Deer Oh Deer:

Seasonal Diet Variation

In Northeastern White-Tailed Deer

Princeton University

Ecology & Evolutionary Biology

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Abstract:

In Princeton, New Jersey, lives a population of *Odocoileus virginianus,* or Northeastern white-tailed deer, a species which is currently negatively impacting North American ecosystems due to overabundance. The expansion of invasive species is another ecological concern, so we set out to address how these two processes interact, and their impacts on the environment. We performed a survey of Princeton residents to validate that deer overabundance is a locally relevant concern. The survey revealed that residents generally held a negative opinion about deer impacts locally, both on human and natural spaces. I then used DNA metabarcoding on 169 Northeastern white-tailed deer fecal samples to characterize the composition of deer diets and how they vary seasonally. I tested the hypothesis that deer diets primarily rely on native understory vegetation, including grasses and forbs, as a result of the abundance and nutritional quality, but would switch to eating non-native trees and shrubs in the winter. We found that deer diet components vary seasonally, and this variation entails changes in group size patterns and diet quality. From elemental composition of 50 deer dung samples, we found that C:N of deer dung increased in the winter, suggesting that deer diet quality declines in the winter in accordance with seasonal changes in diet composition. Additionally, individuals in larger groups had lower quality diets, though this was not as a result of intraspecific competition. This research worked with an accessible study system to provide insight about regional environmental and social concerns, with the goal of assessing overpopulation, invasive species, and climate change on these scales.

Keywords: deer overabundance, DNA Metabarcoding, invasive species, Northeastern white-tailed deer *(Odocoileus virginianus),* seasonality.

Introduction:

Within the past few decades, North American ecosystems have begun to face major concerns regarding the degradation of natural plant life and wildlife, two of which play a significant role: deer overpopulation and the proliferation of invasive species.[4,6,14,27,30,33,34,36,37,40,42,45,52] *Odocoileus virginanus*, or Northeastern white-tailed deer, have caused widespread ecological damage due to their rapid population growth.[4,30,37,40] Meanwhile, invasive plant species also cause environmental harm by competing with native plants for resources and decreasing biodiversity.[4,11,14,33] This research addresses the influence that deer overabundance has on the growth of invasive species by looking at deer seasonal dietary patterns and components.

Northeastern white-tailed deer are ruminants, a type of ungulate which relies on a cyclical process of chewing and fermentation in order to extract nutrients from their food.[19] Deer have generalist diets, meaning they consume a wide variety of plants rather than specializing on a single species.[8,19] Deer graze, selecting herbaceous understory vegetation, and browse, eating from bushes or trees.[8,19]

Historically, white-tailed deer faced extreme declines in population sizes, at one point reaching only 3 individuals per square kilometer, leading to reintroduction efforts that sought to restore their populations.[36,52] Since then, deer densities have dramatically increased and now average greater than 60 individuals per square kilometer.[36,52] Deer overabundance has disrupted many ecosystem processes; for example, deer are known to prevent the growth of many woody or herbaceous species through their browsing.[30,36,40,42] Vegetation is unable to regenerate quickly enough to outpace deer consumption, meaning that some plant species cannot reestablish themselves and thus decline.[30,36,40,42] Furthermore, deer can damage agricultural crops, reduce flowering and fruit production, and prevent forest regrowth efforts which destabilizes forest succession.[30,36,40,42]

White-tailed deer populations have been able to prosper in many areas since they lack adequate levels of predators.[4,21,30,36,40,42] As such, population sizes are not limited by predation factors, but instead by the availability of resources in a given area. Wildlife management efforts have turned to sterilization, regulated hunting, and other conservation techniques to attempt to reduce deer overpopulation.[12,23,30,32,36,40,42] However, these methods have not yet efficiently addressed these issues, leading many to propose investigating diet composition because plants are the limiting factor to deer growth.[12,23,30,32,36,40,42]

Additionally, wildlife administration covers the management of invasive plant species. Invasive species are most commonly known as non-native plants that invade an area and grow unregulated.[4,6,11,14,33,34,35] In terms of direct impacts on deer, non-native species are less nutritious because they contain more carbon.[4,6,11,14,33,34,35] These plants are sometimes introduced by humans and then naturally continue to grow in their new environment.[4,6,11,14,33,34,35] Without adequate predation or regulation, invasive species can dominate and exploit an area for resources.[4,6,11,14,33,34,35] This causes biodiversity to decline, as invasive species compete with native plants.[4,6,11,14,33,34,35]

Forest ecosystems can only contain a limited amount of resources, which help determine the abundance of species like deer.[36] With such high deer densities in forest ecosystems, deer populations have exceeded the carrying capacities of these habitats.[36] This means that deer populations face periods of resource scarcity, particularly during winter when resources are at their minimum.[36,42,48] Resource scarcity further influences the bounds of the population size in an area, and requires deer to adapt their behavioral strategies in order to persist during these periods of limited resource availability.[36,42,48] In turn, deer may resort to less nutritious resources, such as invasive species because these plants are generally thought to be faster growing and more nutrient-efficient than native plants.[7,11,14,33,35] Invasives tend to have higher carbon to nitrogen (C:N) ratios, a measurement which indicates the quality of the diet or of a component.[7,11,14,33,35] As such, deer may switch to eating invasive species during periods of scarcity.

Beyond ecological aspects, white-tailed deer and their behavioral patterns have numerous impacts on human society. Millions of vehicle collisions with deer occur each year, costing estimates of billions of dollars in insurance claims and healthcare costs, as well as increasing human and deer mortality rates.[12,23,40] White-tailed deer can also transmit diseases to humans and livestock, including COVID-19, Lyme disease, bovine tuberculosis, chronic wasting disease (CWD), and other bacterial or tick-borne diseases.[36,40,44] Rates of disease transmission between deer and humans have also been modeled to show that disease transmission increases with increasing deer.[44,51] Therefore, it is evident that deer overpopulation threatens multiple social, economic, and ecological structures.

Vehicle collisions, transmission of infectious diseases such as Lyme disease or COVID-19, and plant maintenance are a few deer-related concerns in the Municipality of Princeton in New Jersey.[38] Nowadays, population reduction is the primary strategy for deer-related concerns. As of 2001, the Municipality of Princeton adheres to an up-to-date Community Based Deer Management Plan (CBDMP) as required by the state.[38] The predominant method of population reduction in Princeton is via culling, or lethal removal of individuals, due to the ineffectiveness of nonlethal methods.[38] In 2022, $133,000 were invested in deer management efforts, including culling, removal, and female sterilization procedures.[24] Yet, despite how costly this program is, deer are prominent in Princeton, so by understanding the existing plant composition, we can evaluate what role deer play in this environment. Using five data streams that assess deer behavior, plant composition, and local opinion, this research will allow for wildlife operations to be improved in Princeton, New Jersey.

This research provides scientific groundings behind the importance of studying deer, their behaviors, and diet, and reveals social gaps where policy changes are necessary. What do Princeton residents think about deer? Are there areas in which the Municipality's policies have been lacking regarding wildlife management? Furthermore, this research will be presented to the Princeton Township Council, highlighting the public's perception of current local wildlife policies, their concerns, and informing them of our findings to adjust landscape management procedures.

The present work connects the impacts of invasive species and deer overpopulation by looking at seasonal trends in deer diet. Prior research, such as that of Averill et al. (2017), has found that deer overabundance causes increases in invasive species growth, since deer selectively avoid invasive species.[4] Despite this avoidance, resource scarcity and heightened competition during winter may motivate the consumption of invasive species. The current research tests this prediction, specifically looking at how seasonal changes impact deer utilization of invasive plants.

Seasonal changes play a role in deer diet because as the weather changes, so too does the plant life in a given area.[55] Researchers have investigated how seasonal variation impacts consumptive behaviors in deer, and have found that deer modify their diets to preferentially select plants that will support their overall digestive health and survival.[15,26,29,31,47,49] These studies have highlighted how during the winter, deer became less selective and maximized their energy usage to fit any external conditions impacting their typical dietary patterns.[15,29,31] Deer diets are influenced by the changing levels of resource availability and access throughout the year.[29,54] Prior studies have pointed out that deer change their consumption strategy, or choose whether to browse or graze, depending on what is available in their environment.[8,15,16,24,29,31,41,49] Unlike prior research, however, I set out to question whether non-native species are nutritionally helpful or harmful for Northeastern white-tailed deer and evaluate what proportion of their diet is made up of non-native plants.

Seasonal changes also contribute to shaping deer group dynamics, closely tied to their life history. Northeastern white-tailed deer are social animals, known to form groups that tend to feed together.[44] During winter, larger groups form to improve foraging efficiency and survival, while deer become more solitary in the spring to prioritize reproduction and territorial independence.[15,16,49] This seasonally variable grouping behavior should impact how much they compete with one another for food; not only is food generally scarce in winter, but within-group competition for the available food should be more severe due to larger group sizes. For instance, Morrison et al. (2002) demonstrated a specific seasonal case in which competition occurred, where deer would compete when high snow caused restricted movement.[26,31] Indeed, while prior literature has indicated that white-tailed deer do not compete strongly for food, competition can occur in cases where resources are running low and resource exploitation is necessary.[15,16,49]

Both deer overabundance and the spread of invasive species are impacted by climate change.[7,17] Increasing global temperatures are predicted to increase the number of invasive species in the Northern hemisphere.[7,17] This is because invasives will be able to take advantage of increased space availability, as other species, including natives, decline in high temperatures.[7,17] The effects of climate change on deer populations are less well-established. One perspective suggests deer may suffer from a changing climate, becoming more susceptible to thermal stress and disease transmission in warmer weather, and it is hypothesized that their foraging patterns will change as a result.[7,17] Felton et. al (2024) attribute these potential changes to energy expenditure, as it is more costly for deer to forage in the summer when it is warmer, and that shorter winters will be less costly, as deer would not have to navigate deep snow as often.[17] However, shorter winters might alleviate resource scarcity and cause deer to form smaller groups, both of which might alleviate forage competition.[17] Thus, how deer respond to seasonality might also allow us to project how climate change might have a significant impact on deer populations in the future.

By applying these prior understandings, I examine the ramifications of seasonality on deer grouping behavior, diet composition, and diet quality. Combining direct behavioral observations, camera trap data, DNA metabarcoding data, and stable isotope data, I investigate the following questions: How do white-tailed deer diets change throughout the seasonal cycle? What types of plants are these Northeastern white-tailed deer consuming through the seasons and in what proportions? What are the nutritional impacts of these diet changes? I also set out to understand what role, if any, plant status has on nutrition. Are there seasons in which non-native plants are more frequently consumed than native plants? I hypothesize that deer primarily rely on herbaceous plants, including grasses and forbs, because of the availability of these plant types throughout the year and their increased nutritional value. Furthermore, I hypothesize that group size influences how much competition deer experience, thereby impacting the quality of their diets. These hypotheses suggest the following specific, testable predictions: (1) Deer will be observed spending a disproportionate amount of time grazing as compared to other behaviors, especially browsing. (2) Proportions of herbaceous foliage in deer diets are greater than that of browse resources. (3) Diets composed of more herbaceous vegetation will be of higher quality, with lower C:N. Additionally, (4) deer group size impacts diet quality, with deer in larger groups exhibiting lower quality diets (see Table 1).

Methods:

Princeton Resident White-Tailed Deer Survey:

In March of 2021, our research team designed a survey using Qualtrics XM to gauge local perceptions of deer on the environment. The survey intended to assess attitudes about deer and determine whether these perspectives connected with other deer-related concerns. The ultimate goal was to integrate responses regarding deer consumptive behavior and make correlations between local observations and the impact of the deer population in Princeton, New Jersey. I predicted that if attitudes towards deer and deer-related impacts (such as vehicle collisions, yard damage, and disease transmission) were correlated, then those most disturbed by deer behavior would have a negative opinion about the species. The survey worked towards gaining a clear picture of local opinion about deer to uncover whether adjustments to plant composition in the environment would improve public perceptions and reduce deer-related concerns. With these analyses in mind, we hoped to characterize the impact of deer on native plants for the Princeton Township Council to support their work with U.S. Fish and Wildlife. In particular, this would be most effective with any non-native plants that were introduced through landscaping efforts.

Furthermore, we wanted to evaluate the available plant life being offered by Princeton residents and the correlation between deer encounters, to ascertain whether deer were attracted to places with particular species. The survey set out to answer the following research question: is there an association between deer presence and the presence of particular plants? All of the plant species listed on the survey can be eaten by deer, but our suggestions will focus on what we expected deer to eat based on prior deer diet preferences. We did not include any plants toxic to deer, such as daffodils or lavender.[14,18,22,35] We grouped plants as deer-attractive or deer-resistant based on evidence from prior literature, including local plant guides.[14,18,22,35] Deer-resistant plants were included to capture a more diverse range of plants consumed. It is our hope to provide information regarding deer behaviors and diet composition to support future wildlife management policies.

The survey was approved by the Princeton University Institutional Review Board (IRB), which granted the survey the ability to be made public. The survey was reviewed by members of the Princeton Township Council prior to circulating it to the public. No identifiable data were collected, and the survey was entirely voluntary. No incentives or benefits were provided for completing the survey. After reviewing an IRB approved consent form, survey participants had to confirm that they were residents of Princeton, New Jersey, to access the survey. Optionally, respondents could list the name of the street they lived on, whether they wanted to be contacted with the results of the survey afterwards, and if so, providing a means of contact (via email or phone number).

In the first half of the survey, respondents were asked a series of multiple-choice questions: (1) How often do you encounter deer on or near your place of residence? (2) How many individual deer do you typically see on or near your place of residence? (3) When do you normally see deer on or near your place of residence? (4) What is your attitude towards the deer in Princeton? (5) Are you concerned about any of the following deer-related issues? The options listed were yard damage, deer-vehicle collisions, deer scat, disease transmission, impacts on the local ecosystem, presence in public areas, or other. (6) In your opinion, how do deer impact the plant life in your yard? In the second half of the survey, respondents were shown ten specific plants available in the area with both the common name and scientific name, and asked whether respondents did or did not have these plants around their residence. These plants were: (1) *Ambrosia artemisiifolia* or Asters (2) *Symphyotrichum novae-angliae* or Common Ragweed (3) *Euonymus alatus* or Euonymus (4) *Alliaria petiolata* or Garlic Mustard (5) *Ilex aquifolium* or Holly (6) *Impatiens walleriana* or Impatiens (7) *Hedera helix* or Ivy (8) *Taxus cuspidata* or Japanese Yew (9) *Toxicodendron radicans* or Poison Ivy (10) *Lactuca virosa* or Wild Lettuce. Of these, holly and Japanese yew are deer-resistant, meaning that deer tend to avoid consuming these plants if other options are available instead, while deer generally consumed other options.[27,31]

Observational Data:

Observations of *Odocoileus virginanus* were made during random chance encounters in the areas surrounding Princeton University's central campus in the Municipality of Princeton where a substantial population of Northeastern white-tailed deer reside. We collected this data on a Google Spreadsheet which was accessible voluntarily by members of the Department of Ecology & Evolutionary Biology at Princeton University. On the first page of this spreadsheet, we annotated the following information during encounters: (1) Observer's full name. (2) Date in m/dd/yyyy format. (3) Time by 24-hour clock rounded to the nearest fifteenth minute. (4) Location. (5) Canopy cover as either "Open" or "Closed." (6) Weather in regards to cloud cover in terms of "Clear" or "Overcast."(7) Latitude. (8) Longitude. (9) Total group size. (10) Number of males, determined by visible antlers. (11) Number of juveniles, determined by visible spots. (12) Behavior or observable actions. (13) The sample code number, if one was collected during the sighting. (14) Observer comments about the sighting. The present results rely on data collected from September 8th, 2019, to December 29th, 2024. As of August 25th, 2024, a total of 141 fecal samples were collected by observers from random chance encounters with Northeastern white-tailed deer. The seasons were categorized by the following months: March to May as Spring, June to August as Summer, September to November as Fall, and December to February as Winter.

Camera Trap Data:

To assess the viability of the random chance encounters and the timing of those observations, camera trap data produced by Bakos Princeton Wildlife Monitoring Project (BPWMP) led by Professor Gaspar Bakos were integrated. From 2019 to 2022, Professor Bakos collected over 30,000 media images, photos or videos, over various locations in the Municipality of Princeton. The entirety of this dataset was transferred onto an 8TB LaCieRugged RAID Shuttle USB 3.1 Gen 2 Type-C External Hard Drive. This data was organized in a Google Spreadsheet by annotating: (1) Folder location and title on the hard drive. (2) The video name. (3) Date as reported by the camera, in mm/dd/yyyy. (4) Observation of the animal in the image or video. (4) Time of the capture as reported by the camera down to the minute in 24-hour time. (5) Temperature as recorded by the camera, in both degrees Fahrenheit and Celsius. (6) Notes about the analysis or observation. (7) Location of the camera trap. Separate pages of the sheet were created to distinguish between camera folders, while one page kept a compiled running list of all interpretations. Data containing images or videos of deer were extracted to draw patterns regarding deer group size, foraging activity, and other noticeable behaviors. For the camera trap footage containing deer, the information was categorized in a new page of the sheet as follows: (1) Observation date in mm/dd/yyyy. (2) Observation time in 24-hour time. (3) Deer group size. (4) The number of present males or juveniles, if visible. (5) Behavior of the deer.

DNA Metabarcoding & Diet Analysis:

On a new page of the same Google Spreadsheet as the observations, we tracked fecal sample collection from encounters with Northeastern white-tailed deer. The information was categorized as follows: (1) The Pringle Lab inventory number 1, in the format of PRN_##_ODVI_##, which represented the lab, final two digits of the year, the shorthand for *Odocoileus virginianus*, and the sample number. (2) Pringle inventory number 2, or a shortened version using PRN_##_##, with the year and the sample number. (3) Date of DNA extraction. (4) Control extraction IDS number. (5) Sample ID in the format WTD###, shorthand for white-tailed deer. (6) The season with December to February being “Winter,” March to May being “Spring,” June to August being “Summer,” and September to November being “Fall.” (7) Date of sample collection in m/dd/yyyy. (8) Time in the 24-hour format, rounded to the nearest fifteenth minute. (9) Latitude. (10) Longitude. (11) Standard of the sample, listed as either “Gold,” “Silver,” or “Bronze.” A gold standard sample meant defecation was observed during the encounter and the sample could be associated with a specific individual. Silver represented a fresh sample found near a group of deer, but where defecation was not observed, such that the sample could not be attributed to a specific individual. Finally, bronze meant a fresh fecal sample was found but there were no deer present in the vicinity. (12) Demography of the individual from whom the sample was collected. (13) Group size during the encounter. (14) Behavior during the encounter. Grazing of the understory and browsing in the overstory plants were distinguished by the position of the head, with head up (90° or above) indicating browsing and head down (90° or below) indicating grazing. (15) Notes from the observer about the collection. Fecal samples were collected to be analyzed using DNA metabarcoding, wherein the DNA in a sample is extracted, amplified, and sequenced. The resultant DNA sequences are then compared to reference DNA sequences ('barcodes') to identify the organisms present in the sample.

Upon locating a fresh fecal sample, observers used nitrile gloves to pick up the sample and place it in a labeled quart-sized Ziploc Bag. In order to avoid contaminating the sample, the observer would take only a portion of the sample that was towards the top of the pile, so that it was not touching any plants or the ground directly. When uncontaminated material was readily accessible, such as when the dung sample was in the form of a fumet, the sample was homogenized within the plastic bag by massaging the bag for >30 seconds until the material was well-mixed within the bag. A pea-sized amount of the sample was then transferred 2 mL BashingBead tube with 750 μL of DNA preservation buffer (BashingBead buffer from Quick-DNA™ Fecal/Soil Microbe Kits by Zymo Research) with a never-used plastic knife. When uncontaminated material was difficult to collect, such as when dung was in the form of discrete pellets, intact pellets were collected; during processing, the centers of multiple pellets (>5) were extracted with bleach-rinsed, UV-sterilized plastic tweezers and placed into tubes filled with DNA preservation buffer. Samples were refrigerated prior to processing for no more than 24 hours whenever possible. However, if the sample could not be processed within 24 hours, samples were frozen until the sample could be processed. Any excess material not used for DNA metabarcoding was transferred to a paper bag and oven dried for a minimum of 72 hours at *ca.* 60°C.

After processing, DNA was extracted from the samples using Quick-DNA™ Fecal/Soil Microbe Kits by Zymo Research. Extractions were conducted in the Pringle Lab at Princeton University and followed established extraction protocols from the extraction kits. Samples were extracted in small batches of 5-29 samples along with one sample-free extraction to serve as an extraction control to allow for the identification of contaminants introduced during lab work. After extraction, we conducted polymerase chain reaction (PCR) on all samples (in triplicate, to enable quality assessment via comparison of technical replicates) as well as on extraction and PCR controls (DNA-free water instead of DNA extract). We amplified the P6 loop of the chloroplast trnL(UAA) region using indexed primers trnL(UAA)g and trnL(UAA)h. Since uniquely indexed primer pairs were used for each sample for PCR, all PCR products were pooled and purified using MinElute™ purification kits (Qiagen, MD, USA). Purified samples were then pooled into a single library for sequencing on an Illumina MiSeq 600nt 2×150 (20-25M reads) at Princeton Lewis-Sigler Institute Genomics Core Facility.

Of the total 169 samples that underwent both DNA extraction and PCR testing, fifty samples were submitted for nutritional analysis at the UC Davis Stable Isotope Facility. The fifty samples were selected to capture the seasonal, interannual, and demographic variation present in the dataset. For nutritional analysis, the oven-dried material was hand ground into powder using a mortar and pestle. Powdered samples were then transferred to glass vials and oven dried at *ca.* 60°C for another 24 hours. 2-4 ug of powdered material was then encapsulated in tin and sent to UC Davis for dual ^{13}C carbon and ^{15}N nitrogen analysis. Encapsulated samples were run on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.), which measured the total mass of carbon and nitrogen in all the samples as well as the isotopic composition of both. The mass of carbon was divided by the mass of nitrogen to yield sample C:N. Standard deviations of repeated measurements of laboratory standards were <0.1%.

Deer Gastrointestinal Tract Samples:

Additional samples were collected for DNA metabarcoding along the gastrointestinal tract of 27 Northeastern white-tailed deer. Deer were culled during the Township's annual deer culling efforts: deer were baited with corn (*Zea mays*) and then humanely killed either by skilled bowhunters or drop nets (electrified nets dropped onto the deer). Samples were collected from the gastrointestinal tract of the deer, while deer carcasses were processed into venison for donation to local food pantries. For each individual, samples of digesta were collected from at most four positions along the gastrointestinal tract: rumen, small intestines, large intestines, and rectum. As many of these samples were collected as possible, but for some individuals there was no material available at a particular gastrointestinal tract location. This data was reported on a third page of the same observational Google Spreadsheet, listing the following information: (1) AnimalID in the format PTN-##-m/f-ODVI-##, displaying the location of Princeton, the last two digits of the year, the sex of the individual, an abbreviation for *Odocoileus virginianus*, and the sample number. (2) Sex of the individual. (3) Age class as either "adult" or "juvenile." (4) Date in the format yyyy/mm/dd. (5) Chest measurement in centimeters. (6) Body length in centimeters. (7) Hindfoot measurement in centimeters. (8) Number of fetuses present. (9) The weight of the kidney in milligrams. (10) The kidney fat weight in milligrams. (11) The calculated ratio of the kidney weight to fat. (12) The ID number of collected tongue tissue. (13) The ID for collected liver tissue. (14) Rectum contents represented with the ID number. (15) Rumen contents by ID number. (16) Small intestine contents by ID number. (17) Large intestine contents by ID number. (18) Notes from the collector. All the gastrointestinal samples underwent the same processing and DNA extraction as the opportunistically collected samples.

Data Analysis:

Data analyses were performed in R version 4.4.1 using the following packages across all dataset analyses: ade4, ape, BiodiversityR, bipartite, colorspace, corrplot, data.table, deSolve, dplyr, EcoSimR, , flextable, geosphere, ggmap, ggplot2, ggpubr, ggspatial, ggtext, grid, gridExtra, gt, indicspecies, kableExtra, knitr, lattice, leaflet, leaflet.extras, lubridate, magick, nicheROVER, nlme, officer, patchwork, permute, plyr, primer, vegan, Rcmdr, RColorBrewer, readr, readxl, reshape2, RinSp, rJava, sf, StereoMorph, stringr, svglite, tcltk, tidyr, tidyverse, webshot2, xlsx. Of these packages, leaflet and leaflet.extras were used to plot a heatmap of the observational encounters through the latitude and longitude coordinates collected. This allowed us to visualize where the majority of deer sightings occurred.

First, sequence data resulting from DNA metabarcoding were categorized into molecular operational taxonomic units (mOTUs) using established bioinformatics protocols in the lab. Next, the data were turned into relative read abundances (RRAs), by calculating the proportion of the total reads for a given sample mapped to each mOTU. Finally, we also measured diet quality in samples using a carbon to nitrogen ratio (C:N ratio). Samples with a lower C:N ratio indicated a high-quality diet, whereas a higher C:N ratio indicated a low-quality diet. This ratio developed out of dual 13C & 15N natural abundance sampling performed by the UC Davis Stable Isotope Facility.

We also performed non-metric multidimensional scaling (NMDS) using our Bray-Curtis dissimilarity matrix to visualize the relationships between the variables in a two-dimensional ordination space. This would allow us to see patterns within the data, particularly how samples are clustered. In the vegan package, we then calculated the Bray-Curtis dissimilarity between every possible pair of samples. On this Bray-Curtis dissimilarity matrix, we ran a Permutational Multivariate Analysis of Variance (PERMANOVA) to assess whether season, collection type, or collection standard had a significant effect on diet composition. A PERMANOVA test was also used to look into diet composition using Julian day. Additionally, for all of these factors, we also ran indicator analyses to figure out which specific plant species may have primarily contributed to diet differences.

Besides these, we also used Analysis of Variance (ANOVA) tests to explore two other dietary characteristics – diet richness and diet diversity. Both diet richness and diet diversity were calculated using the vegan package. Diet richness is the total number of unique plant species in each sample, while diet diversity takes into account both the richness and evenness of the diet components in each sample. We used the Shannon diversity index to quantify diet diversity; a high Shannon diversity value indicates more spread and the presence of multiple species, while a low Shannon diversity value could indicate that the sample had fewer species or was primarily composed of one particular species.

ANOVAs were also performed on many other variables in order to understand the effects that one factor may have on another. In any instance in which we wanted to assess the relationship between two variables, we used an ANOVA to find the significance of the correlation. ANOVAs were performed on the following correlations for the observational set: Julian day against group size, season against average group size, sample standard against season, standard against frequency. For the camera trap data, we performed ANOVAs for: season by frequency, time slot by total group size, and season by total group size. For the DNA metabarcoding results, we calculated ANOVAs for: season by group size, season by C:N ratio, Julian day against C:N ratio, status by C:N ratio, status group by C:N ratio, group size against C:N ratio, and sex by C:N ratio.

Regarding the survey data, Chi-Squared Tests of Independence were performed on the response data from each question to assess the associations between variables. The chi-square tests would allow us to test the null hypothesis, meaning that there is no relationship between the variables, or the alternative hypothesis, meaning that there is an association between the variables. The chi-squared tests report a p-value, just as the ANOVAs or PERMANOVA tests, or a measurement that quantifies the probability that the relationship between variables is the result of random chance. For p-values <0.05, the null hypothesis is rejected meaning that it is likely that the variables are significantly associated. In contrast, p-values 0.05 mean that the null hypothesis must be rejected, as the association between the variables is likely made due to chance.

Results:

Princeton Resident White-Tailed Deer Survey Results:

From April 2024 to October 2024, the survey received 142 responses (n=142). The most commonly reported attitude regarding the presence of Northeastern white-tailed deer in the area shows that Princeton residents feel somewhat negative about the deer (Graph 1; n=142; χ^2p-value<0.05). Out of 142 respondents, there were a total of 54 respondents who selected a negative opinion, in contrast to only 33 respondents who had positive attitudes towards the deer, while 20 were ambivalent (Graph 1; n=142; χ^2 p-value<0.05). Residents reported that they typically saw groups of 3-4 individuals per sighting, and sightings frequently occurred 1-2 times per week, with 3-4 times per week being the second most prominent number of encounters (Graph 2, Graph 3; n=142; χ^2 p-value<0.05). These sightings primarily occurred in the late afternoon to early evening time, around 4pm-8pm, followed by the mid-morning from 6am-11am (Graph 4; n=142; χ^2 p-value<0.05).

In terms of deer-related issues, respondents were predominantly concerned with deer-vehicle collisions, followed by yard damage, then disease transmission from deer to humans, for example, with Lyme disease or COVID-19 (Graph 5; n=142; χ^2 p-value<0.05). Additionally, respondents reported feeling that deer are somewhat harmful to plant life in their yard (Graph 6; n=142; χ^2 p-value<0.05). The majority of respondents felt negatively about the impact of deer life on their yards, with somewhat harmful being the dominant perspective, followed by extremely harmful, then neutral (Graph 6; n=142; χ^2 p-value<0.05). Only one respondent selected that the deer are extremely helpful towards plant life around their property (Graph 6; n=142; χ^2 p-value<0.05), and no respondents reported feeling that deer were somewhat helpful towards their property (Graph 6; n=142; χ^2 p-value<0.05). Ivy, *Hedera helix*, was found to be the most reported plant grown on or around a resident's property, followed by garlic mustard, *Ilex aquifolium*, and poison ivy, *Toxicodendron radicans* (Graph 7, Graph 8, Graph 9, Graph 11; n=142; χ^2 p-value<0.05). Residents reported having Japanese yew, *Taxus cuspidata*, impatiens, *Impatiens walleriana*, and asters, *Ambrosia artemisiifolia*, least (Graph 7, Graph 8, Graph 9, Graph 11; n=142; χ^2 p-value<0.05). In terms of the association between deer presence and plant species presence, only wild lettuce was significantly associated with deer presence (Graph 13; Table 4). We found that there was no relationship between attitude towards deer and the group size residents observed (Table 5). Table 3 displays the results of Chi-Squared Tests of Independence for all of the aforementioned questions from the survey (Table 3).

Observational Data Results:

Deer sightings were biased by researcher observations, most concentrated along Faculty Road, near the Carnegie Lake region (Graph 15). Group sizes varied significantly across the seasonal cycle, both when season was treated as a continuous and categorical predictor (Graph 14, Graph 16, Graph 17; n=1526; p-value<0.05). Regarding seasonal group size information, in the spring, the average group size was 3.26, with a range of groups of 1 to 22 individuals, coming from a total of 377 observations. The average group size of the summertime was 1.97, with group sizes ranging from 1 to 9 individual deer, out of 399 observations. In the fall, the average group size was 2.15 individuals, with a range of group sizes from 1 to 15, out of 451 total observations. Finally in the winter, the average group size consisted of 4.59 individuals, with ranges of 1 to 19 members per observed group, with 299 total wintertime observations. (Graph 16, Graph 17; n=1526; p-value<0.05).

Camera Trap Data Results:

Using the Bakos Princeton Wildlife Monitoring Project video files, we observed that the summer had the most frequent deer encounters on camera, at 366 sightings, while the winter displayed the least amount of deer sightings, at 127 observations (Graph 19; n=903; p-value = 0.451). Of the total 903 observations, the majority occurred from 4pm-9pm, at 417 total sightings, while the least were observed from 9pm-1am (Graph 20; n=903; p-value = 0.824). We also analyzed deer group sizes in the video data using the same seasonal categories by month above. With 248 observations in the spring, groups ranged from 1 to 5 individuals, with an average of 1.5202 deer (Graph 21; n=903; p-value = 0.0174). In the summer, 366 observations were noted, including a range of 1 to 5 members per group, and an average of 1.4426 (Graph 21; n=903; p-value = 0.0174). From the 162 observations made in the fall, the group size ranged from 1 to 3 individuals, with an average of 1.3148 (Graph 21; n=903; p-value = 0.0174). Finally, in the winter, the range remained 1 to 5 individuals, with a mean of 1.4567, out of 127 sightings. (Graph 21; n=903; p-value = 0.0174). The sampling of videos was biased by the time frame, as the cameras were only operational for a little over two years, from March 2020 to June 2022, and by the distribution of cameras which was haphazard and not systematic.

DNA Metabarcoding & Diet Analysis Results:

In the summer, the plant family Vitaceae, the grape family, was the most commonly consumed plant family. A similar relative read abundance (RRA) of around 0.25 was found for plant families of other taxonomic groups. The top five most consumed plant families were: Rosaceae, Vitaceae, Poaceae, Celestraceae, and Cupressaceae. (Graph 22; n=145; $p<0.05$). The distribution of consumed plant families differed by sex, as juveniles relied primarily on Vitaceae. It is important to note that the majority of collected juvenile samples were collected in the spring and summer, which is when Vitaceae consumption peaked, such that diet differences between adults and juveniles may be an artifact of temporal sampling biases. Meanwhile, the consumed plant taxa were similar between males and females. (Graph 23; n=145; p-value<0.05). Regarding MDS analysis of sex and sex groups, juvenile samples were the most clustered, while female and male samples overlapped (Graph 34; n=145; p-value<0.05). This was also true for NMDS analysis of these categories as well (Graph 35; n=50; p-value = 0.00727).

Deer diets were least diverse in the fall, while individual deer diets were more diverse in the summer, though this pattern was only marginally significant (Graph 24; n=145; p-value = 0.0821). When comparing diet quality using the ratio of carbon to nitrogen within the samples, sample C:N was greatest in the winter at 20.61 and lowest in the summer at 12.91. (Graph 26; n=50; p-value<0.05). This suggests that deer in the winter had lower quality diets than deer in the summer due to the higher proportion of carbon in the diet. Additionally, the winter had the largest range, spanning from 12.917 to 29.232, while the summer had the shortest range, from 9.718 to 16.683 (Graph 26; n=50; p-value<0.05). After performing NMDS analyses for the standard and season, the spring displayed the widest spread, indicating that the samples were more dissimilar, while the summer was the most condensed, meaning the samples were more similar (Graph 27; n=1000; p-value<0.001). Using Julian day in lieu of season did not yield any additional insights, though Julian day, like season, was a highly significant predictor of compositional variation (Graph 28; n=1000; p-value<0.001).

Diet quality varied significantly over the course of the year: Julian day was parabolically associated with dung C:N, with diet quality cycling from being lower quality to higher quality, back to low quality again through the year (Graph 29; n=50; p-value<0.05). During the winter, diet quality is lowest, as evidenced by a high C:N ratio, while in the summer, diet quality is highest, displayed by a low C:N ratio. This indicates that seasonal variation does indeed impact diet quality.

Furthermore, when dissecting the diet components, plant types were categorized as either native to the Princeton area, introduced, invasive, or uncertain. While most samples indicated minimal consumption of invasives (RRA<0.1), a handful of samples had considerable amounts of invasive plant DNA, as much as 0.75. Contrary to our predictions, diets with greater proportions of introduced plants were of higher quality (lower C:N ratios), while the proportion of invasive and native plants in the diet was not significantly related to C:N ratio (Graph 30; n=50; p-value introduced = 0.00139; p-value invasive = 0.684; p-value native = 0.495). Since the distinction between introduced and invasive plants is somewhat arbitrary, we also summed the proportion of invasive and introduced plants in the diet together into non-native diet fraction. There was indeed a significant relationship between non-native diet fraction and C:N ratio, but not between the fraction of native plants and C:N ratio (Graph 31; n=50; p-value non-native = 0.0158; p-value native = 0.495). Again, the relationship was in the opposite direction of what we had predicted, however, dung C:N declined with increasing non-native diet fraction, indicating that diet quality increases with the amount of non-native plants consumed. There was also a significant relationship between group size and C:N ratio from the dung samples (Graph 32; n=50; p-value<0.05). As predicted, dung C:N declined with increasing group size, indicating that individuals in larger groups have lower quality diets. There was no relationship between diet quality and sex (Graph 33; n=50; p-value = 0.912).

Deer Gastrointestinal Tract Results:

DNA metabarcoding was also used to characterize composition of digesta along the gastrointestinal tracts of 27 deer. Within all gastrointestinal tract locations, the rumen, rectum, large and small intestines, the Poaceae plant family was found to be the most prevalent; additionally, the rumen contained the largest proportion of Poaceae in comparison to the other three tract locations (Graph 36; n=27; p<0.001). It is important to note that this component was almost entirely made up of *Zea mays*, or corn, which was used as bait during the deer culling efforts. While an encouraging validation that DNA metabarcoding recapitulates what an animal consumes, this likely biased the composition of the samples towards being primarily made up of Poaceae, particularly those collected from sites located earlier within the gastrointestinal tract. Furthermore, the rumen had the lowest average diet richness, while the small intestine had the highest average diet richness, likely also deriving from the high prevalence of corn in the data from rumen samples (Graph 37; n=27; p-value = 0.0035). The largest standard deviation of diet richness was in large intestine samples, though all samples had a range of about 15 (Graph 37, Graph 38; n=27). When analyzing the relative read abundances and the relationships between locations, ANOVA testing revealed a significant positive relationship between the small intestine and large intestine RRAs, small intestine and rectum RRAs, and the large intestine and rectum RRAs, indicating that digesta composition is correlated within the gastrointestinal tract (once digesta leaves the rumen, at last) (Graph 39; n=27). Additionally, for these locations MDS analysis demonstrated that the samples were all relatively similar to one another, and that the rumen had the most dissimilarity (Graph 40, Graph 41; n=27; p-value<0.01). The MDS distribution did not indicate any clustering by group size, though it was a significant predictor of compositional variation (Graph 42, Graph 43; n=100; p-value<0.001). When grouped by sex, the gastrointestinal samples from males were more dissimilar than those of females (Graph 44; n=27).

Discussion:

The current research uncovered many compelling results which address a range of issues concerning deer overpopulation, invasive species growth, and seasonal diet quality. To begin, the resident survey indicated largely negative attitudes towards deer locally, underscoring that deer overabundance is a locally relevant issue in Princeton, New Jersey. Behavioral encounters displayed that deer group sizes varied seasonally, being smallest in the summer and largest in the winter, as anticipated. Deer diets also varied seasonally, both compositionally and quality-wise; deer had lower quality diets in the winter, as predicted. Furthermore, there was a link between group size and diet quality, in which individuals in larger groups had lower-quality diets, as predicted. Additionally, non-native plants seem to play a critical role in determining diet quality. Most interestingly, dung C:N increased with increasing consumption of non-native plants, indicating that individuals who consumed more non-native plants actually had higher quality diets, contrary to our initial expectations.

Northeastern white-tailed deer, or *Odocoileus virginanus*, are natural inhabitants of North American woodlands and occupy an integral niche, serving as both an herbivore and as prey to secondary predators.[1] Deer populations serve as accessible and informative study systems, reflecting local herbivory and the status of other organisms interacting in an ecosystem. With over 1527 physical deer observations recorded from September 2019 through December 2024, and another 904 video observations captured by the Bakos Princeton Wildlife Monitoring Project from March 2020 to June 2022, it is clear that Northeastern white-tailed deer are extremely accessible and can provide a wealth of information reflecting the status of their ecosystem. This research was driven by an interest in local dynamics, considering the impacts on everyday life by common animals. By eating plants, deer regulate the growth of surrounding wildlife, meaning that their dietary patterns can convey information about environmental conditions.

Motivated by the issues posed in the resident survey, we did find that deer overpopulation is a public concern in Princeton. Residents were most concerned about vehicle collisions followed by yard damages. It is possible that if landscaping policies change to better address deer overabundance, Princeton residents may have a more positive outlook on deer and their impact in the surrounding environment. We examined the deer population of the Municipality of Princeton in New Jersey to consider how their diet, which varies seasonally, represents surrounding plant growth. Regarding behaviors and deer patterns, it is conceivable that if residents modify the plants on or around their property, then they might alter the frequency of deer encounters.

The results highlight which plants are consumed by Northeastern white-tailed deer throughout the year, the proportions of these within the diet, and confirmed that diet quality does vary seasonally (Graph 22, Graph 23, Graph 24, Graph 26, Graph 29). This is interesting because deer managed to supplement their diets nutritionally throughout the year, regardless of changing ecosystem components (Graph 29; $n=50$; p-value<0.05). Diets were high quality in the spring and summer, shown by a low C:N ratio, which follows in line with Hypothesis 1. Accordingly, deer diet relied upon seasonal vegetation because of their availability throughout the year, and this included the consumption of more invasives during the warmer months. In both the observational and camera trap datasets, grazing was much more frequently observed over browsing, following Prediction 1 of Hypothesis 1. This supports the finding that foraging behavior changed depending on resource availability.

Diet components change seasonally, while nutrition worsens during the fall and winter. This reinforces our prediction since resource scarcity occurs during cooler months. Additionally, in terms of plant status, only introduced plants had a significant relationship with the ratio of C:N in the samples, when compared to invasive and native plants (Graph 30; $n=50$; p-value introduced = 0.00139; p-value invasive = 0.684; p-value native = 0.495). This may mean that introduced plants differ compositionally in C:N than invasive and native plants. There appeared to be a negative correlation between introduced plants and diet quality, meaning that if the diet proportion included more introduced plants, diet quality decreased (Graph 30; $n=50$; p-value introduced = 0.00139). When invasive and introduced plants are grouped together, this correlative relationship is still present (Graph 31; $n=50$; p-value non-native = 0.0158; p-value native = 0.495).

Altogether, this means that introduced and invasive plants are lower in carbon than nitrogen, making them more nutritious. We observed that deer had higher quality diets in the summer than in the winter, correlating with our finding that deer ate more invasives in the summer than in the winter. The higher proportion of carbon than nitrogen in the diet in the cooler seasons suggests that deer relied less upon introduced and invasive plants, making their diets less nutritious, as a result of limited resources. If deer were able to maintain a consistent diet quality throughout the seasons, then it would be evident that deer are not resource limited. However, this was not the case, as we did find that deer diet declines in the winter, when fewer invasives are consumed, implying that they switch to eating lower quality foods in the winter due to resource scarcity. This validates that there are limits on deer nutrition from seasonal resource availability.

With future shifts in climate change and increasing global temperatures, the winter and fall periods will become shorter, as the spring and summer seasons will become longer. During these times, it may be possible that deer will regulate invasive species growth since deer relied more on invasive species during the summer rather than in the winter. As such, deer may alleviate concerns about biodiversity decline and stabilize ecosystems by consuming more invasive species in warmer months. This finding is provocative as it indicates that deer may counteract invasive growth. This points to a new direction for invasive species management and encourages further research investigating how deer may restrict invasion from non-native plants.

We predicted one mechanism for changes in diet quality to be that of group sizes. More specifically, we predicted that in the winter, groups would become larger. This led us to believe that increased intraspecific competition may impact deer diets. We expected to see that if deer do form larger groups in the winter, then individuals would eat less due to limited resource availability and would also eat lower quality resources. Although we did observe that group sizes did in fact get larger in the winter, deer diets were low in quality regardless of group size in the fall and winter seasons. Regarding the impact of group size on consumptive behavior, it seemed that with an increase in group size, diet quality decreased (Graph 32; $n=50$; $p\text{-value}<0.05$). This rejects the Alternative Hypothesis and predictions that group competition may be causing declining diet quality. Therefore, we did not observe group size impacts on diet, meaning that competition was not a mechanism driving dietary changes. This means that deer overabundance may not influence diet quality due to competition, but that overabundance may cause a strain on nutrition within a population due to fewer resources being available seasonally.

Limitations:

It is important to acknowledge some limitations that we encountered with this research. Firstly, the observational data collected was solely based on random chance encounters rather than structured observational routines or strategies. We also did not track individuals using GPS collars or set up camera traps of the areas that we regularly encountered deer. Additionally, observations were biased by researcher location and convenience. This meant that late night encounters were less frequently recorded as it is difficult to see deer or dung samples at night. Demographic and behavioral information may have also faced human errors, as we relied upon our sight. The work was also limited by the amount of consistent engagement with the research, as only one researcher worked directly on this project prior to my involvement.

Dung samples may have been contaminated during the collection process if collected incorrectly or by picking up plant matter from around the sample. Of the gastrointestinal samples from the culled individuals, in some individuals, rectum samples were not found as the culling may have resulted in a potential gut shot. In another few gastrointestinal individuals, only rectum samples were collected. Future research may benefit from stricter guidelines regarding sample collection.

The resident survey was distributed by word of mouth, flyering, email, and by the Princeton Township Council. Therefore, more advertising efforts could have been made to receive more responses to the survey, increasing the sample size. It is important to note that such surveys often contain biased sampling, as those who feel most strongly about deer are most likely to complete the survey. We also recognize that residents may not have been able to accurately identify which plant species are on or around their property. This may have led to a high number of responses of "Unsure" for that portion of the survey. Additionally, with the question “Are you concerned about any of the following issues? Other (please specify):,” we noted that we received a few responses mentioning a general concern about deer welfare and wellbeing, which were not directly addressed in the survey.

With the BPWMP dataset, categorizing and analyzing the data posed a few challenges. With such a large data set, it took a significant amount of time to extract all of the deer-related videos. Initially, we attempted to use Zamba Cloud to sort out any blank videos and photos prior to searching for deer videos, however, the software could not handle processing large video sets rapidly. So, I began sorting each video by the species that I observed. However, this method of annotating each video one by one proved inefficient, so I switched to screening the videos quickly and only annotating videos containing deer. This meant that it is possible that some videos of deer may have been missed in our collection. Despite these challenges with data collection for all of these datasets, the data remains reliable as we maintained consistent research parameters throughout the study development.

Future Directions:

In the future, it will be advantageous to interpret how climate change will influence consumptive habits of Northeastern white-tailed deer. The effects of climate change and environmental adaptation can cause diet fluctuation by impacting the availability of resources, as well as the length of the seasons, causing deer behavior to change. From another perspective, it is valuable to compare and contrast diet patterns by geography or other spatiotemporal facets. Furthermore, this work can have ramifications for larger woodland plant conservation efforts, particularly when examining the connections between herbivory and primary producers, and the role of invasive species within this.

By analyzing deer populations, researchers can indirectly gather information about the vegetation they consume, providing a clearer understanding of local plant communities and their dynamics. Regarding plant life, seasonal deer consumptive behavior and diet composition can point to concerns regarding plant conservation and biodiversity. Understanding deer diets could help question ways in which these organisms could be utilized against the growth of non-native plant species. Examining the behavior of Northeastern white-tailed deer can reveal valuable connections to the surrounding ecosystem and create opportunities to improve efforts to combat both deer overpopulation and invasive species concerns along the East Coast.

Conclusion:

By integrating observational, experimental, and statistical methods of inquiry, this study uncovered dietary behavior and patterns in Northeastern white-tailed deer located in Princeton, New Jersey. Throughout the year, the deer must adapt to seasonality, adjusting their diets in order to reflect the composition of the surrounding environment. The subject of how deer diets vary seasonally was addressed by combining camera footage data, DNA metabarcoding analysis, and the observation of patterns and behaviors during chance encounters. This research shed light on the interdependent maintenance of plant species that deer consume through an initial ecological examination with Northeastern white-tailed deer as an accessible study system.[4] Collectively, these approaches addressed inquiries concerning deer activity patterns and the effects of that behavior on both humans and flora in the Princeton region.

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Tables:

Table 1: Experimental Breakdown

I. **Question:** How do white-tailed deer diets change throughout the seasonal cycle?

   A. *Research Questions:*

      1. What types of plants are these Northeastern white-tailed deer consuming through the seasons and in what proportions?
      2. Does diet quality vary seasonally?

   B. ***Hypothesis 1:*** Deer diets primarily rely on understory vegetation, including grasses and forbs, because of the availability of these plant types throughout the year.

      1. <u>Prediction 1:</u> Deer will spend the most time eating or performing consumptive behaviors, and of that time, spend more time grazing than browsing.
      2. <u>Prediction 2:</u> Proportions of grazing foliage in deer diets are greater than browse resources.

   C. Tests:

      1. <u>Test 1:</u> To perform seasonal observations of white-tailed deer, including behaviors and other characteristics. Data will be compiled in a collaborative Google Spreadsheet so that observers can annotate when deer encounters occur. This database will be constantly accessible, so that observations can cover all seasons. Camera trapping data provided by Bakos Princeton Wildlife Monitoring Project (BPWMP) will also be analyzed to track deer encounters and behaviors.
      2. <u>Test 2:</u> To identify the species and proportions of plants consumed by white-tailed deer from the DNA in deer dung samples, using a technique

known as DNA metabarcoding. Collected dung samples will be prepared for DNA extraction and submitted the extracted samples to the Princeton Lewis-Sigler Institute Genomics Core Facility for DNA sequencing. The sequenced DNA will be compared against reference plant DNA barcodes; for example, using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) software to match extracted plant DNA to known organisms.

3. Test 3: To perform nutrient assays on collected deer dung samples and analyze the nutritional content to recognize the impact on deer diet. Once samples have been collected, 50 samples will be submitted to the UC Davis Stable Isotope Facility to undergo stable isotope analysis. This will identify proportions of carbon and nitrogen within the samples and can be used to determine the nutritional composition of the diets.

D. ***Alternative Hypothesis:*** Deer consume foliage predominantly because of their group size during the seasons and the nutritional quality of those resources.

1. Prediction 1: Deer diets are proportionally higher in herbaceous vegetation.
2. Prediction 2: Diets composed of more herbaceous vegetation will be of higher quality, with lower C:N.
3. Prediction 3: Deer group size impacts diet quality, and individuals in larger groups will exhibit lower quality diets.

Table 2: Experimental Breakdown Summary

Category	Description
Question	How do white-tailed deer diets change throughout the seasonal cycle?
Research Questions	(1) What types of plants are these Northeastern white-tailed deer consuming through the seasons and in what proportions? (2) Are the deer able to consume enough during the winter to sustain themselves, and does that diet provide an adequate amount of nutrition, in comparison to the diet of the spring?
Hypothesis 1	Deer diets primarily rely on understory vegetation, including grasses and forbs, because of the availability of these plant types throughout the year.
Prediction 1	Deer will be observed eating or performing consumptive behaviors.
Prediction 2	Deer will be observed spending more time grazing than browsing.
Prediction 3	Proportions of grazing foliage in deer diets are greater than mast or browse resources.
Tests	(1) Seasonal observations of white-tailed deer behaviors. (2) DNA metabarcoding on collected dung samples.(3) Nutritional assays on collected samples.
Alternative Hypothesis	Deer consume foliage predominantly because of their group size during the seasons and the nutritional quality of those resources.
Prediction 1	Deer diets are proportionally higher in herbaceous vegetation because they are higher quality.
Prediction 2	Deer group size impacts diet quality.
Prediction 3	Individuals in larger groups will exhibit lower quality diets.
Tests	(1) DNA metabarcoding of dung samples.(2) Analysis of group size using ANOVA and correlation testing.(3) Nutritional assays on collected samples.

Table 2: Experimental Breakdown Summary. This table displays a condensed version of Table 1, depicting the hypothesis development of this study.

Table 3: Princeton Resident Deer Survey Chi-Squared Test of Independence Results

Chi-Squared Test Results

Variable	Chi-Squared Statistic	Degrees of Freedom	P-Value
Attitude Towards Deer	12.22535	5	3.182706e-02
Group Sizes	44.47887	4	5.102241e-09
Encounter Frequency	17.85915	4	1.314815e-03
Timing of Encounters	218.84507	20	2.029976e-35
Deer-Related Concerns	279.77465	30	2.475228e-42
Resident's Experience	36.52113	4	2.260513e-07

Table 3: Princeton Resident Deer Survey Chi-Squared Test of Independence Results. The chart above lists the calculated results from chi-squared (χ^2) testing.

Table 4: Association between Deer Presence and Plant Species

Association between Deer Presence and Plant Species Chi-Square Test Results	
Plant Species	P-Value
Asters	0.207
Common Ragweed	0.695
Euonymus	0.084
Garlic Mustard	0.572
Holly	0.188
Impatiens	0.903
Ivy	0.517
Japanese Yew	0.217
Poison Ivy	0.161
Wild Lettuce	0.003

Table 4: Association between Deer Presence and Plant Species. Results from chi-squared (χ^2) testing comparing the number of weekly encounters, treated as deer presence, and the plant species analyzed.

Table 5: Association between Attitude and Group Size

Association between Attitude and Group Size Chi-Square Test Result	
Comparison	P_value
Attitude vs Group Size	0.620

Table 5: Association between Attitude and Group Size. This chart illustrates results from chi-squared (χ^2) testing of an association between the attitude towards deer and the group size of sightings.

Table 6: Summary of Relationships between Diet Richness and State Variables

Summary of Relationships between Diet Richness and State Variables Coefficients, p-values, and statistical details			
		Coefficients and P-values	
Model	Variable	Estimate	P-value
X[[i]]	(Intercept)	13.50000000	1.310803e-10
X[[i]]	Sex - Male	1.96666667	2.672953e-01
X[[i]]	(Intercept)	23.38238840	6.529304e-02
X[[i]]	Body Length (cm)	-0.06434246	4.759520e-01
X[[i]]	(Intercept)	15.16666667	4.944418e-04
X[[i]]	Fetus	-1.11111111	5.355414e-01
X[[i]]	(Intercept)	14.50838297	6.287460e-07
X[[i]]	Kidney Ratio	0.19511087	9.340456e-01

Table 6: Summary of Relationships between Diet Richness and State Variables. This table contains the p-values and estimated coefficients for various diet richness data points.

Table 7: Summary of Relationships between Diet Diversity and State Variables

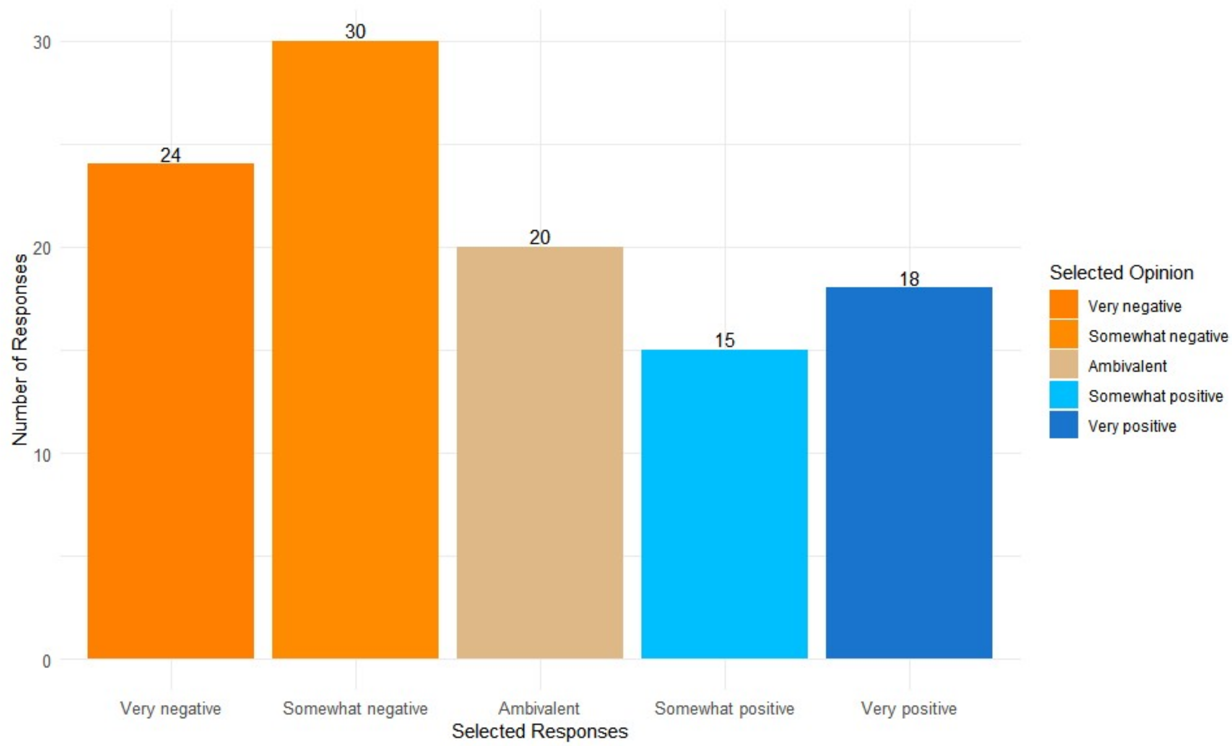
Summary of Relationships between Diet Diversity and State Variables Coefficients, p-values, and statistical details			
		Coefficients and P-values	
Model	Variable	Estimate	P-value
X[[i]]	(Intercept)	1.879265189	3.214813e-12
X[[i]]	Sex - Male	0.074529555	7.158206e-01
X[[i]]	(Intercept)	2.170250360	1.381550e-01
X[[i]]	Body Length (cm)	-0.001744346	8.681200e-01
X[[i]]	(Intercept)	2.114016141	3.064441e-04
X[[i]]	Fetus	-0.156500635	5.053415e-01
X[[i]]	(Intercept)	1.798245203	1.874882e-07
X[[i]]	Kidney Ratio	0.167296042	5.380368e-01

Table 7: Summary of Relationships between Diet Diversity and State Variables. Of the culled individuals, certain data points are measured, with their p-values and estimated coefficients presented.

Figures

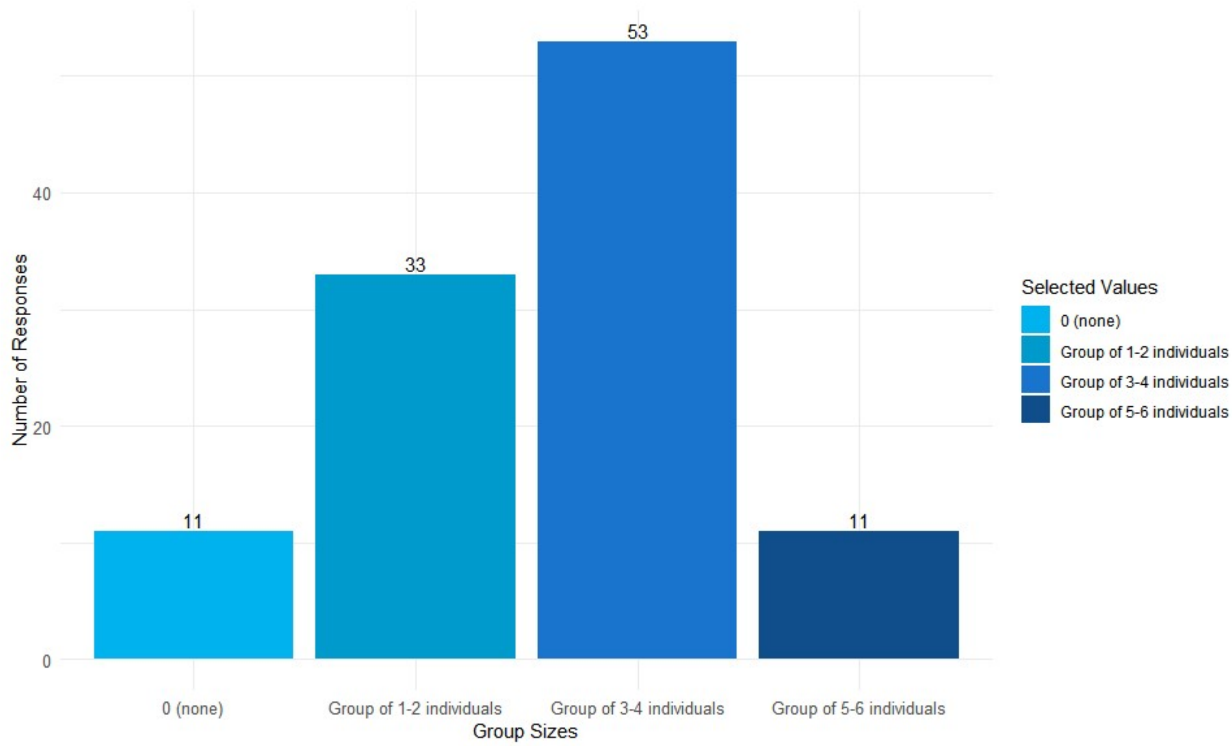
Princeton White-Tailed Deer Survey Figures:

Graph 1: Deer Attitude



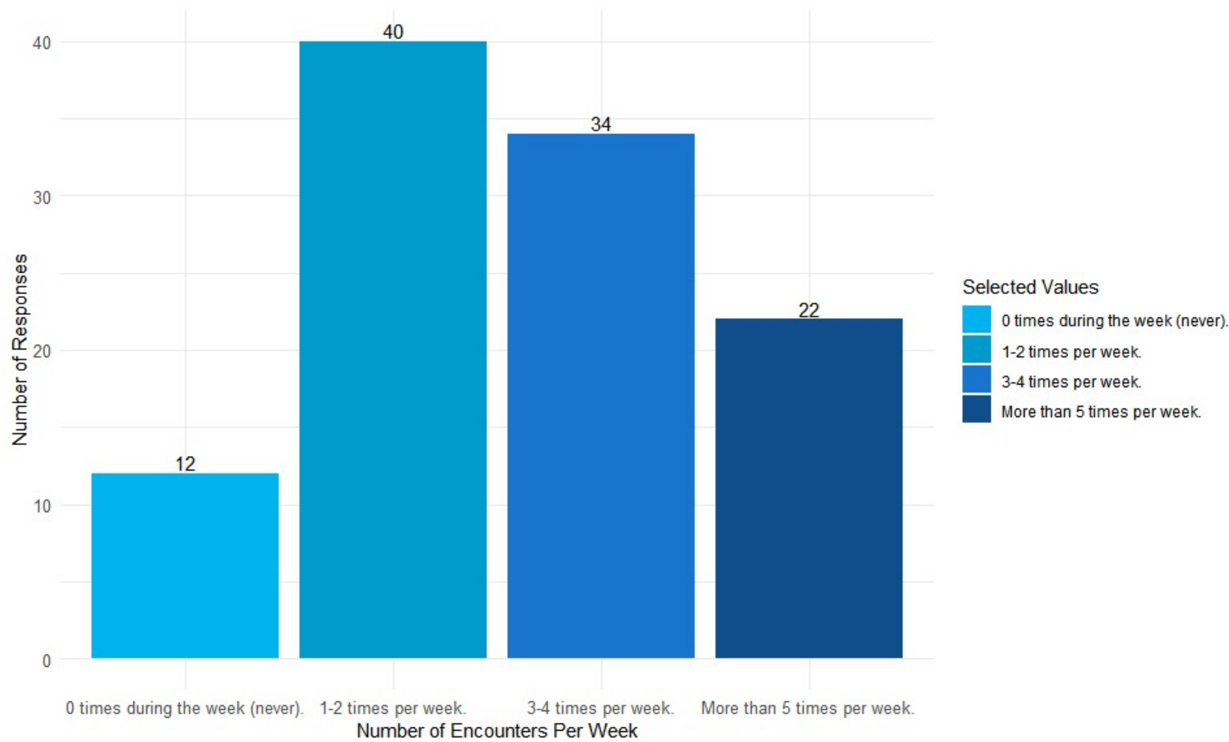
Graph 1: Deer Attitude. This histogram uses responses from the Princeton Resident Deer Survey and demonstrates respondent opinion regarding deer in Princeton, New Jersey with the question, “What is your attitude towards deer in Princeton?” (n=142; χ^2p-value<0.05).

Graph 2: Observed Group Size by Princeton Residents



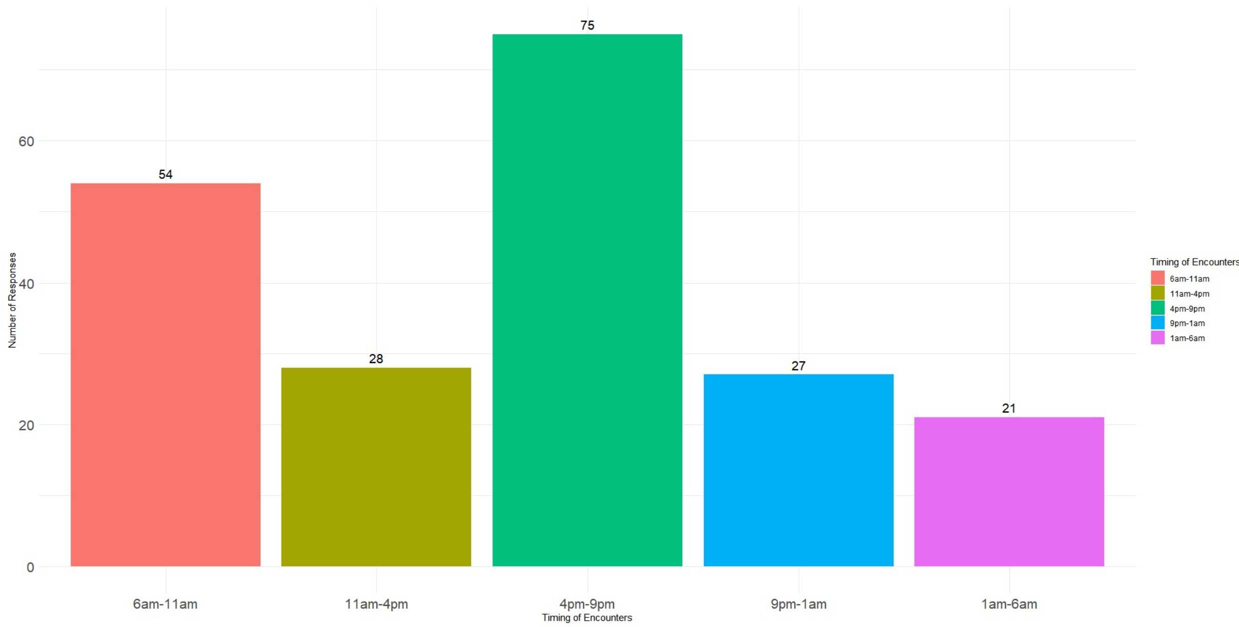
Graph 2: Observed Group Size by Princeton Residents. The histogram above shows the aggregated number of responses per average deer group size (n=142; χ^2p-value<0.05). This references the most common group size per sighting or encounter when considering the question, “How many individual deer do you typically see on or near your place of residence?.”

Graph 3: Observed Number of Encounters by Princeton Residents



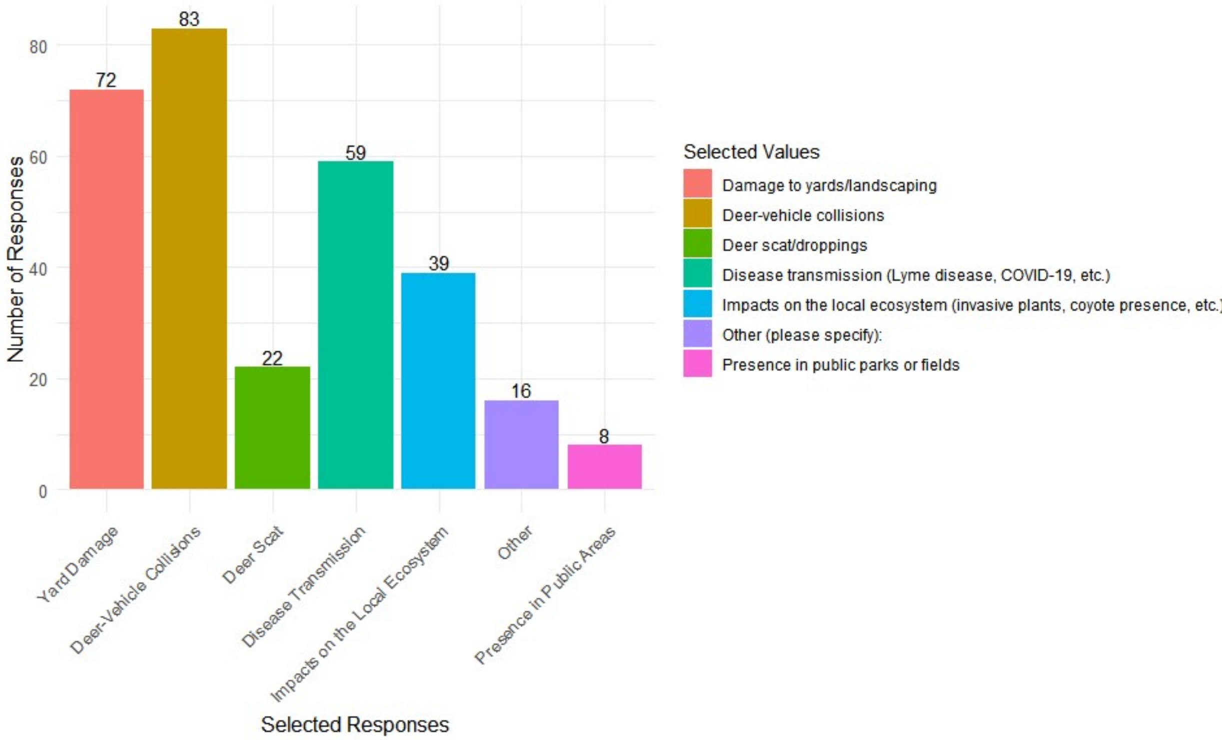
Graph 3: Observed Number of Encounters by Princeton Residents. This histogram reports the number of observations made on average per week by Princeton residents, considering the question, "How often do you encounter deer on or near your place of residence?" (n=142; χ^2p-value<0.05).

Graph 4: Timing of Princeton Resident Deer Encounters



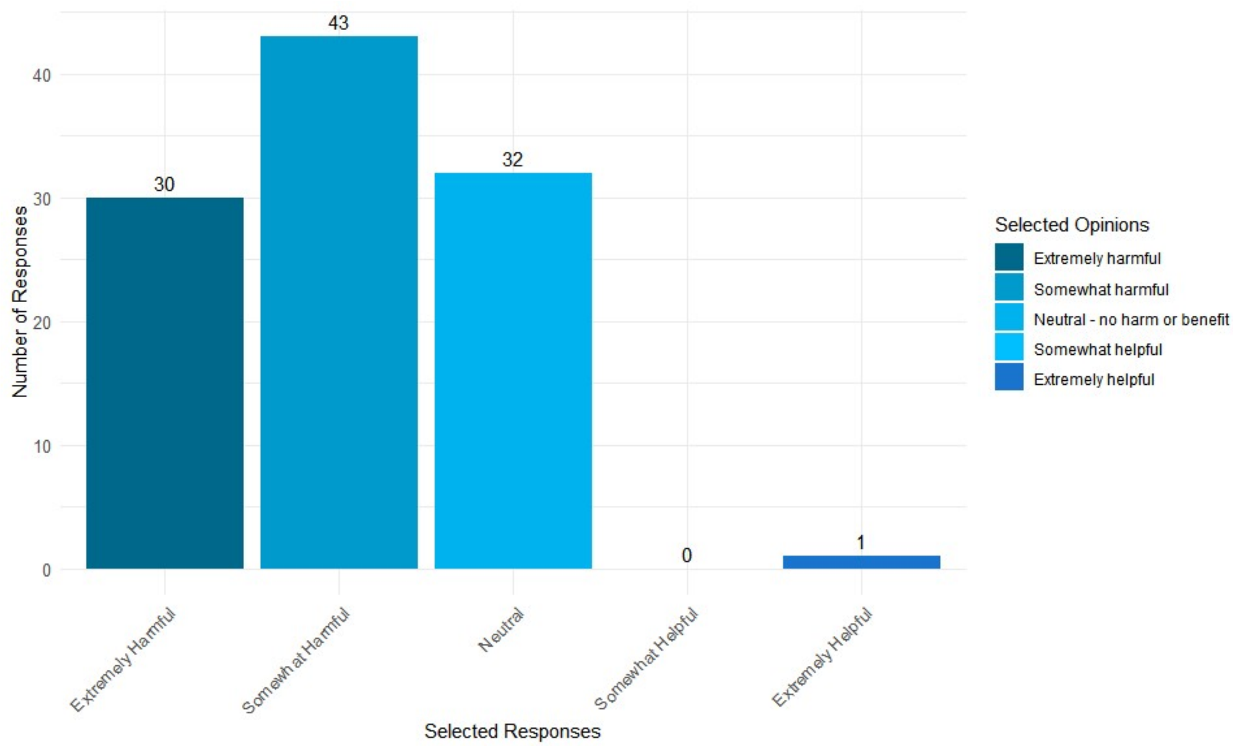
Graph 4: Timing of Princeton Resident Deer Encounters. The histogram above depicts the average time of deer encounter by Princeton residents (n=142; χ^2p-value<0.05). This corresponds to the question, "When do you normally see deer on or near your place of residence?."

Graph 5: Deer-Related Concerns of Princeton Residents



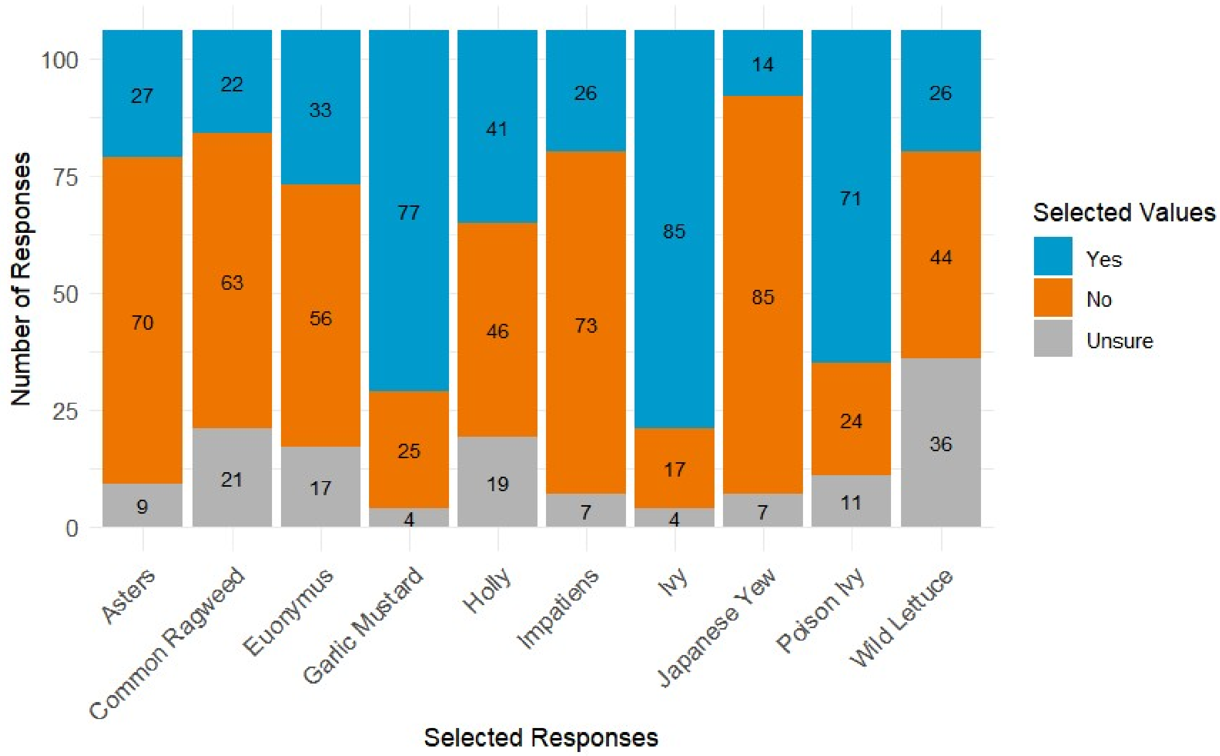
Graph 5: Deer-Related Concerns of Princeton Residents. This histogram lists the distribution of deer-related concerns as selected by survey respondents (n=142; χ^2p-value<0.05). Respondents were asked, "Are you concerned about any of the following deer-related issues?."

Graph 6: Resident Experience of Deer-Impacts on Plants



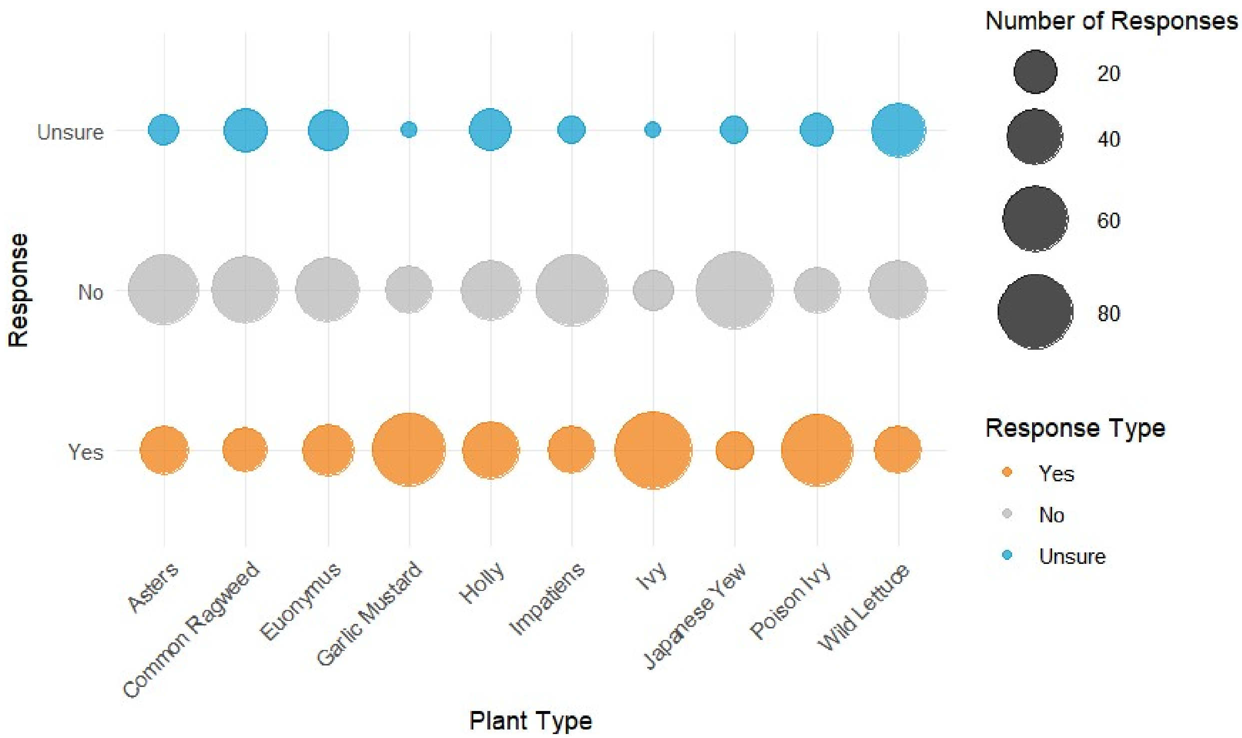
Graph 6: Resident Experience of Deer-Impacts on Plants. This histogram demonstrates the opinions of Princeton residents on the impact of deer on surrounding plant life (n=142; χ^2p-value<0.05). This corresponds to the question, "In your opinion, how do deer impact the plant life in your yard?."

Graph 7: Plant Selection from Survey



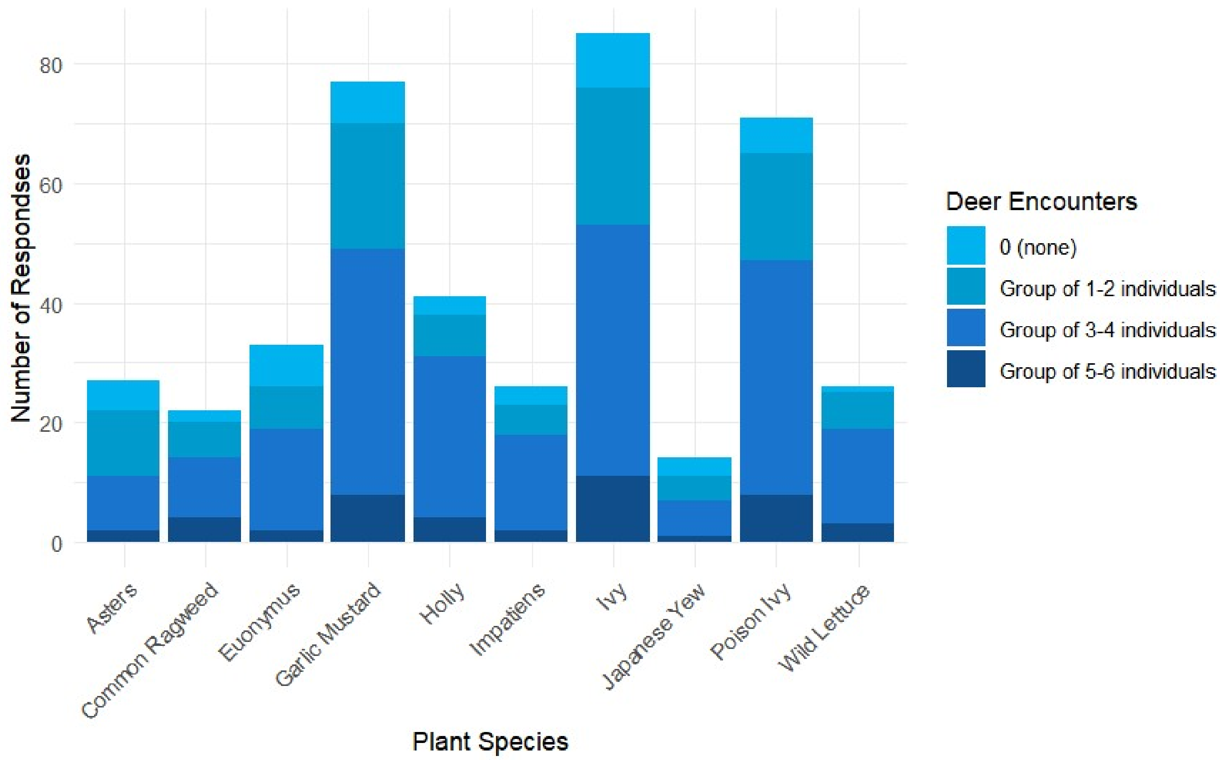
Graph 7: Plant Selection from Survey. The bar graph above displays the plants near residential property of survey respondents (n=142; χ^2p-value<0.05). Residents were asked, “Which of these plants do you have on and/or around your property?.”

Graph 8: Residential Property Plant Composition



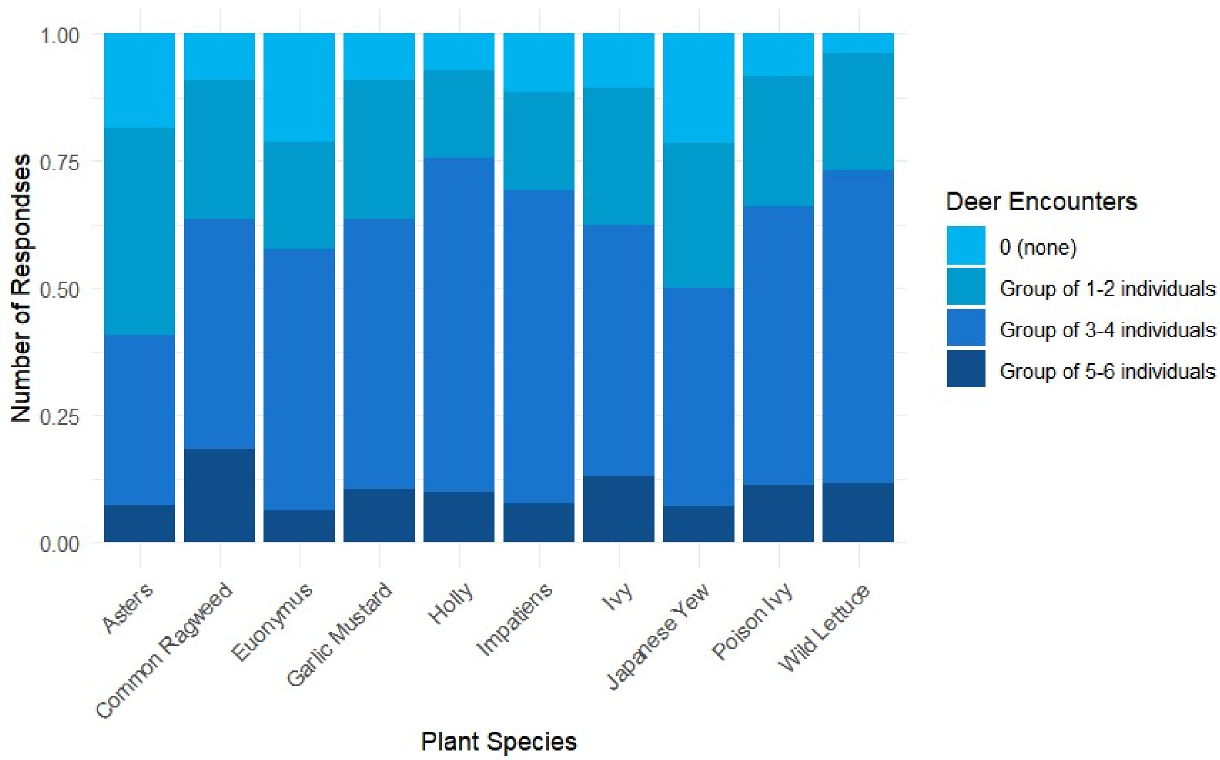
Graph 8: Residential Property Plant Composition. The bubble plot illustrates the plant composition of respondent property by using the density of the responses to show a distribution (n=142; χ^2p-value<0.05).

Graph 9: Plant Presence by Deer Encounter Frequency



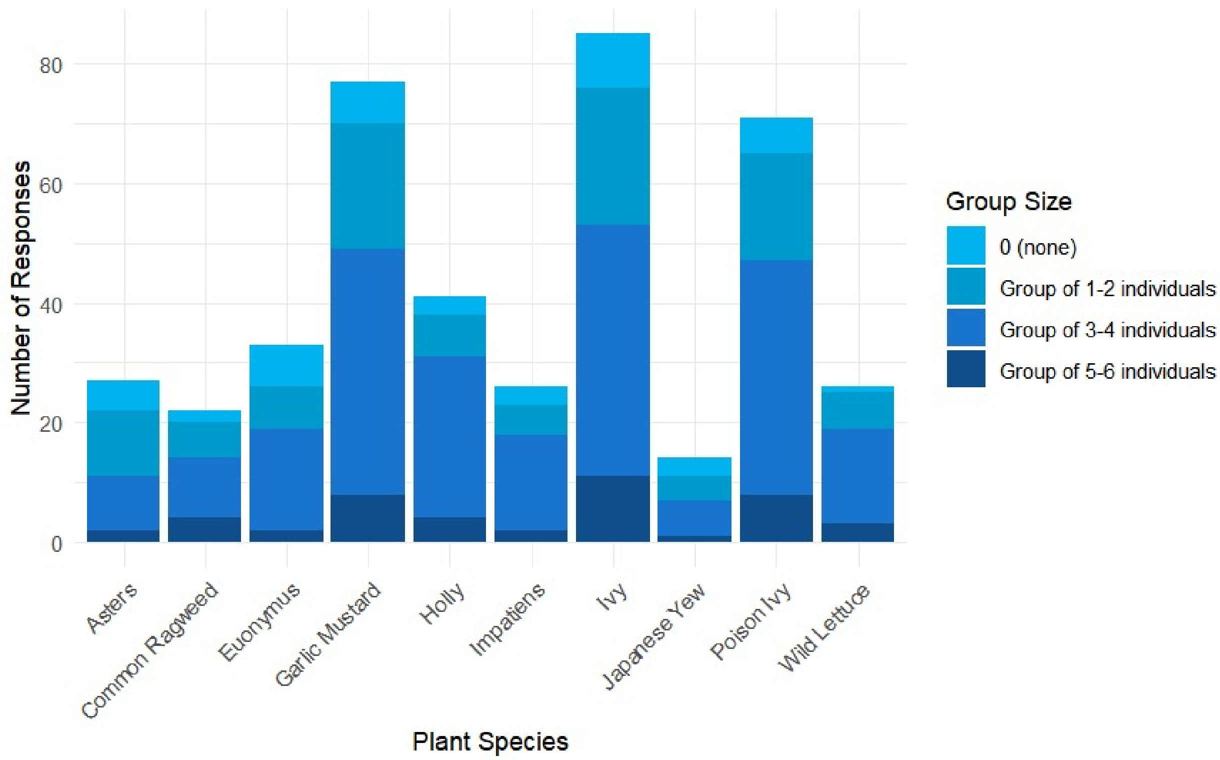
Graph 9: Plant Presence by Deer Encounter Frequency. This bar graph presents the correlation between reported plant species and the count of deer observations from each respondent (n=142).

Graph 10: Relative Plant Presence by Deer Encounter Frequency



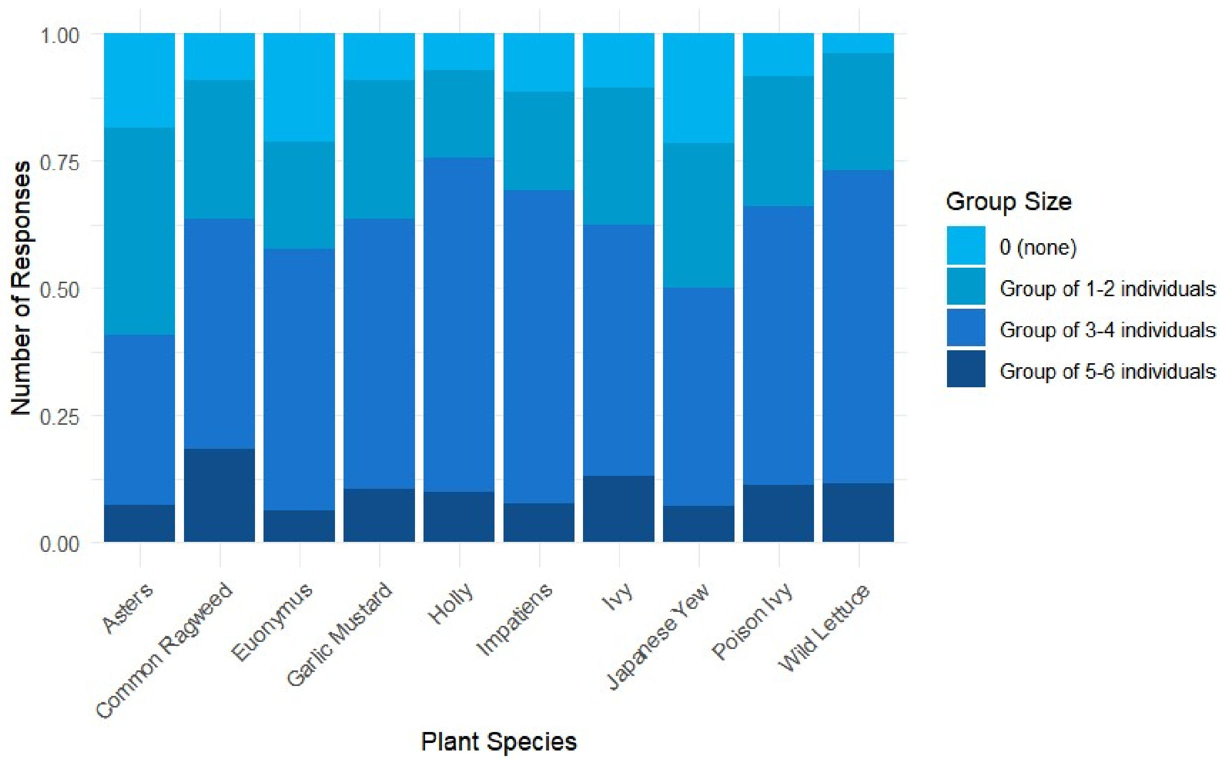
Graph 10: Relative Plant Presence by Deer Encounter Frequency. Using the reported levels of deer encounters with the plants identified by respondents, proportions were made so that these frequencies were relativized (n=142).

Graph 11: Plant Presence by Group Size



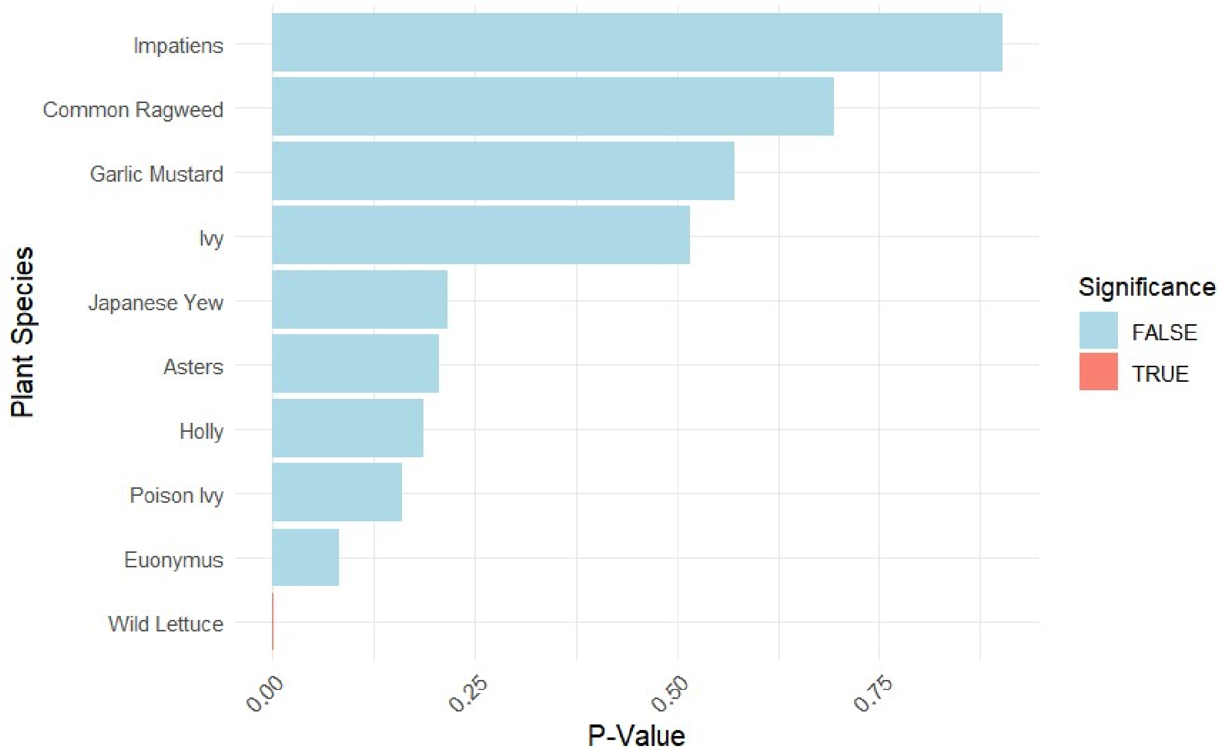
Graph 11: Plant Presence by Group Size. This chart demonstrates the reported plants from respondents in relation to deer group size averages in encounters (n=142).

Graph 12: Relative Plant Presence by Group Size



Graph 12: Relative Plant Presence by Group Size. Taking the proportions of the plant species with the group sizes from observational responses, this graph illustrates the relative ratios of this relationship (n=142).

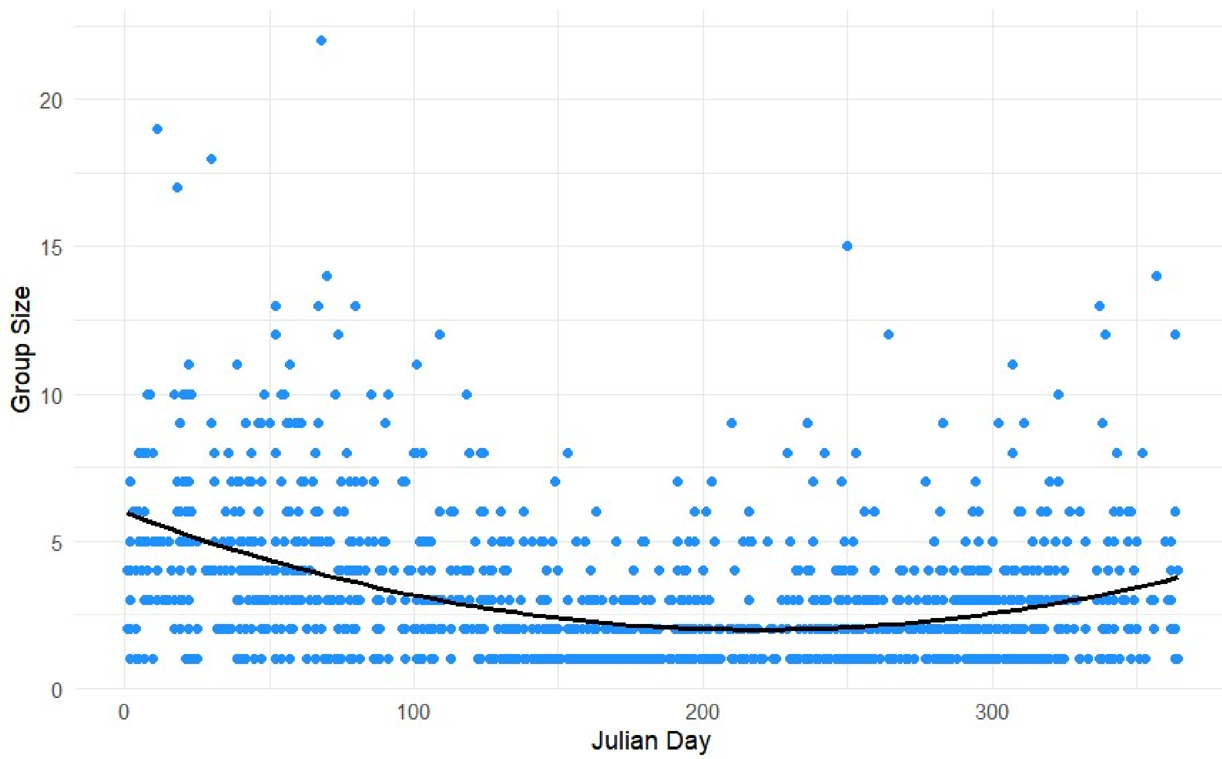
Graph 13: Chi-Square Testing of Association between Deer Presence and Plant Species



Graph 13: Chi-Square Testing of Association between Deer Presence and Plant Species. The above visualizes the chi-square testing p-values to assess the relationship between deer presence per week and the plant species reported (n=142; χ^2p-value <0.05).

Observational Data Figures:

Graph 14: Observations of Deer Group Size in Princeton

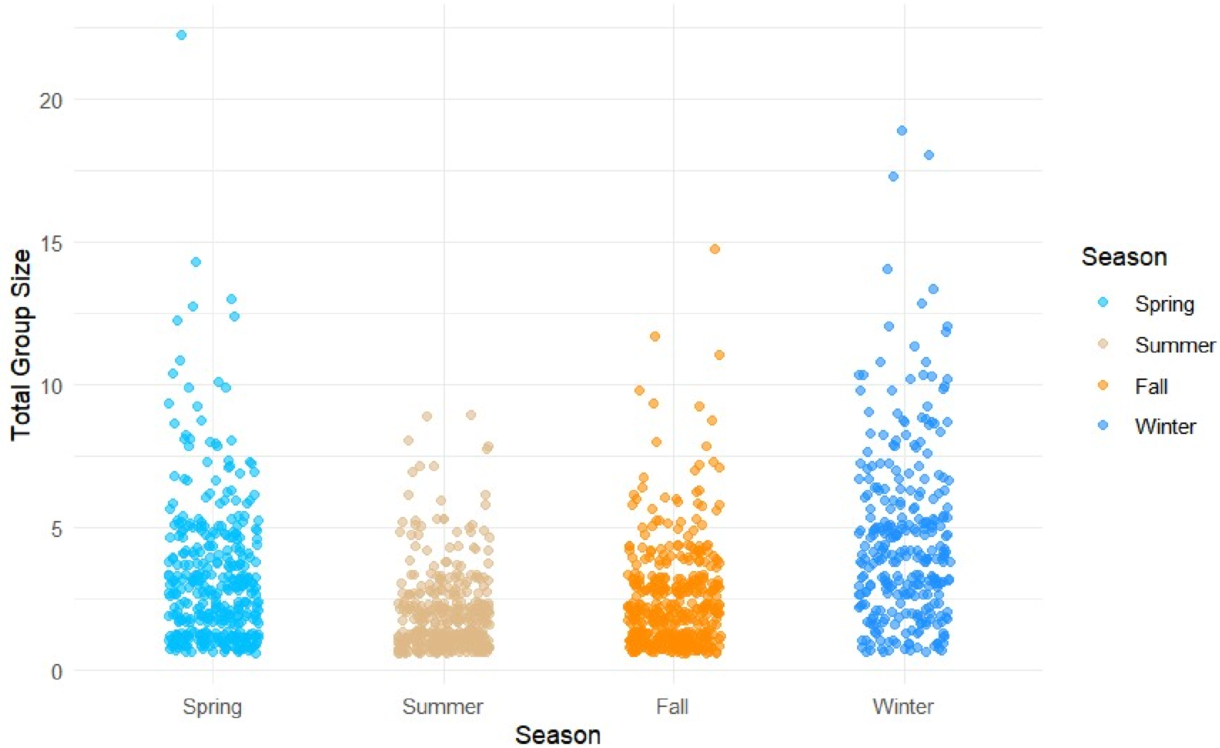


Graph 14: Observations of Deer Group Size in Princeton. This histogram displays the number of reported deer observed by group size over time by Julian day (n=1526; p-value = 0.0966).

Graph 15: Heat Map of Observed Deer Encounters

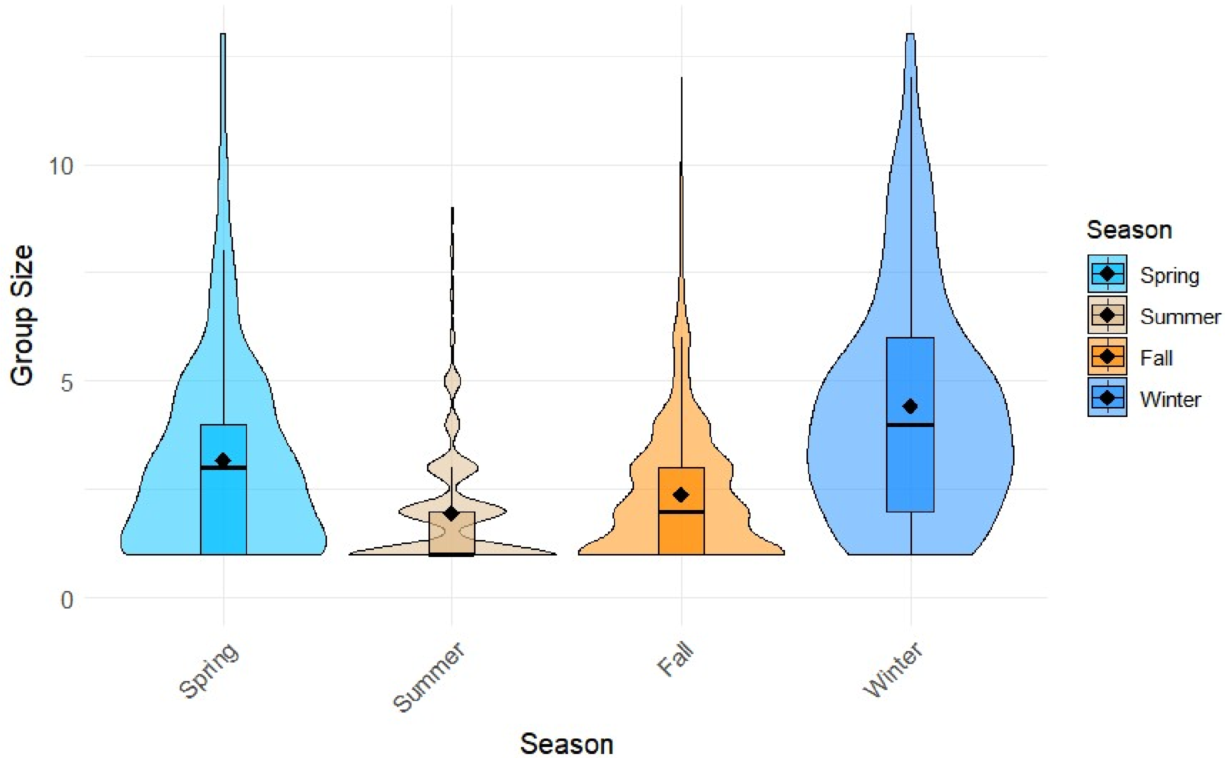
Graph 15: Heat Map of Observed Deer Encounters. This map utilizes latitude and longitude measurements of deer sightings to display areas of deer presence in Princeton, NJ (n=1526). The first image represents the sightings at a scale of 38 m/px and the second image is at a scale of 4.8 m/px.

Graph 16: Observations of Group Size by Season



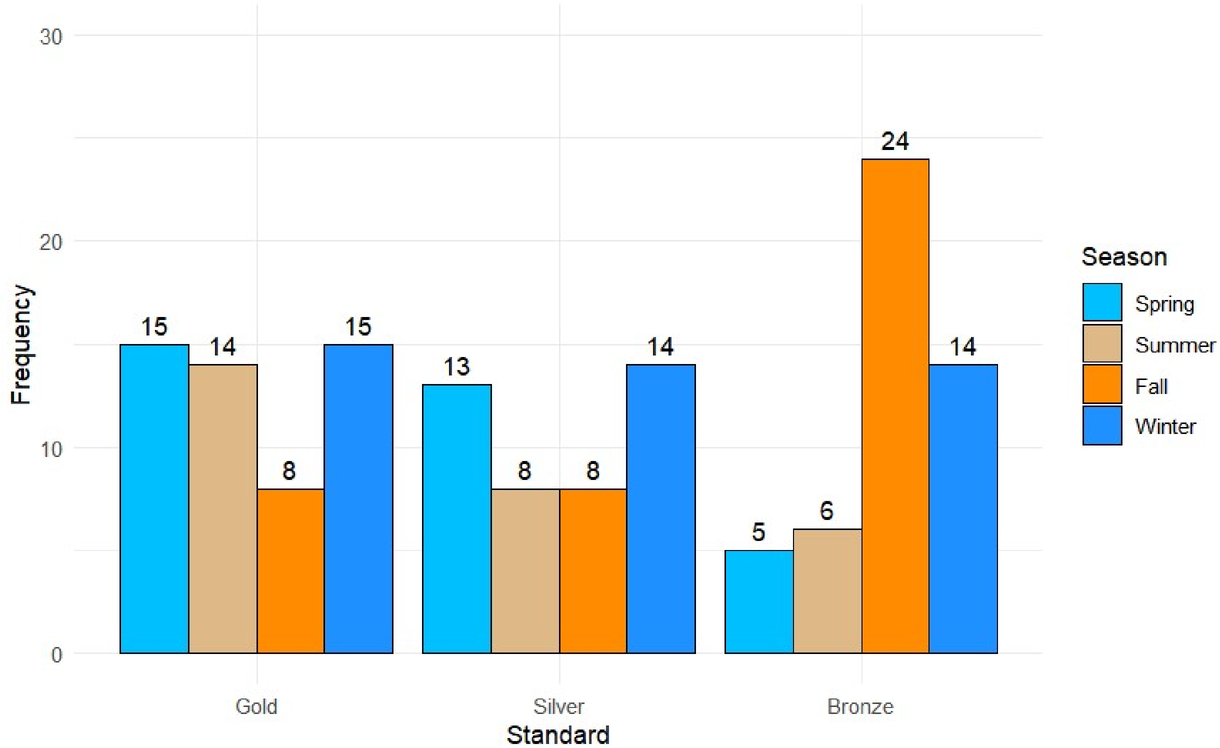
Graph 16: Observations of Group Size by Season. This visualization demonstrates the seasonal distribution of encounters and their group sizes at those times (n=1526).

Graph 17: Average Group Size by Season



Graph 17: Average Group Size by Season. These violin boxplots compile the seasonal group size distributions (n=1526; p-value <0.05).

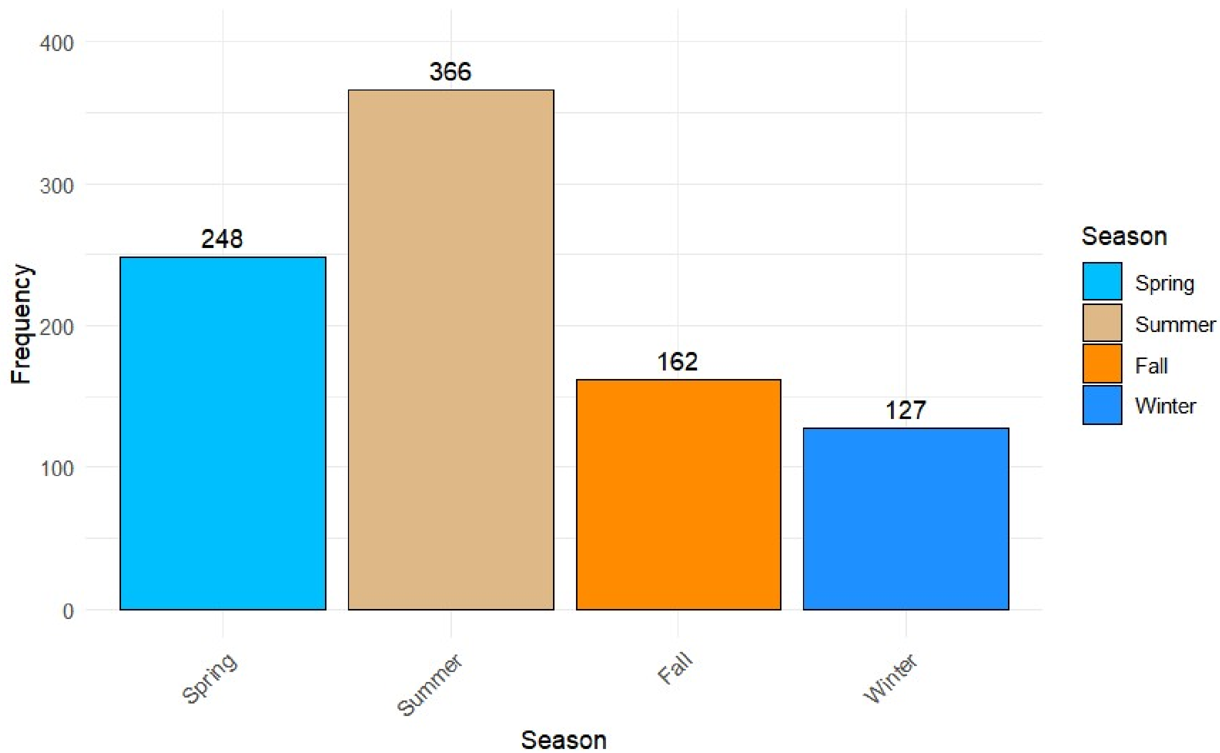
Graph 18: Comparison of Standard by Season for Deer Dung Samples



Graph 18: Comparison of Standard by Season for Deer Dung Samples. The bar graph displays the standard of the dung collected with the season in which it was collected (n=145; p-value Standard = 0.885, p-value Season = 0.781).

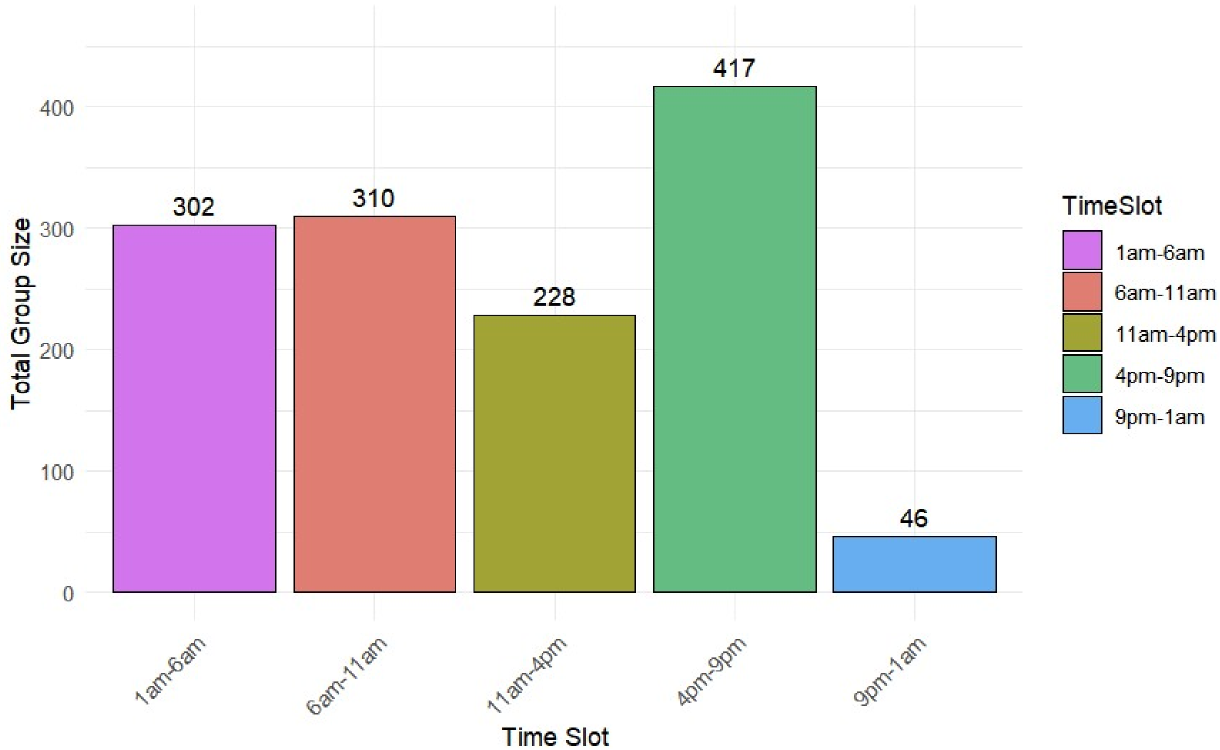
Camera Trap Data Figures:

Graph 19: Observation Frequency by Season



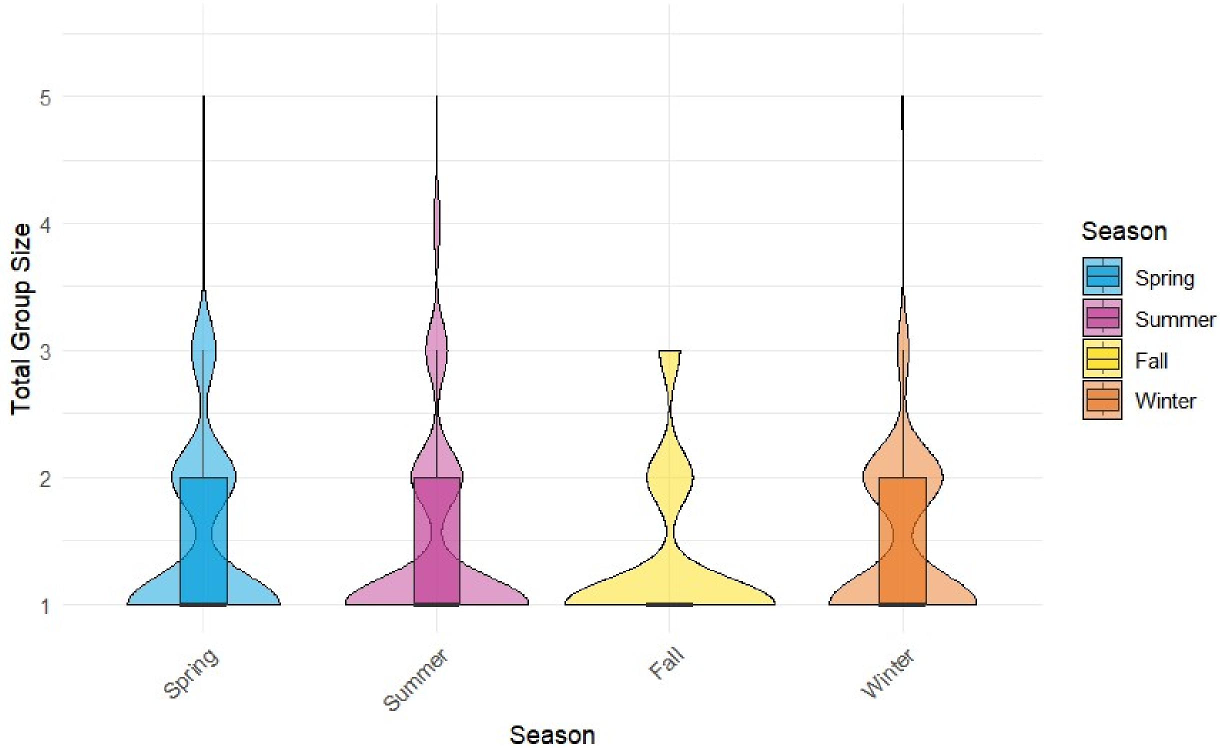
Graph 19: Observation Frequency by Season. This histogram portrays the seasonal frequency of deer individuals observed in the Bakos Princeton Wildlife Monitoring Project video dataset (n=903; p-value = 0.451).

Graph 20: Observation Frequency by Time Slot



Graph 20: Observation Frequency by Time Slot. The video data is sorted by time slot and the total of individuals seen at that time (n=903; p-value = 0.824).

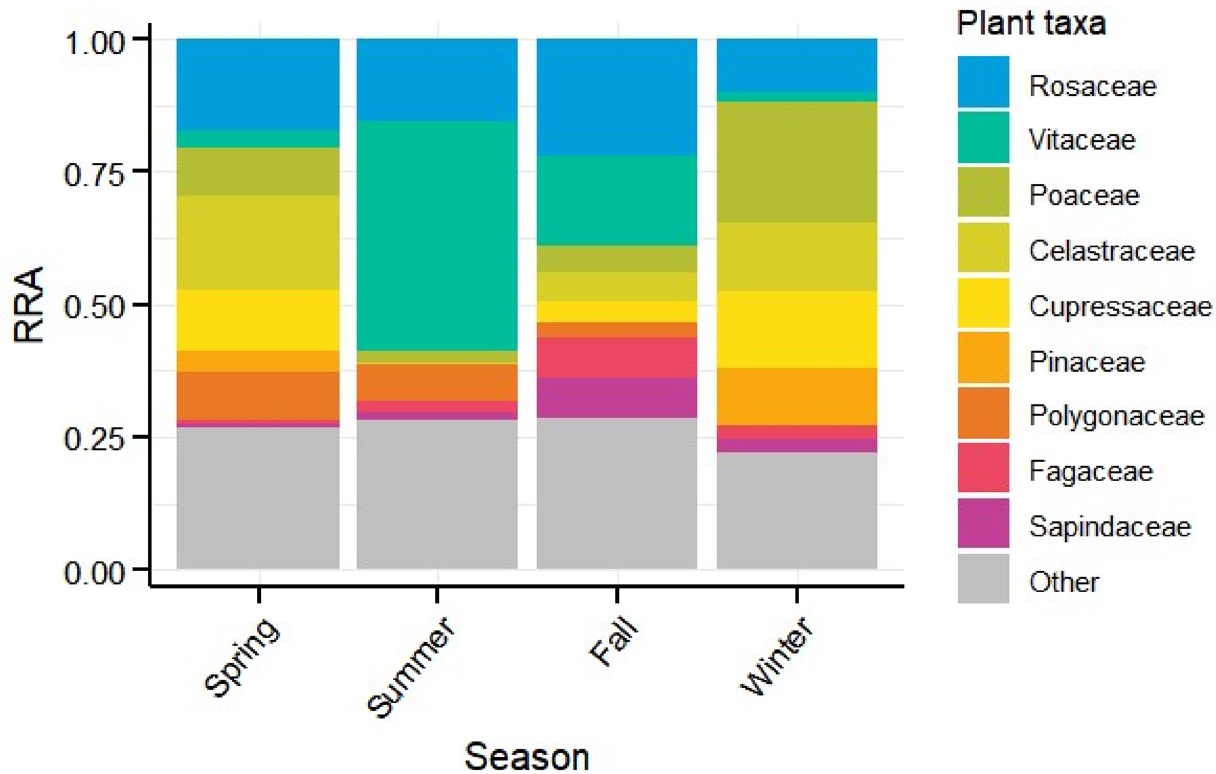
Graph 21: Observation Frequency by Time Slot



Graph 21: Observation Frequency by Time Slot. The video data is sorted by time slot and the total of individuals seen at that time (n=903; p-value = 0.0174).

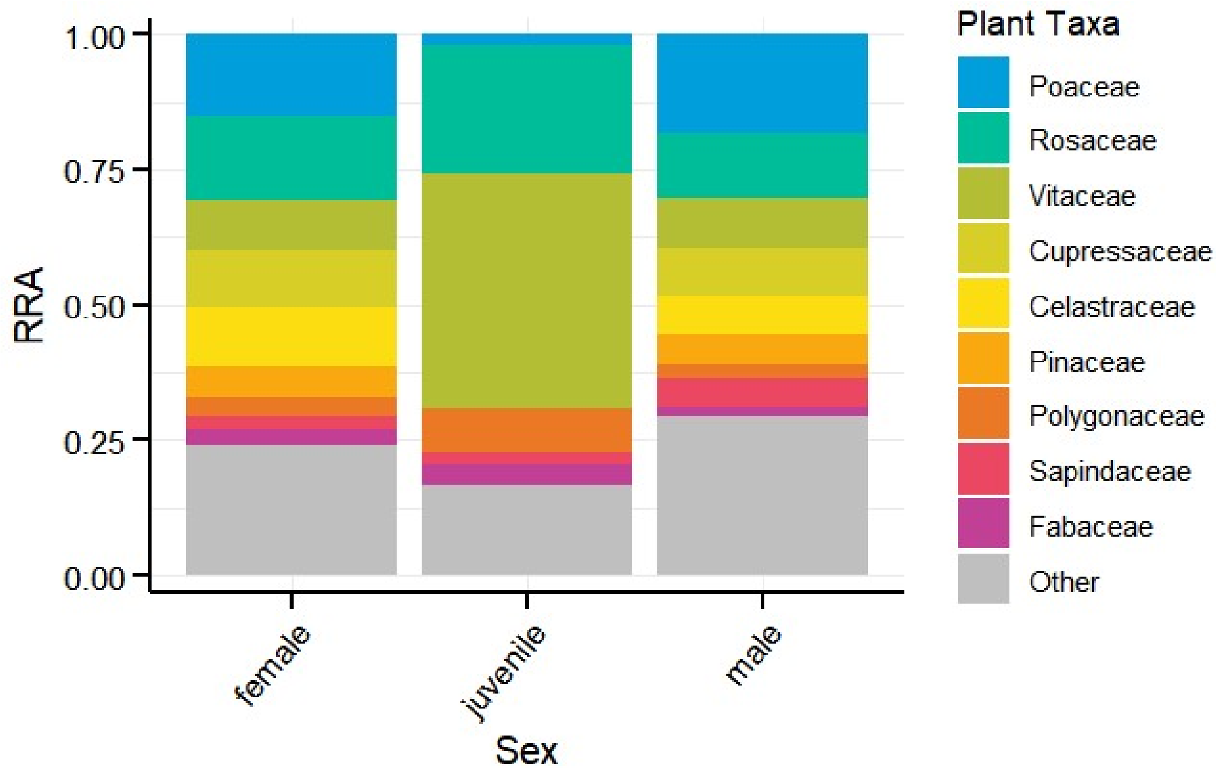
DNA Metabarcoding & Diet Analysis Figures:

Graph 22: Seasonal Plant Family Abundances



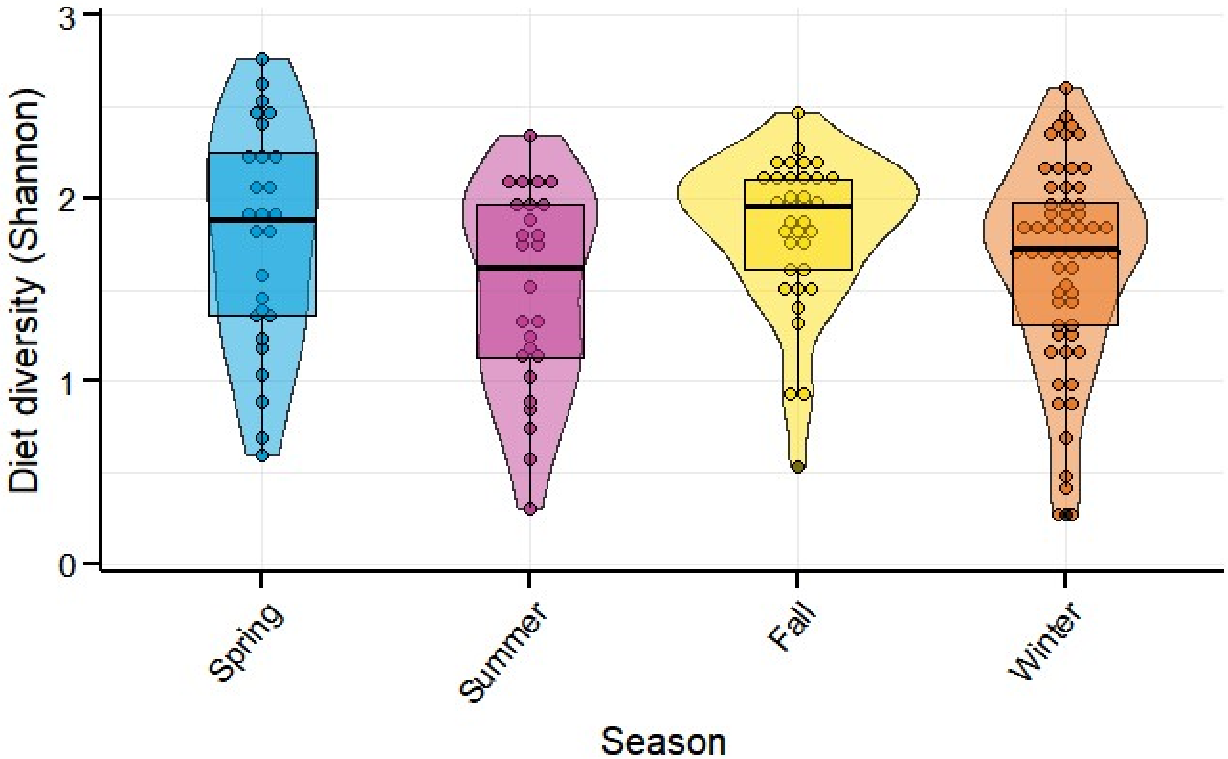
Graph 22: Seasonal Plant Family Abundances. The gradients above portray the diet composition by plant taxa across the seasons from DNA metabarcoding (n=145; p-value<0.05).

Graph 23: Diet Composition by Sex



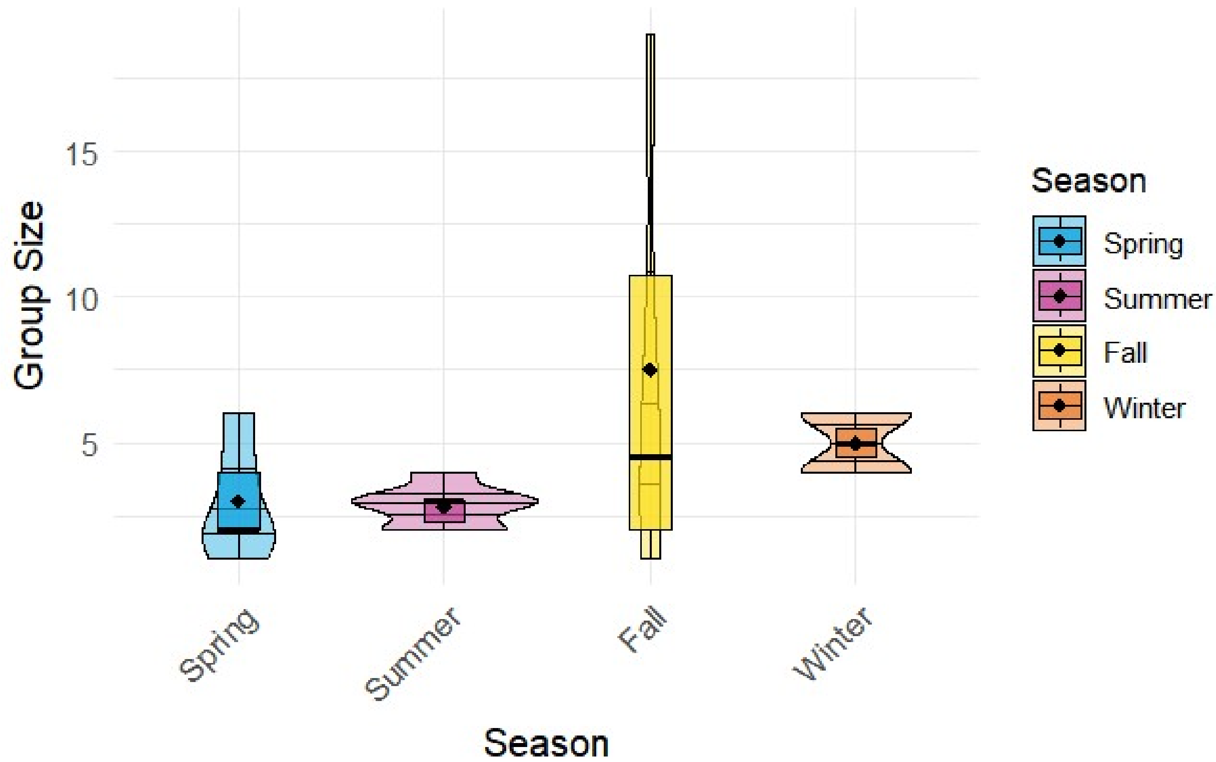
Graph 23: Diet Composition by Sex. Using DNA metabarcoding of the samples tested, this diagram illustrates the demography of the individual and their diet composition by plant taxa (n=145; p-value<0.05).

Graph 24: Diet Diversity Across Seasons



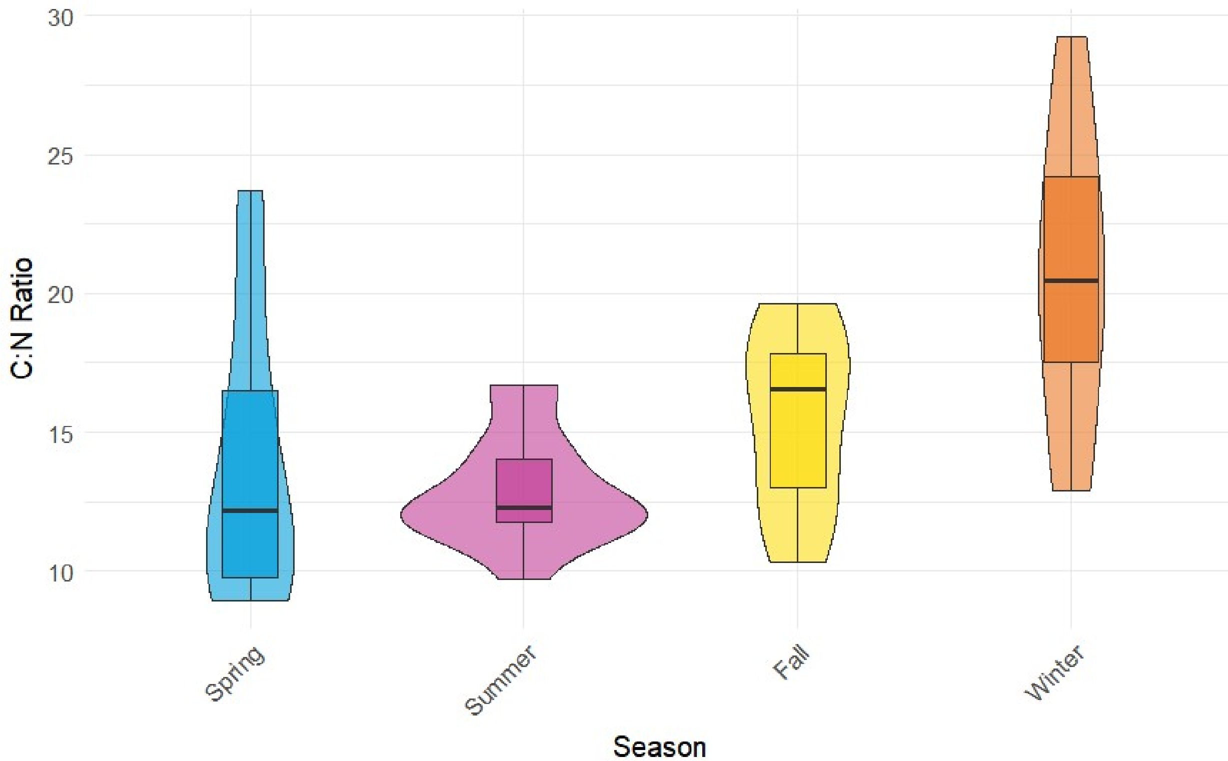
Graph 24: Diet Diversity Across Seasons. The violin plots above present the calculated diet diversity by the Shannon Diet Diversity Index seasonally (n=145; p-value = 0.0821).

Graph 25: Group Size by Season



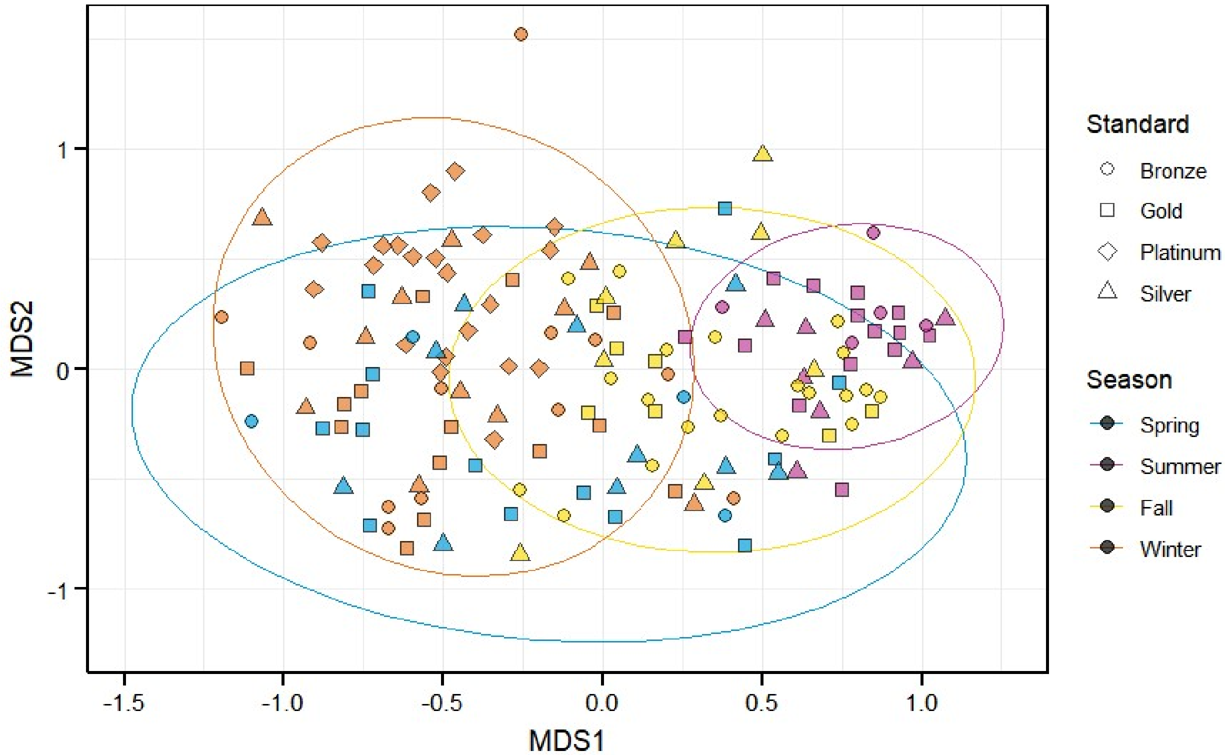
Graph 25: Group Size by Season. The bar graphs reveal the average group size by season (n=145; p-value<0.0001).

Graph 26: C:N Ratio Across Seasons



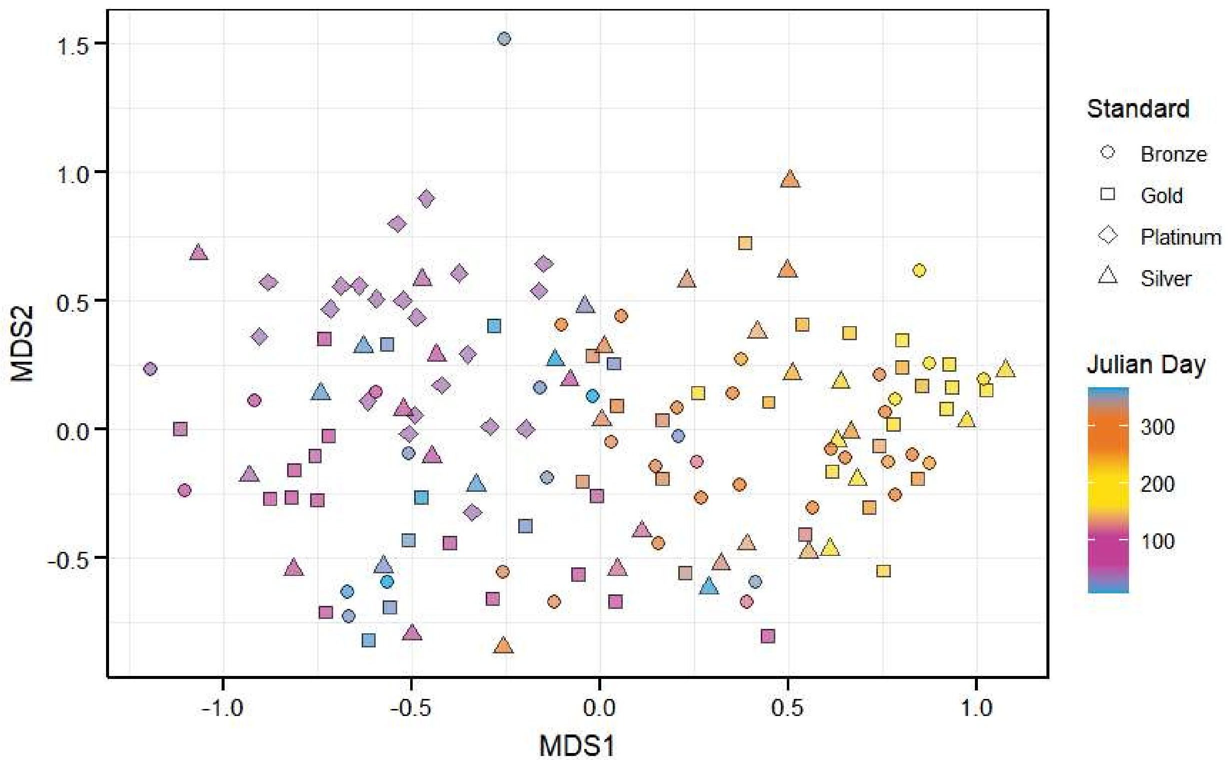
Graph 26: C:N Ratio Across Seasons. Using violin plots, the diet quality is determined using the C:N ratio and compared across the seasons (n=50; p-value<0.05).

Graph 27: MDS Plot of Deer Samples by Season and Standard



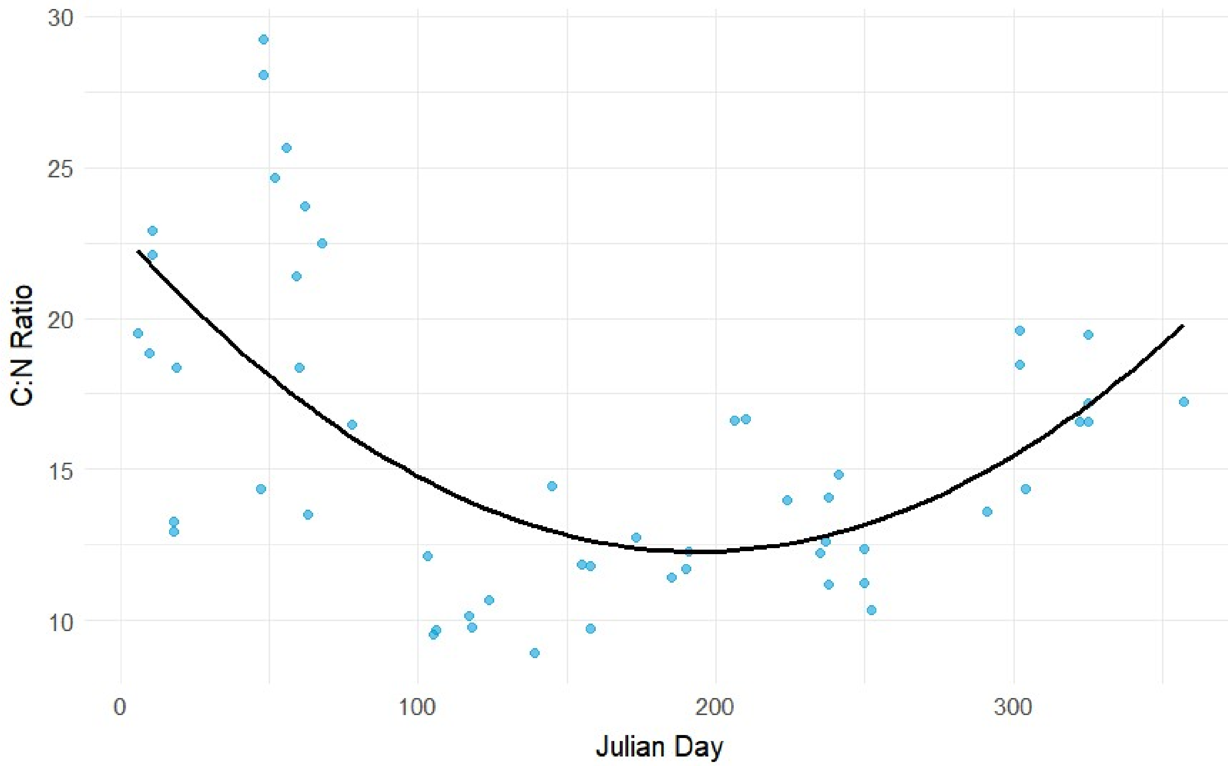
Graph 27: MDS Plot of Deer Samples by Season and Standard. Via multidimensional scaling testing, the similarities and dissimilarities between standard and season are compared (n=1000; p-value<0.001).

Graph 28: MDS Plot of Deer Fecal Samples



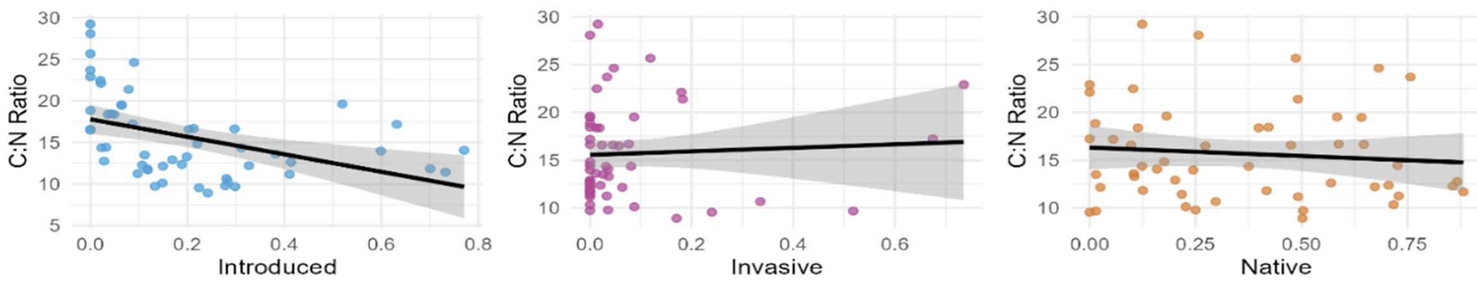
Graph 28: MDS Plot of Deer Fecal Samples. This chart illustrates the gradient of multidimensional scaling testing by standard for the fecal samples (n=1000; p-value<0.001).

Graph 29: Changes in Diet Quality by Julian Day



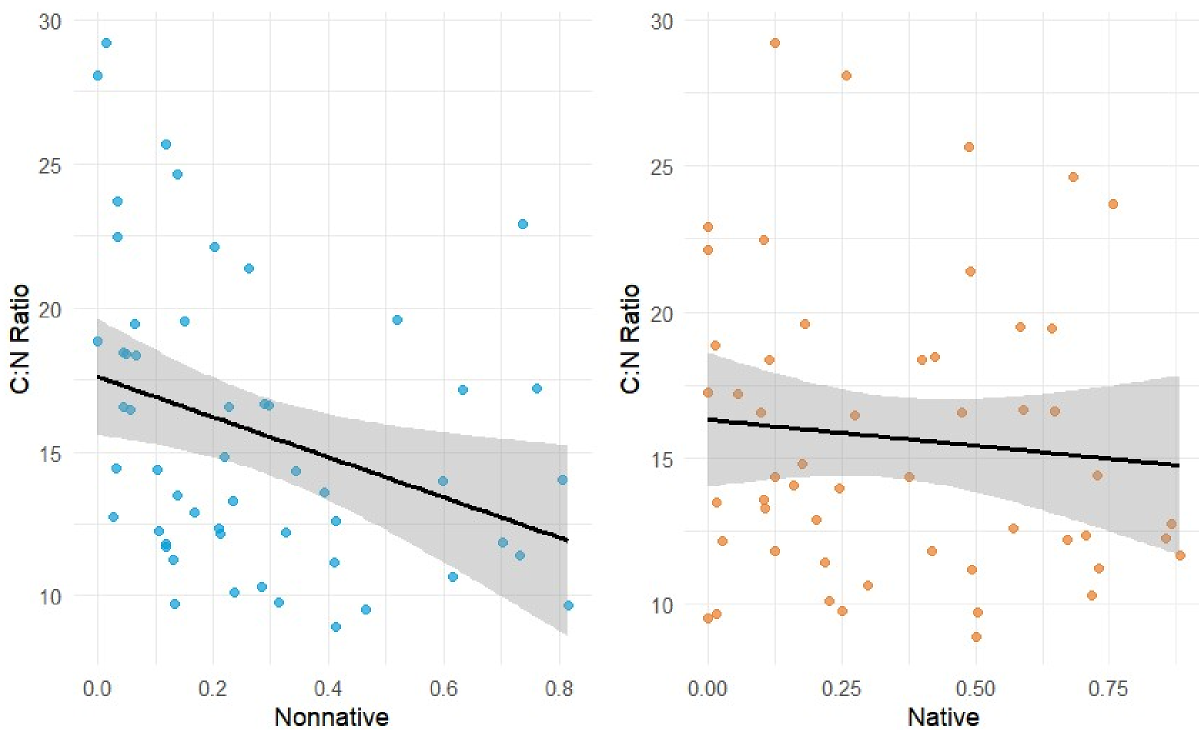
Graph 29: Changes in Diet Quality by Julian Day. The scatterplot above compiles the diet quality, as determined by the C:N ratio, against Julian Day (n=50; p-value<0.05).

Graph 30: Diet Quality by Status



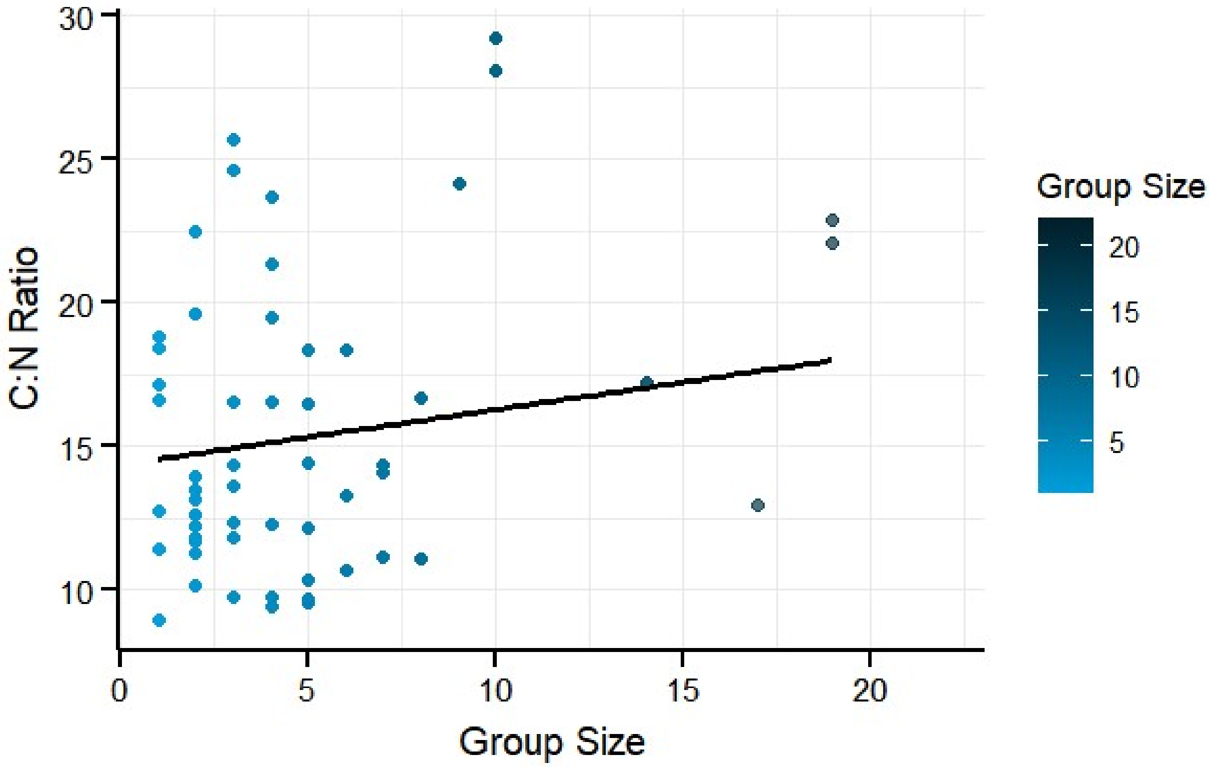
Graph 30: Diet Quality by Status. The charts above compare the status of the plants consumed as introduced, invasive, and native against the diet quality, as portrayed by the C:N ratio (n=50; p-value introduced = 0.00139; p-value invasive = 0.684; p-value native = 0.495).

Graph 31: C:N Ratio by Status Group



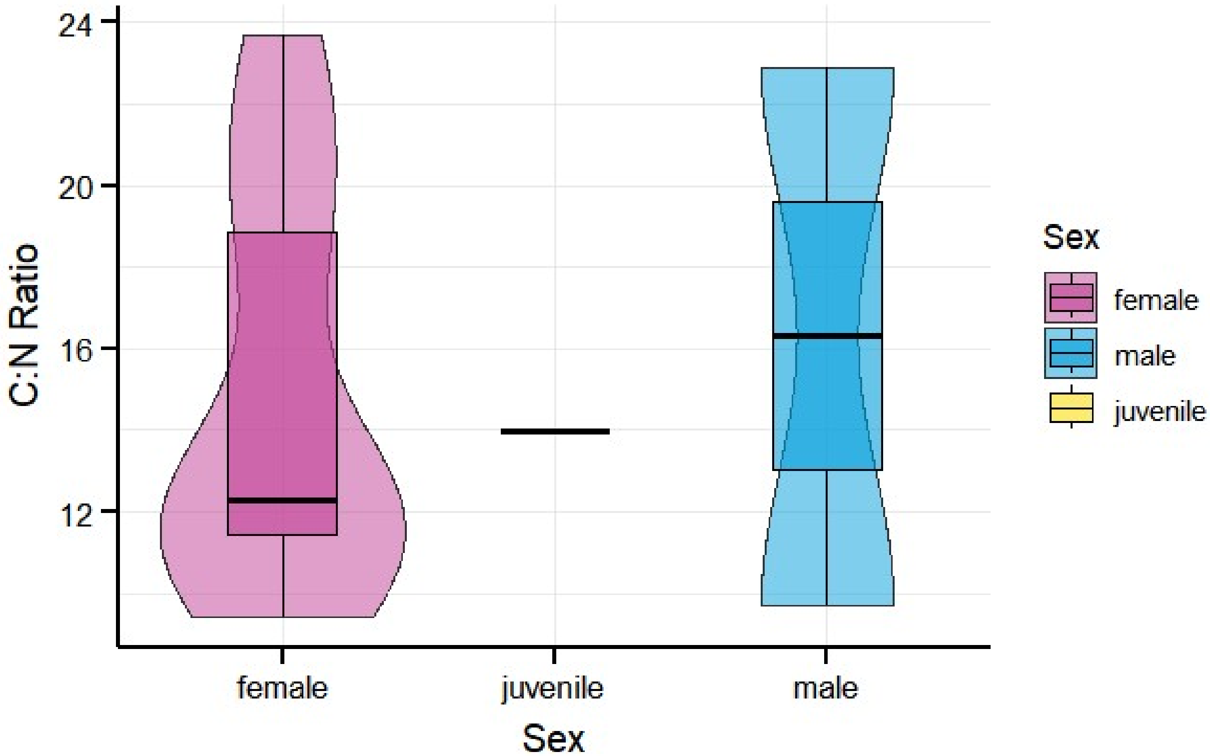
Graph 31: C:N Ratio by Status Group. The graphs above included the statuses of non-native, which includes both invasive and introduced plants, as well as native plants by the C:N ratio to purse through diet quality information (n=50; p-value non-native = 0.0158; p-value native = 0.495).

Graph 32: C:N Ratio by Group Size



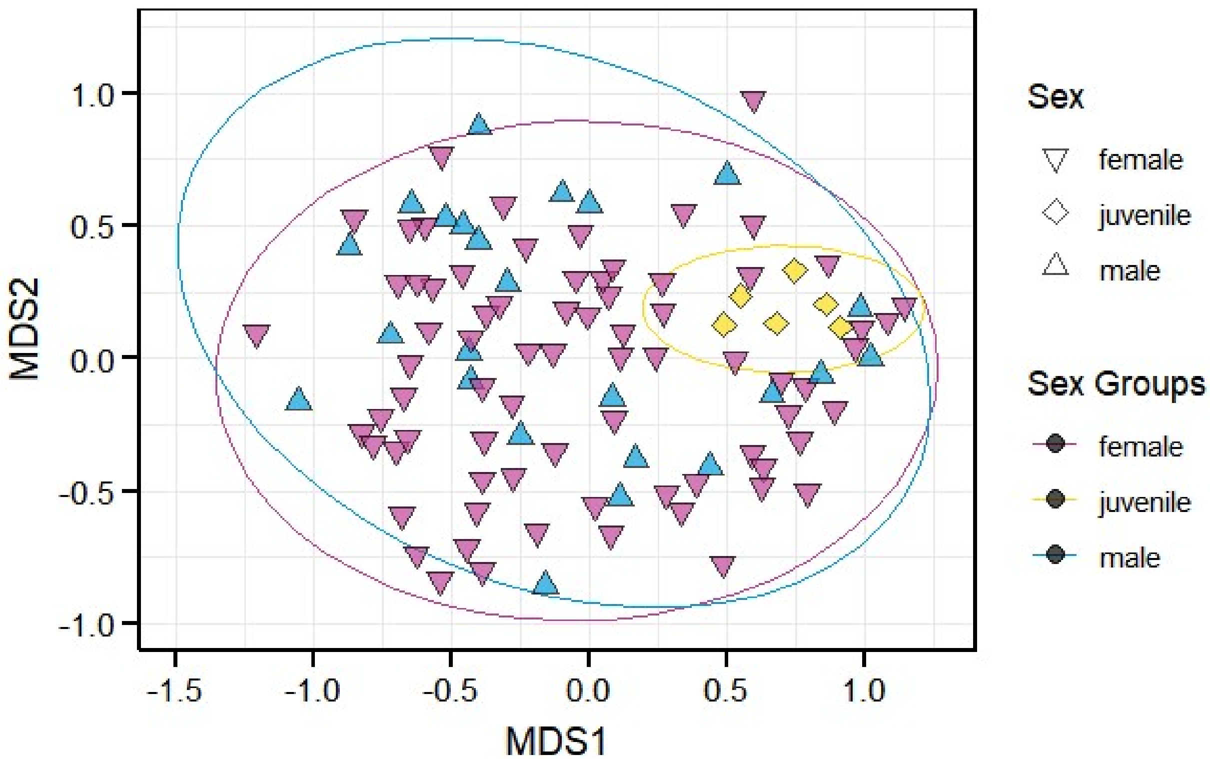
Graph 32: C:N Ratio by Group Size. The scatter plot includes a line of best fit when considering the diet quality by C:N ratio and group size (n=50; p-value<0.05).

Graph 33: Diet Quality by Sex



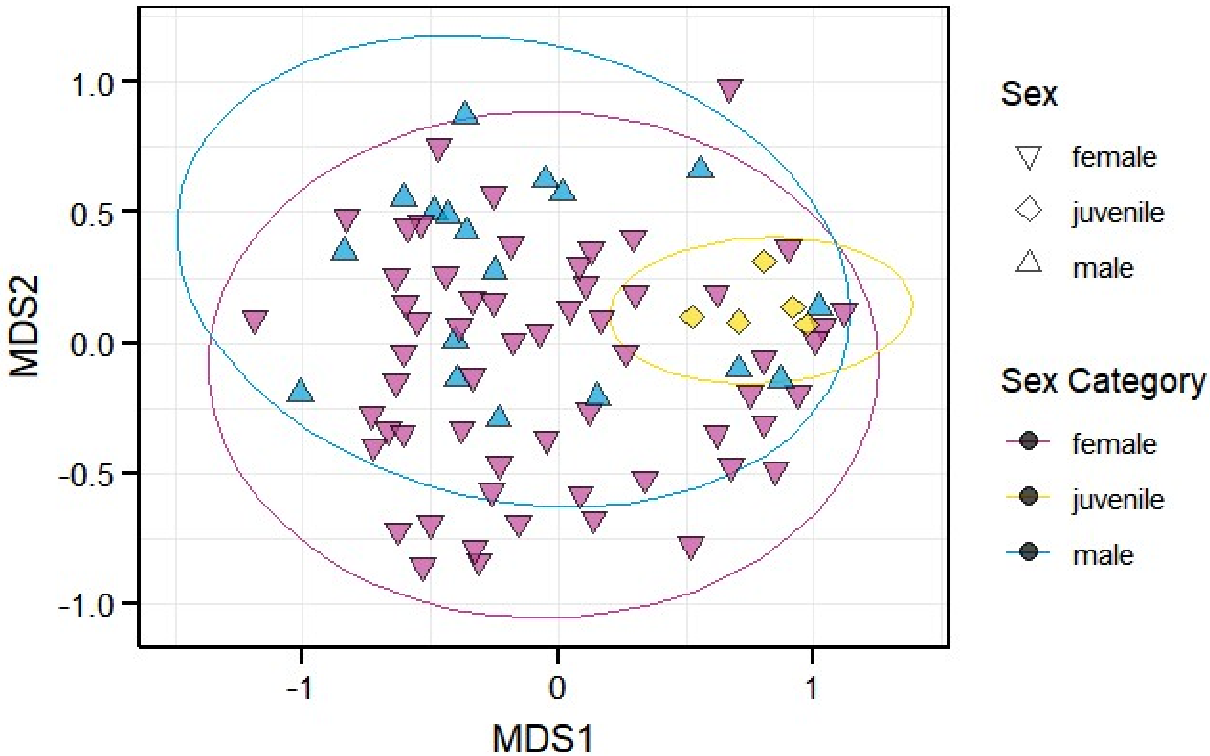
Graph 33: Diet Quality by Gender. The violin plot represents the demography of the deer sample against the diet quality determined using C:N ratio (n=50; p-value = 0.9118).

Graph 34: MDS Analysis of Sex Differences



Graph 34: MDS Analysis of Sex Differences. This diagram portrays the multidimensional scaling testing of sex patterns by sex group (n=145; p-value<0.05).

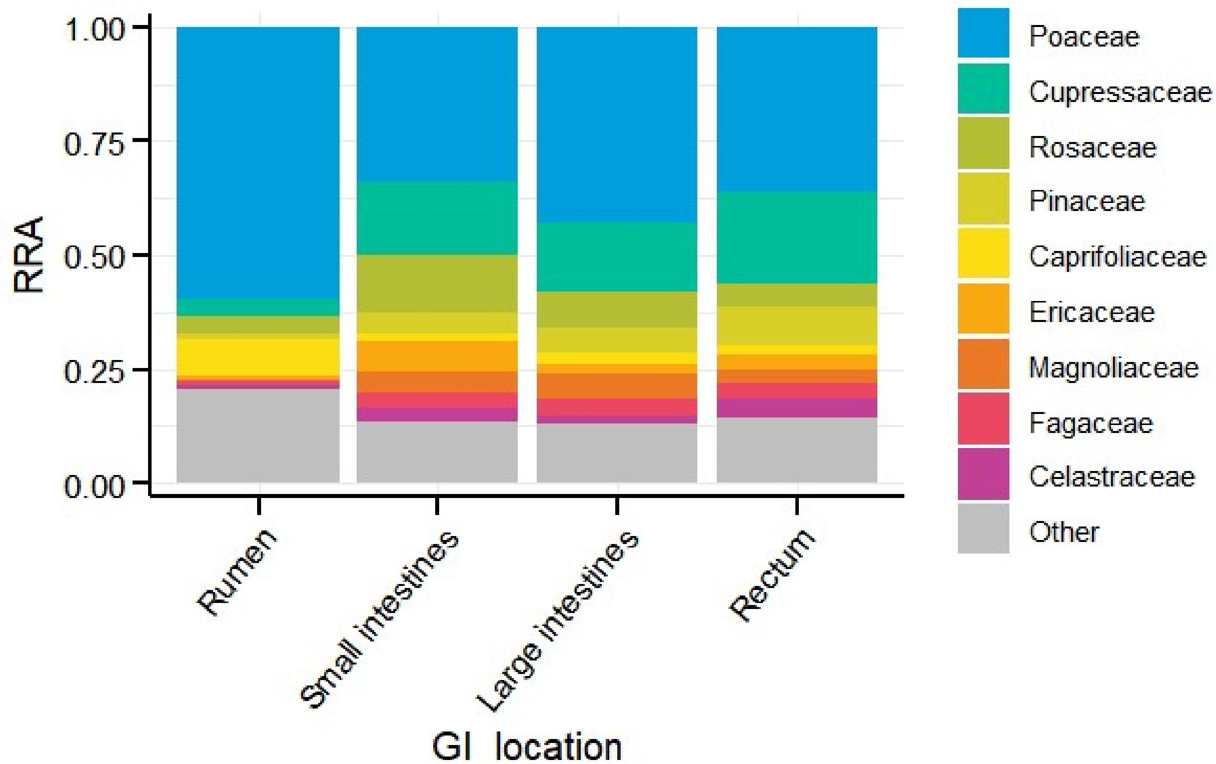
Graph 35: NMDS Plot Based on Sex Differences



Graph 35: NMDS Plot Based on Sex Differences. Demography of sex and sex group are analyzed using non-metric multidimensional testing (n=50; p-value = 0.00727).

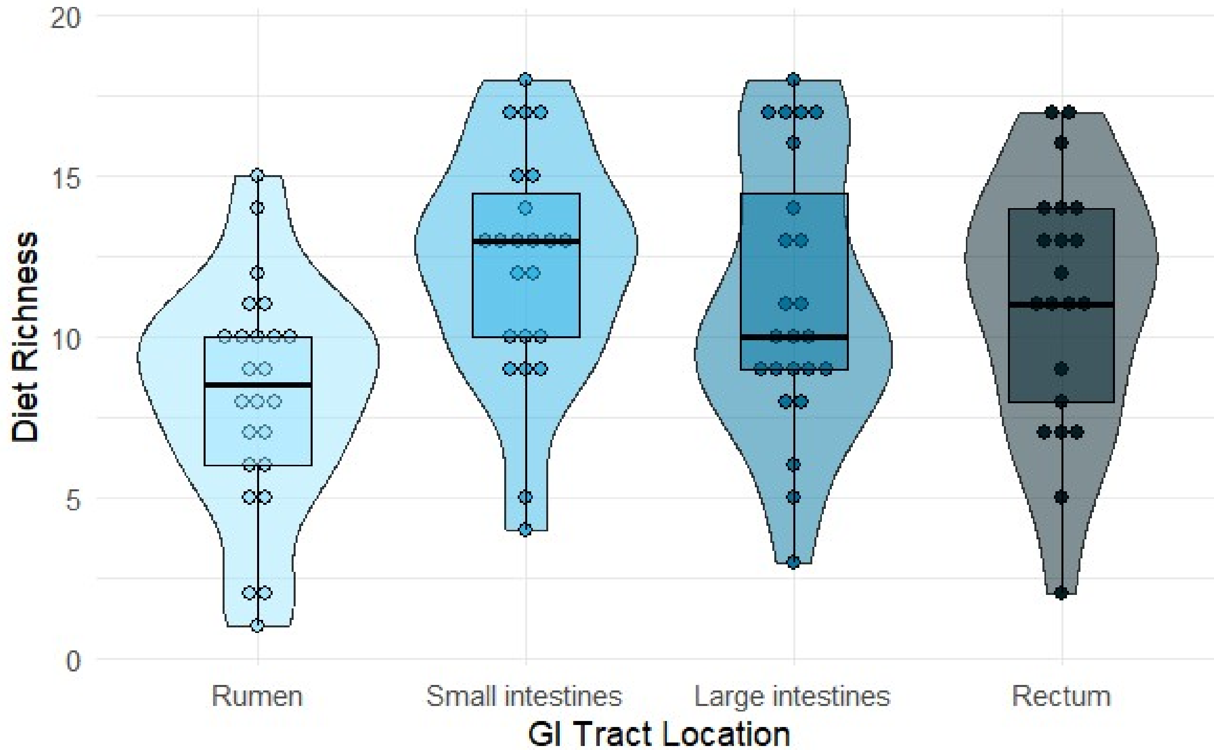
Deer Gastrointestinal Tract Figures:

Graph 36: Relative Abundances of Taxa by Location



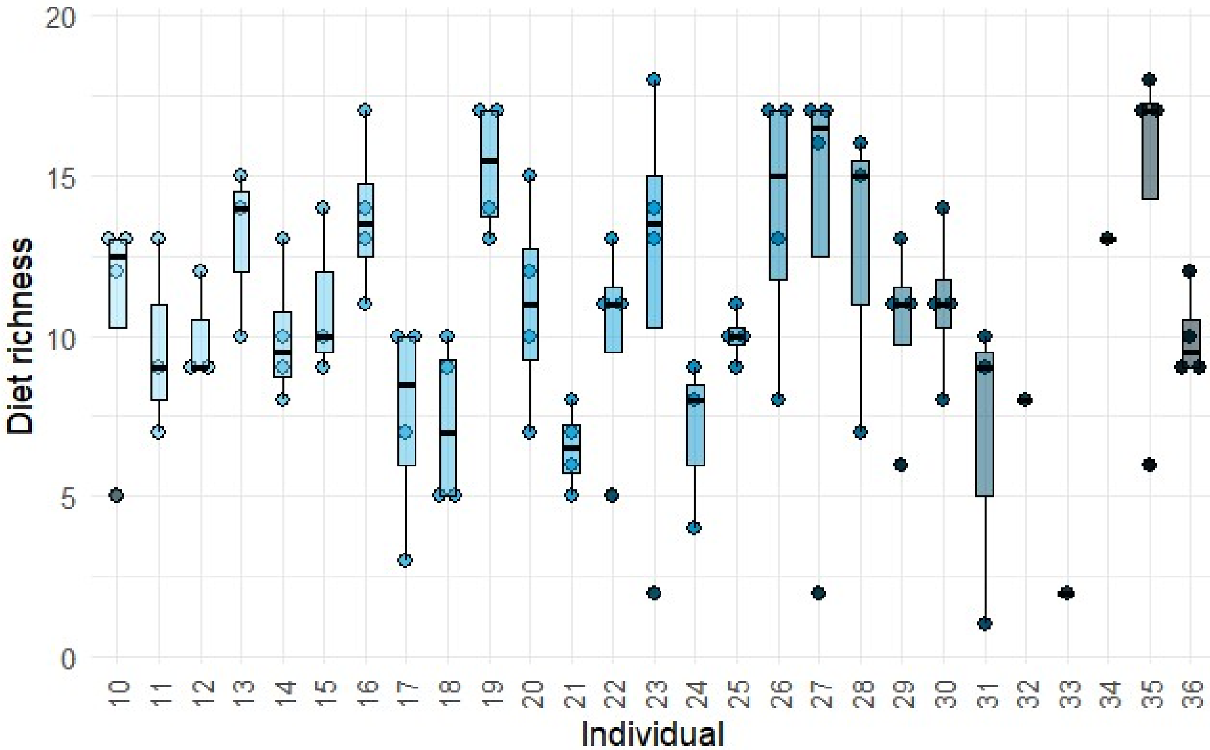
Graph 36: Relative Abundances of Taxa by Location. With relative read abundance measurements, the composition of deer culling samples is distinguished by the locations in the gastrointestinal tract where they are found (n=27; p-value<0.001).

Graph 37: Diet Richness by GI Tract Location



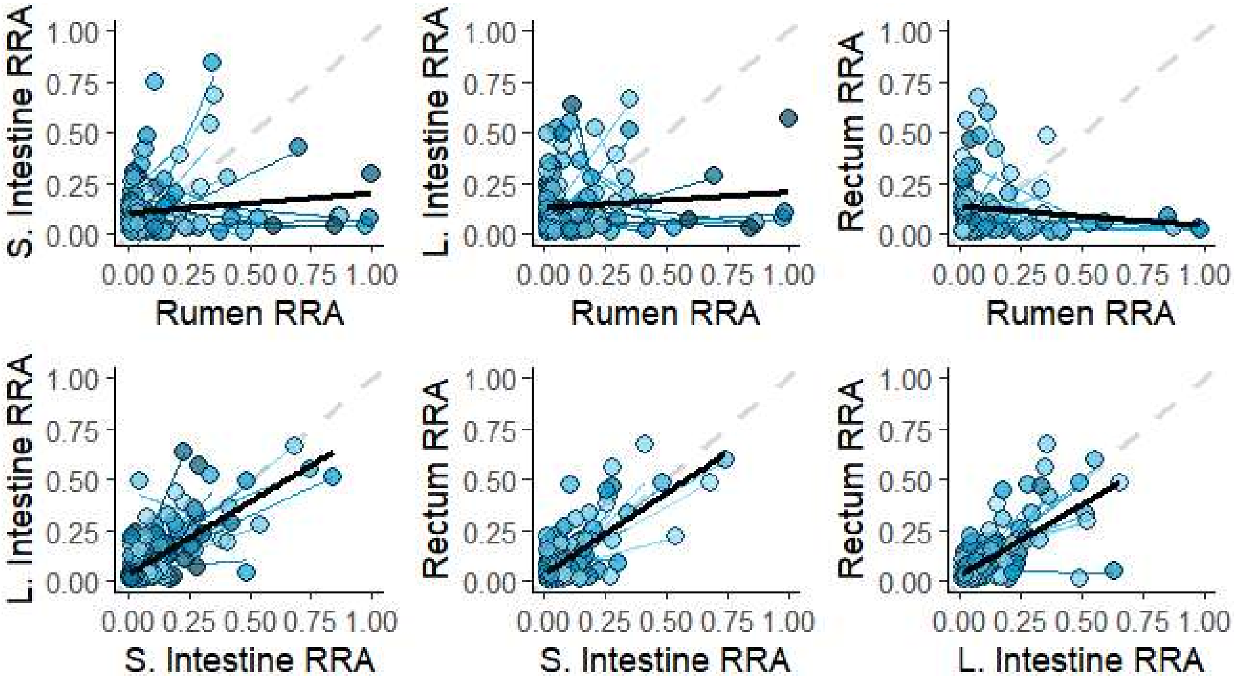
Graph 37: Diet Richness by GI Tract Location. The violin plot demonstrates the diet richness from various locations within the culled deer gastrointestinal tracts (n=27).

Graph 38: Diet Richness of Individual Animals



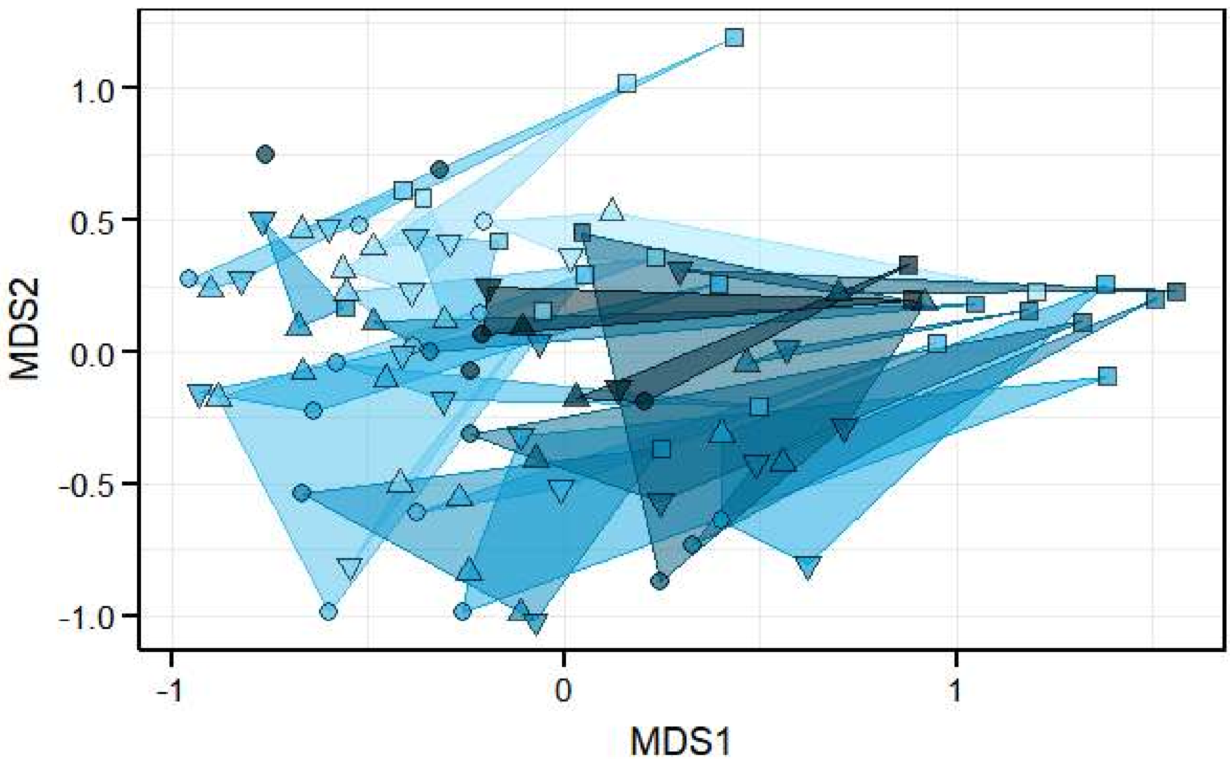
Graph 38: Diet Richness of Individual Animals. The histogram displays the variation across individuals based on the diet richness (n=27; p-value = 0.0035).

Graph 39: RRA Analyses for GI Locations



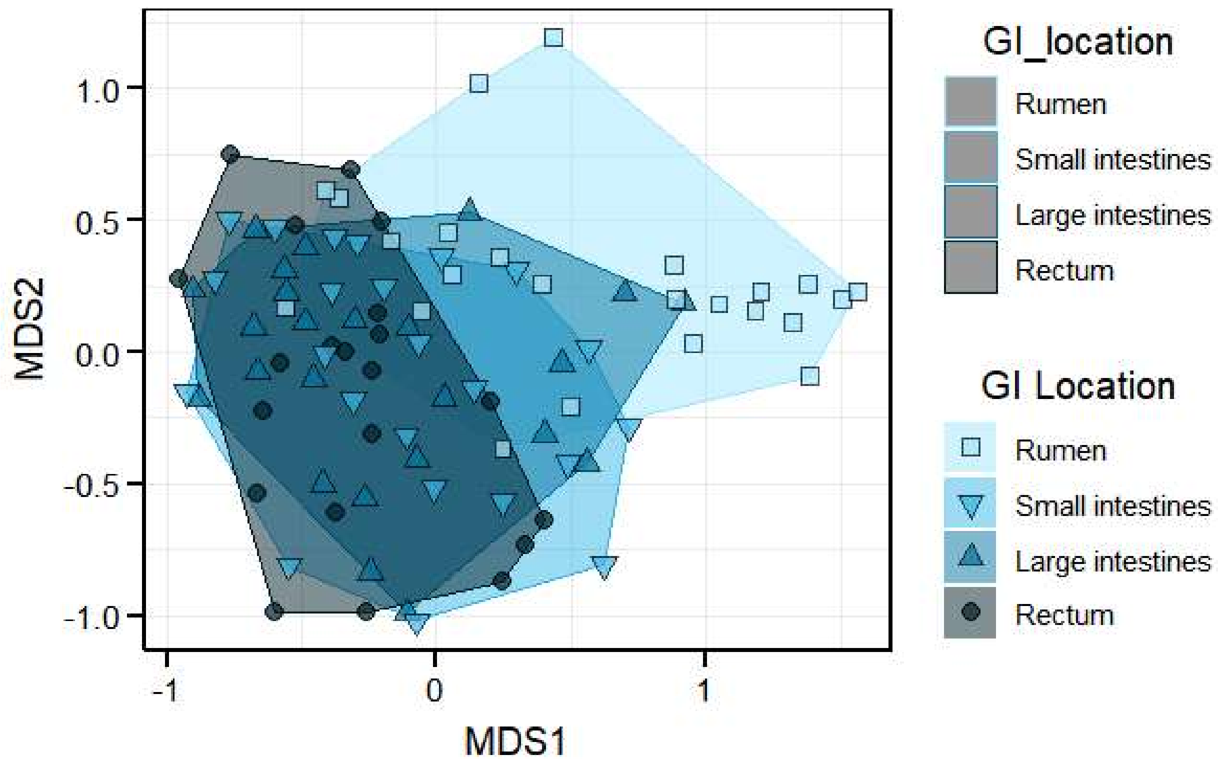
Graph 39: RRA Analyses for GI Locations. This grid contains information of scatterplots for each of the gastrointestinal locations with the relative read abundance (n=27).

Graph 40: MDS Analysis of Animal GI Locations



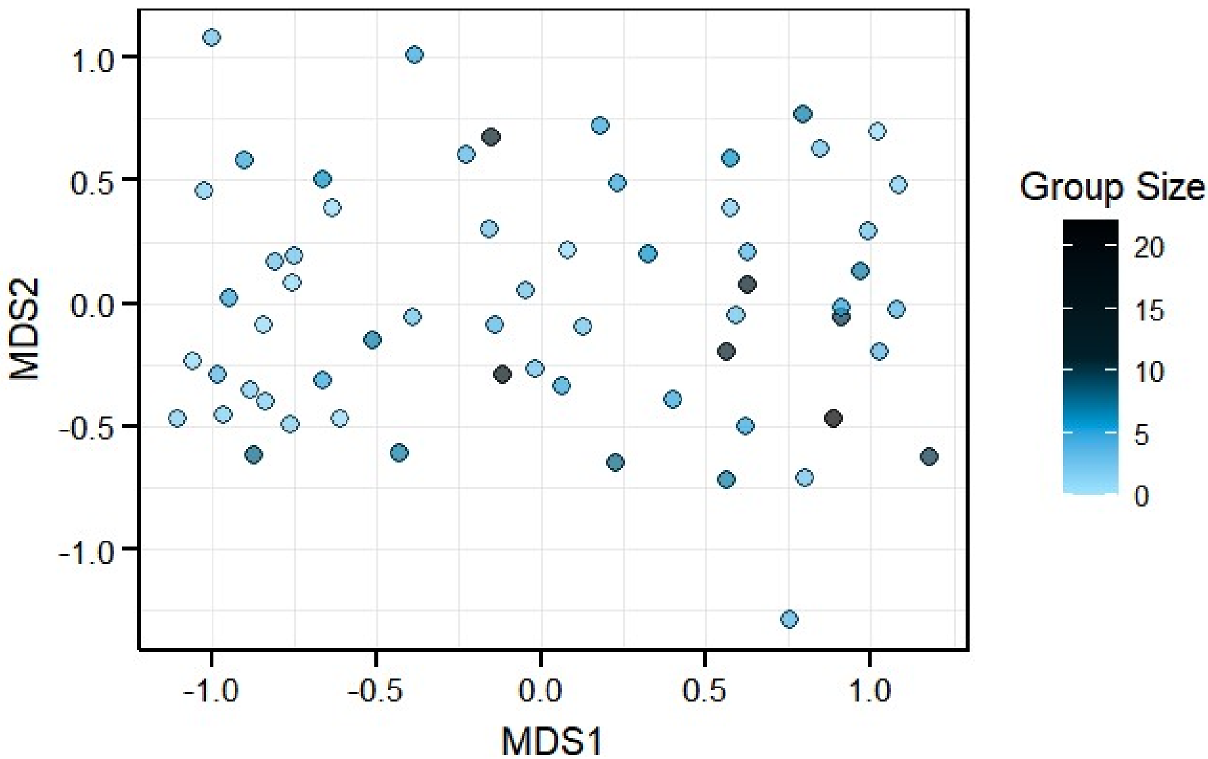
Graph 40: MDS Analysis of Animal GI Locations. This scatterplot portrays the multidimensional scaling analysis of the gastrointestinal samples from culled individuals (n=27; p-value<0.01).

Graph 41: MDS Analysis of GI Locations



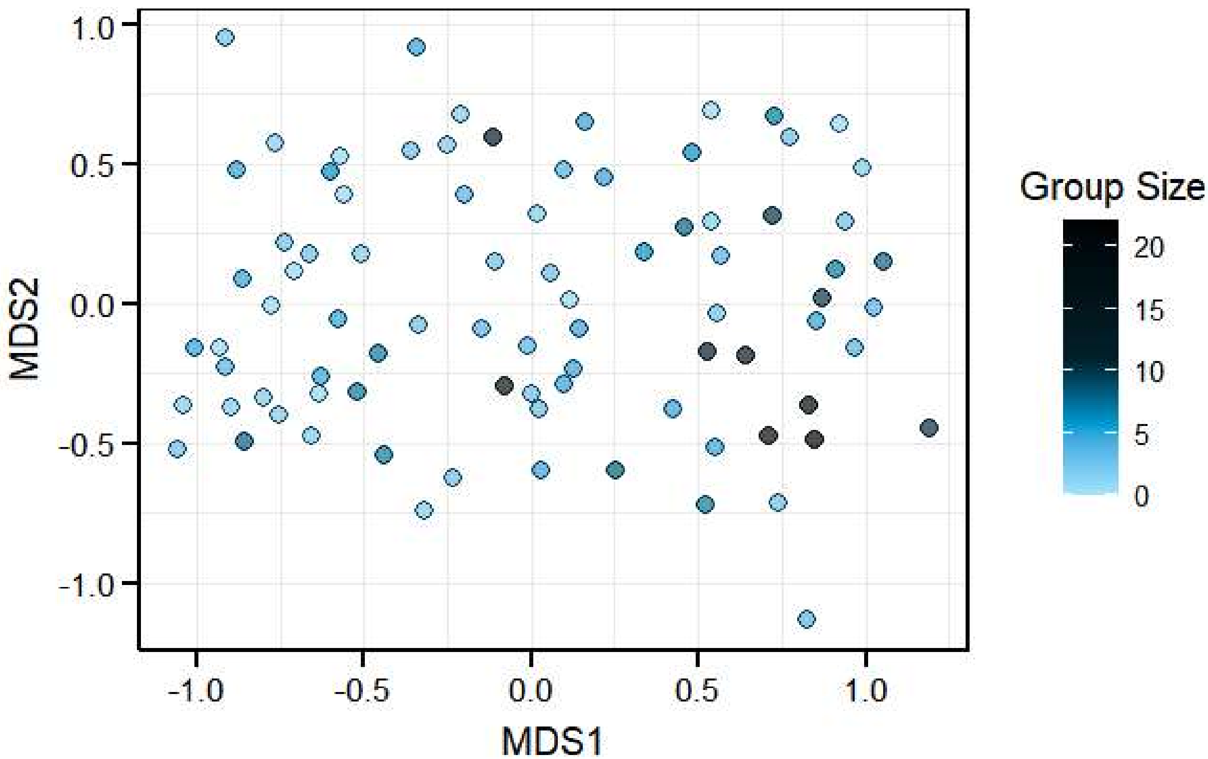
Graph 41: MDS Analysis of GI Locations. Categorized by GI tract location, this multidimensional scaling analysis portrays the distribution of similarity between the samples (n=27; p-value<0.01).

Graph 42: Group Size and MDS Relationship



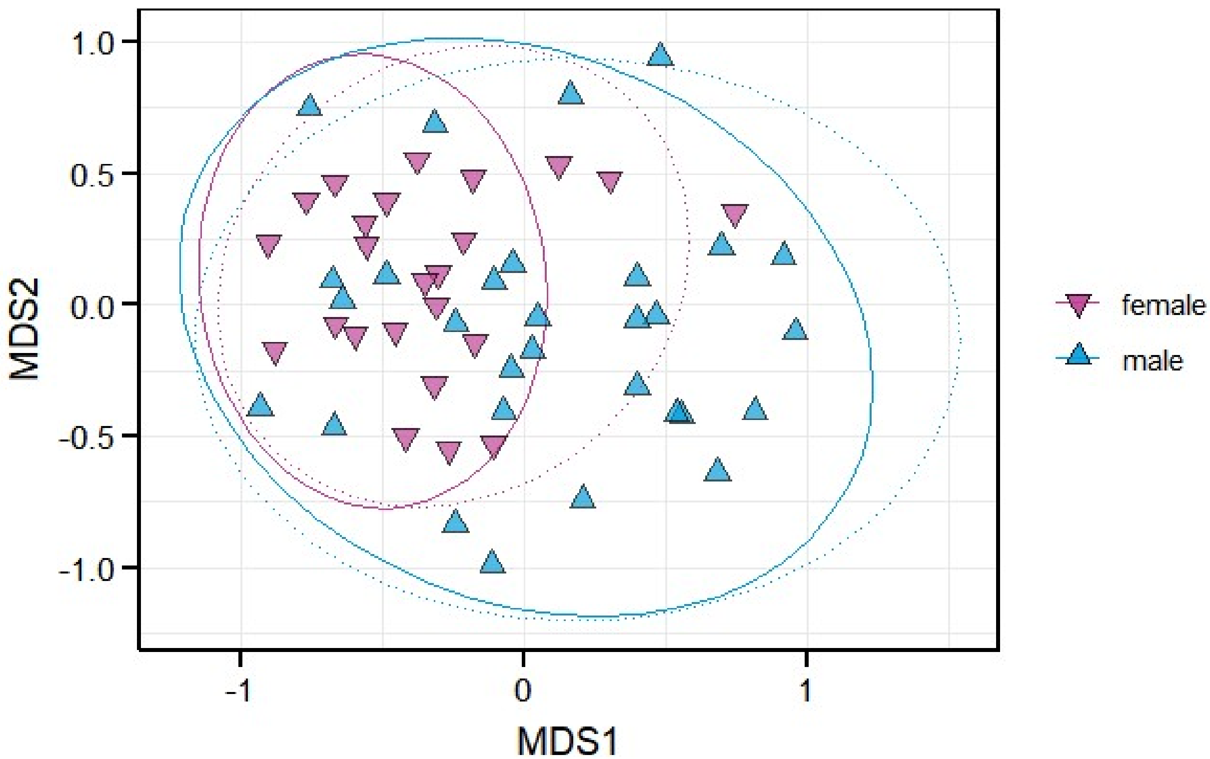
Graph 42: Group Size and MDS Relationship. This multidimensional analysis points to the group sizes by the level of similarity (n=100; p-value<0.001).

Graph 43: Group Size and NMDS Relationship



Graph 43: Group Size and NMDS Relationship. This multidimensional analysis points to the group sizes by the level of similarity (n=100; p-value<0.001).

Graph 44: Relationship between MDS1 and MDS2



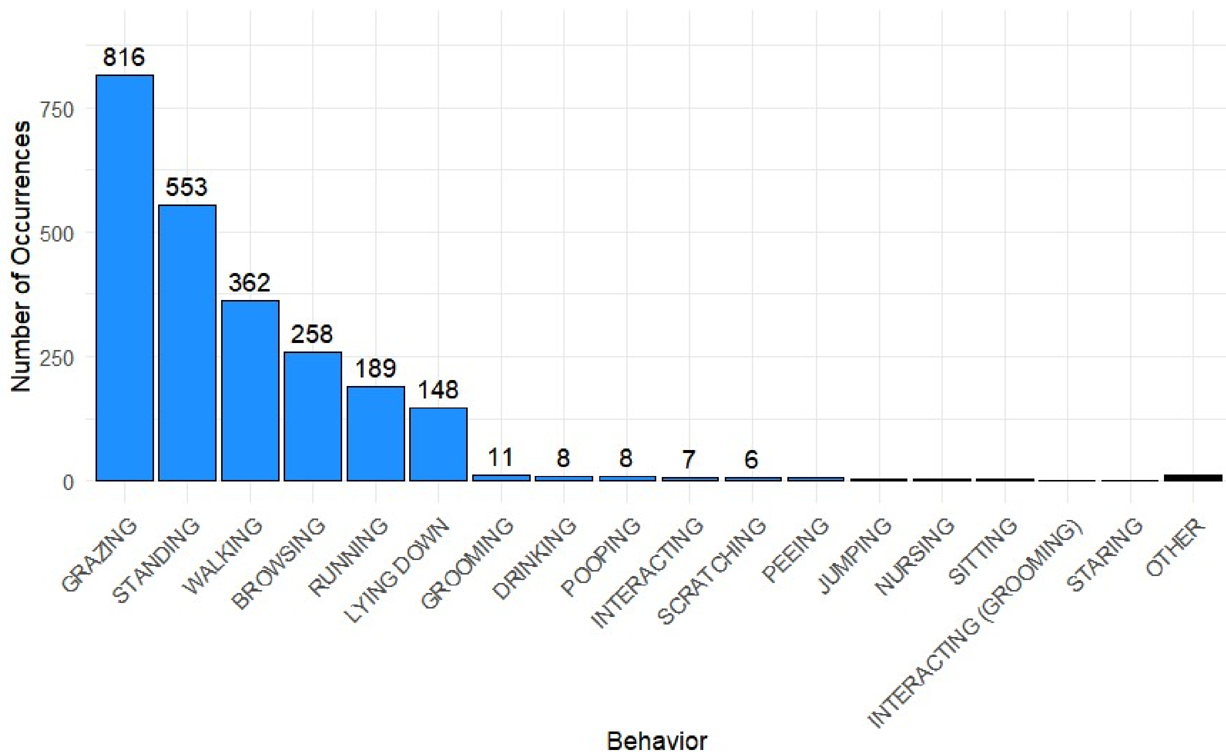
Graph 44: Relationship between MDS1 and MDS2. Using multidimensional analysis, this scatterplot groups differences by sex (n=27). The dotted ellipses represent a confidence level where 95% of the points are found to lie, assisting the distributions of males and females.

Appendix:

Observational Data Appendix:

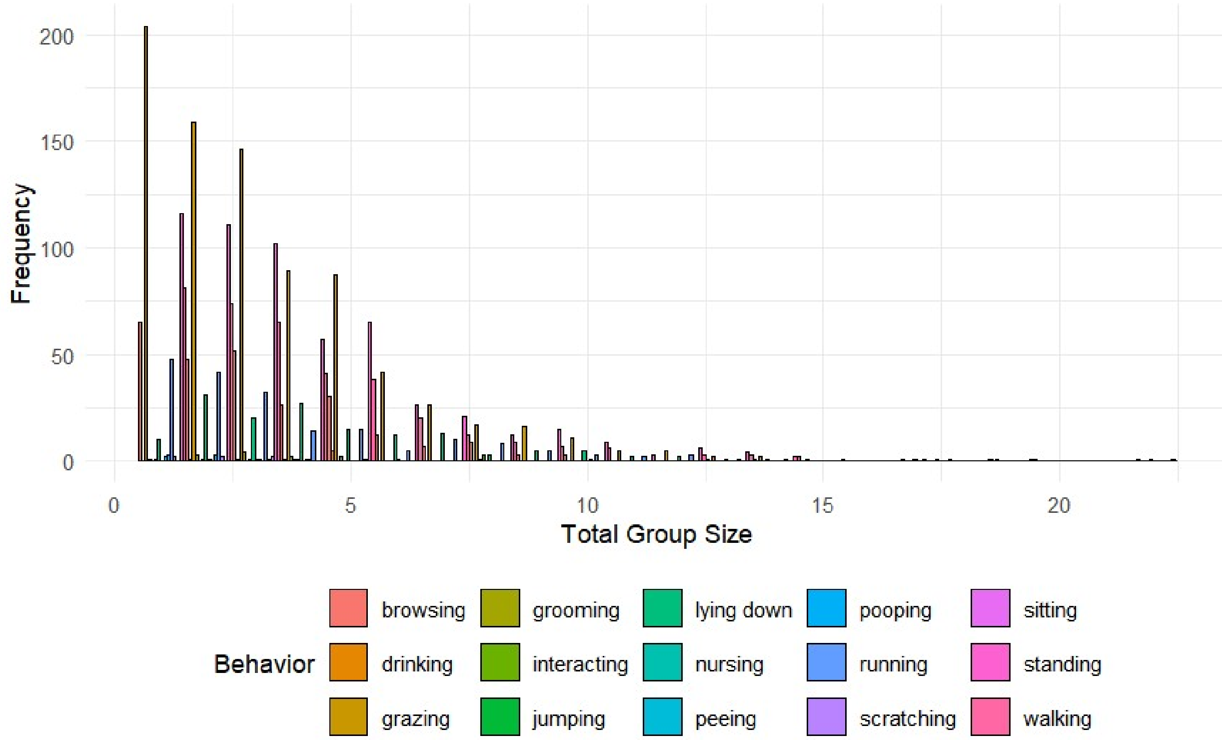
Regarding the 145 fecal samples collected, the most common sample was a bronze sample found in the fall, while the most infrequent encounter was a bronze sample in the spring. During the winter, samples were evenly distributed amongst standard types, while spring samples saw the most variation in collection. (Graph 18; n=145; p-value<0.05).

Graph 45: Observations of Deer Behaviors in Princeton



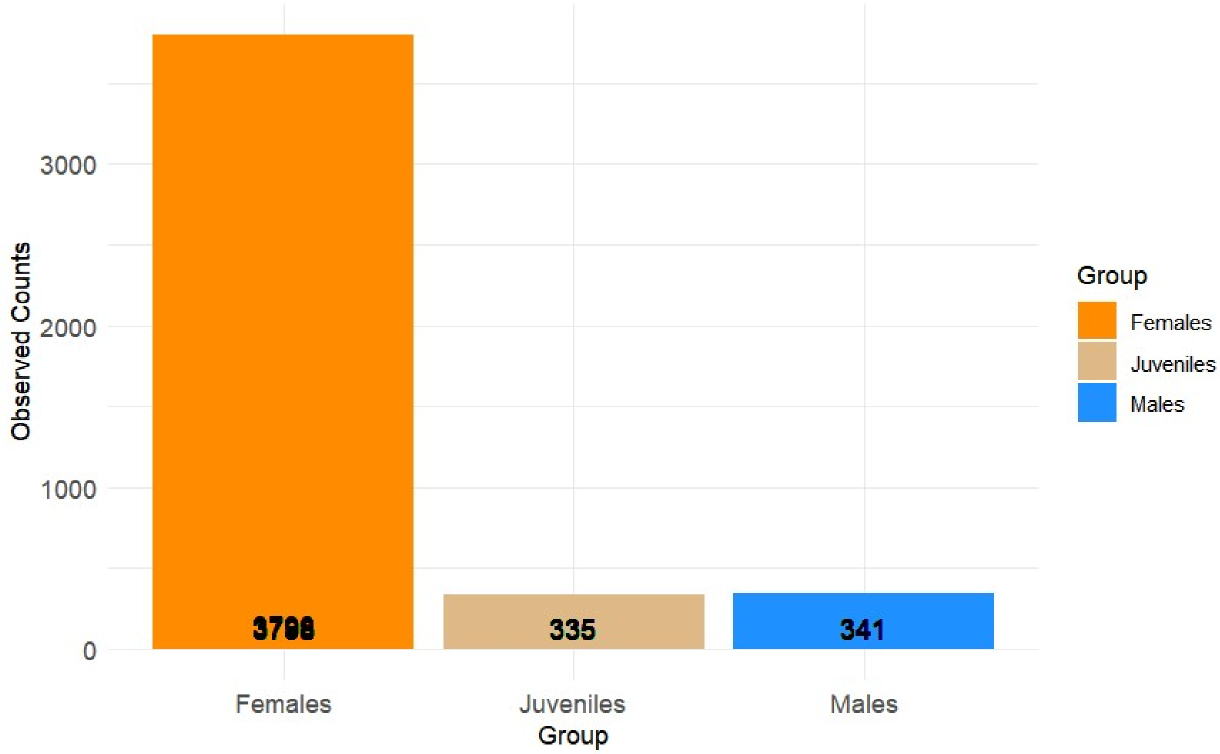
Graph 45: Observations of Deer Behaviors in Princeton. Using observer reports, this histogram represents the frequency of deer behaviors witnessed (n=1526).

Graph 46: Total Group Size by Behavior



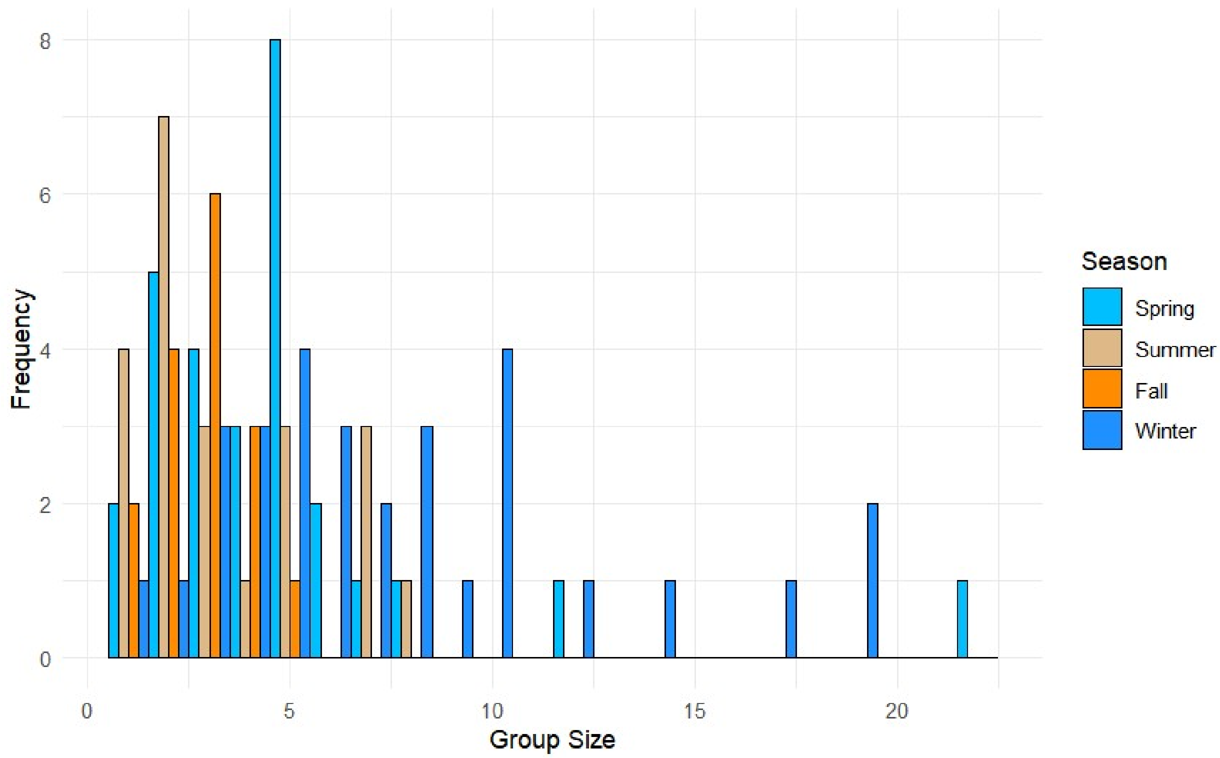
Graph 46: Total Group Size by Behavior. This histogram categorizes the behaviors seen by the group size at the time (n=1526; p-value<0.05).

Graph 47: Group Composition by Gender



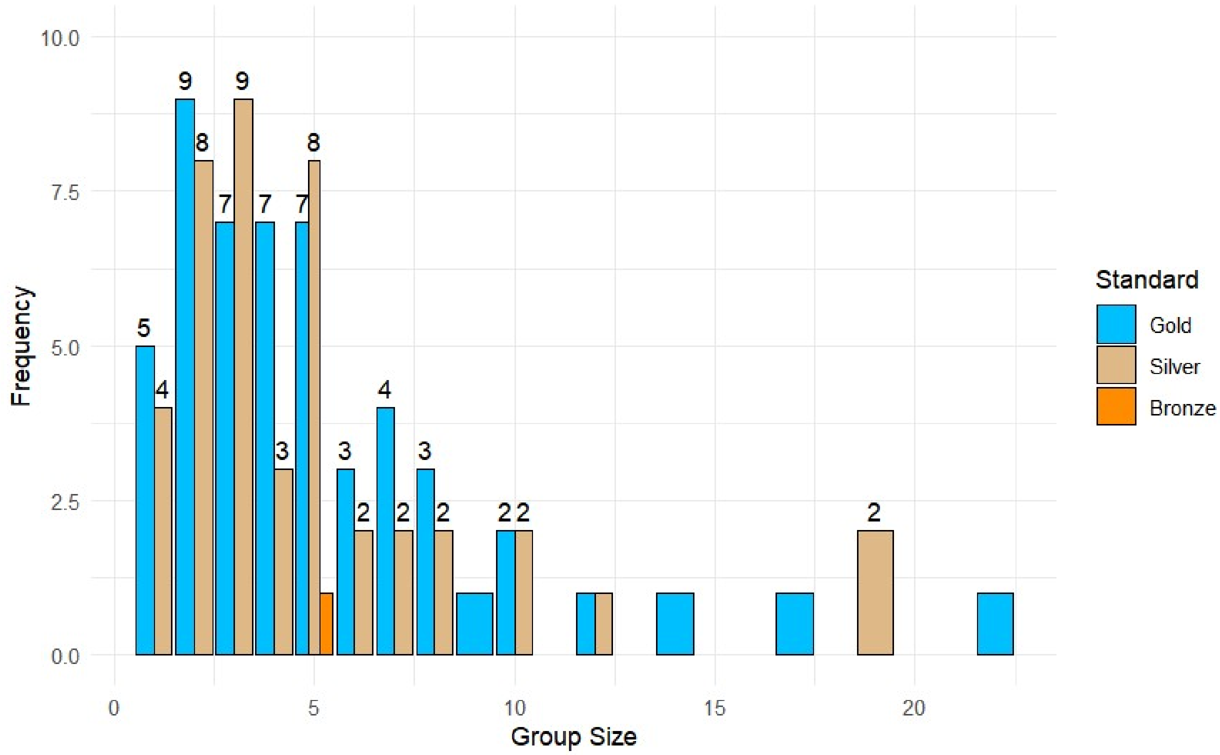
Graph 47: Group Composition by Gender. This diagram reports the demography of the deer individuals from all observed encounters (n=1526; p-value<0.05).

Graph 48: Dung Collection Season by Group Size at Observation



Graph 48: Dung Collection Season by Group Size at Observation. This bar graph portrays the group size against season for the encounters where dung samples were collected (n=145; p-value<0.05).

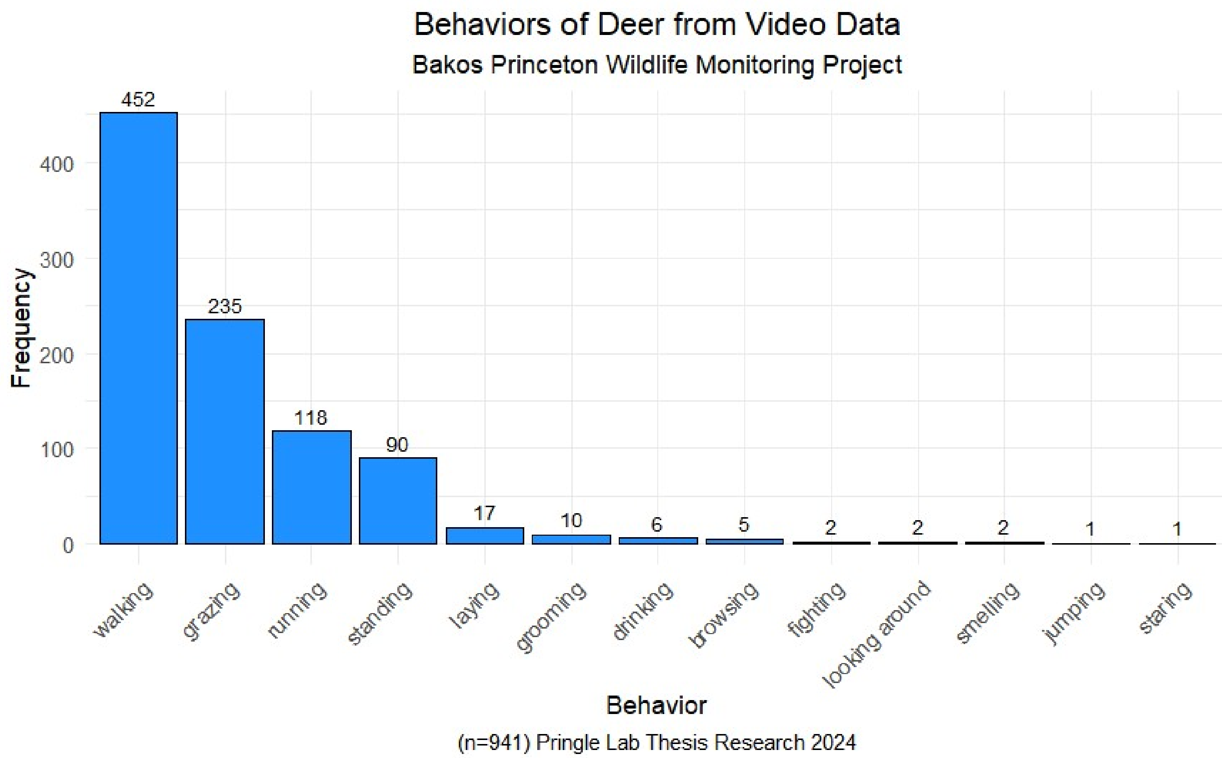
Graph 49: Comparison of Group Size by Standard for Deer Dung Samples



Graph 49: Comparison of Group Size by Standard for Deer Dung Samples. The visualization above contains information regarding the standard of the sample and the group size at the time of collection (n=145; p-value<0.05).

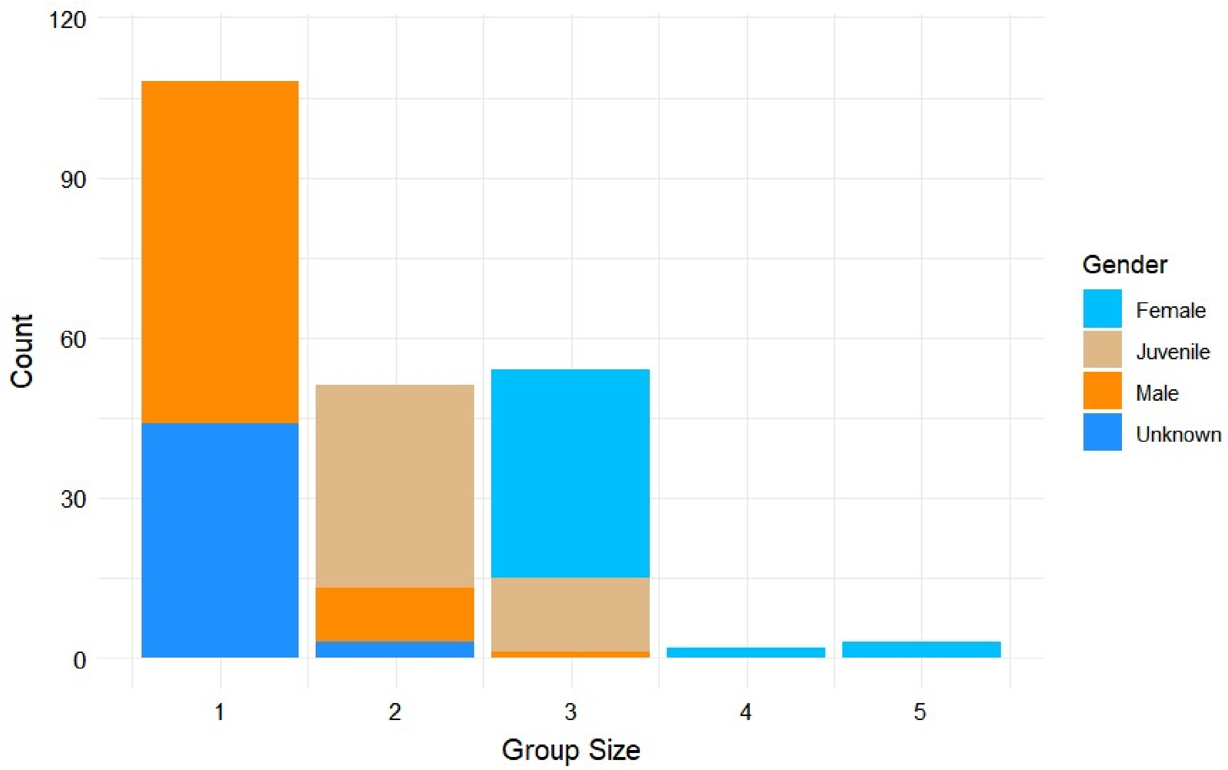
Camera Trap Data Appendix:

Graph 50: Behaviors of Deer from Video Data



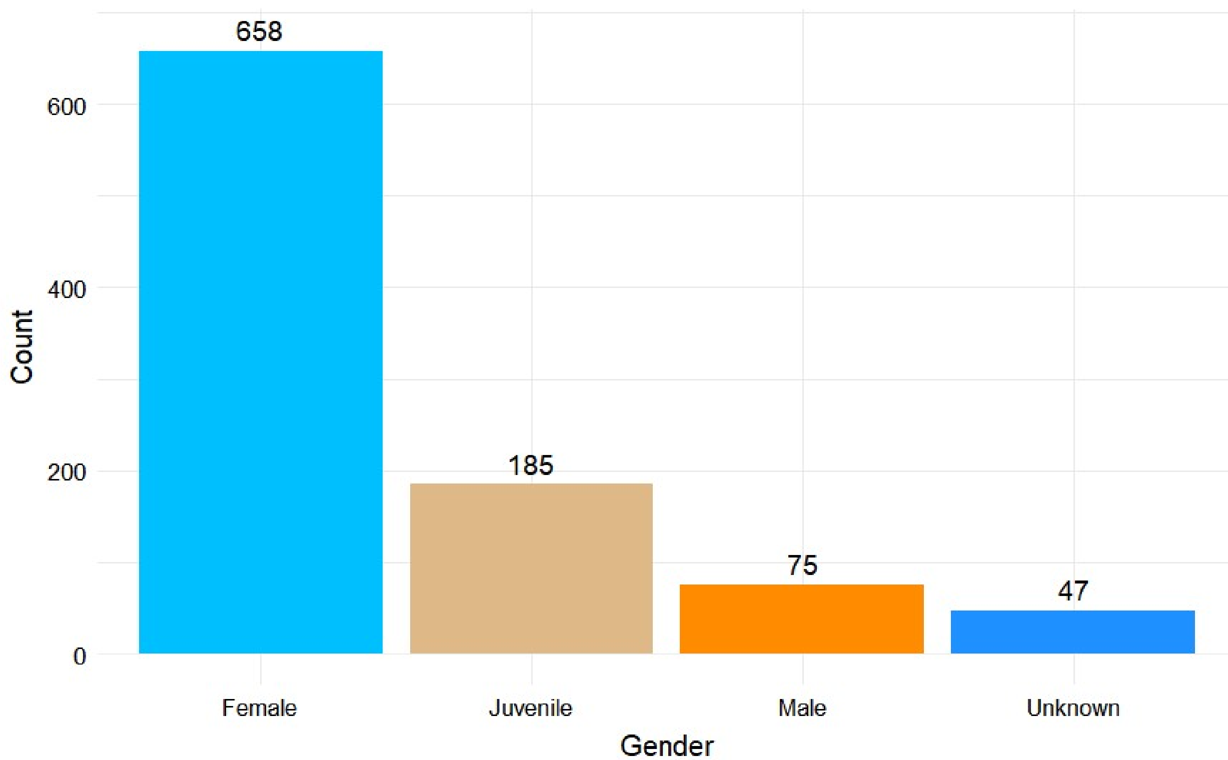
Graph 50: Behaviors of Deer from Video Data. From the observed videos, the frequency of various deer behaviors are delineated above (n=941).

Graph 51: Group Composition by Gender



Graph 51: Group Composition by Gender. Comparing gender and group sizes, this histogram presents information from the Bakos Princeton Wildlife Monitoring Project (n=903; p-value<0.05).

Graph 52: Group Composition by Gender



Graph 52: Group Composition by Gender. Compiled by gender, the frequency of deer individuals seen in the Bakos Princeton Wildlife Monitoring Project video dataset is displayed (n=903).

Honor Code:

This thesis is my own work in accordance with University regulations.