*–specific anti–PGL1 antigen or through the histopathological examination of ear and reticuloendothelial tissues. Leprosy prevalence in the included studies ranged from 10.1% to 23.4% with an average of 15.8%, as detected using ELISA. The lowest prevalence detected through histopathological examination was 1.5%, with an average of 4% across the studies and study sites (Truman *et al.* 1991; Paige *et al.* 2002).

All included studies evaluated population dynamics, except for Paige et al. (2002), who instead evaluated the prevalence rates from year–to–year and calculated an incidence density for the observed population. Five of the six papers also evaluated spatiotemporal patterns of infection, with the exception of Oli et al. (2017) whom only estimated impacts on population dynamics. The years of the study, study site locations, diagnostic methods, and average prevalence of leprosy were summarized for each included record (Table 1.1.).

Table 1.1. Summary of included articles listed in reverse chronological order of the publication date with information about study years, location, sample size, and leprosy prevalence based on enzyme–linked immunoassay (ELISA) or histopathological examination. 'ND' represents that there was no data reported.

Citation	Study years	Location(s), USA	Average disease prevalence as detected by ELISA PGL–1 for adults (*n*)	Average disease prevalence as detected by histopathology	Incidence density (new cases/ 1,000 animal days)
(Oli *et al.* 2017)[A]	2005–2010	Yazoo National Wildlife Refuge in Western Mississippi	17.8% (454)	ND	ND
(Perez–Heydrich *et al.* 2016)	2005–2010	Yazoo National Wildlife Refuge in Western Mississippi	15.9% (466)	ND	ND
(Andrew and Loughry 2012)[B]	2005–2010	Yazoo National Wildlife Refuge in Western Mississippi	16.4% (469)	ND	0.12 – 0.61
(Morgan and Loughry 2009)[A]	2007–2008	Yazoo National Wildlife Refuge in Western Mississippi	10.1% (317)	ND	ND
(Paige *et al.* 2002)[C]	1987–1989, 1997	Point Coupee Parish, Louisiana	19.1% (414)	3% (165)	0.47 – 3.5
(Truman *et al.* 1991)[D]	1984–1989	(1) Tensas NWR, Tallulah, Louisiana; (2) Sherbourne WMA, Krots Springs, Louisiana[E]; (3) Lacassine NWR, Lake Arthur, Louisiana; (4) Welder Wildlife Refuge, Sinton, Texas	(1) 23.4% (77) (2) 13.7% (386) (3) 20.6% (78) (4) 17.1% (35) x̄=16% (576)	(1) 6.4% (77) (2) 3.2% (349) (3) 1.5% (78) (4) 5.7% (35) x̄=3.5% (539)	ND

A The total prevalence rate for the population was not provided in the paper but was calculated from the number of leprous and non–leprous individuals reported.
B Total sample size was cumulative over the 6 study years.
C Histopathologic examination done only on tissue samples collected in 1997.
D Used histopathologic examination on tissue samples collected only at the Louisiana sites. Leprosy prevalence was recorded for the Welder Wildlife Refuge population, but the site was not evaluated for population dynamics or spatiotemporal patterns.
E Sherbourne WMA area was sampled consecutively for 5 years whereas the other areas were not. The total number of animals that were seropositive and sampled were averaged for the ELISA prevalence result. The same method was used to find a prevalence for the histopathology of the armadillos at the Sherbourne WMA, however, no data was available for the autumn/winter of 1988.

Population dynamics

The recorded effects of leprosy infection on wild armadillo population dynamics in the included papers were summarized (Table 1.2.). There were nine main findings related to demographic effects of leprosy infection, which can be further subdivided into reproduction, age structure, sex ratio, and mortality effects.

Firstly, effects on reproduction included 1) reproducing females were more likely to be seropositive than non–reproducing females and 2) the probability that a seropositive female would reproduce did not vary significantly from females that were seronegative.

The effects of leprosy infection on age structure were 3) no yearlings or juveniles were seropositive and 4) older males exhibiting increased phenotypic damage were more likely to be seropositive.

The effects on sex ratio were 5) for seropositive individuals, there was no difference in survival probability between the sexes and 6) there was no significant difference in seropositivity between males and females. However, the latter finding was contradicted by Morgan and Loughry (2009) who found that significantly more females were seropositive than males.

Lastly, for the effects of leprosy infection on mortality, it was found that 7) leprosy overall reduced the population survivability by 14.5%, 8) seropositive individuals persisted in the population for no more than three years and 9) seropositive individuals had no possibility of recovery from infection.

Table 1.2. Summary of the findings for how leprosy impacts population dynamics of wild armadillo populations. Findings highlighted in grey are contrary to findings reported in other records.

Findings	Citation(s)
Reproduction	
1. Lactating— and therefore reproducing— females were much more likely to be seropositive than non–lactating females[A]	(Truman et al. 1991; Morgan and Loughry 2009; Perez–Heydrich et al. 2016)
2. No decrease in the probability for a seropositive female to reproduce	(Oli et al. 2017)
Age structure	
3. No yearlings/juveniles were seropositive	(Morgan and Loughry 2009; Andrew and Loughry 2012; Perez–Heydrich et al. 2016; Oli et al. 2017)
4. Seropositive males exhibited more phenotypic damage than seronegative males[B]	(Morgan and Loughry 2009)
Sex ratio	
5. There was no evidence that survival probability of seropositive individuals differed between the sexes	(Andrew and Loughry 2012; Oli et al. 2017)
6. There was no significant difference in seropositivity between sexes	(Truman et al. 1991; Andrew and Loughry 2012)
More females were positive than males[C]	(Morgan and Loughry 2009)
Mortality	
7. Leprosy reduced survival of adults by 14.5%	(Oli et al. 2017)
8. Infected individuals persisted in the population for no more than 3 years	(Andrew and Loughry 2012)
9. Infected individuals have no possibility of recovery from leprosy	(Oli et al. 2017)

A This finding could also be classified as age structure since reproducing females are typically older females (Morgan and Loughry 2009)
B Phenotypic damage is often used as a proxy for age in male armadillos (Loughry and McDonough 2013)
C This was statistically significant in one year of their study, 2008, and when both 2007 and 2008 were combined.

Spatiotemporal patterns of infection

There were four main findings about infection patterns in wild armadillos with three being categorized as temporal and one as spatial. Two temporal findings were contradictory with 1) there being no significant annual fluctuations in prevalence and 2) there being annual fluctuations in infection prevalence. The last temporal finding that was found by Truman *et al.* (1991) was 3) a significant seasonal variation in infection prevalence which was not evaluated by the other included studies. One spatial finding was recovered and was supported by four of the six studies which was that 4) seropositive individuals were randomly distributed with no significant clumping or clustering of individuals in the areas of study. The spatiotemporal patterns of leprosy infection in wild armadillo populations were summarized (Table 1.3).

Table 1.3. Summary of the spatiotemporal patterns of leprosy infection in wild armadillos that were reported in the included studies. Findings highlighted in gray are contrary to findings reported in other records.

Findings	Citation(s)
Spatial	
1. Seropositive individuals were randomly distributed with no significant clumping or clustering[A]	(Truman et al. 1991; Paige et al. 2002; Morgan and Loughry 2009; Perez–Heydrich et al. 2016)
Temporal	
1. There were annual fluctuations in infection prevalence	(Andrew and Loughry 2012)
2. Infection prevalence was relatively stable year–to–year	(Truman et al. 1991)
3. There were significantly higher prevalence rates in the winter and summer months than in the spring	(Truman et al. 1991)

A Morgan and Loughry's (2009) findings suggested that there was clumping but it was not statistically significant.

Discussion

Previous literature on leprosy in nine–banded armadillos has been limited to basic prevalence studies and clinical laboratory trials. A better understanding of the epidemiology of leprosy in wild armadillo populations not only benefits scientific understanding, but also informs safe management practices of nuisance armadillos. Additionally, armadillos are often encountered, handled, and consumed by people at several parts of their range. Therefore, understanding patterns of infection in wild armadillos can improve public health and prevent the zoonotic transmission of leprosy. Our systematic review of the over 150 records in the global literature recovered only six relevant papers, indicating a significant research gap in the current understanding of leprosy's impacts on population dynamics and spatiotemporal patterns in wild armadillos.

The six articles included in this review recorded leprosy prevalence and, when compared to other prevalence studies that were not included in this review, the prevalence was higher overall (Figure 1.2.). For instance, leprosy prevalence was higher in each locale compared to Loughry *et al.* (2009) and Sharma *et al.* (2015), except for populations in the Merritt Island National Wildlife Refuge in Florida, USA (25%) and Pinebloom Plantation in Georgia, USA (10.5%). It should also be noted that the leprosy prevalence documented in these other studies were based on sample sizes that were overall smaller than those in the six included studies, which had an average sample size greater than four–hundred individuals.

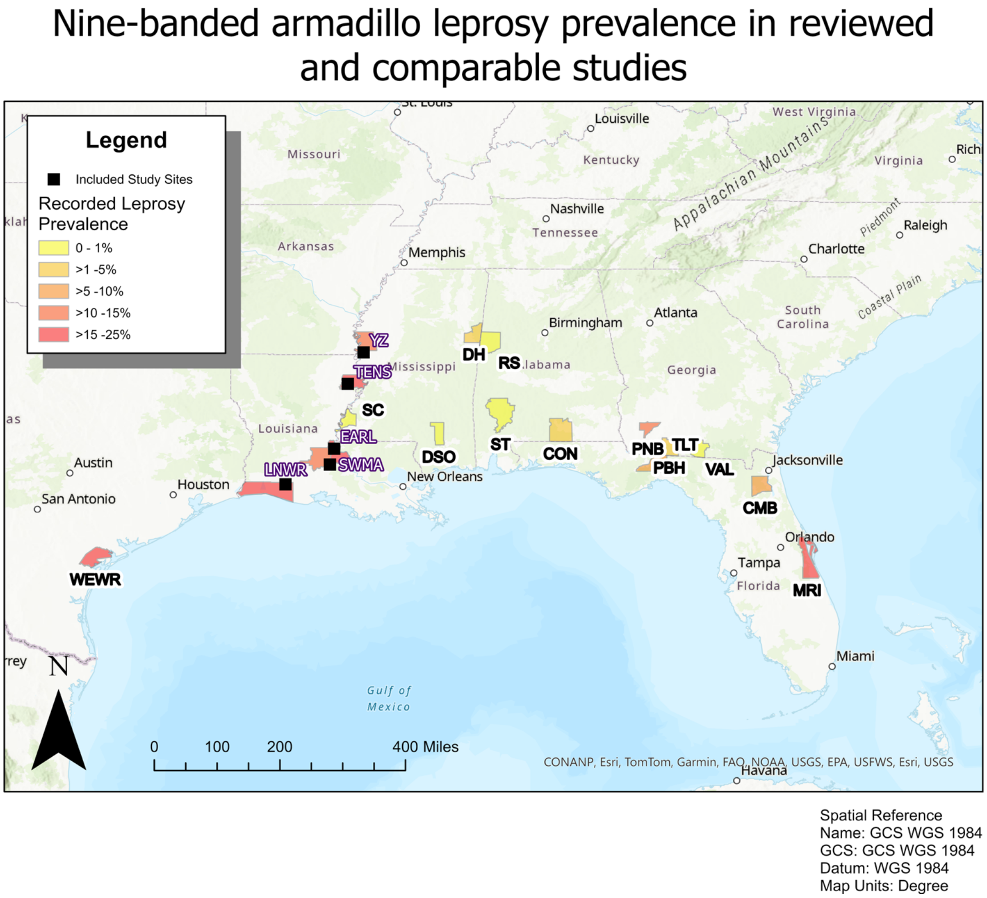

Figure 1.2. The locations of sampled armadillos in the six included papers from our global systematic review; the included records all conducted research in the southeast United States. Leprosy prevalence documented in the included studies is compared with other studies in the southeast United States. (Sharma *et al.* 2015): (DSO) DeSoto National Forest, (CON) Conecuh National Forest, (PBH) Pebble Hill Plantation, (VAL) Valdosta, GA, (TLT) Tall Timbers Research Station and Land Conservancy, (CMB) Camp Blanding, (MRI) Merritt Island National Wildlife Refuge. (Loughry *et al.* 2009): (RS) Riverside, (SC) St. Catherine's Creek National Wildlife Refuge, (ST) Stimpson Wildlife Sanctuary, (YZ) Yazoo National Wildlife Refuge. Both Loughry *et al.* (2009) and Sharma *et al.* (2015) had two of the same study sites and the prevalence they found was averaged in this map: (DH) DeWayne Hayes Recreational area and (PNB) Pinebloom Plantation. Sharma *et al.* (2015) used only ELISA (enzyme–linked immunoassay) for diagnostic testing whereas Loughry *et al.* (2009) used both PCR (polymerase chain reaction) and ELISA testing. The papers included in this review are also provided on the map and include: (YZ) Yazoo National Wildlife Refuge (Morgan and Loughry 2009; Andrew and Loughry 2012; Perez–Heydrich *et al*. 2016; Oli *et al.* 2017), (EARL) East Atchafalaya River Levee (Paige *et al.* 2002), (TENS) Tensas River National Wildlife Refuge (Truman *et al.* 1991), (SWMA) Sherbourne Wildlife Management Area (Truman *et al.* 1991), and (LNWR) Laccasine National Wildlife Refuge (Truman *et al.* 1991) (WEWR) Welder Wildlife Refuge; the latter was not included in the review paper study sites since population dynamics were not evaluated at this site by Truman *et al.* (2015). Outside of the included studies in the review, only more recent prevalence studies were included since they use more updated diagnostic techniques such as ELISA and PCR that are more accurate in estimating prevalence.

Reproductive effects

In non–leprous wild populations, no costs of reproduction for females has previously been demonstrated (Loughry *et al.* 2013). In the findings reviewed here, lactating or reproducing females were more likely to be seropositive for leprosy than non–lactating females (Truman *et al.* 1991; Morgan and Loughry 2009; Perez–Heydrich *et al.* 2016). This finding, however, may be due to older females being more likely to reproduce and also acquire *M. leprae* since the bacteria has a relatively long incubation period and is commonly detected in older adults (Andrew and Loughry 2012; Loughry and McDonough 2013). Still, the findings support that leprous females are still able to reproduce and infection does not decrease the probability of them reproducing (Oli *et al.* 2017). However, whether leprous females face additional costs of reproduction that decreases the number of successful reproductive efforts requires further study.

Whether leprosy infection in males affects reproductive effort was not evaluated. However, the reviewed papers did find that seropositive males had more carapace damage (Morgan and Loughry 2009). Carapace damage is often considered to be correlated with age and occurs as a result of conspecific aggression, as well as other environmental causes (Loughry *et al.* 2002). Therefore, it appears that, like females, leprous males were likely to be older. However, further research will need to be done on whether leprosy infection influences overall male reproductive effort and success.

Age structure effects

A finding of leprosy's impacts on age structure that was consistent across several studies was that juveniles and yearlings were not seropositive (Morgan and Loughry 2009; Andrew and

Loughry 2012; Perez–Heydrich *et al.* 2016; Oli *et al.* 2017). This further supports the finding that leprosy affects older animals as a result of the bacteria's long incubation period (Andrew and Loughry 2012). This finding also suggests that vertical transmission does not occur in armadillo populations (Morgan and Loughry 2009). However, armadillos may live to be eight to twelve years old in the wild, and the studies here only evaluated, at most, six years of population data. Therefore, long–term studies of leprous females and subsequent litters will need to be done to better understand potential transmission dynamics (Loughry *et al.* 2013).

Sex ratio effects

Differences in the sex ratio of leprosy–positive individuals were observed by Morgan and Loughry (2009), with there being more seropositive females than males; this finding was also significant across both years of their study. Other papers, however, observed no significant difference in sex ratio as it relates to leprosy infection (Truman *et al.* 1991; Andrew and Loughry 2012). Since other studies reviewed here demonstrated no sex ratio difference, it is probable that the observed sex ratio by Morgan and Loughry (2009) was a result of a small number of leprous individuals. However, more studies should be done to evaluate whether there is a significant difference in sex ratio in a population with observed leprosy prevalence.

Mortality effects

Leprosy's impact on armadillo mortality was a 14.5% reduction in adult survival, with no possibility of adults recovering (Oli *et al.* 2017). Adult mortality is typically less common than juvenile mortality since juveniles are depredated at a higher frequency than adults (McDonough and Loughry 1997). Without a decrease in juvenile mortality, an increase in adult mortality caused by infection with *M. leprae* can thus impact the population growth rate, which was observed by Oli *et al*. (2017) with a 13% decrease. In addition, Oli *et al.* (2017) concluded that mortality from leprosy, in addition to natural mortality, has a substantial effect at the population level. The other included studies did not evaluate mortality rate; therefore, future studies should evaluate how leprosy may impact a population's mortality rate over a longer time period using multistate capture–mark–recapture modeling, similar to Oli *et al*. (2017).

Spatiotemporal infection patterns

The second main objective of this review was to summarize the spatiotemporal patterns of leprosy infection observed in wild armadillo populations. Across four of the six studies, it was found that seropositive individuals were not spatially clustered and instead, were randomly distributed throughout the population (Truman *et al.* 1991; Paige *et al.* 2002; Morgan and Loughry 2009; Perez–Heydrich *et al.* 2016). Morgan and Loughry (2009) documented that they observed clustering in a portion of their population, but it was not statistically significant.

The spatial patterns of infection observed may be due to an equal risk of any one susceptible individual becoming infected when encountering the bacteria; in this case, a susceptible individual is an adult armadillo, since no juveniles or yearlings have tested positive to date (Morgan and Loughry 2009). According to Truman *et al.* (2014), roughly 15% to 20% of adult armadillos appear to be immune in laboratory settings. Future studies of armadillo immunity to leprosy and genetic variation in armadillo populations are required to understand how it may impact spatial patterns of infection. The other explanation for the spatially homogenous patterns of infection observed in the reviewed studies is widespread contamination of the habitat in which armadillos live, making it equally likely to become infected. Transmission of leprosy in the environment is still not well understood, and the impact of direct or indirect transmission patterns require further study (as reviewed in: Ploemacher *et al.* 2020).

Similar to spatial patterns of infection, the timing of infection also influences wildlife disease systems with changes in prevalence lagging behind changes in the timing of certain events (Brearley *et al.* 2013). Fewer included studies evaluated temporal patterns of infection. Truman *et al.* (1991) found that prevalence rates were higher in the winter and summer months compared to the spring, however, other studies did not sample across different seasons and thus could not support this finding. Still, a reasoning for this observation during the summer and winter months may be the higher rate of transients and more accessible females since they are not weaning young (Loughry and McDonough 2013).

Regarding annual variation in infection, there were conflicting results with Andrew and Loughry (2012) finding that infection prevalence fluctuated annually and Truman *et al.* (1991) finding the opposite with infection prevalence being stable annually. Long–term research will need to be done by sampling leprosy in an armadillo population annually as well as seasonally to further evaluate if there are temporal variations in infection in a population where leprosy is present.

Limitations

Despite the previously discussed findings of leprosy's impact on wild armadillo populations, the reviewed papers also had several limitations. Firstly, the papers were geographically limited to the southeast United States and the review revealed no records evaluating leprosy's impacts in South or Central American populations. In the southeast United States, Louisiana and

Mississippi were the only two states in which population dynamics and spatiotemporal patterns were evaluated.

A study of leprosy's impacts on Alabama, Texas, Georgia, Florida, and other populations east of Mississippi, USA has not yet been done, despite the observed prevalence of leprosy in these states (Figure 1.2.) (Loughry *et al.* 2009; Sharma *et al.* 2015). Since Sharma *et al.* (2015) found that there was a distinct strain of *M. leprae* (3I–2–v15) in south Florida that has not been found elsewhere in the southeast United States, the lack of studies evaluating leprosy's impact on Florida populations as well as other states in the region is a significant gap in the research. In addition, it is believed that armadillos originated in Texas, USA with a separately introduced population in Florida merging near the state of Alabama in the mid twentieth century (Talmage and Buchanan 1954; Taulman and Robbins 1996). The studies reviewed in this paper all sampled armadillo populations west of the Mississippi River. Therefore, sampling east of the Mississippi River would allow a further evaluation of how other strains of *M. leprae* may impact armadillo populations with potentially distinct genetics due to a unique history of introduction and expansion in this region.

In addition to being limited to two states, four of the six papers utilized the same population of armadillos at the Yazoo National Wildlife Refuge and the studies were conducted in overlapping years, which may have conflated the recorded prevalence rates (Morgan and Loughry 2009; Andrew and Loughry 2012; Perez–Heydrich *et al.* 2016; Oli *et al.* 2017). Further, the Yazoo National Wildlife Refuge as well as the other study sites included in this review sampled live armadillos along roadways or trails. However, roadkill have been demonstrated to be representative of adults within the population (Loughry and McDonough 1996), and therefore, only sampling and recapturing live armadillos along roadways may not be representative of all individuals.

All the sites included in these studies were also natural areas in wildlife refuges or management areas. Therefore, the impacts that leprosy may have on more suburban and urban populations has not been evaluated, despite their expansion into urbanized areas over the last several decades (Hohbein and Mengak 2018). Many zoonotic disease systems have demonstrated higher pathogen prevalence in urban populations compared to rural populations such as woodchucks with *Toxoplasma gondii* in the Midwest, USA (Lehrer *et al.* 2010) and mule deer with chronic wasting disease (CWD) in Colorado, USA (Farnsworth *et al.* 2005). However, the relationship between wildlife diseases and more human–dominated landscapes, in general, is complex and requires further study (Brearley *et al.* 2013).

Additionally, the included studies used the same detection method for *M. leprae*, enzyme–linked immunosorbent assay (ELISA) to detect IgM antibodies of the phenolic glycolipid–I (PGL1) antigen. Although ELISA can detect antibodies specific to *M. leprae*, it is limited in detecting early stages of the disease and paucibacillary–type infections, which are considered to be a less severe manifestation of leprosy (Gama *et al.* 2020). Polymerase chain reaction (PCR), on the other hand, detects DNA sequences specific to the *M. leprae* genome, specifically the 18 kDA protein gene, in infected tissues, at small quantities (100 bacteria) such as with multibacillary infections (Williams *et al.* 1990). In one study, 16 of the 30 armadillos sampled were leprosy positive with PCR, whereas only two of the 30 armadillos were seropositive via ELISA (Job *et* *al.* 1991). Due to the higher specificity of PCR, repeating the methods described in the reviewed papers with PCR detection would be more sensitive, providing a better understanding of the disease's impacts on wild nine–banded armadillos at different stages of infection.

The papers reviewed also did not aim to detect *M. lepromatosis,* a recently discovered bacterium that is a causative agent of diffuse lepromatous leprosy (Han *et al.* 2008). A recent study found that armadillos sampled from eight sites in the southeast United States from 2003 to 2012 were negative for *M. lepromatosis* (Sharma *et al.* 2019). However, there is the possibility that over the last decade, armadillos have acquired *M. lepromatosis* from people infected with this bacterium, like red squirrels in the UK (Meredith *et al.* 2014). If possible, the detection of *M. lepromatosis* should not be excluded in future studies as it is likely that wild armadillos could serve as a reservoir for this bacterium.

This systematic review underscores the impact that leprosy may have on wild armadillo populations. The ecology of the disease in wild populations is complex given the long incubation time of *M. leprae*. Although leprosy appears to impact older adults in armadillo populations and lead to an increased risk of mortality, other important components of population dynamics like reproduction and sex ratio does not appear to be impacted. Spatially, the disease appears to be homogenous, which may be due to the number of susceptible individuals or the route(s) of transmission. The effects that leprosy has on wild armadillo populations, especially at other locales and in human–modified landscapes, requires further study.

As nuisance wildlife, armadillos require direct and indirect management, which may put people at risk of zoonotic transmission. In addition, leprosy's impacts on armadillo populations requires consideration when developing management recommendations for property owners. Common management techniques for many homeowners in the southeast United States are to trap and relocate or shoot armadillos (Gammons *et al.* 2009). These techniques might 1) increase a person's interaction with a potentially infected individual and 2) result in the translocation of an infected individual into an unaffected population. With additional studies that evaluate how leprosy impacts wild armadillo populations, current management methods can be refined to improve efficacy while ensuring public health.

Literature cited

Andrew JW, Loughry WJ (2012). Temporal aspects of leprosy infection in a wild population of nine–banded armadillos. *Southeastern Naturalist* **11**, 173–182. doi:10.1656/058.011.0202

Antunes JMAP, Deps PD (2003). Pesquisa de *Mycobacterium leprae* em tatus selvagens da espécie *Dasypus novemcintus* do Estado do Espírito Santo. Thesis, São Paulo, Brazil. xviii, 145.

Balamayooran G, Pena M, Sharma R, Truman RW (2015). The armadillo as an animal model and reservoir host for *Mycobacterium leprae*. *Clinics in Dermatology* **33**, 108–115. doi:10.1016/j.clindermatol.2014.07.001

Brearley G, Rhodes J, Bradley A, Baxter G, Seabrook L, Lunney D, Liu Y, McAlpine C (2013). Wildlife disease prevalence in human–modified landscapes. *Biological Reviews* **88**, 427–442. doi:10.1111/brv.12009

Center for Disease Control and Prevention (1976). Leprosy–like disease in wild–caught armadillos– Louisiana. *Center for Disease Control and Prevention* **25**, 18–23.

Clark WK (1951). Ecological life history of the armadillo in the Eastern Edwards Plateau region. *The American Midland Naturalist* **46**, 337–358. doi:10.2307/2421982

Deps PD, Alves BL, Gripp CG, Aragao RL, Guedes B, Filho JB, Andreatta MK, Marcari RS, Prates I, Rodrigues LC (2008a). Contact with armadillos increases the risk of leprosy in

Brazil: a case control study. *Indian Journal of Dermatology, Venereology and Leprology* **74**, 338–342. doi:10.4103/0378–6323.42897

Deps PD, Antunes JMAP, Faria C, Bührer–Sékula S, Camargo ZP, Opromola DV, Tomimori J (2008b). Research regarding anti–PGL–I antibodies by ELISA in wild armadillos from Brazil. *Revista Da Sociedade Brasileira De Medicina Tropical* **41 Suplemento 2**, 73–76. doi:10.1590/s0037–86822008000700015.

Deps P, Antunes J, Santos A, Collin S (2020). Prevalence of *Mycobacterium leprae* in armadillos in Brazil: A systematic review and meta–analysis. *PLOS Neglected Tropical Diseases* **14**. doi:10.1371/journal.pntd.0008127

Duthie MS, Truman RW, Goto W, O'Donnell J, Hay MN, Spencer JS, Carter D, Reed SG (2011). Insight toward early diagnosis of leprosy through analysis of the developing antibody responses of *Mycobacterium leprae*–infected armadillos. *Clinical and Vaccine Immunology: CVI* **18**, 254–259. doi:10.1128/CVI.00420–10

Farnsworth ML, Wolfe LL, Hobbs NT, Burnham KP, Williams ES, Theobald DM, Conner MM, Miller MW (2005). Human land use influences chronic wasting disease prevalence in mule deer. *Ecological Applications* **15**, 119–126.

Frota CC, Lima LNC, Rocha A da S, Suffys PN, Rolim BN, Rodrigues LC, Barreto ML, Kendall C, Kerr LRS (2012). *Mycobacterium leprae* in six–banded (*Euphractus sexcinctus*) and nine–banded armadillos (*Dasypus novemcinctus*) in Northeast Brazil. *Memórias do Instituto Oswaldo Cruz* **107**, 209–213. doi:10.1590/S0074–02762012000900029

Gama RS, Leite LA, Colombo LT, Fraga LA de O (2020). Prospects for new leprosy diagnostic tools, a narrative review considering ELISA and PCR assays. *Revista da Sociedade Brasileira de Medicina Tropical* **53**, e20200197. doi:10.1590/0037–8682–0197–2020

Gammons DJ, Mengak MT, Conner LM (2009). Translocation of nine–banded armadillos. *Human–Wildlife Conflicts* **3**, 64–71.

Han XY, Seo Y–H, Sizer KC, Schoberle T, May GS, Spencer JS, Li W, Nair RG (2008). A new *Mycobacterium* species causing diffuse lepromatous leprosy. *American Journal of Clinical Pathology* **130**, 856–864. doi:10.1309/ajcpp72fjzzrrvmm

Haywood CJ, Jordon AM, Pena M, Nielsen CK, Jiménez FA (2022). Tissue and gastrointestinal parasites of colonizing nine–banded armadillos at the edge of their northern range. *Journal of Parasitology* **108**, 57–63. doi:10.1645/21–76

Hohbein RR, Mengak MT (2018). Cooperative extension agents as key informants in assessing wildlife damage trends in Georgia. *Human–Wildlife Interactions* **12**, 243–258.

Howerth EW, Stallknecht DE, Davidson WR, Wentworth EJ (1990). Survey for leprosy in nine–banded armadillos (*Dasypus novemcinctus*) from the southeastern United States. *Journal of Wildlife Diseases* **26**, 112–115. doi:10.7589/0090–3558–26.1.112

Job CK, Sanchez RM, Hastings RC (1985). Effect of repeated lepromin testing on experimental nine–banded armadillo leprosy. *Indian Journal of Leprosy* **57**, 716–727.

Job CK, Drain V, Williams DL, Gillis TP, Truman RW, Sanchez RM, Deming AT, Hastings RC (1991). Comparison of polymerase chain reaction technique with other methods for detection of *Mycobacterium leprae* in tissues of wild nine–banded armadillos. *Leprosy Review* **62**, 362–373. doi:10.5935/0305–7518.19910042

Joshi G, Quadir SS, Yadav KS (2021). Road map to the treatment of neglected tropical diseases: Nanocarriers interventions. *Journal of Controlled Release : official journal of the Controlled Release Society* **339**. doi:10.1016/j.jconrel.2021.09.020

Lehrer EW, Fredebaugh SL, Schooley RL, Mateus–Pinilla NE (2010). Prevalence of antibodies to *Toxoplasma gondii* in woodchucks across an urban–rural gradient. *Journal of Wildlife Diseases* **46**, 977–980. doi:10.7589/0090–3558–46.3.977

Loughry WJ, McDonough CM (1996). Are road kills valid indicators of armadillo population structure? *The American Midland Naturalist* **135**, 53–59. doi:10.2307/2426871

Loughry WJ, McDonough CM (2013). 'The nine–banded armadillo: a natural history'. (University of Oklahoma Press).

Loughry WJ, McDonough CM, Robertson EG (2002). Patterns of anatomical damage in a population of nine–banded armadillos *Dasypus novemcinctus* (Xenarthra, Dasypodidae). *Mammalia* **66**, 111–122. doi:10.1515/mamm.2002.66.1.111

Loughry WJ, Truman RW, McDonough CM, Tilak M–K, Garnier S, Delsuc F (2009). Is leprosy spreading among nine–banded armadillos in the southeastern United States? *Journal of Wildlife Diseases* **45**, 144–152. doi:10.7589/0090–3558–45.1.144

Loughry WJ, Perez–Heydrich C, McDonough CM, Oli MK (2013). Population ecology of the nine–banded armadillo in Florida. *Journal of Mammalogy* **94**, 408–416. doi:10.1644/12–mamm–a–198.1

McDonough CM (1997). Pairing behavior of the nine–banded armadillo (*Dasypus novemcinctus*). *The American Midland Naturalist* **138**, 290–298. doi:10.2307/2426822

McDonough CM (2000). Social organization of nine–banded armadillos (*Dasypus novemcinctus*) in a riparian habitat. *The American Midland Naturalist* **144**, 139–151. doi:10.1674/0003–0031(2000)144[0139:SOONBA]2.0.CO;2

McDonough CM, Loughry WJ (1997). Patterns of mortality in a population of nine–banded armadillos, *Dasypus novemcinctus*. *The American Midland Naturalist* **138**, 299–305. doi:10.2307/2426823

McDonough CM, McPhee SA, Loughry WJ (1998). Growth rates of juvenile nine–banded armadillos. *The Southwestern Naturalist* **43**, 462–468.

Meredith A, Pozo JD, Smith S, Milne E, Stevenson K, McLuckie J (2014). Leprosy in red squirrels in Scotland. *Veterinary Record* **175**, 285–286. doi:10.1136/vr.g5680

Monot M, Honoré N, Garnier T, Araoz R, Coppée J–Y, Lacroix C, Sow S, Spencer JS, Truman RW, Williams DL, Gelber R, Virmond M, Flageul B, Cho S–N, Ji B, Paniz–Mondolfi A, Convit J, Young S, Fine PE, Rasolofo V, Brennan PJ, Cole ST (2005). On the origin of leprosy. *American Association for the Advancement of Science* **308**, 1040–2. doi:10.1126/science/1109759

Morgan RE, Loughry WJ (2009). Consequences of exposure to leprosy in a population of wild nine–banded armadillos. *Journal of Mammalogy* **90**, 1363–1369.

Oli MK, Loughry WJ, Caswell H, Perez–Heydrich C, McDonough CM, Truman RW (2017). Dynamics of leprosy in nine–banded armadillos: Net reproductive number and effects on host population dynamics. *Ecological Modelling* **350**, 100–108.

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo–Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Systematic Reviews* **10**, 89. doi:10.1186/s13643–021–01626–4

Paige CF, Scholl DT, Truman RW (2002). Prevalence and incidence density of *Mycobacterium leprae* and *Trypanosoma cruzi* infections within a population of wild nine–banded armadillos. *The American Journal of Tropical Medicine and Hygiene* **67**, 528–532. doi:10.4269/ajtmh.2002.67.528

Pedrini SCB, Rosa PS, Medri IM, Mourão G, Bagagli E, Lopes CA de M (2010). Search for *Mycobacterium leprae* in wild mammals. *The Brazilia Journal of Infectious Diseases* **14**, 47–53.

Perez–Heydrich C, Loughry WJ, Anderson CD, Oli MK (2016). Patterns of *Mycobacterium leprae* infection in wild nine–banded armadillos (*Dasypus novemcinctus*) in Mississippi, USA. *Journal of Wildlife Diseases* **52**, 524–532.

Ploemacher T, Faber WR, Menke H, Rutten V, Pieters T (2020). Reservoirs and transmission routes of leprosy; A systematic review. *PLoS Neglected Tropical Diseases* **14**, 1–27. doi:10.1371/journal.pntd.0008276

Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Avise JC (1996). Molecular documentation of polyembryony and the micro–spatial dispersion of clonal sibships in the nine–banded armadillo, *Dasypus novemcinctus*. *Proceedings. Biological Sciences* **263**, 1643–1649. doi:10.1098/rspb.1996.0240

Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL (2006). The continuing challenges of leprosy. *Clinical Microbiology Reviews* **19**, 338–381. doi:10.1128/CMR.19.2.338–381.2006

Scollard DM (2016). Infection with *Mycobacterium lepromatosis*. *The American Journal of Tropical Medicine and Hygiene* **95**, 500–501. doi:10.4269/ajtmh.16–0473

Sharma R, Singh P, Loughry WJ, Lockhart MJ, Inman WB, Malcolm S. Duthie, Maria T. Pena, Luis A. Marcos, David M. Scollard, Stewart T. Cole, Richard W. Truman (2015). Zoonotic leprosy in the southeastern United States. *Emerging Infectious Diseases* **21**, 2127–2134. doi:10.3201/eid2112.150501

Sharma R, Singh P, McCoy RC, Lenz SM, Donovan K, Ochoa MT, Estrada–Garcia I, Silva–Miranda M, Jurado–Santa Cruz F, Balagon MF, Stryjewska B, Scollard DM, Pena MT, Lahiri R, Williams DL, Truman RW, Adams LB (2019). Isolation of *Mycobacterium lepromatosis* and development of molecular diagnostic assays to distinguish *Mycobacterium leprae* and *M. lepromatosis*. *Clinical Infection Diseases* **71**, e262–e269. doi:10.1093/cid/ciz1121

da Silva M, Portela J, Li W, Jackson M, Gonzalez–Juarrero M, Hidalgo A, Belisle J, Bouth R, Gobbo A, Barreto J, Minervino A, Cole S, Avanzi C, Busso P, Frade M, Geluk A, Salgado C, Spencer J (2018). Evidence of zoonotic leprosy in Para A, Brazilian Amazon, and risks associated with human contact or consumption of armadillos. *PLoS Neglected Tropical Diseases* **12**. doi:10.1371/journal.pntd.0006532

de Souza DK (2016). Avaliação da prevalência de patógenos zoonóticos de importância para a saúde pública em tatus de vida livre – Mato Grosso do Sul – Brasil. Thesis, Universidade de São Paulo, Brazil. doi:10.11606/D.99.2017.tde–24012017–081133

Stefani MMA, Rosa PS, Costa MB, Schetinni APM, Manhães I, Pontes MAA, Costa P, Fachin LRV, Batista IMFD, Virmond M, Pereira E, Penna MLF, Penna GO (2019). Leprosy survey among rural communities and wild armadillos from Amazonas state, Northern Brazil. *PloS ONE* **14**, e0209491. doi:10.1371/journal.pone.0209491

Steuber, J. G. 2007. The cost of an emerging disease: *Mycobacterium leprae* infection alters metabolic rate of the nine-banded armadillo (*Dasypus novemcinctus*). Thesis, University of Akron, Ohio, United States.

https://etd.ohiolink.edu/apexprod/rws_olink/r/1501/10?clear=10&p10_accession_num=akron1195236347. Accessed 14 Feb 2023

Storrs EE, Walsh GP, Burchfield HP, Binford CH (1974). Leprosy in the armadillo: new model for biomedical research. *Science* **183**, 851–852. doi:10.1126/science.183.4127.851

Talmage RV, Buchanan GD (1954). The armadillo: A review of its natural history, ecology, anatomy and reproductive physiology. *The Rice Institute Pamphlet* **41**. Available at: https://scholarship.rice.edu/handle/1911/62727 [accessed 10 April 2022]

Taulman JF, Robbins LW (1996). Recent range expansion and distributional limits of the nine–banded armadillo (*Dasypus novemcinctus*) in the United States. *Journal of Biogeography* **23**, 635–648.

Taulman JF, Robbins LW (2014). Range expansion and distributional limits of the nine–banded armadillo in the United States: an update of Taulman & Robbins (1996). *Journal of Biogeography* **41**, 1626–1630. doi:10.1111/jbi.12319

Tompkins DM, Dunn AM, Smith MJ, Telfer S (2011). Wildlife diseases: from individuals to ecosystems. *Journal of Animal Ecology* **80**, 19–38. doi:10.1111/j.1365–2656.2010.01742.x

Truman R (2005). Leprosy in wild armadillos. *Leprosy Review* **76**, 198–208. doi:10.47276/lr.76.3.198

Truman RW, Kumaresan JA, McDonough CM, Job CK, Hastings RC (1991). Seasonal and spatial trends in the detectability of leprosy in wild armadillos. *Epidemiology and Infection* **106**, 549–560. doi:10.1017/S0950268800067613

Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz–Mondolfi A, Kapopoulou A, Brisse S, Scollard DM, Gillis TP, Cole ST (2011). Probable zoonotic leprosy in the southern United States. *New England Journal of Medicine* **364**, 1626–1633. doi:10.1056/NEJMoa1010536

Truman RW, Ebenezer GJ, Pena MT, Sharma R, Balamayooran G, Gillingwater TH, Scollard DM, McArthur JC, Rambukkana A (2014). The armadillo as a model for peripheral neuropathy in leprosy. *ILAR Journal* **54**, 304–314. doi:10.1093/ilar/ilt050

Vijayaraghavan R (2009). Nine–banded armadillo *Dasypus novemcinctus* animal model for leprosy (Hansen's Disease). *Scandinavian Journal of Laboratory Animal Science* **36**, 167–176.

Williams D, Gillis T, Booth R, Looker D, Watson J (1990). The use of a specific DNA probe and polymerase chain reaction for the detection of *Mycobacterium leprae*. *The Journal of Infectious Diseases* **162**. doi:10.1093/infdis/162.1.193

Chapter 2: Prevalence of leprosy in Alabama nine-banded armadillos (*Dasypus novemcinctus*)

Abstract

Previous studies have examined the prevalence and impact of leprosy on nine-banded armadillo (*Dasypus novemcinctus*) populations, predominantly in natural habitats of the southeast United States. This study evaluated leprosy prevalence of armadillo populations in Lee and Mobile County, Alabama. Armadillos from rural, suburban, and urban zones were evaluated for leprosy infection, shedding light on the disease's prevalence in human-dominated landscapes where there may be a concern of zoonotic transmission. This study detected the first evidence of *Mycobacterium leprae* in a wild juvenile armadillo, suggesting possible vertical or environmental transmission routes, which warrant further investigation. Additionally, 83 adult armadillos in Mobile County exhibited a significantly higher leprosy prevalence (18.1%) compared to Lee County (1.6%, n = 64). Females had a higher infection rate (13%, n = 41) compared to males in Mobile County. The low prevalence in a central Alabama county compared to one along the gulf coast may be a result of environmental conditions as the counties predominantly exist in two different ecoregions. Therefore, this study presents findings that may support the previously described ecological-constraints hypothesis when applied to leprosy's northward expansion. Future research should investigate leprosy prevalence across different ecoregions and land use types to elucidate transmission dynamics. Although no difference between residential zones or significant spatial clustering was observed in this study, the potential zoonotic nature of leprosy underscores the importance of understanding infection patterns in urban and suburban environments and requires further study. This research

contributes valuable insights into the distribution and prevalence of leprosy in wild armadillo populations, informing public health strategies and wildlife management efforts in the southeast.

Introduction

The nine-banded armadillo

The nine-banded armadillo (*Dasypus novemcinctus)* is a medium-sized mammal native to South and Central America (Taulman and Robbins 1996). The armadillo expanded and was introduced in parts of the southeast United States, resulting in a present-day range that extends from the southern tip of Florida to central Illinois (Taulman and Robbins 2014). In Alabama, it is believed that expansion from an introduced population in Florida and a naturally-expanding Texas population resulted in a newly unified population within the state (Talmage and Buchanan 1954; Taulman and Robbins 2014).

Today, the armadillo can be found throughout the state of Alabama and, although they typically prefer bottomland mesic habitats, they can easily adapt to various habitats, including urban and suburban environments (Loughry and McDonough 2013). As evidenced by extension agents in the southeast, armadillos are becoming a common management concern for homeowners in more urban neighborhoods (Armstrong 1991; Hohbein and Mengak 2018). Additionally, armadillos inhabit a home range that is estimated to vary in size between 0.50 ha (Suttkus and Jones 1999) and 20 ha (Fitch *et al.* 1952), although this wide variation in the size of observed home ranges may be a result of the estimation methods used. On average, their home range has been estimated to be eleven hectares, or roughly 27 acres (Gammons *et al.* 2009).

Behaviorally, armadillos are a burrowing species and within their home range, they are likely to utilize multiple burrows. As a relatively solitary mammal, individuals usually den alone, with the exception of females and their young in the spring and early summer (Loughry and McDonough 2013). The armadillo is also mainly nocturnal but this active period has been found to vary seasonally and spatially with more armadillos found to be diurnal in the winter and in natural habitats compared to areas with more human disturbance, such as suburban areas (DeGregorio *et al.* 2021; DeGregorio *et al.* 2023). Although active for only a short period, they spend most of the active time foraging for food which includes mainly grubs and other insects (Ancona and Loughry 2010).

In the summer, individuals breed in non-monogamous pairs where males approach receptive females and exhibit courtship behaviors (McDonough 1997; McDonough 2000). In the spring, a litter of four identical offspring are born (Prodöhl *et al.* 1996). Female and male armadillos appear very similar to each other as the species does not exhibit sexual dimorphism (Ancona and Loughry 2010). A distinguishing characteristic in females is the presence of nipples which can also help infer reproductive status depending on their size and appearance; the reproductive status of males cannot be determined in the field, however, an age classification of "adult" can be used to estimate reproductive maturity (Loughry and McDonough 1996).

Using a combination of carapace measurements and weights, individuals can typically be classified into three broad age classes: adult (> 2 years), yearling (1-2 years), or juvenile (< 1 year) (Loughry and McDonough 1996). Additionally, the presence of phenotypic damage in the form of a scarred carapace, missing tail segments, and frayed ears has been measured in previous studies since the amount of damage is likely correlated with age (Loughry *et al.* 2002; Morgan and Loughry 2009). Carapace damage often results from fighting with conspecifics which has been observed in denser armadillo populations and during the breeding season when competing for mates (McDonough 2000; Loughry *et al.* 2002; Loughry and McDonough 2013).

Leprosy

Interactions between armadillos during territorial conflicts or mating may influence the spread of leprosy. *Mycobacterium leprae* and *Mycobacterium lepromatosis* are the causative bacterial agents of leprosy, a chronic infectious disease that mainly affects the peripheral nerves, skin, and mucous membranes (Han *et al.* 2008; Scollard 2016). Armadillos are the only known natural reservoirs of the bacterium, outside of red squirrels in the UK and humans (Meredith *et al.* 2014; Avanzi *et al.* 2016). The bacteria itself is an obligate intracellular pathogen that prefers colder temperatures such as the lower extremities in humans (Martin *et al.* 2017). Since armadillos have a lower body temperature than most mammals, the infectability of the pathogen is highly effective, making armadillos very susceptible to infection with only roughly 20% estimated to be resistant (Kirchheimer and Sanchez 1981). Around 65% of armadillos experimentally infected with the bacteria will develop a fully disseminated infection, requiring 18-24 months before symptoms begin to develop (Scollard *et al.* 2006; Truman *et al.* 2014). Symptoms include neurological involvement, granulomatous response, and histopathological responses, similar to how the disease presents in humans, and results in the death of an infected armadillo after a year and a half to two years (Truman *et al.* 2014).

The route of transmission between hosts is not well understood, however, it is believed that *M. leprae* is transmitted via infectious aerosols over an extended period (Araujo *et al.* 2016). Intraspecific and interspecific transmission could also occur indirectly through exposure to bacteria in soil and water (Holanda *et al.* 2017; Turankar *et al.* 2019) and vector transmission via an amoeba (Lahiri and Krahenbuhl 2008; Wheat *et al.* 2014), kissing bugs (Neumann *et al.* 2016), or ticks (Ferreira *et al.* 2018). The transmission of *M. lepromatosis* is less understood due to its more recent discovery in 2008 (Han *et al.* 2008; Scollard 2016).

Potential zoonoses

Zoonotic transmission of leprosy from armadillos has recently been demonstrated through the identification of a strain of *M. leprae* unique to armadillos and humans of the southeast United States (Truman *et al.* 2011). In addition, through interviews with leprosy patients native to the United States, it was found that although respondents could not recall direct contact with armadillos, they did report their familiarity with the species and that they engaged in outdoor activities such as gardening, which may have provided indirect contact with infectious armadillos (Truman *et al.* 2011; Sharma *et al.* 2015). In areas of South America considered to be endemic with leprosy, research has also shown that direct contact with armadillos through hunting and consumption of meat— a common cultural and subsistence practice in many communities of the Americas— increases the risk of zoonoses (Deps *et al.* 2008; Deps *et al.* 2020). With the exact routes of transmission being unknown and recent genetic analyses demonstrating a zoonotic source, human interactions with armadillos either directly or indirectly in shared areas may be a public health risk throughout the armadillo's range (Truman *et al.* 2011).

Leprosy prevalence

The study of disease prevalence has been used to better understand the spread of leprosy in wild armadillo populations. The prevalence of leprosy in Brazilian armadillos, detected via polymerase chain reaction (PCR) has ranged from 0% (Pedrini *et al.* 2010) to 63% (da Silva *et al.* 2018), although the latter study included a relatively small sample size of sixteen armadillos. In addition, sampling twelve armadillos in the Coari municipality of the Brazilian Amazon revealed that all twelve were negative via quantitative PCR detection of the multicopy repeat sequence (qPCR-RLEP) in *M. leprae*, but this study also had a small sample size (Stefani *et al.* 2019). More studies need to be conducted in South and Central America to record disease prevalence using consistent diagnostic testing and larger data sets (Deps *et al.* 2020).

Although there have been some studies in South and Central America, the majority of leprosy prevalence studies have been conducted in the southeast United States. Prevalence rates in wild populations of the southeast have ranged from 0% to 25%, using a combination of serologic, molecular, and histopathologic detection. As a result, positive armadillos have been found in every state in the southeast U.S. (Howerth *et al.* 1990; Loughry *et al.* 2009; Truman *et al.* 2011; Sharma *et al.* 2015).

Populations sampled west of the Mississippi River have revealed a prevalence that is variable and confined to low-lying coastal areas. Truman (2005) reasoned that differences could be due to ecological-constraints of *M. leprae* transmission such as humid soils, environmental agents affecting transmission or susceptibility, and higher densities of armadillos in these more attractive habits (Truman 2005). Additionally, Loughry *et al.* (2009) sampled populations east of the Mississippi River to evaluate whether leprosy had spread eastward and the applicability of the ecologic-constraints hypothesis proposed by Truman (2005). Loughry *et al*. (2009) found that leprosy was prevalent in several eastern populations, suggesting that prevalence may instead be explained by an epidemic model where leprosy steadily spreads as armadillos disperse. The ecologic-constraints hypothesis may still explain some patterns of prevalence in wild armadillos, however, if ecologic-constraints do exist, they may not be as restrictive as Truman (2005) had originally proposed. In addition, a recent finding by Haywood *et al.* (2022) who sampled armadillos at the most northern part of their range in Illinois, found that 25 individuals all tested negative, and this finding could be explained by both hypotheses.

The first prevalence study done in Alabama tested 144 armadillos from 15 counties and found no incidence of the disease (Howerth *et al.* 1990). Armadillo populations were later tested using both serologic and molecular diagnostic techniques at three Alabama sites: Fred T. Stimpson Wildlife Sanctuary (31.383333, -87.85), Riverside Recreation Area (32.9, -88.183333) and Conecuh National Forest (32.12714, -86.75290) (Loughry *et al.* 2009; Sharma *et al.* 2015). At the Riverside site in western Alabama, 10% of the 68 adults sampled were found to be positive via enzyme-linked immunosorbent assay (ELISA) of the phenolic glycolipid 1 antigen (PGL1) and qPCR-RLEP, whereas only 1% were positive out of the 63 adults sampled in the Stimpson Wildlife Sanctuary, located just northeast of Mobile County, AL (Loughry *et al.* 2009). At Conecuh National Forest, near the southeast border of Alabama, there were two armadillos serologically positive using ELISA for PGL1, seven serologically positive via ELISA for an anti-natural octyl disaccharide-leprosy IDRI diagnostic (LID), and five positive via qPCR-RLEP out of 38 armadillos sampled, resulting in a 13% prevalence (utilizing the PCR detection result and assuming the armadillos tested were all adults) (Sharma *et al.* 2015).

The prevalence of leprosy in armadillo populations has thus varied widely between populations sampled throughout the western and southern portions of Alabama. The geography also varies drastically from the coastal areas of the south to the piedmont region and Appalachian plateau of the central and northeast (Sapp and Emplaincourt 1975). Therefore, there may also be ecological constraints to disease transmission within the state from north to south that could be evaluated by recording leprosy prevalence in different ecoregions of Alabama. To further evaluate the ecological constraints hypothesis and the epidemic models of infection, Loughry *et al.* (2009) stressed the importance of screening armadillos from central and eastern Alabama, which has not yet been done, to evaluate how the disease is expanding with armadillo populations.

In this study, the prevalence of leprosy in two counties within east-central and southwest Alabama were evaluated to address knowledge gaps related to the geographic variation in leprosy prevalence. In previous studies, an armadillo population has been arbitrarily defined using the confines of a study site such as a national refuge or park. In this study, the armadillos within a county were considered a population. Genetic analyses were not used to create population boundaries, which is often a more precise method, however, there is potential to do this in the future for Alabama populations and subpopulations (Loughry and McDonough 2013).

Leprosy in urban and suburban areas

Leprosy is believed to be a density-dependent disease, and depending on the transmission route, may require direct or indirect contact between individuals which could be exacerbated in more human-dominated areas (Andrew and Loughry 2012). For instance, individual's home ranges may overlap in more attractive habitats like backyards, golf courses, and gardens (Mackenstedt *et al.* 2015). In addition, behavioral changes in the timing of armadillo activity has been documented in areas with human activity which may also increase contact rates between individuals and impact disease transmission (DeGregorio *et al.* 2021).

Several zoonotic disease systems have demonstrated higher pathogen prevalence in urban populations compared to rural populations such as woodchucks (*Marmota monax*) with *Toxoplasma gondii* in the Midwest (Lehrer *et al.* 2010) and mule deer (*Odocoileus hemionus*) with chronic wasting disease (CWD) in Colorado (Farnsworth *et al.* 2005). The mechanism for higher wildlife disease prevalence in more urbanized areas may be an increase in contact rates through the clumping of individuals and resources, which is necessary in directly transmitted, density-dependent disease systems (Brearley *et al.* 2013). For instance, the increased prevalence of CWD in mule deer may have been a result of smaller home ranges, confined food resources, fewer predators, and an increased concentration of mule deer in human-dominated landscapes compared to more natural areas (Farnsworth *et al.* 2005). An increased population density in urban areas has also been observed for some species such as raccoons (*Procyon lotor*), which may lead to a higher prevalence of wildlife diseases (Prange *et al.* 2003; Bradley and Altizer 2007).

Despite the potential for urban and suburban armadillo populations to have different spatial patterns of infection, only two studies have included populations from these areas and neither evaluated leprosy prevalence (DeGregorio *et al.* 2021; DeGregorio *et al.* 2023). The patterns of infection that have been evaluated instead come from more rural armadillo populations in natural areas. For instance, the armadillo population in the Yazoo National Wildlife Refuge, Mississippi, was observed to have a spatially homogenous pattern of infection with no clustering of infected individuals over the study's six-year duration (Perez-Heydrich *et al.* 2016). There is thus a knowledge gap in how urban and suburban armadillo populations may differ from populations in natural areas, including the prevalence of leprosy. This study aimed to sample armadillo populations across an urban to rural gradient to address this knowledge gap in leprosy prevalence.

Demographic patterns of infection

In armadillo populations, leprosy appears to infect older individuals including lactating females and individuals with increased amounts of phenotypic damage; the disease has also not previously been detected in yearlings or juveniles (Morgan and Loughry 2009). Some previous studies have identified more females infected than males (Morgan and Loughry 2009) whereas other studies have not found a significant difference (Truman *et al.* 1991; Andrew and Loughry 2012). The sex, age, location, and reproductive status of each individual collected for this study were recorded following previous methods to contribute to scientific understanding of both armadillo population demographics and patterns of infection (Loughry and McDonough 1996; Loughry *et al.* 2013).

Methods

Study sites

Study sites were limited to two Alabama counties: Lee County and Mobile County. Lee County is predominantly within the piedmont ecoregion and the southern portion is within the southeastern plains; Mobile County, on the other hand, resides completely within the southeastern plains ecoregion (US EPA 2015). Each county was subdivided into urban, suburban, and rural zones to evaluate leprosy prevalence along an urban to rural gradient. The suburban or peri-urban zone is considered to be the area that lies between rural and urban areas and is often a highly interconnected transitional zone (Žlender 2021). Urban and suburban areas can be difficult to define, however, land imperviousness was used in this study since armadillos require pervious ground to forage and burrow (Talmage and Buchanan 1954).

Land cover data from the 2019 National Land Cover Database (NLCD), maintained by the Multi-Resolution Land Characteristics (MRLC) consortium, was used in this study (Detwitz 2021). Areas in the NLCD database classified as "Developed, Open Space" and "Developed, Low Intensity" were used to define suburban zones and areas classified as "Developed, Medium Intensity" and "Developed, High Intensity" were used to define urban zones in each county. All remaining land cover types were classified as rural for the purposes of this study. Road data for map creation was retrieved from the USGS National Transportation Dataset for Alabama (U.S. Geological Survey 2023). County boundaries from the 2020 U.S. Census codes were acquired through the Environmental Systems Research Institute (ESRI) (ESRI 2023).

The literature has recorded various home range sizes for armadillos; 11 hectares was found to be the average size by Gammon *et al.* (2009), which was similar to findings summarized by Loughry and McDonough (2013). Therefore, 11 hectares was used to define an individual armadillo's estimated home range in this study. The square root was taken of the home range value, in square meters, to provide a buffer distance of 331.66 meters for the suburban and urban zones. ArcGIS Pro (v. 3.0.3, ESRI, Redlands, CA, USA) was used to generate the maps and for all spatial analyses in this study. Map creation steps are listed in Appendix I. The final maps for Lee and Mobile County, AL were created on February 8th, 2023 (Figure 2.1.). The coordinate of each collected armadillo was mapped and the zone layer it intersected was its designated residential zone for the purposes of this study.

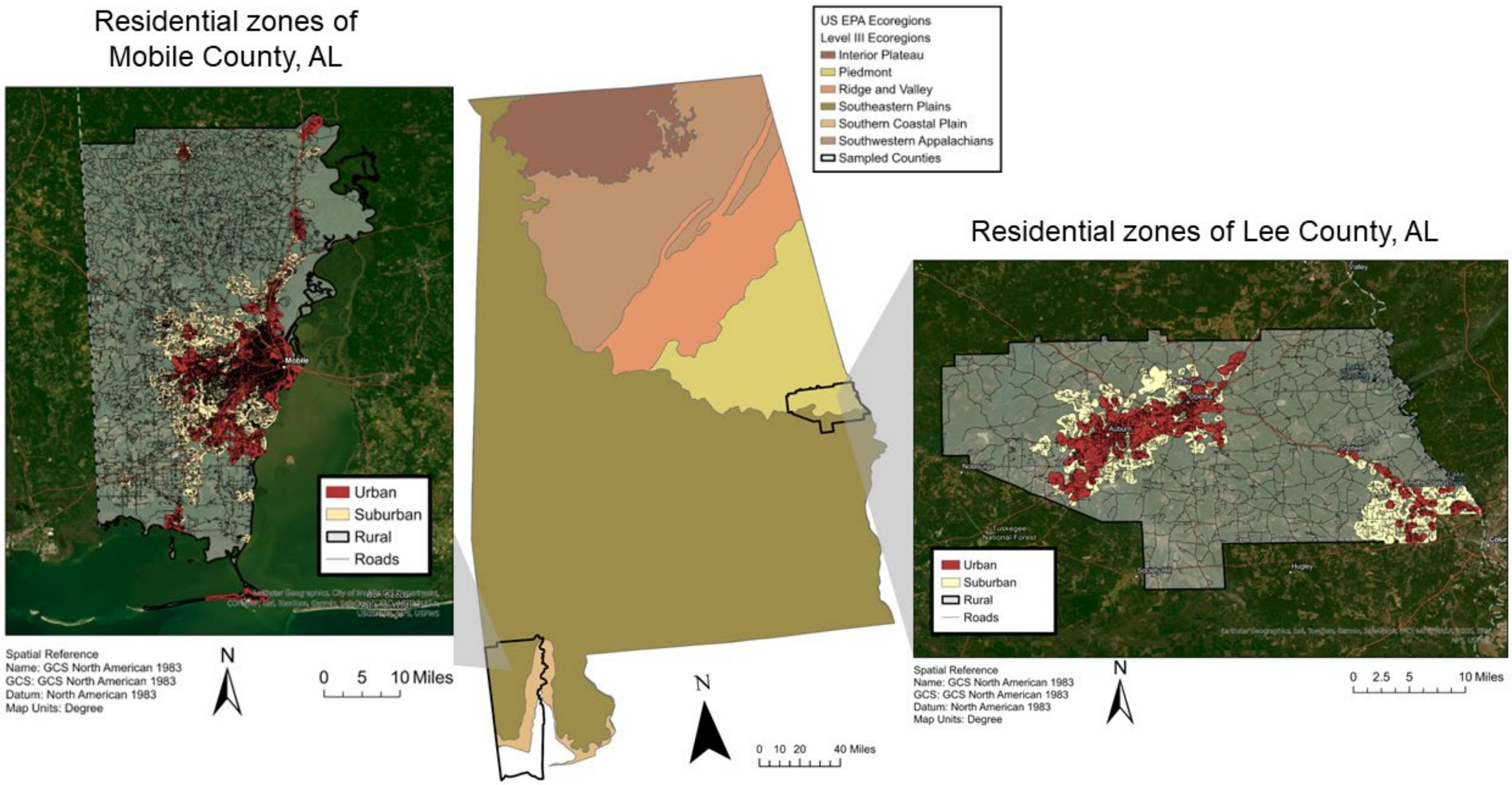

Figure 2.1. The residential zones of Lee County and Mobile County, AL. The ecoregions of Alabama are also mapped using the US EPA Level III GIS data. Residential zones were rural, suburban, and urban and were defined using land cover data from the National Land Cover Database. The maps were created using ArcGIS Pro (version 3.0.3). The coordinate of each collected armadillo was mapped and the residential zone layer it intersected was the one it was designated.

Armadillo collection

Lee County armadillos were collected in the summer of 2022 and Mobile County armadillos were collected in the summer of 2023. Private properties were the primary study sites in both counties. Homeowners with armadillos on their property could contact me through Facebook, a social media application, as well as by calling or texting a telephone number designated for the study. Additionally, community members who observed roadkill armadillos within the counties of interest could provide me with the location and time of their sighting(s). Information was shared through community groups on Facebook, flyers, and Alabama Cooperative Extension Service (ACES) newsletters. Some participating libraries and local businesses also hung fliers with information about the study and my research telephone number. Once homeowners made contact, I responded to them as soon as possible to set traps. In both counties, there were an influx of homeowners with armadillos on their property in which they desired removal, therefore, I visited properties in the order they contacted me while also ensuring I dispersed traps throughout the residential zones of interest.

Each property was visited and carefully inspected to correctly identify armadillo damage and/or burrows that were reported by homeowners. Once physical damage or other evidence— such as camera footage— was confirmed, a box cage trap was placed on the property. The traps used were either single-door 32” Havahart traps or 36” Tomahawk traps. If an armadillo burrow could be identified, the trap was placed directly in front of the burrow. Additionally, a trap was placed in front of any gaps under fences or buildings that could be identified and were evidenced to be caused by an armadillo. If there were no burrows or other suspected entrances, a trap was placed along a nearby building or path where armadillos appeared to travel. If armadillo tracks were observed, traps were placed in those areas. In some instances, homeowners had traps of their own that were placed alongside the ones that I placed to increase trap success. Traps were placed with cage wings on either side of the trap entrance. Cage wings were often 2 x 6” wood planks that were staked into the ground, and in some cases, other materials that the homeowner had available such as cement blocks.

The traps were placed in the afternoon and checked each morning. Traps were left for several nights and up to two weeks at some properties. After three unsuccessful nights, traps were re-arranged according to subsequent signs observed, if any. When an armadillo was trapped, the decision to lethally remove armadillos from a property or return them where found was left to individual homeowners. For homeowners that desired removal, armadillos were humanely euthanized using carbon dioxide at a 20% displacement rate which is in accordance with the American Veterinarian Medical Association’s 2020 guidelines for humane euthanasia (Leary *et al.* 2020). A chamber was constructed for the purpose of euthanasia with a regulator and viewing screen on the lid to monitor individuals and adjust air flow. If the homeowner did not want a trapped armadillo euthanized, they were released exactly where they were trapped.

In addition to trapping, some rural properties with widely dispersed armadillos benefited from me and a research assistant walking at night or in the early morning with long-handled dip nets. Spotlighting with flashlights and listening for sounds of foraging armadillos was used to locate individuals. Any armadillos captured were treated similarly to those caught in box traps; if the homeowner desired the removal of an armadillo caught in a dip net, the armadillo was transferred to the chamber and euthanized. Within the counties of interest, I also drove along roads looking for roadkill armadillos. Research assistants and I searched in the morning on travelled roads as well as responded to community sightings of roadkill armadillos. Roadkill individuals were collected along roadsides if they were safe to acquire and still viable. An armadillo was considered viable if the individual's organs of interest (liver and spleen) were generally intact and still encased by the carcass.

Sample collection

The GPS location of each armadillo was recorded at the site of capture or collection. Armadillos that were euthanized were placed in the euthanasia chamber while still in the trap to eliminate unnecessary stress to the animal. Once mortality was confirmed, the individual was transferred to the bottom of the chamber, which was cleaned with bleach after each armadillo. Tissue was collected by snipping a small tip of the ear and was subsequently affixed in 70% ethanol. Tissue samples were kept in a cooler on ice until transferred to a freezer. A cardiac puncture using a syringe was also performed to collect liquid blood. Blood was expressed onto Nobuto blood filter strips (Advantec 800700 Cole-Parmer).

The sex of each armadillo was recorded by examining external genitalia. The lactation status of females was also characterized as either definitely lactating, possibly lactating, or not lactating by observing the state of the nipples, according to previous studies (Morgan and Loughry 2009). The width of the front carapace, front band, back band, tail base, and the length of the tail were measured using a cloth measuring tape to the nearest centimeter. Lastly, the armadillo was placed in a garbage bag and a spring scale was used to collect its weight to the nearest kilogram;

the weight of the garbage bag was deducted from the final weight. The age classes of armadillos and the weight ranges that have been used in previous literature include: adult (≥ 3.5 kg), yearling (2.5-3.5 kg), and juvenile (≤ 3.5 kg) (Loughry and McDonough 2013; Oli *et al.* 2017). Since leprosy has not previously been detected in yearlings or juveniles, it was a conscious effort to sample more adults, however, all armadillos, regardless of age, were sampled when captured or found (Morgan and Loughry 2009).

If the armadillo was not euthanized, the methods of sample collection varied slightly. Armadillos were transferred out of the live trap into a burlap sack and the weight was measured using the same spring scale; the weight of the sack was deducted from the final weight. The weight was collected first, followed by the collection of blood and tissues. A small tip of the ear was collected using sterile scissors and if the site bled any, this was collected on a Nobuto strip. If the ear did not bleed enough for a full strip, a single front toenail was clipped using a veterinary clipper. The site of the ear collection and/or toenail clipping had a styptic pen applied to stop any excessive bleeding and was sprayed with an antiseptic. Similar to the euthanized armadillos, measurements of the carapace were taken. For sites where I planned to continue trapping on the property or nearby area, I adhered reflective tape using super glue to the front carapace which allowed for short-term long-range identification. Reflective tape has been used in past studies and there are no known survival costs; additionally, reflective tape may even be beneficial as it may increase an armadillo's visibility to motorists (Loughry and McDonough 2013; Loughry *et al.* 2013).

Many of the sampling techniques used for live armadillos were used to sample roadkill, however, instead of ear tissue, small (1g) samples of liver and spleen tissue were collected using disposable scalpel blades. Liver and spleen tissue were affixed in 70% ethanol and kept on ice until placed in a freezer. Blood was collected on Nobuto strips from inside the body cavity or on external orifices like the nose and mouth that had bled during collision. The weight was also taken by placing the armadillo in a garbage bag and using a spring scale; however, it was noted if the weight appeared to be partial due to the collision.

In addition to the previously described measurements, I also recorded any phenotypic damage on the carapace or tail of the individual (Loughry *et al.* 2002). However, it should be noted that phenotypic damage was documented as presence or absence and was not assigned a score, as previous studies have done (Morgan and Loughry 2009), due to the use of roadkill armadillos where phenotypic damage would be more difficult to quantify. Any skin lesions, or signs of leprosy like dermatitis of the feet, nose, or eyes were documented, however, this is not a reliable measure of disease due to the carapace covering most of the armadillo's body and some leprosy-positive individuals exhibiting few to no skin lesions (Truman *et al.* 2014). All the techniques previously described to capture and sample live armadillos were consistent with previously published protocols (Loughry and McDonough 2013). The protocol used in this study was also reviewed and approved by the Auburn University Institutional Animal Care and Use Committee (2022-5002).

Diagnostic testing

Prior to shipment, all tissue samples were kept frozen at or below -17°C and blood samples were refrigerated in Ziploc bags. Tissue and blood samples were sent in batches to the National Hansen Disease Program (NHDP) in Baton Rouge, LA, USA where the lab diagnostic testing was performed. Both qPCR-RLEP and ELISA for PGL1 have been proven to effectively and accurately diagnose leprosy (Gama *et al.* 2020) and were thus used to detect infection with *M. leprae* from the tissue and blood samples I collected. To minimize the amount of sampling and materials needed, the blood of each individual armadillo, where available, were first analyzed using ELISA to detect IgM antibodies against PGL1; ELISA testing determines if an individual is seropositive or seronegative, in other words, whether antibodies are detected in the blood which is indicative of a previous or current infection with the bacteria and has been proven to be an effective method for detecting infection in wild armadillos (Truman *et al.* 1986).

If serologically positive, tissues were then tested for *M. leprae* DNA using qPCR-RLEP; molecular testing has been proven to be an effective diagnostic method for *M. leprae* DNA that is correlated with the number of bacilli in infected tissues (Truman *et al.* 2008). For roadkill and other individuals where tissue from the liver and spleen were the only samples that could be collected, tissues were analyzed using only qPCR-RLEP.

Statistical analyses

Variables that may impact prevalence in armadillos including age, weight, sex, location, and method of collection (roadkill or live capture) were analyzed with seroprevalence to compare with the findings of previous studies done in the southeast. Additionally, the Lee and Mobile population demographics and leprosy prevalence were compared. Descriptive statistics were calculated for the Lee and Mobile County populations using Microsoft Excel. Chi-Square tests of independence were also done in Excel to evaluate whether there were significant associations between the variables of interest. R (version 4.3.2) was used for two-way ANOVA tests for quantitative variables of interest (R Core Team 2022). For additional comparisons, post-hoc tests were used to evaluate significant associations.

Results

Location of sampled armadillos

A total of 94 armadillos were sampled in Lee County and 93 in Mobile County. Roughly half (51%) of the armadillos in Lee County were roadkill compared to the 35% in Mobile County, however, there was not a statistically significant difference between the counties in regard to the collection methods ($\chi 2 = 5.65$, $p > 0.10$). In Lee County, 46 armadillos were collected from 27 private properties and in Mobile County, 57 armadillos were collected from 33 properties. There were more Lee County armadillos sampled from the urban zone (47%) compared to the other residential zones whereas the most represented zone in Mobile County was suburban (43%). There was a statistically significant association between the county and residential zone in which armadillos were sampled with more rural and fewer urban armadillos sampled in Mobile compared to Lee County ($\chi 2 = 20.01$, $p < 0.01$). The capture methods and residential zones of armadillos sampled in both counties are summarized in Table 2.1.

Table 2.1. A summary of the armadillos sampled in Lee and Mobile County, AL by residential zone and method of collection. Live-caught armadillos include live animals that were either released or euthanized. Roadkill includes animals that were found along roadsides or were deceased when donated. Rural, suburban and urban zones were designated using 2019 land cover data from the National Land Cover Database for each county.

Residential Zone	Lee			Mobile		
	Live-Caught	Roadkill	**Total**	Live-Caught	Roadkill	**Total**
Rural	7	4	11	26	7	33
Suburban	26	13	39	24	16	40
Urban	13	31	44	10	10	20
Total	46	48	**94**	60	33	**93**

Demographics of sampled armadillos

There was roughly the same ratio of males to females sampled in both counties (1.5:1 in Lee and 1.3:1 in Mobile) and there was not a significant difference between the counties ($\chi2 = 0.43$, $p > 0.10$). There was a slight significant difference in the sexes sampled between residential zones in Lee County with more males sampled in suburban areas than females ($\chi2 = 6.19$, $p < 0.05$), however, there was no significant difference between the sexes and zones in Mobile County ($\chi2 = 2.36$, $p > 0.10$). There was no significant difference in the ages of armadillos sampled in each residential zone for Lee County ($\chi2 = 5.76$, $p > 0.10$) or Mobile County ($\chi2 = 1.74$, $p > 0.10$).

Armadillos were also given an age classification from their measured weight. Most of the armadillos that were classified as yearlings were not near the minimum adult weight (3.5 kg) nor were missing a significant amount of body mass when weighed. However, there were two females in Lee County that were close to the minimum adult weight but had been observed to be previously lactating; these two females were categorized as adults since biologically, females would not have previously lactated prior to two years of age (Loughry and McDonough 2013). It should also be noted that although weight has been shown to be a reliable measure in distinguishing adults from juveniles, it is less accurate in distinguishing adults from yearlings, therefore, armadillos categorized as yearlings in this study may be adults, and vis-versa (Loughry and McDonough 1996).

There was a significant difference in the age classes sampled in both counties with a significantly greater number of juveniles sampled in Lee County compared to Mobile ($\chi2 = 17.40$, $p < 0.001$). In both counties, there were more adults sampled than yearlings or juveniles (68% were adults in Lee and 89% in Mobile). The body condition of armadillos in each age class for Lee and Mobile Counties were compared. The weight of armadillos did not significantly differ for the three age classes between the counties according to a two-way ANOVA and post-hoc Tukey's Range test ($p > 0.10$). Regarding sex, there was not a significant difference in the weight of adult females between Lee and Mobile County, however, adult males in Lee weighed 0.47 kg greater ($p < 0.01$) than males in Mobile County. Additionally, within each county, the adult weights between sexes were not significantly different in Mobile County (T-score = 0.53, $p > 0.50$), however, they were in Lee County with adult females weighing less than males (T-score = 3.6, $p < 0.01$). There was also a difference in the number of reproductive females in Lee and Mobile County with more in Mobile, however, this was not statistically significant ($\chi2 = 2.70$, $p > 0.05$). Additionally, reproductive females weighed more than non-reproductive females in Mobile County (T-score = 4.5, $p < 0.01$).

There was a significant difference in the presence of phenotypic damage between the counties with more damage observed in Mobile armadillos ($\chi2 = 15.80$, $p < 0.001$). Within each county, there was not a significant difference between the presence of phenotypic damage and sex which is consistent with previous studies (Loughry *et al.* 2002) (Lee: $\chi2 = 0.93$, $p > 0.10$; Mobile: $\chi2 = 0.59$, $p > 0.10$). However, inconsistent with Loughry *et al.* (2002), there was not a significant difference in age and the presence of phenotypic damage (Lee: $\chi2 = 1.72$, $p > 0.10$; Mobile: $\chi2 = 1.32$, $p > 0.10$). Additionally, there was not a significant association between female reproductive status and the presence of phenotypic damage for either population (Lee: $\chi2 = 3.12$, $p > 0.10$; Mobile: $\chi2 = 2.18$, $p > 0.10$). A summary of the population demographics of armadillos sampled in Lee and Mobile County is given in Table 2.2.

Table 2.2. Demography of the Lee and Mobile County, Alabama sample populations. Females were defined as "reproducing" if their nipples were possibly or definitely lactating. The total number of armadillos sampled from each county by sex and age class are summarized.

	Male			Female				
	Total	Average Weight (± SE)	Phenotypic Damage Present	Total	Average Weight (± SE)	Phenotypic Damage Present	Non-Reproducing	Reproducing
Lee	57	4.10 (±0.18)	14	37	3.80 (±0.16)	6	24	13
Adult	40	4.90 (±0.09)	12	24	4.30 (±0.14)	4	11	13
Yearling	6	2.73 (±0.07)	1	7	2.77 (±0.12)	1	–	–
Juvenile	11	1.96 (±0.09)	1	6	2.17[A] (±0.14)	1	–	–
Mobile	52	4.44 (±0.10)	27	41	4.35 (±0.11)	18	19	22
Adult	47	4.61 (±0.06)	23	36	4.56 (±0.08)	16	14	22
Yearling	4	3.00 (±0.14)	3	5	2.92 (±0.15)	2	5	0
Juvenile	1	1.88[B]	1	0	–	–	–	–

A The weight was not collected from three of the juvenile females, therefore, n=3.

B n=1

Leprosy prevalence

An armadillo was considered to be leprosy-positive if they were seropositive for PGL1 via ELISA or if *M. leprae* DNA was detected via qPCR-RLEP. Since juveniles and yearlings have not been found to test positive for *M. leprae,* the prevalence and remaining analysis was done with the total number of adults sampled in each county (n = 64 for Lee and n = 83 for Mobile). There was only one armadillo in Lee County found to be positive via qPCR-RLEP, resulting in a prevalence among adults of 1.6%. Mobile County, on the other hand, had 15 adult armadillos that tested positive, resulting in a prevalence of 18.1% (Table 2.3.). Therefore, there was a significant difference in leprosy infection between the counties ($\chi2 = 10.91$, $p < 0.001$). Outward lesions indicative of leprosy infection was not observed on any of the armadillos sampled in Mobile or Lee County, however, this was expected since gross skin lesions are not a typical sign of disease in armadillos, as described by Truman *et al.* (2014).

Table 2.3. A summary of the leprosy infection results for adult armadillos sampled in Lee and Mobile County, Alabama. Only the adults are summarized here, however, one juvenile in Mobile County, AL did test positive via qPCR-RLEP as well.

County	Adults sampled	Number PGL1[A] (%; *n*)	Number qPCR-RLEP[B] (%; *n*)	Total Leprosy-positive
Lee	64	0 (0%; 50)	1 (1.2%; 64)	1 (1.6%)
Mobile	83	10 (12.7%; 79)	9 (11.0%; 82)	15 (18.1%)

A Seroprevalence detected via ELISA for PGL1
B qPCR using a specific RLEP sequence and heat shock protein 18 gene fragments

Lee County

The individual armadillo that was positive in Lee County was an adult male with a weight of 5.60 kg. The individual was a roadkill collected in a suburban area of the county (Figure 2.2.). The demographics of the positive armadillo in Lee County could not be reliably compared to other leprosy-negative armadillos due to there being only one positive. Additionally, spatial analysis could not be done comparing the three residential zones and leprosy infection in Lee County.

Leprosy-Positive and Leprosy-Negative Armadillos in Lee County, AL

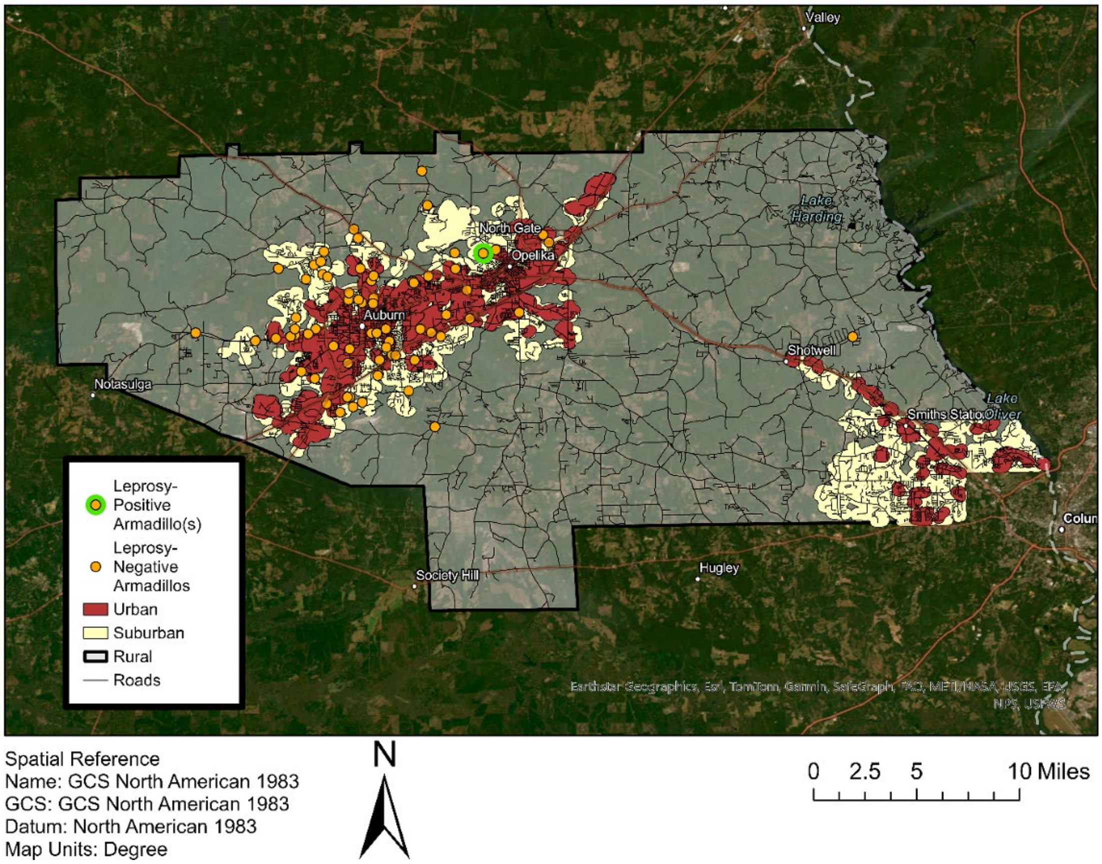

Figure 2.2. The armadillos sampled in Lee County, AL. The leprosy-positive individual, as confirmed by ELISA and qPCR-RLEP, is highlighted in green and was a roadkill collected from a suburban area of the county. There were 64 adults and a total of 94 armadillos in the Lee County sample population.

Mobile County

The armadillos that tested positive for leprosy in Mobile County were all live-caught or euthanized on private property; there were no roadkill armadillos that tested positive. For Mobile County, the demographics of leprosy-positive adult armadillos could be compared to leprosy-negative adults due to a greater number of leprous individuals. Firstly, there was a significant association between sex and leprosy infection with more females (13%) than males (5%) being infected despite a greater number of adult males sampled overall ($\chi2 = 6.69$; $p < 0.01$). Among females, there was also a significant association between reproductive status and leprosy infection with more reproductive females being leprous ($\chi 2 = 8.28$; $p < 0.01$). Additionally, there was a significant association between leprosy infection and presence of phenotypic damage among females with eight leprosy-positive individuals having phenotypic damage and three individuals lacking damage ($\chi 2 = 5.13$; $p < 0.05$). Males, on the other hand, did not have a statistically significant association between phenotypic damage and leprosy infection ($\chi 2 = 1.19$; $p > 0.10$).

There was no significant difference in weight between leprosy-negative and leprosy-positive individuals for both males and females (T-Score = 0.7; $p > 0.10$). Additionally, there was no significant difference in body measurements between leprosy-positive and leprosy-negative armadillos ($p > 0.10$). The averages of the body measurements of leprosy-positive and leprosy-negative armadillos are summarized in Table 2.4.

Table 2.4. The average body size and condition of leprous (either positive via ELISA or PCR) and non-leprous adult male and female nine-banded armadillos in Mobile County, AL. n = 83, however, all measurements could not be obtained from each sampled individual. Therefore, the total sample size in which the averages were calculated is noted for each measurement.

Average measurements (± SE)	**Males**		**Females**	
	Leprosy-positive (SE) [*n*]	Leprosy-negative (SE) [*n*]	Leprosy-positive (SE) [*n*]	Leprosy-negative (SE) [*n*]
Weight (kg)	4.26 (±0.27) [4]	4.45 (±0.10) [48]	4.61 (±0.11) [11]	4.26 (±0.14) [29]
Front carapace length (cm)	19.9 (±0.6) [4]	20.2 (±0.2) [39]	20.8 (±0.3) [11]	20.2 (±0.3) [24]
Front band length (cm)	32.2 (±1.0) [4]	33.1 (±0.4) [42]	33.8 (±0.4) [11]	32.2 (±0.8) [25]
Back band length (cm)	36.1 (±0.7) [4]	36.9 (±0.4) [41]	38.1 (±0.4) [10]	36.9 (±0.5) [25]
Tail base (cm)	15.6 (±0.4) [4]	15.5 (±0.2) [47]	16 (±0.2) [11]	15.3 (±0.2) [28]
Tail base length (cm)	34.9 (±1.5) [4]	32.8 (±0.6) [46]	32.9 (±1.3) [11]	33.4 (±0.3) [28]

There was one juvenile male in a suburban area that tested positive for *M. leprae.* The individual weighed 1.88 kg and was released where it was captured. There was also some phenotypic damage present on the individual's carapace. Since it was the only juvenile sampled that tested positive in either Lee or Mobile County, it could not be compared to other leprous juveniles. Additionally, it was the only juvenile sampled in Mobile County since the rest were either adults or yearlings. The littermates of the individual could also not be captured and sampled; however, they were present near the burrow where the juvenile was captured since the homeowner had observed multiple juveniles simultaneously foraging.

Almost half of the positive adult armadillos were rural (47%) followed by 33% suburban and 20% urban (n = 15) (Figure 2.3.). The positive individuals were then considered as a proportion of the number sampled in each zone to calculate prevalence by zone. Rural leprosy prevalence was 24%, urban was 17%, and suburban was 14%. However, no significant association was found between leprosy infection and residential zone via a Chi-Square test of independence ($\chi2$ = 1.17, $p > 0.10$).

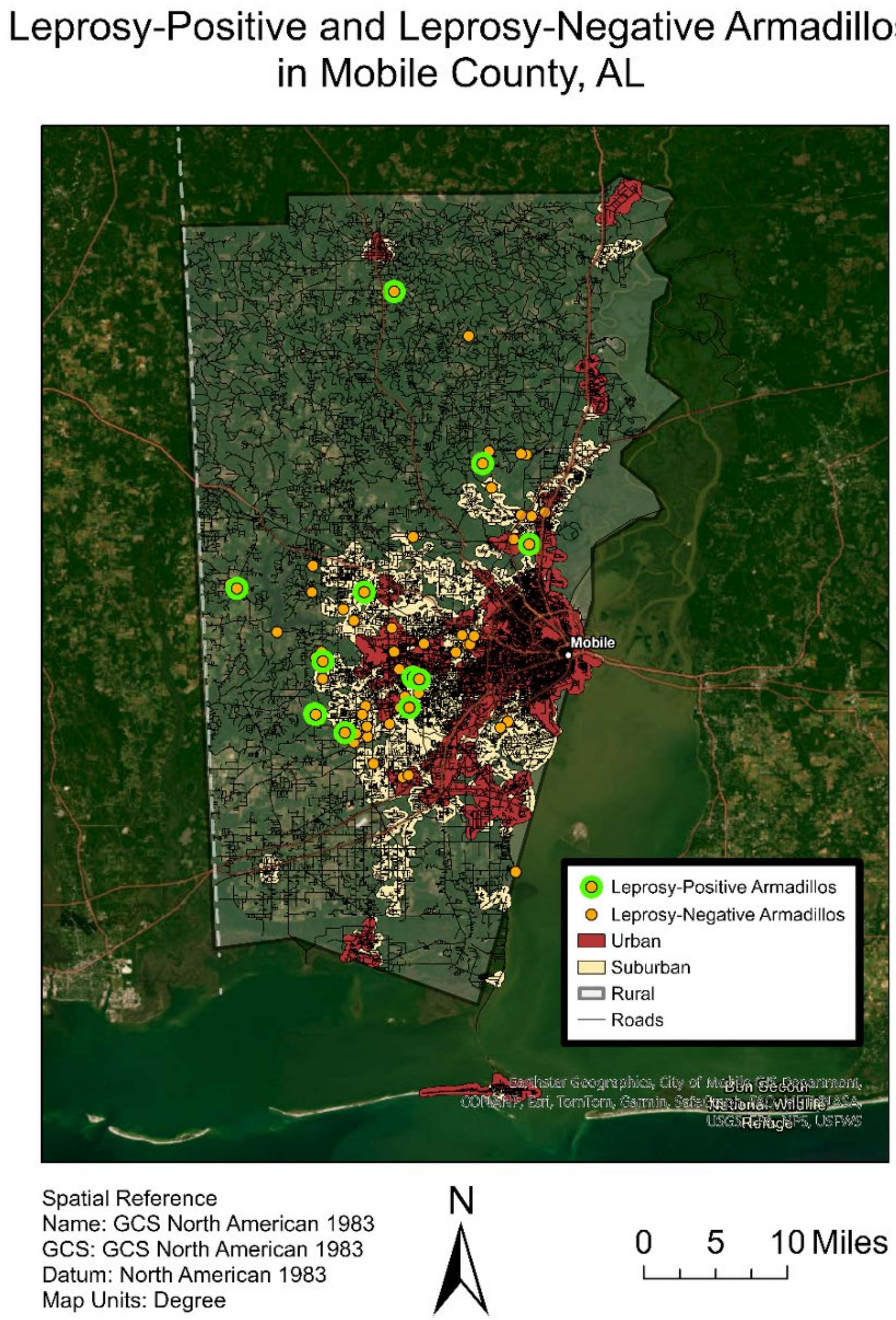

Figure 2.3. All the armadillos sampled in Mobile County, AL and their residential zones. The leprosy-positive adult armadillos, as confirmed by ELISA for PGL1 and qPCR-RLEP, are highlighted in green. There were 83 adults sampled and a total of 93 armadillos were in the Mobile County sample population.

Although there were properties with a combination of leprosy-negative and leprosy-positive adults sampled, it is interesting to note two specific properties due to there being multiple adults with positive and negative leprosy infection. The first property was in a rural area where five males and three females were sampled, all of which were adults. Out of the eight individuals, three were leprosy-positive, two females (M58 and M88) and one male (M91). Traps were placed in different areas of the property, either at burrows or near fences with entrances underneath. The locations of the leprosy-positive and leprosy-negative armadillos are displayed in Figure 2.4.

Leprosy-Positive and Leprosy-Negative Armadillos Sampled at a Rural Property

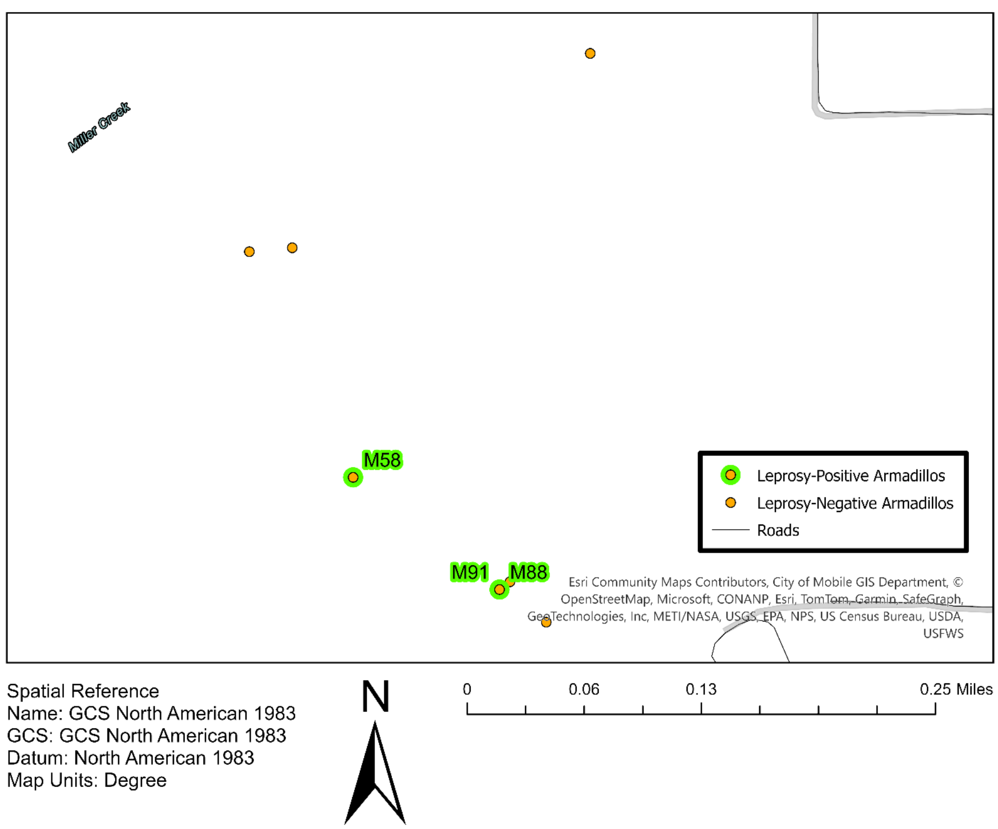

Figure 2.4. A summary of the armadillos sampled at a rural property in Mobile County, AL and their leprosy infection status which was confirmed using qPCR-RLEP and ELISA for PGL1. $n = 8$

Additionally, a suburban property had a total of six armadillos sampled, five adults and one yearling. Four of the adults were males and two were females but only one adult male tested positive that was caught at the end of the sampling period (August 2023). At this property, the trap did not move locations and was placed near an opening underneath a fence. Although formal spatial analysis was not done on either property and the sample size of individuals is small, the spatial patterns of infection at properties in different residential zones are interesting to compare and will require further study.

Discussion

Previous studies have evaluated leprosy's consequences on armadillos (Morgan and Loughry 2009), prevalence in the southeast United States (Loughry *et al.* 2009) and Illinois (Haywood *et al.* 2022), and the potential for leprosy to be zoonotic throughout the nine-banded armadillo's range (Truman *et al.* 2011; Sharma *et al.* 2015). Previous prevalence studies of armadillo populations, however, have been limited to those in more natural areas, such as wildlife refuges. This is the first study of its kind to evaluate leprosy prevalence of armadillos captured on private properties and along roadways in residential areas of the southeast United States. Additionally, armadillos collected in this study were evaluated for leprosy among three residential zones based on land imperviousness: rural, suburban, and urban.

With the increasing evidence of leprosy as a zoonotic disease and recent news stories highlighting leprosy as a potentially endemic disease in parts of the southeast United States (Mandavilli 2023), understanding the prevalence of leprosy in human-dominated landscapes is increasingly important. Leprosy may also be environmentally transmitted, resulting in the infection of people, which is not well understood and may be exacerbated by armadillos (Bhukhan *et al.* 2022). This study also contributes to our understanding of leprosy prevalence in wild armadillos of Alabama which have recently been limited to sites in the eastern and southern portion of the state, including Stimpson Wildlife Sanctuary (1%), Riverside, AL (10%), (Loughry *et al.* 2009) and the Conecuh National Forest (13%) (Sharma *et al.* 2015). With fewer prevalence studies having recently been conducted east of the Mississippi River, evaluating leprosy prevalence in Alabama informs how the disease may be spreading with the armadillo's quickly expanding range (Taulman and Robbins 2014).

The two armadillo populations evaluated in this study, Lee and Mobile County, AL, were comparable in regard to population structure and sex distribution. Both populations had more adults sampled overall since juveniles and yearlings have not been found to be leprosy-positive via PCR or ELISA in previous studies (Morgan and Loughry 2009). However, one juvenile in Mobile County did test positive for *M. leprae* via qPCR-RLEP. The detection of *M. leprae* in a juvenile may be the result of an infection with a low dose of the bacteria. This transmission may have occurred from the mother who may have been leprosy-positive, however, leprosy has not previously been considered to be vertically transmitted (Morgan and Loughry 2009). An additional possibility is that the juvenile was infected through the environment resulting in an ear tissue that contained the bacteria. Evidence of possible environmental transmission has been found (Lahiri and Krahenbuhl 2008; Wheat *et al.* 2014; Holanda *et al.* 2017; Turankar *et al.* 2019) and although it requires further study, the environment could be a potential route of transmitting bacteria to a juvenile. It is also possible that *M. leprae* has existed in wild armadillo juveniles, but they have not been sampled as frequently as adult armadillos in previous studies.

Additionally, qPCR-RLEP is highly specific for *M. leprae* (Truman *et al.* 2008), and it is unlikely that there was cross-contamination, however, it is a possibility that there was sampling error during collection. Although only one individual from the litter could be sampled and one juvenile of the two populations tested positive, it would be beneficial to continue to include juveniles, including all littermates, and yearlings in future prevalence studies.

Overall, there were more adult armadillos sampled in Mobile compared to Lee County. There was no difference in the age of individuals and the method used to collect them (i.e. roadkill or live capture). Adult females in Mobile County had more phenotypic damage and were reproductive compared to Lee County, however, the latter finding was not statistically significant. Still, these findings may indicate an older population in Mobile County as reproductive status and phenotypic damage have been shown to be correlated with age (Loughry *et al.* 2002).

Prevalence of leprosy infection among adult armadillos was found to be significantly higher in Mobile County compared to Lee County. The low prevalence in Lee County resulted in an inability to compare demographic and spatial patterns of infection, however, this could be done for Mobile County. Significantly more females were leprosy-positive compared to males despite a greater number of males represented in the population; this is a finding consistent with Morgan and Loughry (2009), however, other studies have observed no significant difference in leprosy infection between the sexes (Truman *et al.* 1991; Andrew and Loughry 2012). It has been observed that armadillo populations consist of long-term residents, likely reproductive females, and a significant amount of transient individuals who move through an area (Loughry and

McDonough 2001; Loughry *et al.* 2013). These transient individuals are more likely to be younger individuals, including males looking for mates. Since armadillos were sampled during peak breeding season (June – July), there is a possibility that the males sampled were younger individuals who are less likely to be leprosy-positive, which would explain the observed difference in leprosy prevalence between the sexes. However, there is currently no reliable method to determine the exact age of an armadillo, and this is especially difficult for males where reproductive status cannot be accurately estimated in the field.

Despite the differences in leprosy infection in males and females, it has been found that population-level impacts are unlikely to be significant due to leprosy infecting older females who have previously reproduced (Oli *et al.* 2017). My findings support that reproductive females are more likely to be leprosy-positive compared to non-reproductive females. Individual armadillo body condition also did not appear to be greatly impacted in infected individuals since there was not a significant difference in body weight or measurements; the latter finding is contrary to previous findings (Morgan and Loughry 2009) and may be due to leprous individuals in Mobile County experiencing an early dissemination of infection that had not yet affected their body condition.

The low prevalence among adults in Lee County (1.6%), compared to Mobile County (18.1%) may be due to fewer older adults sampled. Additionally, Lee County had significantly fewer rural adult armadillos sampled than Mobile County, which had a rural prevalence rate of 24%. However, it is unlikely that this had a significant impact on observed prevalence since the status of leprosy infection was not significantly associated with the residential zone of collection in

Mobile County. Furthermore, the one leprous individual that was detected in Lee County was collected from a suburban zone.

The varying prevalences between Lee and Mobile County may instead be a result of spatial limits in the disease's expansion. When evaluating leprosy spread eastward from the Mississippi River, Loughry *et al* (2009) had suggested two hypotheses for the disease's seemingly limited eastward expansion and provided some support for both: the ecologic-constraints hypothesis and the epidemic hypothesis. In their study, they found armadillos positive for *M. leprae* in western Alabama (Riverside) at a prevalence of 10%, therefore, although there may be some ecologic-constraints to infection in certain sites, leprosy appeared to still be dispersing eastward (Loughry *et al.* 2009). Since Loughry *et al.*'s 2009 study, Sharma *et al.* (2015) has documented *M. leprae* in armadillos in southeast Alabama, south Georgia, and north Florida, indicating that leprosy has indeed spread eastward, well past the Mississippi River. This study provides additional evidence of leprosy prevalence in the southeast with a relatively high prevalence in a southwestern Alabama county and the detection of leprosy in an east-central county.

This study also evaluated the potential for leprosy's northward expansion in eastern Alabama. There have been four sites east of the Mississippi River and north of Lee County, AL, where prevalence has been recorded: 1) the Yazoo National Wildlife Refuge (NWR), MS (16.4%) (Perez-Heydrich *et al.* 2016) 2) DeWayne Hayes Recreational Area, MS, (3%) (Loughry *et al.* 2009), 3) Riverside, AL (10%) (Loughry *et al.* 2009), and 4) a recent study in southern Illinois, now the northward portion of the armadillo's range, which found that 25 samples screened for *M. leprae* were all negative (0%) (Haywood *et al.* 2022). The only other site north of Lee County was Desha County, AR which is west of the Mississippi River and had a relatively high prevalence of 21% (Truman 2005), although this was calculated from a smaller sample size of individuals (*n* = 42). In short, results from past studies north of Lee County indicate a variable leprosy prevalence. However, although there have been prevalence studies east of the Mississippi River in Alabama and Georgia, these areas have been limited to the southeastern plains, Mississippi Valley loess plains, and Mississippi alluvial plain ecoregions (US EPA 2015). To the best of my knowledge, this is the first recent prevalence study since Howerth *et al.* (1980) that has evaluated leprosy prevalence in a county that is primarily in the piedmont ecoregion (US EPA 2015). Further, this study is the first known detection of *M. leprae* in the piedmont ecoregion of Alabama.

Although Loughry *et al.*'s (2009) ecologic-constraints hypothesis may not be applicable for leprosy's expansion eastward, it may provide an explanation for the lower prevalence observed in Lee County. In other words, there may be ecologic-constraints restricting the spread of leprosy northward across ecoregions in the southeast United States. As Loughry *et al.* (2009) and Truman (2005) described, there may be humid soil conditions, population densities, or other environmental agents that facilitate disease transmission in wild armadillo populations. Although population densities are an unlikely explanation since armadillos were encountered at the same rate in Lee County compared to Mobile, the vegetation type and finer-textured soils in the Piedmont ecoregion may influence leprosy transmission. Alternatively, the epidemic model hypothesis posits that leprosy will steadily spread as armadillos expand, therefore, Lee County prevalence will increase as more diseased armadillos spread northward. To further evaluate these hypotheses, future prevalence studies should be done in the piedmont and ridge and valley ecoregions of north Alabama and Georgia. There are established armadillo populations in these areas, according to extension professionals (Armstrong 1991; Hohbein and Mengak 2018) and my conversations with homeowners in north Alabama.

An additional objective of this research was to evaluate prevalence across an urban to rural gradient. This gradient could not be evaluated for Lee County due to only one armadillo testing positive, however, it was evaluated for Mobile County where the number of leprosy-positive individuals was higher. Leprosy prevalence was found to be higher in rural Mobile County, however, this difference was not statistically significant. Although a difference in prevalence was not observed between the three residential zones of interest in this study, future studies should continue to evaluate spatial patterns of infection in more human-dominated landscapes. Additionally, studies of armadillo populations should be done in locales with existing high leprosy prevalence and variable land imperviousness to evaluate differences between rural, suburban, and urban areas.

This study did not incorporate site-specific statistical analyses of the spatial patterns of leprosy infection, however, previous studies have found that armadillos are relatively dispersed across the landscape and do not exhibit significant clustering (Truman *et al.* 1991; Paige *et al.* 2002; Morgan and Loughry 2009; Perez-Heydrich *et al.* 2016). These studies were also limited to more natural areas, therefore, there is a significant gap in our current understanding of leprosy infection patterns in urban and suburban areas. With the potential for leprosy in wild armadillos to be zoonotic (Truman *et al.* 2011), evaluating populations and the spatial patterns of infection in more human-dominated landscapes is crucial to better understand the disease and its distribution in the southeast United States.

Literature Cited

Ancona KA, Loughry WJ (2010). Sources of variation in the time budgets of wild nine-banded armadillos. *Mammalia* 74, 127–134.

Andrew JW, Loughry WJ (2012). Temporal aspects of leprosy infection in a wild population of nine-banded armadillos. *Southeastern Naturalist* 11, 173–182. doi:10.1656/058.011.0202

Araujo S, Freitas LO, Goulart LR, Goulart IMB (2016). Molecular evidence for the aerial route of infection of *Mycobacterium leprae* and the role of asymptomatic carriers in the persistence of leprosy. *Clinical Infectious Diseases* 63, 1412–1420. doi:10.1093/cid/ciw570

Armstrong JB (1991). Extension wildlife damage management in Alabama. 5 - *Fifth Eastern Wildlife Damage Control Conference* (1991). Available at: https://digitalcommons.unl.edu/ewdcc5/1

Avanzi C, del-Pozo J, Benjak A, Stevenson K, Simpson VR, Busso P, McLuckie J, Loiseau C, Lawton C, Schoening J, Shaw DJ, Piton J, Vera-Cabrera L, Velarde-Felix JS, McDermott F, Gordon SV, Cole ST, Meredith AL (2016). Red squirrels in the British Isles are infected with leprosy bacilli. *Science* 354, 744–747. doi:10.1126/science.aah3783

Bhukhan, Aashni, Charles Dunn, and Rajiv Nathoo. “Case report of leprosy in Central Florida, USA, 2022 - Volume 29, Number 8—August 2023 - Emerging Infectious Diseases Journal - CDC.”. https://doi.org/10.3201/eid2908.220367.

Bradley CA, Altizer S (2007). Urbanization and the ecology of wildlife diseases. Trends in *Ecology & Evolution* 22, 95–102. doi:10.1016/j.tree.2006.11.001

Brearley G, Rhodes J, Bradley A, Baxter G, Seabrook L, Lunney D, Liu Y, McAlpine C (2013). Wildlife disease prevalence in human-modified landscapes. *Biological Reviews* 88, 427–442. doi:10.1111/brv.12009

DeGregorio BA, Gale C, V.Lassiter E, Massey A, Roberts CP, T.Veon J (2021). Nine-banded armadillo (*Dasypus novemcinctus*) activity patterns are influenced by human activity. *Ecology and Evolution* 11, 15874–15881. doi:10.1002/ece3.8257

DeGregorio BA, McElroy MR, Johansson EP (2023). Occupancy and activity patterns of nine-banded armadillos (*Dasypus novemcinctus*) in a suburban environment. *Diversity* 15, 907. doi:10.3390/d15080907

Deps PD, Alves BL, Gripp CG, Aragao RL, Guedes B, Filho JB, Andreatta MK, Marcari RS, Prates I, Rodrigues LC (2008). Contact with armadillos increases the risk of leprosy in Brazil: a case control study. *Indian Journal of Dermatology, Venereology and Leprology* 74, 338–342. doi:10.4103/0378-6323.42897

Deps P, Antunes JM, Santos AR, Collin SM (2020). Prevalence of *Mycobacterium leprae* in armadillos in Brazil: A systematic review and meta-analysis. *PLoS neglected tropical diseases* 14, e0008127. doi:10.1371/journal.pntd.0008127

Detwitz J, U.S. Geological Survey (2021). National Land Cover Database (NLCD) 2019 Products (ver. 2.0 June 2021): *U.S. Geological Survey data release*. doi:https://doi.org/10.5066/P9KZCM54

ESRI (2023). USA Counties - Overview. Available at: https://www.arcgis.com/home/item.html?id=a00d6b6149b34ed3b833e10fb72ef47b [accessed 26 January 2024]

Farnsworth ML, Wolfe LL, Hobbs NT, Burnham KP, Williams ES, Theobald DM, Conner MM, Miller MW (2005). Human land use influences chronic wasting disease prevalence in mule deer. *Ecological Applications* 15, 119–126.

Ferreira, Jéssica da Silva, Diego Augusto Souza Oliveira, João Pedro Santos, Carla Carolina Dias Uzedo Ribeiro, Bruna A. Baêta, Rafaella Câmara Teixeira, Arthur da Silva Neumann, et al. Ticks as potential vectors of *Mycobacterium leprae*: use of tick cell lines to culture the bacilli and generate transgenic strains." *PLOS Neglected Tropical Diseases* 12, no. 12 (December 19, 2018): e0007001. https://doi.org/10.1371/journal.pntd.0007001.

Fitch HS, Goodrum P, Newman C (1952). The armadillo in the southeastern United States. *Journal of Mammalogy* 33, 21–37. doi:10.2307/1375638

Gama RS, Leite LA, Colombo LT, Fraga LA de O (2020). Prospects for new leprosy diagnostic tools, a narrative review considering ELISA and PCR assays. *Revista da Sociedade Brasileira de Medicina Tropical* 53, e20200197. doi:10.1590/0037-8682-0197-2020

Gammons, DJ, MT Mengak, and LM Conner (2009). Translocation of nine-banded armadillos. *Human-Wildlife Conflicts* 3:64–71.

Han XY, Seo Y-H, Sizer KC, Schoberle T, May GS, Spencer JS, Li W, Nair RG (2008). A new *Mycobacterium* species causing diffuse lepromatous leprosy. *American journal of clinical pathology* 130, 856–864. doi:10.1309/ajcpp72fjzzrrvmm

Haywood CJ, Jordon AM, Pena M, Nielsen CK, Jiménez FA (2022). Tissue and gastrointestinal parasites of colonizing nine-banded armadillos at the edge of their northern range. *Journal of Parasitology* 108, 57–63. doi:10.1645/21-76

Hohbein RR, Mengak MT (2018). Cooperative extension agents as key informants in assessing wildlife damage trends in Georgia. *Human-Wildlife Interactions* 12, 243–258.

Holanda MV de, Marques LEC, Macedo MLB de, Pontes MA de A, Sabadia JAB, Kerr LRFS, Almeida RLF, Frota CC (2017). Presence of *Mycobacterium leprae* genotype 4 in environmental waters in Northeast Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* 50, 216–222. doi:10.1590/0037-8682-0424-2016

Howerth EW, Stallknecht DE, Davidson WR, Wentworth EJ (1990). Survey for leprosy in nine-banded armadillos (*Dasypus novemcinctus*) from the southeastern United States. *Journal of wildlife diseases* 26, 112–115. doi:10.7589/0090-3558-26.1.112

Kirchheimer WF, Sanchez RM (1981). Intraspecies differences of resistance against leprosy in nine-banded armadillos. *Leprosy in India* 53, 525–530.

Lahiri R, Krahenbuhl JL (2008). The role of free-living pathogenic amoeba in the transmission of leprosy: a proof of principle. *Leprosy Review* 79, 401–409. doi:10.47276/lr.79.4.401

Leary SL, Underwood W, Anthony R, Cartner S, Grandin T, Greenacre C, Gwaltney-Brant S, McCrackin MA, Meyer R, Miller D, Shearer J, Turner T, Yanong R (2020). 'AVMA guidelines for the euthanasia of animals: 2020 edition'. (*American Veterinary Medical Association*) Available at: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf [accessed 5 April 2022]

Lehrer EW, Fredebaugh SL, Schooley RL, Mateus-Pinilla NE (2010). Prevalence of antibodies to *Toxoplasma gondii* in woodchucks across an urban–rural gradient. *Journal of Wildlife Diseases* 46, 977–980. doi:10.7589/0090-3558-46.3.977

Loughry WJ, McDonough CM (1996). Are road kills valid indicators of armadillo population structure? *The American Midland Naturalist* 135, 53–59. doi:10.2307/2426871

Loughry WJ, McDonough CM (2001). Natal recruitment and adult retention in a population of nine-banded armadillos. *Acta Theriologica* 46, 393–406. doi:10.1007/BF03192446

Loughry WJ, McDonough CM (2013). 'The nine-banded armadillo: a natural history.' (University of Oklahoma Press).

Loughry WJ, Mcdonough CM, Robertson EG (2002). Patterns of anatomical damage in a population of nine-banded armadillos *Dasypus novemcinctus* (Xenarthra, Dasypodidae). *Mammalia* 66, 111–122. doi:10.1515/mamm.2002.66.1.111

Loughry WJ, Truman RW, McDonough CM, Tilak M-K, Garnier S, Delsuc F (2009). Is leprosy spreading among nine-banded armadillos in the southeastern United States? *Journal of wildlife diseases* 45, 144–152. doi:10.7589/0090-3558-45.1.144

Loughry WJ, Perez-Heydrich C, McDonough CM, Oli MK (2013). Population ecology of the nine-banded armadillo in Florida. *Journal of Mammalogy* 94, 408–416. doi:10.1644/12-mamm-a-198.1

Mackenstedt U, Jenkins D, Romig T (2015). The role of wildlife in the transmission of parasitic zoonoses in peri-urban and urban areas. *International Journal for Parasitology: Parasites and Wildlife* 4, 71–79. doi:10.1016/j.ijppaw.2015.01.006

Mandavilli, A. 2023. Leprosy may be endemic in Central Florida, scientists report. *The New York Times* (Digital Edition). 2 August 2023.

Martin RD, Gomez IF, Spies LA (2017). Burden of leprosy. *The Journal for Nurse Practitioners* 13, 538–545. doi:10.1016/j.nurpra.2017.06.010

McDonough CM (1997). Pairing behavior of the nine-banded armadillo (*Dasypus novemcinctus*). *The American Midland Naturalist* 138, 290–298. doi:10.2307/2426822

McDonough CM (2000). Social organization of nine-banded armadillos (*Dasypus novemcinctus*) in a riparian habitat. *The American Midland Naturalist* 144, 139–151. doi:10.1674/0003-0031(2000)144[0139:SOONBA]2.0.CO;2

Meredith A, Pozo JD, Smith S, Milne E, Stevenson K, McLuckie J (2014). Leprosy in red squirrels in Scotland. *Veterinary Record* 175, 285–286. doi:10.1136/vr.g5680

Morgan RE, Loughry WJ (2009). Consequences of exposure to leprosy in a population of wild nine-banded armadillos. *Journal of Mammalogy* 90, 1363–1369.

Neumann, A. da S., F. de A. Dias, J. da S. Ferreira, A. N. B. Fontes, P. S. Rosa, R. E. Macedo, J. H. Oliveira, R. L. de F. Teixeira, M. C. V. Pessolani, M. O. Moraes, P. N. Suffys, P. L. Oliveira, M. H. F. Sorgine, and F. A. Lara. 2016. Experimental infection of *Rhodnius prolixus* (Hemiptera, Triatominae) with *Mycobacterium leprae* indicates potential for leprosy transmission. *Plos One* 11:e0156037.

Oli MK, Loughry WJ, Caswell H, Perez-Heydrich C, McDonough CM, Truman RW (2017). Dynamics of leprosy in nine-banded armadillos: Net reproductive number and effects on host population dynamics. *Ecological Modelling* 350, 100–108.

Paige CF, Scholl DT, Truman RW (2002). Prevalence and incidence density of *Mycobacterium leprae* and *Trypanosoma cruzi* infections within a population of wild nine-banded armadillos. *The American journal of tropical medicine and hygiene* 67, 528–532. doi:10.4269/ajtmh.2002.67.528

Pedrini SCB, Rosa PS, Medri IM, Mourão G, Bagagli E, Lopes CA de M (2010). Search for *Mycobacterium leprae* in wild mammals. *The Brazilian Journal of Infectious Diseases* 14, 47–53.

Perez-Heydrich C, Loughry WJ, Anderson CD, Oli MK (2016). Patterns of *Mycobacterium leprae* infection in wild nine-banded armadillos (*Dasypus novemcinctus*) in Mississippi, USA. *Journal of Wildlife Diseases* 52, 524–532.

Prange S, Gehrt S, Wiggers E (2003). Demographic factors contributing to high raccoon densities in urban landscapes. *Journal of Wildlife Management* 67, 324–333. doi:10.2307/3802774

Prodöhl PA, Loughry WJ, McDonough CM, Nelson WS, Avise JC (1996). Molecular documentation of polyembryony and the micro-spatial dispersion of clonal sibships in the nine-banded armadillo, *Dasypus novemcinctus*. Proceedings. *Biological Sciences* 263, 1643–1649. doi:10.1098/rspb.1996.0240

R Core Team (2022). R: A language and environment for statistical computing. Available at: htpps://www.R-project.org/

Sapp CD, Emplaincourt J (1975). Physiographic regions of Alabama: Alabama Geological Survey Special Map 168.

Scollard DM, Adams LB, Gillis TP, Krahenbuhl JL, Truman RW, Williams DL (2006). The continuing challenges of leprosy. *Clinical Microbiology Reviews* 19, 338–381. doi:10.1128/CMR.19.2.338-381.2006

Scollard DM (2016). Infection with *Mycobacterium lepromatosis*. The American Journal of Tropical Medicine and Hygiene 95, 500–501. doi:10.4269/ajtmh.16-0473

Sharma R, Singh P, Loughry WJ, Lockhart MJ, Inman WB, Malcolm S. Duthie, Maria T. Pena, Luis A. Marcos, David M. Scollard, Stewart T. Cole, Richard W. Truman (2015). Zoonotic leprosy in the southeastern United States. *Emerging Infectious Diseases* 21, 2127–2134. doi:10.3201/eid2112.150501

da Silva, M., J. Portela, W. Li, M. Jackson, M. Gonzalez-Juarrero, A. Hidalgo, J. Belisle, R. Bouth, A. Gobbo, J. Barreto, A. Minervino, S. Cole, C. Avanzi, P. Busso, M. Frade, A. Geluk, C. Salgado, and J. Spencer. (2018). Evidence of zoonotic leprosy in Para A, Brazilian Amazon, and risks associated with human contact or consumption of armadillos. *Plos Neglected Tropical Diseases* 12.

Stefani MMA, Rosa PS, Costa MB, Schetinni APM, Manhães I, Pontes MAA, Costa P, Fachin LRV, Batista IMFD, Virmond M, Pereira E, Penna MLF, Penna GO (2019). Leprosy survey among rural communities and wild armadillos from Amazonas state, Northern Brazil. *PloS One* 14, e0209491. doi:10.1371/journal.pone.0209491

Suttkus RD, Jones C (1999). Observations on the nine-banded armadillo, *Dasypus novemcinctus*, in southern Louisiana. *Tulane Studies in Zoology and Botany* 31, 1–22.

Talmage RV, Buchanan GD (1954). The armadillo: a review of its natural history, ecology, anatomy and reproductive physiology. Rice Institute Pamphlet - Rice University Studies 41. Available at: https://scholarship.rice.edu/handle/1911/62727 [accessed 10 April 2022]

Taulman JF, Robbins LW (1996). Recent range expansion and distributional limits of the nine-banded armadillo (*Dasypus novemcinctus*) in the United States. *Journal of Biogeography* 23, 635–648.

Taulman JF, Robbins LW (2014). Range expansion and distributional limits of the nine-banded armadillo in the United States: an update of Taulman & Robbins (1996). *Journal of Biogeography* 41, 1626–1630. doi:10.1111/jbi.12319

Truman R (2005). Leprosy in wild armadillos. *Leprosy Review* 76, 198–208. doi:10.47276/lr.76.3.198

Truman RW, Morales MJ, Shannon EJ, Hastings RC (1986). Evaluation of monitoring antibodies to PGL-I in armadillos experimentally infected with *M. leprae*. *International Journal of Leprosy and Other Mycobacterial Diseases: Official Organ of the International Leprosy Association* 54, 556–559.

Truman RW, Kumaresan JA, McDonough CM, Job CK, Hastings RC (1991). Seasonal and spatial trends in the detectability of leprosy in wild armadillos. *Epidemiology and Infection* 106, 549–560. doi:10.1017/S0950268800067613

Truman RW, Andrews PK, Robbins NY, Adams LB, Krahenbuhl JL, Gillis TP (2008). Enumeration of *Mycobacterium leprae* using real-time PCR. *PLOS Neglected Tropical Diseases* 2, e328. doi:10.1371/journal.pntd.0000328

Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, Kapopoulou A, Brisse S, Scollard DM, Gillis TP, Cole ST (2011). Probable zoonotic leprosy in the southern United States. *New England Journal of Medicine* 364, 1626–1633. doi:10.1056/NEJMoa1010536

Truman RW, Ebenezer GJ, Pena MT, Sharma R, Balamayooran G, Gillingwater TH, Scollard DM, McArthur JC, Rambukkana A (2014). The armadillo as a model for peripheral neuropathy in leprosy. *ILAR Journal* 54, 304–314. doi:10.1093/ilar/ilt050

Turankar RP, Lavania M, Darlong J, Siva Sai KSR, Sengupta U, Jadhav RS (2019). Survival of *Mycobacterium leprae* and association with *Acanthamoeba* from environmental samples in the inhabitant areas of active leprosy cases: A cross sectional study from endemic pockets of Purulia, West Bengal. *Infection, Genetics and Evolution* 72, 199–204.

US EPA (2015). Level III and IV ecoregions of the continental United States. Available at: https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states [accessed 7 February 2024]

U.S. Geological Survey (2023). USGS National Transportation Dataset (NTD) for Alabama shapefile.

Wheat WH, Casali AL, Thomas V, Spencer JS, Lahiri R, Williams DL, McDonnell GE, Gonzalez-Juarrero M, Brennan PJ, Jackson M (2014). Long-term survival and virulence of *Mycobacterium leprae* in amoebal cysts. *PLOS Neglected Tropical Diseases* 8, e3405. doi:10.1371/journal.pntd.0003405

Žlender V (2021). Developing a spatially explicit method for delineating peri-urban landscape. *Urbani izziv* 32, 98–110. doi:10.5379/urbani-izziv-en-2021-32-02-03

Chapter 3: Wildlife acceptance capacity of nine-banded armadillos (*Dasypus novemcinctus*) and management needs in Alabama

Abstract

The expansion of the nine-banded armadillo (*Dasypus novemcinctus)* in the southeast United States over the past fifty-years has led to novel human-armadillo interactions, resulting in management challenges for residents. Armadillo populations are also not limited to rural areas and are increasingly found in human-dominated areas. Several management methods are currently recommended to residents by Alabama Extension and other wildlife professionals to mitigate human-armadillo conflicts. However, there has yet to be a study on residents' perceptions of these methods nor their experiences of the species as a whole. In this study, residents who own or maintain a property in Alabama (*n*=341) were surveyed and a subset of survey respondents (*n*=15) were included in focus groups or interviews to evaluate tolerance and perceptions of armadillos using a wildlife acceptance capacity (WAC) model. Surveys revealed that Alabama residents exhibit a low tolerance for armadillos and notably, residents in the north expressed lower tolerance levels than other regions. The perception variables of benefits and damage risks were the greatest predictors of tolerance, and a refined WAC model was developed from survey results. In terms of management, it was found that residents perceived low levels of capacity to control armadillos, with concerns regarding the efficacy, convenience, and legality of several common management methods. Recommendations include the development of community resources for humane armadillo removal and continued provision of science-based management education by Extension services. By addressing residents' management needs and

enhancing their understanding of armadillos, human-wildlife conflicts can be effectively managed, ensuring the well-being of both people and wildlife in the southeast United States.

Introduction

In the United States, human population growth and redistribution to previously non-metro areas over the last thirty years have increased the wildland-urban interface, where previously natural areas now contain an increasing number of structures to accommodate a growing human population (Carlson *et al.* 2022). Significant ecological impacts and declines in biodiversity can result from such drastic landscape changes (Grimm *et al.* 2008). Urbanized areas for some species, however, can be favorable, especially for generalists (Ducatez *et al.* 2018).

Nine-banded armadillos (*Dasypus novemcinctus)* are habitat and prey generalists that are relatively undisturbed by humans, making them highly adaptable to urban and suburban areas (Loughry and McDonough 2013). As a result of their adaptability, armadillo populations have increased alongside the growing human population; although a formal census has not been done, it was estimated in 1995 that the armadillo population in the United States was between thirty to fifty million (Gilbert 1995). In addition, due to their expanding range and limited predators, it is probable that armadillo populations have only grown over the last thirty years (Loughry and McDonough 2013; Taulman and Robbins 2014). Although a recent estimate of their population size in the United States is not available, we do know that the armadillo has expanded into other states over the past three decades including Kansas, Illinois, Indiana, Kentucky, and Tennessee (Taulman and Robbins 2014).

Urbanization, human population growth, and an increasing armadillo population has resulted in an increase in human-armadillo interactions in the southeast United States (Hohbein and Mengak 2018). Human-armadillo interactions are a type of human-wildlife conflict (HWC) which refers to situations in which the goals of wildlife and the goals of humans interfere, leading to negative consequences for people, wildlife, or both (Madden 2004; Attia *et al.* 2018). As a result of HWC, wildlife can experience consequences including population declines, ecosystem alterations that reduce available habitat, and extinction (Nyhus 2016). Negative impacts to human populations are often considered as either direct or indirect impacts. Direct impacts include human injuries or death, depredation of livestock, depredation of game species, damage to crops, damage to personal property, and disease transmission. Indirect impacts include the financial and opportunity costs associated with wildlife and their management (Woodroffe *et al.* 2005).

Negative human-wildlife interactions, whether direct or indirect, can lead to the consideration of certain wildlife species as a "nuisance", causing a person adverse effects, whether real or perceived (Colautti and MacIsaac 2004). Armadillos are frequently identified as nuisance wildlife according to resident complaints to state extension agents in the southeast (Armstrong 1991; Bruggers *et al.* 2002; Hohbein and Mengak 2018). With the armadillo rapidly expanding into new areas, complaints have only increased. For instance, Hohbein and Mengak (2018) found that the amount of armadillo complaints in Georgia had increased drastically over an approximately 35-year period with extension agents in 1980 reporting fewer complaints than in subsequent surveys in 2002 and 2016.

In Alabama, like most parts of the species' range, armadillos have expanded northward over the past fifty-years to cover the entire state (Taulman and Robbins 2014). In 1990, a survey of county extension agents in the state revealed that armadillos were, on average, 12.5 of the complaints that they received the previous year. Additionally, there was an observed geographical difference in armadillo complaints with more in the south and west compared to the north and east (Armstrong 1991). Extension agents respond to these complaints by recommending management methods that mitigate or prevent conflict. The Alabama Cooperative Extension System (ACES) website currently hosts an article that recommends several management methods for armadillos including trapping and relocating, shooting, and use of an electric fence (Armstrong 2019). The actual methods that residents are using, concerns, and potential improvements, however, have not been extensively evaluated. There has been some testing of effective baits (Ober *et al.* 2011; Mengak *et al.* 2017) and evaluating the efficacy of translocation (Gammons *et al.* 2009), but previous studies have not incorporated resident perceptions to further explore how management recommendations may be improved.

Wildlife acceptance capacity

Wildlife acceptance capacity (WAC), sometimes referred to as wildlife stakeholder acceptance capacity (WSAC), is a framework used to evaluate tolerance by understanding what is considered a maximum acceptable population size for a species. As described by Decker and Purdy (1988), WAC is similar to the idea of a biological carrying capacity— in which there are environmental limits to wildlife population growth— since it posits that there is a cultural carrying capacity, and when a species exceeds this population size, a person may be motivated to take action to reduce the population. Acceptance or tolerance can thus be determined by the desired current and future population size of a species in an area (Riley and Decker 2000).

There are several factors that can influence acceptance as it is dependent on the species of interest and stakeholder perceptions, beliefs, and past experiences (Decker and Purdy 1988; Zinn *et al.* 2000). Factors that have been previously described to influence acceptance include demographics and perceptions of benefits, risks, costs, and control (Bruskotter *et al.* 2009; Goodale *et al.* 2015); not only do these factors influence acceptance, but these factors often influence each other. For instance, if a person perceives a species as having benefits, it can influence acceptance by making the perception of costs and risks more acceptable, as was seen by Goodale *et al.* (2015) with white tailed-deer and farmers in Canada.

WAC has been used in several studies involving predators to describe the factors that affect tolerance for species such as black bears (Zajac *et al.* 2012), wolves (Bruskotter *et al.* 2009), cougars (Riley and Decker 2000), and panthers (Rodgers and Pienaar 2017). However, despite the utility of the WAC framework, fewer studies have applied the framework to non-predator nuisance wildlife such as armadillos. In one study, Goodale et al. (2015) evaluated Canadian farmers' tolerance for nuisance wildlife that may either pose a threat or a damage risk. A similar framework has also been used to observe tolerance of other nuisance wildlife like prairie dogs in Colorado (Zinn *et al.* 2000), beaver in New York and Massachusetts (Siemer *et al.* 2013) and bandicoots in urban Sydney, Australia (Dowle and Deane 2009).

My research objectives were to evaluate Alabama residents' WAC for armadillos in Alabama and discern variables that predict tolerance. The goal was to also develop a framework for assessing tolerance of armadillos and comparable nuisance wildlife that incorporates perceived disease risk. Due to the ability for armadillos to be reservoirs for the bacteria that causes Hansen's disease, or leprosy (Kirchheimer 1975), and the potential negative HWCs, I sought to incorporate a predictor variable of perceived disease risk. Lastly, I sought to evaluate common management methods used by residents to identify community needs and help managers mitigate human-armadillo conflicts.

From anecdotal experiences and conversations with Alabama residents, I expected tolerance of armadillos to be low overall. Additionally, I expected the perception of control, costs, and benefits to be directly related to tolerance whereas perception of risks would be inversely related (Figure 3.1.). I also expected perceived disease risk of leprosy to be high and inversely related to tolerance. Demographic factors such as education, age, and gender have also been found to impact risk perceptions and overall acceptance of wildlife (Vaske *et al.* 2001; Manfredo and Zinn 2011; Hanisch-Kirkbride *et al.* 2013; Goodale *et al.* 2015; Cross *et al.* 2018). For instance, Goodale *et al.* (2015) observed that older respondents were less tolerant of nuisance species. Therefore, I expected to observe similar patterns related to demographics and tolerance in this study. Location of the respondent in Alabama, residential zone, and frequency of armadillo observations were also evaluated to observe how these additional place dimension factors may impact tolerance, as this has not been extensively done for nuisance wildlife in previous studies. The expected relationship between the WAC factors and tolerance are summarized in Figure 3.1.

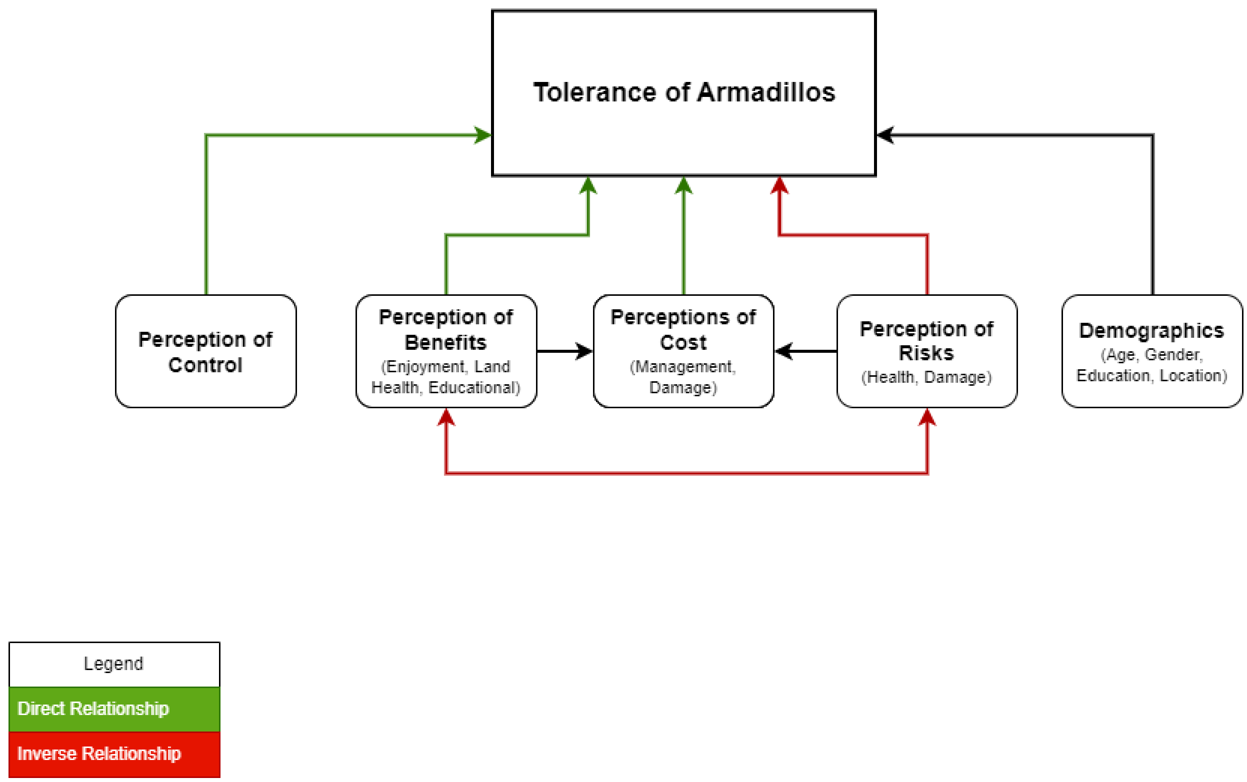

Figure 3.1. Predicted wildlife acceptance capacity (WAC) model for Alabama residents which measures acceptability or tolerance of armadillos and is based on perception variables and demographics that are commonly incorporated. This model also incorporates the perception of health or disease risks as it relates to armadillos serving as natural reservoirs for leprosy bacteria to evaluate how this factor may influence tolerance of the species.

Methods

The study population included Alabama residents, ages 19 or older, who own or maintain a property within the state. The latter parameter was included since a person who maintains a property would be more familiar with potential human-armadillo conflicts and management. A questionnaire was first distributed to target residential tenants across the state of Alabama, with the goal to reach every county and an equal proportion of suburban, urban, and rural residents. Additionally, focus groups and interviews were conducted to further evaluate concepts assessed in the questionnaire. All methods and questions were approved by the Institutional Review Board at Auburn University (22-143 EX 2205).

Questionnaire development

The questionnaire consisted of thirty-four questions (Appendix II). The first three questions confirmed that the respondent was an adult over the age of 19, an Alabama resident, and either owned or maintained a property within the state. If the respondent did not pass the qualifying questions, the survey would end and not be counted. Questions four through eight collected demographic information including education, income, race, age, and sex. Respondents were then asked questions about the location of their residence and the characteristics of the property they maintain. Respondents were asked to report their ZIP-code, self-designate their residence as being in a rural, suburban, or urban area, and provide an estimate of their lot size (in acres). Lastly, the length that respondents have resided at their current residence was collected through a multiple-choice question.

Respondents were then provided with a picture (Figure 3.2.) and statement: "The nine-banded armadillo (hereafter referred to as "armadillo") is the only species of armadillo in the United States.". On the Qualtrics survey, the picture and statement were placed on the front page.

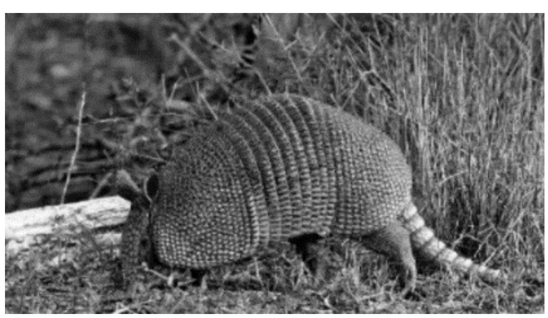

Figure 3.2. Nine-banded armadillo foraging in grass which was shown to respondents at the beginning of the survey. Source: Armstrong (2019).

After reading the statement and viewing a picture of an armadillo, they were asked if they had seen an armadillo or evidence of one in the form of tracks or burrows where they currently live; if a respondent answered “yes” or “not sure”, they were shown the remainder of the questions. If the respondent answered “no”, the survey concluded. The remaining respondents were asked about the frequency of their encounters over the previous twelve months of taking the survey.

A knowledge check was used to assess a person’s understanding of armadillo life history, diet, and behaviors. The survey also assessed variables of tolerance, perceived risk, benefit, damage risk, control, cost and disease risk using multi-item scales which is effective in assessing latent psychological variables that cannot be observed (Zajac *et al.* 2012). Additionally, many of the items included in this survey instrument were adapted from previous studies evaluating similar psychological parameters (Gore *et al.* 2009; Zajac *et al.* 2012; Goodale *et al.* 2015).

Tolerance, the main dependent variable, was evaluated through three questions that were adapted from previous studies: 1) consideration of armadillos as a nuisance which was defined to the respondent as “causing me inconvenience or annoyance” 2) perception of current armadillo population size and 3) desired future population trend (Zajac *et al.* 2012; Hayman *et al.* 2014). To evaluate leprosy risk perception, the survey utilized five questions that separately evaluated dread, severity, and susceptibility, which is a common practice in risk literature and has been utilized in similar studies of perceived risks from wildlife diseases (Sjöberg 1998; Gore *et al.* 2009; Hanisch-Kirkbride *et al.* 2013). The perceived risk of an armadillo damaging personal property was similarly evaluated through three questions that assessed a respondent’s perceived dread, susceptibility, and severity of the risk.

The perceived benefits that armadillos provide were assessed through a five-point Likert scale of the agreement to three statements, which were adapted from Goodale et al. and evaluate cultural services including educational value, sense of place, and aesthetic values (Goodale *et al.* 2015). Armadillos may also provide ecosystem services through the creation of burrows (DeGregorio et al. 2022), control of insect pests, and dispersing of seeds (Rodrigues *et al.* 2020), to name a few. The ability for a person to know how to control armadillos on their property was assessed through one question with a five-point Likert scale. Prior to the perceived control question, respondents were given the statement: “For the below questions, "control" refers to the deliberate prevention or removal of armadillos”.

In addition to assessing the perception of control, the survey also evaluated the type of management methods a respondent would consider using through a question with eight common management methods and a five-point Likert scale (“definitely” to “definitely not”). The management methods were shoot with a gun, trap and relocate, trap and euthanize, use a physical barrier (e.g. fence), use a repellent, use poison, treat yard for grubs and other insects, and “other” where the respondent could provide a write-in response.

Some management methods require disposal such as shooting with a gun or poison. In addition, there may be the occurrence where a person has a roadkill or dead armadillo on their property that they desire to remove. Since zoonotic disease transmission from an armadillo may be a potential public health concern, how a dead armadillo is disposed of by residents is an important consideration (Truman *et al.* 2011). Therefore, a question collected information on the methods of disposal that would be considered for a dead armadillo on a respondent’s property.

The frequency of armadillo control in the previous twelve months was also collected to better understand how often management is being done by residents. Since the current management options often cost money to implement, the perception of this cost may be a predicting variable of tolerance. Repairing damage caused by armadillos is costly and is also a component of the costs incurred by people. Therefore, the respondent was also asked to estimate how much money (USD) an armadillo has cost them in the past twelve months. A separate question assessed their acceptability of the dollar amount that they reported through a five-point Likert scale.

The final question on the distributed questionnaire collected the names and contact information of respondents who were interested in participating in a focus group about armadillos. If a respondent was not interested in participating in a focus group, the final page of the online survey was an infographic about armadillos, management methods, and potential diseases. For respondents that did want to participate in a focus group, the infographic was sent after the completion of the focus group or via the email that was provided if they did not participate. The Qualtrics survey, both digitally and physically, was accompanied by an informative letter and completion of the survey was an indication of consent to participate.

Questionnaire administration

The questionnaire was inputted to Qualtrics and was pre-tested by six graduate students and faculty at Auburn University during August 2022. The feedback received was incorporated and the finalized surveys were distributed to potential respondents. Several ACES county extension offices shared the survey via newsletters and flyers, it was posted on Facebook community groups, and shared via the Nextdoor application. The survey was available on Qualtrics from September 1, 2022, to February 28, 2023.

In an effort to reach more of the state, I also contacted each library in Alabama which had public email addresses that were available and deliverable. There was a total of 24 libraries that agreed to help distribute surveys and an additional seven libraries agreed to share a flyer with a QR code that directed potential respondents to the Qualtrics survey. Twenty paper copies of the surveys were sent in mid-November with a paid return envelope to each of the 24 participating libraries. Surveys were displayed in libraries after receipt of the package and until the end of February 2023. Respondents had the opportunity to pick up a survey, complete it, and mail it back to the Auburn University College of Forestry, Wildlife and Environment. Each paper copy of the survey also included a QR code to the Qualtrics survey if they preferred to complete it digitally instead.

Focus Groups

The names of potential focus group participants were pulled from the survey responses on January 15, 2023. From these respondents, their answers to the three scale items evaluating tolerance were scored and adjusted to a scale from 0-1. Those with a neutral or high tolerance (≥ 0.33) and those with a low tolerance for armadillos (< 0.33) were divided into two groups. Respondents were further subdivided based on their residential zone: suburban, urban, or rural. I desired to hold three focus groups for each category with a maximum of four respondents in each session. Selecting participants for the focus group categories was done using a random number generator.

Participants were emailed to participate in the focus groups at the email they provided on their survey submission. An email was sent to confirm that they were still interested in participating. If a response was received, a follow-up email was sent with the online meeting information and informed consent letter. Information was also included about the opportunity to win one of three $20 Amazon gift cards if they participated in a focus group. If the potential participants did not respond to the email within a week and provided a phone number on their survey, they were also contacted via a phone call. If selected respondents were no longer interested in participating in the focus group, more respondents from the same category were subsequently selected using a random number generator and contacted.

Each of the focus groups were around 60-minutes in length and occurred over Zoom, an online meeting platform. Several of the focus groups became semi-structured interviews with single participants due to lack of group attendance. Each meeting was audio recorded using Zoom and detailed notes were taken throughout the discussion. Participants were also welcome to participate with or without their video camera on. Each group or interviewee were asked a series of questions which were slightly different for each of the high tolerance and low tolerance groups. The questions for the high tolerance groups were tailored more to perceptions of armadillo benefits and risks with less of a focus on the management methods that would be used. On the other hand, low-tolerance participants were asked questions about the utility of common management methods on their properties (Appendix III). Additionally, the low-tolerance group was subdivided by residential zone, however, the questions to participants did not differ between the zones.

Statistical procedures

Survey analysis

I reviewed each online survey and manually entered physical surveys into Qualtrics. The Qualtrics software was then used to remove respondents who did not pass the qualifying questions. Then, all respondents were exported, and summary statistics were calculated in a Microsoft Excel spreadsheet. All comparable demographics variables were analyzed against the population of Alabama according to the U.S. Census Bureau 2022 American Community Survey (ACS) dataset using Z-score calculations with a 95% confidence level (U.S. Census Bureau 2022). A 95% confidence level was also used for the standard errors of all averages calculated.

Respondents who answered "no" to observing armadillos or their evidence in the previous twelve months of taking the survey were separated from those who responded "yes" or "not sure". Respondents provided their ZIP-code, and these were classified as "northern", "central" or "southern"; the regional extension SETs of Alabama used by ACES were used to determine county boundaries for each region. ArcGIS Pro was used to create a map of the ZIP-codes and counties represented by survey respondents using a WGS 1984 geographic coordinate system (GCS). Data for county and ZIP-code boundaries were accessed through the global ArcGIS Pro directory via ESRI (ESRI 2023).

Respondents also provided their lot size and if they did not provide the size in acres, the responses were reviewed and manually converted to acres. All perception variables and knowledge were assigned scores and adjusted to be on a scale from 0 to 1 for easy interpretation of results. Three separate multiple linear regression models were used to evaluate how demographic, place dimensions, and perception and experience variables each predicted tolerance. The lm() function in R Studio was used to evaluate the predicted relationships (R Core Team 2022). For the three main models testing the predicted WAC, tolerance was treated as a continuous dependent variable and the relevant perception variable scores were categorized into groups (i.e. perceived benefit scores categorized into high, neutral, and low). The association between the perception variables of interest and willingness to use lethal management was also analyzed using a logit regression in R Studio.

Relationships between categorical variables were analyzed using a Chi-Square test of independence which was calculated in Microsoft Excel. Lastly, there were two frequency variables that were analyzed as predictors of tolerance using an ordinal logit model: residence length and frequency of armadillo sightings. The ordinal logit model was analyzed in R Studio using the "MASS", "brant" and "ordinal" R packages; the clm() function was used and compared to a null hypothesis to determine if the model was a good fit. Additionally, statistical significance was assessed using the polr() and brant() functions.

Focus group analysis

The audio from the focus groups and interviews were recorded in Zoom. The auto-transcription feature was used and then manually reviewed. The full recordings were transcribed for each participant and focus group. The transcripts were then coded using a classical content analysis approach (Onwuegbuzie *et al.* 2009). The frequency of concepts related to the questions asked were counted and compared between the three categories of participants. Differences in perceptions between residential zones and tolerance levels were compared to identify general trends and patterns.

Results

Survey

A total of 444 surveys were returned, of which 95 were paper surveys (19.8% response rate) and 349 were submitted via Qualtrics. After removing respondents that did not satisfy the qualifying questions, there were 388 respondents. A power analysis with an effect size of 0.2, alpha of 0.05, and power of 0.80 predicts a desired sample size of 399 respondents. Although the sample size was under what is statistically suggested, it is sufficient to observe small to medium-sized effects relevant to my research objectives (Sullivan and Feinn 2012).

There were sixteen respondents who had not observed armadillos in the previous twelve months. Of these sixteen respondents, the majority (62%) had been at their property for more than ten years, were within central Alabama (75%), and were in suburban areas (56%). The locations of the respondents who had not recently observed armadillos are summarized in Table 3.1.

Table 3.1. A summary of the locations of respondents who had not observed armadillos or evidence of armadillos within the previous 12 months. *n*=16

	Southern	Central	Northern
Rural	0	2	0
Suburban	1	7	1
Urban	1	1	0
Not Sure	1	1	1

For the remaining respondents (*n* = 372) who had observed armadillos within the previous 12 months of completing the survey, surveys were reviewed for completeness. Respondents who did not answer all relevant questions used to assess perceptions and the demographics included in the model analysis were removed (*n* = 31). Therefore, the remaining respondents (*n* = 341) were those that passed the qualifying questions and answered all questions to sufficiently estimate their perceptions of armadillos. Lastly, the question assessing perceived cost was removed from the final model analysis since 106 survey respondents did not provide an answer.

Demographics

The average age of survey respondents was 58 years old (± 0.82 SE; 95% CI), and the median age was 62 with 43% of respondents being 65 or older; compared to the population of Alabama, there were significantly more survey respondents who were 65-74 years-old (Z-score = 2.80). There were also significantly more females (66%) than males (34%) compared to the Alabama population (Z-score = 2.08).

A small percentage of respondents who provided a yes or no answer were of Latino, Hispanic, or Spanish origin (0.9%; *n* = 323), however, this was not significantly different from the 2022 ACS survey (Z-score = 0.65). There were, however, significantly fewer black or African American survey respondents (3.1%; *n* = 323) compared to the Alabama population (27.3%) (Z-Score = 3.90). The other races that respondents reported were not statistically different from the 2022 ACS.

There were several respondents who preferred not to provide their average household income (23%). Out of the respondents who did respond (*n* = 262), many (37%) reported earning an average household income greater than \$100,001. The median income of survey respondents was \$80,001-90,000 which is higher than the median income reported by the 2022 ACS (\$59,674).

Most survey respondents who answered the education question (n = 329) were college educated with either a two-year or four-year degree (69%). The most commonly reported education level was graduate or professional school (40%; n = 329). There were no respondents who had not graduated high school. Compared to the 2022 ACS estimates, there were significantly more respondents with graduate or professional degrees and bachelor's degrees than the Alabama population (Z-Scores = 9.398; 4.96). A summary of respondent demographics is provided in Appendix IV.

Location of respondents

All respondents maintained a residence in Alabama. Most respondents, based on the ZIP-code they provided, were located in northern Alabama (40%), followed closely by the central region (38%). In total, 61 of Alabama's 67 counties were represented (Figure 3.3). For respondents who did not answer "not sure", there were more respondents in rural areas (62%), followed by suburban (31%), and urban areas (5%) (Table 3.2.). However, there was not a significant difference between residential zone and region ($\chi 2 = 4.42$; $p > 0.10$; $n = 334$).

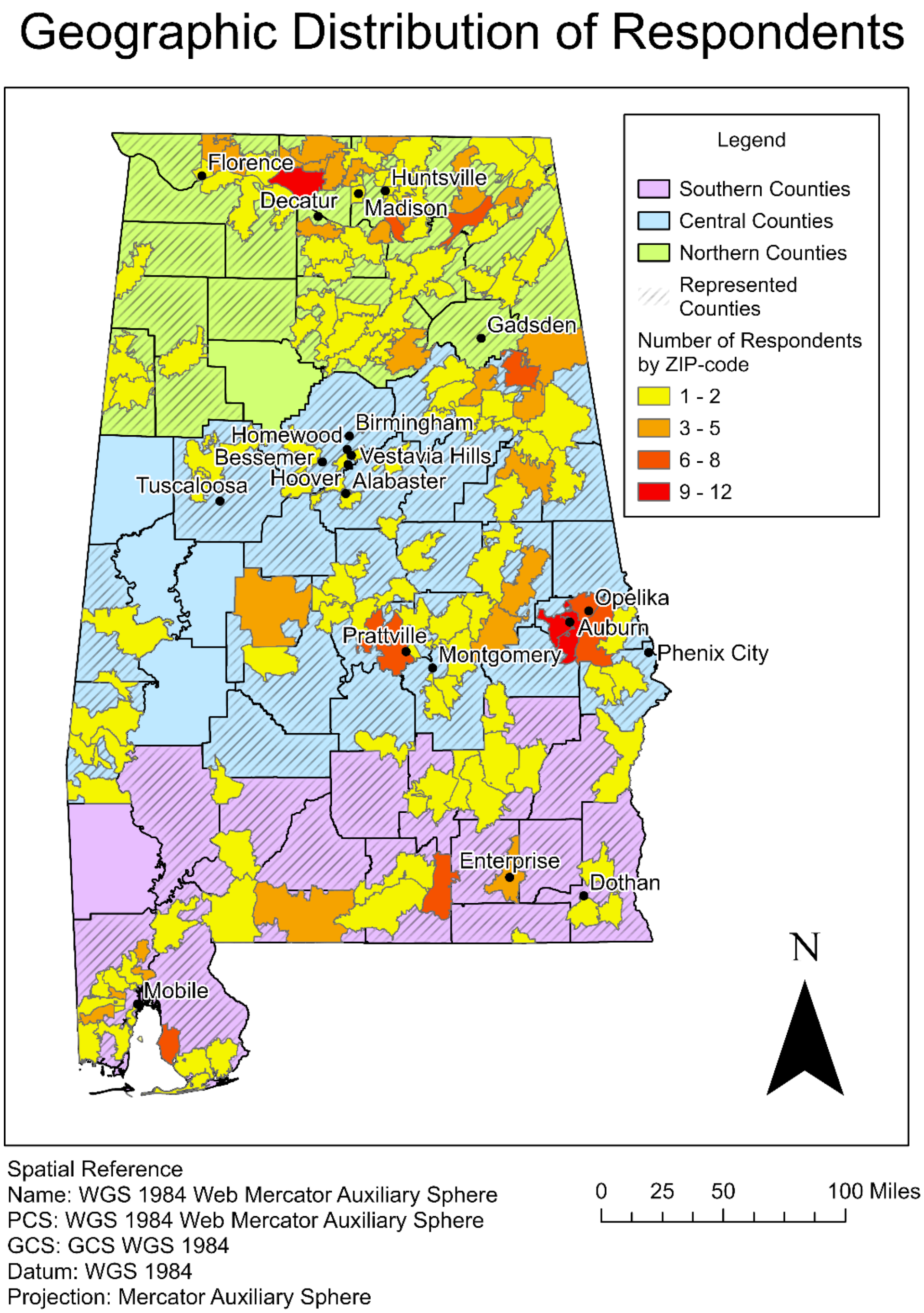

Figure 3.3. The represented regions and counties based on respondent's self-reported ZIP-codes. Regions of Alabama were delineated according to the regional extension sets of the Alabama Cooperative Extension System. $n = 341$

Table 3.2. The regions and self-designated zones that survey respondents provided. ZIP-codes were categorized into northern, central, and southern Alabama based on the Regional Extension SETs of the Alabama Cooperative Extension System. *n*=341

Region	Residential Zone			
	Rural	Suburban	Urban	Not Sure
Northern	87	38	10	1
Central	82	39	5	4
Southern	43	28	2	2
Total	212	105	17	7

Respondents also owned or maintained an average lot size of 34.22 ± 143.48 acres (n = 305) with the median being 3 acres. The largest lot size was 2,000 acres and the smallest lot size was 0.09 acres. There were 36 respondents who did not provide a lot size. Most respondents had also been at their property for more than ten years (60%; n = 341). Only 10 respondents (3%) had been at their current property for less than one year.

Armadillo encounters

Respondents reported a variety of armadillo sighting frequencies. Over half of the respondents (55%) had observed armadillos or their evidence at a rate of every other month or greater (e.g. monthly, weekly, or daily). Respondents were also asked how frequently in the past twelve months they had to control armadillos. Half of the respondents said they had not controlled armadillos. Some (20%) respondents had controlled armadillos at a frequency that was either every other month or greater.

Perceptions

Tolerance for armadillos was evaluated through three scale items (Cronbach's alpha = 0.82) (Table 3.3.). The average tolerance of armadillos was negative (0.23 ± 0.014) (Figure 3.4.).

Respondents felt most negatively about the future armadillo population in their area with 68% of respondents desiring a decrease and only 32% of respondents desiring that populations either stay the same or increase. Additionally, the majority (63%) of respondents felt that the current armadillo population size was more than they would like. Lastly, 65% of respondents felt that armadillos are a “nuisance”.

Table 3.3. Responses to the three questions that were used to determine tenants’ tolerance for armadillos. Question 16 (Q16) had five available answer choices and questions 17 (Q17) and 18 (Q18) had three answer choices each. All respondents were assigned a score, and it was adjusted to fit a scale of 0-1. *n*=341.

Item	x̄	Standard Error (±)	Agree	Neutral	Disagree
Q16 Armadillo as a Nuisance	0.30	0.017	221 (65%)	56 (16%)	64 (19%)
Armadillo Population Trends					
	x̄	Standard Error	Decrease/ More Than I Would Like	Stay The Same/ About Right	Increase/ Less Than I Would Like
Q17 Current Population	0.20	0.015	215 (63%)	117 (34%)	9 (3%)
Q18 Future Population	0.18	0.015	232 (68%)	96 (28%)	13 (4%)
Total	0.23	0.014			

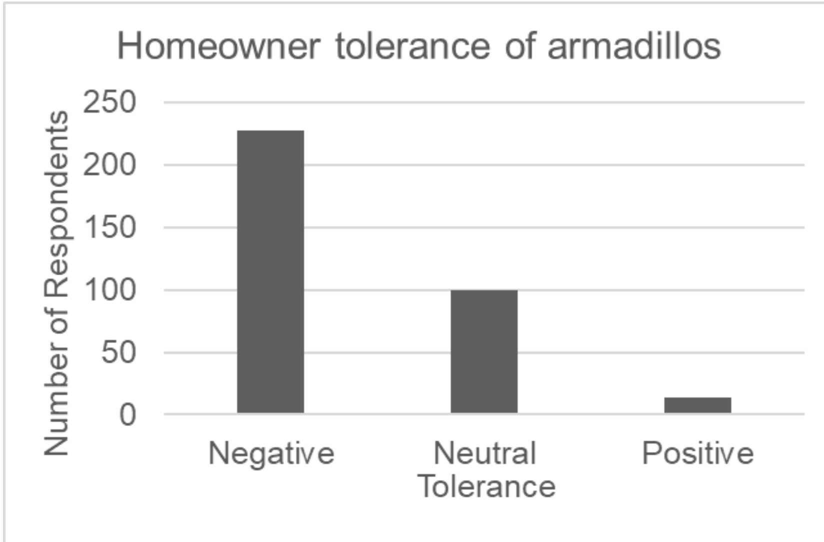

Figure 3.4. Residents' tolerance scores for armadillos on their property. "Negative" is considered scores < 0.33, "neutral" is ≥ 0.33 and ≤ 0.67, and "positive" are scores > 0.67.

A separate question evaluated respondents' knowledge of armadillos. An answer choice was originally included of "they are nocturnal (active at night)". In some areas, however, armadillos may be more active during the day, especially juveniles (DeGregorio et al. 2022). Therefore, the nocturnal choice was removed due to the subjectivity of the question. The remaining answer choices were used to gauge knowledge of armadillos depending on the number of correct or incorrect choices selected: if no answers were selected correctly (1), if one was selected correctly (2), if two were selected correctly (3), and if all three were selected correctly (4). These scores were then adjusted to a scale of 0-1 for interpretation.

On average, respondents were somewhat knowledgeable of armadillos with an average knowledge score of 0.60 (± 0.017). Most respondents knew that armadillos burrow (83%). Most respondents also correctly selected that armadillos do not consume plants as the majority of their diet (74%). It was expected that respondents would be more aware that armadillos carry leprosy, however, only 53% of respondents selected this answer choice.

The respondents who selected that armadillos can carry leprosy were provided with a series of questions to assess disease risk perception ($n = 182$). If a respondent answered that armadillos do not carry leprosy ($n = 159$), they were given a disease risk perception score of zero since it was assumed that if they were unaware that armadillos are reservoirs for the bacteria, they would have no risk perception related to disease. To evaluate perceived susceptibility of leprosy, respondents were asked to estimate the leprosy prevalence in armadillo populations of their surrounding area. However, this question was removed from calculating the perception score due to a low response rate (49%). For those that did respond to this question, 25% was the most answered prevalence rate (43%; $n = 169$). Perception of disease risk was thus measured through four scales with one each evaluating severity and dread, and two evaluating susceptibility (Cronbach's alpha = 0.74).

On average, the perceived leprosy disease risk was low (0.16 ± 0.012). The question evaluating dread had the lowest average score (0.11 ± 0.013) and the question evaluating severity had the highest score (0.51 ± 0.017). Therefore, on average, respondents had a low amount of dread about obtaining leprosy from an armadillo, but the severity of leprosy was considered higher if they were to contract it.

Similar to leprosy risk perception, the survey also evaluated how residents perceived the risk of damage to their property. Respondents were first asked to rate the likelihood of an armadillo damaging infrastructure, landscaping, crops, and livestock to evaluate the susceptibility of damage occurring. If a respondent answered “very likely” to at least one scenario, they were given a score of 3, if they answered “somewhat likely” to at least one, they were given a score of 2, and if they answered “not likely” or “not applicable” to all of them, they were given a score of 1. Several respondents did not answer each susceptibility scenario, however, the scoring system I used required that they only answer at least one scenario.

The two damage scenarios that were considered very likely or somewhat likely to occur were to tenant’s landscaping, yards, or gardens (92%), and crops (60%). The scenario perceived least likely to occur was damage to livestock (11%). Lastly, 31% of respondents felt it was very or somewhat likely for armadillos to damage infrastructure. With all damage scenarios combined, the average perceived susceptibility score was 0.80 (± 0.02). In other words, respondents felt that the likelihood of an armadillo doing some type of damage to their property was fairly high.

The severity score of armadillo damage was, on average, low (0.26 ± 0.016). Therefore, although armadillos are likely to damage property, many respondents felt that this damage would not be very severe. Respondents were also asked if they worry about their property getting damaged to assess the dread surrounding damage risk which was, on average, 0.47 (± 0.017). In other words, respondents felt a neutral amount of dread or worry, on average, about armadillos damaging their property.

A total damage risk perception score was calculated by combining respondents’ perception scores for dread, severity, and susceptibility (Cronbach’s alpha = 0.80). The average damage risk perception score was 0.51 (± 0.014). Therefore, respondents, on average, had a neutral or moderate perception of damage risk to their property.

The perceived benefits of armadillos were assessed through three statements that assessed education, aesthetic enjoyment, and ecosystem services benefits (Cronbach's alpha = 0.89). The highest average perceived benefit was the educational benefit received from watching armadillos (0.40 ± 0.018) which is considered a low to neutral perception. The second highest perceived benefit was improving ecosystem health (0.38 ± 0.017), followed last by enjoying the sight of armadillos (0.31 ± 0.018). The average total perceived benefit was low (0.37 ± 0.016). Therefore, on average, respondents perceived armadillos to have low benefits overall with the highest being educational benefits.

Half of the respondents had not controlled armadillos in the previous twelve months at the time of the survey. As a result, many respondents (43%) that answered the cost question said that armadillos had cost them $0 ($n$ = 338). Some respondents (19%) were not sure how much money they had spent, 15% of respondents reported spending anywhere between $1 and $50, and 24% of respondents spent more than $50.

Fewer respondents provided their perception of the costs they had incurred from armadillos over the previous twelve months (n = 235). Of the respondents that did respond to the cost acceptability question, 44% of respondents had a fair or high perception of cost followed by 34% who had a neutral perception. The average score was thus a fair perception of the costs (0.60 ± 0.021). The cost perception question was removed from the model analysis, however, due to the low response rate.

The average perceived control score was 0.47 ± 0.016, therefore, many respondents (41%) had a low perception of control on their property. For respondents who were controlling armadillos on their property, they were most commonly controlling them at a rate of two to three times per year (13%), followed closely by four to five times a year (10%). Overall, respondents were not frequently managing armadillos on their property. All the scale items used to evaluate tenant perceptions, the Cronbach's alpha, and average scores for each perception are provided in Appendix V.

Management

Respondents were asked which management methods they would consider using. Methods were categorized as lethal if they would cause a mortality (shoot with a gun, trap and euthanize, and use poison) and non-lethal if they would not (use a repellent, use a physical barrier, treat yard for grubs and other insects, and hire a wildlife removal company, which can be nonlethal at the resident's request). There was a greater number of respondents (60%) that would consider using lethal control measures compared to nonlethal measures. Most respondents (87%) would consider using direct removal methods like relocating or shooting instead of preventative methods like fencing or grubicide application. Out of all the methods, shooting with a gun was the most commonly reported control measure that would be considered (35%). The control measures that would not be considered by most respondents were the use of poison (80%) and hiring a wildlife removal company (67%).

Respondents also volunteered other management options that they would consider using, if not listed. A total of 21 respondents selected the "other" option but some just left comments and did not provide a management method. There was thus a total of 14 responses where management options were provided. Five respondents provided a management option of doing nothing, including “wait for them to move on” and “watch them!”. Of the remaining nine responses, seven of them mentioned the use of dogs to deter or kill armadillos. Responses related to using dogs as a management method included “dogs harass them until they leave”, “my dogs kill them weekly”, and “big yard dog”. The remaining responses mentioned cayenne pepper and the use of a crossbow; for the former, a respondent more specifically mentioned that they would sprinkle their property line with cayenne pepper (Table 3.4.).

Table 3.4. The number of respondents who would or would not use common management methods for armadillos on their property. There were five answer choices that were categorized into three groups for interpretation of results: “definitely or probably” (would use), “possibly” (may use), and “probably not” or “definitely not” (would not use). *n* = 341

Management method	Would use	May use	Would not use	**Total**
Shoot with gun	121	44	167	332
Trap and relocate	84	91	157	332
Trap and euthanize	78	42	210	330
Use a physical barrier	97	83	149	329
Use a repellent	84	103	142	329
Use poison	30	24	273	327
Hire a wildlife removal company	26	74	231	331
Treat yard for grubs and other insects	110	93	129	332

I was also interested in the types of disposal methods an individual would consider using for a dead armadillo since that is part of some lethal management methods and may have public health implications. Out of the options given, burying (47%), disposing of the carcass in the garbage

(27%), and leaving above ground (27%) were the most selected options. Respondents could also provide another disposal option they would consider using. A total of 43 respondents selected the "other" option but six offered comments that were not relevant to armadillo disposal. Of the respondents that did answer "other" and provided a response (n = 37), they offered moving the armadillo into the woods (37%), leaving it for other wildlife to consume (26%), and calling the city or animal control (19%).

The willingness to use lethal or nonlethal management was evaluated as a predictor variable for all the perceptions of interest, including tolerance (Table 3.5.). For overall acceptability, I found that respondents who were willing to use lethal management resulted in a 0.29 (± 0.05) decrease in tolerance score ($p < 0.001$; $r^2 = 0.28$). Those willing to use lethal management were also more likely to have a significantly higher perception of control and damage risk but a lower perception of costs and benefits.

Table 3.5. A summary of the differences between the willingness to use lethal and nonlethal management methods and its ability to predict each perception and knowledge variable of interest. n=341 ***p<0.001

Perception/ Variable of Interest	Coefficient	Standard Error
Control	0.13***	0.03
Benefit	-0.35***	0.03
Damage Risk	0.28***	0.02
Disease Risk	0.07***	0.02
Knowledge	0.03	0.03
Cost[A]	-0.23***	0.04
Tolerance	-0.29***	0.03

A n=232

I also evaluated the willingness of respondents to use lethal or nonlethal management methods based on geographic location in the state, residential zone, and lot size. There was not a significant difference in the use of lethal or nonlethal methods among the different residential zones, however, there was a significant difference among geographic regions with a higher proportion of respondents in the north willing to use lethal management ($\chi 2 = 15.3$; $p < 0.001$). Additionally, there was a significant association between the type of management method respondents were willing to use and lot size. Respondents willing to use lethal management had a 40.6 ±16.9 acres greater lot size than those that were not.

Model results

The predicted wildlife acceptance capacity model was evaluated through three multiple linear regression models. Tolerance was the dependent variable with different independent variables of interest in each model including 1) demographic variables 2) location within the state 3) and perception and knowledge variables. Separate ordinal regression models were run to evaluate how sighting frequency and residence length predicted tolerance.

The first model incorporated the demographic variables of interest. A multiple linear regression was used to assess demographic variables, and all were categorical with the exception of age. For each 10-year increase in age, a respondent's tolerance decreased by 0.04 (± 0.02; $p < 0.001$), and although significant, the effect size was relatively small. Sex did appear to predict tolerance with males having a 0.07 (± 0.06) lesser tolerance than females, however, the effect size was also small. Respondents with a four-year degree had a 0.10 (± 0.09) lesser tolerance score than those with some college education, and high school graduates had a 0.16 (± 0.14) lesser score ($p <$

0.05); therefore, education was inconclusive and may be due to fewer respondents in the sample population having less than a four-year degree. However, it may also indicate that education has a lesser effect on tolerance compared to the other demographic variables. For instance, compared to households that had an annual income of $100,001+, households with $20,001–30,000 had a 0.25 (± 0.14) greater tolerance score (p < 0.001). In summary, income was the greatest predictor of tolerance in the model whereas age, education, race, and sex had a less significant association (Table 3.6.).

Table 3.6. Summary of the demographic variables that predicted tolerance in the WAC model for armadillos. Income levels above $20,000 did not have a statistically significant relationship with tolerance. n = 341 ***p < 0.001; **p < 0.05; *p < 0.10

	Coefficient	Standard Error
Age		
	-0.04***[A]	0.009
Race (Compared to "White")		
American Indian or Alaskan Native	0.03	0.10
Asian	-0.11	0.18
Black or African American	0.08	0.08
Sex (Compared to "Female")		
Male	-0.07*	0.03
Income[B] (Compared to "$100,001+")		
$20,000 – 30,000	0.25***	0.07
Education (Compared to "Some College")		
Four-year degree	-0.10*	0.05
High School Graduate	-0.16*	0.07

A Age was scaled by a factor of 10-years to aid in interpretation.
B Income levels above $20,000 did not have a statistically significant relationship with tolerance.

The second model evaluated how tolerance could be predicted by a respondent's geographic region and residential zone. Respondents in rural areas had a tolerance score that was 0.07 (±

0.13) lesser than urban areas, however this result was not statistically significant ($p > 0.10$). The variable that was a significant predictor was the region in Alabama that the respondent resided in. Respondents in the central region had a tolerance score that was 0.18 (± 0.06) and the southern region had a score that was 0.10 (± 0.07) greater than the northern region ($p < 0.01$) (Table 3.7.).

Table 3.7. Summary of how respondent geographic variables including region in Alabama and residential zone predicted tolerance in model analysis. $n = 341$ ***$p < 0.001$; **$p < 0.05$

	Coefficient	Standard Error
Region (Compared to "Northern")		
Central	0.18***	.03
Southern	0.10**	.04
Residential Zone (Compared to "Urban")		
Not Sure	-0.14	.12
Suburban	-0.07	.06
Rural	-0.04	.07

From the final model, it was found that respondents who were either very or somewhat knowledgeable about armadillo behavior, biology, and ecology had a 0.15 (± 0.11) lesser tolerance score than those who were unknowledgeable ($p < 0.05$). There was not a statistically significant association between tolerance and perceived disease risk, however, there was a significant association for perceived damage risks and benefits. Respondents who had a high perceived damage risk had a 0.35 (± 0.03) lesser tolerance score than those who had a low perceived damage risk ($p < 0.001$). Additionally, respondents who had a high perception of benefits had a 0.30 (± 0.05) greater tolerance score than those who had a low perception of benefits ($p < 0.001$). Lastly, the perception of control was found to have a statistically significant association ($p < 0.05$), however, the effect size was small on tolerance (-0.04 ± 0.04) and insignificant in the model compared to the other variables of interest (Table 3.8.).

Table 3.8. The influence of independent perception and knowledge variables of residents on the overall acceptability (or tolerance) of armadillos. The knowledge variable is compared to "unknowledgeable" respondents. The perception variables are compared to respondents who had low or negative perceptions. n = 341 ***$p < 0.001$; **$p < 0.05$; *$p < 0.10$

	Coefficients	Standard Error
Knowledge		
Very	-0.15*	0.06
Somewhat	-0.15*	0.06
	Perceptions	
Disease Risk		
High	-0.007	0.04
Neutral	0.003	0.02
Damage Risk		
High	-0.35***	0.03
Neutral	-0.26***	0.02
Benefit		
High	0.30***	0.03
Neutral	0.08***	0.02
Control		
High	-0.04*	0.02
Neutral	0.02	0.02

An ordinal logistic regression was used for the two variables that were ordered by length of time or frequency of occurrences: residential length and sighting frequency. There was a significant inverse association between residence length and tolerance ($\beta = -1.13$; ± 0.3871; $p < 0.05$; -1.89 to -0.37 CI; ± 95% CI); the final (AIC = 804.05) model was a significantly better fit than the null model (AIC = 810.55; $\chi 2 = 8.50$; df = 1; $p < 0.05$). In other words, as tolerance scores increased, the length of residence at the property decreased.

There was also a significant inverse association between sighting frequency and tolerance (β = -2.26; ± 0.37; $p < 0.001$; -3.01 to -1.54; ± 95% CI); The final (AIC = 1327.6) model was a significantly better fit than the null model (AIC = 1364.1; $\chi 2 = 38.536$; df = 1; $p < 0.001$). In other words, as tolerance scores increased, the frequency in which armadillos were observed decreased.

With all models evaluated, the WAC framework used in this study was redefined with the observed relationships between tolerance and the variables of interest. Additionally, it was expected that perceived damage risk and benefit would be inversely related, which has been found in previous studies. In this study, a low perceived damage risk resulted in a 0.51 ± 0.06 increase in a respondent's perceived benefit score compared to a high perceived damage risk ($p < 0.0001$). Perceived disease risk was also expected to have a significant inverse relationship with perceived benefits; however, this relationship was not statistically significant ($p > 0.05$). The updated WAC framework, according to my findings, is shown in Figure 3.5.

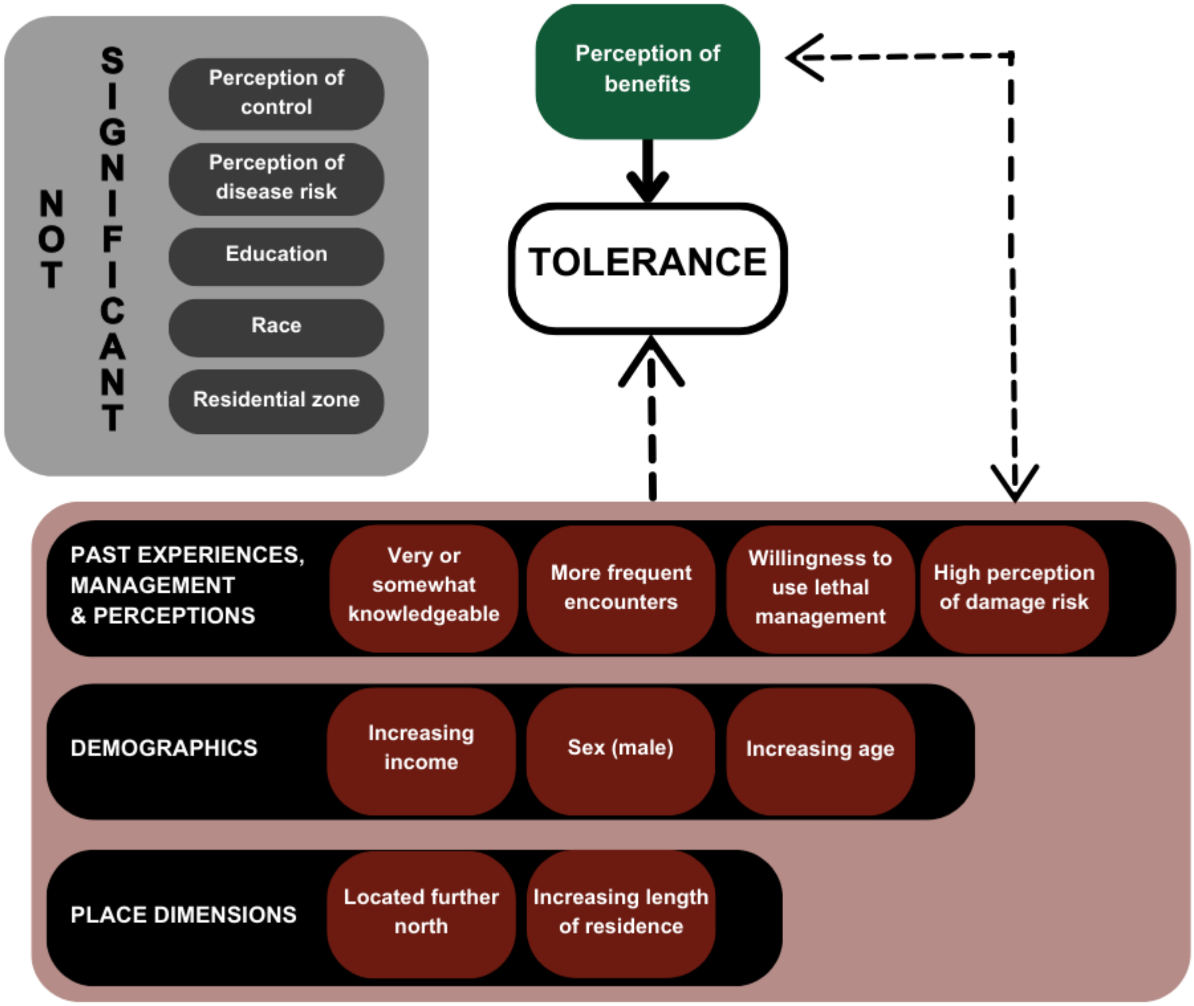

Figure 3.5. Refined WAC framework with the variable relationships observed in this study. Variables that were not significant had either a small effect size or a $p > 0.05$. Dashed arrows indicate an inverse relationship whereas a solid arrow indicates a positive, direct relationship.

Focus Groups

Respondents who agreed to participate in a focus group at the end of the survey ($n = 82$) were assigned a tolerance score based on their survey responses. There were few potential participants who had a high or neutral tolerance ($n = 23$); even fewer of these potential participants responded to the email inquiry or phone call about joining a focus group or interview. Therefore, the high tolerance category was not further subdivided by residential zone. Additionally, only four potential participants out of those who responded to the final survey question were in an urban area; therefore, urban, and suburban areas were combined into one category when organizing the focus groups, hereafter referred to as "urban".

There were nine total focus groups that were organized, three for each group: high tolerance, low tolerance urban, and low tolerance rural. However, several respondents did not attend the virtual focus groups and in these cases, semi-structured interviews were conducted instead with the same set of questions used in the focus groups (Appendix III). There was a total of 15 participants who were either interviewed or participated in a focus group, depending on their tolerance score and residential zone. Most of the participants (60%) were from north Alabama with the remainder equally being from south and central Alabama (20%). The number of participants, type of interview, and average tolerance score is summarized (Table 3.9.).

Table 3.9. A summary of the focus groups and interviews conducted with select survey respondents. The low tolerance group was subdivided into rural and urban or suburban residents. The high tolerance group was not further subdivided due to the few respondents in this category. An average tolerance score is also provided for participants of each group. *n*=15

Residential zone	Total number of participants	Interview type (number of participants)	Average tolerance score (± SE)
		High Tolerance	
	3	• 3 interviews (1)	0.80 (± 0.10)
		Low Tolerance	
Urban	7	• 2 focus groups (2) • 1 focus group (3)	0.07 (± 0.05)
Rural	5	• 2 interviews (1) • 1 focus group (3)	0.017 (± 0.015)

Armadillo encounters

Concepts were coded and summarized after reviewing transcripts from each interview and focus group. First, to further elaborate on the survey, I wanted to understand how tenants are experiencing armadillos on their property. The first set of questions evaluated what time of year and day participants had observed armadillos over the previous twelve months and what past encounters have been. There was not an observable difference in responses among the three group categories for armadillo encounters. Most participants responded that they see more armadillos in the summer than any other season, however, several participants also reported seeing them in the fall. There were also three participants who reported seeing armadillos in the winter but mentioned that they don't see them as often when the weather is cold. Lastly, there were two participants who noted that they see them year-round and that this is novel compared to their past experiences observing them seasonally; it should be noted that these participants were from central and southern Alabama. For instance, one participant who resides in south Alabama commented that they are experiencing more year-round encounters with armadillos than when they first moved to their property: "When they first showed up, it was the spring and late summer, beginning of fall. But this last year, it's pretty much year-round" (low tolerance rural).

The time of day that most people were observing armadillos was at night, although many noted that they don't often see armadillos themselves, but instead the evidence that they have been foraging overnight the following day. I did not specifically ask about roadkill armadillos, but several respondents mentioned that they often see more roadkill armadillos than they do live armadillos near their property. Two of the participants with a high tolerance for armadillos reported seeing “evidence” where they were foraging. There were several respondents with a low tolerance who described a typical encounter with armadillos as “evidence”, however, more respondents with a low tolerance used more charged words related to “damage” like “tearing soil” or “digging up plants” (Table 3.10.):

> “*It was a constant battle with ‘em, tearing up the soil that I’d come out in the morning, and the yard would l– it, if you’ve ever seen feral hogs, what they do, the armadillo is probably just behind a feral hog on how bad it can tear up a yard”* (low tolerance urban).

Table 3.10. A summary of focus group and interview participants' interactions with armadillos, including time of year, time of day and the type of encounter. Select quotes and a count of the codes mentioned by participants are included. *n*=15

Question	Code	Count	Quotes
What time of year do you tend to see armadillos where you live?	Summer/Spring	5	*"I haven't seen any activity, just in the summertime and then it stopped and haven't seen anything since."* (low tolerance urban)
	Fall	4	*"–they were there in the early fall, but once it got cold, they weren't there."* (low tolerance rural)
	Winter	3	*"If you get some warm streaks in the winter, I might see occasionally one, maybe two that will come out."* (low tolerance urban)
	Year-round	2	*"At first, when they first showed up, it was the spring and late summer, beginning of fall. But this last year, it's pretty much year-round."* (low tolerance rural)
What time of day do you observe armadillos?	Dusk	3	*"And then, in the evening, like as it starts to get dark, I've heard them rustling in the- in the leaves in the wooded area that we have."* (low tolerance rural)
	Night/ "Nocturnal"	11	*"I think they're active at night. I see the evidence that they've been out at night. But I don't stay up and watch for them at night."* (low tolerance urban)
	Roadkill	5	*"Roadkill or evidence where they've dug in, you know some of my- you know, looking for grubs and things."* (high tolerance)
	Daytime	3	*"If it's an overcast day- they really- it really messes with them, and they will come out even in daylight hours."* (low tolerance urban)
	Morning	1	*"They've sort of been early morning for me when I would have seen that armadillo"* (low tolerance urban)

What is a typical encounter with an armadillo on your property?	Evidence	7	*"Just the holes but- my neighbor would call it damage. I just call it nature."* (high tolerance) *"Lakeside and streetside looked like it had been plowed up. It was dangerous to walk through the yard without fear of twisting an ankle."* (low tolerance urban)
	Damage	6	*"They'll get into perennials, trees, shrubs and digging around the roots where the where the soil is a little more moist, or there's a little more biological action at the roots. That's where, besides the lawn, they tend to go in. They'll dig up and even knock over plants."* (low tolerance rural)

Perception of armadillos

The participants who had a high tolerance were asked if there was anything about armadillos that they liked (n = 3). One interviewee reported that they cycle the nitrogen in the soil. Two responses mentioned a sense of liking their aesthetics: “I think they’re cute” and “beautiful little beast”. Two high tolerance interviewees mentioned that armadillos eat insect pests, with responses that they eat “yellow jackets”, “fire ants”, and “fall army worms”. Several of the low tolerance participants also mentioned potential benefits including one low tolerance interviewee in an urban area: “I don’t mind them because I have a problem here with yellow jackets and they love to eat yellow jackets.” It was also mentioned that they are “prehistoric” or “ancient” and these are reasons to like them and keep them around: “I think they’re cute, I think they kind of remind me of little piglets in a prehistoric almost way. So, I’ve always been fascinated with them and their hairy bellies” (high tolerance). In short, a certain level of fascination for armadillos was expressed by participants with a high tolerance as well as several with a low tolerance.

One high tolerance participant argued that they are native and that the land belongs to them “Now, people may argue that point that they dig up their land, that they forage, but one has to remember, that land was originally theirs”. However, one-third of low tolerance participants mentioned the opposite and that they are “invasive”: “No, they’re not a native species, they don’t belong in this environment. No, I don’t give them a fighting chance (laughs). If I catch an armadillo, he will not survive,” (low tolerance urban). Still, there were other respondents with a low tolerance who, when discussing with other focus group members, were surprised to learn that they were a non-native species to the southeast United States: “I did not realize that they were not native, that they were like kudzu” (low tolerance urban).

When asked what was the biggest risk that armadillos pose, low tolerance participants mentioned predominantly damage to landscaping in the form of digging up plants and leaving small holes in their yard. Another common response was damage to the foundation of a house or other infrastructure on their property due to burrowing underneath. There was some discussion about people or livestock being injured but most interviewees commented that this was probably unlikely to occur. Additionally, there was a concern for other wildlife and the ecosystem if armadillos were to consume ground-nesting bird eggs or beneficial invertebrates like earthworms. All responses pertaining to risks armadillos pose were coded and summarized (Table 3.11.).

Table 3.11. Low tolerance participants' responses to the biggest risks they feel that armadillos pose were coded. The frequency of codes was totaled and subdivided by the residential zone they resided in. Select quotes are also provided for each included code. *n* = 10

Code	Urban	Rural	Quotes
Foundation/ Infrastructure	3		*"Undermining the foundation of the house would be a problem. But we keep a close eye on it. So, like I said, we filled up the place where they were burrowing under the porch which is a concrete slab type porch"* (urban)
Financial	1		*"Well, they're doing financial damage. I mean, it looks like my beautifully landscaped yard that I pay a service to mow and to upkeep– it looked like someone had plowed my yard when they came into our yard."* (urban)
Landscape	2	3	*"They just need to quit digging up my plants"* (rural)
Personal Injury	1	1	*"-Throughout the yard there were holes. Some were deep enough that our landscaper, who mows for us every week, was very careful, because they were deep enough that he could have twisted his ankle if he had stepped on one of them"* (urban)
Ecosystem Impacts	2		*"So, I imagine they're eating quail eggs and turkey eggs and meadowlarks and kill any– any eggs they can get their- bird eggs they can get their little lips on."* (urban)

Perceived disease risk

Overall, participants were not concerned about acquiring leprosy from an armadillo, as evidenced by the average disease risk score of survey respondents. High tolerance interviewees commented that they feel bad for the armadillos and worry leprosy could eliminate them. One participant commented: "Leprosy is curable, I'm just not worried about it. As far as I can tell, I think they're the victims. Leprosy is an Old-World disease and they got it from us. So, maybe this is payback time (laughs). I don't− I don't worry about it" (high tolerance). Another high tolerance interviewee mentioned that leprosy is one of the things that are mentioned to turn people *against* armadillos: "It [leprosy] does not concern me. I know that is one of the things that are out there and publicized against armadillos".

Other participants were not as concerned because they did not believe they could actually carry leprosy: "Everybody talks about leprosy, they talk about, you know, diseases and all that. But I don't personally buy into that. I mean I think if that was an issue it would have already been documented well. I can't find anywhere− I've looked online, I've researched, I can't find anywhere where it really shows that they carry anything that would be a concern from my perspective" (low tolerance rural). All of the coded responses relating to disease risk are summarized in Table 3.12.

Table 3.12. A summary of coded focus group responses when asked about the perceived disease risk of contracting leprosy from an armadillo. The frequency in which the code occurred is subdivided by the type of focus group or interview. Select quotes are also included for each code. *n* = 14

Code	Low Tolerance		High Tolerance	Quote
	Urban	Rural		
Infection unlikely	1	3	2	*"There is essentially no human-armadillo contact on my property."* (low tolerance rural) *"No, I know they can carry leprosy, and I know that most of the population is immune* (high tolerance)
Unaware			1	
Cautious	1	1	1	*"But, now having this concept of disease carrying, I think I would be even more cautious"* (high tolerance)
Not a concern	5	1	1	*"Yeah it's not really been- the leprosy part has not really been an issue to me. Anytime I do– if I do shoot one, I handle it with gloves. But the actual catching of leprosy has not been an issue, it's more just a– the digging the and the eating the Earthworms."* (low tolerance urban)

Discussion of leprosy risk from an armadillo did reveal perceived transmission routes of acquiring leprosy, although participants overall felt it was unlikely. One participant believed there was a possibility to acquire from claw scratches: "Yeah the only thing I've found out about Hansen's disease, it said if they got you with their claws or anything, then you know, call your doctor and your doctor would monitor you for a while" (low tolerance urban). Another participant felt that there was the possibility to acquire from fleas: "I don't want the fleas because my understanding is the fleas are where the trans- if anything's there that's bad, the fleas are one of the most- best factors for anything that they carry" (low tolerance rural). A high tolerance participant felt it was possible to transmit leprosy from bites: "–I also have not really seen any statistics that say that it has been prevalent, or you know, of all the people I know and have lived in the country, I've known no one that's gotten bit by an armadillo". Lastly, one respondent mentioned a worry about environmental transmission: I don't think I worry about it a lot, but I mean it's, you know you- you do realize it's a possibility that some armadillo could have it, you know, could have infested your soil with it" (low tolerance rural). After the discussion about leprosy, several participants mentioned that although they felt it was unlikely to get the disease, they would still wear gloves when handling a dead armadillo to limit the possibility of transmission.

Management perceptions

Participants with a high tolerance score were asked what they would do if an armadillo was digging small holes in their yard. Two of the interviewees commented that they would do nothing while one participant commented that they would call animal control or wildlife services since they own pets: "–if they were in my area being a nuisance, hmm, I think because I have animals, yes, I would generally call the wildlife and see if they can put them back to their natural habitat. That would be my first interest. But I would not kill or maim an armadillo or set a trap that the armadillo would suffer." None of the high tolerance participants said their answer would change if an armadillo was burrowing on their property or if it was a reproductive female with a litter, on the contrary, they would be excited: "I've never seen one [a juvenile] in person. I think that I would just die and go to heaven if I got to see that".

When asked if they have ever managed armadillos before, one high-tolerance interviewee said that they had called the wildlife services in their city to remove an armadillo, but that their city's wildlife services do not always respond. Another high-tolerance interviewee that had managed for armadillos did not remove them from their own property but regularly removed them from their neighbor's yard and relocated them onto their own property to preserve the armadillo: "I try to educate my neighbor that they are there because she has grubs, good eating and (sigh) it doesn't work. So, I shut up, collect the box and, and leave". The previous interviewee was the only one familiar with direct removal management methods and commented that their neighbor placed the box traps, and they only released them on their property.

Participants with a low tolerance for armadillos had a range of responses from the use of grubicide to shooting. Rural respondents with a low tolerance score were more likely to use shooting as a management method compared to other methods. Other management methods that are less common were also mentioned including bludgeoning armadillos, altering the burrow by blocking the entrance, placing grain to deter them, and using dogs to either deter or dispatch armadillos. Most respondents reported having moderate success with previous trapping methods. Some difficulties with managing armadillos were discussed and is detailed in the following section. One urban participant commented: "As a homeowner, I feel woefully unprepared [to manage armadillos]". The management methods that had been previously used by residents are summarized in Table 3.13.

Table 3.13. The armadillo management methods mentioned by participants when asked about what they have either used or considered using on their property in the past. The frequency that each method occurred is subdivided by the categories in which participants were grouped. Select quotes are also included for each code. *n* = 14

Code	Low tolerance		High tolerance	Quotes
	Urban	**Rural**		
Burrow alteration	1	1		"– [an armadillo] *was digging up underneath the porch and so we just piled rocks in there to keep him from ruinin' the foundation of the house–"* (low tolerance urban)
Use of grain	1			
Grubicide	2			*"I called my lawn service and had a very expensive treatment done for the entire yard that was supposed to kill the grub worms which were there food source. At that point I saw the diminishing of the damage in the yard."* (low tolerance urban)
Do nothing	2	1	1	*"It seems too complicated to me."* (low tolerance urban) *"No, I- I live with them. Tolerate them."* (low tolerance rural)
Trap and euthanize	1	1		
Trap and relocate	1	1	1	*"I will put out live traps, and when I've caught them then I have just taken them and relocated them to the woody area about 5 miles from my house. I'm not going to kill them"* (low tolerance urban)
Shoot	2	5		*"I shoot them on my property, very quietly– you really can't shoot it in the city limits of [redacted] but I have a little rifle where I have a subsonic.*

				They're very quiet, very low impact rounds. They don't make a lot of noise, and won't travel very far" (low tolerance urban)
Use of fertilizer	1			*"I found that fertilizer in the spring, mid-summer and late fall has turned them off from into the main yard"* (low tolerance urban)
Contact animal services			1	
Fencing	1	1		*"I have a fence in my backyard, but it was there before we had a problem with the armadillo, but they don't seem to get in the backyard."* (low tolerance urban) *"They were digging my husband's− right near his blueberry bushes, and he was getting frustrated with that. So we had to put a fence up around his blueberry bushes.* (low tolerance rural)
Dogs		1		*"It's usually the dogs that will harass them. I guess that was my most popular technique, that's for the dog to go out and grab 'em and take care of 'em."* (low tolerance rural)
Bludgeoned		1		*"In fact, I bludgeoned one to death."* (low tolerance rural)

Management needs

A main objective of focus groups and interviews was to identify potential concerns residents had about management techniques. Each management method that is commonly recommended by experts was discussed in a focus group or asked of each interviewee who exhibited a low tolerance for armadillos. Common concerns for all management methods were that they were perceived as difficult or inconvenient and ineffective or unreliable. The expressed concerns are summarized in Table 3.14. by the type of management method and the zone that participants resided in; hiring a wildlife removal company was not included in the coding, however, since participants mentioned that companies may provide lethal or nonlethal removal.

Table 3.14. A summary of the coded responses of participants when asked about armadillo management methods that they would consider using on their property. The frequency that the code occurred is subdivided by the categories in which participants were grouped. Select quotes are also included for each code. *n* = 14

Concerns	*Lethal Removal*		*Nonlethal Removal*		*Preventative*		**Total**
	Urban	Rural	Urban	Rural	Urban	Rural	
Legality	5		2	1			8
Uncomfortable	3	1	3				7
Morally wrong	1		3	1			5
Difficult/ Inconvenient	2	6	2	1		4	15
Ineffective/ Unreliable	2	2	1	2	3	7	17
Disease			1				1
Safety (of people or pets)	1	2	1		2		6
Cost					6	6	12
Environmental impacts	2	2			3	4	11

A common concern of urban participants with lethal removal methods (trap and euthanize, shooting, and poison) was the legality, particularly for shooting. One participant in an urban area commented:

> *"Well, we can't use fire- I live in the city limits. But the end of my street, four houses down begins the county and I, I do have firearms, and I could very easily shoot them. But it is illegal where I am, and I'm afraid my neighbors would probably have a fit. So, I have not done that."*

The legality of using lethal removal methods was not mentioned by rural respondents, however, there was more concern about the difficulty of using such methods. It was commented by a rural participant how inconvenient shooting would be: "As far as, you know, if there's six of them here and I wanted to get rid of them in a month, it would be a lot more out at midnight walking trying to get rid of them". Most of the rural respondents, however, felt that trapping and euthanasia would be most inconvenient due to difficulty trapping them and finding a safe euthanasia method: "I kept reading horror stories about .22 bullets bouncing off of them after- if they were in the trap". There was also expressed concern that shooting through a trap may damage the trap, which is costly.

A euthanasia method for armadillos already in a trap could involve placing them in a constructed chamber and using carbon dioxide according to the American Veterinary Medical Association guidelines (Leary *et al.* 2020) (Figure 3.6.). Although most urban participants would not use a gun due to being uncomfortable or it being illegal within their city, when asked whether they would use an euthanasia chamber, four of the seven participants said they would consider using it but were unlikely to build it themselves. Instead, they recommended having it as a community resource that could be checked out for use: "Like a city, the city animal control you know, had a site like, I say− I mean you still have to haul it, but if they had something you could borrow, or a site where it'd be a community-based thing, I could see that, as a real strong possibility, yeah" (urban).

Figure 3.6. A constructed euthanasia chamber with an attached five-gallon carbon dioxide tank and regulator. Not pictured is the box trap inside of the chamber that can be placed without physically translocating the animal.

Other participants felt hesitant about the effectiveness of getting an armadillo in a trap in the first place for either euthanasia or relocation. One urban participant commented that "Yeah, they can be trapped but there's not easy− I'm just− I have trapped some, but they're just− you have to wander into the trap, as I understand it, you really can't bait 'em". Other comments included concerns about having to regularly check them, trapping unintended wildlife or pets, and potential safety concerns about handling animals in traps. The difficulties with using live traps to trap an armadillo was summarized by a comment from one rural participant who had previous experience using Havahart traps, which are a common brand of live box traps:

> *"Not really. You have to- one, you have to find where their hole is, and then you have to set your trap up and you have to funnel them into the trap. They don't go for bait like a*

raccoon or an opossum would. So it's pretty much you have to funnel them into your trap. And again, I'm catch and release. Which means I have a Havahart trap and they'll go in there and I'll have to pick up the trap, and then let them go. The one thing I have found is that they are stronger and tougher, like a tank, in getting out of a Havahart trap. They have broken two Havahart traps that I have caught numerous very large raccoons and opossums in and that those animals could not get out but the armadillos were big enough that they just bulldozed and bent the hard wire that was holding the Havahart trap, so I have pretty much given up on that."

With relocation in particular, some participants were concerned about the legality as well as some confusion about the laws: "We talked about trapping it, but it gets hard to relocate them, once you trapped them. There's a lot of rules about letting animals loose after you've trapped them. I think you have to keep them within your county, and we didn't know what all those ins and outs were*"* (low tolerance urban). Additionally, relocation being immoral or unethical was also mentioned several times in the discussion of relocation: "−I can't imagine relocating them, why would you do that, go give somebody else some problems, you know?" (low tolerance rural).

There were no participants who agreed that they would use poison, if there was one developed for armadillos, listing common concerns of unintended consequences for pets or other wildlife. Although not coded in Table 3.14., participants also provided concerns they would have about hiring a wildlife removal company. Most participants (55%), including both urban and rural participants, felt that it would be costly to hire a wildlife removal service. The effectiveness was also called into question by some participants: "It wouldn't be worth it. It would be a one-time thing and the armadillos would be right back" (rural). There were three participants who said they might consider it as a last-ditch effort if all other management methods failed. A general consensus by all participants was that they would not want hired professionals to relocate a removed armadillo and instead would prefer that the animal was humanely euthanized.

Common concerns for preventative methods including fencing, grubicide, and repellents were that they would be costly or ineffective. With fencing, all participants except one felt that this would be costly, and they would not consider it to keep armadillos out of their property. In addition to being costly, it was also considered to be ineffective: “We’ve had dog pens and stuff that were fenced in pretty good, and they would get in those also” (rural). For repellents, a common concern was that it would be ineffective or not species-specific. Additionally, there was concern about re-application and cost, however, it would generally be considered by participants if a repellent was developed that was cost-effective and shown to be effective in preventing armadillos.

The final preventative method was the use of a grubicide applied to lawns and gardens. Both rural and urban participants were relatively evenly divided on using grubicide to deter armadillos (50%, n = 4; 40%, n = 5). Common concerns were that grubs are beneficial for their yard health and for the ecosystem at large and would not want to jeopardize that at their residence. In addition to cost, which was also a common concern, the effectiveness of grubicides were called into question. One urban participant had previous experience and said they would not use grubicide again because: “everybody says they're going after grubs and I did the spraying, I did the particle drops, I did everything and it did not diminish the numbers. I mean they were still coming in and just tearing the yard up”. On the contrary, another urban participant had the opposite experience and had found it to be effective in preventing armadillos with plans to reapply over the entirety of their property despite it being costly: “I've already had my landscape company out here last week and I talked to them about that and this summer they’re going to apply that grub treatment to every square inch of soil that we have. Now, it is going to be thousands of dollars”.

There were additional comments that participants made during the discussion, although not in response to any particular question. It is interesting to note that some participants, although they had a low tolerance score, reported that they did not feel strongly against armadillos. For instance, one participant from a rural area, when asked by a fellow member of the group if they would remove all armadillos if they could, said: “No, I tend to adapt around animals who have been around for 50 million years. We haven't been around that long” (low tolerance rural). Additionally, other low-tolerance respondents commented that they may be returning to a “natural state”:

> *“The environment is changing, and we're starting to see things that we normally didn't see. And I think part of that, from my perspective is, you have to decide, okay at- at what point are they invasive versus returning back to a natural state of what used to be before we had all the pesticides and all that-”* (low tolerance urban).

However, there was still the sentiment that armadillos do not belong in Alabama or the southeast ecosystems and should be eliminated. A common sentiment was that most of the participants do not recall armadillos being in their area of northern Alabama until more recently: “They are moving so rapidly from the south to the north, and they are multiplying so quickly that I feel like they probably ought to be eliminated” (low tolerance urban). A long-time resident of Alabama also commented on their experience with armadillos over the past several decades:

> *"I would travel down to the coast, and I would see the signs or runover armadillos mostly. And every year, as I would go down to the coast and then start my way back, they would move their way north. I would see run over armadillos on I-65, a little bit further north. So first it was Mobile, and then it became Montgomery, and then it became Birmingham. And then I thought the Tennessee River would stop them, but it didn't, and they've been here [north AL] at least 10 years"* (low tolerance rural).

Finally, participants were asked where they typically get information about armadillos and their management. Most respondents (53%) reported that they use local extension agents or "ACES" as a source for information, including the online armadillo article that is currently available (Armstrong 2019). Other responses mentioned referencing university pages of the southeast like Auburn University and University of Florida, Google.com, local game wardens, and word-of-mouth from family or friends.

Discussion

Nine-banded armadillo (*Dasypus novemcinctus*) expansion has resulted in novel human-armadillo interactions in Alabama over the past fifty years. Similar to other quickly-expanding nuisance wildlife in the southeast United States, such as wild pigs (Ellis *et al.* 2023), armadillos may cause significant damage to property. Additionally, armadillos are habitat generalists and are not only limited rural or natural areas as they are increasingly being found in human-dominated landscapes (Loughry and McDonough 2013). For instance, Hohbein and Mengak (2018) found that armadillo damage was increasing in Georgia, alongside deer and wild pigs, resulting in an increase in public calls to local extension agents. In this study, it was found that rural, urban, and suburban residents encounter armadillos at comparable frequencies. With the propensity for armadillos to expand into urban areas and cause damage to private property, residents require solutions to control nuisance armadillos.

The main management methods that are often provided through extension websites and county agents are: fencing, live trapping, humane euthanasia or relocation, and spotlighting and shooting at night (Armstrong 1991; Schaefer 2019). An Internet search reveals additional management methods that a resident may utilize including applying capsaicin (e.g. cayenne pepper) to their yard as a repellent or an insecticide to remove the food source. With the many available management options, there have been few studies on the efficacy of management methods for nine-banded armadillos. One study found that double-door traps were the most efficient method to trap armadillos, especially for populations that are not well-established (Haywood *et al.* 2021). It has also been found that several traps and cage wings used simultaneously can increase trapping success (Mengak *et al.* 2017), however, this can be costly for residents. Other studies have not revealed reliable attractants that are effective for armadillos (Gammons *et al.* 2009; Ober *et al.* 2011). Additionally, although relocation is often suggested to people, Gammons *et al.* (2009) found that it was highly ineffective with some translocated armadillos even returning to the site of capture.

Previous findings of armadillo management methods and their efficacy are limited and has been identified by the Wildlife Services National Advisory Committee as a future research priority (Bruggers *et al.* 2002). The lack of reliable and efficient management methods currently available may run counter to residents' expectations when searching for solutions. Additionally, the behaviors of armadillos make their management even more difficult as they are mainly active at night and can be elusive even for the trained biologist to capture (Loughry and McDonough 2013). Spotlighting and shooting can be time intensive, requiring residents to stay up late at night, and local regulations may legally prevent the discharge of firearms. Trapping may take multiple nights and may result in broken or empty traps.

Despite the complexities of armadillo management, resident perceptions related to the species and its management in the southeast has not previously been evaluated. In addition, residents in urban areas of the United States have not been regularly included in perception studies of similar small or medium-sized nuisance mammals as stakeholders are mainly limited to two groups: farmers and hunters (McIvor and Conover 1994; Goodale *et al.* 2015; Liordos *et al.* 2017). Therefore, management options may be offered to tenants who are dealing with nuisance armadillos, however, people's willingness to execute these methods and their potential concerns are not well understood.

Respondents in this study were generally representative of the Alabama population, however the sample population consisted of significantly more females, college-educated individuals, those with a high income, and fewer African American or Black respondents compared to the statewide population (U.S. Census Bureau 2022). Respondents were geographically represented with a survey received from nearly every county within the state. There were more rural respondents surveyed than urban or suburban which resulted in a larger average lot size and should be considered when contextualizing the management concerns of respondents; additionally, the residential zone was self-designated, with no description of the zones provided, therefore, it was left to the interpretation of the individual.

The perception of control is where an individual believes that they have control over conflicts and risks associated with wildlife (Bjerke *et al.* 2000). My study revealed that residents in

Alabama had a low perception of control over armadillos and the majority had not recently controlled for armadillos at the time of the survey. Qualitative analysis revealed tenant concerns about management methods including them being ineffective, difficult or inconvenient to use, and costly. In urban areas, there was additional concern about the legality of management methods, particularly lethal methods that require a firearm within city or county jurisdictions. How a person perceives the amount of control they have over a risk depends on the frequency of previous similar situations and what the outcomes were in the past (Zinn *et al.* 2008). Many residents, both rural and urban, expressed difficulties they have had with managing nine-banded armadillos and several commented that they have "given up" or feel "woefully unprepared" due to their failed attempts at previously managing the species.

Although perception of control was overall low, it was found that most respondents would consider the use of lethal methods. In general, lethal control has been found to be a more acceptable method for species that cause damage to personal property such as deer (Urbanek *et al.* 2015). In addition to damage from wildlife, lethal management is also considered acceptable when wildlife diseases are involved (Koval and Mertig 2004). Respondents who would consider lethal methods were also more likely to have a significantly higher perception of control and damage risk but a lower perception of benefits and costs, which was similar to the findings of Goodale *et al.* (2015).

Most respondents had not recently controlled armadillos, therefore, reported costs accrued from an armadillo were low with a median response of $1 to $50. Other studies have not evaluated the costs that armadillos can cause a resident, however, Goodale *et al.* (2015) did find that damaging species that cost farmers more money in damage reparations or management resulted in a lower tolerance for the species. Future studies should further evaluate the costs that residents incur as a result of armadillo damage and management as well as how it may impact tolerance for the species.

Tolerance was determined by evaluating the perception of the current and future population size as well as the consideration of the armadillo as a nuisance. The tolerance for armadillos was, on average, low and respondents desired a decrease in current and future armadillo populations. It was also observed that tenants' perceptions of control and costs did not significantly predict tolerance. Additionally, the perception of disease risk from leprosy did not significantly predict tolerance for armadillos as was predicted, however, almost half of the respondents were unaware that armadillos can even carry the bacteria that cause leprosy. Therefore, the low perceived disease risk may instead be due to a lack of knowledge as interviewees were also unaware of how the disease is transmitted and questioned if it is actually prevalent in armadillo populations. However, prevalence rates in wild populations of the southeast have occurred at a rate up to 25% and positive armadillos have also been found in every state in the southeast U.S. (Howerth *et al.* 1990; Loughry *et al.* 2009; Truman *et al.* 2011; Sharma *et al.* 2015). Zoonotic transmission of leprosy from armadillos has also recently been demonstrated through the identification of a strain of *M. leprae* unique to armadillos and humans of the southeast United States (Truman *et al.* 2011). Due to the potential zoonotic nature of leprosy in wild armadillos and their increasing expansion into human-dominated areas, educating residents of the southeast about the disease's prevalence and transmission is necessary when discussing safe management methods.

The perceived damage risk of armadillos was, instead, a higher concern for residents and was not only confined to rural areas since respondents in each residential zone felt their property was susceptible to armadillo damage. Lawn and garden damage from armadillo foraging and burrowing behaviors was expected as this is often the main reported concern of nuisance armadillos to local extension agents (Armstrong 1991; Hohbein and Mengak 2018). However, the majority of respondents felt armadillos were also likely to damage their crops. There is currently no scientific literature that has observed armadillos causing significant damage to crops and may instead be the result of other nuisance wildlife like the raccoon (*Procyon lotor)* (Beasley and Rhodes 2008). Additionally, a higher concern for crops was also likely reported due to having significantly more rural respondents represented in the sample population.

The perception of benefits was found to be inversely related to the perceived damage risks which is comparable to previous studies (Goodale *et al.* 2015). Respondents who had a higher perception of ecosystem services, education, and aesthetic benefits from armadillos resulted in a higher tolerance and a lower perception of damage risks. However, there were few people overall who perceived armadillos to have benefits. Armadillos may provide ecosystem services as they are considered ecosystem engineers, constructing burrows that may be used by other species (Lawton and Shachak 1994, DeGregorio *et al.* 2022). Additionally, armadillos may control insect pest species (Rodrigues *et al.* 2020), however, research related to nine-banded armadillo diet has been limited.

Many respondents with both low and high tolerance scores, when discussing benefits or the armadillo in general, mentioned that the nine-banded armadillo is an ancient species that has been in the southeast for millions of years. However, the nine-banded armadillo is often referred to as an invasive non-native species and only entered the United States 150 years ago. Instead, the consideration of armadillos as an ancient or "prehistoric" species may be due to the animal's physical appearance which is unlike many other mammals in the southeast United States. Additionally, it may be a reference to *Dasypus bellus,* a closely-related species that went extinct in North America nearly eleven-thousand years ago (Shapiro *et al.* 2015).

Respondents overall exhibited a moderate amount of knowledge of armadillo behavior, biology, and ecology which is likely due to increased familiarity with the species as it has expanded within the state. Additionally, the sample population was generally well-educated, and the majority said they have used extension services and read ACES articles while researching armadillos. In general, survey findings indicated that a person who is more knowledgeable of armadillos is more likely to have a lower tolerance.

Geographically, residents' tolerance for armadillos did not vary between residential zones in this study, indicating that residents' low tolerance for armadillos persists across an urban to rural gradient. However, tolerance was significantly associated with the region in the state that a tenant resided in. Since the armadillo has expanded northward throughout Alabama over the past thirty years (Taulman and Robbins 2014), the result has been novel interactions for tenants in the north, resulting in a significantly lower tolerance score for respondents compared to central and south Alabama.

For a comparable nuisance wildlife species, the opposite was found in terms of tolerance as residents living in areas of New York and Massachusetts with a high beaver density had a lower tolerance score compared to those in a low-density beaver area (Siemer *et al.* 2013). On the contrary, people living near high populations of the black bear in Oklahoma had a higher tolerance when compared to people in the northeast where the bear population was smaller and there were fewer interactions (Cleary *et al.* 2021). Therefore, the findings on whether people's novel interactions with nuisance wildlife results in a higher or lower tolerance have been inconsistent and may be both region and species-specific. Additionally, many studies that use WAC to evaluate people's tolerance of nuisance wildlife do not incorporate place dimensions such as region or residential zone nor control populations with no or limited interactions with the species of interest. Future research evaluating WAC of nuisance wildlife should include people in areas where target species are expanding or may expand in the near future. The expansion of nuisance wildlife and their interactions with people are increasingly relevant to the field of wildlife management as climate change and other environmental factors shift species' ranges globally (McCarty 2001).

Analogous to novel interactions with a species, past experiences have been shown to impact tolerance as previous interactions often impact affect, or one's emotional response to a stimulus, which can drive the perception of a species' benefits and risks (Damasio 1996; Johansson and Karlsson 2011). For instance, Zinn and Andelt (1999) found that previous negative experiences with prairie dogs in Fort Collins was associated with negative attitudes towards the species and greater acceptance of lethal control methods when compared to the general public and those living at a further distance from prairie dog colonies. It was also observed that Virginia residents' attitudes toward deer were predicted by previous experience with their damage, resulting in a lower acceptance and higher acceptability of lethal management (West and Parkhurst 2002). This study supports previous findings related to tolerance and past experience since the longer residents resided at their property, the lower their tolerance for armadillos; a low tolerance is likely due to more frequent past experiences with armadillos and potential negative interactions. Additionally, lethal management was supported by the majority of respondents, which is likely an emotional response due to past experiences with armadillos.

There were also some demographic variables that predicted tolerance of armadillos including having a higher income, being male, and older. Education and race, however, did not significantly predict tolerance. Similar to the finding in this study that older individuals had a lower tolerance, Goodale *et al.* (2015) found that the age of farmers was found to be a predictor of wildlife acceptance for many damaging species that they evaluated. In addition to the significant demographic variables, residents' wildlife acceptance capacity was mainly predicted by perception of damage risks, benefits, and willingness to use lethal management methods. Other factors including being further north in Alabama and the length of residence also predicted tolerance. A novel WAC framework was developed from my findings and can be applied in other human dimensions studies of comparable nuisance wildlife. Additionally, although not a significant predictor of tolerance, the variable of perceived disease risks was incorporated in my revised WAC model which can be applied to other nuisance wildlife situations in which zoonotic disease may impact stakeholder tolerance.

This study revealed several management needs of residents in Alabama and in the remainder of the armadillo's range in North America. More than half of the residents surveyed in this study are encountering armadillos or their evidence at a frequency of every other month or more. Most respondents also felt that their property was susceptible to damage by an armadillo, which is a type of HWC. Additionally, respondents in the north and areas where armadillos have not resided in recent years are facing a novel management issue. Many residents feel worried and unprepared to protect their property from damage, despite access to Extension resources. Future studies should continue to evaluate effective management techniques for armadillos that are both accessible to people and legal to employ. In the meantime, Extension services in Alabama and the greater southeast region should recognize the management needs identified in this study and continue to provide science-based management education to the public. The potential for community resources that would make humane removal of armadillos more accessible should also be considered by local municipalities and extension agents. The tolerance for armadillos by residents in Alabama and their perception of control is low, however, with education and improved management techniques, HWCs can be mitigated to improve the lives of people in the southeast while ensuring humane and effective management.

Literature Cited

Armstrong JB (1991). Extension wildlife damage management in Alabama. *5 - Fifth Eastern Wildlife Damage Control Conference (1991)*. Available at: https://digitalcommons.unl.edu/ewdcc5/1

Armstrong JB (2019). Controlling armadillo damage in Alabama. *Alabama Cooperative Extension System*. Available at: https://www.aces.edu/blog/topics/forestry-wildlife/controlling-armadillo-damage-in-alabama/ [accessed 11 May 2022]

Attia TSN, Martin T. N, Forbuzie TP, Angwafo TE, Chuo MD (2018). Human wildlife conflict: causes, consequences and management strategies in Mount Cameroon National Park South West Region, Cameroon. *International Journal of Forest, Animal and Fisheries Research* **2**, 34–49. doi:10.22161/ijfaf.2.2.1

Beasley JC, Rhodes OE (2008). Relationship between raccoon abundance and crop damage. *Human-Wildlife Conflicts* **2**, 248–259.

Bjerke T, Vittersø J, Kaltenborn BP (2000). Locus of control and attitudes toward large carnivores. *Psychological Reports* **86**, 37–46. doi:10.2466/pr0.2000.86.1.37

Bruggers RL, Owens R, Hoffman T (2002). Wildlife damage management research needs: perceptions of scientists, wildlife managers, and stakeholders of the USDA/Wildlife Services program. *International Biodeterioration & Biodegradation* **49**, 213–223. doi:10.1016/S0964-8305(02)00042-2

Bruskotter JT, Vaske JJ, Schmidt RH (2009). Social and cognitive correlates of Utah residents' acceptance of the lethal control of wolves. *Human Dimensions of Wildlife* **14**, 119–132. doi:10.1080/10871200802712571

Carlson AR, Helmers DP, Hawbaker TJ, Mockrin MH, Radeloff VC (2022). The wildland–urban interface in the United States based on 125 million building locations. *Ecological Applications* **32**, e2597. doi:10.1002/eap.2597

Cleary M, Joshi O, Fairbanks WS (2021). Factors that determine human acceptance of black bears. *The Journal of Wildlife Management* **85**, 582–592. doi:10.1002/jwmg.21999

Colautti RI, MacIsaac HJ (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions* **10**, 135–141. doi:10.1111/j.1366-9516.2004.00061.x

Cross M, Heeren A, Cornicelli LJ, Fulton DC (2018). Bovine tuberculosis management in Northwest Minnesota and implications of the risk information seeking and processing (RISP) model for wildlife disease management. *Frontiers in Veterinary Science* **5**, 190. doi:10.3389/fvets.2018.00190

Damasio AR (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. doi:10.1098/rstb.1996.0125

Decker DJ, Purdy KG (1988). Toward a concept of wildlife acceptance capacity in wildlife management. *Wildlife Society Bulletin (1973-2006)* **16**, 53–57.

DeGregorio, Brett A., John T. Veon, and Andrhea Massey. “Wildlife Associates of Nine-banded Armadillo (Dasypus Novemcinctus) Burrows in Arkansas.” *Ecology & Evolution (20457758)* 12, no. 5 (May 2022): 1–10. https://doi.org/10.1002/ece3.8858.

Dowle M, Deane EM (2009). Attitudes to native bandicoots in an urban environment. *European Journal of Wildlife Research* **55**, 45–52. doi:10.1007/s10344-008-0212-9

Ducatez S, Sayol F, Sol D, Lefebvre L (2018). Are urban vertebrates city specialists, artificial habitat exploiters, or environmental generalists? *Integrative and Comparative Biology* **58**, 929–938. doi:10.1093/icb/icy101

Ellis HE, Jaebker LM, Bright AD, Smith MD, Carlisle KM (2023). Alabama agricultural producers’ experiences with wild pigs (*Sus scrofa*) and their preferences concerning wild pig management. *Human Dimensions of Wildlife* **0**, 1–5. doi:10.1080/10871209.2023.2273506

ESRI (2023). USA Counties - Overview. Available at: https://www.arcgis.com/home/item.html?id=a00d6b6149b34ed3b833e10fb72ef47b [accessed 26 January 2024]

Gammons DJ, Mengak MT, Conner LM (2009). Translocation of nine-banded armadillos. *Human-Wildlife Conflicts* **3**, 64–71.

Gilbert B (1995). That `little armored thing’ doesn’t get by on looks alone... *Smithsonian* **26**, 142.

Goodale K, Parsons GJ, Sherren K (2015). The nature of the nuisance—damage or threat—determines how perceived monetary costs and cultural benefits influence farmer tolerance of wildlife. *Diversity* **7**, 318–341. doi:10.3390/d7030318

Gore ML, Wilson RS, Siemer WF, Wieczorek Hudenko H, Clarke CE, Sol Hart P, Maguire LA, Muter BA (2009). Application of risk concepts to wildlife management: special issue introduction. *Human Dimensions of Wildlife* **14**, 301–313. doi:10.1080/10871200903160944

Grimm NB, Faeth SH, Golubiewski NE, Redman CL, Wu J, Bai X, Briggs JM (2008). Global change and the ecology of cities. *Science* **319**, 756–760. doi:10.1126/science.1150195

Hanisch-Kirkbride SL, Riley SJ, Gore ML (2013). Wildlife disease and risk perception. *Journal of Wildlife Diseases* **49**, 841–849. doi:10.7589/2013-02-031

Hayman, Harvey R, Mazzotti, Israel, Woodward (2014). Who complains about alligators? cognitive and situational factors influence behavior toward wildlife. *Human Dimensions of Wildlife* **19**. doi:10.1080/10871209.2014.918218

Haywood CJ, Nielsen CK, Jiménez FA (2021). Live-capture techniques for colonizing nine-banded armadillos. *Northeastern Naturalist*, 169. doi:10.1656/045.028.0206

Hohbein RR, Mengak MT (2018). Cooperative extension agents as key informants in assessing wildlife damage trends in Georgia. *Human-Wildlife Interactions* **12**, 243–258.

Howerth EW, Stallknecht DE, Davidson WR, Wentworth EJ (1990). Survey for leprosy in nine-banded armadillos (*Dasypus novemcinctus*) from the southeastern United States. *Journal of wildlife diseases* **26**, 112–115. doi:10.7589/0090-3558-26.1.112

Johansson M, Karlsson J (2011). Subjective experience of fear and the cognitive interpretation of large carnivores. *Human Dimensions of Wildlife* **16**, 15–29. doi:10.1080/10871209.2011.535240

Kirchheimer WF (1975). Experimental leprosy in the nine-banded armadillo. *Public Health Reports* **90**, 483–485.

Koval JH, Mertig AG (2004). Attitudes of the Michigan public and wildlife agency personnel toward lethal wildlife management. *Wildlife Society Bulletin* **32**, 232–243. doi:10.2193/0091-7648(2004)32[232:AOTMPA]2.0.CO;2

Lawton J, Shachak M (1994). Organisms as ecosystem engineers. *Oikos* **69**, 373–386.

Leary SL, Underwood W, Anthony R, Cartner S, Grandin T, Greenacre C, Gwaltney-Brant S, McCrackin MA, Meyer R, Miller D, Shearer J, Turner T, Yanong R (2020). 'AVMA guidelines for the euthanasia of animals: 2020 edition'. (American Veterinary Medical Association) Available at: https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf [accessed 5 April 2022]

Liordos V, Kontsiotis VJ, Georgari M, Baltzi K, Baltzi I (2017). Public acceptance of management methods under different human-wildlife conflict scenarios. *Science of the Total Environment* **579**, 685–693. doi:10.1016/j.scitotenv.2016.11.040

Loughry WJ, Truman RW, McDonough CM, Tilak M-K, Garnier S, Delsuc F (2009). Is leprosy spreading among nine-banded armadillos in the southeastern United States? *Journal of wildlife diseases* **45**, 144–152. doi:10.7589/0090-3558-45.1.144

Loughry WJ, McDonough CM (2013). 'The nine-banded armadillo: a natural history'. (University of Oklahoma Press)

Madden F (2004). Creating coexistence between humans and wildlife: global perspectives on local efforts to address human–wildlife conflict. *Human Dimensions of Wildlife* **9**, 247–257. doi:10.1080/10871200490505675

Manfredo MJ, Zinn HC (2011). Population change and its implications for wildlife management in the New West: A case study of Colorado. *Human Dimensions of Wildlife* **1**, 62–74.

McCarty JP (2001). Ecological consequences of recent climate change. *Conservation Biology* **15**, 320–331. doi:10.1046/j.1523-1739.2001.015002320.x

McIvor DE, Conover MR (1994). Perceptions of farmers and non-farmers toward management of problem wildlife. *Wildlife Society Bulletin (1973-2006)* **22**, 212–219.

Mengak MT, Gammons DJ, Conner LM (2017). Evaluation of attractants for live-trapping nine-banded armadillo. *University of Georgia Outreach* **WSFNR-17-37**, 1–4.

Nyhus PJ (2016). Human-wildlife conflict and coexistence. In 'Annual Review of Environment and Resources, Vol 41'. (Eds A Gadgil, TP Gadgil.) pp. 143–171. (Annual Reviews: Palo Alto) doi:10.1146/annurev-environ-110615-085634

Ober HK, DeGroote LW, McDonough CM, Mizell RF, Mankin RW (2011). Identification of an attractant for the nine-banded armadillo, (*Dasypus novemcinctus*). *Wildlife Society Bulletin* **35**, 421–429. doi:10.1002/wsb.79

Onwuegbuzie AJ, Dickinson WB, Leech NL, Zoran AG (2009). A qualitative framework for collecting and analyzing data in focus group research. *International Journal of Qualitative Methods*. doi:10.1177/160940690900800301

R Core Team (2022). R: A language and environment for statistical computing. Available at: htpps://www.R-project.org/

Riley SJ, Decker DJ (2000). Wildlife stakeholder acceptance capacity for cougars in Montana. *Wildlife Society Bulletin (1973-2006)* **28**, 931–939.

Rodgers P, Pienaar E (2017). Amenity or nuisance? Understanding and managing human-panther conflicts in exurban Southwest Florida. *Human Dimensions of Wildlife* **22**, 295–313. doi:10.1080/10871209.2017.1318322

Rodrigues TF, Mantellatto AMB, Superina M, Chiarello AG (2020). Ecosystem services provided by armadillos. *Biological Reviews* **95**, 1–21. doi:10.1111/brv.12551

Schaefer J (2019). Dealing with unwanted wildlife in an urban environment. Available at: https://sfyl.ifas.ufl.edu/natural-resources/nuisance-wildlife/ [accessed 14 February 2024]

Shapiro B, Graham RW, Letts B (2015). A revised evolutionary history of armadillos (*Dasypus*) in North America based on ancient mitochondrial DNA. *Boreas* **44**, 14–23. doi:10.1111/bor.12094

Sharma R, Singh P, Loughry WJ, Lockhart MJ, Inman WB, Malcolm S. Duthie, Maria T. Pena, Luis A. Marcos, David M. Scollard, Stewart T. Cole, Richard W. Truman (2015). Zoonotic leprosy in the southeastern United States. *Emerging Infectious Diseases* **21**, 2127–2134. doi:10.3201/eid2112.150501

Siemer WF, Jonker SA, Decker DJ, Organ JF (2013). Toward an understanding of beaver management as human and beaver densities increase. *Human-Wildlife Interactions* **7**, 114–131.

Sjöberg L (1998). Worry and risk perception. *Risk Analysis: An Official Publication of the Society for Risk Analysis* **18**, 85–93. doi:10.1111/j.1539-6924.1998.tb00918.x

Sullivan GM, Feinn R (2012). Using effect size—or why the p value is not enough. *Journal of Graduate Medical Education* **4**, 279–282. doi:10.4300/JGME-D-12-00156.1

Taulman JF, Robbins LW (2014). Range expansion and distributional limits of the nine-banded armadillo in the United States: an update of Taulman & Robbins (1996). *Journal of Biogeography* **41**, 1626–1630. doi:10.1111/jbi.12319

Truman RW, Singh P, Sharma R, Busso P, Rougemont J, Paniz-Mondolfi A, Kapopoulou A, Brisse S, Scollard DM, Gillis TP, Cole ST (2011). Probable zoonotic leprosy in the southern United States. *New England Journal of Medicine* **364**, 1626–1633. doi:10.1056/NEJMoa1010536

Urbanek RE, Nielsen CK, Davenport MA, Woodson BD (2015). Perceived and desired outcomes of suburban deer management methods. *The Journal of Wildlife Management* **79**, 647–661. doi:10.1002/jwmg.871

U.S. Census Bureau (2022). ACS Demographic and Housing Estimates. Available at: https://data.census.gov/table/ACSDP1Y2022.DP05?q=alabama [accessed 19 January 2024]

Vaske JJ, Donnelly MP, Williams DR, Jonker S (2001). Demographic influences on environmental value orientations and normative beliefs about national forest management. *Society & Natural Resources* **14**, 761–776.

West BC, Parkhurst JA (2002). Interactions between deer damage, deer density, and stakeholder attitudes in Virginia. *Wildlife Society Bulletin (1973-2006)* **30**, 139–147.

Woodroffe R, Thirgood S, Rabinowitz A (2005). 'People and wildlife, conflict or co-existence?' (Cambridge University Press)

Zajac RM, Bruskotter JT, Wilson RS, Prange S (2012). Learning to live with black bears: A psychological model of acceptance. *The Journal of Wildlife Management* **76**, 1331–1340. doi:10.1002/jwmg.398

Zinn, Harry C., and William F. Andelt. “Attitudes of Fort Collins, Colorado, Residents toward Prairie Dogs.” *Wildlife Society Bulletin (1973-2006)* 27, no. 4 (1999): 1098–1106.

Zinn H, Manfredo M, Vaske J (2000). Social psychological bases for stakeholder acceptance capacity. *Human Dimensions of Wildlife* **5**, 20–33. doi:10.1080/10871200009359185

Zinn HC, Manfredo MJ, Decker DJ (2008). Human conditioning to wildlife: steps toward theory and research. *Human Dimensions of Wildlife* **13**, 388–399. doi:10.1080/10871200802427972

Appendices

Appendix I

The final Lee County and Mobile County residential zone maps were created February 18th, 2023

Lee County

1. GCS and PCS of NAD 83
2. Uploaded Data Sets
    a. County GIS boundary data were imported to define Lee County (ESRI 2023)
    b. Land cover type data were imported (Detwitz 2021)
        i. 30 x 30m Pixel dataset
    c. Major and minor road data were imported (U.S. Geological Survey 2023)
        i. All road segments clipped to Lee County border using “Clip” tool
3. Clipped the NLCD raster to Lee County
    a. Used “Clip Raster” Tool to clip it to the Lee County border
4. Used “Raster to Points” tool on clipped NLCD raster
    a. Used the NLCD land cover class as the field
5. Used “Create fishnet of raster” on NLCD Raster
    a. Used values of raster as template extent
6. “Feature to Polygon” tool using fishnet output as an input and labeled features using the NLCD-to-point output
    a. Resulted in the creation of a polygon that was the same shape of each cell

7. Merged road segments from the USGS dataset and clipped to the county extent using "Clip" tool
8. Used "select by location" to select cells on the NLCD that intersected merged road datasets
    a. Inverted spatial relationship and created a new layer from selection
9. Used "select by attributes" to select cells for each zone
    a. 21 and 22 (open space and low development) for suburban
    b. 23 and 24 (medium and high development) for urban
    c. All other zones that weren't 21, 22, 23, and 24 for rural
10. Aggregated and buffered urban cell layer
    a. Aggregated urban cells with a minimum area of 4500m^2 (900m^2-area of one cell * 5), 900m^2 hole size, 1 m aggregation distance, and "Preserve orthogonal shape"
        i. Aggregated the aggregation result with a 300m aggregation distance, 18,000 square meter minimum area, and 9,000 m^2 minimum hole size
        ii. Buffered the result with a 30m distance
        iii. Urban polygons that were isolated or part of roads that were not included in the road layer were manually removed
11. Aggregated and buffered suburban cell layer
    a. Aggregated urban cells with a minimum area of 4500m^2, 900m^2 hole size, 1m aggregation distance, and "Preserve orthogonal shape"
        i. Suburban polygons that include roads not accounted for on the road layer and isolated polygons were manually removed

b. Buffered with a distance of 331.66 m (SQRT of average armadillo home range of 11,000 m^2) with full, geodesic, dissolve all output features into a single feature

Mobile County

1. Same steps as 1-3 for Lee County.
2. Did an INT function to convert each pixel value of NLCD raster into an integer
3. Used the "Subdivide Polygon Tool"
4. Separated into Rural, Suburban, and Urban using "Select by Attributes" tool (step 9 from "Lee County" map creation)
5. Merged road layers from the USGS database and clipped to county extent using "Clip" tool
6. Used "select by location" to select cells on the NLCD that intersected merged road dataset.
7. Aggregated and buffered urban cell layer
    a. Aggregated urban cells with a minimum area of 4500m^2 (900m^2-area of one cell * 5), 900m^2 hole size, 1 m aggregation distance, and "Preserve orthogonal shape"
        i. Deleted outlying cells that were isolated
        ii. Aggregated the aggregation result with a 300m aggregation distance, 18,000 square meter minimum area, and 9,000 m^2 minimum hole size
        iii. Buffered the result with a 331.66 m distance
8. Repeated Step 7 for the suburban layer

Appendix II

Please select one answer for each of the following questions unless otherwise directed.

1. What is your age?

   ____

2. Are you a resident of Alabama?

   ☐ Yes

   ☐ No

3. Does your current residence include a property or lot that is maintained by your household?

   ☐ Yes

   ☐ No

   ☐ Not Sure

4. Are you of Latino, Hispanic, or Spanish origin?

   ☐ Yes

   ☐ No

   ☐ I prefer not to answer

5. Please indicate your race

   ☐ American Indian or Alaskan Native

   ☐ Asian

   ☐ Black or African American

   ☐ White

   ☐ Other/Unknown

   ☐ I prefer not to answer

6. Please indicate your gender.

   ☐ Male

   ☐ Female

   ☐ Other ___________

   ☐ I prefer not to answer

7. What was your approximate household income in 2021?

   ☐ $0 – 20,000

   ☐ $20,001 – 30,000

   ☐ $30,001 – 40,000

☐ $40,001 – 50,000

☐ $50,001 – 60,000

☐ $60,001 – 70,000

☐ $70,001 – 80,000

☐ $80,001 – 90,000

☐ $90,001 – 100,000

☐ $100,001+

☐ I prefer not to answer

8. What is your highest level of education?

☐ Did not graduate high school/no GED

☐ High school graduate/GED

☐ Technical/Vocational School

☐ Some College

☐ Two-year Degree (AA or AS)

☐ Four-year Degree (BA or BS)

☐ Graduate or Professional School

☐ I prefer not to answer

9. What is the zip code of your current residence? (5-digit zip code)

____

10. How would you describe the location of your residence?

☐ Rural

☐ Suburban

☐ Urban

☐ Not Sure

11. What is the estimated size of the lot you currently live on (in acres)? (If you do not know, please write “N/A”)

____

12. How long have you lived at your current residence?

☐ Less than one year

☐ 1-3 years

☐ 4-5 years

☐ 5-10 years

☐ More than 10 years

The nine-banded armadillo (hereafter referred to as "armadillo") is the only species of armadillo in the United States.

13. Have you ever seen an armadillo or evidence of one (burrows, tracks, etc.) where you live in Alabama?

☐ Yes

☐ No

☐ Not Sure

14. In the past 12 months, how frequently have you observed an armadillo or evidence of one (burrows, tracks, etc.) where you live?

☐ Never

☐ Once

☐ 2-3 times

☐ 4-5 times

☐ Every other month

☐ Monthly

☐ Weekly

☐ Daily

15. Which of the following is true about armadillos? (Please select all that apply)

☐ The majority of their diet consists of plants

☐ They are burrowing animals

☐ They are nocturnal (active at night)

☐ They can have leprosy

16. I consider armadillos to be a nuisance (causing me inconvenience or annoyance).

☐ Strongly Agree

☐ Agree

☐ Neither Agree nor Disagree

☐ Disagree

☐ Strongly Disagree

17. The number of armadillos where I live is
    ☐ More than I would like
    ☐ About right
    ☐ Less than I would like

18. In the future, I would prefer for the number of armadillos in my area to
    ☐ Increase
    ☐ Decrease
    ☐ Stay the same

Please answer Questions 19-23 ONLY if you selected "they can have leprosy" in Question 15.

19. Which of the following is true about leprosy? (Select all that apply)
    ☐ it is curable
    ☐ it is deadly
    ☐ it is highly infectious
    ☐ none of the above

20. Out of the total armadillos in your area, please estimate how many have leprosy.
    ☐ 100%
    ☐ 75%
    ☐ 50%
    ☐ 25%
    ☐ 0%

21. If you were within three feet of an armadillo, how concerned would you be about getting leprosy?
    ☐ Extremely Concerned
    ☐ Moderately Concerned
    ☐ Slightly Concerned
    ☐ Not at all Concerned

22. If you were within three feet of an armadillo **with leprosy**, how likely would it be to contract the disease yourself?
    ☐ Not likely
    ☐ Somewhat likely
    ☐ Very likely

23. If you were to contract leprosy, do you think the consequences would be:

☐ Very serious

☐ Serious

☐ Somewhat serious

☐ Not at all serious

24. On your property, do you feel it is likely for an armadillo to:

	Very Likely	**Somewhat Likely**	**Not Likely**	**Not Applicable**
Damage Infrastructure (fences, decks, etc.)	☐	☐	☐	☐
Damage landscaping, yard, or garden	☐	☐	☐	☐
Damage crops	☐	☐	☐	☐
Damage livestock	☐	☐	☐	☐

25. Do you worry about armadillos damaging your property?

☐ Always

☐ Often

☐ Sometimes

☐ Rarely

☐ Never

26. If an armadillo visited your property, do you feel the consequences would be:

☐ Very severe

☐ Severe

☐ Somewhat severe

☐ Not at all severe

27. Please rate your level of agreement with each of the following:

	Strongly Agree	**Somewhat Agree**	**Neutral**	**Somewhat Disagree**	**Strongly Disagree**
I enjoy seeing armadillos where I live	☐	☐	☐	☐	☐

Armadillos provide an educational opportunity for me or my family	☐	☐	☐	☐	☐
Armadillos improve the ecosystem health where I live	☐	☐	☐	☐	☐

For Questions 28-31, "control" refers to the deliberate prevention or removal of armadillos.

28. I know how to control armadillos on my property. (Please rate your level of agreement)

☐ Strongly agree

☐ Agree

☐ Neutral

☐ Disagree

☐ Strongly disagree

29. Would you consider using the below control methods for armadillos on your property?

	Definitely	**Probably**	**Possibly**	**Probably Not**	**Definitely Not**
Shoot with a gun	☐	☐	☐	☐	☐
Trap and relocate	☐	☐	☐	☐	☐
Trap and euthanize	☐	☐	☐	☐	☐
Use a physical barrier (i.e. fence)	☐	☐	☐	☐	☐
Use a repellent	☐	☐	☐	☐	☐
Use poison	☐	☐	☐	☐	☐
Hire a wildlife removal company	☐	☐	☐	☐	☐
Treat yard for grubs and other insects	☐	☐	☐	☐	☐

Other (Please Explain)	☐	☐	☐	☐	☐

30. Please select the methods of disposal that you would consider for a dead armadillo on your property (Select all that apply).

☐ Prepare and consume meat

☐ Bury

☐ Dispose of in the garbage

☐ Leave above ground

☐ Sell

☐ Keep as trophy

☐ None of these

☐ Other (Please Explain) ____

31. Within the past 12 months, how frequently have you needed to control armadillos on your property?

☐ Once a year

☐ 2-3 times a year

☐ 4-5 times a year

☐ Every other month

☐ Monthly

☐ Weekly

☐ Daily

☐ I have not

32. How much would you estimate armadillos have cost you in total in the past 12 months (USD)?

☐ $0

☐ $1-50

☐ $51-100

☐ $101-200

☐ $201-300

☐ $301-400

☐ $401-500

☐ > $500

☐ I am not sure

33. How would you rate the costs estimated in the previous question?

☐ Completely Acceptable

☐ Somewhat Acceptable

☐ Neutral

☐ Somewhat Unacceptable

☐ Completely Unacceptable

☐ Not Applicable

34. (OPTIONAL) If you are interested in participating in a focus group about armadillos on your property, please write your first name and an email we can reach you at with additional information. We will only use your contact information to contact you about participating in this project. Focus groups will be conducted in the following months. They will occur remotely over an online meeting platform. Groups will consist of roughly 6-8 Alabama residents and questions will be asked about nine-banded armadillos where you live.

First Name: ___________________________________

Email: ____

(Optional) Phone Number: (____) - _____ - _______

Appendix III

High tolerance focus group script

[Welcome] Good evening and welcome everyone. Thanks for taking the time to join our discussion about armadillos in Alabama. My name is Olivia Sciandra, and I am a graduate student at Auburn University.

[Topic Overview] We are conducting this group discussion because we are interested in understanding how homeowners interact with armadillos on their property. We would like to better understand the interactions people have with these animals and improve our understanding of their current management. This discussion will contribute to my research thesis and the results of this research may also be published in a scientific journal.

[Ground Rules] There are no wrong answers here and we expect that each of you will have a different point of view. Please share your point of view even if it differs from what others have said.

We're audio recording the session because we don't want to miss any of your comments. No names will be included in any reports and your comments are confidential. Your video will not be saved, and you can participate with or without your video camera on.

You also don't have to respond to each question I ask. If you also want to follow up on something that someone has said, you want to agree, or disagree, or give an example, please feel free to do that. This is a conversation, and we are interested in hearing from each of you.

We will also include your first names here on our Zoom meeting to help us remember each other's names. Before we begin, I am going to ask that each of you say your first name so I can change your name on the screen [change each display name].

[Opening Question] Alright, let's get started. Let's find out more about each other. Tell us how long you have lived in Alabama. (5 minutes)

[Transition Question] Great, so from here on out, we won't be going around one-by-one so please feel free to jump into the discussion at any point.

1. What time of year do you tend to see armadillos where you live? (5 minutes)

Key Questions

2. What is a typical encounter with an armadillo on your property? (5 minutes)
3. What do you like about armadillos? (5 minutes)
4. If an armadillo was digging small holes on your property, what would you do? (10-15 minutes)
    a. (Probe Question) Would your answer change if they were burrowing on your property?
    b. (Probe Question) Would your answer change if it was a female armadillo with her litter?
5. Have you ever removed an armadillo from your property? If so, how? (10 minutes)
    a. (Probe Question) Would you say it was successful?

b. (Probe Question) Do you have anything on your property that prevents armadillos?

6. Do you ever worry about getting diseases from an armadillo? (10 minutes)

Ending Questions

Provide a brief summary about the topics (done by assistant moderator). (2 minutes)

7. Is there anything else you would like to add about your experiences with armadillos? (5 minutes)

Ending Statements

Great, thank you very much for such a great discussion this evening. We appreciate the time you took out of your evening to talk with us. If you have any questions or concerns after this focus group, please feel free to email us. We will be in touch with all of you about the winner of the gift cards after the conclusion of our focus groups in March. Thank you again for your time and have a great rest of your evening.

Low tolerance focus group script

[Welcome] Good evening and welcome everyone. Thanks for taking the time to join our discussion about armadillos in Alabama. My name is Olivia Sciandra, and I am a graduate student at Auburn University.

[Topic Overview] We are conducting this group discussion because we are interested in understanding how homeowners interact with armadillos on their property. We would like to better understand the interactions people have with these animals and improve our understanding of their current management. This discussion will contribute to my research thesis and the results of this research may also be published in a scientific journal.

[Ground Rules] There are no wrong answers here and we expect that each of you will have a different point of view. Please share your point of view even if it differs from what others have said.

We're audio recording the session because we don't want to miss any of your comments. No names will be included in any reports and your comments are confidential. Your video will not be recorded, and you can participate with or without your video camera on. You also don't have to respond to each question I ask. If you also want to follow up on something that someone has said, you want to agree, or disagree, or give an example, please feel free to do that. This is a conversation, and we are interested in hearing from each of you.

We will also include your first names here on our Zoom meeting to help us remember each other's names. Before we begin, I am going to ask that each of you say your first name so I can change your name on the screen [change each display name].

[Opening Question] Alright, let's get started. To find out more about each other, tell us how long you have lived in Alabama.

[Transition Question] Great, so from here on out, we won't be going around one-by-one so please feel free to jump into the discussion at any point.

1. What time of year do you tend to see armadillos where you live? (5 minutes)

Key Questions

1. What is a typical encounter with an armadillo on your property? (5 minutes)
2. How have you removed armadillos from your property in the past? (10 minutes)
    a. (Probe Question) Was it successful?
    b. (Probe Question) Do you have anything on your property that prevents armadillos?
3. What do you feel is the biggest risk that armadillos pose? (5 minutes)
4. Do you ever worry about getting diseases from an armadillo? (5 minutes)
5. The survey you completed discussed a range of management techniques. I would like to go through some of them and discuss why you would or wouldn't use them. (20 minutes)
    a. Shoot with a gun
    b. Trap and relocate
    c. Trap and euthanize
    d. Use a fence
    e. Use a repellent
    f. Use poison
    g. Hire a wildlife removal company
    h. Treat yard for grubs
6. Where do you get information about removing or preventing armadillos on your property? (5 minutes)
    a. Probe: If "internet" is said, ask for websites that are frequented.

Ending Questions

Provide a brief summary about the topics.

7. Is there anything else you would like to add about your experiences with armadillos? (5 minutes)

Great, thank you very much for such a great discussion this evening. We appreciate the time you took out of your evening to talk with us. If you have any questions or concerns after this focus group, please feel free to email us. We will be in touch with all of you about the winner of the gift cards after the conclusion of our focus groups in March. I would also like to share an infographic that provides some information about armadillos and provides resources about their management [Put infographic in chat]. I will also email this resource to everyone. Thank you again for your time and have a great rest of your evening.

Appendix IV

A summary of survey respondent demographics including age range, gender, race, income, and education by proportion of the sample population represented. *n* = 341 unless otherwise indicated.

	N	Proportion	Margin of Error
Age Range	**341**		
19	3	0.01	0.0099
20-24	7	0.02	0.015
25-34	20	0.06	0.025
35-44	38	0.11	0.033
45-54	49	0.14	0.037
55-59	26	0.08	0.028
60-64	51	0.15	0.038
65-74	101	0.30	0.049
75-84	46	0.13	0.036
85+	0	–	–
Gender	**332**		
Female	216	0.633	0.05
Male	116	0.340	0.05
Race	**323**		
White	304	0.94	0.03
American Indian or Alaskan Native	6	0.019	0.01
Black or African American	10	0.031	0.02
Other/Unknown	1	0.0031	0.01
Asian	2	0.0062	0.01
Latino, Hispanic or Spanish Origin	**323**		
Yes	3	0.01	0.01
No	320	0.99	0.01
Income Category	**262**		
$0-20,000	9	0.026	0.017
$20,001-30,000	15	0.044	0.022

$30,001-40,000	13	0.038	0.020
$40,001-50,000	18	0.053	0.024
$50,001-60,000	17	0.050	0.023
$60,001-70,000	26	0.076	0.028
$70,001-80,000	21	0.062	0.026
$80,001-90,000	26	0.076	0.028
$90,001-100,000	21	0.062	0.026
$100,001+	96	0.282	0.048
Education Level	**329**		
Did not graduate high school/no GED	0	0.000	0.000
High school graduate/GED	17	0.052	0.024
Technical/Vocational School	8	0.024	0.017
Some College	41	0.125	0.036
Two-year Degree (AA or AS)	27	0.082	0.030
Four-year Degree (BA or BS)	104	0.316	0.050
Graduate or Professional School	132	0.401	0.053

Appendix V

Summary of the variables evaluated through the questionnaire and the reliability of the assessment scales used. The results for the calculated scores are also reported on a scale from 0 to 1 which was adjusted for each perception variable. *n* = 341 unless otherwise indicated.

Constructs of Interest	Questionnaire Items	Cronbach's Alpha (α)	x̄ (*n*)	Standard Error
Knowledge		NA	0.60 (341)	0.017
	Q15 Which of the following are true about armadillos? (Please select all that apply) • The majority of their diet consists of plants: False • They are burrowing animals: True • They can have leprosy: True • They are nocturnal (active at night)- REMOVED	–	–	–
Tolerance		0.82	0.23 (341)	0.014
	Q16 I consider armadillos to be a nuisance (causing me inconvenience or annoyance): *Strongly Agree – Strongly Disagree*	–	0.30	0.017
	Q17 The number of armadillos where I live is: *Less than I would like, About right, more than I would like*	–	0.20	0.015
	Q18 In the future, I would prefer for the number of armadillos in my area to: *Increase, Stay the Same, Decrease*	–	0.18	0.015
Perceived Disease Risk		0.74	0.16 (341)	0.012
Severity		–	0.51 (182)	0.017
	Q19 Which of the following is true about leprosy? ***It is curable*** *(not selected),* ***It is deadly*** *(selected)*	–	–	–
	Q23 If you were to contract leprosy, do you think the consequences would be:	–	–	–

	Very serious, serious, somewhat serious, not at all serious			
Susceptibility		–	0.27 (182)	0.016
	Q19 Which of the following is true about leprosy? ***It is highly infectious*** (*selected)*	_	_	_
	Q22 If you were within three feet of an armadillo **with leprosy**, how likely would it be to contract the disease yourself? *Very likely, Somewhat likely, Not likely*	–	–	–
	Q20 Out of the total armadillos in your area, please estimate how many have leprosy. *0%, 25%, 50%, 75%, 100%*	**Not Included in Total Score** –	 –	 –
Dread		–	0.12 (182)	0.013
	Q21 If you were within three feet of an armadillo, how concerned would you be about getting leprosy? *Extremely concerned, Moderately concerned, Slightly concerned, Not at all concerned*	_	_	_
Perceived Damage Risk		0.80	0.51 (341)	0.014
Susceptibility		–	0.80	0.017
	Q24 On your property do you feel it is likely for an armadillo to: • Damage infrastructure • Damage landscaping, yard, or garden • Damage crops • Damage livestock *Very likely, Somewhat likely, Not likely, Not applicable*	_	_	_
Dread		–	0.47	0.017
	Q25 Do you worry about armadillos damaging your property? *Always, Often, Sometimes, Rarely, Never*	_	_	_
Severity		–	0.26	0.016
	Q26 If an armadillo visited your property, do you feel the consequences would be:	_	_	_

	Very severe, Severe, Somewhat severe, Not at all severe			
Perceived Benefit Risk		0.89	0.37 (341)	0.016
	Q27 Please rate your level of agreement with each of the following: *Strongly Agree-Strongly Disagree*	–	–	–
	• I enjoy seeing armadillos where I live [Enjoyment]	–	0.32	0.018
	• Armadillos provide an educational opportunity for me or my family [Education]	–	0.41	0.018
	• Armadillos improve the ecosystem health where I live [Ecosystem Services]	–	0.39	0.017
Perceived Control		–	0.47 (341)	0.016
	Q28 I know how to control armadillos on my property. (Please rate your level of agreement) *Strongly Agree – Strongly Disagree*	–	–	–
Perceived Cost		–	0.60 (235)	0.021
	Q33 How would you rate the costs estimated in the previous question [Q32: How much would you estimate armadillos have cost you in total in the past 12 months (USD)?] *Completely Acceptable – Completely Unacceptable*	–	–	–