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Impacts of Two Temporal Rotations from a Nontoxic Bait to a Cholecalciferol Rodenticide on Wild House Mouse *Mus musculus* L. Consumption, Bait Station Interaction, and Movements

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ABSTRACT

Commensal rodents, including the house mouse (*Mus musculus* L.), pose a substantial risk of damage and disease towards human kind, threatening the infrastructure and food supply on which it depends. The implications of rodent infestations have become more significant as issues of public health and food safety become elevated priorities. Effective control of house mice relies heavily on rodenticides of which, the efficacy is known to be impacted by a variety of factors including palatability, social interaction and the development of behavioral aversions. In this study, analysis of consumption rates, recorded video, and capture-mark-recapture (CMR) data were utilized to investigate changes in house mouse feeding behavior, population demographics, interactions with bait stations, changes in movements, and spatial distribution in response to two sequential temporal rotations from nontoxic bait to a cholecalciferol rodenticide.

Compared to nontoxic bait, consumption of cholecalciferol bait fell to 45% within two days of introduction and continued to decline to approximately 2% within seven days. When nontoxic bait was returned, consumption levels rebounded to approximately 25% of original nontoxic levels, while abundance estimates indicated a 62% population reduction. When cholecalciferol bait was returned, consumption trends were similar to the original baiting, resulting in a reduction in abundance to 3% of the original population.

Analysis of mouse activity in and around bait station locations suggest that mice visited areas where bait stations were to be placed less frequently ($P = 0.0620$) before placement than after. The percentage of visits initially entering empty stations was

significantly less than all other phases. Mice visited stations significantly less (frequency and duration) and consumed significantly less bait during phases offering cholecalciferol compared to phases offering nontoxic bait. The majority of the time mice spent in observation areas (35 x 95 cm area around the station) was inside a bait station and mice were seldom observed to cohabitate a bait station. When nontoxic bait was returned, mouse activity and consumption rebounded then subsequently declined when nontoxic bait was replaced with cholecalciferol the second time, showing similar trends to the initial placement.

Analysis of CMR data revealed that no significant changes were observed in the distance of mouse movements following the initial rodenticide treatment which reduced population abundance by 62%. The location of mouse movements was not significantly impacted by the rodenticide treatment. Juveniles were observed to have significantly greater movements than adults. Typical movements were expansive enough to include multiple bait stations spaced at 2.5 m to 3.5 m intervals. Immigration and emigration were not identified as a significant factor leading to incomplete control after the initial treatment. Demographic analysis of captured individuals and recovered mortality indicated that the cholecalciferol treatment caused greater female mortality, as the male to female ratio increased over twofold from the studies inception to completion.

DEDICATION

I would like to dedicate this body of work and the culmination of my academic career to my friends and family. To those that came before me, my great granddaddy, Maxcy P. Nolan Sr., who walked away from picking that last row of cotton and hitchhiked to Clemson in the 1930's from Pee Dee, SC. Equipped with only the shirt on his back and a few dollars in his pocket, he forged a path across the Palmetto State that would ultimately lead to a Clemson diploma and a better way of life for his descendants. To my grandparents, who have encouraged and supported me as I have journeyed through my educational career. To my parents, who have always pushed me to be my best but encouraged and supported me when I was far from it. My dad, Dr. Maxcy P. Nolan III, who was willing to take me along from an early age to work by his side and learn more about the world around me and pest management principles than any classroom could ever offer in spite of my incessant questioning. It was through his guidance and passion for helping others solve their pest problems that instilled in me a desire to pursue this field of study. To my mother, who has always offered her unconditional love, unwavering support, and a lifelong commitment to making sure we are all cared for. My brother, Dr. Maxcy (Brett) P. Nolan IV, who has been along my side from the beginning providing all the life lessons big brothers are good for and an example of the effort required to obtain a Ph.D. To my extended family and friends who give life meaning and enjoyment making this journey bearable.

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CHAPTER 1 - Review of Literature

House mouse origins and phylogeny

The house mouse (*Mus musculus* Linnaeus) is a small mammalian species in the order Rodentia. Numerous subspecies have been identified over the last 50 years with consistent debate over the correct phylogeny resulting in various changes in classification. However, most all sources are in agreement that *M. musculus* originated from areas in the north of the Indian subcontinent around 900,000 years ago.[1] The earliest recorded association of *M. musculus* with early urban development is dated back to around 6000 BC at the Çatal Hüyük in Turkey.[2] Over time, mice have evolved to be commensal inhabitants with man in nearly a ubiquitous manner across the world. A detailed overview of the phylogenetic lineage of the house mouse is presented by R.J. Berry outlining the deviation of *M. musculus* spp. from four wild sub-species (*M. m. wageri* Eversmann, *M. m. spicilegus* Petenyi, *M. m. manchu* Thomas, and *M. m. spretus* Lataste).[3-5] Of these four sub-species, only *M. m. spretus* has remained solely in a wild form while the other three have developed commensal associations with humans. These commensal associations resulted in the migration of mice around the world following the alteration of habitat by human dwellings and cultivation of the land. The *M. m. wageri* lineage is responsible for the majority of commensal forms radiating from central Asia to the four corners of the earth. This lineage has giving rise to *M. m. brevirostris* Waterhouse the primary commensal form inhabiting southern Europe, Latin America, and southern portions of the United States and *M. m. domesticus* Rutty, the primary

inhabitant of the northern half of North America. Another important commensal sub-species, *M. m. musculus,* is derived from the *spicilegus* lineage and inhabits central Europe, with its range extending north into parts of Sweden and Denmark. While crosses between sub-species have been proven to be fully fertile in the laboratory, they do not interbreed at random in wild settings, maintaining some separation in the genetics of wild populations.

General biology

Habitat.

Although *M. musculus* is generally commensal in nature compared to other small mammals, it is a common misconception that mice are restricted solely to habitation coinciding with man. *M. musculus* persist in non-commensal populations on every continent except Antarctica. In line with their high degree of adaptability and genetic variation they have successfully colonized a vast array of habitat types and environmental conditions spanning "from coral atolls in the Pacific to near-Antarctic conditions on South Georgia, from coastal tussock grass in temperate regions to 4000 metres above sea-level in the Andes, from central heating ducts to refrigerated stores."[5] While their ability to persist in the wild under a variety of environmental conditions demonstrates their adaptive nature, it is in the commensal environments where *M. musculus* thrive in that they are most likely encountered and where they cause serious conflicts with man. Even the derivation of their very name is said to be based on this clashing relationship. The Latin word *Mus* was likely derived from the ancient Sanskrit verb (*mush*), meaning "to

steal" *musculus* literally translates to "little thief," often a very applicable name.[6] Mice will colonize basically any environment man inhabits where food is available, including homes, offices, hospitals, hotels, even our modes of transportation from cars to ships to planes. They are exceedingly successful and problematic in areas with excessive food and harborage such as food processing facilities, confined animal facilities, food stores, and restaurants.

Across the literature, mouse populations are often categorized based on the degree of human interaction present in the habitat that they persist. This distinction is important to convey as mouse behavior, demographics, and movements can fluctuate in response to the degree of these interactions.[5] Attempts to differentiate these varying conditions has resulted in the use of a variety of terminologies. Mouse populations under human containment resulting from captive breeding are generally referred to as laboratory mice.[7-10] Some studies keep mice (usually sourced from wild populations) contained in fabricated enclosures to allow for mice to interact with the environment and each other while ensuring the population stays isolated.[11, 12] It is when describing populations outside of human captivity where the nomenclature begins to get less consistent. Feral,[13-20] wild,[21-26] and free-living[27, 28] are the most common terms used to denote populations that are not under direct human containment. Within these conditions, mouse populations are sometimes further described as being commensal or non-commensal, and a combination of these terms with feral, wild, and free-living is sometimes encountered. The use of feral to describe these populations should refer to a population that was sourced from a population in captivity that now persists in the wild; however, this is

rarely the case. Wild seems to be the most appropriate description of populations outside of direct human containment. Commensal or non-commensal further describes the degree of human interaction the population experiences. Commensal rodent populations are in close association with humans and non-commensal populations exist in habitats that are more removed from human interaction.

Size and appearance.

In relation to the genetic diversity of *M. musculus*, physical size, appearance, fecundity, and behavior also vary slightly in relation to sub-species and environmental gradients displaying the high degree of adaptability and versatility they possess. In general, mature mice weight between 14 and 30 g with wild females being slightly heavier than males. Body length (head + body) typically ranges from 70 to 100 mm. Individuals towards the upper range in size tend to be in cooler environments and/or more isolated from man, while smaller mice tend to be in warmer climates and/or more commensal settings. This variation in size related to environmental conditions is thought to be an adaptation to help regulate internal temperature with larger mice having a lower body surface to volume ratio.[29] The ears of mice are distinct from the skull, rounded, nearly hairless, and moderately large. The skull is relatively slender (~6 mm) and is the limiting factor for the size of an opening mice can navigate. Once their skull is able to penetrate through an opening, their flexible and agile body can follow. The eyes are black and protrude from the skull appearing nearly hemispherical in shape. The tail is semi-naked with obvious scale rings along its length and nearly as long as the body (70 to

90 mm). The length of the tail and its growth rate are also directly related to environmental conditions.[30] The tail is a heat regulating organ and its length is generally expressed along a gradient with increased length as the temperature of the developmental environment elevates.[31]

Mice are born altricial: pink, blind, hairless (except for short vibrissae), and weigh around 0.8 g.[6] Eyes open at around 14 days and they grow adult pelage by 18 days ranging in pigmentation depending on genetic factors. The majority of mice in commensal environments possess coats of varying shades of grey to brown with a white to cream colored belly. However, certain populations have drastically different pelage coloration; including colors ranging from the light sandy hue expressed by mice of Bull Island in Dublin Bay,[32] to nearly black individuals, and of course the familiar albino strains that are commonly used in laboratory settings.

Reproduction.

Female mice become sexually mature at around six weeks of age, although maturity can be delayed under cold environmental conditions or in overcrowded situations.[33, 34] Males reach sexual maturity slightly after females and age of maturation is not effected to the same degree as females by environmental conditions. Breeding intensity in commensal populations and stable environments is fairly constant year-round but can be suppressed if overcrowding occurs.[35] Non-commensal populations display a more defined breeding season dictated by seasonal environmental fluctuations with the majority of breeding occurring from late Spring to early Autumn. The estrus cycle

fluctuates from four to six days with estrous itself (i.e. the period of willingness to mate) occurring for less than one day during the cycle. Gestation lasts from 18-21 days, although implantation may be postponed one to two weeks for nursing females. Parturition generally takes place at night and is followed by a postpartum estrus that occurs within 12-18 hours after birth, leading to mice that are often pregnant while nursing young.[3] The sex ratio of litters is generally 1:1 male to female. Litter size is primarily determined by the size of the female in commensal populations and generally ranges from 5 to 8 pups, although litter sizes from two to 13 have been observed.[6] Litter sizes in wild mice tend to fluctuate seasonally with the largest clutches coinciding with elevated breeding intensity during mid to late Summer.[36] Long-lived female mice can produce anywhere from 6 to 10 litters over their lifetime. Due to the combination of rapid sexual maturity and a short gestation period, mouse abundance has the potential to explode over a relatively short amount of time, making them extremely problematic in pest situations. Berry[5] calculated that a single pair of mice could theoretically expand to a population of 2,688 mice in only 6 months assuming no mortality and a litter size of six young.

Life history.

Newborn mice are altricial and survival is extremely dependent on their mother's ability to provide an adequate nesting location, appropriate nesting material, protection from predation by natural predators and other mice, and milk for nourishment. Juvenile mortality can often be substantial if these conditions are not provided, such as in times of

harsh weather or overcrowded conditions. Southwick[37] found that "the most important factor related to survival of the young was the condition of the nest at and shortly after parturition. This nest condition was due largely to the amount of activity in the nest area by other mice". While in the nest, nursing mice are exposed to various flavors and odors brought back to the nest from the mother's trips to forage for food. Mice use these initial exposures to these flavors and scents to develop an understanding of what types of forage are appropriate to consume.[26, 38] Mice have a highly-developed sense of touch, taste, and smell but very poor eyesight, making these interactions key to survival. Young mice begin their first explorations outside of the natal nest around four to five weeks postpartum. These short excursions are generally accompanied by the mother and/or siblings and traverse along established travel corridors to nearby food sources. Within two weeks of these initial excursions, mice fledge from the nest and become sexually mature. The lifespan of mice is highly variable and depends on a variety of factors including the time of parturition, population density, environment, and genetics. Laboratory mice may live from two to three and a half years; however, the majority of mice in naturally occurring populations do not typically survive past a year. Studies in Stockholm have failed to record any wild mice that have survived more than two winters.[39] DeLong reported that adult mice present before population expansion had an increased survival rate compared to mice that were born during times of population expansion from studying a wild populations in Richmond, California. Rates of survival in breeding populations were also negatively affected by increased population densities with juveniles being impacted to a greater degree than adults.[13]

Home range, movements, and densities.

The degree to which mice move and the timing of these movements is dependent on the environment in which they persist. Studies have estimated movements through a variety of methods including trapping,[13, 14, 16, 17, 21, 40-44] radio telemetry,[12, 15, 21] track boards,[45, 46] census baiting, and fluorescent powders.[12] In commensal populations, if the environment provides an adequate supply of all the necessary resources, mice tend to not move great distances and can occupy home ranges in the order of 2-10 m^2.[47-50] In non-commensal populations, however, home ranges can extend to much larger sizes depending on the distribution of food, cover, competing species, population density, and other environmental factors. Reports in the literature for home range sizes in non-commensal populations rage from 35 to 80,000 m^2 across a variety of these conditions.[15-17, 19, 21, 50-52] In general, males have been shown to have larger home ranges than females and site fidelity is maintained throughout the breeding season. Upon the completion of breeding season, home ranges have been reported to increase from 3 times[21] to 10-fold[15] that of breeding levels and site fidelity diminished. Home range was also not significantly impacted by population density,[13, 15, 21] although mice have been shown to traverse greater distances when population levels are near extinction.[16] The ranges of males and females overlap extensively but intrasexual home ranges tend to be more exclusive but not always separate.[51] Chambers[21] observed that the home ranges of males in a wild population were more exclusive during the breeding season but transitioned to a more gregarious nature once breeding ceased. Krebs et al.[15] came to a different

conclusion using radio telemetry, reporting that home ranges of 90-100% of breeding males overlapped.

Movements of mice can be broken down into two main categories: local movements and dispersal movements.[13] The nature of these two categories of movements can be affected by environmental factors, seasonal variation, food availability, distribution of cover, presence of other species, and a variety of biological factors (e.g. breeding vs non-breeding) but tend to adhere to a general framework.

Local movements consist of trips made by mice to carry out necessary daily activities such as feeding, collecting nesting material, guarding territories, and interactions with other deme members. Estimates of these movements in commensal habitats include, but are not limited to, reports of 1 m[41] (corn crib), 4 m[40] (building), 8.5 m and 7.8 m[50] (males and females respectively; barn), and 5.7 m[41] (barn). Local movements of non-commensal populations include reports of 30 m[41] (field), 10.7 (breeding) to 24.4 m[13] (non-breeding; field), 10 to 30 m[14] (fence lines), 8-35 m[43] (reed-beds), 88.1 m[50] (agricultural fields), and 9.5 m[16] (small island; grassland). Local movements by mice are generally correlated to the time of day with most movement occurring during the night with activity concentrated in short spurts (1-4 h) related to gastrointestinal activity.[48] Mice in natural habitats seek shelter during the day and are less active, although in commensal situations, activity patters of mice can shift and become based more on the need to feed and carry out other necessary activities. Nights with increased illumination such as times when the moon is full or unobstructed have been shown to decrease trapping success presumably from a suppression of movement.[3]

Dispersal movements can be considerably longer than local movements and are generally the response to environmental disturbance (e.g. harvesting (wild) and cleaning[44] (commensal) or social pressures (e.g. subordinate and juvenile dispersal).[53] This means that the majority of dispersing individuals in undisturbed environments are immature or subordinate males and females (with relative equal distribution among the sexes).[17] In general, non-commensal populations of mice have greater rates of dispersal following seasonal variability in habitat.[53] Dispersal movements of the following magnitudes have been reported across a variety of habitats: 9 m to 116 m[41] (livestock facilities and fields), 34 m to 43 m[40] (building), 48.8 m[17] (field), 45 to 300 m[43] (reed-beds), 10 to 190 m[44] (farm), 189 m and 210.3 m[50] (males and females respectively; agricultural fields), and 300-1,000 m[21] (wheat lands). When these dispersal movements occur in and around buildings, it is not common for them to incorporate successful translocation from one building to another, with estimates of this occurring 2-7%[53, 54] of the time.[47] Dispersal movements of considerable distance have also been observed in times when weather patterns changed or in high density populations (particularly by the juveniles of both sexes and by sub-adult males).[13] Maximum movements reported in the literature range from 2^3 to 2.4^6 km but such movements are not typical.

The density of mouse populations is directly correlated to the presence and abundance of food resources, adequate cover, environmental conditions, competing species, and available nesting sites. Some of the highest population densities recorded have been in poultry barns with estimates of 2.1 mice/m^2 [55] and 7 mice/m^2.[47] More typical reports of commensal densities have included reports of 0.14-0.17 mice/m^2

(barn),[50] 1.7 mice/m^2 (seed house),[56] 0.77 mice/m^2 (vivarium),[57] and 0.47 mice/m^2 (vivarium).[57] In more non-commensal situations, extreme abundances have been observed in so called "mouse plagues" that occur periodically in accordance to elevated survival through generally unfavorable seasons. One such outbreak in Lascelles, Australia resulted in the collection of 544 tons of mice (and estimated 32 million individuals) with density estimates of approximate 875 mice per hectare.[5] Densities reported in more common non-commensal situations show that population densities follow a typical pattern correlated to breeding activity with low density in the spring, increasing numbers over the summer (with a peak generally reached in late summer or early fall), and a subsequent decline through winter.[3, 13, 50] Density estimates for non-commensal populations include reports of 11.1 (spring) to 53 mice/ha[50] (fall, agricultural fields), ~25-270 (late winter) to ~175-715 mice/ha[13] (summer, range across multiple fields), ~500-0 mice/ha[16] (island with population declining to extinction), and ~50 (early spring) to 623 mice/ha (late summer, Australian reed-beds).

Mice are known to exhibit thigmotaxic behavior as they travel about, meaning they have a strong tendency to keep their body in constant contact with walls and other objects present in their environment. Due to this phenomenon, room corners and areas where environmental objects intersect are common areas where mice can be observed resting, grooming, and are often chosen as nesting sites. Knowledge of this behavior can be useful when performing inspections for mice and when setting bait stations and traps. Mice have also developed the ability to move about their environment using a kinesthetic sense, a type of muscle memory, to aid them when startled while moving along familiar

travel routes helping overcome their poor eyesight.[6] When startled, mice are agile and quick (3.7 m/s) for their small size allowing them to escape into nearby cover to evade predation and detection.

Social structure.

The social structure of house mice varies depending on the distribution of food,[58] habitat availability,[59] and population density.[60] In most commensal situations, mouse populations form social groups called demes comprised of anywhere from 5 to 80 individuals with the average being around 10 individuals.[18, 47, 54, 61] These demes generally consist of a dominant male, several breeding females (including daughters of the breeding male), and other subordinate males that are presumably prevented from breeding by the dominate male.[11, 62] These demes are primarily constructed around a vigorously defended territory by the dominant male;[63] however, females and subordinates also actively participate in the defense of established territories. Individuals within this social group show little aggression towards each other.[11] Deme territory can be maintained over successive generations if ample food and habitat are provided and the dominant male is replaced by a suitable offspring. Selander[47] genetically confirmed the establishment of these demes in chicken farms in Texas by showing both interbarn and intrabarn heterogeneity in allele frequency across samples taken from various barns on the same farm (interbarn) and various locations within individual barns (intrabarn). Lidicker[11] observed the fate of weanlings in a semi-natural environment and made the following observations pertaining to the ultimate fate of these individuals. After

weaning, a substantial proportion of the population explore areas adjacent to their natal home range in search of vacant habitat they can establish as their territory or a vacant niche in an already established deme. Of the sexes, females were more likely to be accepted into an adjacent deme while males are more likely to continue exploratory attempts throughout their lifetime.[62] If they are unsuccessful in establishing a residence over a relatively short period of time, they will almost always return to their natal territory where they will remain as member of that deme. Some reports have described the return of males to the original deme as more problematic, making their ultimate fate less certain.[41] These exploratory trips are hazardous and can often lead to increased mortality when population densities are elevated and territorial behavior is on full display.

Social behavior.

As with the variation exhibited in the physical attributes, life history, and social structure of *M. musculus*, the behavior is also variable dependent on a variety of ecological, environmental, and genetic factors. However, some generalizations and trends in mouse behavior are applicable across the majority of these conditions.

Crowcroft and Rowe[58, 64] spent a considerable amount of time examining the behavior of wild caught mice placed in an artificial enclosure with minimal disturbance by observing their behavior through holes in the floor above the experimental population. When introduced into a new environment, mice were exploratory but cautious, traveling along walls while they examined each new object they encountered by smell and touch.

If the mouse was disturbed, the outcome was always an instant retreat to the nearest nest box, validating the importance of these early interactions with objects in the environment. When two unfamiliar mice encountered each other after being released into an unfamiliar environment at the same time, the immediate response was always a mutual retreat by both individuals regardless of sex. Over subsequent encounters, dominance began to develop with one mouse holding its ground and ultimately making aggressive runs at the subordinate. These early interactions are the precursors to the social relationship that is likely to develop between the two individuals. When a mouse was released into an environment where another mouse of either sex had already resided for over 24 hours, the resident mouse would immediately rush at the newcomer. Over the course of a few hours, mice of the opposite sex and non-pregnant females would develop a cordial relationship. To the contrary, male mice would fight viciously until one established dominance over the other with the dominant male continuing to pursue the subordinate until it retreated. In general, the larger male became dominant.[25] Subsequently, the subordinate's behavior was influenced by the dominant individual, with the subordinate avoiding the dominant and primarily staying at its nest until the dominate male was inactive, allowing it to roam although still avoiding the dominant's nest.

Resident mice of an established deme displayed little aggression towards each other. However, the introduction of an intruder would elicit an immediate search of the area by all deme members except for very young individuals. During the search, resident mice would seek to identify each other through smell and attack the stranger when it was identified. When an attack ensued, it could be problematic for mice to accurately identify

each other with mice often mistakenly attacking members of the own deme temporarily until proper identification was established.

Justification of control

Damage.

Damage caused by *M. musculus* is wide reaching and difficult to quantify due to the varied nature of detrimental conditions they cause and the frequent failure to correctly associate a loss with the presence of mice.[65] It is also rarely the case that reported damage by rodents are species specific and often losses by any commensal rodent (*Rattus norvegicus* Berkenhout, *Rattus rattus* Linnaeus, and *M. musculus*) are lumped together.

Damage to food crops through direct consumption and contamination by rodents is challenging to quantify, but generally regarded as significant. While direct feeding by commensal rodents on foodstuffs (particularly rats) can be significant, the greatest concern is often the contamination of stored foodstuffs and processing equipment through direct rodent contact or via droppings and urine. A single mouse can excrete between 40 and 100 fecal pellets a day as well as excreting drops of urine indiscriminately as they move about.[6] In Asia alone, it is estimated that in one year rodents eat enough rice from the fields to feed 200 million people.[66] In 1982 the Food and Agricultural Organization of the United Nations reported that "rats" (most likely meaning commensal rodents) destroyed in excess of 42 million tons of food worth $30 billion dollars.[67] In 2000, Pimentel et al.[68] reported the estimated economic cost of commensal rodent damage from the destruction of foodstuffs in the United States at $19 billion/year, far outpacing damages caused by any other invasive species. Corrigan[6] estimated the total cost of

rodent control programs in 2001 at $337 million for all commensal species and $223.7 million for *M. musculus* specifically.

Livestock damage.

Some of the most severely impacted industries by commensal rodents worldwide are confined animal operations. These facilities offer an ideal environment for commensal rodents to thrive, providing a plethora of sources for food, water, harborage, and protection from predation often leading to substantial populations. In 1998, USDA estimated the rodent population on poultry farms in the United States was in excess of 1.4 billion individuals.[69] Estimates of damage and attempts to control rodents has been presented for a variety of these facilities with the most severely impacted being poultry[55, 70-74] and swine.[75-77] The damage rodents cause in these facilities is extensive and includes damage via contamination, gnawing on structural, electrical, mechanical and utility components, and undermining foundations and concrete slabs with their burrowing activities.[55] Damage caused by burrowing and gnawing on insulation in these building can be substantial leading to increased utility cost.[78] Perhaps the greatest threat posed by infestations in livestock facilities is the vector potential *M. musculus* present, being known vectors of several livestock pathogens (Table 1.1). The Layers '99 study[79] by the USDA identified poultry houses with an elevated rodent index as being nearly nine times more likely to be positive for *Salmonella enterica* serovar Enteritidis (SE) than houses with a lower index. Controlling mice in these facilities is challenging due to the inability

to exclude mice from animal feed, abundant habitat, lack of predators, and complications between farm activities and rodent control efforts.[55, 76]

Table 1.1. Common pathogens of livestock in North America that rodents may harbor or disseminate

Disease	Agent	Livestock affected	Rodents implicated
Bordetellosis	bacteria	poultry, swine	*Rattus* spp.
Encephalomyocarditis	virus	swine	*Rattus* spp. *M. musculus*
Erysipelas	bacteria	poultry, swine	*Rattus* spp.
Fowl cholera	bacteria	poultry	*Rattus* spp. *M. musculus*
Fowl pox		poultry	
Leptospirosis	bacteria	cattle, poultry, swine, equine	*Rattus* spp. *M. musculus*
Pseudorabies	virus	swine	*Rattus* spp.*
Salmonellosis	bacteria	poultry, swine	*Rattus* spp. *M. musculus*
Swine Dysentery	bacteria	poultry, swine, equine	*Rattus* spp. *M. musculus*
Toxoplasmosis	protozoan	swine	various rodents
Trichinosis	nematode	swine	*Rattus* spp.

Adapted and modified from Timm et al.[80] and Corrigan.[6]
*Opinions differ on the significance of rodents as the reservoir or disseminator.

Mice have been identified as a primary vector and reservoir for *Salmonella* spp. in poultry houses, particularly serotypes of human health concern.[73] Welch et al.[81] demonstrated that mice can be infected with as few as 15 SE cells and then rapidly replicate the infection internally ultimately shedding them in excreta. This excreta was able to sustain infection for at least 148 days and transmission between mice was observed. Davies[70] identified isolates of *Salmonella* spp. from 61of 85 (71.8%) bulked samples of mouse droppings and 8 of 13 (61.5%) mouse carcasses in poultry houses.

Henzler[73] identified *M. musculus* as the primary agent of infection on poultry houses and reported an average shedding 2.3 x 10^5 SE organisms per fecal pellet, a dose sufficient to inoculate adult hens. Perhaps the most concerning finding of this study was the persistence of SE in mice for 10 months following a thorough disinfection of the farm showing the ability for mice to reservoir this pathogen between flocks if populations are not controlled.

Zoonotic disease.

M. musculus is known to be a natural reservoir and vector for a variety of zoonotic pathogens. While in general it is considered that *M. musculus* plays a limited role in the transmission of human infectious disease,[3, 6, 82] the importance of commensal rodents in the transmission and harborage of these pathogens cannot be overlooked and is often underestimated.[83] Due to similarities between rodent and human physiology, rodents poses the ability to amplify[72, 73, 84] zoonotic pathogens, making them a critical component to disease systems. Several authors including Battersby,[85] Blackwell,[82] and Corrigan[6] have provided reviews of some zoonotic diseases associated with commensal rodents including *M. musculus*. A summary of prominent zoonotic pathogens specific to *M. musculus* is presented in Table 1.2. While the role of *M. musculus* in the dissemination of zoonoses has been reported as limited, many additional diseases are transmitted by other commensal rodents (e.g. *R. rattus* and *R. norvegicus*) and semi-commensal rodents (e.g. *Peromyscus leucopus* spp.) that are often times partially mitigated with control efforts targeting *M. musculus*. In one of the more recent reviews

(2015) of rodent zoonoses, Battersby[85] comments that our understanding of the role rodents play in etiology is still lacking in many aspects and rodent-borne diseases are often misdiagnosed and under correlated. This complicates and impedes the establishment of accurate risk assessment, but it is highly probably that the risk is likely considerable in many situations. Battersby[85] concludes by stating that what is certain, in many situations, is that the role rodents play in etiology for both man and animals is often the most compelling motive for effective rodent control.

Table 1.2. Significant zoonotic pathogens of *M. musculus*

Disease	Agent	Vector
Hymenolepiasis	*Hymenolepis* spp.	various Coleoptera spp. can be involved
Leptospirosis	Spirochete	
Lymphocytic choriomeningitis (LCM)	Arenaviridae	
Murine typhus	Rickettsiae	fleas: *Xenopsylla cheopis* and others or directly from mice.
Plague	*Yersinia pestis*	fleas: *Nosopsyllus fasciatus* and others
Rat-bite fever	*Streptobacillus moniliformis* *Spirillum minus*	
Rickettsialpox	Rickettsiae	mites: *Liponyssoides sanguineus* or directly from mice
Salmonellosis	*Salmonella enterica* serovars	
Trichinosis	*Trichinella spiralis*	
Tularemia	*Francisella tularensis*	ticks: *Dermacentor variabilis* *Dermacentor andersoni* *Amblyomma americanum* flies: *Chrysops* spp.

Adapted and modified from Corrigan[6]

Rodent integrated pest management (IPM)

IPM overview.

Effective rodent control programs follow an integrated pest management (IPM) approach to address problems caused by rodents. IPM practices that were originally developed by entomologists to address issues involved with indiscriminate pesticide usage provide the foundation for IPM strategies.[86] While the general underlying principles of rodent IPM are consistent with traditional IPM programs targeting conventional agricultural pests, Marsh[87] explains that the "principles and parameters used in IPM programs for vertebrate pest differ substantially from those of other crop pest…" meaning that many practices inherent to traditional IPM will have to be adapted and altered to be effective. Rodent IPM takes into account the various facets of rodent control, incorporating effective monitoring with various control methods including cultural, physical, biological, and chemical to prevent rodent detriments by the most economical means possible while being mindful of both environmental and public health concerns. Colvin and Jackson[88] relay the need for modern rodent IPM programs to be flexible and adaptive to address new challenges and situations as they arise or are identified through periodic evaluation. Commensal rodents are intelligent, persistent, and cunning, meaning that effective control programs must be constantly reviewed, evaluated, and adjusted. Although the science and technology are often available to successfully mitigate a rodent infestation, many control efforts succumb to improper coordination, follow-through, and leadership.[88]

Methods for monitoring abundance.

Methods to effectively monitor abundance are fundamental to ecology[89] and critical in implementing and evaluating control programs[90] or efficacy trials targeting *M. musculus* and other commensal rodents. However, accurate abundance estimates are difficult to obtain and often cost prohibitive for use outside of scientific research.[91] Due to these constraints, two general categories of monitoring have been developed: indices (e.g. tracking plates, capture rates, fecal deposition) and methods that seek to estimate true abundance (e.g. capture-mark-recapture (CMR) and line transects).[92] While methods that seek to estimate true abundance have the ability to provide accurate estimation under ideal circumstances, they are frequently degraded by the inability to adhere to stringent analytical assumptions under real world conditions, resulting in estimates of questionable quality.[92, 93] While indexing procedures still operate under various assumptions, these methods have been refined to offer methods to provide index parameters that are reflective of the true abundance and are more efficient to employ.[92, 94]

Population indices.

An overview of methods utilized to index rodents was assembled by Engeman and Whisson[92] who identified the following qualities inherent to desirable indices: practicality, sensitivity, robustness, prior validation, and precision and variance estimation. A variety of techniques (bait consumption,[55, 77, 95, 96] tracking plates,[91, 95] trapping,[55, 95] sightings of active animals, signs,[95] gnaw sticks,[91] remote photography[77])

have been developed to index rodent populations. While all of these techniques have the ability to provide presence/absence information, few have been further developed into a method to give reasonable estimates of abundance or provide a reliable index of the population under praxis conditions. These methods developed to index populations are not equipped to provide actual population estimates but are implemented to make relative comparisons between populations or to monitor population trends.[92] Whisson et al.[91] remarks that the sensitivity of these monitoring techniques would be enhanced and they would provide more meaningful information to inform management decisions if they were further developed to provide continuous measurements and not just occupancy information. Quy et al.[45] expanded upon this idea by taking three primary techniques (CMR, tracking, and census baiting) utilized to monitor *M. musculus* populations to determine each methods ability to estimate populations of known size. Of these methods, CMR estimates and census baiting were determined to provide reasonable estimates of population size, with census baiting being favored over CMR due to the extensive requirements of time, money, and skilled personnel needed to conduct the CMR analysis. These indirect indices (methods that do not require direct handling of animals) are generally favored for industry application due to their cost effectiveness and avoidance of possible human health concerns;[46] whereas, CMR studies are more involved but provide additional demographic data allowing for more detailed analysis of the population.

Methods for estimating true abundance and demographics.

In situations were more detailed information of a population is required beyond what indices can provide, various implementations of CMR are utilized. CMR designs cover a wide range of methods[97] that differ in how data collection is structured (e.g. timing, number of captures, placement of traps) and how it is analyzed (e.g. open vs. closed populations, varying capture and recapture probability, inclusion of auxiliary data, inclusion of covariates). The two primary categories CMR models fall under are closed and open designs. Closed designs assume demographic closure (no births or deaths) and geographic closure (no emigration or immigration) during sampling periods; whereas, open designs allow for these demographic and geographic changes to occur. The first use of modern CMR principles in estimating closed animal populations was developed by Petersen[98] and Lincoln[99] giving rise to the Lincoln-Petersen method thoroughly described by Seber[100] and the basis for modern models of greater complexity. Pollock et al.[97] provides an inclusive overview of CMR design, analysis, and interpretation of these models. These models rely heavily on satisfying the following assumptions for valid inference identified by Lindberg.[93]

- Marked individuals are representative of the entire population of interest and parameters estimated from these marked individuals can be inferred on the population at large.
- Methods used to mark individuals do not alter their behavior or fate.
- Marks are not lost or misread.
- All marked animals have the same probability of capture.

- The fate of marked individuals is independent of other marked individuals.
- The population is closed (no births, deaths, emigration or immigration) between samples (for closed models).

Under most practical circumstances, there are potential violations of these assumptions and the inability to satisfy these assumptions is often partially mitigated though either experimental design or analytical approaches.[93] Although these early closed CMR models provided reasonable estimates of population demographics such as population size, age structure, and sex ratio, they only provided estimates reflecting a snapshot in time and often violated closure assumptions for short lived species such as mice. The development of open model designs involving CMR techniques were first independently described by Cormack,[101] Jolly,[102] and Seber[103]. This open model structure allowed for sampling periods to traverse times of demographic and geographic fluctuations and allowed for the estimation of additional parameters of interest such as apparent survival and recruitment into the population between periods.[93] This basic open model framework is often referred to as the Cormack-Jolly-Seber (CJS) model. While this CJS model framework was useful in obtaining improved estimates of parameters associated with open populations, it did not allow for very detailed estimation in population abundance, particularly for short lived species such as mice. Pollock[104] developed a method known as Pollock's robust design that combined the techniques of the early closed Lincoln-Petersen method with the open CJS sampling scheme to create a framework to address these issues and a platform to estimate all of the parameters associated with both prior models.[93] This "robust design" was one of the first combined

designs (i.e. a design that utilizes the techniques of at least two other model frameworks).[93] This robust design framework involves a framework with two or more open primary occasions each of which are comprised of two or more closed secondary occasions (Fig. 1.1). This robust design framework allowed for parameters estimated under an open model structure to be derived between primary occasions (e.g. apparent survival, temporary emigration, recruitment) and more specific abundance estimates for each closed secondary occasion.[93]

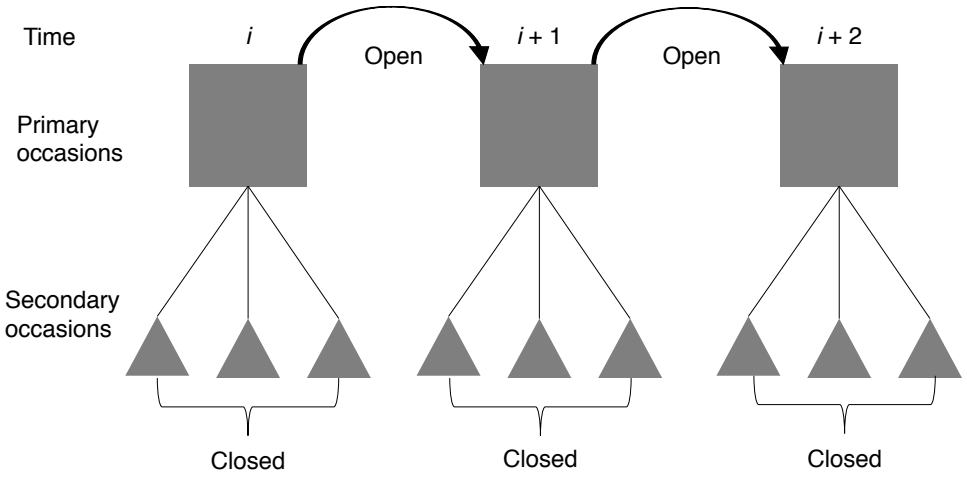

Figure 1.1. Pollock's robust design sampling method. The population is considered demographically and geographically open between primary occasions and demographically and geographically closed between secondary occasions.
Adapted from Lindberg.[93]

To extract more information from data being collected for purposes outside of traditional CMR studies, researches began including auxiliary information into CJS

model structures to estimate population parameters.[105] Types of auxiliary data that have been implemented into these models include: band recoveries by Burnham,[106] ancillary sightings by Barker,[105] and dead recoveries by Kendall el. al.[107] Lindberg et al.[108] took the model structure from these designs that included auxiliary observations and combined them with the robust design framework to allow estimations of parameters for open and closed models. The inclusion of auxiliary data in these models has been shown to improve parameter estimation beyond what is offered by more simplistic models so long as adequate sample sizes are taken.[105, 107] The use of these robust models that include auxiliary observations show promise for being particularly applicable for improving parameter estimation for short lived species such as *M. musculus* when limited time is available to collect data and multiple sources of information can be utilized.

Methods of control

Colvin describes the "modern rodent control era" as beginning with the advent of World War II.[88] The push for more advanced methods of control was driven by the need to address many of the more complex rodent-human conflicts developing as a result of damaged infrastructure, large numbers of transient people, and a scarce food supply.[88] Initial control efforts relied heavily on acutely toxic rodenticides and were often implemented without much consideration for rodent ecology, behavioral response, cultural control, or human and environmental health. During the 1940s and 1950s, investigators also began focusing more attention towards the biology and ecology of commensal rodents and started to implement knowledge gleaned from these studies to

improve rodent control practices.[88] Early studies establishing the foundation for these principles were conducted by Davis and others (Davis;[109] Davis et al.;[110] Davis and Fales;[111, 112] Emlen et al.[57, 113]) in the United States and Chitty and Southern[48] in the United Kingdom. In more recent years, Singleton et al.[114] argued that effective modern rodent control programs should follow more of an "ecologically-based" approach to rodent pest management and remarked on the need for further research into rodent ecology from a pest management perspective. Current rodent IPM framework often looks at mitigating issues from an ecological perspective and consists of a combination of tools to reduce rodent populations including cultural control practices (e.g. proper sanitation, habitat management), rodenticides, rodent proofing, timing of treatments, and traps.[6, 114, 115]

Rodenticides.

Gratz[116] states that although almost any mammalian poison can be considered a potential rodenticide, only a select few compounds remain viable options after applying the highly selective criteria needed for a suitable candidate. Characteristics inherent to effective rodenticides include a high toxicity to rodents, high degree of bait acceptance, failure to induce "bait shyness", high degree of reacceptance, and a specificity towards rodents to reduce impacts on non-target species.[116] Today, rodenticides are broadly categorized by their mode of action into the following categories: acute rodenticides, fumigants, first-generation anticoagulants, and second-generation anticoagulants.[115]

Initial efforts to control rodents into the 1950s relied heavily on single-dose or acute rodenticides (e.g. arsenic, phosphorus (yellow), strychnine, sodium fluoroacetate (1080), red squill, and zinc phosphide) that generally included compounds targeting the central nervous system and were in many cases also highly toxic to man and non-target species.[116] An overview of these compounds is provided by Gratz,[116] where it is mentioned that although the use of these compounds declined significantly after the introduction of anticoagulants in the early 1950s, some of these compounds still provide viable options in situations where resistance to anticoagulants has developed or in time sensitive cases where single dose control might be needed.

In the late 1940s, rodenticides took a significant step forward with the discovery and development of the first anticoagulant compounds dicoumarin and warfarin by the Wisconsin Alumni Research Foundation.[117, 118] The recognition of anticoagulants as possible candidates for a rodenticide occurred rapidly, and early trials lead to the discovery that these compounds offered significant advantages over previously available acute rodenticides.[119] Anticoagulant compounds are effective as rodenticides due to their delayed onset of action (reducing bait shyness), reduced toxicity to non-target species, availability of a suitable antidote (Vitamin K), and increased palatability due to their high potency which requires less active ingredient.[117, 120] These early forms of anticoagulants later deemed "first-generation" anticoagulants included the compounds chlorophacinine, coumafuryl, diphacinone, isovaleryl indandione, and pindone. Due to their impressive efficacy, eradication programs relied heavily on baiting, and indiscriminant use of these compounds gave way to resistance issues by the late 1950s.[121] To address this resistance

issue, and to decrease the time and amount of consumption needed for mortality to develop, more potent forms of these anticoagulant compounds were derived by the late 1970s.[122, 123] These "second-generation" anticoagulants were up to 100 times more potent than their predecessors.[117] Even with the increased efficacy of these second-generation compounds, reports of resistance were being reported in the UK for *R. norvegicus* by 1976[124] and *M. musculus* by the early 80s.[125] Lund[126] provides an early overview of resistance to these second-generation compounds in Denmark and Sweden warning that reliance on these compounds would not be effective in eliminating warfarin-resistant rodents and recommended a push for the development of other rodenticides.

Detailed reviews on the development, chemical structure, use, and various anticoagulant compounds developed as rodenticides were compiled by Hadler and Buckle,[117] Jackson and Ashton,[118] and Witmer and Eiseman.[115] Fisher[120] also provides a review on susceptibility to anticoagulants specific to *M. musculus*.

Calciferols as rodenticides.

Facilitated by the development of resistance to anticoagulant compounds, calciferols were investigated for possible use as a rodenticide in the early 1970s.[127] Calciferol is a general term that encompasses a family of compounds more commonly referred to as Vitamin D. Within this class of sterol compounds, Vitamin D_2 and Vitamin D_3 (ergocalciferol and cholecalciferol, respectively) are the two "activated" forms of physiological importance.[128] Upon ingestion, calcium is mobilized in the body resulting in hypercalcemia and mineralization of major organs.[8] Following ingestion of a lethal

dose, death usually starts to occur within three days with the average time-to-death ranging from four to five days.[129] Death is usually the result of heart failure due to blood vessel blockage in the heart by calcification.[129, 130] Studies by Greaves et al.[127] and Rennison[131] demonstrated that calciferols were effective against anticoagulant-resistant rodents including *R. norvegicus, R. rattus,* and *M. musculus*. Calciferols are generally described as having low secondary toxicity, making them an attractive alternative in situations where impacts on non-target species and human safety are a concern.[130-132] The decreased sensitivity of *M. musculus* towards anticoagulants compared to *Rattus* spp. is also not observed in calciferols, with LD_{50} values for cholecalciferol being more proportional for *M. musculus* and *R. norvegicus* (i.e., 42.5 and 43.6 mg/kg, respectively) making the compound particularly appealing for mouse control.[129] Calciferol was formulated into a commercial form at 0.1% (1000 ppm) along with warfarin at 0.025% (250 ppm) and marketed in Europe and Canada under the trade name Sorexa® by Sorex, Ltd., London. Cholecalciferol was formulated at 0.075% (750 ppm) into commercial rodenticides under the trade names Quintox® and Rampage® by Bell Labs, Inc. in the 1980s.[20, 130]

Efficacy.

While early laboratory studies showed efficacy of nearly 100% for calciferol rodenticides, a prominent behavioral effect was observed after rodents consumed a sizable amount of treatment baits.[127, 130] Within one to three days following a sizable ingestion, affected rodents ceased feeding on the treatment bait and any other sources of

food, presumably due to toxicosis associated with calciferol poisoning.[127, 128, 133, 134] This phenomenon, referred to as "stop feed effect" by Prescott et al.,[128] seems to be inherent to all calciferol rodenticides and is even mentioned as a positive characteristic by limiting non-target exposure[129] through preventing excess consumption so long as a lethal dose is achieved in the target species. Early choice feeding studies[127, 130] showed this stop feeding effect was delayed to an extent where cessation in feeding arrived after ingestion of a lethal dose, subsequently preventing this reduction in feeding from affecting the efficacy of treatment baits. However, Greaves et al.[127] warned that this phenomenon might lead to the development of "bait shyness," or a conditioned taste aversion in field situations, if rodents did not consume a lethal dose within the period of time before this reduction in feeding began to occur and suitable alternative sources of food were available.

There is limited research available on the efficacy of calciferols in field applications, particularly pertaining to *M. musculus*. Rowe et al.[135] investigated the efficacy of calciferol formulated with warfarin at various rates in controlling wild *M. musculus* populations in rural and urban setting across the United Kingdom. Results showed that calciferol formulated with warfarin, each at 0.025% (250 ppm) in an oatmeal bait, was not effective in controlling six of seven populations. Formulations of both calciferol and warfarin at 0.05% (500 ppm) in a dehusked canary seed base with 5% corn oil reduced census consumption by 94.2-97.4% from pre-treatment levels. Highest efficacy (97-100%) was achieved with a formulation containing 0.1% (1000 ppm) calciferol and 0.025% (250 ppm) warfarin in a whole canary seed base. Preference and

efficacy of treatments baits was also notably impacted by base constituents. Brown and Marshall[95] started a field efficacy study in 1985 to assess the effectiveness of Quintox® in a variety of environments and habitats across the Unites States. This test involved one indoor test in each of five designated "regions" of the United States and one outdoor test (two for Norway rats) in each region for Norway rats (*R. norvegicus*), roof rats (*R. rattus*), and house mice (*M. musculus*). While details on these tests are not specific and census techniques varied from location to location, efficacy results showed an average of 90% or greater population reduction in each of the five regions for each species. This study also mentioned that no bait shyness or aversion was noted in any of the field trials, but did not describe how these claims were evaluated. In Australia, Twigg and Kay[20] tested the efficacy of two Quintox® formulations (wheat and pellets) across three different seasons (early summer, late summer-autumn, and winter) in outdoor penned enclosures to determine its potential as a broadcast rodenticide for agricultural use. Efficacy results were highly variable between formulations and across seasons. Greatest efficacy (92% wheat, 72% pellets) was achieved during winter and the poorest reduction occurred during late summer (33% wheat, 5% pellets). Over all three trials, wheat produced a significantly greater reduction in mouse abundance than the pelleted formulation. Efficacy was reported to be inversely related to the availability of alternate food sources and an aversion to Quintox® was mentioned as a contributing factor. In trials with the greatest efficacy, survival was biased towards males and was significantly different in one trial. Although primarily targeting rock squirrels (*Spermophilus variegatus* Erxleben), Beard et al.[136] observed poor efficacy towards *M. musculus* using

Quintox® in the southwestern United States reporting a 45% population reduction after baiting for six days during the early summer. In Australia, Redhead et al.[137] observed a 23% population reduction after baiting for seven days in the winter on agricultural land using Quintox® in bait stations.

Conditioned taste aversion.

Zeinelabdin and Marsh[133] describe the phenomenon of conditioned taste aversion as the learned avoidance of a food or drink that has been associated with previous illness. There are mixed reports of conditioned taste aversion to calciferol rodenticides in the literature. Marsh and Tunberg[129] observed little evidence of taste aversion in *R. norvegicus*. In this study, 18 individuals that survived exposure to Quintox® and an untreated natural food in a simulated field test were trapped and housed individually in the laboratory. Seven days later, they were offered a choice diet between Quintox® or untreated food for 15 days resulting in 17 of the 18 consuming a lethal dose. However, conflicting reports showing a clear conditioned taste aversion in studies by Zeinelabdin and Marsh,[133] Prescott et al.,[128] and Twigg and Kay.[20] Zeinelabdin and Marsh,[133] caused conditioned taste aversion to a novel sucrose solution in Norway rats (*R. norvegicus*) that lasted for 18 days by conditioning with the novel sucrose solution for two hours, immediately followed by intubation with a sub-lethal dose of calciferol. Prescott et al.[128] observed that acceptance of cholecalciferol pellets was significantly reduced in Norway rats (*R. norvegicus*) with one-day exposure to cholecalciferol 18 days previously compared to individuals with no prior exposure. Prescott et al.[128] further investigated this

phenomenon with calciferol to see if the conditioned taste aversion was linked to the rats ability to detect (taste) calciferol in the bait, the taste of the base constituents, or if it was a response to a rapid physiological effect of the active ingredient. Results showed that rats were only developing a conditioned aversion specific to the formulation they originally consumed. Oral intubation of the active ingredient following ingestion of the base constituents did not produce an aversion to the same bait. Twigg and Kay[20] observed that when presented a choice, mice (*M. musculus*) that were previously exposed to Quintox® for 28 days in an outdoor enclosure consumed significantly less cholecalciferol laced wheat compared to non-treated wheat in a lab setting. Less than 25% of the mice consumed any Quintox® on the first day of the trial and only 17% of the mice perished. However, naïve mice were also shown to have a similar aversion to the Quintox® with only 22% consuming Quintox® on day one resulting in 22% mortality.

Baiting strategies.

Since man's first efforts to control rodents with poisons, rodents have been adaptive and often highly successful at avoiding these attempts.[138] As a result, substantial effort has been allocated to try and overcome many behaviors such as neophobia, bait shyness, and conditioned taste aversion that prevent successful control. While many of these efforts are directed towards the composition and formulation of the rodenticide, the methods employed in administering the rodenticide such as timing, presentation, placement, and consideration of the populations' exposure history are also important in maximizing control.

Prebaiting.

To reduce the effect of behavioral avoidance of poisons by rodents such as neophobia and bait shyness, a method knows as "prebaiting" was developed. This technique uses a nontoxic bait preceding the placement of a treatment bait to enhance the acceptance of a rodenticide, however, it has been described as requiring considerable persistence, expertise, and effort.[117, 139] The mechanisms by which prebaiting facilitates the acceptance of a rodenticide are still unclear, but previous studies indicate two theorized pathways: the reduction of an animals' innate suspicion towards novel food items translating to a "momentum to consume" and the suppression of an animal's ability to form learned aversions.[138, 140] Prebaiting is often used preceding treatments with acute rodenticides due to the fact they have an increased propensity to cause aversion.[141] Increased efficacy using prebaiting before acute rodenticide treatments in the field has been observed, although population reductions greater than 60-70% were still reportedly difficult to achieve.[142] Robbins[138] performed laboratory experiments on deer mice (*Peromyscus maniculatus gambelii* Baird) and *M. musculus* revealing that the prebait formulation should mimic the taste of the treatment bait as close as possible even if initial acceptance of the prebait is diminished. These experiments also suggested that prebaiting with an effective toxin-flavored mimic increased the effectiveness of a toxic bait by inhibiting the formation of conditioned taste aversion even when consumption of the toxic-flavored prebait was significantly less. Rowe et al.[135] compared the efficacy of treatments targeting *M. musculus* with and without prebaiting before treating with a

single bait comprised of a mixture of calciferol and warfarin. Results showed efficacy ranging from 97-100% both with and without prebaiting (based on census baiting) making it difficult to conclude if there is any added benefit.

Saturation baiting.

With the advent of the first anticoagulant compound used as a rodenticide (dicoumarin), O'Connor[119] introduced a technique referred to as "saturation baiting" or "sustained baiting" for bait deployment. Saturation baiting as described by Dubock[141] entails providing bait points with a continual supply of a rodenticide that is replenished frequently to ensure excess bait is available to rodents until feeding ceases. This technique allowed for the cumulative ingestion of the slow acting first-generation anticoagulants needed to cause mortality to be obtained before rodents stop feeding on the treatment bait. Efficacy using this technique is generally high but it requires large quantities of bait and labor. Dubock[141] also comments that saturation baiting introduces a substantial amount of active ingredient into the environment and encourages the ingestion of significantly more active ingredient that what is needed to cause mortality. This increased deposition of active ingredient into the environment and over ingestion poses a potential greater risk to non-target species through secondary poisoning.

Pulsed baiting.

In response to the development of the more potent second-generation anticoagulants, Dubock,[143, 144] introduced pulsed baiting as an alternate technique for bait

deployment. This technique consists of periodic "pulses" of bait being deployed instead of the continual surplus of bait supplied by saturation baiting. Due to the increased potency of second-generation anticoagulants compared to their predecessors, less bait is required to be consumed to achieve a lethal dose. The original concept for this baiting technique was the result of comparing efficacy and rapidness of control across several different studies evaluating the effectiveness of the second-generation anticoagulant brodifacoum versus the other less potent anticoagulants. Rennison and Dubock[145] found that saturation baiting with brodifacoum at progressively stronger concentrations did not affect how rapidly control of *R. norvegicus* was achieved. In the same study, brodifacoum placements of various lengths (one, four, and seven nights) were conducted and analyzed for subsequent efficacy. Results showed efficacy of 41%, 51%, and 68% for lengths of one, two, and seven nights, respectively, contrasting with the 100% control observed by Redfern et al.[146] in the laboratory for seven days of baiting and often after just a single feeding. Combining the findings from these studies, Dubock hypothesized that due to the acute toxicity of brodifacoum in conjunction with the delayed time till death, socially dominant rats that had already consumed a lethal dose after the first two days of the study continued to feed for at least part of the remainder of the seven-day study and were subsequently behaviorally excluding subordinates from consuming a lethal dose. This translated into the theory that providing a continual supply of bait was not necessary as the majority of consumption observed during this time was wasted consumption by the dominant mice that had already consumed a lethal dose, but had not died yet (termed "over kill" consumption). The idea of pulsed baiting was to reduce the

placement interval and quantity of bait, thereby optimizing the probability of feeding from subordinate mice and reducing overconsumption by dominant mice. Dubock[141] presents preliminary studies demonstrating the use of this pulsed baiting technique and reports comparable efficacy and rate at which control is achieved with commenting on the added benefits of decreased bait use, decreased labor inputs, decreased environmental impact, and a reduced risk of secondary poisoning to non-target species. Corrigan and Williams[55] utilized a modification of this technique to control *M. musculus* in caged layer poultry houses in Indiana. This modification used a smaller number of specially designed bait stations made of PVC and shaped like an inverted "T" that were filled with a large quantity of bait. These stations were deployed and sequentially repositioned to various predetermined locations following a regimented time schedule with the goal of exposing mice in all areas of the building to the treatment bait for two, two day intervals. This technique followed similar logic to Dubock's pulsed baiting technique but presented bait in "time pulses" as the stations were relocated to various bait points in an attempt curtail behavioral exclusion and minimize the labor and inputs needed to gain control. Corrigan reported population reductions of 78.8% and 74.4% in two poultry houses using this technique and receiving positive feedback from operators on the practicality, convenience, and cost-effectiveness of the program.

Behavioral response to control measures

Neophobia.

Neophobia, or as it is sometimes called "new object reaction", is a behavior exhibited by rodents characterized by the reluctance to approach or interact with a novel item, sound, or odor introduced into an environment.[9, 147] This behavior is thought to be an evolutionary development facilitated by predation, unpredictable and extreme changes in habitat, food quality/availability, and dispersion.[9] Mice have been described as exhibiting limited neophobic behavior compared to the commensal rat species (*R. novegicus* and *R. rattus*), where strong neophobic expression has been observed.[24, 148-150] Some authors have even described mice as being neophilic in certain situations.[24, 48] Crowcroft[24] observed trap response heterogeneity in wild mice in artificial enclosures with some individuals described as trap-prone and others trap-shy and suggested some of the differences might be the result of varying mouse temperament. Results also showed that social interactions and population demographics had a marked impact on trap response with the absence of trap-proneness or trap-shyness in an all-female population and subsequent appearance when males were added. Males tended to be more trap prone than females and female trap response exhibited greater heterogeneity than males. Andrzejewski et al.[151] observed a correlation between social dominance and trap-proneness with male mice in a laboratory setting. Kronenberger[9] observed increased neophobia in wild mice compared to lab strains and suggested this was a result of Darwinian fitness.

Behavioral exclusion.

As mentioned in the previous sections regarding social structure and behavior, *M. musculus* activity is highly influenced by social factors and territoriality. Several authors have commented on how these behavioral factors, such as competitive exclusion, can affect control measures. Spaulding and Jackson[152] observed subsequent peaks in consumption several days after heavy initial feeding of a bromethalin rodenticide had subsided and speculated this was partially caused from the mortality of dominant rodents allowing subordinate individuals access to feed. These authors also commented on the importance of recognizing these delayed increases in consumption and that early termination of baiting before these delayed feedings were observed could result in developing a false conclusion that the product had failed when in fact the remaining rodents were never allowed to feed due to competitive exclusion. In regards to the development of the pulsed baiting technique, Dubock[141] recommended the application of limited amounts of bait separated by several days to prevent overfeeding by dominant individuals of the population and to allow for dominant mortality leading way to subordinate feeding on successive bait placements. This technique was mindful of the social hierarchy Dubock presumed existed in the population referring to this hierarchy as a "gnawing-order." In field trials with the field vole (*Microtus agrestis* Linnaeus), Myllymäki[153] observed that efficacy was dependent on bait point density with significantly higher control achieved by broadcasting bait across the treatment area compared to placing bait in bait stations spaced 5 m apart. The implications of territoriality when deciding on bait placement should be taken into consideration and

maximizing the density of bait placements can allow for bait to be made available for the maximum number of individual rodents.

Territoriality has also been shown to affect the movement of rodents following mortality from control programs. In a study observing ricefield rat (*Rattus argentiventer* Robinson & Kloss) movements following a control program, Temme[154] noted that individuals rapidly immigrated into vacant niches and habitat from surrounding areas of less favorable habitat quality. Lund[139] commented on the need to account for this risk of reinvasion and suggested that control measures should cover an area expansive enough to minimize the risk of reinvasion from surrounding populations and such treatments should be timed to coincide with seasons of low population density. There are conflicting reports to these claims that suggest these changes in movement and home range following population declines are species-specific and dependent on the social structure and habitat of the population. While studying a population of *M. musculus* in a stable indoor environment with ample food, water, and cover, Young et al.[40] did not observe any significant changes in movements before or after treatments with 1080 rodenticide. This result supports the findings of previous studies mentioned in the home range section of this review which indicates that home range size is not dependent on population density but more reliant on habitat type, in addition to resource availability and distribution.

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CHAPTER 2 - Effect of two temporal rotations from a nontoxic bait to an organic rodenticide containing cholecalciferol on consumption by the wild house mouse (*Mus musculus* L.)

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ABSTRACT

BACKGROUND: Effective control of commensal rodents, including the house mouse (*Mus musculus* L.), relies heavily on rodenticides. The goal of this study was to evaluate mouse consumption of an organic rodenticide, compared to a nontoxic bait, both when the population was naïve to the rodenticide and after previous exposure. Consumption rates were analyzed between bait types and exposure history to determine their impact on consumption. Mouse population estimates were calculated to determine rodenticide impact on abundance.

RESULTS: Compared to nontoxic bait, consumption of cholecalciferol bait fell to 45% within two days and continued to decline to ~2% within seven days. When nontoxic bait was returned, consumption levels rebounded to ~25% of original nontoxic levels, while abundance estimates indicated a 62% population reduction. When cholecalciferol bait was returned, consumption trends were similar to the original baiting, resulting in a reduction in abundance to 3% of the original population.

CONCLUSION: The cholecalciferol rodenticide had a significant "stop feeding" effect on the mouse population compared to nontoxic bait but did not show signs of conditioned aversion. Although consumption of the cholecalciferol bait was significantly less than the nontoxic bait, mouse abundance declined by 97% after a reintroduction of cholecalciferol bait.

Keywords: rodent control, *Mus musculus*, organic, pest management, feeding behavior, cholecalciferol

INTRODUCTION

Controlling populations of commensal rodents, such as the house mouse (*Mus musculus* Linnaeus), with rodenticide baits has been a common method of control for over 50 years. Baits have included a variety of physical forms, base constituents, and active ingredients in an effort to find effective combinations that control rodent populations while keeping non-target species safe.[1] Forms of baits have included blocks, chunks, pellets, loose meal, place packs, and others. Base constituents have included a variety of inactive ingredients such as grains, seeds, vegetable oils, and corn meal in an attempt to increase bait attractiveness and consumption.[2] Active ingredients used in rodenticides have traditionally been divided into two classes based on mode of action: anticoagulants (formulations that suppress blood clotting) and neurotoxins (formulations that attack the central nervous system).[3]

In the 1970s and early 1980s, the development of a novel class of active ingredients, calciferols, was explored as an alternative to the existing anticoagulant and neurotoxin compounds after concerns were expressed over anticoagulant resistance observed in some commensal rodent populations.[4-6] Calciferol is a general term that encompasses a family of sterol compounds more commonly referred to as Vitamin D, of which Vitamin D_2 and Vitamin D_3 (ergocalciferol and cholecalciferol, respectively) are the two "activated" forms of physiological importance.[7] Upon ingestion, calcium is mobilized in the body resulting in hypercalcemia and mineralization of major organs.[8] Death is usually the result of heart failure due to blood vessel blockage in the heart by calcification.[9] Calciferols were also found to have low secondary toxicity making them

an attractive alternative in situations where impacts on non-target species and human safety were a concern.[6, 9, 10] Of these two forms of Vitamin D, cholecalciferol was developed into a commercially available rodenticide with the trade name Quintox® by Bell Labs, Inc. in 1984.[9, 11] A more recent interest in cholecalciferol as a rodenticide has been instigated by growth in the organic food production market. The formulation used in this study, Terad$_3$ Ag BLOX®, is the first and only rodenticide registered by the United States Environmental Protection Agency and listed by the Organic Materials Review Institute (OMRI) for use in and around organic production facilities. This rodenticide contains a new formulation and to the best of our knowledge acceptance has not been evaluated in comparison to a nontoxic bait or any other rodenticide.

Behavioral resistance exhibited by commensal rodents to control measures has been identified as a significant factor in loss of efficacy.[2, 12] Previous studies utilizing calciferol rodenticides have exhibited significant decreases in consumption (stop feeding effect) within one to three days following initial exposure resulting in decreased efficacy.[4, 7, 8, 11, 13] Zeinelabdin and Marsh[13] reported conditioned taste aversion to a novel sucrose solution in Norway rats (*Rattus norvegicus* Berkenhout) that lasted for 18 days by conditioning with the novel sucrose solution for two hours, immediately followed by intubation with a sub-lethal dose of calciferol. When presented a choice, Twigg and Kay[11] observed that mice previously exposed to Quintox® for 28 days in an outdoor enclosure consumed significantly less cholecalciferol laced wheat compared to non-treated wheat in a lab setting. While previous studies have identified the presence of behavioral resistance properties associated with calciferol exposure, different base

constituents have been shown to affect acceptance making testing of this novel formulation relevant.[14] Furthermore, little work has been done to evaluate the possibility of reconditioning bait acceptance with a nontoxic formulation followed by the reintroduction of a calciferol rodenticide in a field setting.

This study was designed to evaluate changes in house mouse consumption between a commercially available nontoxic bait and a certified organic rodenticide containing the active ingredient cholecalciferol, both when the population was naïve to the rodenticide and after it had been previously exposed in a field setting. Two temporal rotations from nontoxic to cholecalciferol bait allowed for the analysis of consumption rates dependent on bait type and exposure history. Additionally, population demographic estimations were derived by means of capture-mark-recapture (CMR) and recovery of deceased mice to evaluate the rodenticide's impact on mouse abundance and survival.

EXPERIMENTAL METHODS

Study area.

This study was conducted from 31 March 2014 to 20 August 2014 in a warehouse used to store and process agricultural seeds in Pickens County, South Carolina (34° 65′N, 82° 50′W). The selected test site offered a location with an existing mouse population that was naïve to any prior testing or recent control measures. Previous attempts to control the mouse population included low intensity baiting with anticoagulant formulations which ceased more than 6 months before the study start date. Observational inspections and live trapping prior to the study indicated an active infestation of mice with regular sightings of mice, mouse droppings, damaged bags and seeds, nesting sites, and damage to the building from gnawing.

The warehouse was constructed of steel I-beam framing with metal siding over a concrete slab floor. The dimensions of the building were 46 m long and 24 m wide, with the majority of the building in an open floor plan containing seed processing equipment, pallets of seeds and bags, and other miscellaneous supplies (Fig. 2.1). These goods were distributed around the perimeter of the warehouse leaving a large open expanse of concrete slab in the middle section of the floor that mice were not likely to traverse (verified by video surveillance and mouse sign). An 8 m section on one end of the building contained climate controlled office space and a walk-in cooler set to approximately 5 °C. Packages of seed on pallets were also housed in the cooler, and numerous mice were observed traveling in and out of the cooler doors freely. The remainder of the building had no climate control. There were minimal changes to the

warehouse during the study due to normal management activity. It should be noted that throughout the study access of mice to spilled feed and grains was ubiquitous. Therefore, food availability was not thought to be a limiting factor on the mouse population.

Bait stations.

Sixty-one commercially available Aegis-RP® (Liphatech, Inc., Milwaukee, WI) bait stations were distributed around the inside perimeter of the warehouse. These stations were placed according to manufacturer's recommendations, spaced approximately 2.5 m to 3.5 m from adjacent bait stations, and placed either directly against the wall or as close to the wall as possible (Fig. 2.1). The stations measured approximately 32 cm in length, 20 cm in width, and 10 cm in height, were tamper resistant, and made of high-impact plastic with one entrance hole on each side. Bait stations were placed two weeks prior to bait application to allow mice to habituate to their presence. Each station was uniquely numbered and designated to a specific location throughout the entirety of the study.

Details of baits tested.

Treatment baits in each phase of the study were placed on metal rods included with the stations to prevent premature spoilage, secure the bait in the station, and make bait more accessible. The nontoxic bait used in this study, DETEX®, was selected because it is manufactured by the same company as the treatment bait (Bell Laboratories, Inc., Madison, WI) and was found to be of similar formulation and size. Each block of

nontoxic bait was yellow, rectangular in form measuring 47 mm x 25 mm x 25 mm, and weighed approximately 20.5 g. Individual blocks of cholecalciferol bait, Terad$_3$ Ag BLOX® (Bell Laboratories, Inc., Madison, WI), were also rectangular in form, measured 45 mm x 25 mm x 25mm, weighed approximately 28.5 g, and were brown in color. Previous studies have shown no statistically significant differences in levels of consumption related to bait color.[15] Cholecalciferol was formulated into the bait at a rate of 750 mg × kg^{-1}. LD_{50} for cholecalciferol has been determined for the ICR strain of *Mus musculus* L. at 42.5 mg x kg^{-1} translating to consumption of 1.13 g of the cholecalciferol bait needed to achieve the LD_{50} dose for a 20 g mouse.[9]

CMR protocol.

Eighty-two folding Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) were used to capture mice for CMR analysis. Mice were captured on 2,624 trap nights with trapping occurring twice a week between 31 March 2014 and 18 August 2014. Traps were set at intervals of 7.5 m along interior walls of the warehouse, exterior walls of the warehouse, and surrounding the warehouse on three sides (North side was bounded by a road). Traps surrounding the warehouse were approximately 12 m, 18 m, and 24 m from the East, South, and West sides of the building, respectively. Traps were baited with a mixture of peanut butter and oats that were wrapped in wax paper and formed into packets. Cotton balls were added to traps when ambient temperature was below 10 °C to prevent trap-related mortality. On nights when trapping was to occur, traps were set between 2000 h and 2400 h and checked between 0600 h and 1000 h.

Captured mice were measured from nose to tail, weighed, sexed, aged (as juvenile or adult), and had their reproductive condition noted. Age was determined by visual assessment of captured mouse genitalia. Males with abdominal testes were considered juveniles and those with scrotal testes were considered adults. Females with an imperforate vagina were conserved juveniles and those with a perforate vagina were considered adults. After all morphological data were collected, mice were marked in each ear with an individually numbered size 1 Monel tag (National Band and Tag Co., Newport, Kentucky), and released at the point of capture. Throughout the study, any deceased mice were recorded by logging their discovered time, location, sex, and body weight before being removed from the study area. Although evaluating bait consumption was the primary focus of this study, attempts were made to estimate mortality levels during each phase of the study. Efforts to retrieve deceased mice involved periodic searches of the facility in all accessible areas inside the building and the grounds immediately surrounding it. Personnel working at the facility were also advised to report any deceased mice encountered.

Treatment schedule.

The treatment schedule for the four study phases is illustrated in Fig. 2.2 and was constructed around the temporal deployment of nontoxic and cholecalciferol bait. When bait was present, stations were serviced and bait weighed every two days using procedures described in the following section. Phase 1 began when nontoxic bait was added to the stations on 21 April 2014 and remained in the stations for 19 days until 10

May 2014. On 10 May 2104, the nontoxic bait was removed and stations were left empty for 8 days. Phase 2 began on 18 May 2014 when the treatment bait containing cholecalciferol was added to the stations. Cholecalciferol bait remained in the stations for 21 days until 8 June 2014. On this date, cholecalciferol bait was removed and the stations were left empty for 8 days. Phase 3 began on 16 June 2014 when nontoxic bait was returned to the stations for 36 days until 22 July 2014. On this day, stations were left empty for 2 days until 24 July 2014. Phase 4 began on 24 July when every other bait station was replaced with a Pro-Ketch® multiple catch mousetrap (Kness Mfg. Co., Inc., Albia, IA) for additional study goals outside the scope of this paper. The addition of these traps had a negligible impact on mouse abundance as only two mice were captured. In the remaining stations, cholecalciferol bait was returned and remained in the stations for 27 days until the study was completed on 20 August 2014.

Bait station service procedure.

For all phases of the study, bait stations were serviced using the following procedure. On the first day of each study phase the amount of bait placed in each station was weighed and recorded. Each bait station received two blocks of bait for every deployment (as per the product label), unless consumption in a station exceeded two blocks between service intervals. If consumption exceeded two blocks, additional blocks were added to ensure a sufficient amount of bait was available to last between service intervals. Every two days, stations were collected and the remaining bait weighed to calculate consumption. The remaining bait was returned to the station and weighed with

any additional bait needed to compensate for consumption. After service, stations were returned to their designated location. Bait that was not consumed within two weeks, or determined to be compromised was replaced when servicing the stations. Stations were left empty preceding each study phase to reduce pre-baiting effects and to monitor how mice responded to empty stations for an additional study.

Data analysis.

Analyzing consumption.

Consumption data was converted to mean consumption per station per day to make comparison between and within treatments meaningful. This conversion of consumption was executed by taking the total consumption of all stations from each service interval (~2 days), dividing it by the number of days for that interval, and then dividing that quotient by the number of stations present for that phase of the study (Phases 1-3 = 61 stations, Phase 4 = 32 stations). Unless otherwise indicated, all consumption numbers are reported in mean consumption per station per day. Comparison of consumption data between phases of the study was performed using ANOVA and Fisher's protected least significant difference test. Custom contrasts were constructed to test for significance between specific hypotheses.

All statistical testing assumed a two-sided alternative hypothesis, and $P \leq 0.05$ was considered evidence of statistically significant differences or effects. Analyses were performed using commercially available JMP 12 statistical software (SAS Institute Inc., Cary, NC).

Analyzing CMR data.

CMR data was analyzed using the Barker[16] robust design model with full heterogeneity. This model was selected because it allowed for the inclusion of auxiliary observations, including dead recoveries and re-sightings, and combines them with live captures under Pollock's[17] robust design framework which includes demographically closed primary occasions comprised of live recaptures during secondary occasions (>1). Inclusion of auxiliary data in these models has been shown to improve estimations of survival and abundance over traditional robust designs.[18] Three primary occasions were designated for this model and were constructed around times when the population best met closure assumptions. The three primary occasions adhered to the following timeframe: Occasion one consisted of 13 secondary occasions during Phase 1 including when bait stations were empty, occasion two consisted of nine secondary occasions during Phase 3 including when bait stations were empty, and occasion three consisted of three secondary occasions during the end of Phase 4 after no new deceased mice were recovered and consumption of bait had ceased. Since no re-sightings were collected during this study, the model was modified to set the probability of any re-sightings to 0 by fixing parameters *R* and *R'* to 0. Fixing these parameters allowed the model to functionally resemble a robust version of modeling theory described by Burnham[19] and similar to that used by Lindberg et al.[20] Capture data from the 50 traps surrounding the warehouse showed little evidence of mice immigrating into the population or emigrating from the warehouse to adjacent areas. Of the limited number of outside captures, almost

all were directly against the exterior of the building, and the majority of recaptures from these mice were either in the same location or in adjacent interior traps. Since little evidence of temporary emigration from the population was observed to support a source of immigration, availability parameters (α' and α'') were fixed to 0 and 1 respectively to exclude temporary emigration and immigration from the model. Permanent emigration was also excluded from the model by fixing fidelity (F) to 1.

Fourteen models were considered for analysis (See Table 2.1). Parameters for capture (p) and recapture (c) probability were modeled as either constant over time (ie. p), varying by primary occasion (ie. p_o), varying within primary occasion (ie. p_t), with behavioral effects ($p \neq c$), with heterogeneity (ie. p_h), or combinations thereof (ie. p_{hot}). Models that included heterogeneity used two mixtures and π was constant across all occasions. Support for each model was assessed using Akaike's Information Criterion and corrected for small sample size (AIC_c) and model 6 ($S_o p_h r \pi f 0_o$) was selected as the most supported model (Lowest AIC_c value) of the 14 candidate models (Table 2.1).[21-23] Analysis was done using package RMark ver. 2.2.2[24] to interface with MARK[25] executable files within program R ver. 3.3.2.[26]

Ethical note.

This study was conducted under an Animal Use Protocol approved by the Clemson University Institutional Animal Care and Use Committee.

RESULTS

Consumption results for each study phase are presented in Fig. 2.3 and Fig. 2.4.

Phase 1 nontoxic bait.

Consumption data for the first three service days of this phase was excluded from data analysis because the manufacturer's recommendations of two blocks per station resulted in a significant number of stations with no bait remaining when serviced. It took three service days of adding additional bait before consumption did not exceed the amount of bait present in these stations and consumption could be accurately monitored. During Phase 1 of the study mean consumption per station per day of the nontoxic bait was consistent across the phase and averaged 9.2 g/station/day (SD ± 0.5 g/station/day) (Fig. 2.3). Mortality recovered during Phase 1 totaled one mouse (Table 2.4).

Phase 2 cholecalciferol rodenticide.

Once nontoxic bait was replaced with cholecalciferol bait, consumption rates decreased immediately (Fig. 2.4). The first time stations were serviced after the bait change, consumption decreased to 4.5 g/station/day from 9.9 g/station/day on the last sampling of Phase 1. This is a 49% reduction in consumption when compared to the average consumption of the first treatment phase (Phase 1) with nontoxic bait. Subsequent samplings revealed a continued decline in consumption resulting in a mean consumption of 0.7 g/station/day (SD ± 1.4 g/station/day) (Fig. 2.3) for the treatment phase. Standard deviation during this phase was elevated due to the precipitous drop in

consumption during the first three samplings. After the third sampling, mean consumption leveled off at approximately 0.2 g/station/day and variation among samples was less than 0.1 g/station/day for the remainder of the phase. Mortality recovered during Phase 2 totaled 70 mice (Table 2.4).

Phase 3 return of nontoxic bait.

Nontoxic bait was returned to stations seven days after the removal of cholecalciferol bait. Mean consumption immediately rebounded from 0.2 g/station/day at the last sampling of Phase 2 to 1.4 g/station/day on the first sampling of this phase (Fig. 2.4). This rebound in consumption continued for the remainder of the treatment, resulting in a mean consumption of 2.3 g/station/day (SD ± 0.5 g/station/day) (Fig. 2.3). Mean consumption steadily increased over the first 6 samplings of this treatment from 1.4 g/station/day to 2.6 g/station/day and then leveled off for the remaining seven samplings at approximately 2.7 g/station/day. Mortality recovered during Phase 3 totaled seven mice (Table 2.4).

Phase 4 return of cholecalciferol.

The return of cholecalciferol bait during this phase revealed a similar pattern to the transition from Phase 1 (nontoxic) to Phase 2 (cholecalciferol). Consumption dropped drastically from the last sampling of Phase 3 to the first sampling of Phase 4 with consumption of 2.7 g/station/day to 1.7 g/station/day, respectively (Fig. 2.4). The mean consumption for Phase 4 was 0.2 g/station/day (SD ± 0.5 g/station/day) (Fig. 2.3).

Consumption from subsequent samplings during this phase declined from the first sampling of 1.7 g/station/day to 0.1 g/station/day by the third sampling. Mean consumption per station per day had a range of 0.3 g/station/day to 0.0 g/station/day over the remainder of the phase as consumption continued to decline until the study's completion. Mortality recovered during Phase 4 totaled 24 mice (Table 2.4).

Trends in consumption across phases.

Mean consumption of bait was significantly higher ($F = 393.33$, $P = <0.0001$, $df = 1, 35$) for nontoxic bait (mean = 4.5 g/station/day) relative to cholecalciferol bait (mean = 0.5 g/station/day) during the study. Between the four treatment phases, the only consumption results that were not significantly different ($F = 1.71$, $P = 0.1993$, $df = 1, 35$) were between the two like treatments involving cholecalciferol (phases 2 and 4) with treatment means of 0.72 and 0.24 g/station/day, respectively. When introduced to the nontoxic bait formulation, mice consumed more bait at more constant levels for the entire phase when compared to cholecalciferol bait (Figs. 2.3 and 2.4).

CMR population estimations.

Model estimates.

Model parameters were estimated from 415 captures of 183 unique individuals (Table 2.2). Model 6 ($S_o p_h r \pi f 0_o$) was selected as the most supported model with the lowest AIC_c score of the 14 candidate models (Table 2.1). This model had no behavioral effect but heterogeneity was included in the capture (p) and recapture (c) probability.

Population size (*N*) was estimated at 194, 74, and 6 individuals during Phase 1, Phase 3, and at the conclusion of Phase 4, respectively. Population size was not estimated for Phase 2 because this phase did not meet closure assumptions. Survival from Phase 1 to Phase 3 was estimated at 0.098. Survival estimation from Phase 3 to the end of Phase 4 resulted in a lack of convergence by the model due to insufficient data to estimate this parameter. Naïve survival for this time period was 0, as there were only three captures from two previously uncaptured individuals over the entirety of this final primary occasion.

CMR demographics.

A summary of population demographics for the all captures, including those used for model estimates are presented in Table 2.3. The most noteworthy change in population dynamics occurred with the sex ratio by showing a consistent trend of increasing male captures as phases progressed. Of all mice captured during Phase 1, there were approximately half as many males captured compared to females (m/f ratio of 0.56). By Phase 3 this ratio had changed to equal captures of males and females (ratio of 1.00), and by Phase 4 more males were captured than females (ratio of 1.38).

Recovered mortality demographics are presented in Table 2.4. Recovered mortality demographics reflected similar results from CMR data in respects to sex and age ratios. Markedly more females were recovered dead during Phase 2 (m/f ratio of 0.38) compared to later phases (ratio of 1.00 and 1.38 for Phases 3 and 4, respectively).

This corresponded to CMR findings of a female dominated population transitioning to a male heavy population after heavy female mortality from cholecalciferol treatments.

Known fate.

To provide additional insight into questions regarding conditioned aversion and behavioral avoidance during the study, CMR data was evaluated to investigate realized survival across treatment periods relative to bait exposure history. One hundred seventy individuals were trapped before the final week of Phase 2, suggesting a minimum of one week of potential cholecalciferol exposure if they remained faithful to the population. Of these 170 individuals, 18 were captured again from the start of Phase 3 to the completion of the study. Of these 18 individuals, nine mice were recovered dead after the start of Phase 4, presumably from ingestion of cholecalciferol. While the study design offered no method to be certain these nine individuals previously ingested a sublethal dose of cholecalciferol during Phase 2, they were at a minimum, subjected to one week of previous cholecalciferol baiting and five of the nine mice were present for the entirety of Phase 2.

DISCUSSION

Consumption of the bait containing cholecalciferol was significantly less than that of the nontoxic bait. Both times cholecalciferol bait was introduced, consumption immediately decreased significantly compared to the previous nontoxic phase. Reduction in consumption is expected when administering a lethal treatment that removes individuals from the population. However, the removal of individual mice from the population due to death from the cholecalciferol bait is not sufficient to explain the change in consumption depicted in this study. This is evident when comparing consumption between Phase 2 and Phase 3 (Figs. 2.3 and 2.4) and population estimates before and after Phase 2 (Table 2.2). Consumption levels in Phase 3 rebounded to levels much greater than Phase 2 and abundance estimates were 38% of the Phase 1 population, indicating that not all changes in consumption during Phase 2 were due to mortality. Instead, some other mode of action, such as a stop feeding effect due to cholecalciferol poisoning, likely caused the reduction in consumption.

While comparing total consumption of a rodenticide bait versus a nontoxic offering is important to develop an understanding of bait acceptance, this type of analysis alone is not effective in addressing the response of a population to the introduction of a rodenticide. Suppression of feeding (stop feeding) following ingestion of cholecalciferol and calciferol have been established in previous studies on mice and other commensal rodents.[4, 7, 11] These studies indicate that the stop feeding effect appears within the first several days following cholecalciferol exposure and is sustained throughout the time bait

is present. Results from this study support previous findings of other cholecalciferol rodenticide formulations on wild mice showing poor efficacy and suggest that following the recommended application instructions on the label (maintaining an uninterrupted supply of bait for 15 days or until feeding stops) for this cholecalciferol rodenticide is not an effective method of rodent control.[11, 27] The mechanism behind this behavioral reaction is not entirely known, although it is thought to be the result of mice developing a connection between toxicosis from cholecalciferol poisoning and ingestion of the treatment bait.[7] When rodenticide feeding ceases before target animals have consumed a lethal dose, this stop feeding effect becomes problematic. Sublethal doses of a rodenticide not only fall short of the task of killing the target species, they can also be the precursor to the development of bait shyness including conditioned bait aversion.[7, 12, 28-30] Reintroduction of the nontoxic bait after three weeks of cholecalciferol baiting indicated there was still a substantial number of individuals remaining in the population (approx. 25% of the pre-treatment population based on consumption comparisons and 38% based off CMR analysis from Phase 1 to Phase 3). Due to the availability of alternate food sources in the warehouse and the stop feeding effect, it is unlikely that leaving bait in the stations beyond the recommended two-week period would have been beneficial in obtaining additional consumption of cholecalciferol bait and subsequent mouse mortality. When nontoxic bait was returned to the stations during Phase 3, consumption rebounded indicating the stop feeding effect ceased. When cholecalciferol was returned during Phase 4, mice consumed bait at a similar proportion to when it was first introduced to the naïve population and then transitioned back to the stop feeding trend observed during

Phase 2. This observation of repeated initial consumption rates indicates that the majority of mice feeding during this subsequent cholecalciferol baiting were either naïve to previous exposure or mice originally exposed that did not develop a conditioned aversion. CMR data and mortality recovery analysis suggests that both of these circumstances were likely occurring.

The results from this study failed to support the presence of conditioned aversion to the formulation of cholecalciferol bait used in this study. Phase 4 consisted of a reintroduction of cholecalciferol bait to the mouse population after it had been removed 47 days prior and replaced with nontoxic bait for ~5 weeks. Upon its return, consumption of cholecalciferol bait followed a similar pattern of initial acceptance and subsequent rapid decline (stop feeding) as when it was first introduced to the naïve mouse population. Combining mean consumption per station of the first four sample days cholecalciferol bait was present (Phase 2) resulted in 56% of the consumption from the last sample of nontoxic bait (Phase 1). The same calculation from Phase 4 to Phase 3 revealed that mice were initially consuming the cholecalciferol bait at a rate equal to 84% of the previous nontoxic phase. This result further supports the argument for little conditioned aversion effect, as mice consumed a proportionally greater amount of cholecalciferol bait when it was reintroduced. Comparing total consumption between the same two intervals resulted in a 56% reduction in consumption from Phase 2 to Phase 1 and a 44% reduction from Phase 4 to Phase 3.

While consumption between initial exposure and reintroduction was similar, it must be considered that these results were obtained from a population that was not

completely closed. It is likely that competitive exclusion, births, and shifts in local movements by mice resulted in the addition of some mice naïve to cholecalciferol exposure, subsequently preventing the development of any direct conditioned aversion effect. However, based on comparisons between consumption of Phase 1 and Phase 3 and CMR data it was apparent that a sizable proportion of the original population was still present after the first round of cholecalciferol baiting and immigration from areas surrounding the warehouse was not likely occurring. Eighteen mice were known to have survived a minimum of one week's exposure to cholecalciferol from Phase 2 into Phase 3. The persistence of these pre-exposed mice and the consistency in consumption between the first and second time cholecalciferol bait was introduced indicate little to no evidence of conditioned aversion. It should also be noted that after completion of Phase 4, the mouse population in the test facility was nearly completely eradicated (evident by CMR trapping and sightings) and nine of the 18 mice previously exposed in Phase 2 were recovered dead after cholecalciferol was reintroduced in Phase 4. This makes the case of complete bait aversion highly unlikely since tagged individuals that survived the first treatment were ultimately not encountered after the final treatment phase or recovered dead, presumably from mortality associated with cholecalciferol consumption.

Bait avoidance whether it be from neophobia, a stop feeding effect, conditioned bait aversion, or some other behavioral mechanism is a common issue that often prevents successful rodent control.[2, 12, 31-34] Understanding and developing methods to overcome these types of behavioral avoidance are critical in maximizing control efforts. This study provides evidence that a stop feeding effect was observed after cholecalciferol exposure

but was reversed by rotating to a nontoxic formulation. This "bait and switch" rotation from a nontoxic bait → rodenticide → nontoxic → rodenticide shows promise in addressing some behavioral resistance traits. Possibilities for the ability to regain consumption in this study would be the inability of mice to retain knowledge of bait avoidance over a certain length of time, removal of dominant individuals allowing subordinates to feed,[32] or by reconditioning bait uptake with a nontoxic bait. Previous studies on deer mice (*Peromyscus maniculantus* Wagner) have shown promise in reconditioning previously exposed individuals to a neurotoxin rodenticide (Compound 1080) after delays in time and placebo offerings.[35] It is an established method for certain rodenticides, particularly acute toxicants, to "pre-bait" the population with a nontoxic bait to help offset bait shyness and increase initial uptake of the bait.[6, 36, 37] However, it is less common in practice to introduce a period of nontoxic baiting between two rodenticide applications. Subsequent studies designed to address either this time delay theory, competitor removal, bait reconditioning, or a combination of these factors are needed to further understand the mechanisms at play in this phenomenon.

CONCLUSIONS

Cholecalciferol bait applied according to label recommendations for 21 days reduced the population of *Mus musculus* L. by ~75% based on pre and post consumption data and ~62% based on CMR analysis. The proprietary organic approved formulation tested in this study did exhibit a similar stop feeding effect found by other studies testing previous cholecalciferol formulations and prevented effective control of the mouse population when following the label recommendations.[7, 11, 38] Consumption of the cholecalciferol bait was restored to proportionately similar levels compared to initial consumption after a period of no bait followed by nontoxic baiting. The ability to regain consumption using this "bait and switch" technique could be a method pest control operators utilize when they encounter baits that present a stop feeding effect and little conditioned aversion. During the second round of cholecalciferol baiting, mortality increased, bringing population abundance estimates down to approximately 3% of initial levels. With a limited number of rodent control options available around organic food production facilities, this formulation shows promise and potential for controlling house mouse populations so long as care is taken to address behavioral resistance.

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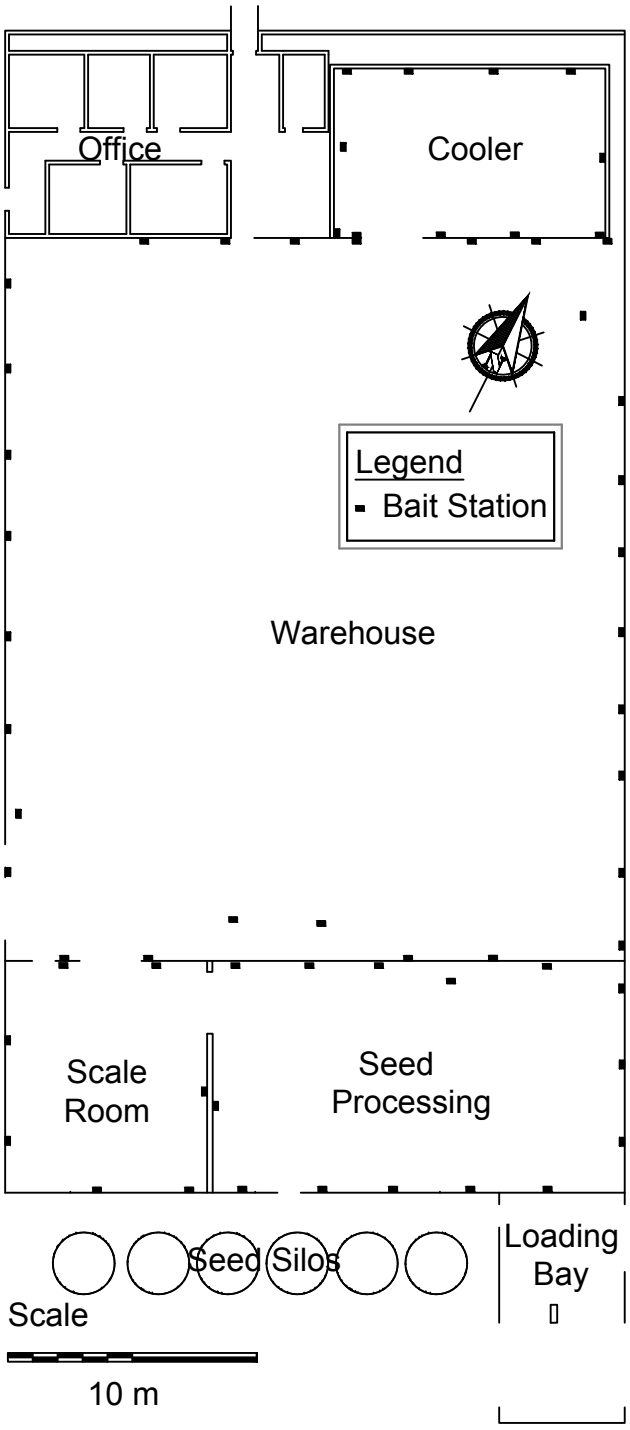

Figure 2.1. Diagram of the study site warehouse located in Pickens County, SC with the location of bait stations depicted along the interior of warehouse walls.

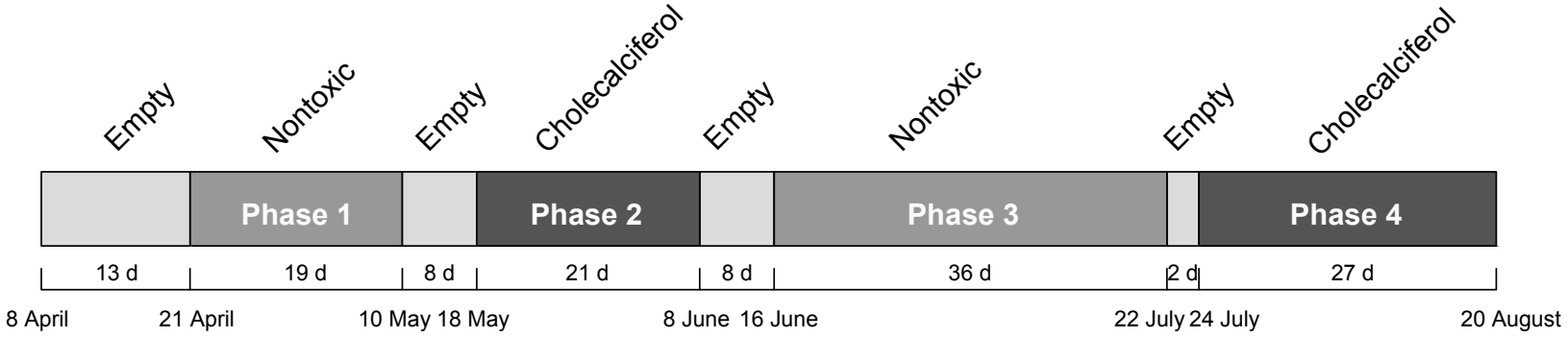

Figure 2.2. Timeline of the treatment schedule indicating the condition of bait stations, the designated study phases, and duration of each phase.

Table 2.1. CMR models considered for use to estimate abundance and survival for a wild *Mus musculus* population undergoing a treatment consisting of two temporal rotations from a nontoxic bait to a cholecalciferol rodenticide in a warehouse in Pickens County, SC

Model no.	Model name	No. parameters	Δ AICc
1	$S_o p r f0_o$	8	110.82
2	$S_o p_o r f0_o$	10	112.67
3	$S_o p_{ot} r f0_o$	33	132.85
4	$S_o p c r f0_o$	9	109.89
5	$S_o p_o c_o r f0_o$	13	114.21
6	$S_o p_h r \pi f0_o$	10	0
7	$S_o p_{ho} r \pi f0_o$	12	1.96
8	$S_o p_h c_h r \pi f0_o$	12	0.51
9	$S_o p_{ho} c_{ho} r \pi f0_o$	16	5.85
10	$S_o p_{ho} c r \pi f0_o$	13	99.90
11	$S_o p_h c r \pi f0_o$	11	100.95
12	$S_o p_h c_{ho} r \pi f0_o$	14	4.35
13	$S_o p_{ho} c_h r \pi f0_o$	14	2.38
14	$S_o p_{hot} r \pi f0_o$	35	19.92

Models used to estimate Survival (*S*), capture (*p*), recapture (*c*), recovery (*r*), and unmarked individual probability (*f0*) of house mice. Subscripts in model names indicate variation among primary periods (*o*) or variation within secondary occasions (*t*). Heterogeneity models used two mixtures and the probability an individual occurs in the mixture (π) was constant across all occasions. If *c* is not mentioned in the model name it is shared with *p* (no behavior effect). Fixed parameters are not mentioned in the model name. The number of parameters and the difference (Δ AICc) in AICc from the model with the lowest AICc are also presented.

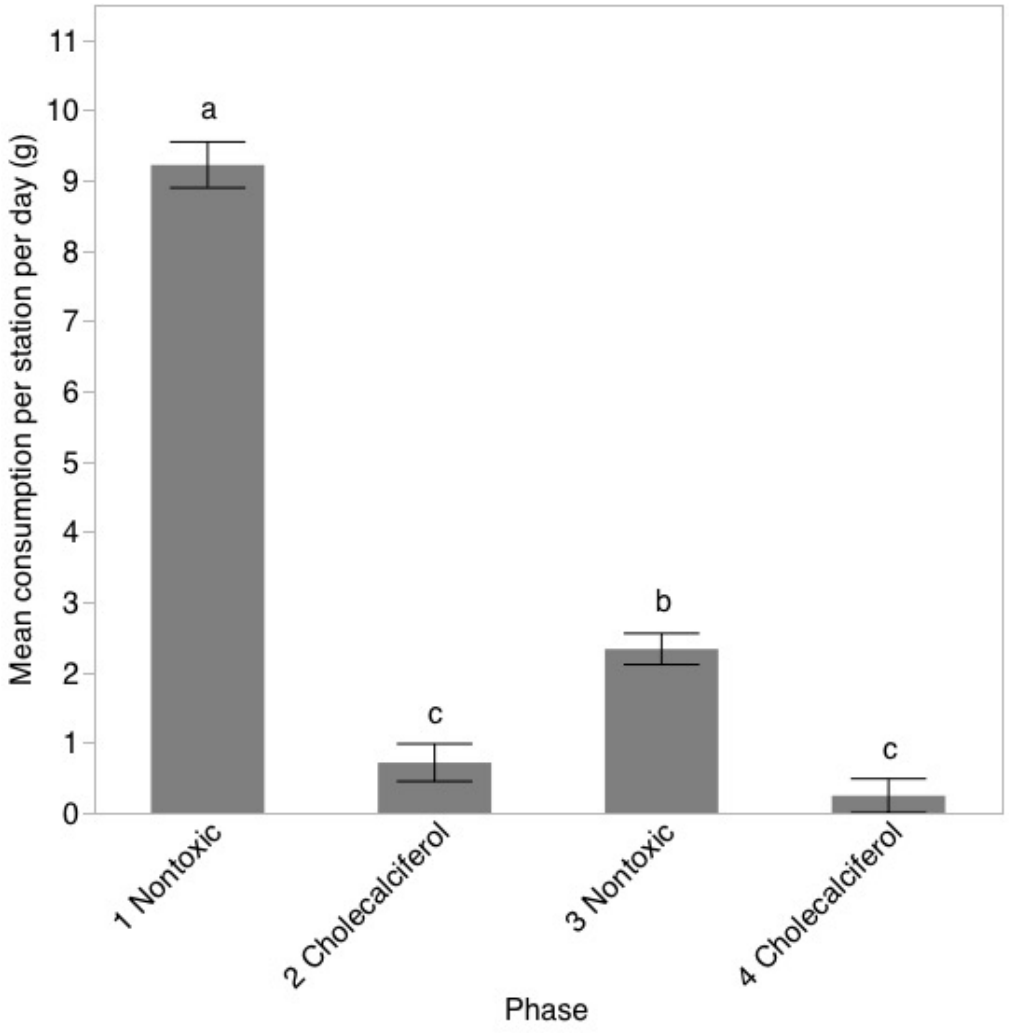

Figure 2.3. Comparison of mean consumption per station per day (± LSM SE) of deployed baits by a wild *Mus musculus* population for each study phase consisting of two temporal rotations from a nontoxic bait to a cholecalciferol rodenticide in a warehouse in Pickens County, SC. Phase means connected by the same letter are not significantly different based on ANOVA and Fisher's protected least significant difference test ($p \leq 0.05$).

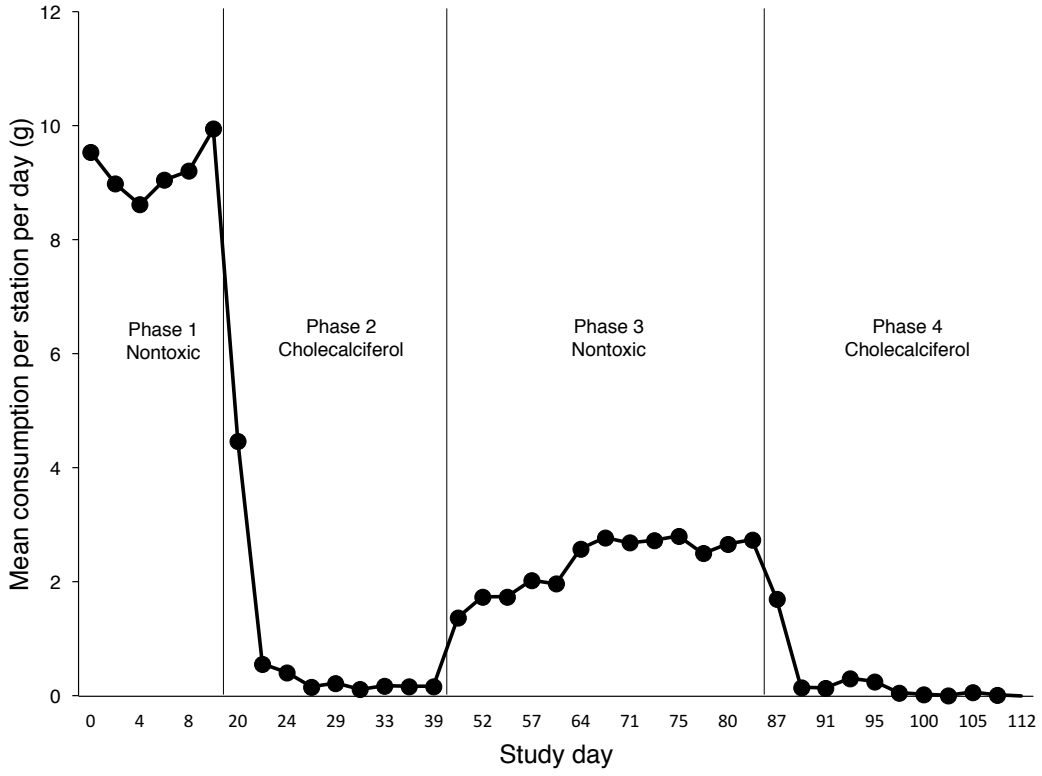

Figure 2.4. Temporal depiction in days from study inception of mean consumption per station per day of deployed baits by a wild *Mus musculus* population for each study phase consisting of two temporal rotations from a nontoxic bait to a cholecalciferol rodenticide in a warehouse in Pickens County, SC from 30 April 2014 to 20 August 2014.

Table 2.2. CMR model parameter estimates derived from model $S_o p_h r \pi f0_o$ using a robust design with three primary occasions including dead recoveries to estimate demographic parameters for a wild *Mus musculus* population undergoing a treatment consisting of two temporal rotations from a nontoxic bait to a cholecalciferol rodenticide in a warehouse in Pickens County, SC. Model $S_o p_h r \pi f0_o$ included survival (*S*) varying across primary occasions (*o*), capture probability (*p*) modeled with heterogeneity (*h*) and shared with recapture probability (*c*), recovery (*r*) and mixture occurrence (π) modeled as constants, and unmarked individuals (*f0*) varied by primary occasion

Parameter	Primary occasion	Estimate	SE	95% CI
S	1-2	0.098	0.030	0.053-0.175
	2-3	†	†	†
p mix 1	1-3	0.492	0.047	0.401-0.583
p mix 2	1-3	0.092	0.011	0.073-0.115
r	1-3	0.271	0.033	0.211-0.340
π	1-3	0.094	0.024	0.056-0.152
f0	1	50	13	24-75
	2	28	8	12-44
	3	4	4	0-11
N	1	194	-	174-227
	2	74	-	62-95
	3	6	-	3-22

Model probability estimates of Survival (*S*), capture (*p*)[‡], recovery (*r*), mixture occurrence (π), unmarked individuals (*f0*), and abundance (*N*) of house mice.

† Estimates are not reported due to lack of model convergence from insufficient data (observed survival was 0)

[‡] Capture probability (*p*) is shared with recapture (*c*) for this model.

Table 2.3. CMR demographics derived from the capture of wild *Mus musculus* undergoing a treatment consisting of two temporal rotations from a nontoxic bait (Phase 1 and 3) to a cholecalciferol rodenticide (Phase 2 and 4) in a warehouse in Pickens County, SC

Phase[†]	No. new captures	No. total captures	Sex ratio m/f	Age ratio j/a	Mean weight g[‡]	Mean body length mm[‡]
1	144	144	0.56	0.31	15.7	72.3
2	35	70	0.75	0.52	13.7	71.1
3	28	46	1.00	0.04	16.3	76.3
4	6	6	1.33	0.40	16.4	76.3

Demographic summary for captured mice.
[†] Phases 1 and 3 include occasions when bait stations were empty.
[‡] Capture weight and body length were taken at initial capture.

Table 2.4. Recovered mortality demographics derived from the recovery of wild *Mus musculus* undergoing a treatment consisting of two temporal rotations from a nontoxic bait (Phase 1 and 3) to a cholecalciferol rodenticide (Phase 2 and 4) in a warehouse in Pickens County, SC

Phase[†]	No. recoveries	No. tagged	Sex ratio m/f	Age ratio j/a
1	1	0	-	-
2	70	34	0.38	0.53
3	7	4	1.00	0.00
4	24	18	1.38	0.12

Not all recoveries were identifiable for sex and age.
[†] Phases 1 and 3 include occasions when bait stations were empty.

CHAPTER 3 - Analysis of wild house mouse (*Mus musculus* L.) interaction with bait stations while implementing two temporal rotations from empty stations to nontoxic bait to a cholecalciferol rodenticide

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ABSTRACT

BACKGROUND: Commensal rodents including the house mouse (*Mus musculus* L.) continue to pose a serious risk of damage and disease. Effective monitoring procedures and a better understanding of behavioral response to control measures have become advantageous as issues of public health and food safety become an elevated priority. The objective of this study was to analyze the behavioral response of house mice to bait stations through various phases of a baiting program. Eight distinct phases were constructed around a temporal progression from no stations to two rotations between empty stations, nontoxic bait, and a cholecalciferol rodenticide. Behavioral impact was estimated by analyzing the frequency and duration of mouse visits to stations during each phase.

RESULTS: Results suggested mice visited areas where bait stations were to be placed less frequently ($P = 0.0620$) than after bait station placement. The percentage of visits initially entering empty stations was significantly less than all other phases. Mice visited stations significantly less (frequency and duration) and consumed significantly less bait during phases offering cholecalciferol compared to phases offering nontoxic bait. The majority of the time mice spent in recording areas was inside a bait station and mice were seldom observed to cohabitate a bait station. When nontoxic bait was returned, mouse activity and consumption rebounded and subsequently declined when it was replaced with cholecalciferol, showing similar trends to the initial placement.

CONCLUSION: Results show that mice were curious to a novel bait station placed in their environment, but may not interact with it to the extent they would after

ample time to habituate. Cholecalciferol causes an aversion to consume bait, which is reflected by a reduction in both the frequency and the duration of visits mice make to a bait station. Although activity decreased, mice still visited bait stations when cholecalciferol was present, indicating that mice had a more pronounced aversion to the bait itself than to the bait station.

Keywords: rodent control, *Mus musculus*, organic, pest management, feeding behavior, monitoring

INTRODUCTION

Monitoring commensal rodent activity and behavioral response to methods aimed at controlling populations has been a crucial area of research since man's first efforts to eradicate these infestations.[1-3] When administering any rodenticide as a method for population control, a variety of factors can affect efficacy including behavioral resistance.[1-3] The adaptability and cunning nature of rodents have resulted in numerous accounts of various forms of behavioral resistance arising in these species.[1, 4] In general, the prominent commensal rat species, the Norway rat (*Rattus norvegicus* Berkenhout) and the roof rat (*Rattus rattus* Linnaeus), are considered to show increased behavioral resistance towards control measures compared to the house mouse (*Mus musculus* Linnaeus). However, there is evidence showing elevated levels of behavioral resistance in wild populations of mice compared to laboratory strains and heterogeneity in individual trapability has been observed.[5, 6] A lack of knowledge on how control measures affect commensal rodent behavior is further exacerbated by the complexities of gathering and interpreting behavioral data which impedes the development of successful control strategies. A better understanding of the behavioral interaction between rodents and the devices used to control their populations should facilitate the development of more effective control programs. Efforts made to observe *R. norvegicus* feeding patterns and behaviors in and around bait stations using video surveillance have yielded intriguing results regarding the frequency, timing, and duration of visits, as well as interactions between rodents.[7-12] A similar study observing *R. rattus* behavior utilized recorded video to monitor visits and feeding habits for various bait station types facilitating the

identification of which type station rats preferred.[13] Efforts to observe *M. musculus* interactions with control devices in naturally occurring populations are limited in the literature and have primarily been employed for general observation or in controlled environments.[14, 15] The use of video as a monitoring tool not only allows for monitoring of the population, but it also provides the unique benefit of real time direct observation of rodent behavior while being non-intrusive.

Beginning in the 1960s, rodenticides targeting commensal rodents were required by the U.S. Department of Agriculture to include labels stating that baits must be contained within "tamper-proof bait boxes" when placed in locations accessible by children and nontarget animals.[16] As a result, the use of tamper-proof bait stations has become a common practice. These bait stations are enclosed boxes generally molded from plastic with holes on each end allowing rodent entry. Internal to the station are usually metal rods or a tray designed to hold the majority of commercially available rodenticides. The design of these stations has been criticized in the literature for a tendency to focus on reducing the risk of pesticide hazards and user friendliness instead of increased efficacy.[16, 17] While efforts have been made to describe the effect bait stations have on rodent behavior, little has been done to quantify the frequency and duration of time *M. musculus* spend in and around these devices using direct observation. In addition to a behavioral response towards empty bait stations, it is also important to observe interactions with these devices, taking into consideration what type of bait is offered inside the station and the exposure history of the population. In this study, a temporal progression from no station to empty stations and then a temporal rotation from

nontoxic bait → rodenticide → nontoxic → rodenticide was implemented to observe the behavioral response of mice to a variety of possible treatment conditions.

Previous studies have observed a "stop feeding" effect following the use of calciferol rodenticides.[18-22] This avoidance behavior is characterized by a drastic reduction in consumption of bait within one to three days post placement and is thought to be the result of mice developing a connection between toxicosis from sublethal calciferol exposure and ingestion of the treatment bait.[19] Decreased efficacy in calciferol rodenticides has been attributed to this suppression of feeding.[19, 22] It has been suggested that mice still visit stations while this stop feeding effect is ongoing, however, no efforts have been made to evaluate the impact this avoidance has on the frequency and duration of visits to bait stations.

Various methods have been utilized to gather information to aid in the development of more effective control programs. The majority of past methods used to monitor rodent activity in and around areas where control programs are underway have relied on collecting information from census techniques that do not allow direct observation of animal behavior. These methods include techniques such as analyzing rodenticide consumption, monitoring movement with tracking boards, fluorescent powders, capture-mark-recapture, PIT tagging, still imagery, and others.[23] These methods are useful in and of themselves, but do not offer the same level of observational information provided through video analysis. Advancements in infrared technology and digital camera sensor resolution have made it possible for these types of observations to be made in more locations at a more accessible price point. To date, limited examples of

commensal rodent behavior monitored though video surveillance have been available in the literature, especially recordings of free-ranging wild populations.

This study was designed to accomplish the following objectives. First, to observe the behavioral response of a naturally occurring mouse population to bait stations and a temporal rodenticide treatment rotation by analyzing frequency of visits, timing of visits, and duration of visits. Second, to examine the practicality of using video surveillance equipment as a method to observe *M. musculus* under praxis conditions.

MATERIALS AND METHODS

Study area.

This study was conducted from 27 February to 20 August 2014. The location of the study was a warehouse used to store and process agricultural seeds in Pickens County, South Carolina (34° 65′ N, 82° 50′ W). The selected test site offered a location with an existing mouse population that was naïve to any prior testing or recent control measures. Previous attempts to control the mouse population included low intensity baiting with anticoagulant formulations which ceased more than 6 months before the study start date. Observational inspections and live trapping prior to the study indicated an active infestation of mice with regular sightings of mice, mouse droppings, damaged bags and seeds, nesting sites, and damage to the building from gnawing.

The warehouse was constructed of steel I-beam framing with metal siding over a concrete slab floor. The dimensions of the building were 46 m long and 24 m wide, with the majority of the building in an open floor plan containing seed processing equipment, pallets of seeds and bags, and other miscellaneous supplies (Fig. 3.1). These goods were distributed around the perimeter of the warehouse leaving a large open expanse of concrete slab in the middle section of the floor that mice were not likely to traverse (verified by video surveillance and mouse sign). An 8 m section on one end of the building contained climate controlled office space and a walk-in cooler set to approximately 5 °C. Packages of seed on pallets were also housed in the cooler, and numerous mice were observed to travel in and out of this area freely. The remainder of the building had no climate control. There were minimal changes to the warehouse during

the study due to normal management activity. Access to spilled feed and grains was ubiquitous throughout the study. Therefore, food availability was not thought to be a limiting factor on the mouse population.

Observation areas.

Four rectangular observation areas 35 x 95 cm each were designated in areas along the interior of the perimeter warehouse walls in locations where mice activity was observed and where bait stations would be placed later in the study (Fig. 3.1). The boundary of each observation area was marked on the floor with blue tape prior to the placement of bait stations. The tape was placed a week prior to data collection and no alteration of mouse activity was observed after it was added. A varifocal video camera was placed a minimum of one meter above each observation area and adjusted so that each observation area appeared similar in scale and aspect on the monitoring display (Fig. 3.2).

Bait stations.

Sixty-one commercially available Aegis-RP® (Liphatech, Inc., Milwaukee, WI) bait stations were distributed around the inside perimeter of the warehouse (Fig. 3.1). The tamper resistant stations were made of high-impact plastic with one entrance hole on each side and measured approximately 32 cm in length, 20 cm in width and 10 cm in height. These stations were placed according to the manufacturers recommendations and spaced approximately 2.5 to 3.5 m from adjacent bait stations and placed either directly

against the wall or as close to the wall as possible if obstructions were present in that location. Care was taken to assure the stations were placed so that the entry holes were adjacent and parallel to the wall. Bait stations were placed two weeks prior to any bait application to monitor the response of mice to their presence. Each bait station was uniquely numbered and was designated a specific location throughout the entirety of the study.

Details of baits tested.

Treatment baits in each phase of the study were placed on metal rods included with the bait stations to prevent premature spoilage of the bait and make it more accessible to mice. The nontoxic bait used in this study, DETEX®, was selected because it was manufactured by the same company as the treatment bait (Bell Laboratories, Inc., Madison, WI) and was found to be of similar formulation and size. Each block of nontoxic bait was yellow, rectangular in form measuring 47 x 25 x 25 mm, and weighed approximately 20.5 g. Individual blocks of cholecalciferol bait, Terad$_3$ Ag BLOX® (Bell Laboratories, Inc., Madison, WI), were also rectangular in form measuring 45 x 25 x 25mm, weighed approximately 28.5 g, and were brown in color. Cholecalciferol was formulated into the bait at a rate of 750 mg $\times$ kg^{-1}. Baits were not altered from their commercially available form, and previous studies have shown no statistically significant differences in levels of consumption related to varying bait colors.[24] LD_{50} for cholecalciferol has been determined for the ICR strain of *M. musculus* at 42.5 mg x kg^{-1}

translating to consumption of 1.13 g of the cholecalciferol bait needed to achieve the LD_{50} dose for a 20 g mouse.[25]

Details of video surveillance.

Six closed-circuit surveillance cameras were used during the study. Initially, four high definition cameras were placed on 27 February 2014, almost a month prior to data collection, to allow for mice to habituate to their presence in the warehouse environment. These cameras were mounted directly above four bait stations that were placed along the inside of the exterior walls of the warehouse and distributed towards the corners of the building where they remained for the entirety of the study (Fig. 3.1). The cameras were IP68 rated weatherproof dome cameras with a resolution of 1920 x 1080 pixels. Each camera was fitted a 2.8-12 mm varifocal lens that allowed for the proper framing necessary to clearly visualize individual mice and provide a similar scaled view for each observation area. Infrared LEDs provided illumination allowing rodent activity to be monitored in all lighting conditions. Two additional cameras were placed on 15 April 2014 to observe additional mouse movements and behavior in various areas of interest around the study area. These cameras were identical except for the resolution of the sensors being 976 x 582 pixels. Cameras were connected via coaxial cable to a central hard disk recording device and monitoring screen located in an office adjacent to the treatment area allowing for discrete observation of the population. Video was recorded on the hard disk within the monitoring device and exported to external storage devices to

be analyzed on a proprietary software program that allowed playback of the recorded footage on a computer.

Video observation schedule.

The study was divided into eight phases based on the presence or absence of a bait station and the type of bait contained within the station during two temporal rotations from a nontoxic bait to a cholecalciferol rodenticide (Fig. 3.3). All phases consisted of 4 sampling nights separated by approximately 4-day intervals except for Phase 4 and Phase 6 which consisted of 2 sampling nights. Only video recorded during the night was selected for analysis due to possible alteration in mouse behavior during the day from warehouse activities. On nights selected to analyze video in each phase, four sampling intervals were derived to analyze each night's activities adhering to the following schedule: 21:00-21:15, 00:00-00:15, 03:00-03:15 and 6:00-06:15. These times were selected after watching several complete nights of video and quantifying mouse activity throughout the entire night. It was determined by analyzing variation in the frequency and duration of mouse visits that these sampling intervals adequately represented mouse activities for the entire night.

Phase 1 started preceding the placement of bait stations into the observation areas. This allowed for observation of mouse activity prior to any alterations of the study site except for the tape on the floor used to designate the observation areas. Phase 2 began when empty bait stations were deployed inside the observation areas. Phase 3 began when nontoxic bait was added to the bait stations. Phase 4 began when bait was removed

from the stations rendering them empty. Phase 5 began when the treatment bait containing cholecalciferol was added to the bait stations. Phase 6 began when the cholecalciferol bait was removed from the stations rendering them empty. Phase 7 began when nontoxic bait was reintroduced into the stations. The final phase, Phase 8, began when cholecalciferol was reintroduced to the stations.

Video analysis.

Video was analyzed by two trained individuals watching recorded footage on proprietary video playback software. Viewers were trained by the same individual and were found to produce nearly identical results after comparing analysis of identical test footage. The software allowed for simultaneous viewing on a single screen of all four stations, two stations, or individual stations. Recorded video could be played as fast as 16x real time for rapid review, to as slow as 1/16x real time, and frame by frame, allowing for detailed observation and analysis of mouse movements. Timestamps accurate down to one-hundredth of a second were overlaid on each viewing screen and were used to calculate timing and duration of events.

Video was analyzed using the following procedure. Duration of a visit was initiated when half the body of a mouse entered into the observation area and ceased when half of the mouse exited the observation area. In addition to recording duration in the observation area, duration was also quantified for time mice spent in a bait station and was defined by the time when half the body of a mouse entered into the bait station until half of the mouse exited the bait station. If a mouse exited the bait station and reentered

the bait station before leaving the observation area, that time was added to the time of previous visits into the bait station for that specific visitation into the observation area for a total duration. If mice present during a sampling interval remained inside the observation area after the time allotted for that interval, the activities for these mice were recorded until they exited the observation area. Analyzing video using the above procedure allowed for several types of data to be calculated including the following:

- Frequency of visits to the observation areas
- Time of visits to the observation areas
- Duration of visits to the observation areas
- Duration of visits inside bait stations
- Duration inside the observation areas but not inside bait stations
- Percentage of visits into the observation areas resulting in bait station entry

Bait station service procedure.

Bait stations were serviced using the following procedure. On the first day of each study phase the amount of bait placed in each station was weighed and recorded. Each bait station received two blocks of bait throughout the study (as per the product label), unless consumption in a station exceeded two blocks between service intervals. If consumption exceeded two blocks, additional blocks were added to ensure a sufficient amount of bait was available to last between service intervals. Every 2 days stations were collected and remaining bait weighed to calculate consumption. The remaining bait was returned to the station and weighed with any additional bait needed to compensate for

consumption. After service, stations were returned to their designated location. Bait that was not consumed within 2 weeks, or determined to be compromised was replaced when servicing the stations.

Deceased mice were recorded by logging their discovered time, location, and body weight before being removed from the study area and disposed of properly. Efforts to retrieve deceased mice involved periodic searches of the facility in all accessible areas inside the building and the grounds immediately surrounding it. Personnel working at the facility were also advised to report any deceased mice encountered.

Data analysis.

Comparison of data for the frequency, duration, and timing of mouse visits during the study was done using ANOVA and Fisher's protected least significant difference test. Custom contrasts were constructed to test for significance between specific hypothesis. All statistical testing assumed a two-sided alternative hypotheses, and $P \leq 0.05$ was considered evidence of statistically significant differences or effects. Analyses were performed using commercially available JMP 12 statistical software (SAS Institute Inc., Cary, NC).

RESULTS

A summary of mouse activity within the observation areas for each phase of the study is presented in Table 3.1.

Frequency of mouse visits.

A summary of the frequency of visits into the observation areas for each phase of the study is presented in Table 3.1 and graphically in Fig. 3.4. Across all study phases, mice entered into the observation areas 437 times. Phase had a significant effect on the frequency of visits by mice into the observation areas ($F = 4.9533$, $P = 0.0022$, $df = 7, 20$). Frequency of visits by bait type differed significantly ($F = 15.8522$, $P = 0.0007$, $df = 1, 20$) with mean visits per night of 20.3 and 8.5 for nontoxic and cholecalciferol baits, respectively. Frequency of visits did not differ between the nontoxic bait and empty bait stations ($F = 0.1088$, $P = 0.7450$, $df = 1, 20$) with mean visits per night of 20.3 and 19.6 for nontoxic and empty bait stations respectively. A specific contrast comparing the frequency of visits between Phase 1 (no stations) and Phase 2 (empty stations) was suggestive of significance ($F = 3.9074$, $P = 0.0620$, $df = 1, 20$) with means of 12.5 and 20.8 visits per night, respectively. Specific contrast between Phase 4 (empty stations) and Phase 5 (cholecalciferol) revealed a significant decrease ($F = 13.0989$, $P = 0.0017$, $df = 1, 20$) in the frequency of visits with means of 28 and 9.5 visits per night for the respective phases. Frequency of visits elevated from a mean of 9 visits per night during Phase 6 (empty stations) to a mean of 17 visits per night during Phase 7 with the reintroduction of the nontoxic bait. However, specific contrast between Phase 6 and

Phase 7 showed that the elevation was only suggestive of significance ($F = 2.4495$, $P = 0.1333$, $df = 1, 20$). The study came to completion after the cholecalciferol bait was added back to the stations during Phase 8, showing an initial sustained number of visits and then quickly falling to no visits by the end of the phase. The mean number of visits for Phase 8 was 7.5 visits per night.

Duration of visits.

The duration of mouse visits into the observation areas were categorized into three different measures: duration spent in observation areas, duration spent in bait stations, and duration spent in observation areas, but not in bait stations. Durations are reported as the mean of all visits for each sampling night and are presented in Table 3.1.

Duration spent in observation areas.

A summary of mean duration spent in the observation areas for each phase of the study is presented in Table 3.1. The mean duration spent in the observation across all phases of the study ranged from 5 (Phase 1, no stations) to 118 s (Phase 7, nontoxic). Contrast revealed that phases offering nontoxic bait (Phase 3 and 7) had a significantly higher mean duration of visits when compared to phases with no station (Phase 1), empty bait stations (phases 2, 4, 6) or cholecalciferol bait (Phase 5 and 8) ($F = 4.6319$, $P = 0.0438$, $df = 1, 20$). The mean duration mice spent in observation areas was 5, 7, 82, and 11 s for phases with no station, empty stations, nontoxic bait, and cholecalciferol bait, respectively. There was no significant difference between the duration mice spent in the

observation areas for phases with empty stations and cholecalciferol bait (F = 0.0267, P = 0.8718, df = 1, 20).

Duration spent in bait stations.

A summary of mean duration spent in bait stations for each phase of the study is presented in Table 3.1, and Fig. 3.5. Contrast revealed that phases offering nontoxic bait (Phase 3 and 7) had significantly higher mean duration of visits when compared to phases with cholecalciferol bait (Phase 5 and 8) and empty stations (Phase 2, 4, and 6) (F = 10.0607, P = 0.0056, df = 1, 17). The mean duration spent in the observation areas was not significantly different between phases with empty bait stations and cholecalciferol bait (F = 0.0004, P = 0.9849, df = 1, 17). Nontoxic phases (Phase 3 and Phase 7) were the only phases where mice remained inside stations for longer than 60 s and visits of this duration comprised approximate 25% of all visits for both of these phases.

Duration spent in observation areas not in bait stations.

A summary of mean duration spent in the observation area and not in a bait station for each phase of the study is presented in Table 3.1. Durations did not differ between study phases (F = 1.1648, P = 0.3696, df = 6, 17). For all study phases mean duration spent in the observation areas but not in a bait station ranged from 3 to 11 s. The vast majority of time mice spent in the observation area they were inside a bait station, represented by a strong correlation between the two durations (r = 0.991).

Percentage of visits entering bait stations.

Rather than conducting and interpreting an overall test of differences among the percentage of visits into the observation areas across all phases (Table 3.1); contrasts were constructed to address the question of a possible neophobic reaction to the presence of a bait station. Specifically, the following scenarios were addressed: First, the percentage of visits entering a bait station when the stations were first placed empty (Phase 2) was compared to all other phases. Second, the percentage of visits entering a bait station during Phase 2 was compared to other phases with empty stations (Phase 4 and 6). Results from these contrasts showed that the percentage of visits resulting in mice entering a bait station was significantly different ($F = 7.7247$, $P = 0.0134$, $df = 1, 16$) between Phase 2 and all other phases and between Phase 2 and other phases with empty stations ($F = 7.7782$, $P = 0.0131$, $df = 1, 16$). Differences in the percentage of visits entering a bait station by bait type were not significantly different ($F = 0.0001$, $P = 0.9908$, $df = 1, 16$).

Co-visitation.

Across all study phases there were only nine times (2% of all visits (437)) when multiple mice (two in all cases) were present in the observation area at the same time. All nine of these events occurred during nontoxic bait phases (3 and 7). Of these nine visits, five resulted in both mice utilizing the bait station at the same time and had durations of 1, 13, 20, 45, and 758 s.

Bait consumption.

Results for mean bait consumed per day is presented in Table 3.1 and visually compared to mean duration of time spent in bait stations in (Fig. 3.6). Phase had a significant effect on mean bait consumed per station per day ($F = 185.2778$, $P = <0.0001$, $df = 3, 35$). Both phases offering nontoxic bait (Phases 3 and 7) showed significantly more consumption ($F = 393.3341$, $P = 0.0001$, $df = 1, 35$) than cholecalciferol phases with treatment means of 9.22 and 2.33 g/station/day, respectively. Between the four phases when bait was present, the only consumption results that were not significantly different ($F = 1.7114$, $P = 0.1993$, $df = 1, 35$) were between the two treatments involving cholecalciferol (Phase 5 and 8) with treatments means of 0.72 and 0.24 g/station/day, respectively.

DISCUSSION

Mice have been described as exhibiting limited neophobic behavior compared to the commensal rat species (*R. novegicus* and *R. rattus*).[1] Some authors have even described mice as being neophilic in certain situations.[6, 26] To the contrary, however, some studies have demonstrated that wild mice have an increased propensity to display neophobic behaviors towards novel food items compared to laboratory strains.[5] Although previous studies have observed an elevated level of neophobia in wild mice when selecting food items, some caution may be necessary in extrapolating that claim towards a novel object containing food (e.g. bait stations). Results from this study demonstrated that mice were not fearful of approaching the bait station (frequency of visits), but were initially more reluctant to actually enter the station (percentage of visits entering the station), i.e., curious but cautious. Furthermore, contrast analysis indicated results suggestive of significance ($F = 3.9074$, $P = 0.0620$, $df = 1, 20$) for an increased frequency in the number of visits during Phase 2 (empty stations) compared to Phase 1 (no stations) supporting arguments describing mice as curious and explorative when presented with novel items. To the contrary, however, results indicate that although mice visited the observation areas at a suggested greater frequency following the placement of bait stations, the percentage of visits entering a bait station (Table 3.1) were the lowest for Phase 2 (56.7%) compared to all other phases, and significantly less than the average of all other phases with empty bait stations ($F = 7.7782$, $P = 0.0131$, $df = 1, 16$) and all other phases ($F = 7.7247$, $P = 0.0134$, $df = 1, 16$). This reveals that while explorative, mice may be reluctant to interact with a novel bait station to the same degree as they may after

having time to habituate. The placement of nontoxic bait inside the stations did not elicit a neophobic response as the frequency of visits to stations remained similar between Phase 2 (20.8 mean visits) and Phase 3 (23.5 mean visits).

The introduction of cholecalciferol bait in Phase 5 caused a significant reduction ($F = 16.9054$, $P = 0.0005$, $df = 1, 20$) in the frequency of visits to observation areas compared to previous phases when bait stations were present (Phase 2, 3, and 4) and in bait consumption ($F = 406.8557$, $P = <0.0001$, $df = 1, 35$) compared to when nontoxic bait was offered previously (Phase 3). This reduction in the frequency of visits can be partially explained by mortality caused by the cholecalciferol bait, although not in entirety as the frequency of visits began to drop before mortality was observed and visits did elevate later during Phase 7 when nontoxic bait was returned. Furthermore, the difference in the mean amount of time mice spent in bait stations (49 to 2 s) from Phase 3 (nontoxic) to Phase 5 (cholecalciferol) respectively, suggests that mice were not only visiting less frequently, but each visit was also of a shorter duration. Proportionally, mice were visiting the stations at a greater frequency during Phase 5 (40% of Phase 3) compared the reduction in mean duration in the stations (4% of Phase 3). This suggests that although mice did visit the stations less frequently after cholecalciferol bait was offered, the duration of visits was reduced to a greater degree than frequency. This same phenomenon was observed when comparing the frequency and duration of visits between the second deployment of cholecalciferol bait (Phase 8) and the preceding nontoxic phase (Phase 7). In essence, mice were less likely to stop and spend time inside stations but were not altogether avoiding them. While there was no direct method to observe mouse

activity inside stations during these visits of varying duration, comparisons between the frequency of visits, duration of visits, and bait consumption (Chapter 2) reveal that the duration of visits was more indicative of the amount of bait consumed than frequency of visits suggesting mice were using some of this additional time to feed.

An overall reduction in mouse activity was observed during phases that offered cholecalciferol bait. This reduction in activity was apparent from analyzing the frequency and duration of visits into the observation areas, bait stations, and in bait consumption. However, the reintroduction of the nontoxic bait between the phases offering cholecalciferol was successful in reversing this reduction in observed activity. The increase in the frequency of visits was suggestive of significance ($F = 3.2293$, $P = <0.0875$, $df = 1, 20$) and the increase in duration of time spent in bait stations was significant ($F = 7.8113$, $P = <0.0124$, $df = 1, 17$) from Phase 5 (cholecalciferol) to Phase 7 when nontoxic bait was reintroduced. This increase in observed activity between these two phases was also associated with a significant elevation in bait consumption ($F = 21.7052$, $P = <0.0001$, $df = 1, 35$) from Phase 5 (cholecalciferol) to Phase 7 (nontoxic).

The use of video surveillance equipment proved to be a reliable and effective method to monitor mice during this study. The system did not require any special knowledge to operate or an excessive investment of time to install. Infrared illumination from the cameras provided ample illumination to clearly monitor rodent activities in low lighting conditions. The inclusion of a variable focus lens on each camera proved to be critical when trying to frame a certain area for monitoring when mounting options were limited. The development of a method to analyze recorded video using object

recognition software would make this method much more practical and effective although initial investments would be significant. Previous studies utilizing lab strains of *R. norvegicus* were successful in analyzing movements via video analysis software using algorithms for movements and image color thresholding.[27] However, further development of these technique into a system to quantify rodent activity and behavior for field use and monitoring are still lacking.

CONCLUSIONS

The introduction of empty bait stations into a wild mouse population resulted in an increase (suggestive of significance (P = 0.0620)) in the frequency of visits by mice into observation areas when compared to a prior phase with no bait stations present, suggesting that mice were curious to the novel item and it was not causing a neophobic response that prevented them from approaching the station. However, although results suggested mice were visiting more frequently after bait stations were deployed, mice were initially entering empty bait stations less frequently than subsequent phases with baited stations and empty stations. This suggests that while mice were inclined to approach a novel item, they may be less likely to interact with it to the same degree as they would after given time to habituate or associate it with a safe food source.

Mice had a clear aversion to consume cholecalciferol bait and the reduction was correlated with both the frequency and duration of visits to bait stations. While the frequency of visits was significantly reduced after cholecalciferol bait was added, the duration of visits was reduced to a greater degree suggesting that the duration of time mice spend inside the station is more indicative of feeding than the frequency of visits. Results also revealed that the precipitous decrease in consumption of cholecalciferol was not reflected to the same degree in frequency of visits, signifying that this reduction in consumption was not entirely caused by mice avoiding the stations but an aversion to consume the cholecalciferol bait and spend time inside the stations. This study also demonstrates that a reduction in frequency and duration of visits was associated with the "stop feeding" activity of the cholecalciferol treatment but could be terminated by a

subsequent nontoxic baiting, thereby increasing the frequency and duration of visits as well as consumption. By analyzing the trend in mouse activity when cholecalciferol bait was returned to the stations, a similar pattern was observed to the first placement, supporting our conclusion that the cholecalciferol bait caused a reduction in frequency and duration of visits with duration being impacted to a greater degree. The results of this study suggest that the reduction in efficacy by the cholecalciferol bait was more closely associated with mice developing an aversion to the bait itself, thus reducing consumption and the duration of time they spend in stations, than an aversion specific to the bait station.

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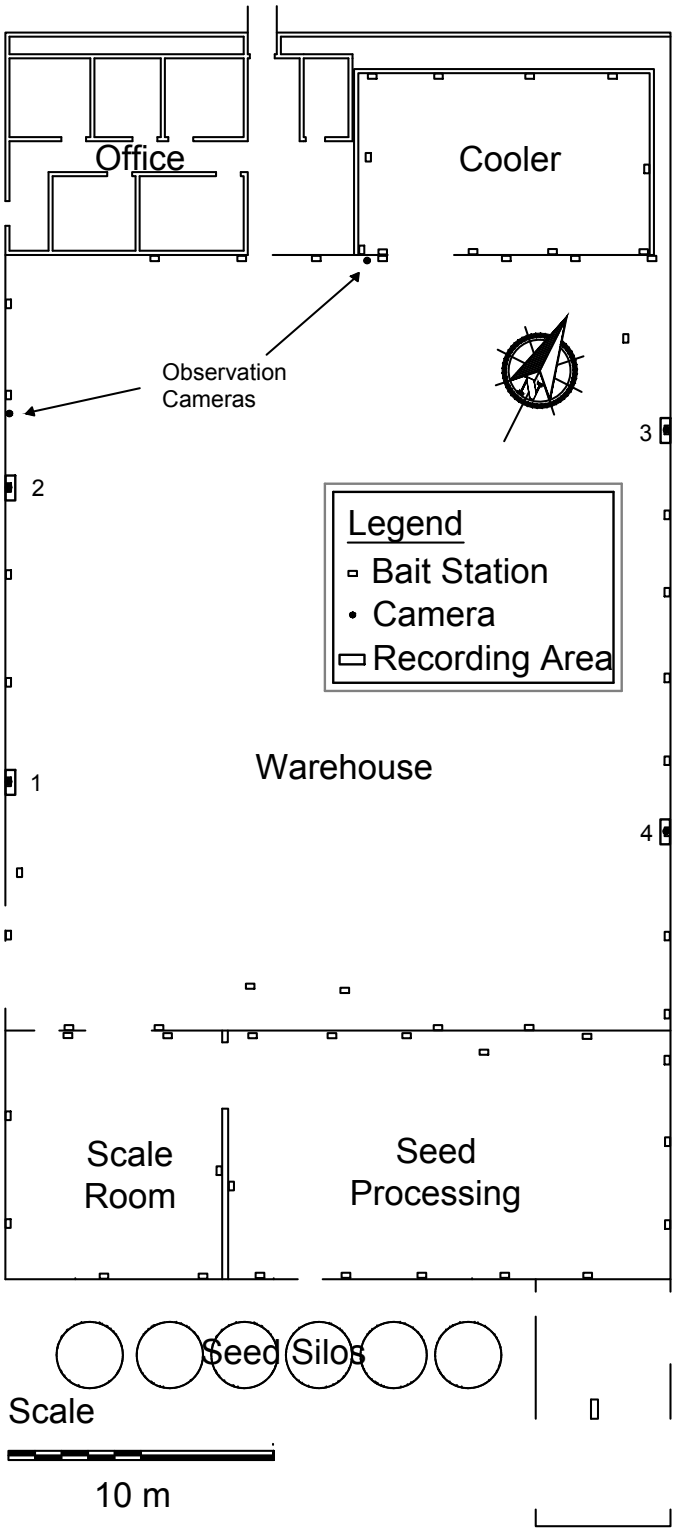

Figure 3.1. Diagram of the study site warehouse located in Pickens County, SC with the location of bait stations, observation areas, and cameras indicated.

Figure 3.2. View of bait station placement in the observation areas on the monitoring device with time stamp overlays used to analyze *Mus musculus* activities inside the observation areas.

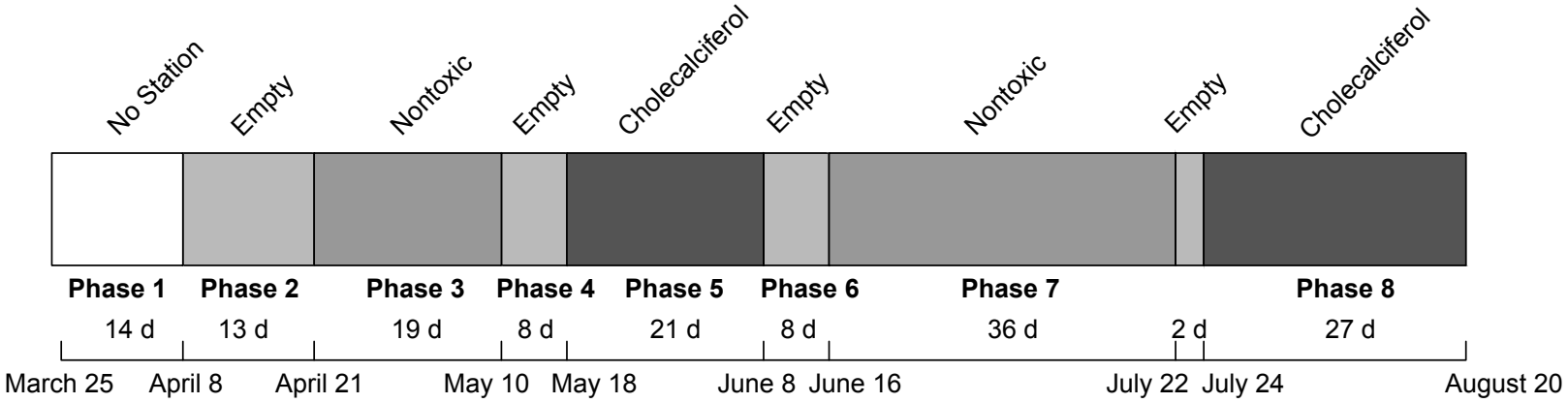

Figure 3.3. Treatment schedule indicating each study phase, condition of bait stations, duration of each phase, and date for a study analyzing wild *Mus musculus* activities around bait stations in a warehouse in Pickens County, SC.

Table 3.1. Summary of results from a study located in a warehouse in Pickens County, SC from March to August 2014 analyzing wild *Mus musculus* activity around bait stations and consumption data during a temporal progression from no stations to empty stations and then two rotations between empty stations, nontoxic bait, and a cholecalciferol rodenticide

Phase	Bait station present	Type of bait		Mean frequency of visits per night		Mean duration (s) in observation areas		Mean duration (s) in bait stations		Mean duration (s) not in bait stations		Percentage of visits entering bait stations		Mean bait consumed (g) per station/day	
1	no	none	Mean (±SE)	**12.5**	±(2.3)	**5**	±(1)	**NA**		**NA**		**NA**		**NA**	
			Standard Dev.	**4.7**	CDE†	**2**	B†	**NA**		**NA**		**NA**			
2	yes	none	Mean (±SE)	**20.8**	±(3.4)	**7**	±(1)	**2**	±(1)	**5**	±(1)	**56.7%**	±(8.0%)	**NA**	
			Standard Dev.	**6.7**	ABC†	**2**	B†	**2**	B†	**2**	A†	**16.0%**	B†		
3	yes	nontoxic	Mean (±SE)	**23.5**	±(2.7)	**59**	±(17)	**49**	±(14)	**10**	±(4)	**74.8%**	±(4.9%)	**9.2**	±(0.2)
			Standard Dev.	**5.4**	AB†	**34**	AB†	**27**	AB†	**7**	A†	**9.9%**	AB†	**0.5**	A†
4	yes	none	Mean (±SE)	**28.0**	±(8)	**6**	±(1)	**3**	±(1)	**3**	±(1)	**81.7%**	±(1.7%)	**NA**	
			Standard Dev.	**11.3**	A†	**2**	B†	**1**	B†	**1**	A†	**2.4%**	A†		
5	yes	cholecalciferol	Mean (±SE)	**9.5**	±(0.3)	**5**	±(0)	**2**	±(0)	**3**	±(0)	**78.6%**	±(4.8%)	**0.7**	±(0.5)
			Standard Dev.	**0.6**	DE†	**1**	B†	**0**	B†	**1**	A†	**9.6%**	A†	**1.4**	C†
6	yes	none	Mean (±SE)	**9.0**	±(2)	**6**	±(2)	**3**	±(2)	**3**	±(0)	**81.2%**	±(9.7%)	**NA**	
			Standard Dev.	**2.8**	DE†	**3**	B†	**2**	B†	**0**	A†	**13.8%**	A†		
7	yes	nontoxic	Mean (±SE)	**17.0**	±(3.6)	**118**	±(63)	**111**	±(64)	**7**	±(1)	**71.9%**	±(8.2%)	**2.3**	±(0.1)
			Standard Dev.	**7.2**	BCD†	**127**	A†	**128**	A†	**3**	A†	**16.5%**	AB†	**0.5**	B†
8	yes	cholecalciferol	Mean (±SE)	**7.5**	±(3.1)	**16**	±(6)	**5**	±(2)	**11**	±(6)	**68.1%**	±(5.2%)	**0.2**	±(0.1)
			Standard Dev.	**6.2**	E†	**13**	B†	**4**	B†	**11**	A†	**9.0%**	AB†	**0.5**	C†

† Phase means in the same column connected by the same letter are not significantly different based on ANOVA and Fisher's protected least significant difference test ($p \leq 0.05$).

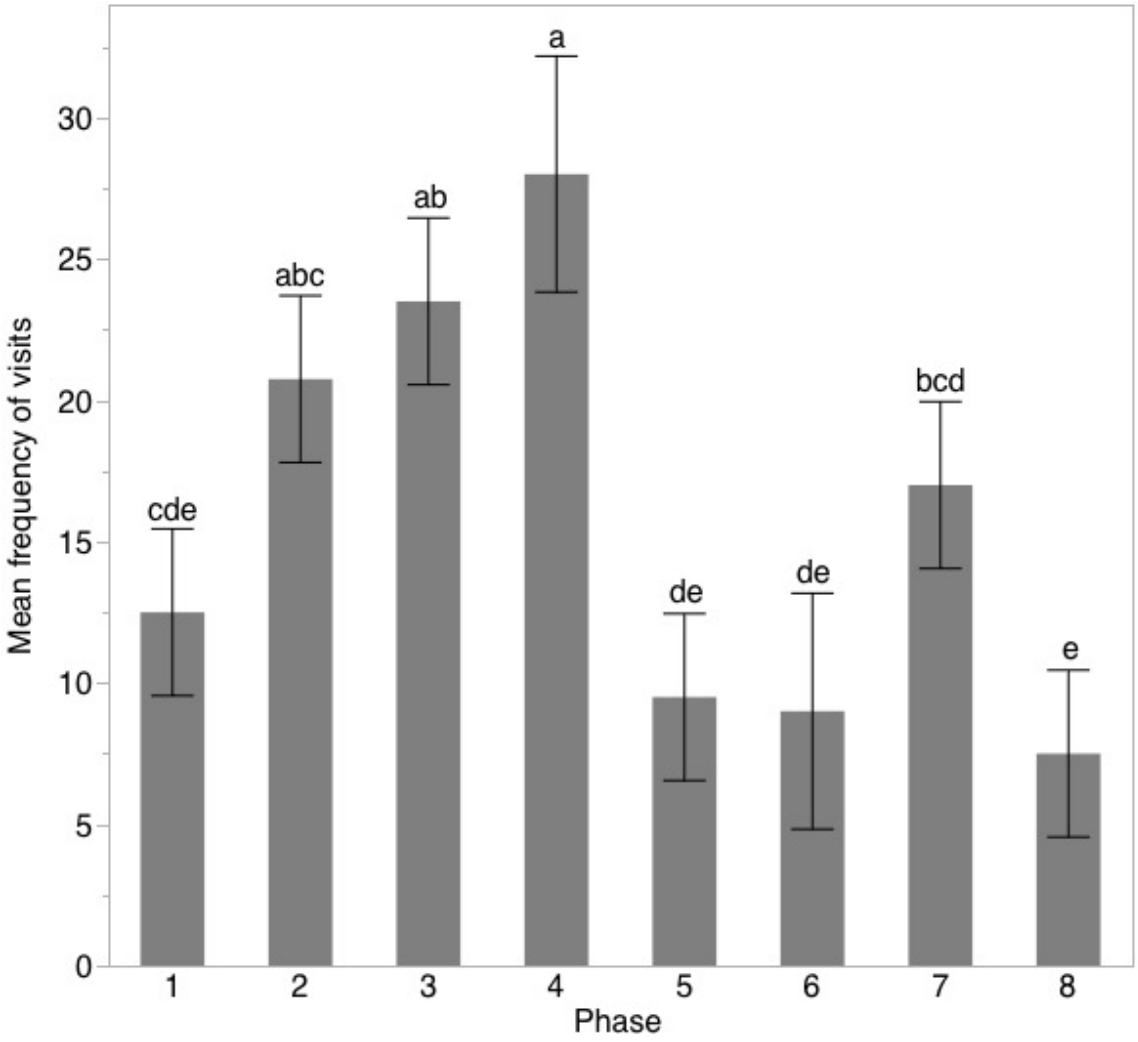

Figure 3.4. Frequency of visits by wild house mice *Mus musculus* (± LSM SE) into observation areas immediately surrounding bait stations for each study phase from 25 March to 18 August 2014 in a warehouse in Pickens County, SC. The study consisted of a temporal progression from no stations (Phase 1) to two rotations between empty stations (Phase 2, Phase 4 and Phase 6), nontoxic bait (Phase 3 and Phase 7), and a cholecalciferol rodenticide (Phase 5 and Phase 8). Phase means connected by the same letter are not significantly different based on ANOVA and Fisher's protected least significant difference test ($p \leq 0.05$).

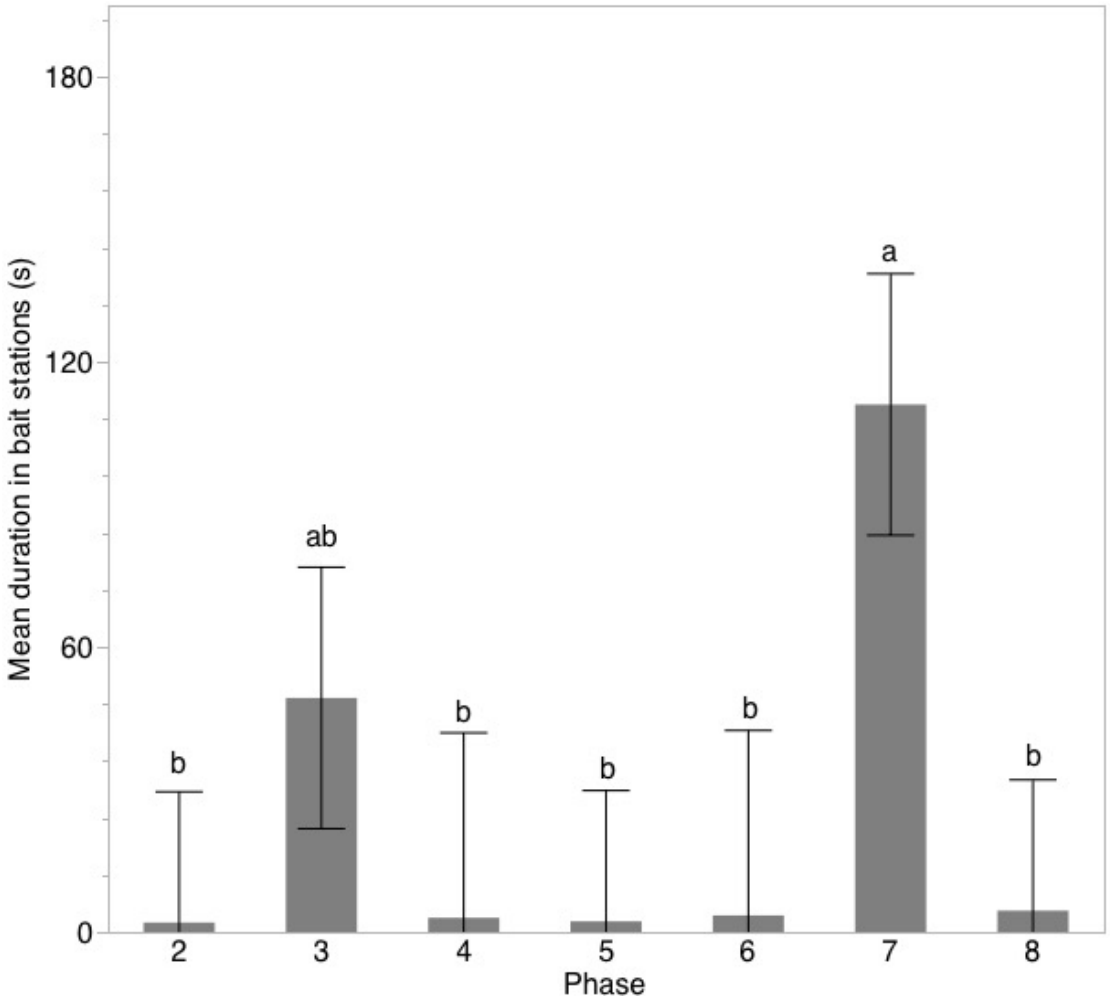

Figure 3.5. Mean duration of visits by wild house mice *Mus musculus* (± LSM SE) into bait stations for each study phase from 25 March to 18 August 2014 in a warehouse in Pickens County, SC. The study consisted of a temporal progression from no stations (Phase 1) to two rotations between empty stations (Phase 2, Phase 4 and Phase 6), nontoxic bait (Phase 3 and Phase 7), and a cholecalciferol rodenticide (Phase 5 and Phase 8). Phase means connected by the same letter are not significantly different based on ANOVA and Fisher's protected least significant difference test ($p \leq 0.05$).

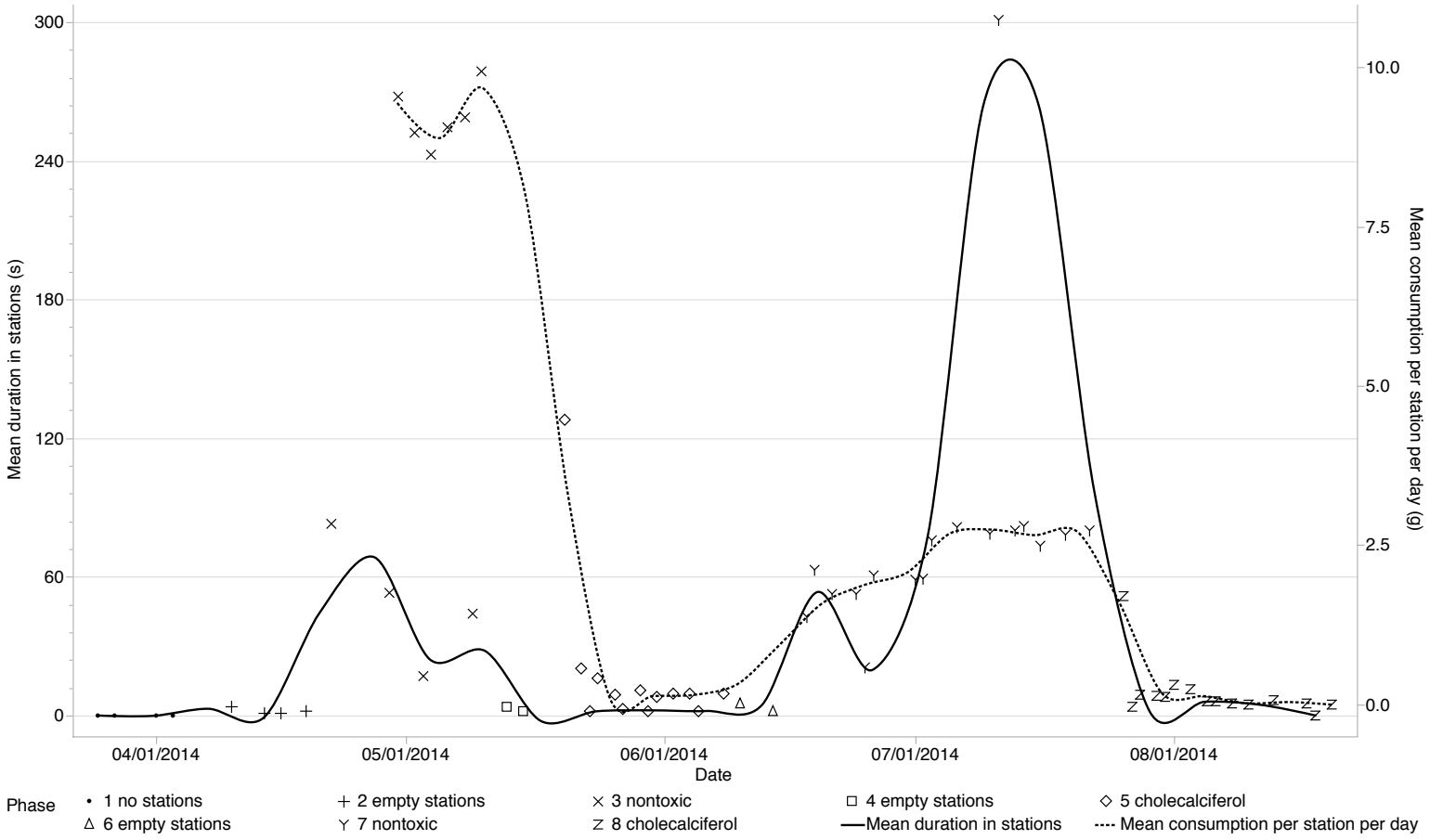

Figure 3.6. Mean duration of time *Mus musculus* spent in bait stations and mean consumption per station per day for each phase in a study consisting of a temporal progression from no stations (Phase 1) to two rotations between empty stations (Phase 2, Phase 4 and Phase 6), nontoxic bait (Phase 3 and Phase 7), and a cholecalciferol rodenticide (Phase 5 and Phase 8).

CHAPTER 4 - Analysis of wild house mouse (*Mus musculus* L.) movements before and after implementing a cholecalciferol rodenticide treatment

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ABSTRACT

BACKGROUND: Movements of the house mouse (*Mus musculus* L.) are known to vary across ecological and social gradients. A better understating of the impact rodenticide treatments have on mouse movements can facilitate the identification of the epidemiological, ecological, and behavioral ramifications involved with these treatments. This study used capture-mark-recapture analysis to identify the magnitude and location of mouse movements before and after the population was subject to treatment with a cholecalciferol rodenticide.

RESULTS: No significant changes were observed in the distance of mouse movements following an initial rodenticide treatment that reduced population abundance by 62%. The location of mouse movements was not significantly impacted by the rodenticide treatment. Juveniles were observed to have significantly greater movements than adults. Typical movements were expansive enough to include multiple bait stations spaced at 2.5 m to 3.5 m intervals. Immigration and emigration were not a significant factor leading to incomplete control after the initial treatment.

CONCLUSION: Results indicate that mouse movements are not significantly altered following a rodenticide treatment that causes a drastic, rapid decline in abundance. Incomplete control of a mouse population can occur with a cholecalciferol rodenticide even when typical movements suggest access to multiple bait stations, indicating that mice either did not consume the bait, are excluded from feeding, or develop an aversion to consume the bait.

Keywords: rodent control, *Mus musculus*, pest management, movement, monitoring

INTRODUCTION

Efforts to control commensal rodents including the house mouse (*Mus musculus* Linnaeus) rely heavily on knowledge of mouse biology and behavior, including their movements within the environments in which they exist. Additionally, an improved understanding of how mice move within their environment may help elucidate the roll mice play in the dissemination of pathogens. Previous studies looking at mouse movements have indicated that movements are highly variable depending on a variety of factors including population density, sex, age, habitat, and environmental conditions.[1-7] Understandably, the majority of ecological studies of mice and other small mammals focus on relatively stable populations that are expected to persist through the entirety of the study. While these studies provide valuable knowledge on movement parameters attributed to populations under typical conditions, they do not always present opportunities to see how the movements and distribution of a population may respond to drastic and rapid fluctuations in abundance. A better understanding of how control measures, such as a rodenticide baiting program, impact mouse distribution and movements not only helps equip pest management professional with tools to better mitigate pest populations, it also helps elucidate the impact of treatments on factors pertinent to epidemiology and vector management.

Mouse movements vary in response to environmental and ecological fluctuations and are generally divided into two primary classes based on the frequency and magnitude they entail: local movements and dispersal movements.[1] Local movements consist of movements made performing normal daily activities such as foraging, defending territories, and interacting with other mice. An individual's home range is comprised of

an aggregation of these local movements. Dispersal movements occur infrequently and are movements of a greater magnitude than local movements away from an individual's home range to a new, non-overlapping range and are generally in response to some environmental or social stress.[8] After dispersal, a new pattern of local movements will ensue, resulting in the creation of a new home range.

Local movements are heavily impacted by the environment in which mice persist and are correlated to the abundance of food, cover, competing species, population density, and other environmental factors. Due to the propensity for commensal habitats to have increased resource density, mice in these settings generally have local movements of a decreased magnitude compared to non-commensal populations.[7, 9-11] Fluctuations in the movement patterns throughout the year also tend to be less dramatic in commensal settings where climatic variability between seasons is less pronounced, breeding is more consistent throughout the year, and resource availability is generally greater.[12]

Dispersal movements in all habitats are highly variable by nature. This makes determining what factors might be impacting the likelihood, frequency, and magnitude of dispersal movements challenging; however, they are generally derived from some type of environmental or social stress. Under stable conditions, fledging juveniles and subordinates generally comprises the majority of dispersers. Larger dispersals across all age groups and sexes can occur in response to dramatic environmental disturbances, such as crop harvesting activities or cleaning in more commensal environments.[13, 14] While a rodenticide treatment does not directly equate to an environmental disturbance, the drastic, rapid reduction in population density could trigger a similar dispersal response as the survivors adapt to changes in population demographics and social structures. Studies

have suggested that immigrants filling in ecological niches created by mortality following rodenticide treatments can be significant and have been blamed for failed control.[15, 16] Lidicker[4] reported mice traversing great distances on an island when the population approached extinction, suggesting movements may become erratic following a rodenticide treatment that leaves few survivors.

When an attempt to control a rodent population with a rodenticide fails, it is generally assumed that one or more of the following three factors are responsible: physiological, ecological, or behavioral.[17] Each of these factors can be solely responsible for failure, or, as is commonly the case, they interact with each other to varying degrees. Efforts to determine to what degree each of these factors contributes to failed control requires a variety of adequate data that is difficult to collect in free-living populations. This study was designed to identify how mouse movements may be involved with ecological and behavioral factors effecting control. A rodenticide with a unique active ingredient, cholecalciferol (Vitamin D_3), was selected because the population was naïve to this active ingredient, thereby reducing the probability of physiological resistance even though no cases of resistance have been reported.[18] In addition, movement and demographic data was collected from capture-mark-recapture (CMR) and bait consumption was monitored. This data was utilized to determine how mouse movements and ranges may change in response to a baiting program and to elucidate any ecological or behavioral implications these fluctuations in abundance may contribute to a loss of efficacy in conventional rodenticide baiting strategies.

The goal of this study was to monitor the movement of mice in response to a control program using rodenticides in a free living commensal population. Movements

were analyzed and compared before and after the population was subject to heavy mortality from a rodenticide baiting program to see if the treatment had any impact on movement parameters. CMR data and the demographics of captured individuals was also utilized to help determine if the location or distribution of mice around the study site was a contributing factor to reduced efficacy through ecological or behavioral factors such as immigration or competitive exclusion.

MATERIALS AND METHODS

Study area.

This study was conducted from 27 February to 20 August 2014. The location of the study was a warehouse used to store and process agricultural seeds in Pickens County, South Carolina (34° 65′ N, 82° 50′ W). The selected test site offered a location with an existing mouse population that was naïve to any prior testing or recent control measures. Previous attempts to control the mouse population included low intensity baiting with anticoagulant formulations which ceased more than 6 months before the study start date. Observational inspections and live trapping prior to the study indicated an active infestation of mice with regular sightings of mice, mouse droppings, damaged bags and seeds, nesting sites, and damage to the building from gnawing.

The warehouse was constructed of steel I-beam framing with metal siding over a concrete slab floor. The dimensions of the building were 46 m long and 24 m wide, with the majority of the building in an open floor plan containing seed processing equipment, pallets of seeds and bags, and other miscellaneous supplies (Fig. 4.1). These goods were distributed around the perimeter of the warehouse leaving a large open expanse of concrete slab in the middle section of the floor that mice were not likely to traverse (verified by video surveillance and mouse sign). An 8 m section on one end of the building contained climate controlled office space and a walk-in cooler set to approximately 5 °C. Packages of seed on pallets were also housed in the cooler, and numerous mice were observed to travel in and out of this area freely. The remainder of the building had no climate control. There were minimal changes to the warehouse during the study due to normal management activity. Access to spilled feed and grains was

ubiquitous throughout the study. Therefore, food availability was not thought to be a limiting factor on the mouse population.

Bait stations.

Sixty-one commercially available Aegis-RP® (Liphatech, Inc., Milwaukee, WI) bait stations were distributed around the inside perimeter of the warehouse (Fig. 4.1). The tamper resistant stations were made of high-impact plastic with one entrance hole on each side and measured approximately 32 cm in length, 20 cm in width and 10 cm in height. These stations were placed according to the manufacturers recommendations and spaced approximately 2.5 to 3.5 m from adjacent bait stations and placed either directly against the wall or as close to the wall as possible if obstructions were present in that location. Care was taken to assure the stations were placed so that the entry holes were adjacent and parallel to the wall. Bait stations were placed two weeks prior to any bait application to monitor the response of mice to their presence. Each bait station was uniquely numbered and was designated a specific location throughout the entirety of the study.

Details of baits tested.

Treatment baits in each phase of the study were placed on metal rods included with the bait stations to prevent premature spoilage of the bait and make it more accessible to mice. The nontoxic bait used in this study, DETEX®, was selected because it was manufactured by the same company as the treatment bait (Bell Laboratories, Inc., Madison, WI) and was found to be of similar formulation and size. Each block of

nontoxic bait was yellow, rectangular in form measuring 47 x 25 x 25 mm, and weighed approximately 20.5 g. Individual blocks of cholecalciferol bait, Terad$_3$ Ag BLOX® (Bell Laboratories, Inc., Madison, WI), were also rectangular in form measuring 45 x 25 x 25mm, weighed approximately 28.5 g, and were brown in color. Cholecalciferol was formulated into the bait at a rate of 750 mg × kg^{-1}. Baits were not altered from their commercially available form, and previous studies have shown no statistically significant differences in levels of consumption related to varying bait colors.[19] LD_{50} for cholecalciferol has been determined for the ICR strain of *M. musculus* at 42.5 mg x kg^{-1} translating to consumption of 1.13 g of the cholecalciferol bait needed to achieve the LD_{50} dose for a 20 g mouse.[20]

Study timeline.

The study timeline is illustrated in (Fig. 4.2) and was constructed around the predicted die-off of the mouse population following cholecalciferol baiting. The study began on 31 March 2014 when traps were first placed to perform CMR analysis. Movements before the cholecalciferol treatment were estimated using 14 nights of trapping from the study start date until 20 May 2014. During this pre-treatment phase, bait stations were either empty or contained nontoxic bait; except for the last night traps were set when cholecalciferol had been in place for two days but before any mortality was observed. The remaining 18 nights of trapping from 22 May 2014 to 22 August 2014 were used to estimate movements after the die-off as the population began to experience mortality from the cholecalciferol rodenticide. During this post-treatment phase, bait stations initially contained cholecalciferol bait for 21 days, then nontoxic bait

for 36 days, and concluded with a reintroduction of the cholecalciferol bait that lasted for 27 days. Between each nontoxic and cholecalciferol phase stations were left empty for at least 3 days to prevent any direct prebaiting effects.

CMR protocol.

Eighty-two folding Sherman live traps (H. B. Sherman Traps, Inc., Tallahassee, Florida) were used to capture mice for CMR analysis. Mice were captured on 2,624 trap nights with trapping occurring twice a week between 31 March 2014 and 18 August 2014. Traps were designated a unique location for the entirety of the study and set at intervals of 7.5 m along interior walls of the warehouse, exterior walls of the warehouse, and surrounding the warehouse on three sides (North side was bounded by a road). Traps surrounding the warehouse were approximately 12, 18, and 24 m from the East, South, and West sides of the building, respectively. Traps were baited with a mixture of peanut butter and oats that were wrapped in wax paper and formed into packets. Cotton balls were added to traps when ambient temperature was below 10 °C to prevent trap-related mortality. On nights when trapping was to occur, traps were set between 2000 h and 2400 h and checked between 0600 h and 1000 h. Captured mice were measured from nose to tail, weighed, sexed, aged (as juvenile or adult), and had their reproductive condition noted. Age was determined by visual assessment of captured mouse genitalia. Males with abdominal testes were considered juveniles and those with scrotal testes were considered adults. Females with an imperforate vagina were conserved juveniles and those with a perforate vagina were considered adults. After all morphological data was collected, mice were marked in each ear with an individually numbered size 1 Monel tag

(National Band and Tag Co., Newport, Kentucky), and released at the point of capture. Throughout the study, any deceased mice were recorded by logging their discovered time, location, sex, and body weight before being removed from the study area. Efforts to retrieve deceased mice involved periodic searches of the facility in all accessible areas inside the building and the grounds immediately surrounding it. Personnel working at the facility were also advised to report any deceased mice encountered.

Population demographics.

Procedures for the estimation of population demographic parameters used in this study are outlined in the methods of Chapter 1 and used a Barker robust design with full heterogeneity. Estimates were calculated for three primary occasions during the following timeframes. Primary occasion one was comprised of 13 secondary occasions (nights of trapping) before any cholecalciferol bait was placed giving a baseline estimate of the population. Primary occasion two was comprised of nine secondary occasions after the completion of the initial 21 days of cholecalciferol baiting providing an estimation of population parameters after the population die-off. The final estimation of population parameters (primary occasion three) was derived from three secondary occasions after no deceased mice were recovered during the second cholecalciferol rodenticide placement.

Data analysis.

Movement data was derived by utilizing capture locations for each mouse based off of unique trap locations. The location of traps and subsequent captures was imported

into a GIS workspace for analysis. Distance between captures was derived by using a model incorporating the unique identification number (UID) for each individual and the specific time and trap location for each capture. The point to line tool was used to connect the captures for each UID in the chronological order in which they occurred. The lines generated from these calculations were then segmented into individual lines representing a linear distance between each capture. These distances were then compared between mice with the main effects being phase (before or after treatment), age (juvenile and adult), and sex (male or female).

The majority of mouse movement was restricted to areas around the perimeter of the building where adequate cover existed. There was a limited amount of mouse movements away from the perimeter in areas where adequate cover was provided by boxes, pallets, or shelving (Fig. 4.3). It is for this reason that mice were not observed (through both mouse sign and video surveillance) to have traversed the open expanse in the middle of the warehouse; thereby limiting their ability to move in a linear fashion from one side of the warehouse to another. Due to this restriction in mouse movement, it is likely that the linear distances between trap locations used to analyze mouse movements would generally underestimate the true distances mice were traveling but would provide an index sensitive enough to detect significant changes in movement patters or centers of mouse activity.

Movement data was processed to provide a mean distance per capture and a mean distance per movement. Mean distance per capture was calculated by taking the sum of the distances between all captures for that individual and dividing it by the number of times that individual was captured. This parameter provides an “on average” movement

estimate as it includes captures for which no movement was recorded (i.e. the mouse was recaptured in the same trap). Mean distance per movement was calculated by taking the mean of all recorded movements for each individual (i.e. distances between all recaptures that were not in the same trap).

To explore the possibility of a shift in centers of activity before and after the rodenticide treatment, a distance between mean center of captures was derived for all individuals that were captured at least twice before and after treatment. This calculation determined the geographical mean center of captures both before and after the treatment and then calculated the distance between these two points to see if they deviated from the distances of an individual's typical movements (i.e. average of movements before and after treatment).

Comparison of data for the distance of mouse movements during the study was done using ANOVA and Fisher's protected least significant difference test. A 2 x 2 x 2 factorial experimental design was used for both mean movement per capture and mean distance per movement. The main effects for these models were phase (before or after treatment), age (juvenile or adult), and sex (male or female). All possible interactions were also considered for significance. Where appropriate, custom contrasts were constructed to test for significance between specific hypotheses. To check for the possibility of individual variation affecting results, paired t-tests were performed on parameters for individuals that had at least two captures before and after the die-off. Results from these paired tests did not deviate from the results of the ANOVA analysis including all individuals so only ANOVA analysis for all individuals was used for statistical inference. All statistical testing assumed a two-sided alternative hypothesis,

and $P \leq 0.05$ was considered evidence of statistically significant differences or effects. Analyses were performed using commercially available JMP Pro 13 statistical software (SAS Institute Inc., Cary, NC).

Ethical note.

This study was conducted under an Animal Use Protocol approved by the Clemson University Institutional Animal Care and Use Committee.

RESULTS

Impact of rodenticide on population demographics.

CMR abundance parameters were estimated from 415 total captures of 183 unique mice. The abundance estimate for the initial population before the cholecalciferol rodenticide was placed was 194 individuals. After 21 days of baiting with cholecalciferol, CMR models estimated a reduction in abundance from 194 individuals to 74 (62% reduction) while pre and post treatment census baiting estimated the reduction at approximately 75%. The population was considered to have remained somewhat stable at this reduced level until the second round of cholecalciferol baiting began on 24 July 2014. This second baiting further diminishing abundance to an estimate of six individuals from this time until the completion of the study. Other noteworthy findings regarding population demographics revealed a trend from more females in the population before the treatment (m/f ratio of 0.54) to a more males in the population following the treatment (m/f ration of 1.03, Table 4.1). This trend was also supported by the demographics of individuals known to have survived and perished following the initial cholecalciferol treatment (Table 4.2).

Rodenticide impact on movement.

One hundred and five mice out of 212 unique individuals were recaptured at least once during the study (72 before and 33 after die-off) allowing for movement parameters to be estimated for these individuals. A summary of results from the estimation of movement parameters before and after the cholecalciferol treatment is presented in Table 4.3.

There was no significant difference before and after the cholecalciferol treatment and subsequent die-off in mean distance per capture ($F = 1.4290$, $P = 0.2347$, $df = 1, 103$) or mean distance per movement ($F = 0.5090$, $P = 0.4772$, $df = 1, 102$). Age was the only main effect of the model indicating significance, revealing that juveniles had significantly greater movements than adults for both mean distance per capture ($F = 4.7767$, $P = 0.0313$, $df = 1, 97$; juveniles M = 12.0 m, SE = 2.13; adults M = 8.0, SE = 0.88) and mean distance per movement ($F = 4.1617$, $P = 0.0441$, $df = 1, 96$; juveniles M = 14.3 m, SE = 2.42; adults M = 9.8, SE = 1.02). No interactions were significant.

Rodenticide impact on centers of activity.

Ten mice were captured at least twice before and after the die-off providing an estimate for the distance between the mean center of their captures. The results from these mean center calculations are presented in Table 4.4. The majority of these calculated shifts did not span a distance larger than those typically noted for each individual's average movement (as indicated by the percent of average movement, Table 4.4) and were likely not indicative of any meaningful shifts in range. Males had a greater overall observed distance between mean captures (11.8 m) compared to females (6.8 m); however, the difference was not significant due to high variation and lack of sample size.

Immigration.

CMR data was analyzed to detect any trends indicating immigrating into the study area. After the first two nights of trapping the population started to become saturated with tagged individuals and the ratio of daily recaptures to total daily captures began to

stabilize (Fig. 4.4). This ratio remained relatively constant even as the number of total daily captures diminished as a result of the die-off from cholecalciferol baiting indicating that immigration was not significant. In addition, total daily captures declined as a result of mortality from cholecalciferol baiting and did not increase during the subsequent 36-day nontoxic phase. Capture results from traps outside the building also revealed the mouse population within the warehouse was isolated and there was no evidence that immigration or emigration was occurring. Thirty-eight individuals were captured in the exterior traps surrounding the warehouse. Of these 38 mice, 13 were recaptured with 10 of these individuals being caught inside the warehouse at one point in the study (5 males and 5 females). These recaptured individuals were often trapped inside and then outside, or vise-versa, indicating they were not immigrating into the population but revealing that these exterior locations were part of their typical home range. The outermost lines of traps that surrounded the warehouse on three sides (one side was bounded by a road) resulted in only four total captures over the entirety of the study. None of these four individuals were ever recaptured and some had a physical appearance that was markedly divergent from mice trapped within the warehouse; presumably attributed to genetic isolation from the warehouse population.

DISCUSSION

Results from this study did not show significant evidence of changes in movement patterns by *M. musculus* before and after the rodenticide treatment. While not in response to a rodenticide treatment, similar results have been reported from multiple sources indicating that *M. musculus* movement parameters generally do not fluctuate based on population density.[1, 21, 22] Evidence from this study, combined with previous findings where alterations in movements were observed, suggest that modifications to habitat and the distribution and availability of food resources may be a greater determining factor for movement behavior than population density.[12] This indicates that population density alone does not impact movements regardless of whether density is changed through natural means or through rapid anthropogenic pressures on the population.

Juvenile mice were observed to have significantly greater measures in both distance per capture and distance per movement than adults. Increased movements in juveniles have been reported in other studies and is often attributed to dispersing juveniles trying to establish territorial boundaries after fledging from their natal range.[1] Some of these fledgling individuals have been observed to remain nomadic and never develop specific territories.[23] These individuals that remain nomadic tend to be smaller in size[24] and are subject to increased mortality under natural conditions.[3] This observed deviation between juvenile to adult movements was consistent before and after the treatment and was not significantly impacted by the population decline.

There was no evidence to show that individuals immigrating into the population from surrounding areas prevented successful control. Data from traps surrounding the

building revealed there were no established mouse populations around the building to serve as a source population for immigrants, neither did they detect any evidence that mice were dispersing from nearby fields into the study area. Of the 38 mice captured in traps outside the warehouse, 13 were recaptured. Of these recaptures, 10 included locations that were both indoors and outdoors revealing they were not immigrants and had established ranges that included parts of the warehouse interior and the immediate external areas.

The office space on the north end of the building was considered as a source of un-trappable individuals that may have subsequently immigrated into the trappable population. There were numerous points of entry into the walls of the office structure that adjoined with the warehouse and cooler floor were traps were placed, but no traps were placed above the drop ceiling or in the offices after inspections of these areas revealed minimal mouse sign and few trappable locations. While it is probable that any mice residing in these areas of the building were less likely to be trapped, there was no sustainable source of food available in these areas requiring any mice residing here to forage out onto the cooler or warehouse floor where they would subsequently be exposed to trapping and the rodenticide. It is for this reason, and after observing capture histories around these areas, that it was determined any mice that may have been living in this space and remaining undetected were not solely responsible for the incomplete control observed after the first cholecalciferol baiting phase.

Analysis of movement data indicated that mice were traversing an area expansive enough to allow the opportunity for them to be exposed to multiple bait stations. The majority of recaptured mice were captured in a different trap than their original capture

(Table 4.3: probability of movement between captures). The mean distance moved per capture (5.3 – 17.0 m) and mean distance per movement (8.5 – 17.0 m) across all sex and age classes greatly exceeded the distance between bait stations (2.5 – 3.5 m). Mouse movement analysis revealed ranges that overlapped with multiple bait stations, proving exposure to bait was not limited by their range of travel. In addition, bait stations were supplied with an abundance of cholecalciferol bait and were never depleted between service intervals assuring the probability of exposure was maximized.

Access to bait stations does equate to all mice having an equal opportunity to feed from stations. Some authors have attributed reduced efficacy in rodenticide baiting programs to issues involving behavioral exclusion and territoriality that are known to impact mouse movements and ranges. It is well documented that mice demes (family groups) are highly territorial towards each other and mouse movements are restricted by these territorial boundaries. Within a deme, movements are less regulated and little aggression has been reported towards individuals of the same deme.[25] To the contrary, some authors have cited intraspecific competition as a significant source of reduced efficacy in situations when population densities are high and bait density low.[15] While this study suggests that on average mice were traversing areas expansive enough to include several bait stations, movement analysis did not provide a method to analyze if local movements around bait stations were being limited by social pressures. Consumption data of nontoxic bait before the initial cholecalciferol treatment revealed that several bait stations were experiencing consumption values ranging from 50 - 60 g/day. Consumption at this rate conservatively estimates that these stations were being visited daily by at least 12 individual mice, assuming mice were feeding solely on bait

from these stations at the highest reported rate (~4 g/day).[26, 27] If any competitive exclusion limiting movements was occurring at stations exhibiting consumption at these elevated levels, it was still allowing for feeding to occur from a significant number of individuals. *M. musculus* deme sizes have been documented from 5-80 individuals demonstrating it could be possible for consumption at this rate to be occurring within a single deme, thereby reducing any competitive interactions.[9, 28-30]

Movement parameters before and after the population die-off were not significantly different suggesting that the movements of mice were not being restricted by social pressures from a hierarchical limit or intraspecific competition as mice were following similar patterns of movement pre and post treatment. In addition, results from shifts in centers of activity, did not provide substantial evidence to support an argument for a significant number of mice relocating and filling empty range gaps left behind from individuals that succumbed to the first treatment. CMR demographic data and recovered mortality demographics did reveal evidence that survival was biased towards male mice indicating that some factor (possibly related to social interaction) was responsible for this biased result. Movement analysis did not provide any information to differentiate why males might be more likely to survive than females as there were no significant differences between the sexes. A similar result favoring male survival after a cholecalciferol treatment was observed by Twigg and Kay[31] although no explanation for this observation was provided. Subsequent laboratory trials with survivors from the field test in the before mentioned study reported 100% efficacy, as have other laboratory trials, indicating no evidence to support an argument for decreased efficacy towards male mice from a physiological prospective.[20, 32] This suggests that some behavioral or ecological

factor that is more pronounced in male mice, possibly enhanced by the nature of cholecalciferol poisoning, is likely to blame.

While analyzing movement and demographics of a population at large via CMR techniques does interject a level of bias that could potentially skew results by failing to detect individuals that might be more or less prone to control measure such as the rodenticide used in this study, it appears that ecological factors related to movement were not the primary factor contributing to the incomplete control observed after the first cholecalciferol baiting. The stop feeding action of the cholecalciferol bait observed by other studies was fully apparent in this study and is likely the principal cause of reduced efficacy. However, results do indicate that survival is biased towards males indicating males may be more prone to survive a cholecalciferol rodenticide treatment indicating that some behavioral or social factor could be a secondary factor attributing to decreased efficacy.

CONCLUSIONS

Results from this study indicate that movements of *M. musculus* are not significantly affected across sex or age following a rapid and drastic reduction in population abundance following a cholecalciferol rodenticide treatment. This study showed evidence that movements of juveniles exceeded that of adults and this trend was constant before and after the population die-off resulting from the rodenticide treatment. There was also little evidence to suggest that mice changed the geographic location of their typical ranges in response to the treatment. Interpretation of capture history and location suggests that a mouse population can existing in an isolated building surrounded by native grassland and graze land and remain isolated from these surrounding areas with the vast majority of mouse movements being contained within the building. *M. musculus* populations in an isolated building may not be subject to immigration or emigration following a rodenticide treatment. Reduced consumption and incomplete control of a mouse population can result from a single cholecalciferol treatment even when typical mouse ranges indicate access to multiple bait stations.

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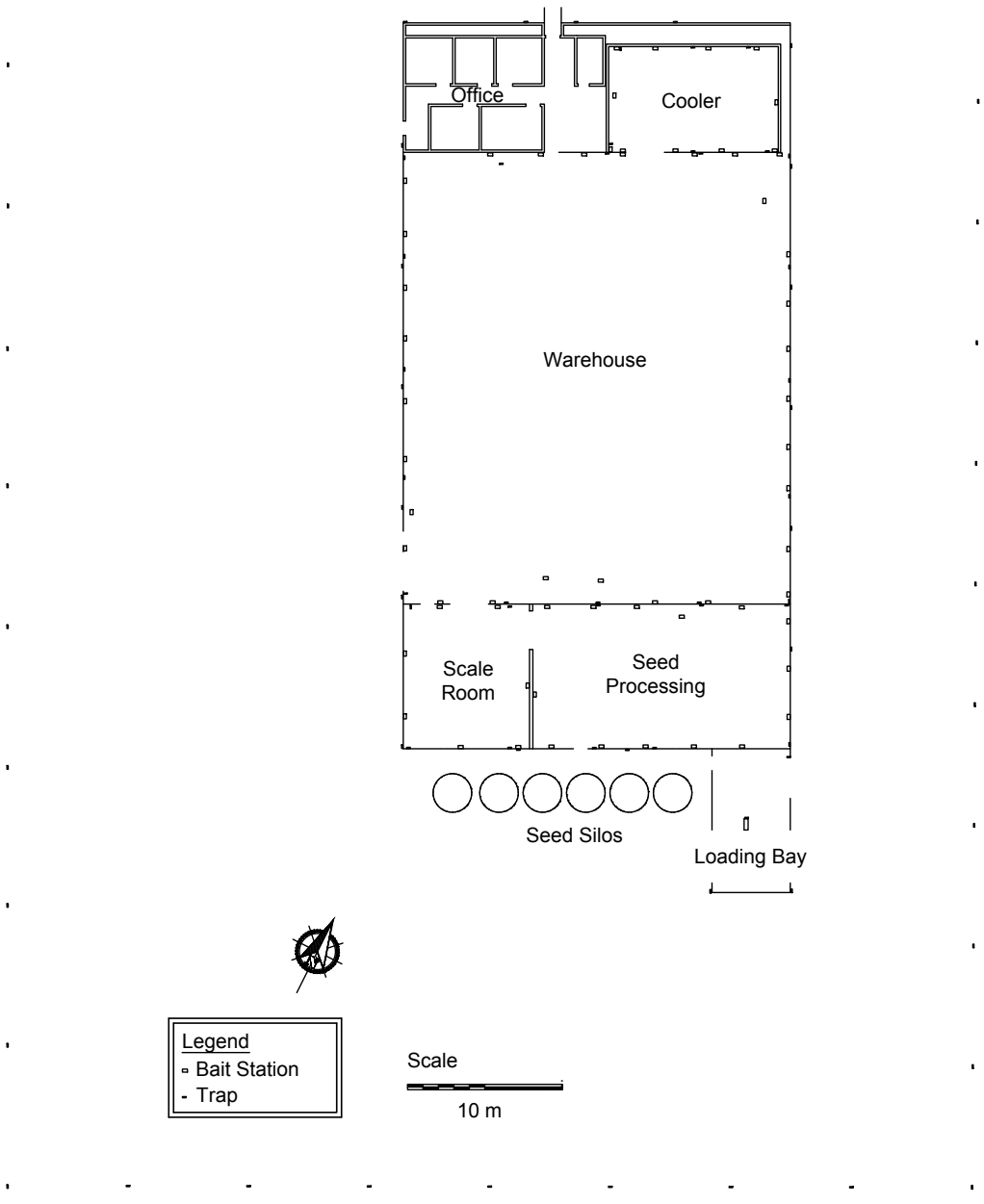

Figure 4.1. Diagram of the study site warehouse located in Pickens County, SC with the location of bait stations and traps depicted to scale.

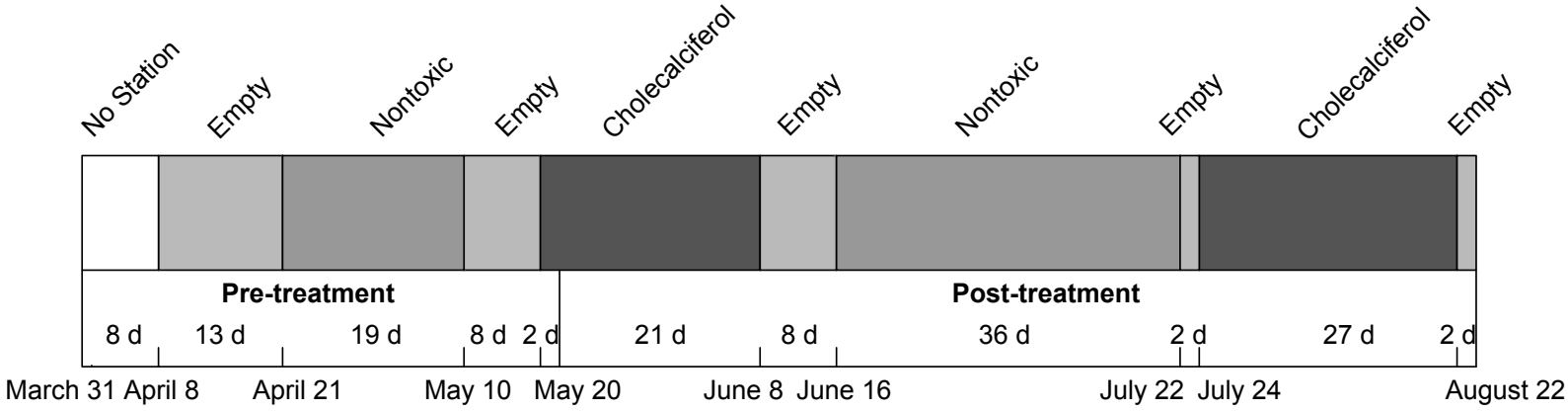

Figure 4.2. Timeline of the treatment schedule for a free-living *Mus musculus* population in a warehouse in Pickens County, SC indicating the study phases, condition of bait stations, and duration of each event.

Figure 4.3. Visual representation of the main warehouse floor, cooler, and seed processing area in a warehouse in Pickens County, SC during a CMR study analyzing the movements of a wild *M. musculus* population. The arrangement of equipment and stored goods resulted in a large expanse of open floor space that mice did not traverse.

Table 4.1. CMR demographics derived from the capture of wild *Mus musculus* undergoing treatment by a cholecalciferol rodenticide in a warehouse in Pickens County, SC

Phase	No. new captures	No. total captures	Sex ratio m/f	Age ratio j/a	Mean weight g‡	Mean body length mm‡
Before	151	151	0.54	0.31	15.7	72.3
After	62	93	1.03	0.26	14.8	73.8

Demographic summary for captured mice.
‡ Capture weight and body length were taken at initial capture.

Table 4.2. Summary of movement analysis for individuals known to have survived or perished following the initial treatment of a cholecalciferol rodenticide in a warehouse in Pickens County, SC

Known fate	No. of captures		Mean distance per capture (m)		Mean distance per movement (m)		Max. movement (m)	Probability of movement between captures	Sex ratio m/f	n
	Mean	Range		SD		SD				
Survived	3.0	2-5	10.6	± 7.7	12.7	± 8.8	30.9	0.8	2.00	6
Perished	4.1	2-12	8.5	± 7.2	10.4	± 7.6	29.1	0.7	0.53	23

Table 4.3. Summary of movement analysis derived from CMR data on a wild *Mus musculus* population before and after treatment with a cholecalciferol rodenticide in a warehouse in Pickens County, SC

Sex	Age group	Phase	No. of captures		Mean distance per capture (m)		Mean distance per movement (m)		Max. movement (m)	Probability of movement between captures	n
			Mean	Range		SD		SD			
♂	A	Before	3.9	2-12	9.2	± 6.3	11.0	± 6.8	30.9	0.69	22
		After	3.6	2-7	7.4	± 4.6	8.5	± 5.0	35.9	0.63	16
	J	Before	3.7	2-4	12.7	± 5.7	13.9	± 6.0	29.1	0.89	3
		After	3.0	2-3	17.0	± 6.5	17.0	± 6.5	30.8	1.00	2
♀	A	Before	3.9	2-12	8.5	± 6.2	10.1	± 7.2	38.6	0.73	38
		After	3.1	2-7	5.3	± 2.2	8.7	± 4.3	37.9	0.68	12
	J	Before	3.3	2-8	11.6	± 7.8	13.7	± 8.2	31.4	0.67	9
		After	3.7	2-4	9.3	± 2.8	15.0	± 5.1	25.9	0.78	3
	Overall	Before	3.8	2-12	9.3	± 8.0	11.0	± 8.4	38.6	0.72	72
		After	3.4	2-7	7.4	± 6.4	9.7	± 8.8	37.9	0.68	33

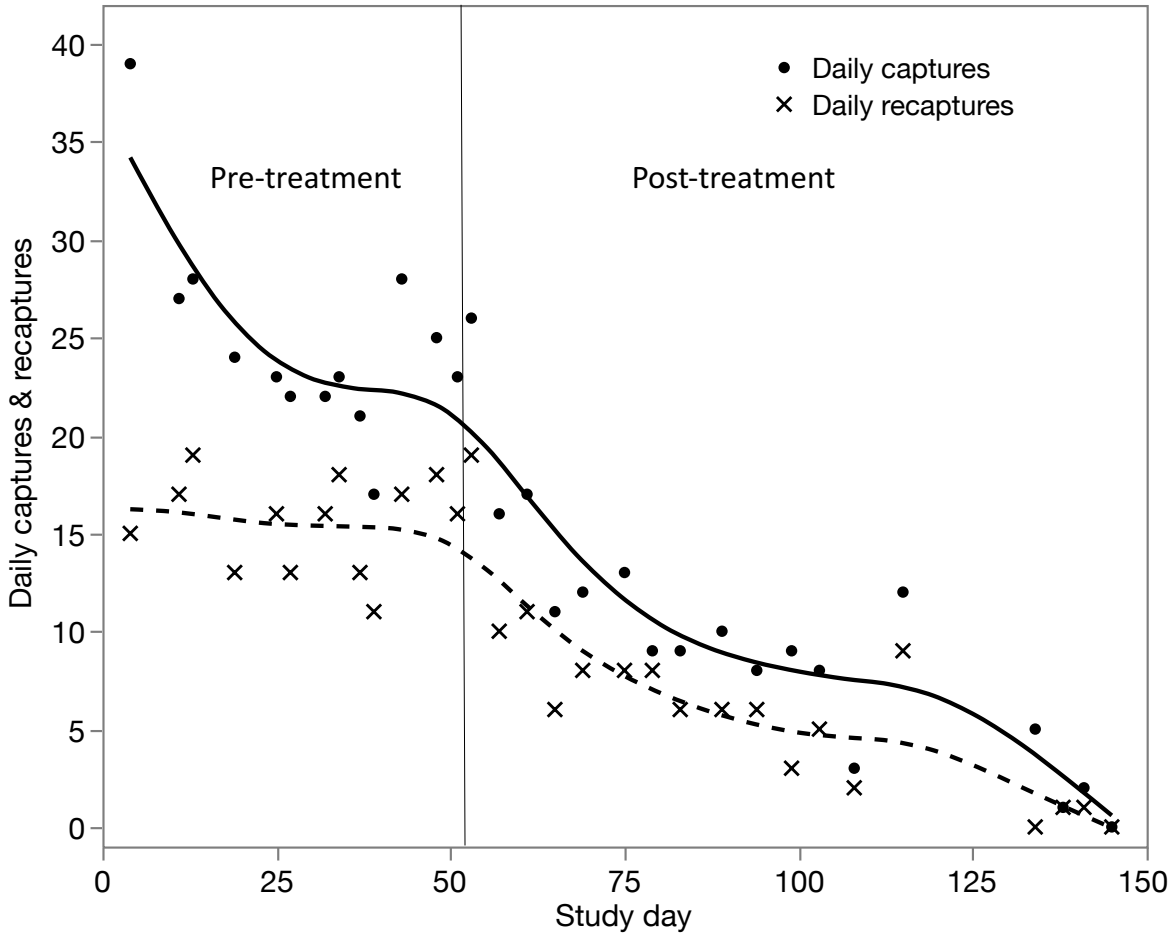

Figure 4.4. Comparison of daily captures vs. recaptures for a wild *M. musculus* population pre and post treatment with a cholecalciferol rodenticide in a warehouse in Pickens County, SC.

Table 4.4. Summary of distances between mean centers of activity for a wild *Mus musculus* L. population before and after treatment with a cholecalciferol rodenticide in a warehouse in Pickens County, SC

Sex	Age group	UID	Distance between mean center (m)	Percent of average movement	Captures before/after treatment
		1024	11.6	77%	3/7
		1082	8.9	185%	4/5
♂	A	1099	5.0	53%	3/2
		1108	14.5	105%	4/2
		1124	15.4	60%	2/5
		1009	3.3	60%	2/7
		1014	1.4	50%	4/2
♀	A	1016	5.0	77%	9/2
		1022	22.6	68%	8/4
		1040	2.1	47%	5/6

FURTHER RESEARCH

The field of rodentology is in need of increased attention and study, especially from the educational sector. Currently, minimal interest has been allocated to this sector and few universities provide programs directed at studying rodents, particularly in relation to pest management. Rodents continue to pose a significant risk of damage to human health, structures, food supply, and our livestock. It is unquestionable that the implications of commensal rodent damage are only expanding as the human population continues to increase and resources become further limited. While the literature to date involving commensal rodents does offer a solid foundation of knowledge on these species, it is becoming antiquated. The majority of research responsible for providing the current knowledgebase on commensal rodents was performed from the 1950s to the 1990s. As a result, the number of individuals that were educated as a result of this research are dwindling.

The results from this body of work have resulted in a number of areas where further research is needed, in addition to some direction where future investigative efforts may be focused. The following are some suggestions and comments on directions that may be pertinent.

- Consumption data provided a reasonable estimate of efficacy and required much less effort and expertise to conduct than CMR analysis. If there is limited aversion by the population to consume census bait following a rodenticide treatment, consumption data alone may be sufficient to estimate efficacy.

- Results from this study indicated that consumption of a rodenticide can be increased after a period of nontoxic baiting. Further research is needed to investigate the importance that each component of this technique had on the ability to regain consumption: duration of the nontoxic period, removal of competing individuals, and what type of nontoxic bait is ideal, if it is needed at all.
- In this study, cholecalciferol proved to be more efficacious towards females than males. Understanding what mechanisms might have been involved to result in this biased efficacy may lead to more effective control measures.
- Video monitoring of a wild mouse population provided a method to index bait station interaction and feeding activity. The development of a similar passive method to automatically analyze and report mouse activity under praxis conditions would be beneficial for monitoring and making management decisions.
- Analyzing movement data via CMR for a mouse population experiencing heavy mortality from a rodenticide treatment was complicated by reduced sample size. Using a method that was able to track more individuals more frequently, such as PIT tagging or radio telemetry would likely provide more statistical power and increase the resolution of movement analysis.