

ABSTRACT

A Comparative Analysis of Fecal Sterol Distribution to Investigate the Disappearance of Mastodon at the Page-Ladson Sinkhole

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The Page-Ladson sinkhole in Florida is a historic site which provides rich stratified deposits of late Pleistocene specimens and artifacts. Evidence from the site indicates coexistence of the prehistoric species of mastodon *M. americanum* and pre-Clovis humans. However, the relationship between the two species is not yet clear, and the disappearance of mastodons in the early Holocene is still being studied. A way to understand the relationship between species over time is to analyze their chemical remains preserved in sediments, and the comparative analysis of fecal sterol/steroid-derived compounds might provide new evidence regarding the extirpation of mastodons over time. In this study, we retrieved fecal samples from different mammals and sediments from the Page-Ladson sinkhole and extracted lipid fractions for GC/MS analysis. The lipid extract analysis revealed a number of sterol and steroid derived compounds, many of which successfully matched with the Page-Ladson compounds. Further interpretation can reveal methods for detecting past ranges of different mammalian species.

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A COMPARATIVE ANALYSIS OF FECAL STEROL DISTRIBUTION TO
INVESTIGATE THE DISAPPEARANCE OF MASTODON AT THE PAGE-LASON
SINKHOLE

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INTRODUCTION

The end of the Pleistocene epoch has shown evidence from bones, teeth, tusks, and human lithic artifacts of mastodon extinction (*Mammut americanum*) in different regions of North America. Before extinction, evidence shows that the spread of the species of mastodon *M. americanum* has been found in areas such as Mexico and as far east as Florida according to evidence from the Page-Ladson sinkhole.^{1,4} Migration to North America through a land corridor in the Bering Strait allowed for the similar species of *Mammuthus columbi* and *Mammuthus primigenius* to spread across the present day United States, inhabiting northern areas such as South Dakota and southern locations including Kansas, California, and even Florida.^{2,3,4} Understanding the habits and characteristics of the different mammoth and mastodon populations (e.g. migration patterns) can allow for a better understanding of their behaviors prior to extinction as well as illuminate a better picture of other species that dwelled in areas at the same time (e.g. different mammals such as early humans).

The Page-Ladson sinkhole has shown traces of both mastodons and humans indicating not only that there was a pre-Clovis human occupation of Florida, but that these humans lived along with the mastodons.⁵ For example, some lithic artifacts were found near a cut-marked mastodon tusk which indicates mastodon butchering.⁶ The significance of these discoveries is rooted in the collective findings of the Quaternary extinction events. The end of the Pleistocene epoch of the Quaternary period has shown

the most evidence of megafaunal species extinctions throughout each continent due to human hunting activity.¹⁰ With that being said, as the Pleistocene epoch progressed towards the Holocene epoch, it has been hypothesized that there was a co-habitation of humans and mastodon at and around the Aucilla River (location of the present-day Page-Ladson sinkhole) followed by evidence of extinction.¹¹ Evidence of pre-Clovis human occupation with mastodon in Florida places humans at the area earlier than previously expected and has encouraged further study of the extirpation of the American mastodon.

Fecal sterol analysis provides a potential mechanisms to study mastodon or mammoth occurrence in the absence of other fossil evidence. Human fecal matter is rich in sterols such as coprostanol¹², and sterols have been used by many researchers to study the influence of human waste in aquatic systems and their subsequent environmental impacts. Argiriadis et al. of The Ca' Foscari University of Venice studied the presence of coprostanol in New Zealand to illuminate mankind's presence in the area and their impact on the environment/climate.⁷ Fecal sterol biomarkers were also used in a study in Brazil to determine factors related to sewage contamination.⁸ Studying the patterns and characteristics of different species can therefore be conducted by comparing and analyzing their fecal sterol distributions. Fecal sterol distributions can be thought of as a unique footprint which can identify a certain species at a certain period in time. A study looking at surface sediment at Sungai Tebrau, Johor found that the fecal sterol distribution of the surface had four principal sterols which were coprostanol, cholesterol, epicoprostanol, and cholestanol. This study used these distributions to see if the surface was contaminated with sewage.⁹

Fecal sterols from non-human mammals have also been studied. For example, fecal biomarkers in pigs, dogs, cows, horses, goats, sheep, and other mammals were studied to distinguish between modern and past environments.¹³ Moreover, this strategy can be used to reconstruct the history of ancient species as well. In a similar way as the previously mentioned studied, identifying the characteristic fecal sterols of a certain mammalian species and charting the distribution of those sterols over time can illuminate the species' appearance and disappearance during a certain period. For instance, the decline of mammalian fecal sterols during a certain period and the rise of mastodon fecal sterols during the same time could indicate a predator-prey relationship. With that being said, sterols from mastodon and mammal samples can be isolated and then compared to the soil from Page-Ladson which can highlight patterns of the species that lived or went extinct during the transition to the early Holocene epoch.

The objectives for this study therefore include (1) analyzing lipid extracts from sediment core samples from the Page-Ladson site for distinctive sterol compounds, (2) identifying the sterol compositions of manure from mammals that might have inhabited Florida during sediment deposition in the Page Ladson sinkhole, and (3) examining chemical transformations during the transfer of mammalian fecal sterols from fresh manure to underlying soil layers. These data and interpretations can help develop methods for detecting past ranges of mammalian species where physical fossils are not found. Interpretation of the timing of the appearance and disappearance of species at the Page-Ladson sinkhole can improve our understanding of how and why the mastodons disappeared in the area near the end of the Pleistocene epoch.

CHAPTER ONE

Materials and Methods

Samples

Samples for sterol extraction in this study were obtained from an array of fecal and sedimentary samples. Four samples from a sediment core with ages of approximately 8500, 9245, 10590, and 11810 years were obtained from Dr. Michael Waters, Texas A&M University. The oldest sample corresponds with sedimentological evidence for the occurrence of mastodons and the younger samples appear to have been deposited after mastodons last appeared in the area. The Page-Ladson sinkhole samples were sent to Baylor in vials which were stored frozen at -20 °C prior to analysis. Appendix A shows that each vial is associated with a number, the higher of which is associated with a deeper sample in the core (i.e. Vial 4 (PL3 – 25) was the deepest sample received).

Six manure samples (E1-E6) from African Elephants (*Loxodonta africana*), representing the closest living relative to *M. americanum*, were provided by the Cameron Park Zoo, Waco, Texas. Additional manure samples from large mammals endemic to North America were also donated, including four samples of bison (*BI-B4*), six samples of white-tailed deer (*WTD1-WTD6*), and six samples of North American Black Bear (*BB1-BB6*). These samples were kindly provided by Manda Butler, the Animal Care Manager of Mammals at Cameron Park Zoo and approved by the Animal Care and Use Committee. The samples were collected by zoo staff in sealed plastic bags which were promptly stored in the freezer at Baylor at -20 °C.

Through the kind cooperation with Dr. Paul and Candace Martens, we acquired domestic mammalian samples including those from domesticated chicken (*Gallus gallus domesticus*), Dexter cattle (*Bos taurus*), Holstein cattle (*Bos taurus*), and American LaMancha goat (*Capra aegagrus hircus*). Soil slabs were also obtained from the site to study the migration of compounds of feces down into the soil after mammal defecation. Within the A soil horizon, we dug down to a maximum of 10.5 inches to obtain the soil slab samples. Three soil samples were obtained at different levels of the 10.5-inch slab by scraping the sides of the slab with a stainless-steel spatula or spoon. The levels included between 0-1 inches, 4-5 inches, and 8-9 inches. While on the farm site, samples were obtained by filling Whirl-Pak[®] bags, sealing them, and labeling them. Methanol and Kimwipes[®] were used to clean and dry the spoon and spatula between each sample. Pictures with a ruler next to each sample were taken to reference as a scale. The samples acquired at Martens farm as a whole comprised of feces from a domesticated chicken (*C*), a fresh sample from a Dexter cow (*DF*), mixed Dexter and Holstein cattle (*DH*), an American LaMancha goat (*LMG*), three Holstein cattle (*H1*, *H2*, *H3*), a sample of older Holstein manure under Dexter cattle manure (*HUD*), as well as well as three soil slabs (*S1*, *S2*, *S3*) (Figure 1) and soil under a Holstein fecal sample (*DUF*). After obtaining the samples they were brought back to the lab and stored at -20 °C in the freezer.



Figure 1: Slabs of dirt obtained from Martins' Farm

Sample Preparation

In order to identify the sterol distribution of each sample, they were first freeze-dried to remove any water. The Labconco FreeZone 4.5L Freeze-Dryer (Lyophilizer) was used to freeze-dry the samples. To begin the freeze-dry process, the drain line on the front of the freeze-dryer was opened and any water that had accumulated in the reservoir was removed. Then the chiller was turned on to the manual settings, and when the temperature reached around $-40\text{ }^{\circ}\text{C}$, it was ensured that all valves on the condensation chamber were closed so that the vacuum pump could be turned on. When the pressure in the condensation chamber reached around 100×10^{-3} mbar, the samples from the Martins farm, Cameron Park Zoo, and the Page-Ladson sinkhole were inserted into the holding

chamber. The pressure inside the chamber was promptly recorded at each stage of the freeze-dry process for each cluster of samples. After opening both of the isolation valves between the condensation chamber and the drying chamber, the pressure was allowed to settle until reaching 100×10^{-3} mbar or lower.

After around a day of freeze-drying, the samples from each respective cluster were ready for grinding to homogenize and prepare them for extraction. Using a mortar and pestle, each sample was ground, sieved (mesh size 20) to remove large chunks of plant material, transferred into glass jars, and labeled so that they could be extracted. Between each grinding, the mortar and pestle were thoroughly washed with soap and water, dried with Kimwipes[®], washed with DCM and then dried with Kimwipes[®] again. This cleaning step was repeated multiple times in order to ensure that there was no cross contamination between different fecal or sedimentary samples.

Lipid Extraction

To extract the fecal sterols from the various samples, we used the Accelerated Solvent Extractor (ASE[™]). The ASE[™] 200 was used by Dionex[™] to extract our samples. Multiple ASE[™] sessions were conducted for each respective cluster of samples. Before extraction, the stainless-steel holding tubes and the glass collection tubes were cleaned to ensure no cross contamination. As a part of the pre-extraction, the stainless-steel holding tubes were washed by putting them through one cycle through the ASE[™] empty at 100 °C and at 1500 psi. The glass collection tubes were cleaned by combusting them 460°C for around 12 hours. For each cluster of samples, the samples were prepared into the stainless-steel holding tubes with 11 mL, 22 mL or 33 mL capacity. First, between half a gram to two grams of the samples were weighed using aluminum foil as a base. This

amount was used so that the rest of the space in the holding tubes could be filled with diatomaceous earth (DE). Still on the aluminum foil, each sample was mixed with DE and then poured into the prepared holding tube. The rest of the space in the tube was filled with DE. The function of DE is to take up space in the steel cells and promote solvent flow through the cells by preventing clump formation. After loading the holding tubes into the ASE™, the extractor was ready for preparation. First the solvent reservoirs were checked, and if any were below one liter they were filled up using HPLC solvents. The solvents used in this extraction were methanol and dichloromethane. The gases used in the ASE™ must also be turned on, including nitrogen as they serve the function of building up pressure. Each gas must be kept at a certain level such as the nitrogen gas chamber at minimum levels of 500 psi. Compressed air must also be turned on before beginning the extraction to increase the pressure in the ASE™ before extraction. The pertinent settings for the extraction are listed in Figure 2.

Temperature	100 °C
Pressure	1500 psi
Flush %	30 vol
Purge	60 sec
Static	5 min
Heat	5 min
Preheat	1 min

Figure 2: ASE™ settings for the extraction of fecal and sedimentary samples

The extractions for each cluster of samples were run for 12 hours. After each extraction, the stainless-steel holding tubes were emptied, washed with soap and water, and then

ultrasonicated using the VWR B2500A - MT for reuse. The holding tubes were ultrasonicated three times, each thirty minutes long, with the first sonication having the tubes immersed in water with Alconox and the other two sonications having the tubes in 90:10 DCM-Methanol solution. The holding tubes can then be pre-extracted again through the ASE™ with fresh solvents again for reuse. The glass collection vials were evaporated using the TurboVap® LV Evaporator in order to concentrate the samples and get rid of any unnecessary volatile organic solvents. The content in the collection vials were then transferred using a pipette to 1.5 mL vials.

Open-column Chromatography

Before the samples were ready for gas chromatography analysis, we first wanted to obtain the total lipid extraction of each. They had to be run through open column chromatographs in order to maximize the concentration of sterols in the vials and eliminate any leftover aqueous material. First, sodium sulfate (Sodium Sulfate, Anhydrous, Granular (12-60 Mesh)) was added to each vial (filled to around a sixth of the vial volume), and in order to mix the contents, the Barnstead Lab Line Multi-Wrist Shaker was used. While waiting for the contents of the vial to mix, open column chromatographs were set up using Pasteur pipettes, which were previously combusted at 460°C to get rid of any organic contamination. DMCS Treated Glass Wool was then inserted into the pipette in order to keep the to be added sodium sulfate from falling to the other side. A longer Pasteur pipette was used to push the glass wool into place at the bottom of the smaller pipette. To handle the glass wool, forceps were used which were cleaned using methanol and dichloromethane (DCM) in order to eliminate organic contamination. After the glass wool was added, approximately 4 centimeters of silica gel

was added to the pipette. VWR Weighing Paper was used to transfer the silica gel from its original container to the pipette. The silica gel acts as the stationary phase of the column which will bind to most polar organic compounds. To condition the column, 1 mL of DCM was run through the silica gel in order to make sure that the interacting sterols don't bind so tightly to the column. Small vials or beakers were added under each pipette so that the initial DCM which ran through the column could drop without spilling. These vials and beakers were promptly removed once the DCM finished wetting the column. The samples from the shaker were then removed and allowed to settle. Using a pipette with a rubber top, the organic layer (containing the sterols) was withdrawn and inserted into each respective pipette. Caution was taken to make sure the sodium sulfate at the bottom of the vials were not taken. For around 20 minutes, the liquid samples were allowed to run through column. Liquid which finally reached the bottom of the column leaked into 1.5 mL gas chromatographic collection vials. As soon as this dripping started, a 1 mL aliquot of DCM was added and allowed to run through the column for around 25 minutes. The DCM acts as the mobile phase of the column as it is relatively nonpolar. After the samples completely ran through the column, the vials were topped with Argon in order to prevent contact with oxygen (avoiding oxidation).

Derivatization

Before gas chromatography-mass spectrometry (GC/MS), the samples must first be derivatized. Specifically, we prepared them using trimethylsilyl (TMS) ethers. The purpose of derivatization is to have the TMS react with alcohols, acids, and amines to make the compounds of interest more volatile. The derivatization protocol we followed used the reagent bis(trimethylsilyl)trifluoroacetamide (BSTFA). We also used pyridine to

act as a base catalyst which can decrease the time of the reaction, especially those including compounds that have steric hinderance. 500 μL of the sample was obtained from the original sample volume using the FlexiVap Work Station. The 500 μL of samples in the GC vials were then transferred to DCM (an aprotic solvent) since protic solvents such as methanol would react with the BSTFA 125 μL of pyridine and BSTFA were added to each sample and were heated at 65°C for around 20 minutes to ensure the reaction goes to completion. After the samples cooled to room temperature, they were stored in the refrigerator.

Gas Chromatography

The batches of samples were then ready for gas chromatography-mass spectrometry (GC-MS) analysis. The HP 6890 GC System was used to analyze the samples in Dr. Bill Hockaday's Organic Geochemistry Lab. Gas chromatography analysis can be used to identify the compounds in the sample by identifying the peaks from the GC and finding their respective mass spectrum. The column type was HP-5 5% Phenyl Methyl Siloxane. The column dimensions were 30.0 m x 250 μm x 0.25 μm nominal. The injection volume was 2.0 μL . Figure 3 shows the temperature program run for the GC.

Oven Ramp	°C/min	Next °C	Hold min	Run Time
Initial	-	70	2.00	2.00
Ramp 1	5.00	210	0.00	30.00
Ramp 2	3.00	300	10.00	70.00

Ramp 3	0.00	-	-	-
Post Run	-	70	0.00	70.00

Figure 3: The temperature program for the GC analysis

A vial of hexane was added first to function as both a blank and for rinsing during the GC analysis. Acetone and DCM were both used as cleaning solvents for the autosampler syringe and the carrier gas was helium. For each session, after loading an aliquots of the samples in the autosampler carousel, the sample identification and GC method were entered into the Chemstation software that controls the GC. After starting the system, the sample program ran for 75 minutes per vial and the results were analyzed using Chemstation data analysis software before running the next batch. Chemstation software linked to the NIST database provides initial identification information for unknown compounds, but without the additional consideration of molecular structural information related to retention time, misidentification is possible.

CHAPTER TWO

Sterol Distribution from Page-Ladson

Distribution Index Analysis (See Appendix C)

The gas chromatograms and mass spectra for the four Page-Ladson site samples were analyzed for compounds of interest, which included those that had a steroid backbone as well as others such as n-alkanes and α -tocopherol. A comprehensive index was made which highlighted the initial identification of a variety of compounds identified using Chemstation software and comparison with the NIST database and their individual chromatographic retention times. Compounds that were found in common among the Page-Ladson samples were checked to see if they had matching retention times. . This is because if they did not have the same retention times, the compounds are not the same, and at least one must have been misidentified

For compounds that had a steroid backbone, the Chemstation software indicated the common presence of Cholest-7-en-6-one, 3-(acetyloxy)-9-hydroxy-, ($3\beta,5\alpha$)-, Pregnane-3,11,20-trione, (5β)-, Pregn-4-ene-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), (11β)-, Urs-9(11)-en-12-one-28-oic acid, 3-acetoxy-, methyl ester ($14\beta,20\beta$), and Pregnane-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), ($5\alpha,11\beta$)- in the Page-Ladson samples. There were also additional compound identifications that were only observed in individual samples. Based on the expected retention time for steroid compounds around 50 to 60 minutes on the GC method, we focused on the later eluting compounds in Figure 4, which displays the

steroid compounds and their retention times. The earlier eluting compounds must have been misidentified by the software.

Pregn-5-en-20-one, 3,16-bis[(trimethylsilyl)oxy]-, (3 β ,16 α)-	51.132 min
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-17-hydroxy-6-methyl-, 3,20-bis(O-methyloxime), (6 α)-	37.069 min
Bilirubin	35.319 min
Cholan-24-oic acid, 3,7-dioxo-, (5 β)-	35.319 min
Chol-7-ene, (5 β)-	35.959 min
Bufa-20,22-dienolide, 3-(acetyloxy)-14,15-epoxy-16-hydroxy-, (3 β ,5 β ,15 β ,16 β)-	35.879 min
1H-Cyclopropa[3,4]benz[1,2-e]azulene-2,5-dione, 9,9a-bis(acetyloxy)-3-[(acetyloxy)methyl]-1a,1b,4a,7a,7b,8,9,9a-octahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 α ,1 β ,4 α ,7 α ,7 β ,8 α ,9 β ,9 α)]-	36.359 min
Phorbol 12,13-dihexanoate	37.085 min
DES-A-ARBORANE	37.537 min
Bufa-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-, (3 β ,5 β ,15 β ,16 β)-	38.143 min
4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, (1 α ,1 β ,1 γ ,2 α ,3 α ,6 α ,6 β ,7 α ,8 β ,8 α)-	39.949 min
4,13,20-Tri-O-methylphorbol 12-acetate	41.435 min
14 β)-3,19-Epoxyandrosta-5,7-diene, 4,4-dimethyl-3-methoxy-17-methylthiomethoxy-	50.728 min
5,16,20-Pregnatriene-3 β ,20-diol diacetate	51.591 min
Cholestane-3,7,12,25-tetrol, tetraacetate, (3 α ,5 β ,7 α ,12 α)-	51.591 min

Figure 4: Retention times for unique compounds in Page-Ladson samples

An important feature to note about Figure 4 is that aside from the first two compounds on the list, the remaining thirteen were uniquely identified just from PL3-25, or in other words the lowest depth sample given to us from the Page-Ladson site which dates back 11,810 years ago.

The compounds without a steroid backbone which were found in all of the samples from the Page-Ladson site included the n-alkanes nonacosane (C₂₉H₆₀), hentriacontane (C₃₁H₆₄), and tritriacontane (C₃₃H₆₈). These long-chain hydrocarbons, which had the strongest MS signals among the compounds eluting near 50 minutes, are derived from plants and are commonly called “leaf waxes” due to their concentration in the cuticle of leaves. The four Page-Ladson samples also included trace concentrations of tocopherols, which are plant-derived oils that have vitamin E activity. Further discussion of the leaf waxes and tocopherol in mammal fecal samples will follow in the later chapters. The PL3-25 sample also included six distinctive chromatographic peaks from trace concentrations of compounds found in mammalian feces that elute between 51 and 60 minutes. These compounds appear to be steroid-related, were detected in diverse mammalian samples, and will be discussed in the following chapters. These still-unidentified compounds might be related to bile acids and have potential mammal biomarker implications. Figure 5 shows the chromatogram for *E2* specifically for the 129.00 ion fragment between 45.00 and 65.00 minutes which indicates the three sterols and their stereoisomers. Figure 6 shows the chromatogram for *E2* in the range 45.00 minutes to 60.00 minutes which identifies n-alkanes, squalene, and α -tocopherols.

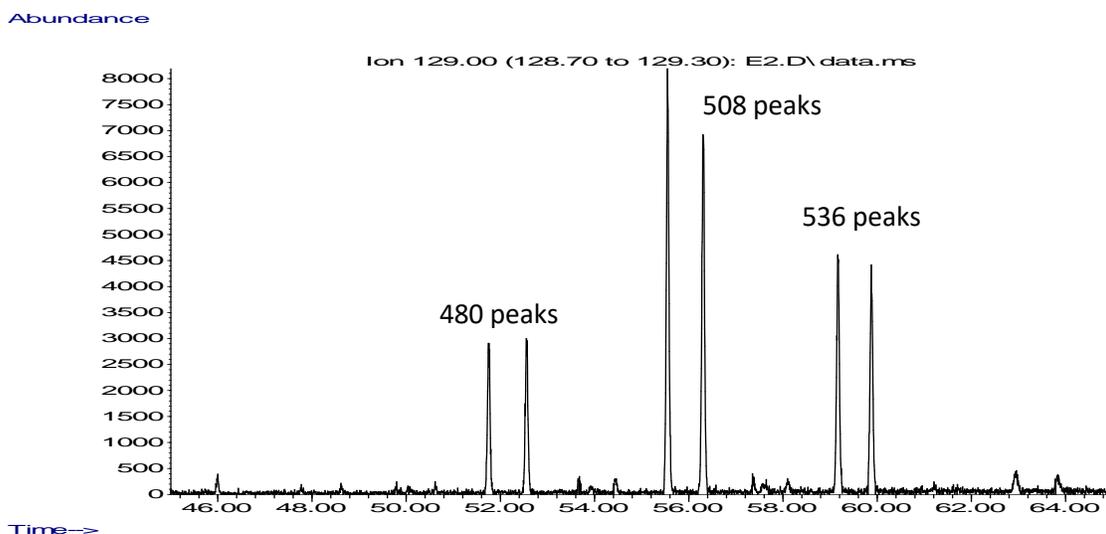


Figure 5: 129.00 Ion fragment chromatogram of *E2* between 45.00 and 65.00 minutes

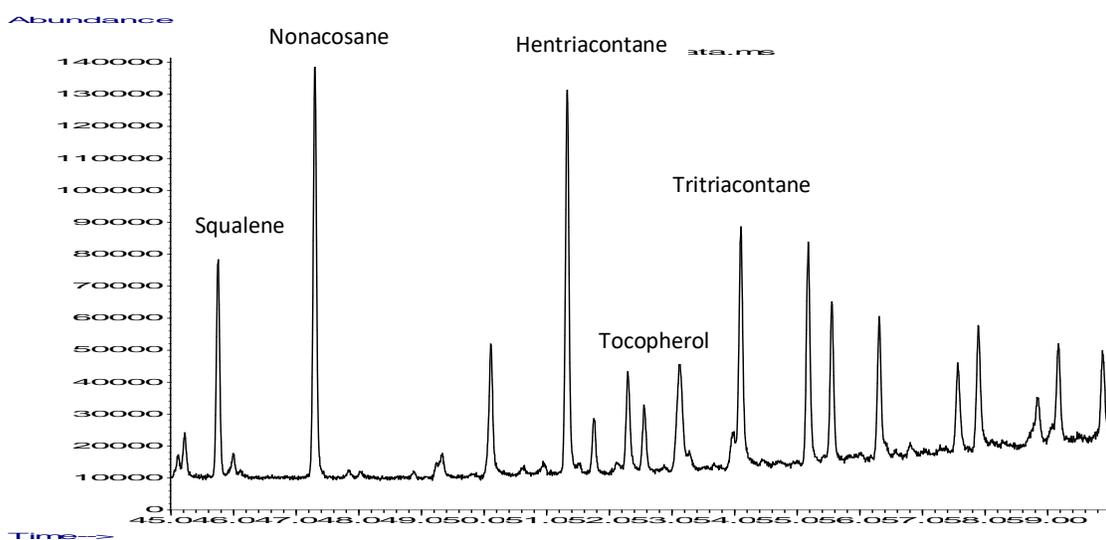


Figure 6: N-alkanes, squalene, and tocopherols from 45.00 to 60.00 minutes of *E2*

Ion Fragment Analysis

When looking at the mass spectra of the four Page-Ladson site samples, there were three distinctive compounds (six peaks total, with two probable stereoisomers for each) which appear to be sterol-related. These chromatographic peaks were first detected on an extracted ion chromatogram for the m/z 129.0 ion fragment, which is commonly

employed for detecting sterols such as cholesterol in a wide range of samples. We did not detect any of the common sterols and stanols that we hypothesized for the Page-Ladson sediments. These six peaks detected as trace components in the PL3-25 sample were relatively abundant in many of the Cameron Park Zoo and Martens Farm samples. However, these peaks were not found in the other three Page-Ladson samples, indicating a distinguishing factor between the lowest depth sample of the Page-Ladson site (PL3-25) and the other samples of higher depths. The three sterol-related compounds of interest include those with m/z 480, 508, and 536 ions. These might be the molecular ion peaks, though higher m/z features (up to m/z 645) with very low signal intensity suggest the compounds could have higher masses in line with bile acids. Figures 7, 8, and 9 show the mass spectra (from *E2* as a reference) for the 480, 508, and 536 ions respectively.

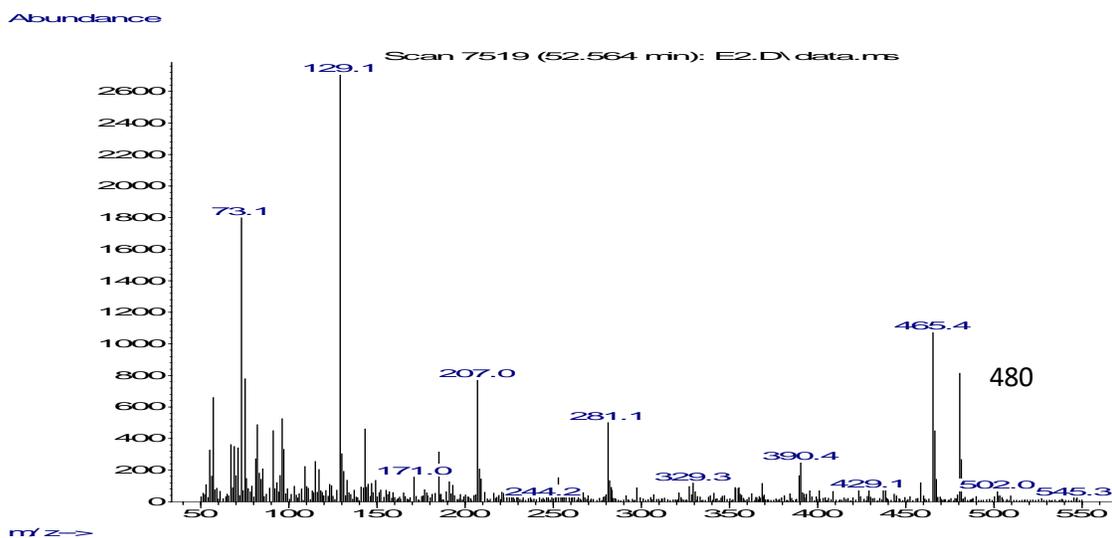


Figure 7: Mass spectrum indicating the 480 m/z ion

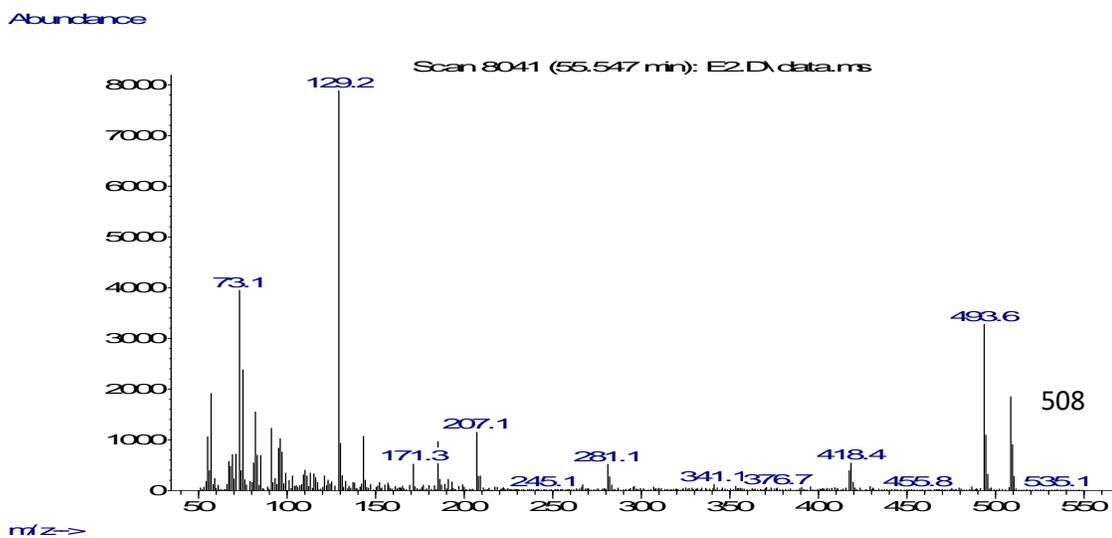


Figure 8: Mass spectrum indicating the 508 m/z ion

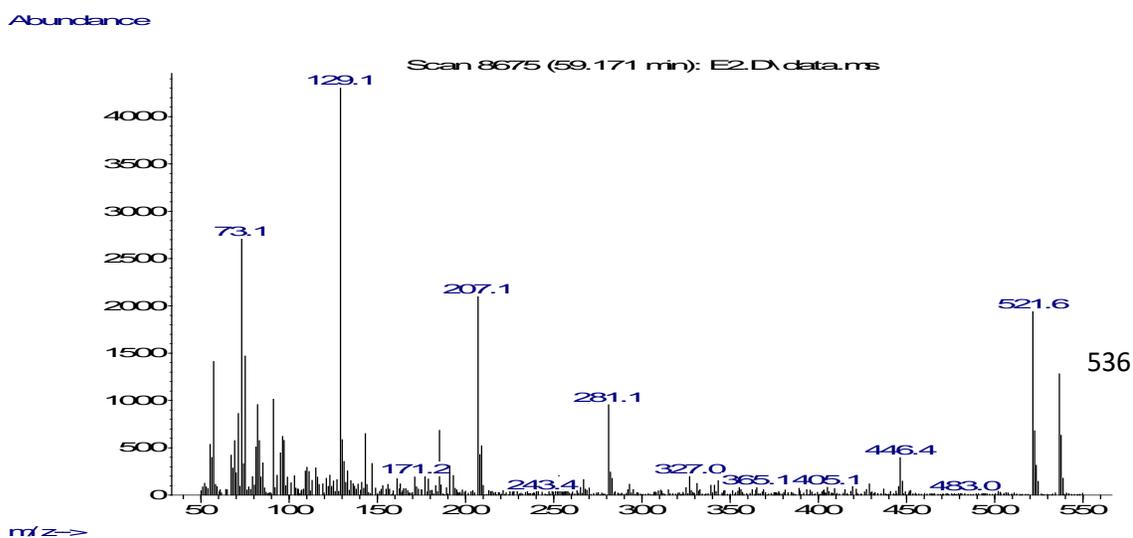


Figure 9: Mass spectrum indicating the 536 m/z ion

For PL3-25, the 480 peak and its stereoisomer were found with retention times 51.745 and 52.568 minutes. The 508 peak and its stereoisomer were found with retention times 55.535 and 56.289 minutes. The 536 peak and its stereoisomer were found with retention times 59.164 and 59.867 minutes. Figure 10 shows information on each peak and its isomer.

Sample	Ion Fragm	Ret Time	Type	Width	Area	Start Time	End Time
PL3-25	480	51.741	rVV	0.086	390	51.7	51.785
PL3-25	480	52.543	rBV	0.154	374	52.454	52.608
PL3-25	508	55.546	rBB	0.12	676	55.489	55.609
PL3-25	508	56.291	rVB	0.171	815	56.209	56.381
PL3-25	536	59.166	rBV	0.171	872	59.09	59.261
PL3-25	536	59.88	rBB	0.154	625	59.81	59.964

Figure 10 – Ion Fragment Information on sample PL3-25.

CHAPTER THREE

Sterol Distribution from Cameron Park Zoo

Distribution Index Analysis (See Appendices D, E, F and G)

The mass spectra for the Cameron Park Zoo samples were analyzed for compounds of interest which included those that had a steroid backbone as well as others such as n-alkanes and α -tocopherol. A comprehensive index was made which highlighted the presence of a variety of compounds while noting each individual retention time. For each species from Cameron Park Zoo, compounds which were found in common among the replicate samples were cross-checked for similar retention times. Characteristic distributions for many compounds were found for individual species and collectively among the mammals.

For compounds identified by the Chemstation software as having a steroid backbone, the Cameron Park Zoo samples showed the common presence of Cholest-7-en-6-one, 3-(acetyloxy)-9-hydroxy-, (3 β ,5 α)-, 9,19-Cyclolanostane-3,7 β -diol, diacetate (20R,14 β), 9,19-Cyclolanostan-7-ol, 3-acetoxy-, Arundoin, 9,19-Cyclocholestan-3-one, 4,14-dimethyl-, Pregn-4-ene-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), (11 β)-, Urs-9(11)-en-12-one-28-oic acid, 3-acetoxy-, methyl ester (14 β ,20 β), Pregnane-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), (5 α ,11 β)-, Cholestan-3-one, 2-[1-hydroxy-2-(3-methylphenyl)ethyl]-, Anthiaergosta-5,7,9-trien-3-one, β -Sitosterol trimethylsilyl ether, and Cholestan-3-ol, 5-chloro-6-nitro-,

(3 β ,5 α ,6 β)-. The compounds that were only observed once are displayed in Figure 11 with their retention times.

Dotriacontane	53.261 min	WTD2
19-Norpregna-1,3,5(10)-trien-20-yne, 3,17-bis[(trimethylsilyl)oxy]-, (17 α)-	45.996 min	B2
Cholest-4-ene-3,6-dione	49.225 min	E6
17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene	40.943 min	BB5
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-3,4a,7b-trihydroxy-1,1,6,8-tetramethyl-, [1aR-(1a α ,1b β ,2 α ,3 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-	48.791 min	BB5
Bufa-20,22-dienolide, 3,5,14-trihydroxy-, (3 β ,5 β)-	50.631 min	BB5
Methanesulfonic acid, 2-(3-hydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthryl)-	50.631 min	BB5
Androst-4,9(11),16-trien-3-one, 16-[2-(methylcarbonyloxy)-1-oxoethyl]-	35.197 min	BB3
Fluoxymesterone	35.512 min	BB3
3,9-Epoxypregn-16-en-14-ol-20-one, 11,18-diacetoxy-3-methoxy-	36.580 min	BB3
Cholesterone	37.609 min	BB3
Cholesterol trimethylsilyl ether	52.549 min	BB3
β -Sitosterol acetate	51.382 min	BB1
Stigmast-5-en-3-ol, oleate	51.382 min	BB1
(22R)-6 α ,11 β ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-3,20-dione	42.083 min	WTD6
3,9-Epoxypregnan-14-ol-20-one, 3,11,18-triacetoxy-1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-triacetate, [1aR-(1a α ,1b β ,4a β ,5 β ,7a α ,7b α ,8 α ,9 β ,9a α)]-	38.531 min	WTD5
9,19-Cyclolanost-6-en-3-ol, acetate	42.172 min	WTD5
9,19-Cyclolanost-24-en-3-ol, acetate	42.903 min	WTD5
22R)-21-Acetoxy-6 α ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-3,20-dione	36.595 min	WTD3
Hyochoic acid	40.195 min	WTD2
9,19-Cycloergostan-3-ol-7-one, 4,14-dimethyl-	50.786 min	WTD2
Betamethasone Acetate	51.163 min	WTD2
1,2,6a,6b,9,9,12a-Heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-eicosahydropicene-4-carboxylic acid, methyl ester	35.898 min	WTD1
D-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 14,15:21,23-diepoxy-7-hydroxy-4,4,8-trimethyl-, (5 α ,7 α ,13 α ,14 β ,15 β ,17 α)-	41.271 min	WTD1

D-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 14,15:21,23-diepoxy-7-hydroxy-4,4,8-trimethyl-, (5 α ,7 α ,13 α ,14 β ,15 β ,17 $\alpha\alpha$)-	41.271 min	WTD1
9,19-Cyclolanost-7-en-3-ol	44.980 min	WTD1
Oleanolic acid	49.859 min	E4
Urs-12-ene-3 β ,11 β -diol, diacetate	52.134 min	E4
β -k-Strophanthin	48.125 min	E3

Figure 11: Retention times for unique compounds in Cameron Park Zoo samples

The compounds without a steroid backbone which were found to match other samples from the Cameron Park Zoo included the n-alkanes Heptacosane (E2-E5, WTD2-WTD6, B1-B4), Octacosane (WTD2, B4), Nonacosane (E3-E6, WTD1-3, WTD5-6, BB1-3, BB5-6, B1-B4), Tetratetracontane (E1-E4, WTD1-WTD6, BB1, BB4, BB6, B1, B3, B4), Triacontane (WTD2), Hentriacontane (E1-E6, WTD1-WTD6, B1, B3, B4), Tetratriacontane (WTD1, WTD4), and Tritriacontane (E2, E4, E6, WTD1-2, WTD4-6, B2-B4). Other noteworthy non-steroid backbone compounds included Squalene (E1, E3, E5-6, WTD1-4, BB3-4, BB6), β Carotene (E2, B4), (+)- α -Tocopherol acetate (E2, WTD1-WTD6, BB1, BB6), and α -Tocopherol (vitamin E), trimethylsilyl derivative (E2, WTD1-WTD6, B2, B4). Both Tocopherol compounds (i.e. Vitamin E) were found in each White-Tailed Deer sample, as well as in other Cameron Park Zoo samples. Dotriacontane was identified in *WTD2* as n-alkane; however, it was only found once so it is in Figure 5.

Ion Fragment Analysis

The peaks that were found in the Page-Ladson sample PL3-25 were similarly found with higher signals in several of the Cameron Park Zoo samples. These include

elephant samples such as E1, E2, and E4 as well as the bison samples B2, B3, and B4. The two mammals actually show the presence of an additional compound derived from the sterol which has a 549 peak and its respective stereoisomer. Finally, several of the deer samples gave strong signals of just the 508 peak and not any of the other noted peaks. For E2 (which had the clearest signal among the elephant samples), the 480 peak and its stereoisomer were found with retention times 51.758 and 52.558 minutes. The 508 peak and its stereoisomer were found with retention times 55.553 and 56.307 minutes. The 536 peak and its stereoisomer were found with retention times 59.171 and 59.874 minutes. The 549 peak and its stereoisomer were found with retention times 62.936 and 63.834 minutes. For B2 (with similar signals to B3 and B4) the 480 peak and its stereoisomer were found with retention times 51.751 and 52.557 minutes. The 508 peak and its stereoisomer were found with retention times 55.546 and 56.306 minutes. The 536 peak and its stereoisomer were found with retention times 59.158 and 59.873 minutes. The 549 peak and its stereoisomer were found with retention times 62.948 and 63.834 minutes. For WTD3 (with similar signals to WTD2, WTD4-6) the 508 peak with its stereoisomer which were found with retention times 55.553 and 56.302 minutes. Figure 12 shows information on each peak and its isomer for the elephant, bison, and deer samples.

Sample	Ion Fragm	Ret Time	Type	Width	Area	Start Time
E2	480	51.751	rVB	0.24	6755	51.648
E2	480	52.558	rBB	0.24	5721	52.454
E2	508	55.546	rBV	0.206	15015	55.437
E2	508	56.302	rVB	0.189	13218	56.209
E2	536	59.162	rBV	0.189	10077	59.055
E2	536	59.869	rBB	0.274	8958	59.707
E2	549	62.931	rBV	0.24	1439	62.793
E2	549	63.838	rVV	0.171	1277	63.771
B2	480	51.751	rVB	0.24	6755	51.648
B2	480	52.558	rBB	0.24	5721	52.454
B2	508	55.546	rBV	0.206	15015	55.437
B2	508	56.302	rVB	0.189	13218	56.209
B2	536	59.162	rBV	0.189	10077	59.055
B2	536	59.869	rBB	0.274	8958	59.707
B2	549	62.931	rBV	0.24	1439	62.793
B2	549	63.838	rVV	0.171	1277	63.771
WTD3	508	55.562	rVV	0.343	304759	55.445
WTD3	508	56.317	rVB	0.36	330674	56.216

Figure 12 – Ion Fragment Information on E2, B2 and WTD3

CHAPTER FOUR

Sterol Distribution from Martens Farm

Distribution Index Analysis (See Appendices G and H)

The mass spectra for the Martens Farm samples were analyzed for compounds of interest which included those that had a steroid backbone as well as others such as n-alkanes. A comprehensive index was made which highlighted the presence of a variety of compounds while noting each individual retention time. As previously mentioned, for each Martens Farm sample, compounds which were found in common between the samples were checked to see if they had similar retention times. This was a cross analysis with compounds from Page-Ladson and Cameron Park Zoo as well.

For compounds that had a steroid backbone, the Martens Farm samples showed the common presence of Cholest-7-en-6-one, 3-(acetyloxy)-9-hydroxy-, (3 β ,5 α)-, 9,19-Cyclolanostane-3,7 β -diol, diacetate (20R,14 β), 9,19-Cyclo-9 β -lanost-7-en-3 β -ol, acetate, 9,19-Cyclolanostan-7-ol, 3-acetoxy-, 9,19-Cyclolanostane-3,7-diol, Cholestane, 2-formyl-3-(2-methylbenzylidene)-, 3,9 β ;14,15-Diepoxypregn-16-en-20-one, 3,11 β ,18-triacetoxy-, and 9,19-Cyclocholestan-3-one, 4,14-dimethyl-. The compounds that were only observed once are displayed in Figure 13 with their retention times.

9,19-Cyclolanostane-6,7-dione, 3-acetoxy-	50.963 min	DUF
3-Heptafluorobutyryloxy-3,5,10-pregnatrien-20-one	55.564 min	HOL 1
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	63.937 min	HOL 1
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3 β ,4 α ,5 α)-	53.991 min	LMG
Pregn-5-en-20-one, 12-(acetyloxy)-3,8,14-trihydroxy-, (3 β ,12 β ,14 β)-	48.456 min	Slab 1
10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxylic acid, methyl ester	55.179 min	Slab 2
Pregn-4-ene-3,20-dione, 11,18,21-tris[(trimethylsilyl)oxy]-, bis(O-methyloxime), (11 β)-	40.726 min	Slab 3

Figure 13: Retention times for unique compounds in Martens Farm samples

The compounds without a steroid backbone which were found to match other samples in Martens Farm included the n-alkanes Heptacosane (DH, DUF, DF, HUD, H1, H3, LMG), Nonacosane (DH, DUF, DF, HUD, H1, H2, H3, LMG), Triacontane (H1), Hentriacontane (DH, DF, HUD, H1, H2, H3, LMG), Tetratriacontane (HUD), Tritriacontane (Same as Hentriacontane), and Pentatriacontane (HUD, LMG). Other noteworthy non-steroid backbone compounds, which were similarly found in the Martens Farm samples, included Squalene (DH, DF, H1, LMG), β Carotene (DH, H1, H3, LMG), (+)- α -Tocopherol acetate (LMG), and α -Tocopherol (vitamin E), trimethylsilyl derivative (DF).

Ion Fragment Analysis

The Martens Farm mammals that shared the similar sterol peaks of 480, 508, 536, and in some cases 549 included *DF*, *DH*, *LMG*, and *H1-H3*. For the Dexter Fresh sample, the 480 peak and its stereoisomer were found with retention times 51.752 and 52.541 minutes. The 508 peak and its stereoisomer were found with retention times 55.530 and 56.301 minutes. The 536 peak and its stereoisomer were found with retention times 59.142 and 59.868 minutes. There was no 549 peak associated with *DF*. These peaks were similarly found in Dexter and Holstein sample as well as the La Mancha Goat sample. For the Holstein sample (*H1*), the 480 peak and its stereoisomer were found with retention times 51.769 and 52.569 minutes. The 508 peak and its stereoisomer were found with retention times 55.564 and 56.319 minutes. The 536 peak and its stereoisomer were found with retention times 59.188 and 59.897 minutes. It is noteworthy to mention that *H1* had the clearest and strongest signal among the Holstein samples (*H2* and *H3*). Figure 14 shows the ion fragment analysis for the Martens Farm samples.

Sample	Ion Fragm	Ret Time	Type	Width	Area	Start Time	End Time
Dex Fresh	480	51.783	rVB	0.257	19065	51.689	51.946
Dex Fresh	480	52.556	rVB	0.377	23182	52.461	52.838
Dex Fresh	508	55.572	rVB	0.292	24989	55.461	55.753
Dex Fresh	508	56.299	rBV	0.137	12006	56.216	56.353
Dex Fresh	536	59.156	rVB	0.274	39030	59.062	59.336
Dex Fresh	536	59.889	rVB	0.171	23650	59.816	59.988
HOL 1	480	52.158	rBV	0.206	41264	52.032	52.238
HOL 1	480	52.574	rVV	0.257	187539	52.478	52.735
HOL 1	508	55.568	rVB	0.257	347442	55.478	55.736
HOL 1	508	56.402	rBV	0.669	367562	56.233	56.902
HOL 1	536	59.173	rVB	0.463	1290246	59.011	59.474
HOL 1	536	60.019	rBV	0.909	881299	59.782	60.691
HOL 1	549	63.197	rBV	0.892	311526	62.869	63.76
HOL 1	549	63.956	rBV	1.2	362545	63.76	64.96

Figure 14 – Ion fragment analysis of Martens Farm samples (*DF* and *H1*)

The three slab samples (*S1*, *S2*, and *S3*) were obtained from under Holstein fecal sample did not show peaks of interest for the sterol with either 480, 508, 536, and 549 peaks.

CHAPTER FIVE

Discussion

The sterol distributions figures and ion fragment analyses from the Page-Ladson site, Cameron Park Zoo, and Martens Farm reveal pertinent information to the characteristics of mammals in the past and the present. First, assumptions will be based off the common compounds found in each dataset. Then, deductions will be made from the ion fragments as to the common sterol found in the various mammals. Finally, using the data, hypotheses will be considered as to the characteristics of the mastodon and bison that inhabited the Page-Ladson site over 10,000 years ago.

Common Compounds in Each Dataset

The compound that was found in common among the Page-Ladson, Cameron Park Zoo, and Martens Farm dataset (26 of the 38 total samples) was Cholest-7-en-6-one, 3-(acetyloxy)-9-hydroxy-, (3 β ,5 α)- which eluted around 46.000 minutes for each samples. This is also known as Viperidone acetate. (Figure 10)

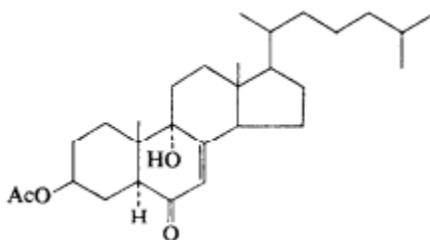


Figure 10: Viperidone acetate

Found common between Page-Ladson and Cameron Park Zoo included both Pregn-4-ene-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), (11 β)- and Pregnane-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime), (5 α ,11 β)-. What is interesting about these two compounds is that these are most likely derivatives of progesterone (pregn-4-ene-3,20-dione). These compounds were found in both Page-Ladson and elephant samples which indicate that this hormone was used by elephant and mastodon as a part of their respective normal bodily processes. In fact, this is the case, as there have been studies reaffirming the ovarian function of elephants including their luteinizing hormone and progesterone cycles.¹⁴ These progesterone derivatives are also found with single retention times (Figures 4,6, and 8) in various mammals further supporting evidence of this hormone. Pregnane-3,11,20-trione, (5 β)-, found in the Page-Ladson dataset, is a derivative of 11-ketoprogesterone or ketogestin which is another important steroid hormone (Figure 11).

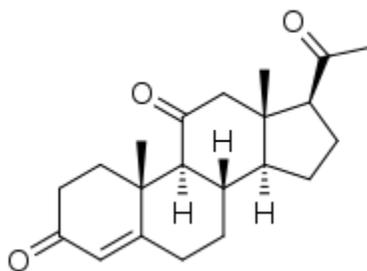


Figure 11: 11-Ketoprogesterone

Another interesting compound which we found in black bears is Anthiaergosta-5,7,9-trien-3-one. The reduction of this compound can result in ergosterol, which through ultraviolet irradiation will turn into ergocalciferol (vitamin D₂) that can promote bone growth in mammals.^{15, 16} The same two black bears which showed the ergostatrienone

also showed signatures of β -Sitosterol trimethylsilyl ether. There was also (+)- α -Tocopherol acetate and α -Tocopherol trimethylsilyl derivative which are different forms of vitamin E. This compound was found throughout each dataset, which is evidence of tocopherols being broadly distributed in all mammalian fecal matter. This is indicative of how mammalian waste incorporates into sediments throughout deposition. There are even traces of these tocopherols in the Page-Ladson samples which is further evidence of the it being distributed in fecal matter. Another manifestation of vitamins in our results includes β Carotene, which is a precursor to vitamin A.

Plant leaf waxes, the long-chain n-alkanes nonacosane ($C_{29}H_{60}$), hentriacontane ($C_{31}H_{64}$), and tritriacontane ($C_{33}H_{68}$) were present in all Page-Ladson samples as well as in all of the mammal fecal matter samples of mammals. (Figure 12).



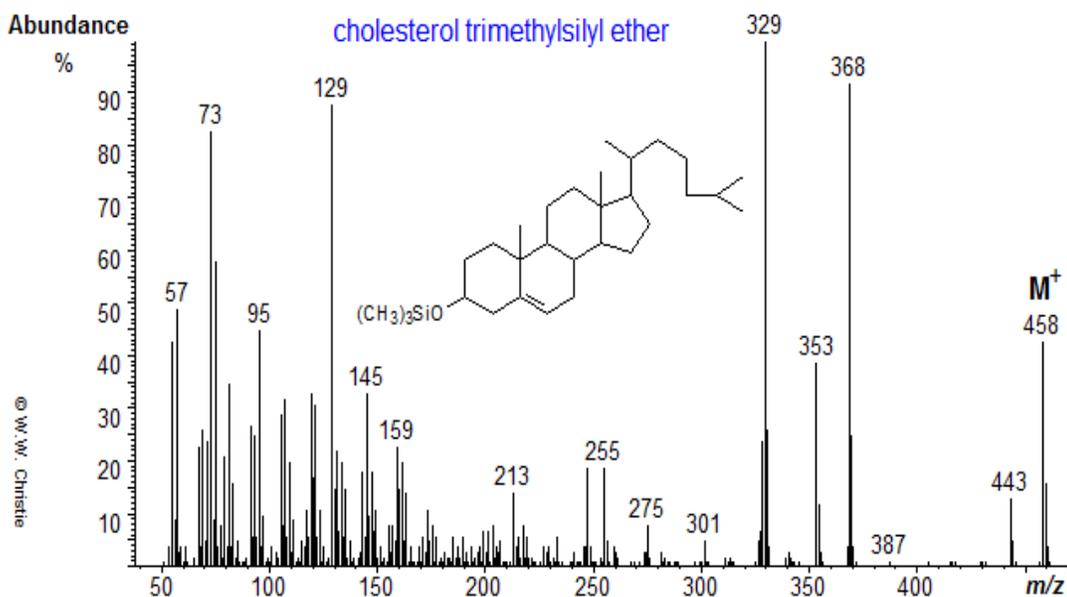
Figure 12: Heptacosane

The plant origin of these compounds is well established, but it is worth noting that mammalian fecal matter can transfer long-chain n-alkanes to depositional environments

like sink holes. This process could bias distributions of specific n-alkanes in sediments if the mammal's dietary preference were for specific plants on the landscape.

Ion Fragment Analysis

The mass spectra for the chromatographic peaks of interest (the sterol-related compounds) included features similar to cholesterol trimethylsilyl ether but lacked distinctive ions with m/z in the 300-400 range as expected for many sterol and stanol derivatives. Figure 13 shows the mass spectrum for this compound.



These potential sterol derivatives included m/z 480, 508, and 536 ions that might be the molecular ions, though they might also have been fragment ions from larger molecules such as bile acids that are found in mammal digesta. The 480 peak with its stereoisomer is 22 Da greater than cholesterol, which can result from several different transformations including additions of carbon and oxygen-containing moieties and additional double

bonds. The 508 peak with its stereoisomer is attributed to the 480 peak plus 28 which comes from an addition of an ethyl group (C_2H_4). The 536 peak with its stereoisomer is attributed to the 508 peak plus 28 which comes from another addition of an ethyl group.

These peaks were found among the mammals with the strongest signal in *E2*, *B2*, *WTD3*, *DF*, and *H1*. The La Mancha Goat also showed a weaker signal of these peaks; however, none of the black bears showed these peaks. Therefore, these cholesterol compounds are unique to specific mammals and are most likely involved in the digestion process, the products of which manifest themselves as cholesterol derivatives such as the bile acids in fecal matter. What makes this finding more interesting is that only the deepest sample from Page-Ladson (PL3-25), which was indicated to have been the time where mastodons lived, showed the same but weak peaks of these cholesterol compounds. The younger Page-Ladson samples did not show these sterols, which indicates that these sterols could be associated with mastodon living near the Aucilla River. If this hypothesis is valid, loss of the sterol peaks signal after PL3-25 indicate that the mastodon went extinct sometime between 10590 and 11810 years ago in the area, and higher resolution sampling could refine this date.

Bison, a species thought to have grazed the area as well during the time, showed these sterol-related peaks as well. The Holstein and Dexter cattle (that are close relatives to the bison species) also showed these sterol-related peaks. With that being said, if bison grazed the area near the Page-Ladson sinkhole long after the mastodon went extinct, there should be signature of these peaks in other Page-Ladson samples. However, since they were not detected, it is possible that either the sterol-related compounds from bison were

oxidized and not preserved, or that the bison disappeared from the area due to migration or hunting activity.

The absence of the sterol-related compounds from the three soil slab samples taken from under aged Holstein manure supports the oxidation hypothesis that the sterols were chemically transformed. Analysis of the ion fragment of the Holstein samples indicate that the sterol-related compounds were present in the Holstein manure but were not transferred to soil during the ca. 3-6 months following deposition on the soil surface. Applying this to the Page-Ladson sinkhole, bison fecal matter might not be detected as it could be chemically transformed in the soil. It is therefore evident that the bison continued to exist in the area of the Aucilla River even after mastodon extirpation.

The other less plausible hypothesis which explains bison disappearing from the area could be entertained if considering the relationship between mastodon and bison. Grazing bison flourish when mastodon maintained the landscape of the valley where the bison lived.¹⁷ If mastodon were hunted to extinction, bison could have left the area which would favor grazing and survival. However, it is unlikely that an extirpation of mastodon would force a mass migration of bison to another region. Additionally, since the major species of bison in the last Pleistocene became extinct later during the mid-Holocene, the hypothesis that they were hunted to extinction when transitioning from the late Pleistocene to early Holocene epoch is not as feasible.¹⁸

CONCLUSION

The Page-Ladson sinkhole in its deepest depths shows evidence of mastodon with a variety of steroid compounds and unique mammalian sterols. The steroid compounds found in each dataset's distribution index give indications to the bodily processes of the mammal's such as progesterone in female ovarian cycles. These sterols can be cross referenced with mammals received from the Cameron Park Zoo and Martens Farm to deduce that mastodons between the period of 10590 and 11810 years ago disappeared due to the hunting activity of pre-Clovis humans during the transition from the late Pleistocene to early Holocene epoch. Bison did not show these sterol peaks due to chemical transformations of the fecal matter when depositing into the soil. Nonetheless, the bison still co-existed with humans and mastodon in the area and most likely continued to graze well into the Holocene epoch following mastodon extirpation. Further study can not only attempt deduce the significance of the various cholesterol derivatives found in the 129.00 ion fragment in mammals, but it can also seek to highlight the chemical transformations of mastodon sterols over time from areas including and excluding the Page-Ladson sinkhole.

APPENDIX

APPENDIX A

Sample Information

Location:

Cameron Park Zoo

Martens Farm

Page-Ladson Site

Elephant #1 (E1)	11/29/2018	0.1
Elephant #2 (E2)	11/27/2018 0.1 Tembo	
Elephant #3 (E3)	11/27/2018 0.1 Tanya	
Elephant #4 (E4)	11/28/2018 0.1 Tembo	
Elephant #5 (E5)	11/28/2018 0.1 Tanya	
Elephant #6 (E6)	11/29/2018 0.1 Tanya	
Bison #1 (B1)	11/30/2018 2 Males 1 Female	
Bison #2 (B2)	11/30/2018 2 Males 1 Female	
Bison #3 (B3)	11/30/2018 2 Males 1 Female	
Bison #4 (B4)	11/30/2018 2 Males 1 Female	
White-Tailed Deer #1 (WTD1)	11/28/2018 Male #1	
White-Tailed Deer #2 (WTD2)	11/28/2018 Male #2	
White-Tailed Deer #3 (WTD3)	11/30/2018 1 Male 3 Females	
White-Tailed Deer #4 (WTD4)	11/28/2018	1.3
White-Tailed Deer #5 (WTD5)	11/30/2018 Male #4	
White-Tailed Deer #6 (WTD6)	11/28/2018 Male	
NA Black Bear #1 (BB1)	11/28/2018	1.0
NA Black Bear #2 (BB2)	11/29/2018	0.1
NA Black Bear #3 (BB3)	11/27/2018	0.1
NA Black Bear #4 (BB4)	11/29/2018	1.0
NA Black Bear #5 (BB5)	11/28/2018	0.1
NA Black Bear #6 (BB6)	11/27/2018	1.0

Sample Name (Abbreviation)	Date Collected	Additional Information
Chicken (C)	9/13/2018	Sample #4
Holstein #1 (H1)	9/13/2018	Sample #1
Holstein #2 (H2)	9/13/2018	Sample #2
Holstein #3 (H3)	9/13/2018	Sample #3
Dirt Under Feces (DUF)	9/13/2018	Sample #7
Dexter Fresh (DF)	9/13/2018	Sample #11
Slab #1 (S1)	9/13/2018	Sample #8
Slab #2 (S2)	9/13/2018	Sample #9
Slab #3 (S3)	9/13/2018	Sample #10
Dexter and Holstein (DH)	9/13/2018	Sample #5
La Mancha Goat (LMG)	9/13/2018	Sample #12
Holstein Under Dexter (HUD)	9/13/2018	Sample #6
Vial #1 (V1)	11/14/2018	14 - 2.33 g
Vial #2 (V2)	11/14/2018	17 - 2.4 g
Vial #3 (V3)	11/14/2018	25 - 2.34 g
Vial #4 (V4)	11/14/2018	6 - 2.35 g

APPENDIX B

Name Index

- Compounds highlighted do not have a steroid backbone
- 1 Glycine, N-[(3 α ,5 β ,7 α ,12 α)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-
methyl ester
 - 2 Heptacosane
 - 3 Cholest-7-en-6-one, 3-(acetyloxy)-9-hydroxy-, (3 β ,5 α)-
 - 4 Lanost-8-ene-3,7-dione, (13 α ,14 β ,17 α)-
 - 5 Octacosane
 - 6 Nonacosane
 - 7 Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandro-8-en-17-yl)-
Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-
8 hexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester
 - 9 9,19-Cyclolanostane-3,7 β -diol, diacetate (20R,14 β)
 - 10 9,19-Cyclo-9 β -lanost-7-en-3 β -ol, acetate
 - 11 9,19-Cyclolanostan-7-ol, 3-acetoxy-
 - 12 9,19-Cyclolanostane-6,7-dione, 3-acetoxy-
 - 13 Tetratetracontane
(5 β)Pregnane-3,20 β -diol, 14 α ,18 α -[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-
14 diyl)]-, diacetate
 - 15 7,8-Epoxy lanostan-11-ol, 3-acetoxy-
 - 16 Squalene
 - 17 Cholestane, 3,5-dichloro-6-nitro-, (3 β ,5 α ,6 β)-
17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-
18 styrylhexadecahydrocyclopenta[a]phenanthren-2-one
 - 19 25-Norisopropyl-9,19-cyclolanostan-22-en-24-one, 3-acetoxy-24-phenyl-4,4,14-
trimethyl-
17 β -Acetoxy-1',1'-dicarboethoxy-1 β ,2 β -dihydro-17 α -methyl-3'H-cycloprop[1,2]-
20 5 α -androst-1-en-3-one
 - 21 Anodendroside E 2
 - 22 Triacontane
 - 23 Lycopene
 - 24 Hentriacontane
 - 25 3'H-Cycloprop(1,2)-5-cholest-1-en-3-one, 1'-carboethoxy-1'-cyano-1,2-dihydro-
26 Dotriacontane

- 27 Tetratricosane
- 28 Tritriacontane
- 29 3-Heptafluorobutyryloxy-3,5,10-pregnatrien-20-one
- 30 Arundoin
- 31 Lup-20(29)-en-3-ol, acetate, (3 β)-
- 32 Pentatriacontane
- 33 β Carotene
4,4,6a,6b,8a,11,11,14b-Octamethyl-
1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-
34 one
- 35 Betulin
- 36 Ethyl iso-allocholate
(22S)-21-Acetoxy-6 α ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-
37 diene-3,20-dione
- 38 Cholestan-3-one, 2-[1-hydroxy-2-(3-methylphenyl)ethyl]-
- 39 Hexatriacontane
Pregn-4-ene-3,11,20-trione, 6,17,21-tris[(trimethylsilyl)oxy]-, 3,20-bis(O-
40 methyloxime), (6 β)-
- 41 8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-
- 42 5-Chloro-6 β -nitro-5 α -cholestan-3-one
- 43 9,19-Cyclocholestene-3,7-diol, 4,14-dimethyl-, 3-acetate
- 44 9,19-Cyclolanostane-3,7-diol
Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-
45 pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-
- 46 Anodendroside A
- 47 Cholesta-8,24-dien-3-ol, 4-methyl-, (3 β ,4 α)-
- 48 a-Homocholest-4a-en-3-one
- 49 Glycine, N-[(3 α ,5 β)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester
- 50 Cycloartenol acetate
- 51 Cholestane, 2-formyl-3-(2-methylbenzylidene)-
Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl- α -D-allopyranosyl)oxy]-1,14-
52 dihydroxy-, (1 β ,3 β)-
(22S)-21-Acetoxy-6 β ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-
53 diene-3,20-dione
(22S)-6 α ,11 β ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-
54 3,20-dione
(22R)-6 β ,11 β ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-
55 3,20-dione
- 56 6-Azacholest-4-en-7-one, 6-benzyl-3 α -hydroxy-
- 57 (+)- α -Tocopherol acetate
- 58 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3 β ,4 α ,5 α)-

2-(16-Acetoxy-11-hydroxy-4,8,10,14-tetramethyl-3-oxohexadecahydrocyclopenta[a]phenanthren-17-ylidene)-6-methyl-hept-5-enoic
 59 acid, methyl ester
 60 3,9 β ;14,15-Diepoxy pregn-16-en-20-one, 3,11 β ,18-triacetoxy-
 Pregnan-20-one, 3,11-dihydroxy-17,21-bis[(trimethylsilyl)oxy]-, O-
 61 methyloxime, (3 α ,5 β ,11 β)-
 62 11 β ,19-Cyclopregn-5-ene-3,20-dione, 11-hydroxy-, cyclic bis(ethylene acetal)
 63 Pregn-5-en-20-one, 12-(acetyloxy)-3,8,14-trihydroxy-, (3 β ,12 β ,14 β)-
 64 Cholestan-3-ol, 5-chloro-6-nitro-, acetate (ester), (3 β ,5 α ,6 β)-
 65 Gamabufotalin
 66 Olean-12-ene-3,15,16,21,22,28-hexol, (3 β ,15 α ,16 α ,21 β ,22 α)-
 [5-(3-Methoxymethoxy-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-
 67 dodecahydro-1H-cyclopenta[a]phenanthren-17-yl)-hex-1-ynyl]-trime
 68 Glycine, N-[(3 α ,5 β ,12 α)-3,12-dihydroxy-24-oxocholan-24-yl]-
 69 Anodendroside E 2, monoacetate
 70 3,9-Epoxy pregn-16-ene-14,20-diol, 7,11,18-triacetoxy-3-methoxy-
 10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-
 1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-
 71 carboxylic acid, methyl ester
 Pregn-4-ene-3,20-dione, 11,17-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime),
 72 (11 β)-
 Pregn-4-ene-3,20-dione, 11,18,21-tris[(trimethylsilyl)oxy]-, bis(O-methyloxime),
 73 (11 β)-
 74 1',1'-Dicarboethoxy-1 β ,2 β -dihydro-3'H-cycloprop[1,2]cholesta-1,4,6-trien-3-one
 D-Glucopyranoside, (3 β ,22 α ,25S)-22,25-epoxy-3-methoxyfurost-5-en-26-yl
 75 2,3,4,6-tetra-O-methyl-
 76 9,19-Cyclocholestan-3-one, 4,14-dimethyl-
 77 (25S)-3Beta-acetoxy-5alpha,22beta-spirost-9(11)-en-12beta-ol
 7-(1,5-Dimethyl-hexyl)-4-(2-methoxycarbonyl-ethyl)-4,10a,10b-trimethyl-5,8-
 dioxotetradecahydro-9-oxa-pentaleno[2,1-a]naphthalene-3-carboxylic acid,
 78 methyl ester
 79 Bufalin
 Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethyl-
 80 hexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester
 81 Anodendroside E 1
 18-Norcholest-17(20),24-dien-21-oic acid, 16-acetoxy-4,8,14-trimethyl-3,11-
 82 dioxo-, methyl ester
 83 Pregnan-11-one, 3,17,20,21-tetrakis[(trimethylsilyl)oxy]-, (3 α ,5 β ,20S)-
 84 **α -Tocopherol (vitamin E), trimethylsilyl derivative**
 85 Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5 β)-
 5H-Cyclopropa(3,4)benz(1,2-e)azulen-5-one, 1,1a- α ,1b- β ,4,4a,7a- α ,7b,8,9,9a-
 86 decahydro-7b- α ,9- β ,9a- α -trihydroxy-3-hydroxymethyl-1,1,6,8- α -tetramethyl-4a-
 methoxy-, 9,9a-didecanoate

- 87 **Tetratriacontane**
- 88 3'H-Cycloprop(1,2)cholesta-1,4,6-trien-3-one, 1'-carboethoxy-1'-cyano-1 β ,2 β -
dihydro-
1',1'-Dicarboethoxy-1 β ,2 β -dihydro-17 β -
89 propionoxy(3'H)cycloprop[1,2]androsta-1,4,6-trien-3-one
Pregn-4-ene-3,20-dione, 11-hydroxy-17,21-bis[(trimethylsilyl)oxy]-, bis(O-
90 methyloxime), (11 β)-
91 4a-Phorbol 12,13-didecanoate
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 3,9,9a-tris(acetyloxy)-3-
[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-4a,7b-
92 dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 $\alpha\alpha$,1b β ,2 α ,3 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-3-
[(acetyloxy)methyl]-2-chloro-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-3,4a,7b-
93 trihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 $\alpha\alpha$,1b β ,2 α ,3 α ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
(22S)-6 β ,11 β ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-
94 3,20-dione
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,4a,9,9a-tetrakis(acetyloxy)-
3,[(acetyloxy)methyl]-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-2,7b-
95 dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 $\alpha\alpha$,1b β ,2 α ,3 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
96 Pregn-5-en-20-one, 3,16-bis[(trimethylsilyl)oxy]-, (3 β ,16 α)-
4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8,8a-
bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-
dodecahydro-6b-hydroxy-3a-methoxy-1,1,5,7-tetramethyl-, [1aR-
97 (1 $\alpha\alpha$,1b β ,1c α ,2a α ,3a β ,6a α ,6b α ,7 α ,8 β ,8a α)]-
98 3,9-Epoxyregnane-11,14,18-triol-20-one, 16-cyano-3-methoxy-, 11-acetate
99 Pregnane-3,11,20-trione, (5 β)-
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-17-hydroxy-6-methyl-, 3,20-bis(O-
100 methyloxime), (6 α)-
Pregn-4-ene-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime),
101 (11 β)-
102 Urs-9(11)-en-12-one-28-oic acid, 3-acetoxy-, methyl ester (14 β ,20 β)
103 3'H-Cycloprop(1,2)-5 α -cholest-1-en-3-one, 1',1'-dicarboethoxy-1 β ,2 β -dihydro-
Pregnane-3,20-dione, 11,21-bis[(trimethylsilyl)oxy]-, bis(O-methyloxime),
104 (5 α ,11 β)-
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,9,9a-tris(acetyloxy)-3-
[(acetyloxy)methyl]-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-
105 1,1,6,8-tetramethyl-, [1aR-(1 $\alpha\alpha$,1b β ,2 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-
1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4a,7b-trihydroxy-3-(hydroxymethyl)-
106 1,1,6,8-tetramethyl-, [1aR-(1 $\alpha\alpha$,1b β ,2 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
107 **Bilirubin**
- 108 Cholan-24-oic acid, 3,7-dioxo-, (5 β)-
109 Lanosta-7,9(11)-diene-3 β ,18,20-triol, 3,18-diacetate, (20R)-

- 110 17 β -Acetoxy-1',1'-dicarboethoxy-1 β ,2 β -dihydrocycloprop[1,2]-5 α -androst-1-en-3-one
- 111 Chol-7-ene, (5 β)-
- 112 Bufo-20,22-dienolide, 3-(acetyloxy)-14,15-epoxy-16-hydroxy-, (3 β ,5 β ,15 β ,16 β)-
- 113 3,9-Epoxypregn-16-ene-14-18-diol-20-one, 7,11-diacetoxy-3-methoxy-1H-Cyclopropa[3,4]benz[1,2-e]azulene-2,5-dione, 9,9a-bis(acetyloxy)-3-[(acetyloxy)methyl]-1a,1b,4a,7a,7b,8,9,9a-octahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 α ,1 β ,4 α ,7 α ,7 β ,8 α ,9 β ,9 α)]-
- 114 Phorbol 12,13-dihexanoate
- 115 DES-A-ARBORANE
- 116 Bufo-20,22-dienolide, 14,15-epoxy-3,16-dihydroxy-, (3 β ,5 β ,15 β ,16 β)-5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-
- 117 4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, (1 α ,1 β ,1c β ,2a β ,3a β ,6a α ,6b α ,7 α ,8 β ,8a α)-
- 118 4,13,20-Tri-O-methylphorbol 12-acetate
- 119 4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6]oxiren-4-one, 8,8a-bis(acetyloxy)-2a-[(acetyloxy)methyl]-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-6b-hydroxy-3a-methoxy-1,1,5,7-tetramethyl-, [1aR-(1 α ,1 β ,1c β ,2a β ,3a α ,6a α ,6b α ,7 α ,8 β ,8a α)]-
- 120 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 3-[(acetyloxy)methyl]-1a,1b,4,4a,5,7a,8,9-octahydro-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1 α ,1 β ,4a α ,5 β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
- 121 Cholest-5-ene-16,22-dione, 3 β ,26-dihydroxy-, 3-acetate, (20S,25R)-14 β)3,19-Epoxyandrost-5,7-diene, 4,4-dimethyl-3-methoxy-17-methylthiomethoxy-
- 122 5,16,20-Pregnatriene-3 β ,20-diol diacetate
- 123 Cholestane-3,7,12,25-tetrol, tetraacetate, (3 α ,5 β ,7 α ,12 α)-1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 9,9a-diacetate, [1aR-(1 α ,1 β ,4a β ,5 β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
- 124 D:A-Friedo-2,3-secooleanane-2,3-dioic acid, dimethyl ester, (4R)-19-Norpregna-1,3,5,7,9-pentaen-21-al, 3,17-bis[(trimethylsilyl)oxy]-, O-methyloxime, (17 α)-
- 125 (22R)-21-Acetoxy-6 β ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-3,20-dione
- 126 3,9-Epoxypregnan-14-ol, 3-methoxy-11,18,20-triacetoxy-
- 127 19-Norpregna-1,3,5(10)-trien-20-yne, 3,17-bis[(trimethylsilyl)oxy]-, (17 α)-Pregn-4-en-18-al, 3-(methoxyimino)-20-oxo-11,21-bis[(trimethylsilyl)oxy]-, 18-(O-methyloxime), (11 β ,17 α)-
- 128 Cholest-4-ene-3,6-dione
- 129
- 130
- 131
- 132
- 133
- 134

- 135 Cholestan-3-one, 2-[1-hydroxy-2-(3-methylphenyl)ethyl]-
- 136 Cholestano[7,8-a]cyclobutane, 3-methoxy-6-oxo-2'-methylene-
Acetic acid, 17-(4-hydroxy-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-
pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-
- 137 tetradecahydrocyclopenta[a]phenanthryl ester
- 138 13,27-Cycloursan-3-ol, acetate, (3 β ,13 β ,14 β)-
17-(2-Hydroxy-1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-
2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-
- 139 cyclopenta[a]phenanthrene
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 2,9,9a-tris(acetyloxy)-3-
[(acetyloxy)methyl]-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro-3,4a,7b-
- 140 trihydroxy-1,1,6,8-tetramethyl-, [1aR-(1 α ,1b β ,2 α ,3 β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
- 141 Bufo-20,22-dienolide, 3,5,14-trihydroxy-, (3 β ,5 β)-
Methanesulfonic acid, 2-(3-hydroxy-4,4,10,13,14-pentamethyl-
2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-
- 142 cyclopenta[a]phenanthryl)-
- 143 Anthiaergosta-5,7,9-trien-3-one
- 144 β -Sitosterol trimethylsilyl ether
- 145 Androst-4,9(11),16-trien-3-one, 16-[2-(methylcarbonyloxy)-1-oxoethyl]-
- 146 Fluoxymesterone
- 147 3,9-Epoxy pregn-16-en-14-ol-20-one, 11,18-diacetoxy-3-methoxy-
- 148 Cholesterone
- 149 Cholesterol trimethylsilyl ether
- 150 Ursa-9(11),12-dien-28-oic acid, 3-(acetyloxy)-, methyl ester, (3 β)-
- 151 β -Sitosterol acetate
- 152 Stigmast-5-en-3-ol, oleate
(22R)-6 α ,11 β ,21-Trihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-diene-
- 153 3,20-dione
- 154 3,9-Epoxy pregnan-14-ol-20-one, 3,11,18-triacetoxy-
1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-
[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5,9,9a-
- 155 triacetate, [1aR-(1 α ,1b β ,4a β ,5 β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
- 156 9,19-Cyclolanost-6-en-3-ol, acetate
- 157 9,19-Cyclolanost-24-en-3-ol, acetate
5H-Cyclopropa[3,4]benz[1,2-e]azulen-5-one, 9-(acetyloxy)-3-
[(acetyloxy)methyl]-1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9a-trihydroxy-
- 158 1,1,6,8-tetramethyl-, [1aR-(1 α ,1b β ,4a β ,7a α ,7b α ,8 α ,9 β ,9a α)]-
- 22R)-21-Acetoxy-6 α ,11 β -dihydroxy-16 α ,17 α -propylmethylenedioxypregna-1,4-
- 159 diene-3,20-dione
- 160 Hyocholic acid
- 161 Cholestan-3-ol, 5-chloro-6-nitro-, (3 β ,5 α ,6 β)-
- 162 4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone
- 163 9,19-Cycloergostan-3-ol-7-one, 4,14-dimethyl-

- 164 Betamethasone Acetate
1,2,6a,6b,9,9,12a-Heptamethyl-10,13-dioxo-
1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-eicosahydricene-4-
165 carboxylic acid, methyl ester
D-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione, 14,15:21,23-diepoxy-7-
166 hydroxy-4,4,8-trimethyl-, (5 α ,7 α ,13 α ,14 β ,15 β ,17 $\alpha\alpha$)-
167 9,19-Cyclolanost-7-en-3-ol
168 Oleanolic acid
169 Urs-12-ene-3 β ,11 β -diol, diacetate
170 β -k-Strophanthin

APPENDIX C

Distribution Index – Page-Ladson

	PL1 - 14	PL2 - 17	PL3 - 25	PL4 - 6
1	40.369 min	38.809 min	45.996 min	35.305 min
2				
3	45.993 min		45.996 min	
4				
5				
6			47.299 min	
7	54.572 min	51.514 min	41.932 min	36.631 min
8				
9				
10				
11				
12				
13	47.296 min		38.863 min	43.106 min
14		48.862 min	49.842 min	
15	39.472 min		36.765 min	49.291 min
16				
17	44.679 min		48.242 min	
18	38.626 min	40.209 min	37.222 min	40.832 min
19	47.582 min	35.723 min	41.435 min	36.768 min
20			35.433 min	55.806 min
21		45.073 min	49.328 min	49.291 min
22				

23	44.467 min	39.306 min	46.133 min	39.917 min
24			51.328 min	
25	50.051 min			36.768 min
26				
27				
28				
29				
30				
31			36.988 min	
32				
33		36.088 min		36.402 min
34				
35			38.125 min	
36				
37		38.180 min	40.063 min	35.716 min
38				
39				
40	40.964 min	37.214 min	40.212 min	38.831 min
41				
42				
43	44.061 min			
44	50.960 min			51.880 min
45	39.466 min		45.121 min	39.397 min
46		45.073 min	46.750 min	
47				
48				
49				
50				
51				
52				
53		38.072 min	38.125 min	
54	46.919 min			

55	46.919 min			50.948 min
56				
57				
58				
59	42.210 min		42.601 min	
60				
61				
62				
63				
64				
65				
66	44.061 min	47.668 min	43.812 min	
67	50.051 min			
68				
69	38.626 min	39.178 min	45.121 min	34.796 min
70				42.186 min
71				
72	39.472 min	36.100 min		39.397 min
73				
74		42.290 min		41.643 min
75	45.993 min			
76				
77				
78	39.632 min	46.106 min		
79				
80				
81				
82				
83	44.679 min		52.734 min	
84				
85				
86	53.898 min	36.494 min	36.628 min	37.362 min
87				

88	38.626 min			42.981 min
89	39.632 min			
90	39.963 min			
91	44.467 min			37.362 min
92	45.742 min	37.974 min	38.994 min	44.187 min
93	45.742 min			
94	46.919 min			
95	46.885 min			50.948 min
96	51.132 min			
97		35.717 min	38.994 min	
98		35.717 min		
99		37.523 min	39.509 min	37.522 min
100		37.069 min		
101		37.780 min		
102		39.506 min		36.402 min
103		40.461 min		41.643 min
104		48.862 min		
105			35.148 min	
106			35.148 min	
107			35.319 min	
108			35.319 min	
109			35.433 min	
110			35.794 min	34.796 min
111			35.959 min	

112	35.879 min	
113	36.359 min	
114	36.359 min	
115	37.085 min	
116	37.537 min	
117	38.143 min	
118	38.994 min	
119	39.949 min	
120	41.435 min	
121	47.693 min	
122	49.242 min	40.832 min
123	50.236 min	
124	50.728 min	
125	51.591 min	
126	51.591 min	
127	54.174 min	36.631 min
128		39.294 min
129		41.912 min
130		44.055 min

APPENDIX D

Distribution Index- Cameron Park Zoo – Elephant

	E1	E2	E3	E4	E5	E6
1	45.995 min	35.057 min	45.993 min	54.723 min	40.228 min	41.407 min
2		43.116 min	43.118 min	43.109 min	43.108 min	
3	45.995 min	45.991 min	45.981 min	45.973 min		45.985 min
4						
5						
6			47.296 min	47.293 min	47.309 min	47.299 min
7	45.572 min	35.297 min	48.125 min		37.204 min	46.128 min
8						
9		53.970 min				
10						
11						
12						
13	38.856 min	38.864 min	38.866 min	38.857 min	40.982 min	38.857 min
14		58.119 min	46.290 min			
15	40.874 min	40.881 min	35.316 min	36.511 min	45.103 min	39.560 min
16	45.743 min		45.747 min		45.737 min	45.733 min
17						
18		35.057 min	36.145 min		45.555 min	45.916 min
19	45.572 min	48.620 min	45.113 min	35.193 min	48.721 min	53.278 min
20			54.400 min		51.744 min	
21						45.099 min

22	36.638	36.132			50.993	41.904
23	min	min			min	min
24	51.321	51.323	51.314	51.322	51.322	51.317
24	min	min	min	min	min	min
25	55.934				38.770	55.952
25	min				min	min
26						
27						
28		55.164		55.163	55.174	
28		min		min	min	
29						
30		57.565				
30		min				
31						
32						
33		59.176	50.948		42.365	
33		min	min		min	
34						
35				49.859		
35				min		
36						
37	39.713		39.700	43.846	35.701	39.560
37	min		min	min	min	min
38						
39	55.174				55.174	
39	min				min	
40	37.204	35.297	35.316	40.074	40.228	41.910
40	min	min	min	min	min	min
41						
42						
43						
44				37.188	43.909	
44				min	min	
45	35.284		35.739	39.285	39.839	40.984
45	min		min	min	min	min
46	41.399		49.239		49.230	
46	min		min		min	
47						49.225
47						min
48						
49						46.128
49						min
50						
51						52.300
51						min

52	39.713 min	47.191 min			35.701 min	
53						
54						
55			36.762 min	38.079 min		38.052 min
56		53.124 min				
57						
58				39.285 min	39.839 min	46.831 min
59						
60						
61		49.294 min				
62						
63						
64						
65						
66	52.550 min	51.078 min	47.176 min	36.211 min	48.721 min	
67			45.113 min			
68						
69	45.995 min	52.135 min	39.912 min	42.360 min	40.628 min	36.754 min
70	39.148 min					
71						
72	38.719 min		36.145 min	45.481 min	36.358 min	
73						
74	49.835 min		36.762 min		45.555 min	36.754 min
75			39.912 min	45.973 min		
76						
77	37.936 min					
78						
79						
80						
81			47.999 min			
82						

83	37.444 min		49.959 min		
84		52.301 min			
85					
86	40.874 min	36.766 min			35.284 min
87					
88			39.157 min		
89			36.762 min		
90	46.120 min				
91	53.928 min	53.278 min	43.930 min	40.977 min	
92	37.947 min	49.883 min	36.145 min		40.862 min
93					
94					
95	38.719 min	48.843 min			41.897 min
96					
97				36.571 min	55.421 min
98					
99					
100					
101				37.188 min	
102			39.723 min		
103	37.044 min				36.633 min
104	48.864 min				
105				35.193 min	
106					
107					
108					
109	52.550 min				
110					
111					
112					

113	49.835 min		
114			
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116			
117			
118			
119			
120			
121			
122	36.766 min	49.304 min	
123			
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129			
130		43.846 min	
131			
132			
133			
134			49.225 min 52.300 min
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150		39.285 min	

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53.265
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49.859
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48.125
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APPENDIX E

Distribution Index – Cameron Park Zoo – White-Tailed Deer

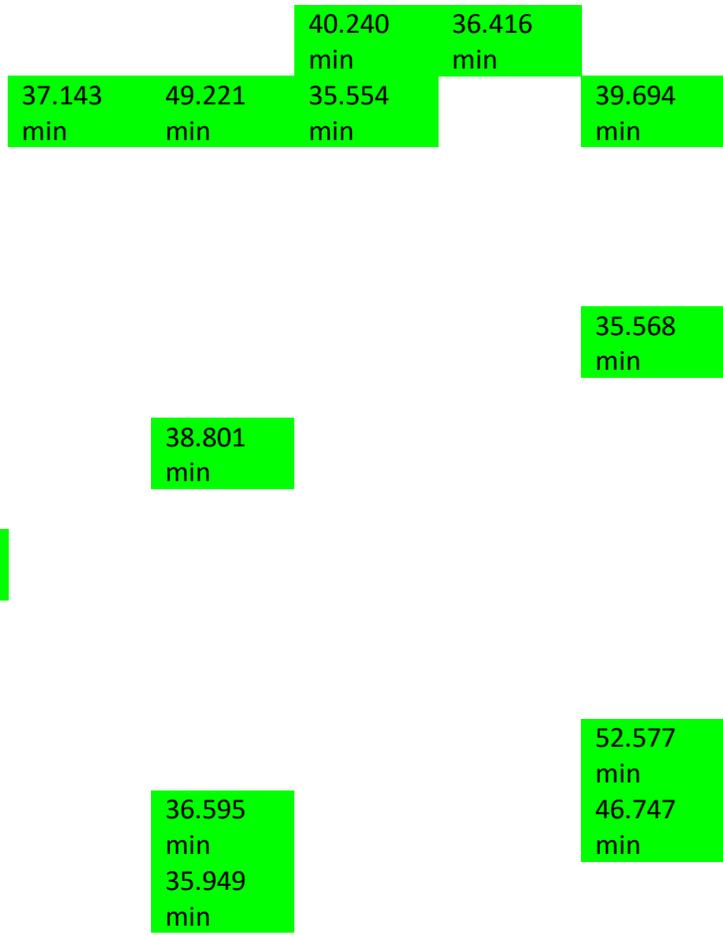
	WTD1	WTD2	WTD3	WTD4	WTD5	WTD6
1		53.924 min	38.801 min	52.111 min		51.897 min
2		43.105 min	43.117 min	43.098 min	43.103 min	43.118 min
3		45.974 min	45.991 min	46.002 min	46.001 min	45.981 min
4						
5		45.214 min				
6	47.306 min	47.317 min	47.317 min	47.787 min	47.310 min	47.296 min
7		53.690 min	43.208 min		50.825 min	
8			49.832 min			
9			50.061 min	50.031 min		
10					41.440 min	
11				50.031 min		
12						
13	38.853 min	38.852 min	38.864 min	38.846 min	38.863 min	38.860 min
14	35.898 min		52.570 min		49.825 min	47.038 min
15	36.647 min	40.864 min	37.378 min	45.121 min	38.194 min	36.888 min
16	45.752 min	36.097 min	45.757 min	45.756 min	36.096 min	36.105 min
17		50.946 min		38.486 min		42.112 min
18	36.333 min	44.173 min	37.950 min	38.280 min	37.199 min	42.083 min
19	57.023 min	51.163 min	47.855 min	52.111 min	53.746 min	38.071 min

20	36.647 min	35.949 min				44.181 min
21	44.506 min	44.368 min			49.825 min	39.694 min
22		49.323 min				
23	36.887 min	54.827 min	35.544 min	36.234 min	42.092 min	36.253 min
24	51.353 min	51.352 min	51.335 min	51.340 min	51.362 min	51.348 min
25	39.728 min				55.929 min	
26		53.261 min				
27	51.353 min			51.340 min		
28	51.353 min	51.352 min		51.340 min	55.175 min	55.166 min
29						
30						
31						
32						
33						
34						
35						
36						
37		47.008 min	41.916 min	35.725 min	35.713 min	39.694 min
38						
39	55.148 min					
40	36.750 min		35.726 min		39.000 min	35.710 min
41						
42					42.903 min	
43						
44						
45		38.063 min				39.231 min
46		35.703 min	35.304 min			
47						
48						
49						
50						

51						
52		50.100 min				
53		49.677 min	44.934 min	35.725 min	36.416 min	44.444 min
54				44.367 min		42.083 min
55						
56	39.231 min					
57	53.096 min	53.118 min	53.119 min	53.094 min	53.128 min	53.103 min
58						
59		40.864 min				37.368 min
60						
61	45.980 min	56.284 min			55.552 min	
62						
63						
64	39.699 min	42.099 min	39.710 min		40.880 min	
65	50.359 min					
66		50.946 min		39.852 min	56.986 min	50.936 min
67	40.722 min				42.709 min	
68						35.568 min
69	44.506 min	37.143 min	39.104 min	39.852 min	49.682 min	35.568 min
70						
71						
72	35.550 min		36.218 min	41.566 min		39.911 min
73						
74			39.104 min		39.000 min	36.745 min
75	36.744 min		41.596 min	38.280 min	37.931 min	
76				41.806 min		
77	35.898 min					
78			36.218 min			

79						
80	36.744 min					
81	44.660 min	45.522 min				42.112 min
82	40.722 min		52.130 min			
83			53.684 min			
84	52.296 min	52.306 min	52.313 min	52.294 min	52.334 min	52.302 min
85		52.124 min		54.820 min		
86	36.750 min	36.217 min	40.990 min		35.839 min	36.465 min
87						
88						38.734 min
89	39.231 min				41.189 min	
90						
91		51.758 min	35.304 min		35.839 min	36.745 min
92		42.253 min		46.516 min	41.286 min	
93		39.601 min				
94						
95						
96						
97	44.506 min	36.623 min		38.486 min	36.416 min	44.444 min
98						52.577 min
99						
100						
101						
102						
103	43.694 min	52.781 min		53.912 min	35.839 min	
104	37.207 min					
105		39.132 min				
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42.083
min
57.864
min

38.531
min
42.172
min
42.903
min
50.825
min

36.595
min

40.195
min
42.099
min
50.786
min
50.786
min
51.163
min

42.094
min
49.821
min

35.898
min
41.271
min
44.980
min

APPENDIX F

Distribution Index – Cameron Park Zoo – Black Bear

	BB1	BB2	BB3	BB4	BB5	BB6
1	37.265 min	35.734 min	37.609 min	36.177 min	40.212 min	35.714 min
2						
3	45.993 min	45.982 min	45.977 min	45.979 min		45.979 min
4						
5						
6	47.313 min	47.297 min	47.297 min		47.310 min	47.322 min
7	45.221 min	51.160 min	53.081 min		43.224 min	40.218 min
8						
9	50.062 min					
10						
11	50.062 min					
12						
13	38.865 min			38.852 min	36.754 min	38.858 min
14	48.748 min	40.975 min			52.540 min	
15	35.122 min	39.907 min	36.466 min	46.150 min	45.093 min	36.480 min
16			45.737 min	45.744 min		45.750 min
17						
18	37.014 min	37.826 min	40.210 min		42.298 min	36.183 min
19	37.265 min	50.692 min	56.882 min	49.265 min	46.110 min	36.183 min
20			62.254 min	47.905 min	51.402 min	48.151 min
21					39.703 min	43.733 min

22	37.214	39.112			44.647	44.462
23	min	min			min	min
24	47.313		47.297			
24	min		min			
25	40.540					
25	min					
26						
27						
28						
29						
30						
31	59.093					58.267
31	min					min
32						
33	36.482	36.597			42.749	
33	min	min			min	
34						
35						
36						
37	35.711	35.671	35.626		41.892	39.715
37	min	min	min		min	min
38						
39						
40	37.214	35.671			37.205	
40	min	min			min	
41						
42						
43			48.812			
43			min			
44						
45	39.117	55.150	44.674	37.588	35.565	
45	min	min	min	min	min	
46	37.014	39.535			46.533	
46	min	min			min	
47						
48						49.243
48						min
49			53.950			
49			min			
50						
51						
52	46.890				40.943	
52	min				min	
53			39.707	46.889	41.892	46.116
53			min	min	min	min

54		50.692 min				
55						
56			40.210 min	36.754 min	51.477 min	
57	53.126 min					53.100 min
58						
59	40.540 min	38.055 min	44.674 min		40.440 min	42.064 min
60						
61						
62						
63						
64	48.793 min		49.846 min	45.190 min		
65						
66	52.548 min	54.287 min				60.708 min
67						
68	38.454 min					
69	39.447 min	49.840 min	39.078 min	45.990 min		45.990 min
70		35.671 min	44.674 min			39.446 min
71						
72		38.563 min			37.034 min	40.418 min
73						
74	38.077 min	51.309 min			35.565 min	41.138 min
75	38.454 min		45.977 min			
76			41.810 min	41.806 min		
77						
78					45.093 min	
79						54.375 min
80		37.197 min				
81		39.706 min				
82						

83						
84						
85			49.315 min			
86	35.493 min	45.245 min	40.410 min	37.045 min	40.989 min	36.766 min
87						
88		36.597 min			35.565 min 40.440 min	
89						
90						
91	46.513 min	40.975 min	40.410 min	37.045 min		52.146 min
92	35.711 min	36.191 min	44.662 min	36.177 min	46.647 min	48.585 min
93	35.711 min		37.443 min	49.545 min		
94	50.216 min		41.810 min			
95	43.804 min				37.034 min	35.714 min
96						
97	39.911 min		37.443 min		39.080 min	49.528 min
98						
99						
100						
101						
102					40.829 min	
103	35.122 min	37.826 min			36.754 min	
104						
105						
106					48.791 min	
107						
108						
109						
110						
111						
112						
113					51.774 min	
114						

115			
116			
117			
118		35.077 min	
119			
120			
121	44.661 min	43.811 min	
122			49.803 min
123			
124			
125			
126			
127			
128			
129			
130			
131			
132			
133		37.826 min	
134			
135			
136	50.719 min	49.840 min	50.694 min
137			53.261 min
138	59.093 min		58.444 min
139			40.943 min
140			48.791 min
141			50.631 min
142			50.631 min
143	50.959 min		50.934 min
144	51.382 min	56.299 min	51.385 min
145		35.197 min	

146		35.512 min
147		36.580 min
148		37.609 min
149		52.549 min
150	35.300 min	
151	51.382 min	
152	51.382 min	

APPENDIX G

Distribution Index – Cameron Park Zoo and Martens Farm – Bison and Holstein

	B1	B2	B3	B4	HOL 1	HOL 2	HOL 3	HUD
1	35.714 min	35.193 min	35.175 min	57.558 min	47.837 min	55.612 min	40.338 min	40.377 min
2	43.116 min	43.115 min	43.116 min	43.121 min	43.122 min		42.312 min	43.109 min
3	45.985 min	45.996 min	45.980 min	45.978 min	57.576 min			57.569 min
4								
5				45.123 min				
6	47.305 min	47.304 min	47.300 min	47.293 min	47.317 min	47.353 min	47.315 min	47.287 min
7	52.300 min	51.517 min	46.117 min			48.902 min	40.891 min	46.435 min
8								
9				53.951 min				
10								
11								
12								
13	38.857 min	45.224 min	38.858 min	38.851 min				41.692 min
14		45.098 min	54.450 min		45.105 min			45.069 min
15	45.110 min	49.236 min	39.138 min	49.236 min	45.105 min	41.020 min	45.115 min	42.840 min
16				45.744 min	45.756 min			
17				42.555 min	48.248 min			
18	39.955 min	38.240 min	35.175 min	38.308 min	48.288 min	45.118 min	41.137 min	48.179 min
19	50.940 min	35.193 min	40.876 min		48.351 min		41.137 min	
20	58.833 min		45.517 min		48.546 min			

21			49.820 min		49.249 min 49.340 min		41.806 min
22							
23	36.114 min	35.559 min	36.103 min	45.464 min	49.934 min	46.718 min	44.446 min 43.961 min
24	51.311 min		51.312 min	51.328 min	51.380 min	51.405 min	51.356 min 51.322 min
25	37.034 min	35.193 min	55.987 min		52.169 min		
26							
27							
28		55.180 min	55.164 min	55.163 min	55.216 min	55.234 min	55.197 min 55.174 min
29					55.564 min		
30		57.564 min			57.576 min		
31					58.290 min	50.047 min	
32					58.862 min		55.174 min
33		41.903 min	59.863 min	59.147 min	59.182 min	59.198 min	
34					63.937 min 67.772 min		
35							
36						41.020 min	
37		35.393 min	38.058 min	36.102 min		41.923 min	52.545 min
38		52.300 min	52.295 min	52.288 min		52.342 min	52.316 min 52.294 min
39				38.851 min		55.234 min	
40	54.941 min	35.731 min	35.538 min	45.464 min			40.338 min 43.452 min
41							41.834 min
42							43.017 min
43							43.017 min
44	45.985 min	57.564 min					43.943 min
45	40.864 min	36.771 min	36.241 min	44.555 min			44.446 min

46			57.621 min		49.836 min
47					50.390 min
48					50.390 min
49					50.630 min
50					50.973 min
51	52.300 min	52.295 min	52.288 min		52.316 min
52					40.531 min
53	37.600 min	35.393 min	44.762 min	36.102 min	
54		39.497 min	41.910 min		
55		39.497 min			
56	39.955 min				
57					
58					
59	50.088 min	44.355 min	35.532 min		48.424 min
60					
61	49.808 min				
62					
63					
64	47.562 min			37.600 min	
65		48.676 min			
66	57.861 min	49.796 min	42.539 min		
67					
68		40.874 min			
69	38.560 min	45.098 min	36.966 min	45.978 min	
70		40.874 min			
71					
72			35.532 min	40.869 min	41.383 min

73					
74		36.771 min	37.904 min		
75					
76					
77					
78	37.954 min		36.538 min		
79					
80					
81	39.721 min				44.664 min
82					
83		37.439 min			
84		52.300 min		52.288 min	
85					
86	36.766 min	49.236 min	40.984 min	58.821 min	45.870 min 51.322 min
87					
88					
89		36.771 min			
90					
91	36.766 min	49.322 min	36.372 min	50.939 min	
92		42.149 min	38.058 min	43.744 min	
93		37.834 min			
94		39.497 min			
95		38.811 min	36.755 min		
96					
97		48.876 min	42.539 min		
98				38.308 min	
99					
100					
101			40.596 min		
102					

103	37.034 min	37.439 min	36.241 min	35.519 min
104				
105	47.562 min		52.112 min	
106				
107				
108				
109				
110	53.718 min			
111				
112				
113		37.834 min		
114				
115				
116				
117				
118				
119				
120				
121				
122	46.671 min		44.922 min	
123			49.546 min	
124				
125				
126				
127				
128				
129				
130				48.019 min
131	38.400 min	48.876 min		
132		45.996 min		
133			38.630 min	

APPENDIX H

Distribution Index – Martens Farm – Remaining

	Chicken	Dex and Hol	DUF	Dex Fresh	LMG	Slab 1	Slab 2	Slab 3
1	35.742 min	35.211 min	41.916 min	40.704 min	43.041 min	40.380 min	40.078 min	40.498 min
2		43.121 min	43.116 min	43.110 min	41.132 min			
3		46.013 min	45.991 min	45.991 min		46.009 min		45.996 min
4			45.991 min					
5								
6		47.316 min	47.300 min	47.294 min	47.310 min			
7	40.240 min	46.316 min	48.877 min		41.920 min	40.208 min	43.565 min	43.213 min
8			48.877 min		58.787 min			
9			50.055 min				50.058 min	
10			50.055 min				50.063 min	
11			50.055 min				50.058 min	50.054 min
12			50.963 min					
13		49.339 min	55.170 min	40.990 min		47.301 min		
14	38.040 min				53.228 min	40.380 min		45.070 min
15	36.497 min	40.903 min		45.105 min	40.223 min	45.215 min	43.588 min	42.955 min
16		45.767 min		45.745 min	45.761 min			
17					44.229 min			
18	38.897 min	43.464 min		48.140 min	41.646 min	40.397 min	45.211 min	41.572 min

19	40.840 min	50.631 min		58.787 min	43.386 min	43.776 min	42.395 min
20			41.390 min		42.220 min	43.445 min	40.521 min
21	45.058 min		43.796 min	44.229 min		43.685 min	
22							
23	38.806 min	42.612 min	49.054 min	44.378 min	43.369 min	43.696 min	43.167 min
24		51.357 min	53.129 min	51.351 min			
25							
26							
27							
28		55.186 min	55.175 min	55.197 min			
29							
30				57.592 min			
31							
32				55.197 min			
33		59.199 min		59.187 min	40.128 min		
34							
35				66.308 min			
36		39.389 min	40.990 min	49.265 min	49.839 min		
37				44.132 min		41.393 min	42.955 min
38		52.323 min					
39							
40			43.453 min		40.191 min		40.223 min
41							41.812 min
42							
43							
44		43.938 min		43.961 min			
45					43.432 min	46.640 min	41.589 min
46		59.536 min				43.685 min	

47								
48								
49						51.982 min	46.114 min	
50						53.991 min		
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72			40.075 min
73			40.726 min
74			40.761 min
75			41.429 min
76			41.812 min
77			43.390 min
78		42.573 min	43.390 min
79			47.316 min
80			49.808 min
81	38.566 min	42.184 min	52.077 min
82		42.727 min	
83		51.758 min	
84		52.301 min	
85		57.004 min	
86	39.720 min		
87			
88			
89			
90			
91			
92	46.973 min		
93			
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95	37.846 min		
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39.932
min

35.211
min
38.566
min

38.040
min

BIBLIOGRAPHY

1. Polaco, O.J., et al. 2001. The American mastodon *Mammut americanum* in Mexico. Pp, 237-242, in: *La Terra degli Elefanti* (G. Cavarretta, P. Gioia, M. Mussi, and M.R. Palombo, eds.). Consiglio Nazionale delle Ricerche, Rome.
2. "About Mammoths." San Jose Mammoth Page, www.ucmp.berkeley.edu/mammal/mammoth/about_mammoths.html.
3. Hodges, Glenn. "Stone Knife and Mastodon Bones Point to Earlier Arrival of First Americans." *National Geographic*, National Geographic Society, 13 May 2016, news.nationalgeographic.com/2016/05/160513-first-americans-clovis-mastodon-florida-page-ladson/.<http://advances.sciencemag.org/content/2/5/e1600375.full>
4. *S. David Webb (ed.), First Floridians and Last Mastodons: The Page-Ladson Site in the Aucilla River*, 403-435 ©2006 Springer
5. "Reconstructing Human History with the Help of Fecal Sterols." *ScienceDaily*, ScienceDaily, 10 Oct. 2018, www.sciencedaily.com/releases/2018/10/181010105539.htm.
6. Fracácio, Renata, et al. "Fecal Sterols in Estuarine Sediments as Markers of Sewage Contamination in the Cubatão Area, São Paulo, Brazil." *SpringerLink*, Springer, 4 Apr. 2012, link.springer.com/article/10.1007/s10498-012-9167-2.
7. Nordin, N., and M. M. Ali. "Distribution of Fecal Sterols in Surface Sediment of Sungai Tebrau, Johor." *Stability of Plane Couette Flow of Carreau Fluids Past a Deformable Solid at Arbitrary Reynolds Numbers: Physics of Fluids: Vol 30, No 7*, AIP Publishing LLC, 27 Nov. 2013, aip.scitation.org/doi/abs/10.1063/1.4858698.
8. *S. David Webb (ed.), First Floridians and Last Mastodons: The Page-Ladson Site in the Aucilla River*, 493-515 ©2006 Springer
9. *S. David Webb (ed.), First Floridians and Last Mastodons: The Page-Ladson Site in the Aucilla River*, 215-246 ©2006 Springer
10. Martin P. S. (1967). "Prehistoric overkill". In Martin, P.S.; Wright, H.E. *Pleistocene extinctions: The search for a cause*. New Haven: Yale Univ. Press.
11. Gorman, James (13 May 2016). "Humans and Mastodons Coexisted in Florida, New

Evidence Shows". The New York Times.

12. Sadzikowski, M R et al. "Cholesterol-reducing bacterium from human feces." *Applied and environmental microbiology* vol. 34,4 (1977): 355-62.
13. Harrault L, Milek K, Jarde´ E, Jeanneau L, Derrien M, Anderson DG (2019) Faecal biomarkers can distinguish specific mammalian species in modern and past environments. *PLoS ONE* 14(2)
14. Plotka, E. D.; Seal, U. S.; Zarembka, F. R.; Simmons, L. G.; Teare, A.; Phillips, L. G.; Hinshaw, K. C.; Wood, D. G. *Biology of Reproduction* 1988, 38 (2), 309–314
15. Heilbron, I. M.; Kennedy, T.; Spring, F. S.; Swain, G. *Journal of the Chemical Society (Resumed)* 1938, 869.
16. Britannica, T. E. of E. Ergosterol. <https://www.britannica.com/science/ergosterol> (accessed Apr 11, 2019).
17. Why Mastodons Matter. <https://mastodonvalleyfarm.com/why-mastodons-matter/> (accessed Apr 11, 2019).
18. McDonald, J. N. (1981). *North American bison: their classification and evolution*. Berkeley: University of California Press.