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COMPARATIVE SUBTIDAL AND SUPRATIDAL TAPHOMONIC CHANGES IN THE ELEMENTAL COMPONENTS OF MARINE VERTEBRATE BONES USING ENERGY DISPERSIVE X-RAY SPECTROSCOPY AND PRINCIPLE COMPONENT ANALYSIS: ECOLOGICAL AND FORENSIC APPLICATIONS

A THESIS SUBMITTED TO THE GRADUATE FACULTY

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By

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COMPARATIVE SUBTIDAL AND SUPRATIDAL TAPHOMONIC CHANGES IN THE ELEMENTAL COMPONENTS OF MARINE VERTEBRATE BONES USING ENERGY DISPERSIVE X-RAY SPECTROSCOPY AND PRINCIPLE COMPONENT ANALYSIS: ECOLOGICAL AND FORENSIC APPLICATION

A THESIS

APPROVED FOR THE DEPARTMENT OF FORENSIC SCIENCE

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THESIS ABSTRACT

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NAME: Caitlyn Briana McElreath

TITLE OF THESIS: Comparative Subtidal and Supratidal Taphonomic Changes in the Elemental Components of Marine Vertebrate Bones using Energy Dispersive X-Ray Spectroscopy and Principle Component Analysis: Ecological and Forensic Applications

DIRECTOR OF THESIS: Wayne Lord, Ph.D.

PAGES: 86

ABSTRACT. Individuals and agencies of multiple disciplines have evolved interests which result in the studies and research of marine mammals as well as other protected marine dwellers such as sea turtles. Aside from wildlife conservation, an exponential increase in interest to these protected animals has been the side effect of trade for profit to fund activities targeted at human populations as acts of terror. Experts believe that wildlife trade and trafficking is being used to finance terrorist and criminal activities (Wyler & Sheikh, 2013). This study focuses on observations made by analysts while examining remains of protected animals that may be involved in trade or trafficking on the black market such as marine vertebrates. According to U.S. Fish and Wildlife Forensic officials, recent observations have found that marine organisms like the sea turtle and harbor porpoise display signs of advanced taphonomy and even premature fossilization following decomposition. Because of this observed advanced decay, analysts find it difficult to effectively and efficiently age marine bone specimens. In an attempt to develop a less costly and time-consuming method for analyzing marine bone specimens,

this study was created in which bone samples were observed over the course of a year. In this study, skeletal remains from submerged marine vertebrates including a porpoise, seal, sea turtle, and a bovine cow control were sampled to determine a plausible explanation for these observations. The specimens were necropsied prior to submersion, and the bones of each vertebrate were segregated with respect to species. Periodic samplings took place over the course of one year. Using the Scanning Electron Microscope (SEM), the bone specimens were examined for topographic changes and analyzed for elemental composition. Among the different elemental aspects of bone composition, a list of elements was compiled and monitored for change throughout the duration of 12 months.

Keywords: wildlife forensics, forensic science, taphonomy, anthropology

THESIS INTRODUCTION

A growing interest in wildlife has led to high societal placement of certain wildlife specimens which have been established as having high worth. Particularly rare wildlife species are greatly valued for a multitude of reasons which can include uniqueness, visual appeal, traditional remedies, and cultural or historical significance. Endangered and protected animals are considered highly valuable primarily due to their rarity and ecological uniqueness. More specifically, marine mammals and other protected marine vertebrates are often unlawfully obtained for trade reasons as mentioned above. All marine mammals are protected under the Marine Mammal Protection Act of 1972 which essentially attempts to maintain populations and to ensure the ecological integrity of marine mammals in the environment (Roman *et al.,* 2013). Other endangered or threatened marine species, such as sea turtles, are further protected by the Endangered Species Act (Act, 1973). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) determines endangerment classification and places animals in protection as necessary. Although these laws are designed to ensure the protection of these species, many individuals and groups specifically target these protected animals due to their high trade value. Following narcotics, experts consider the wildlife trade as among one of the world's largest illegitimate businesses, bringing in between $5 billion and $20 billion US dollars annually (Rosen & Smith, 2010). Because the trade market largely involves protected marine vertebrates to fund other possible criminal and terrorist activity, wildlife investigators and agencies have placed a large interest in the analysis of confiscated evidence containing wildlife parts.

As the trade of these wildlife items surges and enforcement increases, new investigative and analytical issues become more apparent. Monitoring authorities have confiscated illegal items harvested from marine vertebrate animals that appear to be taphonomically advanced such as bone, ivory, or teeth and tusks. This poses a problem during analysis because examiners are unable to determine the age of the specimens. By knowing the age of the specimen, examiners have the capacity to determine approximate time since death which serves as an aid while investigating wildlife crimes. Determining the age of the evidentiary bone specimens is critical in wildlife crime investigations because it is a determining factor on whether individuals have the authority to possess the bones or not. For example, prior to the Marine Mammal Protection Act of 1972, individuals had the authority to possess whale ivory despite the method it was obtained. After the 1972 act was established, individuals possessing whale ivory were to have legal permitting that was issued by federal and state wildlife agencies that granted possession of the specimen material. Permitting is granted as long as the specimen has been lawfully obtained and the individual has established good reason for having possession of the specimen such as for educational or research purposes. With this being said, individuals could theoretically claim that the specimens in their possession were obtained and are aged prior to the 1972 regulation. Investigators face problems with this claim because aging the bone specimens has proved to be difficult due to degenerate diagenesis.

Analysts in wildlife laboratories are able to age the bones to a certain extent; however, current methods being used require the use of expensive instruments and reagents and are time extensive. Furthermore, limited studies are being conducted on the early stages of diagenesis in marine vertebrate specimens so little to no baseline data

exists in the scientific community. In an attempt to find an alternative method that would be more cost and time effective, this study was created to develop a methodology for processing and analyzing marine bone specimens that are commonly observed in wildlife forensic cases.

This study encompassed research in which bone specimens were submerged in a marine or subtidal environment. A terrestrial, also referred to as supratidal, aspect was included as an environmental control in which the bone specimens were placed on land. For both maritime and terrestrial studies, skeletal remains were taken from representative marine vertebrate groups which include a harbor porpoise (*Phocoena phocoena*), two Kemp's Ridley sea turtles (*Lepidochelys kempi*), and three (different) species of seals: harbor seal (*Phoca vitulina*), grey seal (*Halichoerus grypus*), and harp seal (*Pagophilus groenlandicus*). A terrestrial animal control specimen of a domestic cow (*Bos Taurus)* was included in both the subtidal and supratidal environments. These specific specimens were selected for two major reasons: 1) Forensically, these specimens are commonly encountered as victims of wildlife forensic crimes, and 2) Ecologically, each specimen represents unique ecological niches which allows them to represent a large variety of marine vertebrates. It can also be noted that these specimens were already available due to natural demise, and no actions were taken to kill any specimens specifically for this study.

Elemental observation took place over the course of this study to monitor changes over the time of taphonomic bone decay. Comparisons were made and analyzed for significant changes or consistencies. It was hypothesized that: 1) Bone elements in subtidal environment will change differently than elements in supratidal environment and

2) Changes in bone elements will be species specific. The null hypothesis was that no significant changes would exists among the differing species and environments. A number of disciplines were utilized in this project including ecology, oceanography, taphonomy, anthropology, and applied wildlife forensic science. Lastly, a novel statistical analysis was utilized for the statistical analysis of this study which brought to light a new application for Principal Component Analysis (PCA).

Taphonomy

The broad study of how organisms decay and the processes leading up to fossilization is referred to as taphonomy. Coined by Russian paleontologist I.A. Efremoy in 1940, taphonomy is derived from the Greek words *taphos* (burial) and *namos* (laws) (Lyman, 1994). This derivative seems appropriate according to Lyman (1994) who stated, "Taphonomy is the science of the laws of embedding or burial." Lyman (1994) continues to explain that taphonomy is the changes and transitions of a decaying organism as it moves through the biosphere and into the lithosphere. Taphonomic changes can be studied on a short-term as well as on a long-term scale. Much can be learned by studying the processes of decay of an organism and the environment the organism is exposed to during decay. The environment plays a large role in the decay process and can affect the preservation and changes of an organism. The condition of the organism at the time of discovery can also provide valuable information to an investigator. For instance, the rate of taphonomy can be examined by inspecting the articulation of the specimen. Articulation refers to the connectedness of parts or joints. Articulation is a derivative from the Latin word for joint: *articulus* (Haglund & Sorg,

2002). When the bone specimens are discovered disarticulated, this can be a sign of mature decay, scavenging, or rough weathering conditions. Disarticulation can also be indicative of possible foul play to a victim or specimen prior to mortality. Understanding how organisms disarticulate taphonomically allows analysts to interpret and infer the presence of manipulation and may allow them to conclude possible manner of death.

In forensic science, taphonomy aids in the investigation process by helping answer questions regarding the victim's condition both pre-and post-mortem. Forensic investigators can turn to taphonomic applications to analyze individuals at a scene as well as possible evidence within a crime scene to assist an ongoing investigation. Clues within a crime scene can be analyzed taphonomically in order to determine the demise of the specimens in question. Forensic taphonomy is a growing discipline in which experiments are conducted to observe and record short and long term taphonomic changes. This provides a better understanding about the decaying organism and the environment of decomposition.

In regard to experimental taphonomic research, paleontological taphonomic applications are studied and applied to forensic taphonomic experiments. Haglund and Sorg (2002) describe experimental taphonomic research as *actualistic research*. Actualistic experiments typically focus on a specific taphonomic process and involve a controlled setting with independent and observed dependent variables (Haglund & Sorg, 2002). This allows a researcher to model their experiment and examine certain variables that may or may not affect taphonomic changes. This can also aid in discovering cause and effect variables. A second actualistic approach is when a researcher recreates a process seen in the fossil record or forensic setting. This is done by creating the process

in a natural setting and observing as the experiment progresses (Haglund & Sorg, 2002). Another taphonomic research approach is the systematic process of analyzing recorded observations of decomposition as well as the variables affecting taphonomy such as environmental factors and scavenging. Haglund & Sorg (2002) suggests performing terrestrial and marine decomposition case studies on a regionalized basis to account for different climates and weathering patterns that are specific to certain locations in the world. As weather and climate conditions can vary regionally, it is important to note how these different environments affect the decomposition of the specimens. This can be important in cases in which specimens are transported from different parts of the world in regard to wildlife trade. In regional specific studies, the time since death serves as the control variable if that information is available to the researcher.

In wildlife forensics, it is important for the analyst to be familiar with common taphonomic changes in animals. This can be important in determining cause of death which can be indicative of a wildlife crime in some cases. Forensic pathologists are required to necropsy specimens that have been found equivocally deceased or if a crime is suspected. These determinations can be made with a working knowledge in taphonomy and animal pathology. With a working knowledge of taphonomy and bone decay, it was inferred that patterns in observed taphonomic changes would provide insight on how to better analyze the bone specimens as they begin the early stages of diagenesis. Taphonomical patterns also allow scientists to look at environmental influences on the decay of specimens. Certain environments may alter the way specimens decay and undergo taphonomic change.

Bone Structure

When evaluating taphonomic changes in bone specimens, it is important to understand the structure of bone and the changes it undergoes as proteins and bone material in the bone begin to break down. The bone matrix is composed three (different) types of calcified intracellular material: osteocytes, osteoblasts, and osteoclasts (Mescher, 2010). Each type of cell performs specific and necessary functions to maintain bone structure. Osteocytes combine through individual matrices called canaliculi which are densely compacted to form osteons. Neighboring the compact boney matrix are osteoblasts which secrete type I collagen, glycoproteins, and proteoglycans which ultimately make up the osteoid layer. As mineralization occurs, this multicellular layer hardens forming the compact calcified matrix containing cavities with osteocytes (Mescher, 2010). Embedded within these cavities and matrix layers are special opportunities for inorganic elements to be incorporated. Mescher (2010), notes that about 50% of the bone matrix is composed of inorganic material such as bicarbonate, citrate, magnesium, potassium, and sodium. For this study, it is hypothesized that as the bone specimens begin to decay or breakdown, other inorganic material is incorporated in the bone matrix spaces and cavities. Elements bound within the bone matrix will either leach out of the material or bind to elements found within the exposed environment thus incorporating inorganic elements into the matrix. The last compositional bone cells are the osteoclasts which are large multinuclear cells made up from the fusion of bone marrow-derived cells. These cells are motile and can attach to different surface folds of the bone matrix to aid in the resorption and remodeling of bone tissue (Mescher, 2010). Running parallel to the long axis of bone elements are Haversian canals which are known

to be the networks that carry blood and nutrients (Pfretzschner, 2004). Connecting these Haversian canals are a series of matrices connections known as Canaliculi that transport nutrients flowing in from the Haversian canals to the different layers of osteons (Mescher, 2010).

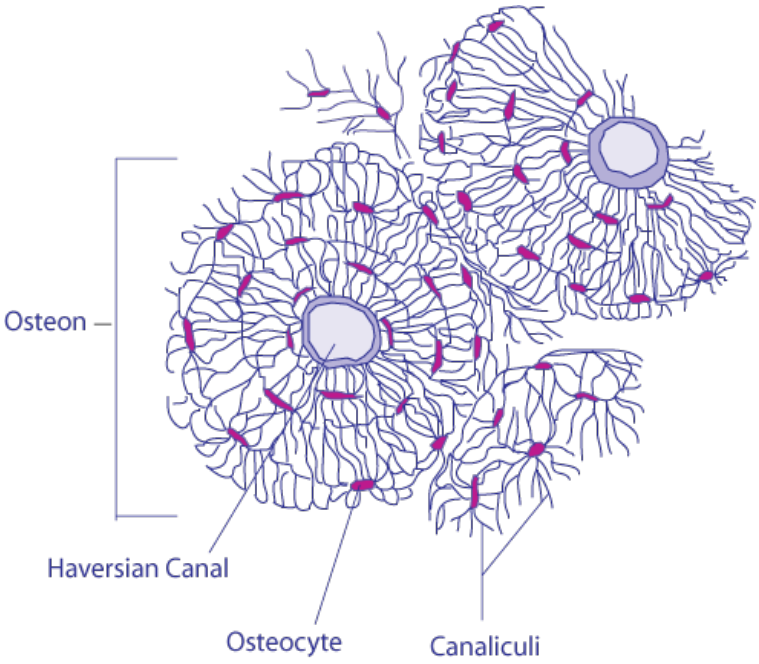


Figure 1: Illustrative diagram of histological bone structure. Source: (Gray, 1918) colored and modified by Wikimedia Commons and BDB 2006

Fossilization and Mineralization

As bones begin to decay and break down, a process referred to as diagenesis commences which is considered to be the replacement of proteins and organic materials with inorganic substances (Pfretzschner, 2004). This process of mineral diffusion is thought to occur both internally and externally. According to Pfretzschner (2004), diffusion occurs through Haversian canals at approximately 0.02 mm^2/day freely and about 11 mm^2/day in free water. As mineral diffusion allows for inorganic substitutes to be incorporated within the boney matrix as the collagen, organic elements, and other

proteins begin to decay and leave the bone material, the beginning stages of early diagenesis initiates (Pfretzschner, 2004). Although the early stages of diagenesis have not been explored in its entirety, it has been accepted that the first stage of diagenesis involves the process of decay of the organic bone material while the incorporation of inorganic minerals in the boney matrix explain the second stage of diagenesis (Pfretzschner, 2004).

The composition of bone is the driving factor on how the process of diagenesis proceeds. The elemental makeup of bone is through a series of calcium phosphate molecules, more specifically hydroxyapatite—$Ca_{10}(PO_4)_6(OH)_2$ (Trueman 1999). The vast amount of phosphate groups present in the bone mineral allow for elemental substitutions giving bone characteristically unstable variations. Explained by Trueman (1999), common chemical and structural modifications are possible with elemental substitutions which can include: Sodium (Na), Strontium (Sr), Magnesium (Mg), Rare Earth Elements (REE) substituting for Calcium (Ca), Carbonate (CO_3) and Hydrogen Phosphate (HPO) substituting for Phosphate (PO_4), and Fluorine (F^-), Chlorine (Cl^-) and CO_3^{-2} substituting for Hydroxide (OH). Trueman (1999) further explains that hydroxyapatite contains very small bone crystals with a high surface area which makes bone highly reactive. As bones decay in the environment, the recrystallization process is driven by the unstable nature and high reactivity of bone. Naturally, as the bone material moves to a more stable form, minerals from the surrounding sediment and environment are incorporated in the pore space of the bone. In regard to the sedimentary environment, the most stable form of apatite is francolite (carbonate flurapatite) which is the primary composition of fossil bone (Trueman, 1999). For stability, bone mineral recrystallizes to

francolite and in the process elements present in the environment are incorporated into the bone material. These elements typically bind from the outside layer and move into the spongy bone.

This study examines the elemental aspect of the beginning stages of mineralization initiating the fossilization process. It is important to note that the bones under study are not classified as fossils because a large portion of the organic material is still present. The idea of examining organic and inorganic changes of bone specimens derived from observations of wildlife officials indicating that some cases of marine vertebrate bones are being submitted to the lab under a prematurely fossilized condition. Considering that the rate of diagenesis is unknown, analysts are having difficulty determining the age of these specimens. Limited literature is available in regard to short term fossil studies; however, ample amounts of research have been conducted on ancient preexisting fossils. Methods used to analyze ancient fossils in previous research were incorporated into this study to examine bones undergoing the fossilization or recrystallization process. In regard to ancient fossil studies, researchers commonly measure the REE concentration to observe taphonomic characteristics in trace elements (Tütkena *et al.,* 2008). Because this study involves short term sampling; however, major elements are being studied instead. In an attempt to measure the rate of taphonomy and the beginning stages of fossilization, elemental concentration measurements will be taken on the bone samples to determine if a significant difference exists between the marine vertebrate bones and terrestrial control both in the water and on land.

A similar study is currently underway with a group associated with the U.S. Fish and Wildlife Service (FWS) in Ashland, Oregon. Their study involves the analysis of

bones that have washed up on shore with premature fossilization characteristics. The target analysis in this study is the sulfur uptake in the bone matrix. FWS will also conduct elemental analysis on the bone specimens. Along with sulfur analysis, other elements will be examined over the course of the study in the bones to determine if there is a significant difference in element uptake. Information from the current study have the potential to aid in the sulfur analysis study being conducted by FWS.

The Scanning Electron Microscope (SEM)

As the bone specimen decay was observed over time, morphological and elemental changes were targeted over time. The Scanning Electron Microscope (SEM) allowed for the analysis of both observations. The SEM was utilized to create visual images of topography and morphological changes on the bone surface as well as measuring the elemental concentration of set elements over time. The SEM provided a two-dimensional scanning image which is obtained through electron emission from both primary and secondary signals (Todokoro & Ezumi, 1999). The sample is placed in a vacuum-sealed chamber in which electrons are emitted at a high acceleration rate through a primary electron beam. As electrons are emitted, the sample is penetrated, and electrons accelerate through the chamber eventually returning to specialized detectors. *Todokoro* and Ezumi (1999) describe the scanning electron microscope as producing "a scan image at high spatial resolution in a high acceleration voltage area." The Scanning Electron Microscope (SEM) also can be equipped with x-ray diffraction which can be utilized for elemental analysis. Seeck and Murphy (2015) note that the atomic distances usually fall within the range between 0.1 and 10 Å. The electromagnetic waves of x-rays fall within

that wavelength range which can be used to determine atomic potential present in a sample through a series of calculations. As x-rays are scattered or diffracted, the electromagnetic wave field yields electric or magnetic potential (Seeck & Murphy, 2015). With the use of formulas and approximations, SEM x-ray diffraction can provide researchers with elemental analysis of a sample as well as percent of elements present within a representative area of the sample.

In a recent study on bone and tooth apatite in fluvial and marine settings, researchers utilized x-ray diffraction patterns to determine the presence of increased recrystallization patterns (Tütkena, Vennemannc, & Pfretzschnerd 2008). The researchers examined the outer rim and central compacta of the bone samples and noted sediment contamination along the outer rim between pore spaces. Most of the outer rim samples contained quartz from the sediment. These studies, along with multiple others, were used as guides for SEM methodology for this study on bone analysis.

The present study used the SEM to take topographical images of the bone surface in order to compare morphological changes over time. Figure 2 shows an example image of the topographical surface of a seal bone. Different areas of the bone were examined which included, but were not limited to, the outer edges, inner spongy bone surface, and exterior bone layers. Along with comparison imagery, x-ray diffraction was utilized to determine elemental composition. Through a series of statistical analyses, a series of differences exists between the elements incorporated in the marine and terrestrial bones over time.

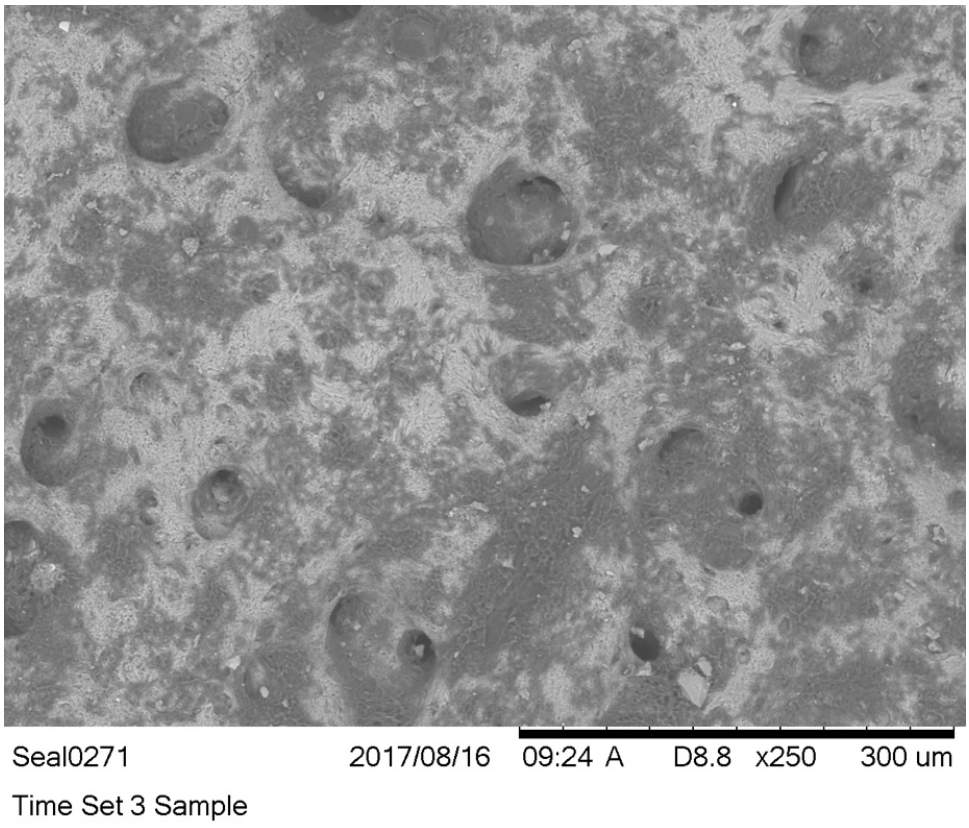


Figure 2: Scanning Electron Microscope Image of Seal Sample

THESIS METHODS

The specimens selected for this study were done so that representatives from different ecological groups were incorporated. Morphological differences are also noticeable between the specimens analyzed. Differences in diet and environment can have lasting effects on bone composition and structure. The harbor porpoise (*Phocoena phocoena*) spends its entire life submerged underwater preying on whiting, sand eels, and other common fish and shrimp (Santos *et al.,* 2004). Kemp's Ridley sea turtles (*Lepidochelys kempi*) are known to spend the primary portion of their lives in marine waters with the exception of birth and females coming to land to nest and lay their eggs. In a dietary study of Kemp's Ridleys, Burke *et al.* (1994) found that crabs serve as the main dietary component in North Atlantic waters. It should also be noted that the density of the sea turtle bones in this study had a cartilage-like consistency—much different from that of the seal or cow bones. This could be reflective of the lifestyle and environment of the sea turtles compared to that of the seal and cow bones. Generally speaking, the seals and cows spend a large portion of their lives on land carrying weighted muscle mass that would otherwise be somewhat alleviated in the water. Thicker and more dense bones are necessary to fit the lifestyle of these terrestrial dwelling organism. The seal representatives prey on squid and a variety of fish such as herring and flounder (Bowen & Harrison, 1996). These specimen representatives divide portions of their life in the water as well as on land. The final specimen group is the terrestrial control, the domestic cow, which serves to represent an ecological group that spends its entire life on land. Domestic cows are typically vegetative eaters and can consume a variety of grasses and vegetables in their primary terrestrial environment (Hanley & Hanley, 1982). The

different lifestyles and diets of these specimens are reflective in bone composition and structure. The make-up of the bones may affect mineralization once diagenesis begins.

Each specimen used in this study was examined and necropsied under the supervision and direction of Inga Sidor, DVM. Recordings of the necropsy examination included observations of body condition, external and internal structures, and abnormalities. After necropsy, the specimen bones were disarticulated and segregated in regard to species (Fig. 3-5). The segregated bones were placed in mesh dive bags that were marked with colored tags that were indicative to respective specimen group. Lobster traps were modified to house the bone specimens to contain and protect the bagged specimens from scavengers and environmental factors.

Figure 3: Disarticulated Phocoena phocoena Bones

Figure 4: Disarticulated Lepidochelys kempi Bones

Figure 5: Disarticulated Phoca vitulina, Halichoerus grypus, and Pagophilus groenlandicus

After the skeletal remains were secured in the modified lobster traps, they were placed in their proposed environments. The maritime traps were placed just off the coast of Appledore Island in Maine. The location of this study was determined due to the collaborative efforts and scientific support of the Shoals Marine Laboratory which is located on Appledore Island. This specific location is important because it is considered a marine sanctuary which eliminates commercial and outside interruptions to marine field studies. The secured bones were submerged in an island cove approximately 3-9 meters underwater for protection against storms and strong current waves. This depth placement was also appropriate because it allowed the traps to remain submerged entirely regardless of the rise and fall of the ocean tide. The terrestrial traps were placed approximately 200 meters inland[1]. Both specimen groups were in actualistic environments in which they were exposed to weathering, scavenging, and other factors involved with natural bone petrification.

Over the course of 12 months, samples were collected from the dive bags in both marine and terrestrial environments. The bones specimens were placed in heat sealed bags labeled with their respective vertebrate group and shipped to the University of Central Oklahoma (UCO) for analysis.

Upon arrival at UCO, the time zero samples were placed with a dermestid beetle colony (*Dermestidae*) on campus for approximately two weeks for tissue removal. For all bone analysis preparation, representative sections of the bones were taken measuring approximately 10.0 x 10.0 mm in maximum dimension and rinsed using deionized water. The representative sections were then dried using compressed air and paced on a SEM

stub secured by adhesive carbon tape. Sections were placed in labeled stub holding containers and stored in a secure location for analysis.

Scanning Electron Microscope (SEM) Analysis

The instrument used for analysis was a Hitachi TM3000. Images from the SEM were obtained using the TM3000 software on a Microsoft computer. Each sample was analyzed by being placed in the vacuum chamber which was then evacuated prior to imaging. Appropriate imaging distance was noted to be between 8.0-9.0 mm for maximum image quality and EDS analysis. Viewing was captured at 250x magnification in 3 different representative areas of the bone. The images obtained were saved and captured in the Quantax 70 viewing frame for EDS analysis. Settings for the SEM and EDS analysis were determined based on the recommendations of the technical support team that represented the company of the instrument.

Energy-Dispersive X-ray Spectroscopy (EDS) Analysis

While simultaneously using the TM3000 viewer, the specimen image was opened in Quantax 70 for scanning. The EDS was run for 140 seconds for each sample for supreme quality. For quantifying options, the program provides a color map which shows colored areas for particular elements present in the sample, a line scan in which a line can be drawn and elements within the line path are quantified, and a circle scan in which a quantification is done within a circumference. For this study, a circle scan was utilized in which a moveable circle was placed and quantified. Because it was known that the bones were composed of major elements such as: Carbon, Calcium, Oxygen, and Phosphate, the

scan was altered to screen for specific elements known to be present in the environments of the bone (Trueman, 1999). The scan was limited to: Zirconium, Iodine, Potassium, Sulfur, Aluminum, Magnesium, Zinc, Copper, Silicon, and Lead. A function known as "%Quantify" was utilized to yield a spectrum, table, and bar graph containing elemental concentration information. Results were captured and saved as PDF files.

Principal Component Analysis (PCA)

This study encompasses multiple variables and changes to track, therefore, finding an appropriate statistical method to measure significant change was initially difficult. Because multiple variables are present within this study, the decision was made to use Principal Component Analysis (PCA) as the best fit analysis for this study. In a study, similar to the chemical change analysis done in this study, Lovett *et al.* (2000) compared watershed variables (elevation, stream length, and area) and chemical variables using PCA. This gave the ability to compare ten chemical variables across 38 different streams using a correlation matrix. In doing so, scientists were able to pin point where the most change was occurring and could investigate further as to determine which chemicals were initiating the most change. Statistical analysis for this study utilized covariant formulas to calculate principle component scores that refer to how much sample data is changing. These scores are plotted through a series of graphs and tables to illustrate changes and patterns of change over time. This allows analyst to calculate predictable patterns for the specimen changes based on their calculated principle component scores and relations of the multiple variables involved.

THESIS RESULTS

Baseline Elemental Composition of Skeletal Tissue of Vertebrate Specimens

Each specimen was analyzed via the SEM for morphological comparison throughout each time series. Subsequently, the elemental composition was measured using the Energy Dispersive X-Ray Spectroscopy (EDS) component. Initially, the EDS will screen for the most concentrated elements above a factory set threshold, however, the EDS allows for selective elemental screening and threshold adjusting so that elements present in lower concentrations can be measured. Because it is known that bones are commonly composed of Calcium, Carbon, Oxygen, and Phosphorous, those elements were eliminated from the screening (Trueman, 1999). Elements found abundantly in the ocean environment that could be incorporated into the bone matrix were selected for at a threshold of 0.1 wt.% (1000ppm) (Friel, 2003). The selectively screened elements included: Zirconium (Zr), Iodine (I), Potassium (K), Magnesium (Mg), Copper (Cu), Zinc (Zn), Silicon (Si), Aluminum (Al), Lead (Pb), and Sulfur (S). These elected 10 elements were screened for in the 4 different animal categories over the course of 15 months. The baseline data shown in Table 1 provides numeric percentages regarding the elemental composition of each specimen at the beginning of the time series. The numerical data is reported to the second decimal place because the EDS program reports the concentration data as such.

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	0.51	0.87	0.04	0.00	3.11	0.77	2.94	7.13	23.21	61.40
Seal	0.67	1.49	1.56	0.16	1.53	2.75	9.48	20.53	17.04	44.79
Turtle	0.36	0.44	1.87	0.00	1.19	0.98	1.52	6.01	22.74	64.88
Porpoise	0.44	0.48	4.37	0.00	0.84	1.41	2.16	8.36	20.02	61.52
Average	0.50	0.82	1.96	0.04	1.67	1.48	4.03	10.51	20.75	58.15

Table 1: Baseline Elemental Composition Mean Percentages[2]

Because these specimens are considered *baseline* specimens, it can be noted that this is the pre-exposed data set that did not undergo any environmental exposure. In the initial time zero set, the elemental compositions remain at a fairly low amount with the exception of Zirconium which averages at about 58.1% followed by Iodine and Potassium with 20.7% and 10.5% respectively. Among the different specimens being analyzed, it can be noted that the seal bones appear to differ in concentrations of Potassium with a mean of 20.5%. This amount differs 12.2% from the next highest Potassium concentration which is 8.3% in the porpoise bones. Sulfur content in the seal bones demonstrations as a noticeable difference with 9.4% mean concentration compared to the next highest being 2.9% in the cow bones. The seal bones also differ from the other specimens in Zirconium content with a low mean concentration of only 44.7% compared to the next low being 61.3% in the cow specimen. The Zirconium content in the seal specimens differs 16.6% from the next closest concentration in the cow. The baseline data shows no measurable amount of Silicon in the turtle and porpoise bones; however, this concentration changes as time continues. From this baseline data, observations can be made on elemental changes among species as time evolves.

Comparative Time-series Analysis of Bones in Subtidal Environment

Tables 2-4 display numerical data referring to the vertebrate bones sampled from the marine or subtidal environment. The numbers represent elemental percentages as they change through time. The previously mentioned elements—Zirconium, Iodine, and Potassium—are present in slightly lower concentrations showing a decreasing trend in overall mean percentage as other elements are incorporated into the bone matrix over time. The overall high concentration of Zirconium shows a decrease in mean percentage as time proceeds from a mean of 58.1% to 49.3%. Comparatively, the Silicon concentration increases from an overall mean of 0.041% at time zero up to 9.67% at the end of the study in the time set 4. Although the seal bones showed differences in elemental concentrations in the baseline data, the proceeding tables provide information showing mean concentrations being consistent with the other specimens with the exception of Silicon uptake towards the end of the study with a mean concentration at 17.3% in the final time set. Silicon is also present in a measurable amount in the turtle and porpoise bones as time advances whereas the time zero data detected no measurable Silicon in those species. The cow bones show a higher uptake in Lead compared to the other specimens collectively within the tables. The Lead uptake in the terrestrial cow specimens shows a slight decline which could indicate that the environmental influence of the cow bones in marine waters could have an effect on certain metal uptake in the bony matrix.

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	0.23	0.48	0.72	1.69	3.98	5.21	3.33	4.28	19.58	60.50
Seal	0.34	0.75	1.36	5.76	3.84	2.10	1.77	4.84	21.03	58.20
Turtle	0.39	1.01	0.96	2.86	4.33	3.25	4.43	4.01	19.71	59.04
Porpoise	0.12	0.55	2.10	4.14	6.01	6.61	1.78	4.33	16.41	57.94
Average	0.27	0.70	1.29	3.61	4.54	4.29	2.83	4.37	19.18	58.92

Table 2: Mean Elemental Composition Subtidal Post 62 Days

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	0.22	0.52	1.73	6.06	3.41	8.56	3.22	5.93	17.20	53.15
Seal	0.12	0.31	0.78	2.86	2.81	3.17	1.01	5.00	22.23	61.72
Turtle	0.27	0.47	0.71	3.47	2.96	1.71	2.48	4.79	22.91	60.24
Porpoise	0.17	0.47	1.73	3.90	4.28	11.46	1.78	5.30	16.73	54.19
Average	0.20	0.44	1.24	4.07	3.36	6.23	2.12	5.26	19.77	57.32

Table 3: Mean Elemental Composition Subtidal Post 335 Days

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	0.27	0.28	1.40	6.25	3.76	12.28	7.38	6.78	15.93	45.66
Seal	0.44	0.12	4.57	17.38	3.35	6.47	3.98	6.13	14.30	43.26
Turtle	0.47	0.24	2.55	9.90	2.64	2.74	3.34	5.83	20.97	51.31
Porpoise	0.14	0.58	1.90	5.19	3.56	5.84	1.92	5.16	18.69	57.02
Average	0.33	0.31	2.60	9.68	3.33	6.83	4.16	5.97	17.47	49.31

Table 4: Mean Elemental Composition Subtidal Post 427 Days

Comparative Time-series Analysis of Bones in Supratidal Environment

Compared to tables 2-4, the below tables (5-7) contain the same axis information except the numerical data corresponds to vertebrate bone specimens in the terrestrial or supratidal environment. The Zirconium trend in the terrestrial data remains constant in the 335 and 427-day collection; however, low mean concentrations in time set of 62 days for the cow and turtle specimens contribute to a lower overall mean concentration of 51.7%. The Iodine concentration remains fairly constant as time proceeds as Potassium decreases by almost half the concentration in the marine vertebrates throughout the time series. A spike in sulfur concentration in at 62 days present in the cow and turtle bones, but a large decrease in all specimens follow in 335 and 427 days. Differing fluctuations in metal (Cu, Zn, Al) concentrations are present, but no noticeable differences arise in the terrestrial or marine datasets. The Lead concentrations in the terrestrial cow bones do opposite as the marine data set. Instead of increasing in mean Lead concentration, the terrestrial cow bones display a decline in mean Lead concentration starting at 5.6% in time set art 62 days and decreasing to 0.55% in the final time set. The Silicon concentrations in the turtle and porpoise specimens remain fairly low with the exception of the time set 62 days turtle with a mean concentration of 4.9%.

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	2.54	1.16	0.96	1.07	3.27	5.67	12.62	13.11	16.84	42.76
Seal	0.51	0.56	1.58	0.08	1.57	1.60	4.79	10.62	19.02	59.68
Turtle	2.48	1.82	5.75	4.94	2.61	2.19	12.46	11.88	15.35	40.50
Porpoise	0.15	1.70	1.38	0.00	1.68	2.34	2.54	7.49	18.47	64.25
Average	1.42	1.31	2.42	1.52	2.28	2.95	8.10	10.78	17.42	51.80

Table 5: Mean Elemental Composition Supratidal Post 62 Days

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	1.33	2.56	0.53	0.68	2.51	1.78	4.69	11.73	20.71	53.48
Seal	0.62	0.83	0.95	0.17	1.37	0.14	0.86	4.43	22.10	68.53
Turtle	0.90	1.19	1.53	0.00	4.59	1.05	1.77	4.81	19.49	64.66
Porpoise	0.31	1.14	1.33	0.52	1.53	0.83	1.45	5.19	22.64	65.04
Average	0.79	1.43	1.09	0.34	2.50	0.95	2.19	6.54	21.24	62.93

Table 6: Mean Elemental Composition Supratidal Post 335 Days

	Cu	Zn	Al	Si	Mg	Pb	S	K	I	Zr
Cow	0.07	2.27	0.32	0.18	1.97	0.56	3.14	11.41	28.70	51.36
Seal	0.18	0.51	1.06	0.49	1.63	0.31	0.69	4.65	21.91	68.56
Turtle	0.45	1.25	0.61	0.22	4.62	1.52	2.25	5.97	19.70	63.42
Porpoise	0.26	0.76	1.08	0.20	1.92	1.38	1.03	5.00	22.67	65.70
Average	0.24	1.20	0.76	0.27	2.53	0.94	1.78	6.76	23.24	62.26

Table 7: Mean Elemental Composition Supratidal Post 427 Days

Novel Statistical Analysis of Patterns and Trends in Bone Taphonomy

Principal Component Analysis was utilized in a novel manner regarding the taphonomic comparison of subtidal versus supratidal environments. The percentage data is plotted using a box plot to illustrate the variability in the mean percentages of the elements. Figure 6 contains a box plot of the cow bones analyzed. Differences, fluctuations, and outlying data can be visualized using this type of graph. For example, the terrestrial cow Zirconium concentration showed a mean percentage fluctuation of a 42.7% low up to a 53.4% high. The green box plot in the Zirconium column corresponds to those low and high percentage figures through a box and whisker design. The black band in the middle of the box represents the 50^{th} percentile of the data. The top and bottom of the box represents the 75^{th} and 25^{th} percentile respectively. The lines protruding from the boxes are referred to as whiskers which are calculated using a formula that plots outlying numerical data. The dots plotted away from the whiskers represent numerical data that differs significantly from the main data set (R Core Team, 2017). This particular box plot for the cow specimen shows little illustration for the metal (Cu, Zn, Al) and Silicon differences. This is primarily due to graphical scaling of the low concentration measurements throughout the time series compared to the higher concentration measurements such as Zirconium and Iodine.

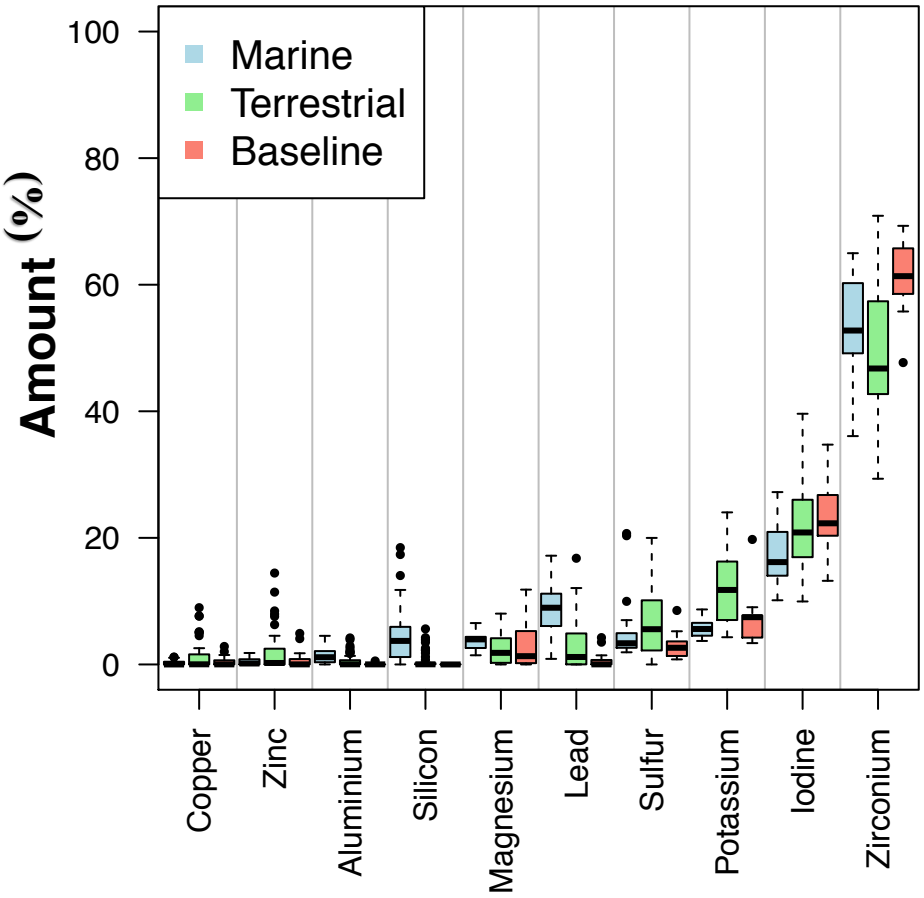


Figure 6: Box Plot of Cow Specimen Illustrating Elemental Concentration Changes and Differences

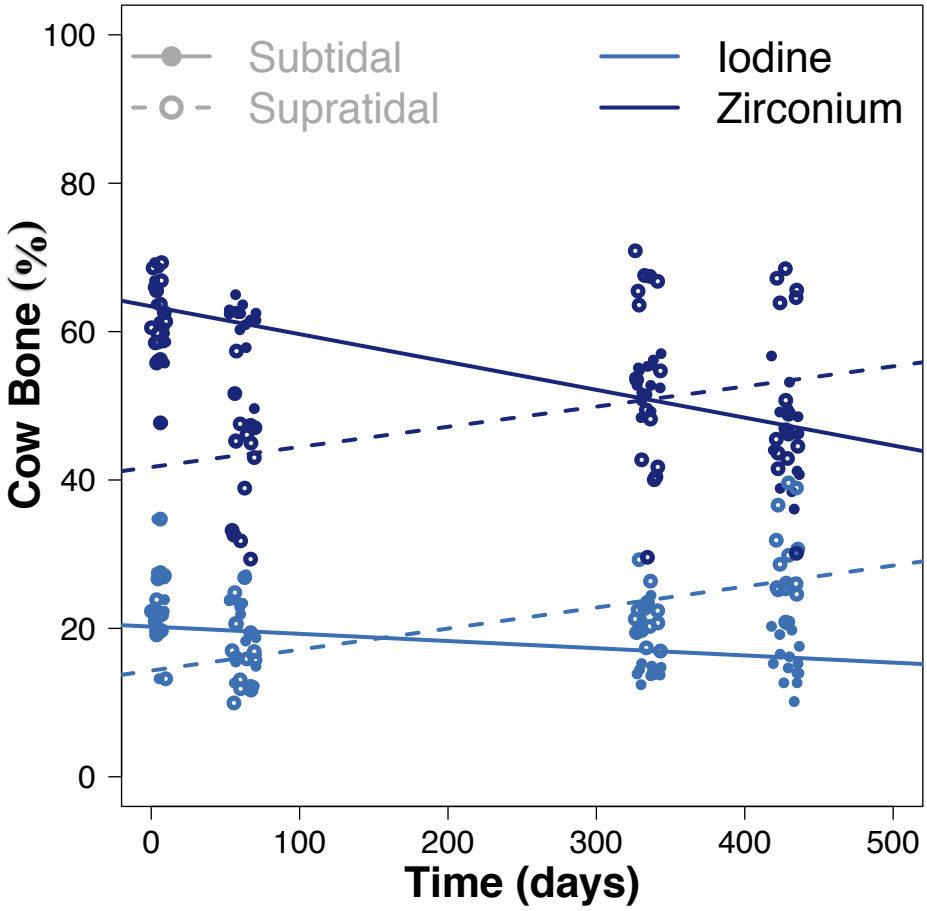


Figure 7: Iodine and Zirconium Mean Concentration Percentages in both the Subtidal and Supratidal Environments. The trend lines were created using post-exposure data. R^2 values are reported as follows: subtidal Zirconium: 0.61, subtidal Iodine: 0.11, supratidal Zirconium: 0.11, supratidal Iodine: 0.39.

The higher concentration elements were compared as time progressed. For example, Figure 7 illustrates the two most abundant elements found in the sample specimens. The mean percent concentrations are plotted over time in the cow specimen. As Zirconium seems to increase in the terrestrial environment, a decrease trend is noticed in the marine environment. Similarly, Iodine has an increasing trend line in the terrestrial samples, whereas, a decrease is noted in the marine environment samples. A plausible environmental influence could explain for the difference in elemental uptake or release in

these specimens. The terrestrial environment may include factors that allow favorability for Zirconium and Iodine uptake, whereas the marine environment may contain variables that aid in the release of these elements. The marine environment may also contain other elements that compete against Zirconium and Iodine leading to a decrease in matrix incorporation. The appendix contains similar plots for all specimens involved in this study. Interestingly to note, the seal specimen displays similar trends in the subtidal and supratidal environments as the cow specimen. As discussed before, these specimens are most similar ecologically among all involved study specimens which can explain the consistency and similarities in the changing trends.

Principal Component Analysis (PCA) combines variables through a series of formulas to create scores known as *z-scores* which are calculated per component (Quinn & Keough, 2002). The differing components incorporate multivariate analysis and can illustrate change or a possible predictable pattern for change over time. The principal component scores for the complete data set are shown in table 8. Notice some of the elements do not contain numerical data due to insignificant contribution to this type of analysis. After preliminary analysis, the complete data was refined to include 6 critical elements to be used in PCA. The critical elements used in analysis include: Iodine, Potassium, Zirconium, Silicon, Lead, and Sulfur.

	PC1	PC2	PC3	PC4	PC5	PC6
Potassium	-0.2589	-0.5402	-0.2325	0.2722	-0.6601	0.2779
Silicon	-0.1899	0.4971	0.5060	0.5049	-0.1179	0.4381
Sulfur	-0.2948	-0.1510	-0.3762	0.0632	0.6410	0.5778
Lead	-0.1308	0.4153	-0.1757	-0.7069	-0.3518	0.3950
Iodine	0.1852	-0.5111	0.6725	-0.3653	0.0973	0.3308
Zirconium	0.8710	0.0677	-0.2555	0.1839	-0.0785	0.3627

Table 8: Principal Component Loadings of Relative Amounts and Symbols for Complete Data. Magnesium, Copper, Aluminum, and Zinc were excluded from the final data analysis due to insignificant contribution to data

Below is a table (9) containing information regarding the standard deviation and proportion of the principal component means as they contribute to the entire data set. The proportion of variance numbers are a fraction of the complete data set. For example, Principal Component 1 represents 68.1% of the variability in the data. The cumulative proportion is a summation of the component scores as the principle component scores increases.

	PC1	PC2	PC3	PC4	PC5	PC6
Standard Deviation	12.509	5.8462	4.4318	3.1856	2.6494	1.4287
Proportion of Variance	0.6818	0.1489	0.0856	0.0442	0.0306	0.0089
Cumulative Proportion	0.6818	0.8307	0.9163	0.9605	0.9911	1.0000

Table 9: Standard Deviation, Proportion of Variance, and Cumulative Proportion for Principal Component Means 1-6

Each calculated component score explains the data set from different variables and different combinations of variables. Some principle components can explain more fractions of the data than others. Figure 8 illustrates the proportion of variance as how much weight the components carry regarding explaining changes or variations in the data

set. With this visual, it can be noted that Component 1 explains the largest proportion of the total data set. Because Principal Component Scores 1 and 2 explain most of the variance in this data set, they were the primary components used in the statistical analysis of this study. Principal Components 3-6 contribute very little information regarding the variance of this study, so they were not included in the final statistical composites.

Another way to graphically explain the component score data is to graph the elemental scores individually. The plots of the elemental scores illustrate change within this multivariate data set. The positive and negative numbers denote where changes deviate from the principle component medians. Figure 9 demonstrates the elemental deviation and direction throughout duration of the study. Similarities in elemental change and direction can be compared. For example, Silicon and Lead are moving negatively from PC1 and PC2 in a similar direction. This similarity can indicate a common trend such as even matrix incorporation or parallel elemental release. Figure 9 illustrates the data in Table 8 as the lines represent trends in the numerical data.

Principal component scores are plotted in an axis rotation. In an attempt to place the multidimensional data into a 2D view, the first component (PC1) becomes the first new axis which is determined from a "line-of-best-fit" from a scatterplot of component 1 data (Quinn & Keough, 2002). The axis for Component 2 runs perpendicular from component 1. Figure 10 plots the mean principal component scores of the species and exposure status. The deviations away from the PC lines show change that can be measured and compared among the different specimens. Contained in the appendix are individual plots of each specimen illustrating the elemental changes over time in both the marine and terrestrial environment.

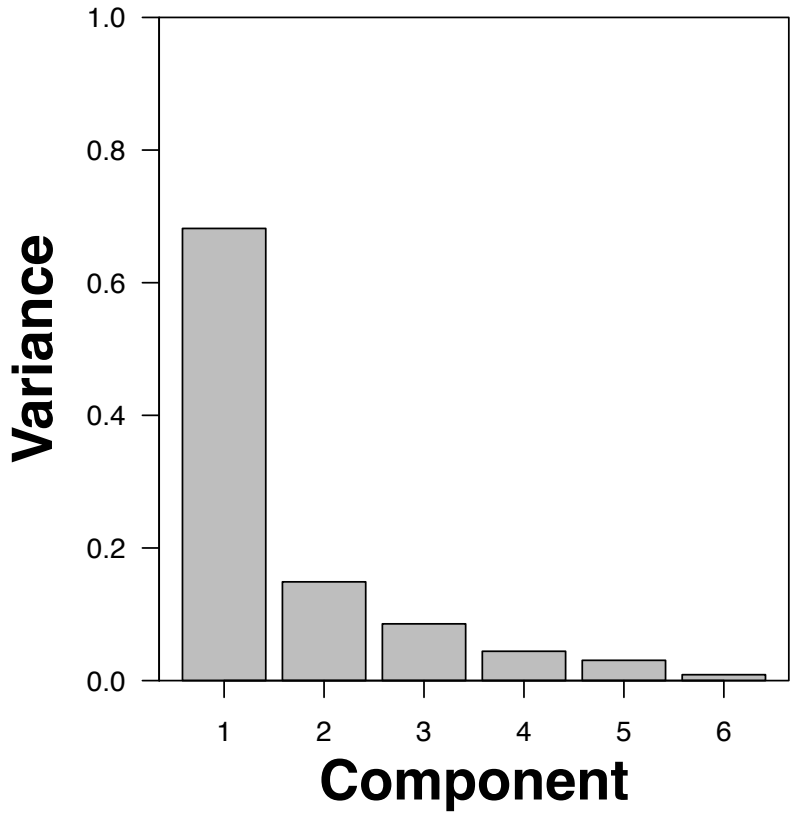


Figure 8: Proportion of Variance plotted for each Component Set. The Proportion of Variance is the amount of data represented throughout the complete data set.

Because time was a factor for this analysis, the means of the specimens for each time set were calculated and plotted against principle Components 1 and 2. Each time set was compared among the individual specimens in both the subtidal and supratidal environments to view changes in principal component scores over time. The plotted points indicate means by species and condition for certain time sets. By following the specimens, it can be visualized how the data changes over time. For example, in Figure 11, each plot line can be followed from time 2 to 3 to 4 to compare changes or patterns over time. The black solid plot referring to the cow specimen shows a counterclockwise

rotation from time set 2 to 4 moving in a positive direction towards PC1. Because a visual pattern can be depicted, predictions could be made if a fifth-time set were added. It would be expected that the cow specimen would continue to move in a positive direction towards PC1.

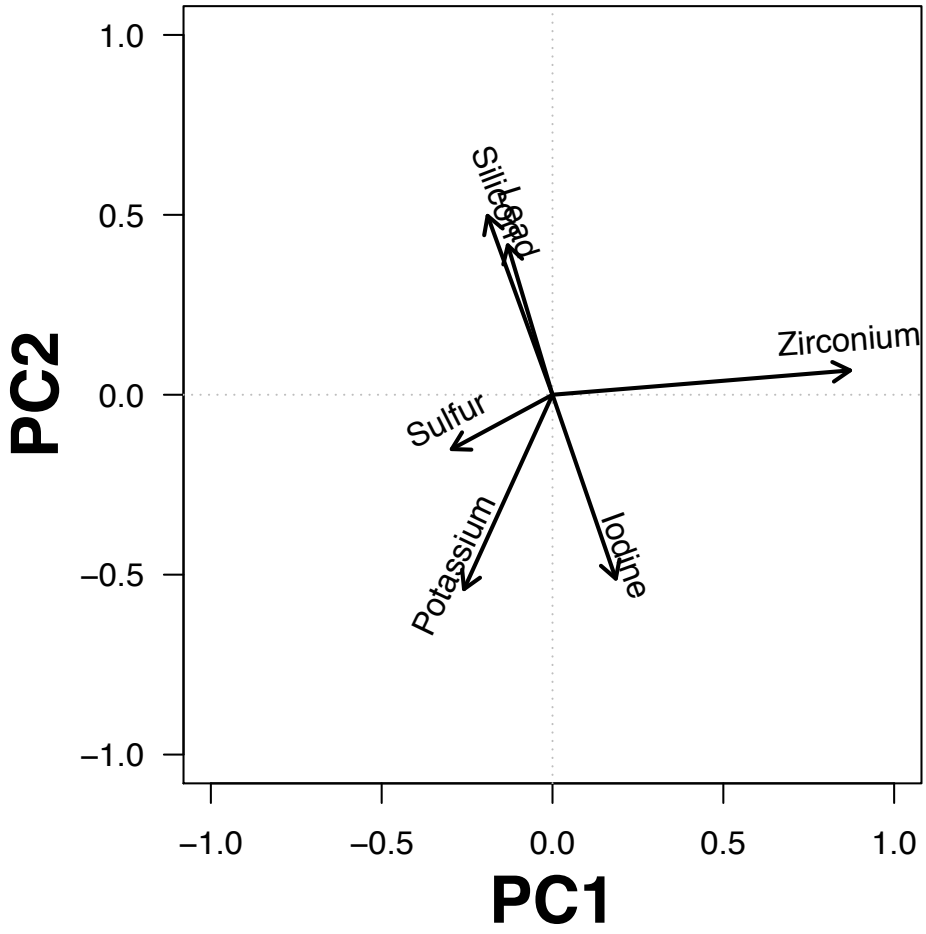


Figure 9: Principal Component Scores 1 and 2 plotted to illustrate direction of elemental movement or change

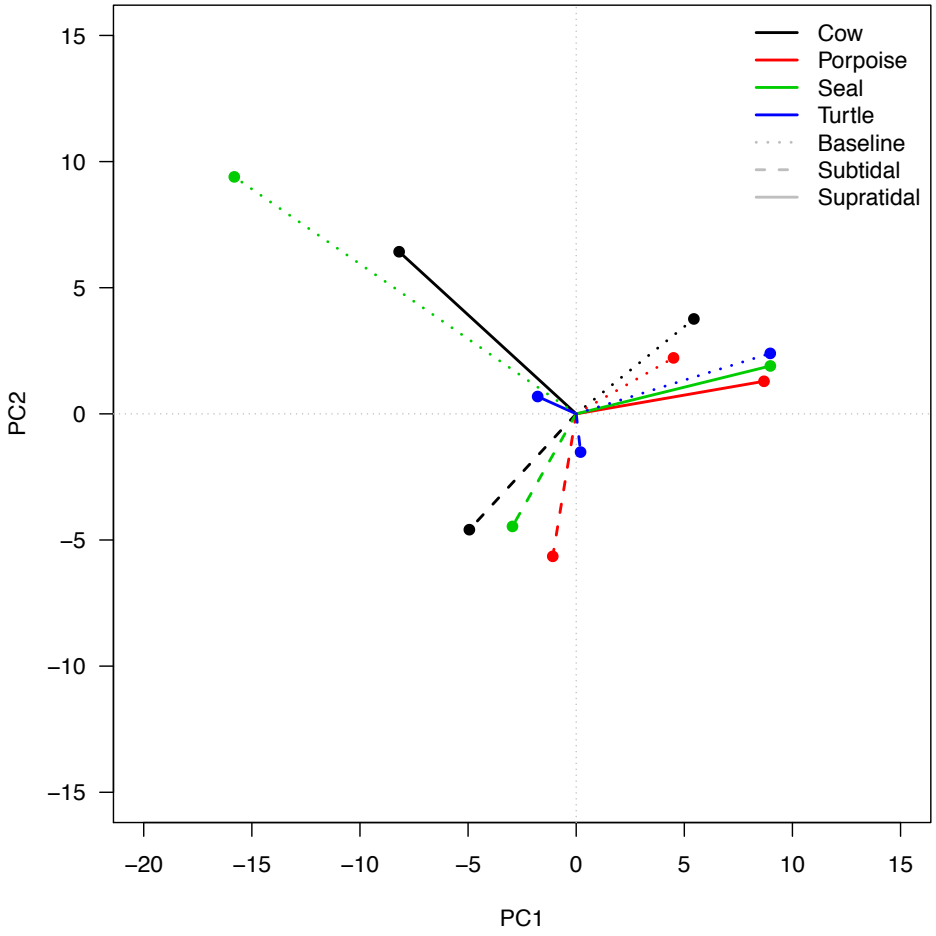


Figure 10: Principal Component Scores 1 and 2 plotted for baseline, ocean, and terrestrial data sets

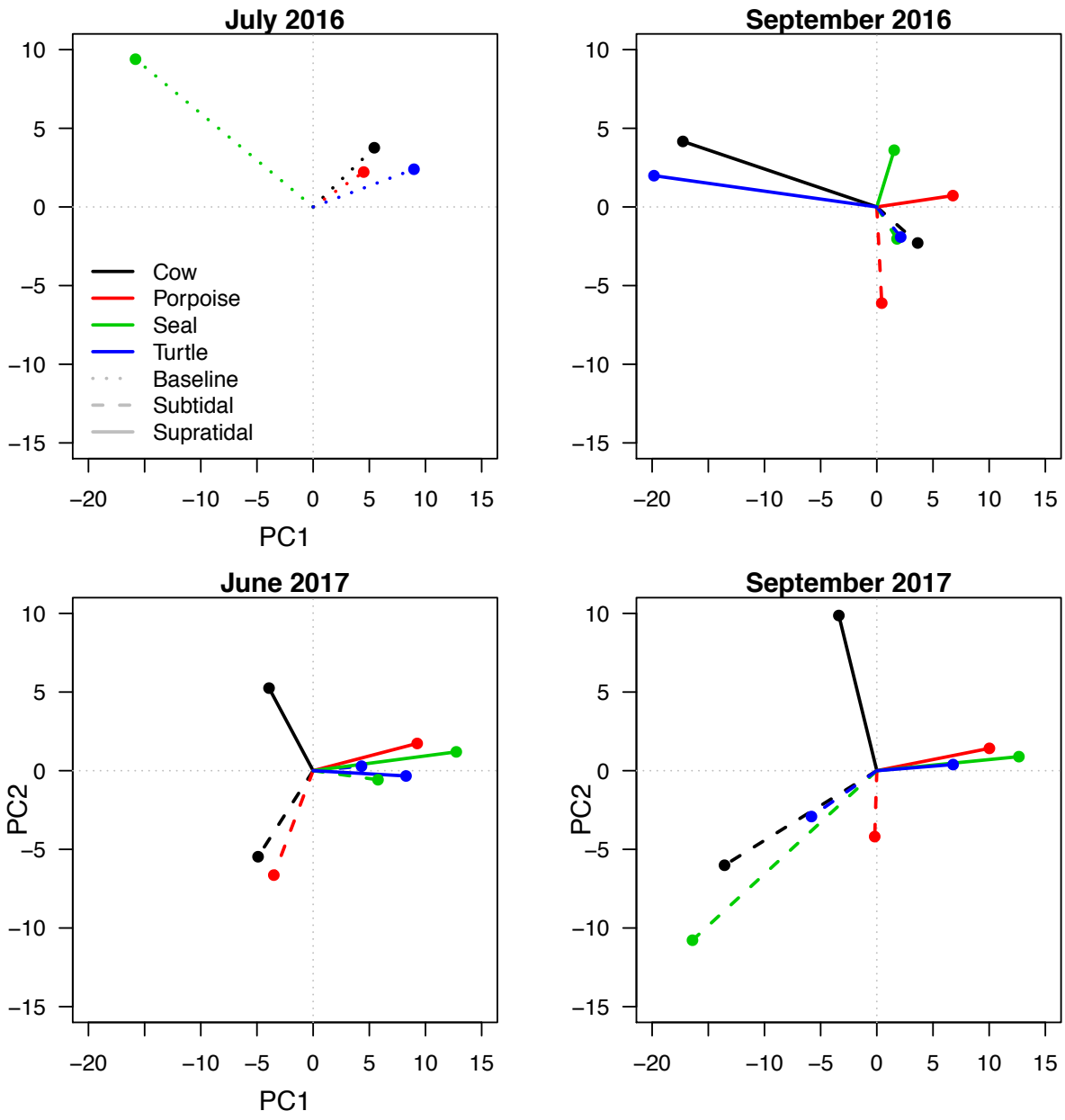


Figure 11: Plotted Principle Component Means for unexposed (July 2016), 62-days exposed (September 2016), 335-days exposed (June 2017), and 427-days exposed (September 2017)

Surface Topography

Each specimen analyzed through the SEM provided an initial surface image before undergoing EDS analysis. These documented photos show surface topography that

goes beyond this current research but is available for future studies. Below are representative examples of images taken from each representative vertebrate specimen before exposure (see Fig. 12-15). Noticeable physical differences were observed with the turtle bones in which the specimen bones had a cartilage-like, rubbery consistency. Specimens that live a large portion of their lives in the water can be expected to have less bone density due to water compensating for gravitational weight. In contrast, the cow bones were much more dense and heavier in weight. Because cows are such bigger specimens that bear large amounts of weight in the terrestrial environment, dense and thick bones can be expected to aid with structural support. The topography and physical appearance of the seal and porpoise bones were very similar with the porpoise bones being slightly less dense. In order of density and firmness of the bones, observations were as follows: turtle, porpoise, seal, and cow (with the later specimens being the densest).

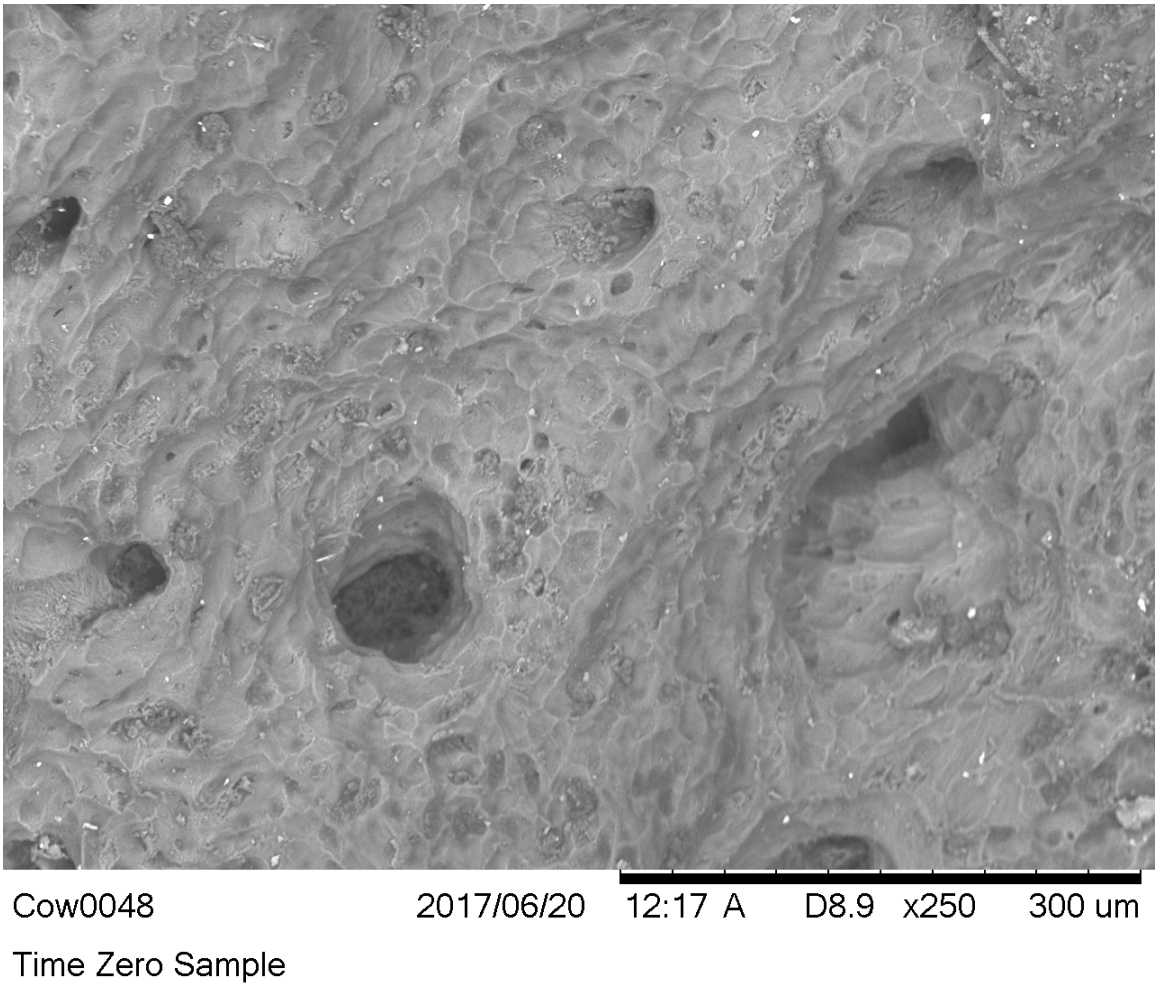


Figure 12: SEM image of topographical surface of cow bone specimen

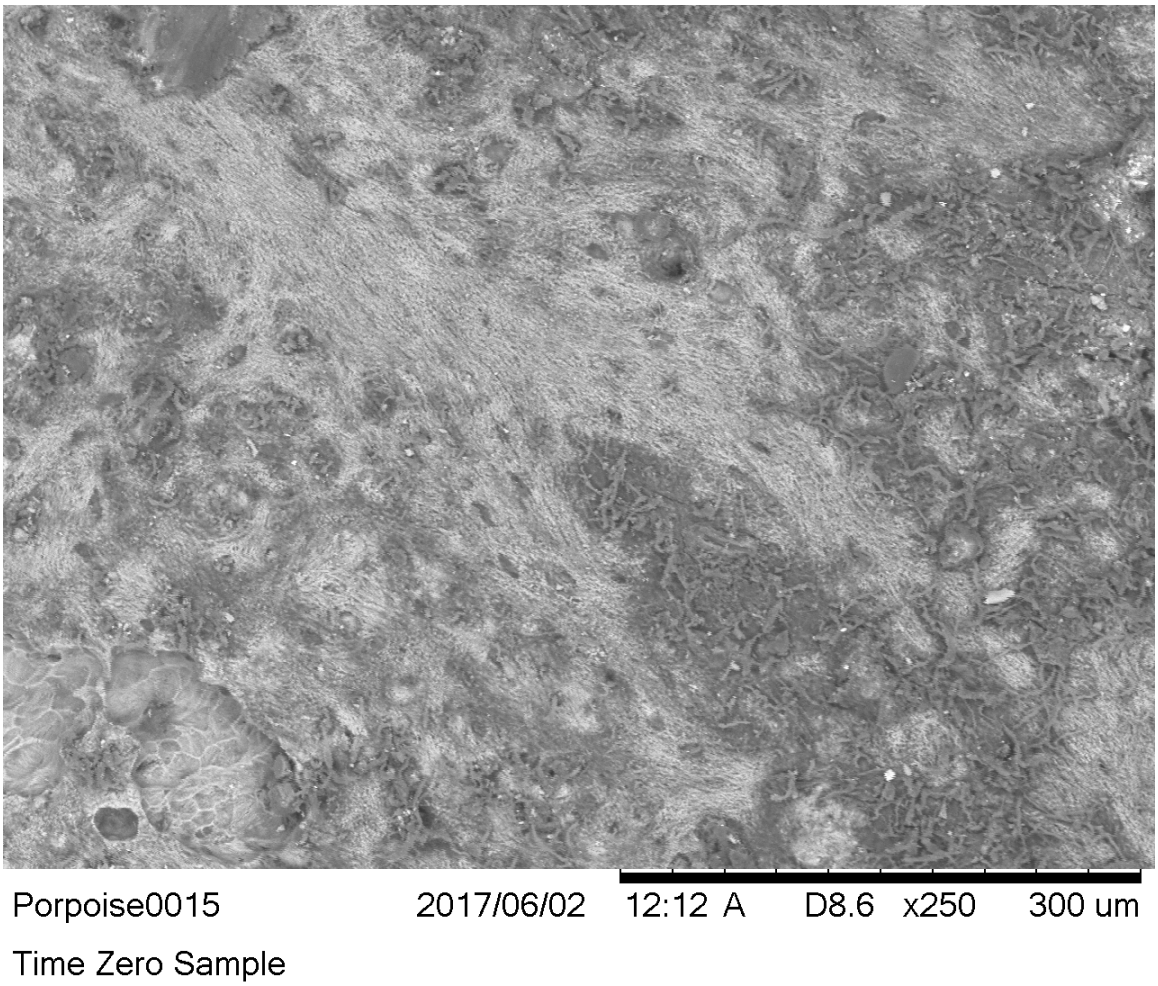


Figure 13: SEM image of topographical surface of porpoise bone specimen

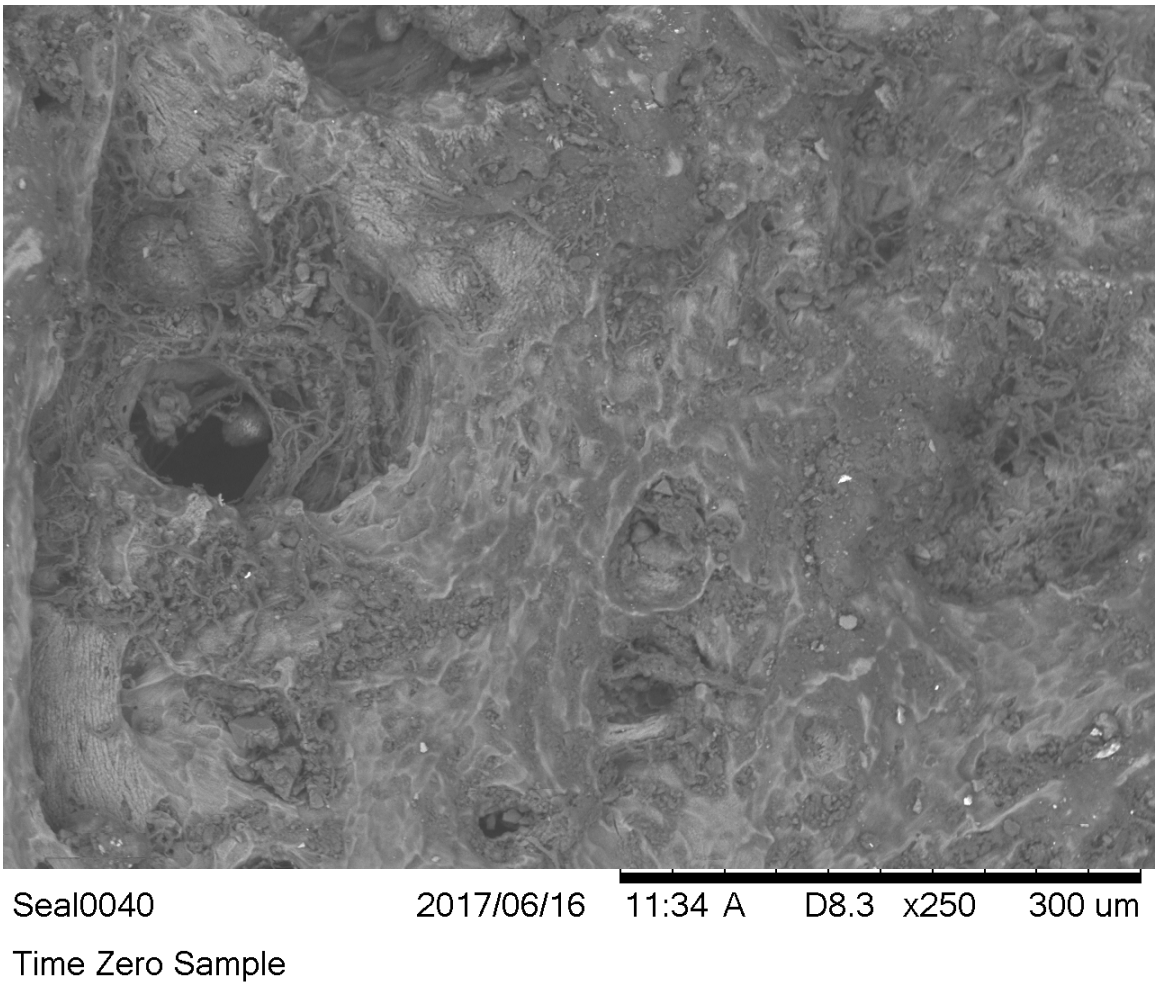


Figure 14: SEM image of topographical surface of seal bone specimen

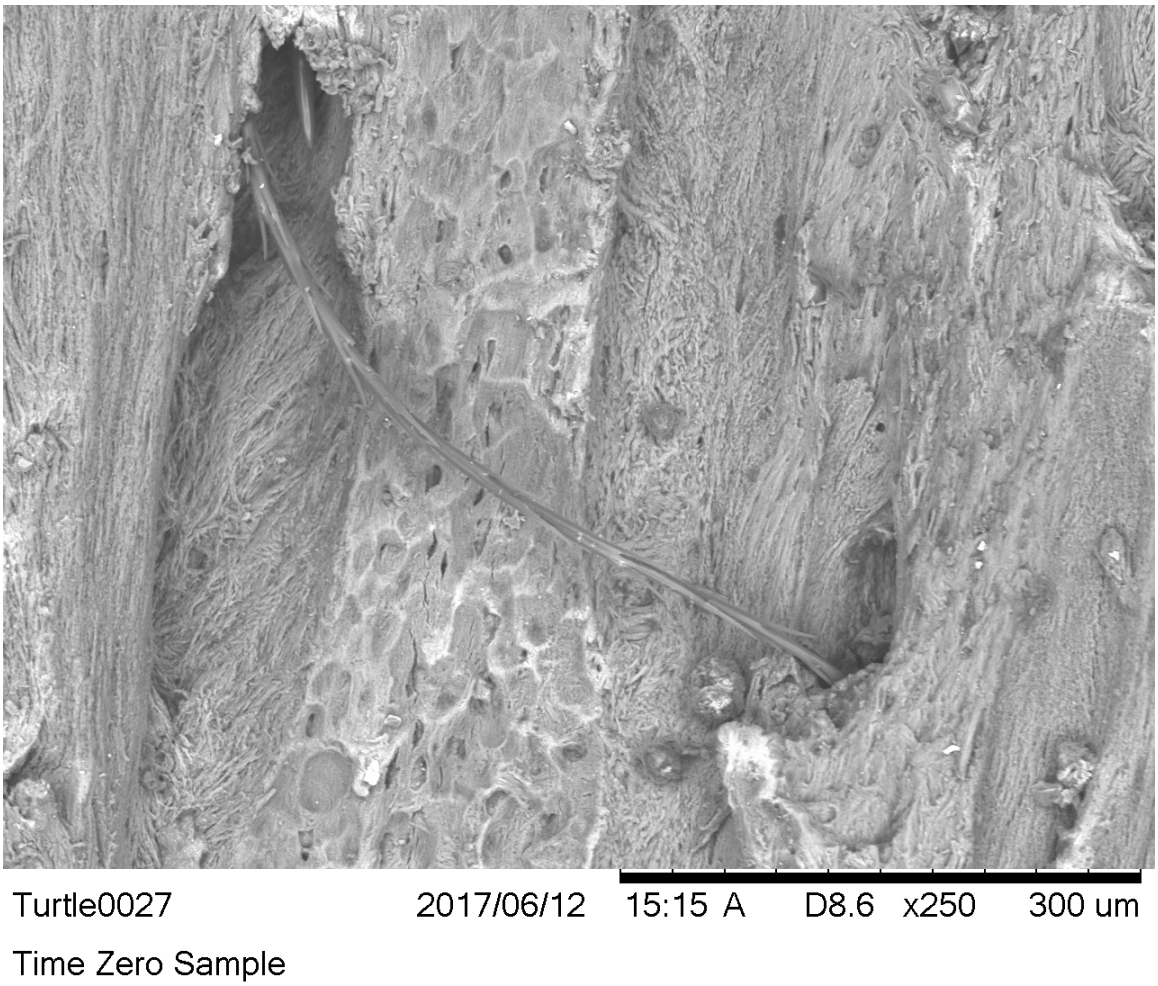


Figure 15: SEM Image of topographical surface of turtle bone specimen

DISCUSSION

In an attempt to fill the existing void in literature regarding the taphonomy of marine vertebrates, this project provided a unique and new outlook to the scientific community from a generated list of inquiries from analysts and investigators that were examining questioned specimens unsuitable for age analysis on bones. As the beginning stages of diagenesis and mineralization have not yet been explored on a short time scale in regard to marine vertebrates, this study provides preliminary data about elemental uptake and release in both the subtidal and supratidal environments.

Analytical methods were developed using adjacent studies on relevant subject matter and then modified to specifically fit this project. The protocol was extracted using suggested methods based on the technical support of the instrument's representatives as well as from Seeck and Murphy (2015). Because this study was so unique, multiple trials were run prior to data analysis to determine the best method appropriate for the analysis needed to complete this research. Multiple adjustments were made in preliminary trial analysis which led to a sound protocol that was carried out for the specimen examination. Current bone research using the SEM and EDS components has been conducted on a variety of terrestrial specimens (Bloebaum *et al.,* 1997). As being one of the first to use marine vertebrate bones from the environment, many modifications were made to best fit this study.

The initial EDS analysis of the bone specimens yielded results that were expected for bone composition. The elements present were Calcium, Hydrogen, Oxygen, and Phosphorous which are known to be the major inorganic elements that make up the bone matrix (Trueman, 1999). These major elements were hidden from the screening to

enhance the analytical presence of other inorganic elements. Secondary analysis included elements commonly found in the ocean environment: Iodine (I), Potassium (K), Magnesium (Mg), Copper (Cu), Zinc (Zn), Silicon (Si), Aluminum (Al), Lead (Pb), and Sulfur (S) (Drever, 1977 & Turekian, 1968). Zirconium (Zr) was noted to be present in the bones in high concentrations on preliminary screening so it was added to the list of elements to screen for over time. The pre-exposed or baseline specimens each contained high amounts of Zirconium (see Table 1). Zirconium is known to act as an inhibitor in the formation of calcium phosphate thus avoiding the problem of bones forming around themselves (Takada *et al.,* 2017). The natural presence of Zirconium in the pre-exposed specimens could serve as a stabilizing and protective mechanism within bones to prevent excessive and unnecessary bone growth within an organism.

A notable concern for the unexplained high concentrations of Zirconium existed on the basis that Zirconium could be an artifact of contamination on the specimens in the instrument chamber from either the handling tools, dremel blade, or placement stub. In an attempt to diminish this concern, runs on the instrument were performed to test the blank chamber, dremel blade, and placement stub for the presence of Zirconium which was nonexistent during these tests. Intertidal snail specimens were also tested for the presence of Zirconium. Energy-dispersive screenings were performed on representative surface areas of 5 intertidal snail shells from the same marine environment as the sample specimens in this study. The methodology for handling the shells was the same as the bone specimens and also similarly, the same 10 elements were selected for when running the EDS program. The shells were found to be composed primarily of high concentrations of Iodine and Potassium. Zirconium was not present in measurable

amounts on the invertebrate snail control specimens which contests the concern of Zirconium being a contamination artifact in this study.

Because Zirconium was present in the baseline specimens at an average of 58.1%, differences and changes within the bone composition were easily followed throughout each time set. The baseline seal bone data differs from other specimens in Zirconium by a low of 44.7% concentration. Noticeable differences in the Sulfur and Potassium concentration are present in the seal bones at 9.4% and 20.5% respectively. A possible explanation for the differences in the seal bones could be that the samples were derived from three different species of seals from differing ecological areas along the Northern Atlantic Ocean coastlines due to foraging or breeding behavior (Thompson, *et al.* 1996). Likewise, the baseline turtle and porpoise samples share similarities in numerical data in almost every screened element. Ecologically, these two specimens spend a majority of their lives in the water occupying similar costal environments (Boyle, 2006 and Bjørge & Tolley, 2009).

The subtidal elemental concentration data shows a trending decrease in higher concentrated elements such as Zirconium throughout the time series. As Zirconium concentrations decrease, with the exception of the seal data, the lower concentrated elements increase in percent concentrations such as Silicon which shows an overall increase of 9.62% (see Tables 2-4). Similarities in decreasing Iodine concentrations can be shown among the cow and seal specimen (see A7 and A9 in Appendix). Silicon, which was not present in measurable amounts in the baseline turtle and porpoise data, shows a steady increase in all specimens as the time series progresses. The metal elements with the exception of Lead (Copper, Zinc, Aluminum), remained at constant concentrations

and showed little change. This can lead to the assumption that certain metallic elements may be more difficult to incorporate in the boney matrix. Relative similarities within the subtidal exposed specimens reveal comparable data between specimens that live similar lifestyles. For example, the specimens that spend more time in the water are more alike than the specimens that spend time in the terrestrial environment.

The supratidal environment reveals data much different from the subtidal environment. To begin, the Zirconium and Sulfur concentrations steadily increase between the cow and seal specimens compared to an increase in subtidal conditions. Although a decreased spike in Zirconium concentration is noted in the cow specimens collected at 62-days, the data still displays an increasing trend over time. Although the data was methodically analyzed according to protocol, this spike decrease could be due to instrumental or technical errors on the day the 62-day exposure samples were examined. Another noticeable low numerical value in the 62-day data is the turtle Zirconium concentration which is 40.5%. Despite the outlying numbers, a pattern of the turtle specimen data can be depicted to predict how the Zirconium concentration is changing over time.

A noticeable spike in sulfur concentration at 62-day exposure in all the specimens is present. Recent studies on aquatic algae producing dimethylsulfide (DMS) and dimethylsulfoniopropionate (DMSP) in environments containing a range of salinities may help explain the large spike in Sulfur concentration (Zhuang *et al.,* 2011). Around this 62-day period, the National Climate Report documented record breaking temperatures along the Northeastern coastlines along with below average rainfall (NOAA National Centers for Environmental Information, 2017). These climate changes could have cause

stress on the algae and bacteria growing on the bone specimens undergoing taphonomic decay. Extreme changes in environmental conditions such as temperature, moisture, light intensity, and salinity can cause the algal cells to lyse resulting in the release of sulfur containing molecules such as DMS and DMSP (Zhuang *et al.* 2011). Above average precipitation and normal temperatures were recorded around the 335-day exposure collection in June 2017 (NOAA National Centers for Environmental Information, 2017). High moisture and optimal temperature likely resulted in a favorable environment for the algae and bacteria thus resulting in a lower recorded Sulfur concentration during this time set.

As before in the subtidal exposed specimens, metal (Cu, Zn, Al) fluctuations are present but remain unremarkable for comparison purposes. The exceptionable lead concentrations in the supratidal exposed cow specimen shows a decline throughout the time series whereas an opposite increase is noticed in the subtidal environment. The increase of lead concentration in the subtidal cow bones is probably due to the high availability of lead found in the marine environment (Drever, 1977 & Turekian, 1968).

The novel statistical analysis utilized for the data in this study allowed for multivariate comparison over a series of time. Different combinations of elemental concentrations were calculated and produced data that could be placed in plots, graphs, and tables for comparisons and pattern analysis. Elemental concentrations were plotted over time to visually depict trending changes or outlying data. For example, plotted data can show tight consistent clusters or spread out diverging concentrations. Again, this data can show possible trends that can be used to find patterns of change. Graphs such as the time series graphs in Figure 10 portray how the specimens vary from the calculated

Component Scores 1 and 2. By following certain specimens and exposure status throughout the time sets, possible patterns or rotations can be noticed. This allows for predictions and possible diagnostic implications to be made for unknown specimens being analyzed in forensic or wildlife laboratories by comparing them to known data sets.

The data provides information in which differences and similarities can be noted among the different specimens. The different representative vertebrate groups show multiple similarities or large differences throughout the time series. The multivariate regressions reveal high R-values for each species between subtidal and supratidal environments over time (see Appendices 7-10). A selection of those correspondences is highlighted with plausible explanations for why those resemblances exist. Lifestyle seems to play a large role in the vertebrate elemental compositional uptake and release. Specimens that spend either vast portions of time on land or in the water parallel characteristics with other analyzed specimens that live in a similar manner. The elements displaying noticeable changes and variations are those that were initially present in higher concentrations. Zirconium displayed noteworthy changes in all specimens as the time series progressed. Following Zirconium, Iodine and Potassium show noteworthy fluctuations among the different environments. A longer time series of this study would likely provide more insightful data regarding the elements of lower concentrations. Conversely, some details exist within the data that does not provide insight on elemental pattern analysis.

Although portions of the data confirm patterns or measurable differences that can be followed throughout the time series, inconsistencies exist that provide knots in the data set. These differences are primarily due to the uneven incorporation and release of the

elements within the specimens as well as in the environments. The highly variable environments contain a large variety of elements which are competing for the bony matrix. As elemental changes occur in chemically unstable environments, uneven distributions of elemental integration and release result in unexplained variability in the data especially when measuring fairly low concentrations. Aside from environmental and biological complexities, the instability of the different bones allows for obvious variations to occur in elemental uptake and release. A longer time series using this data has the potential to provide information that can be used in pattern analysis to predict more accurately how these changes are occurring. On the contrary, short time analysis can provide information as far as minor influential changes that could be occurring in the bones over a matter of days. The expansion of this study would benefit from both shorter and longer termed analysis.

For further applications involving human studies, this information may also be of use to forensic medicolegal investigations in which human bodies are recovered from the maritime environment. Elemental pattern analysis has the potential to aid in the analysis of human remains retrieved from subtidal areas. Further implications of this study can also give scientists the ability to age human remains recovered from shipwreck sites, bones of unknown eras, plane crashes, and other remains being analyzed in equivocal death investigations regarding ocean body disposals. A possibility exists that the subtidal environment itself could have an effect on the mineralization and taphonomy of specimens regardless of the specimen being of maritime origin or not. The scope of this study has the potential to have widespread application in a variety of forensic settings as well as other scientific disciplines. With previously used techniques from adjacent subject

matters, research and analysis can continue to be conducted in hopes of finding answers to areas that have been unexplored.

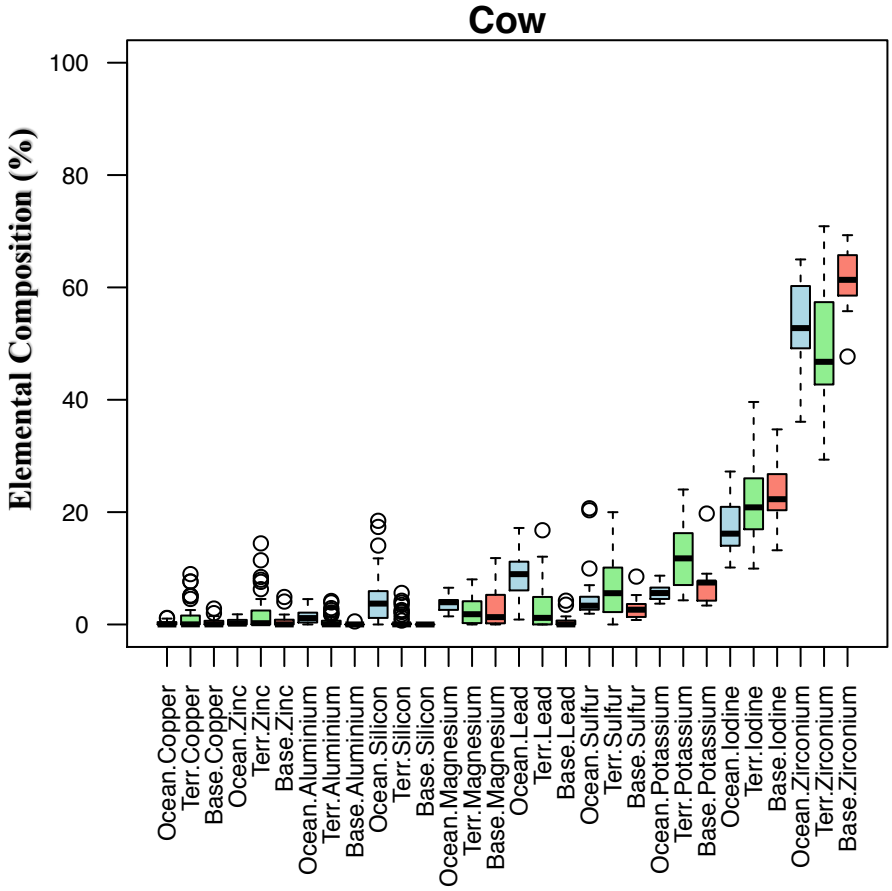
THESIS CONCLUSION

The broad application of this study allows for expansion for future analysis and studies to be performed. A clear lack of research exists regarding the taphonomy and mineralization of maritime vertebrates. Using this preliminary data, future studies can be conducted with a variety of environmental and biological observations being targeted in order to rule out contributing factors to the variations in elemental uptake and release over time. The uniqueness of this particular study encompassed the use of first-hand observations along with supporting elemental and statistical data to examine bone taphonomic changes over time. At the conclusion of this scientific survey, it has been made noteworthy that marine vertebrates are compositionally diverse from terrestrial vertebrates. With the consideration of environmental lifestyle and diet, the bone structure and composition vary between species. Taphonomically speaking, different environments have different effects on the specimens as they proceed to decay and mineralize. Bones submerged in the subtidal environment displayed contrasting elemental uptake and release compared to the bones placed in a supratidal area. The uniqueness of environmental variables has distinct characteristic influences on the bones contained within. Innovative statistical analysis allowed for the application of Principal Component Analysis to compare multiple variables to observe taphonomical changes over time. This analytical method has previously been utilized for a broad spectrum of multivariate analysis such as crime correlation to crime theories as well as chemical changes based on environmental co-variables (Messner & Rosenfeld, 1997 and Wold *et al*., 1987). With this study, PCA allowed for elemental change comparisons to aid in taphonomic analysis.

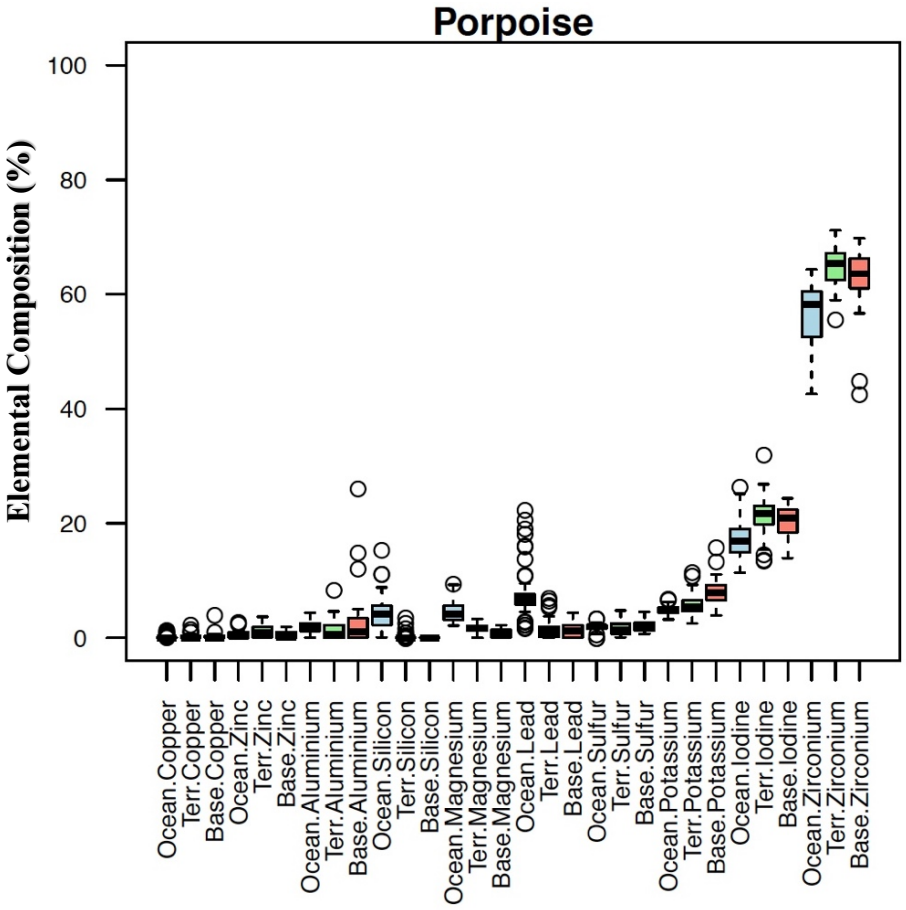
This novel method can be used in pattern analysis in forensic science and wildlife laboratories in which unknown specimens are submitted for examination.

The data collected and analyzed for this study lays foundational information for wildlife investigators to utilize for comparison purposes and to methodologically mirror for evidence bone analysis. Unknown specimens can be analyzed and compared to patterns and component scores created from PCA to give characteristic estimations that aid investigators in estimations on bone examination. Appendices 5 and 6 illustrate patterns of PC score changes over time which can provide predictive power for future studies being compared to this baseline data. By utilizing science and statistical analysis, more advancements can be made to improve forensic science analysis and investigative techniques.

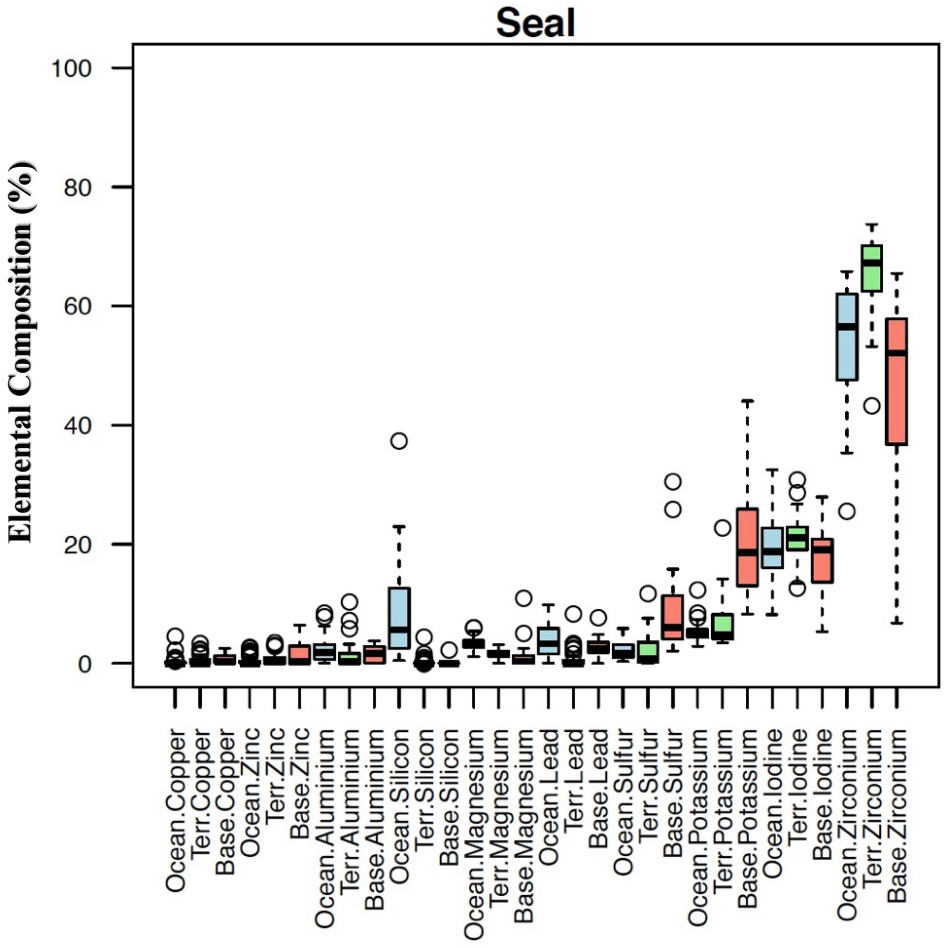
APPENDICIES



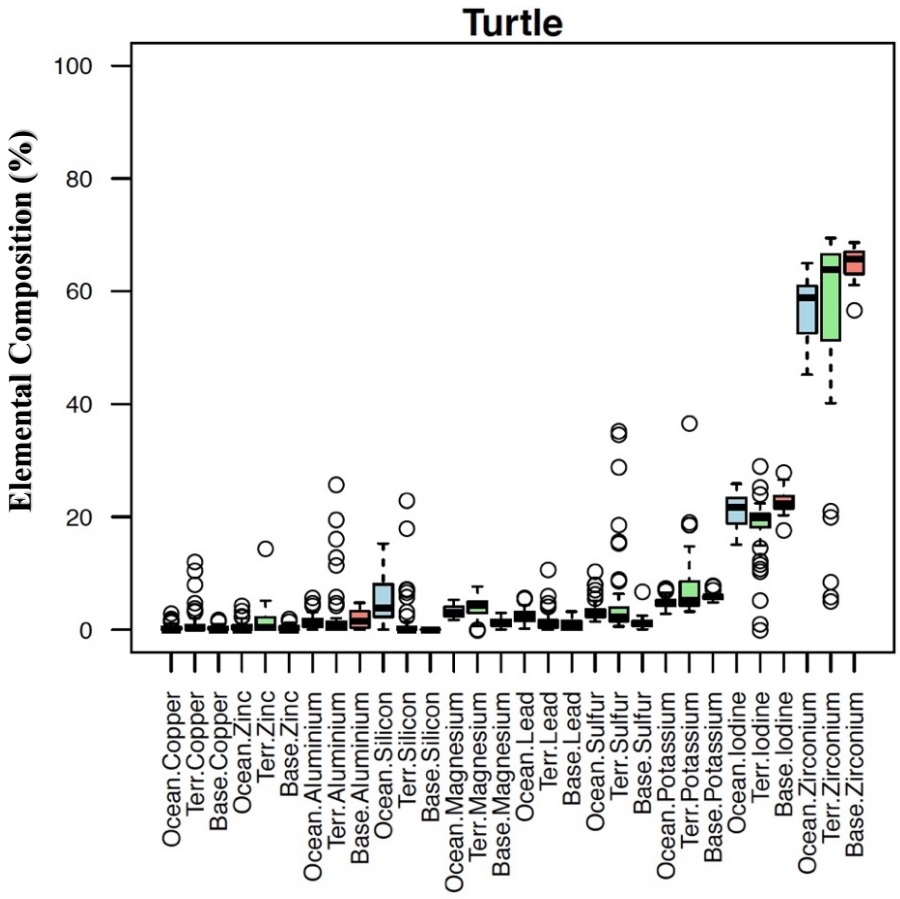
A 1: Box plot of cow specimen illustrating baseline, subtidal, and supratidal data



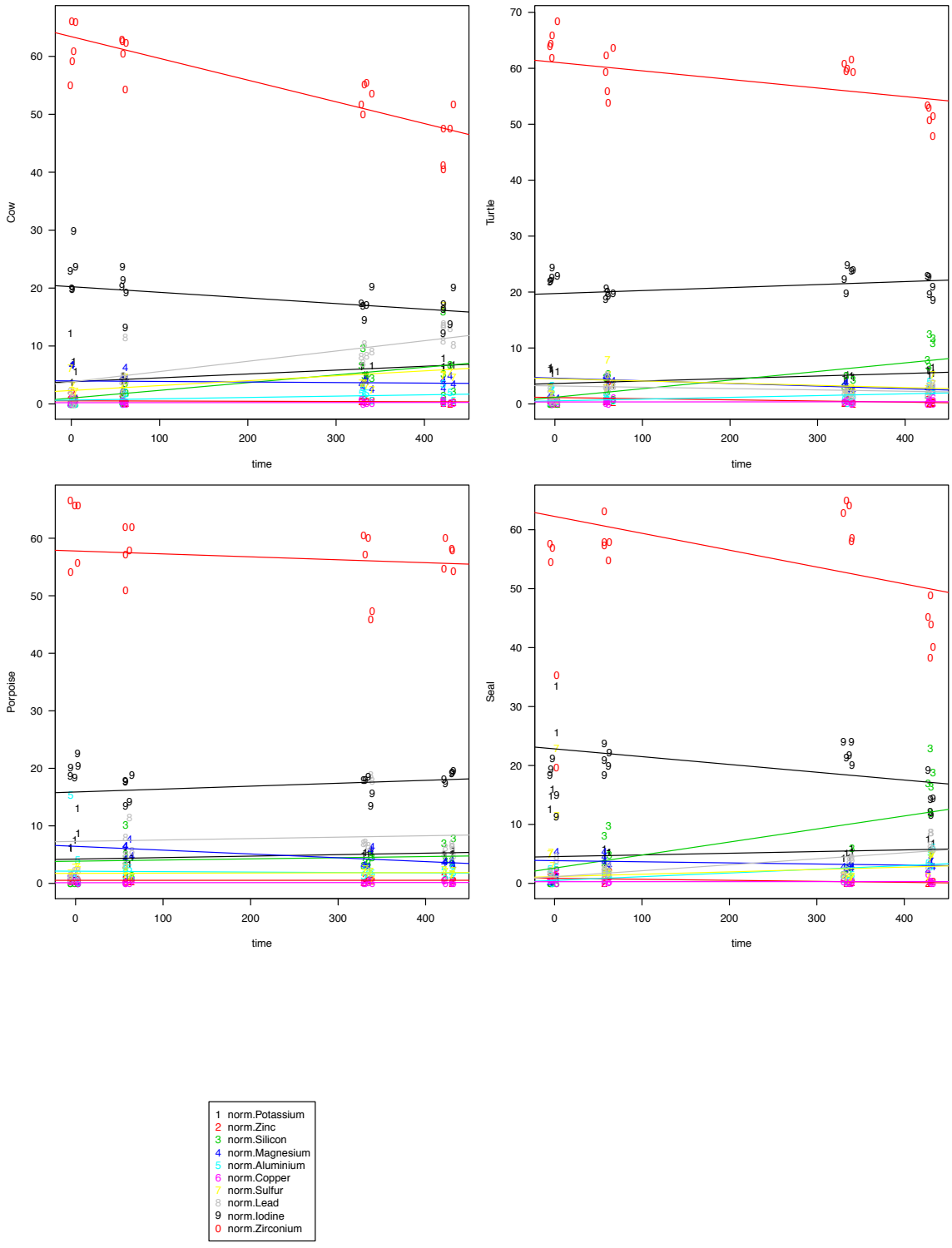
A 2: Box plot of porpoise specimen illustrating baseline, subtidal, and supratidal data



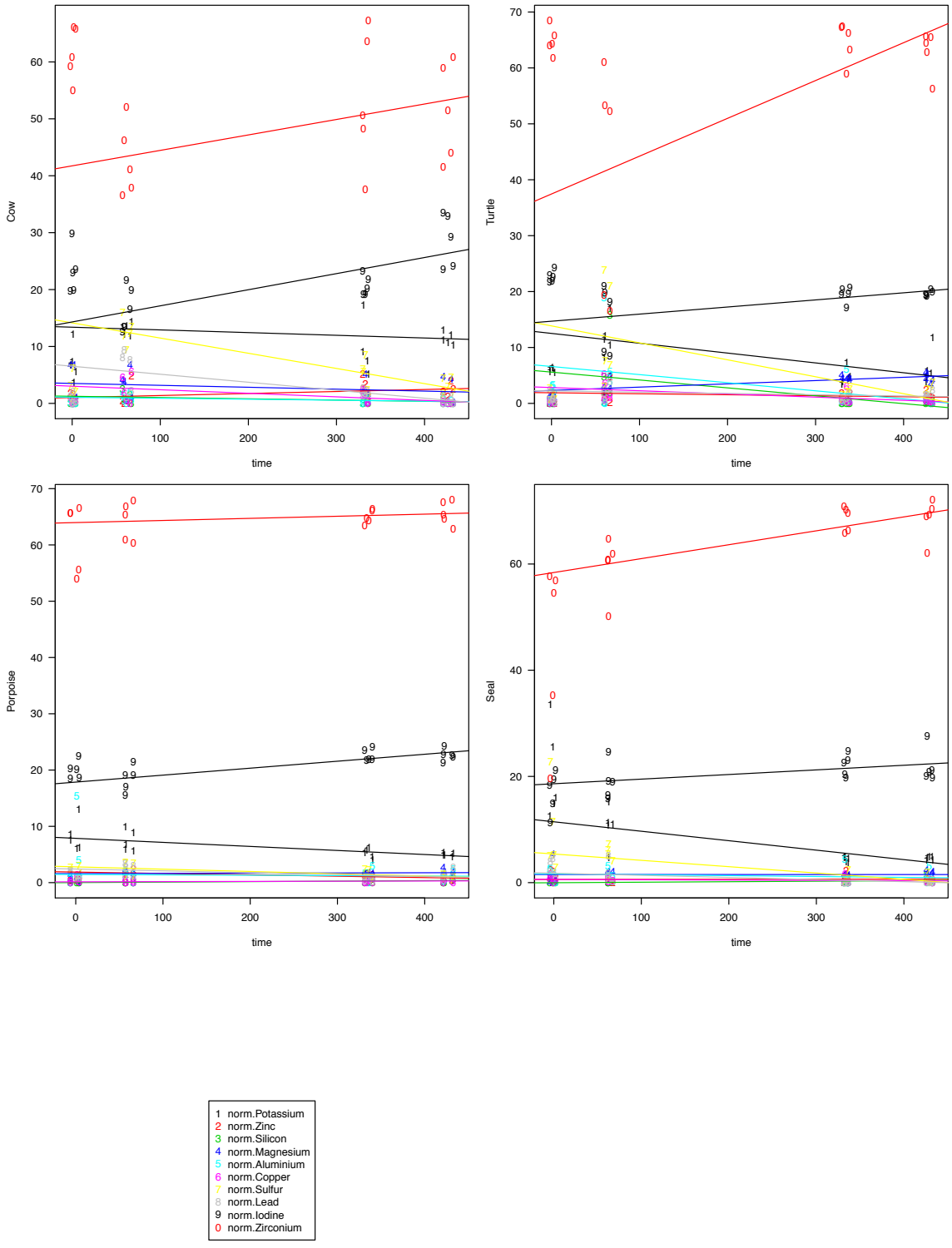
A 3: Box plot of seal specimens illustrating baseline, subtidal, and supratidal data



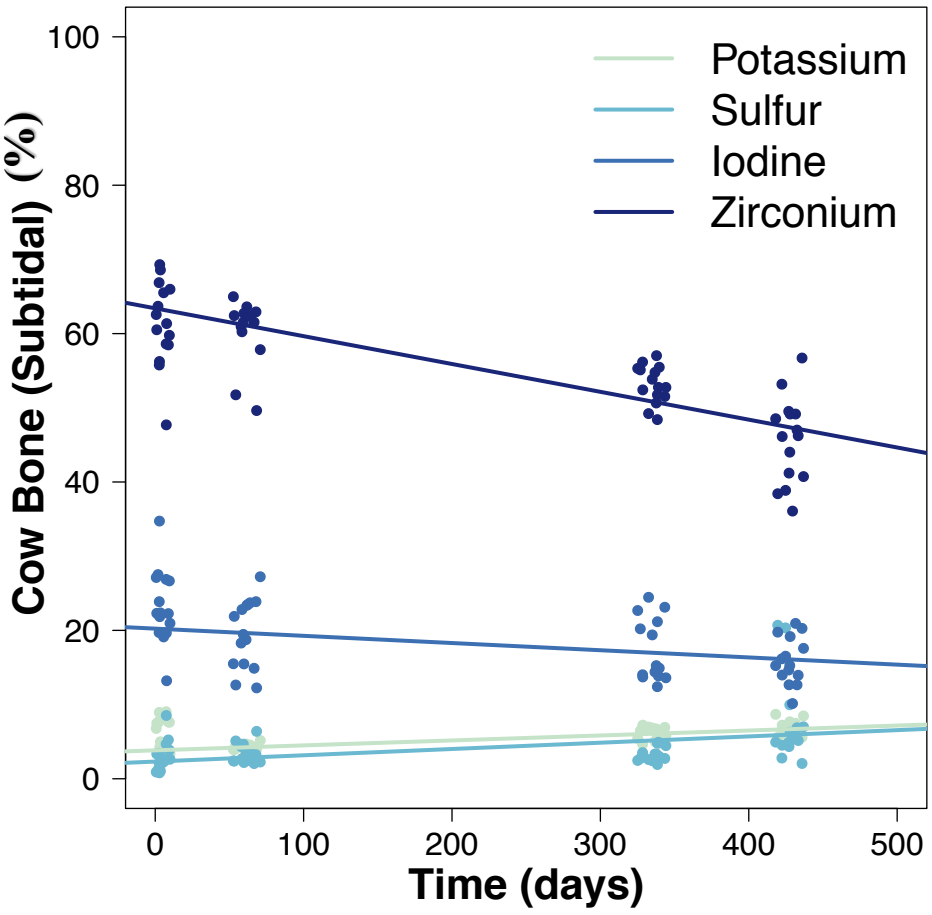
A 4: Box plot of turtle specimen illustrating baseline, subtidal, and supratidal data



A 5: Raw elemental concentration percentages over time for subtidal specimens

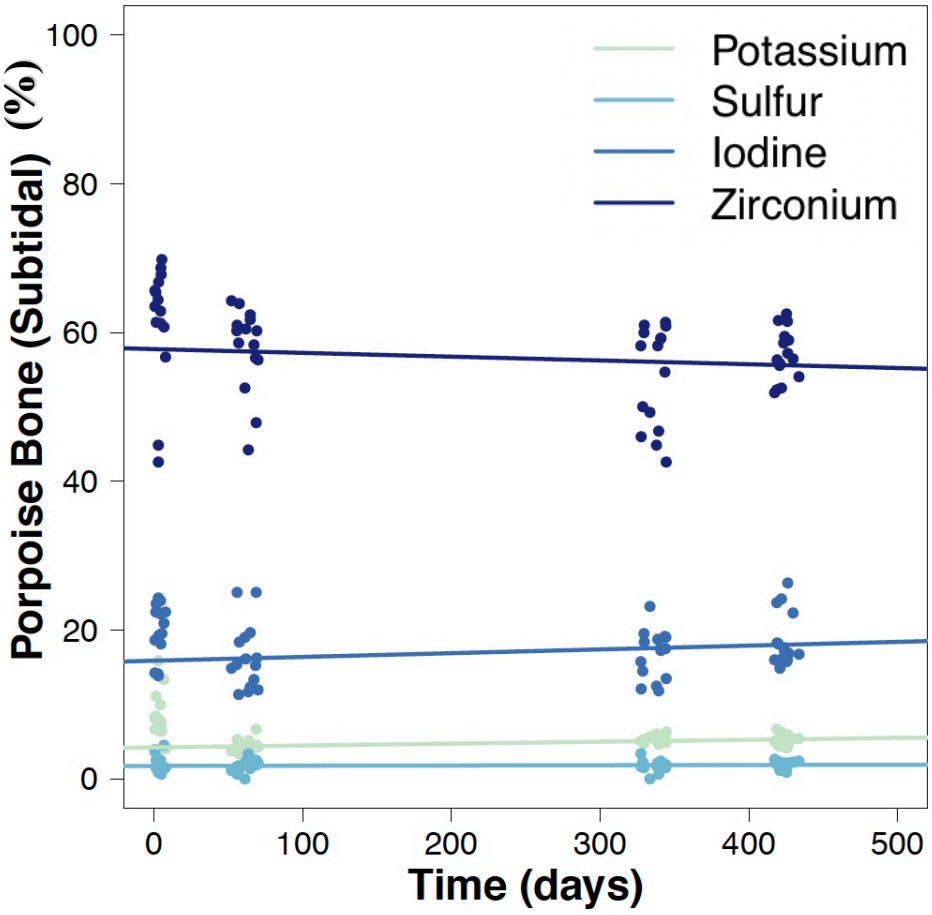


A 6: Raw elemental concentration percentages over time for supratidal specimens



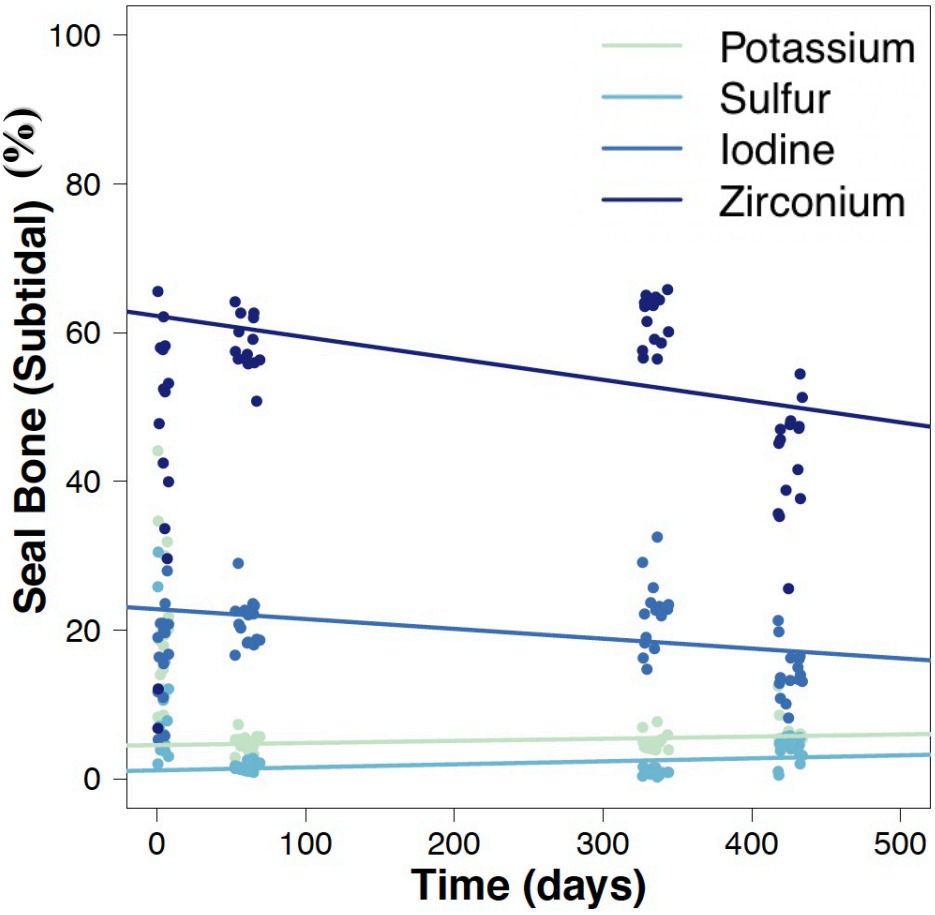
A 7: Elemental concentration percentages for subtidal cow specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time

(Subtidal vs. Supratidal: Residual standard error: 5.606 on 344 degrees of freedom, multiple R-squared: 0.9187, adjusted R-squared: 0.9152, F-statistic: 259.2 on 15 and 344 DF, p-value: < 2.2e-16)



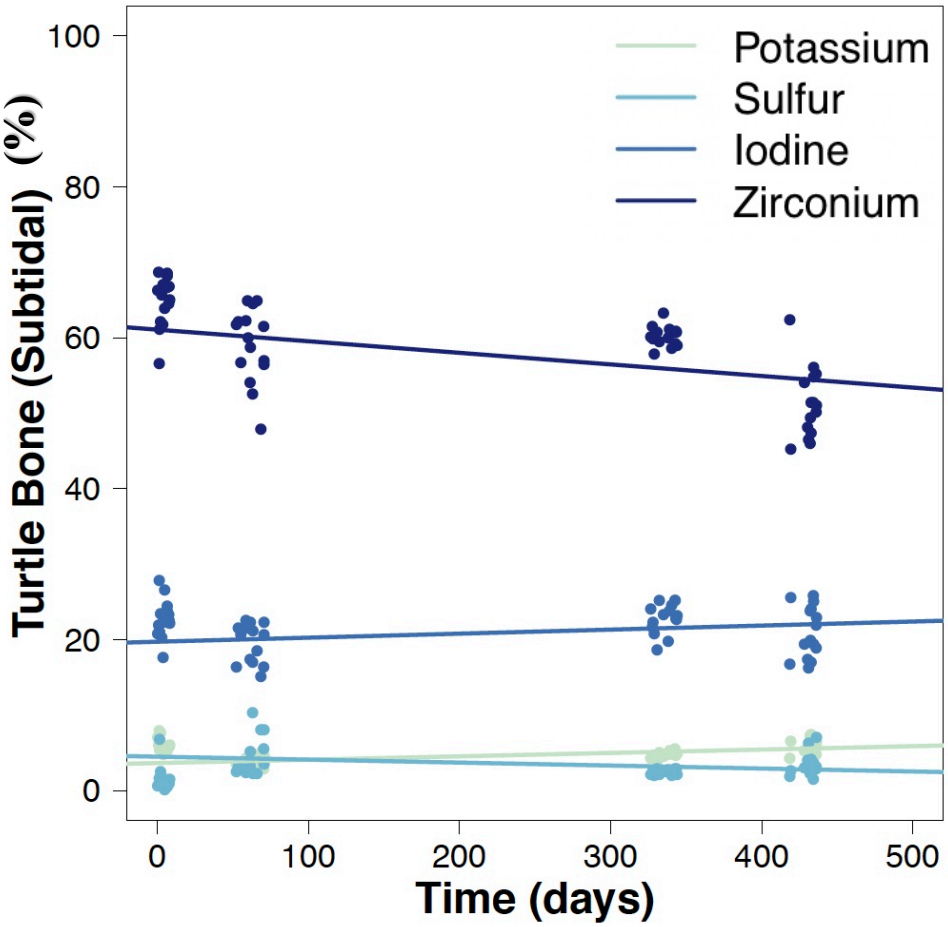
A 8: Elemental concentration percentages for subtidal porpoise specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time

(Subtidal vs. Supratidal: Residual standard error: 2.982 on 344 degrees of freedom, multiple R-squared: 0.9849, adjusted R-squared: 0.9842, F-statistic: 1496 on 15 and 344 DF, p-value: < 2.2e-16)



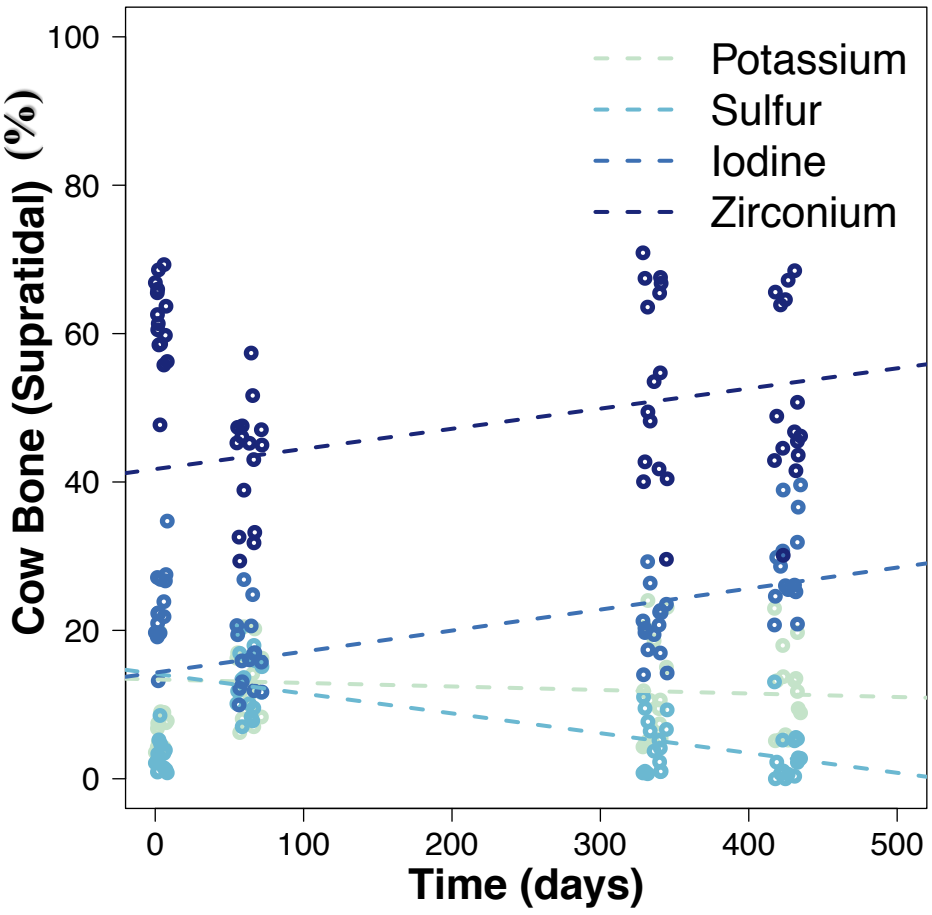
A 9: Elemental concentration percentages for subtidal seal specimens depicting Potassium, Sulfur, Iodine, and Zirconium changes over time

(Subtidal vs. Supratidal: Residual standard error: 4.256 on 344 degrees of freedom, multiple R-squared: 0.9689, adjusted R-squared: 0.9675, F-statistic: 714.5 on 15 and 344 DF, p-value: < 2.2e-16)

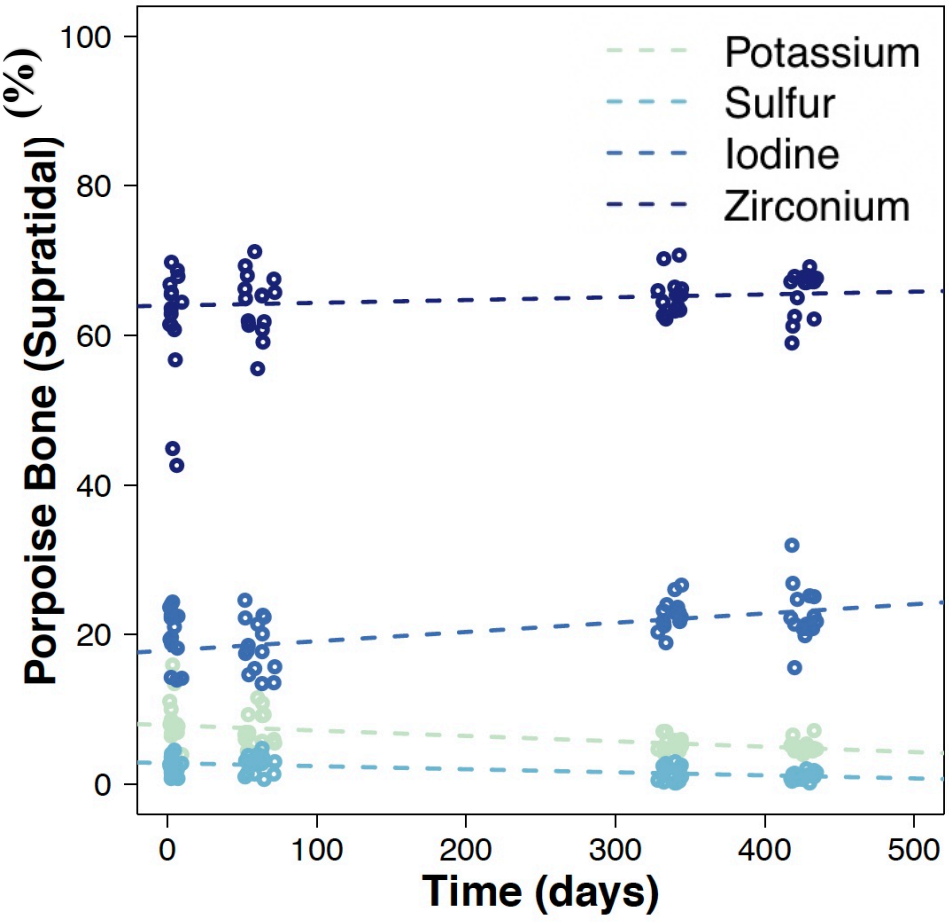


A 10: Elemental concentration percentages for subtidal turtle specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time

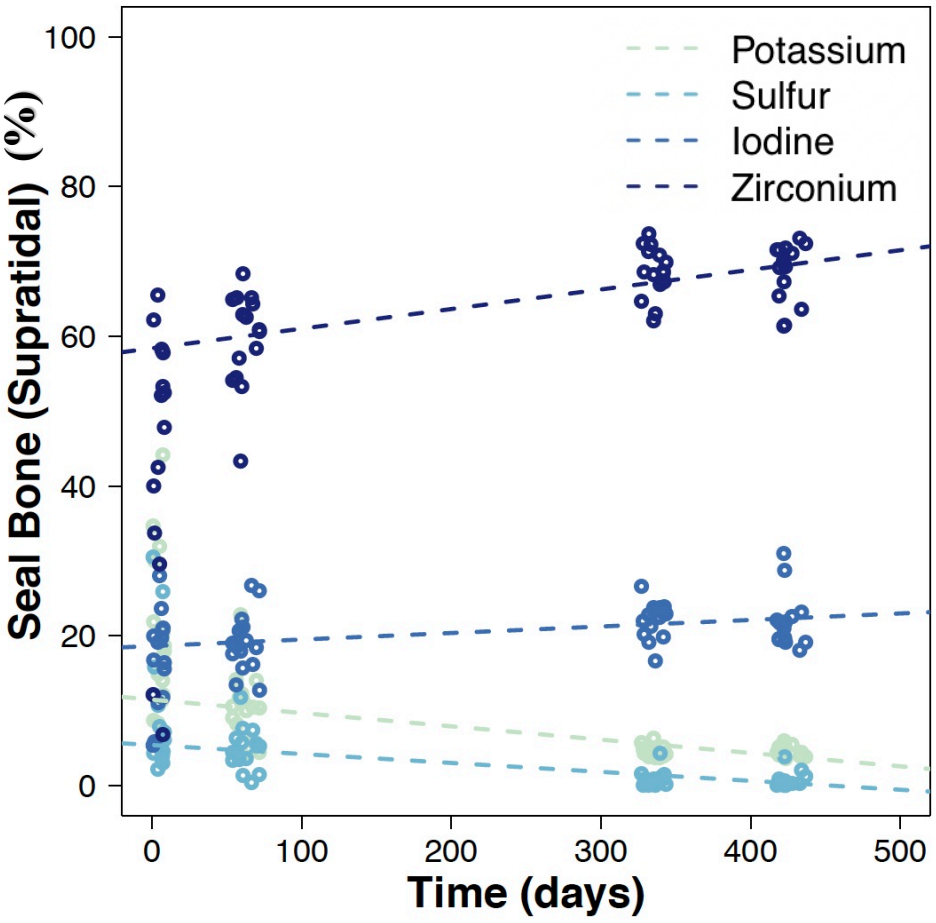
(Subtidal vs. Supratidal: Residual standard error: 6.533 on 340 degrees of freedom, multiple R-squared: 0.9182, adjusted R-squared: 0.9146, F-statistic: 254.5 on 15 and 340 DF, p-value: < 2.2e-16)



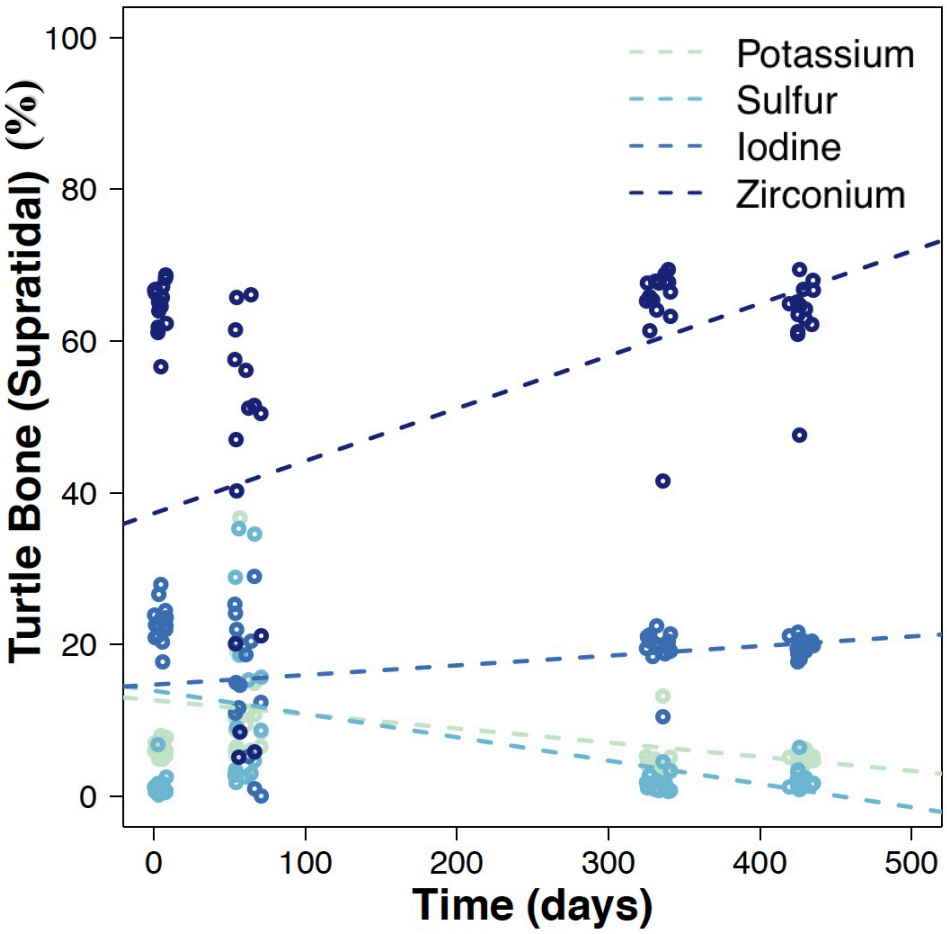
A 11: Elemental conentration for supratidal cow specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time



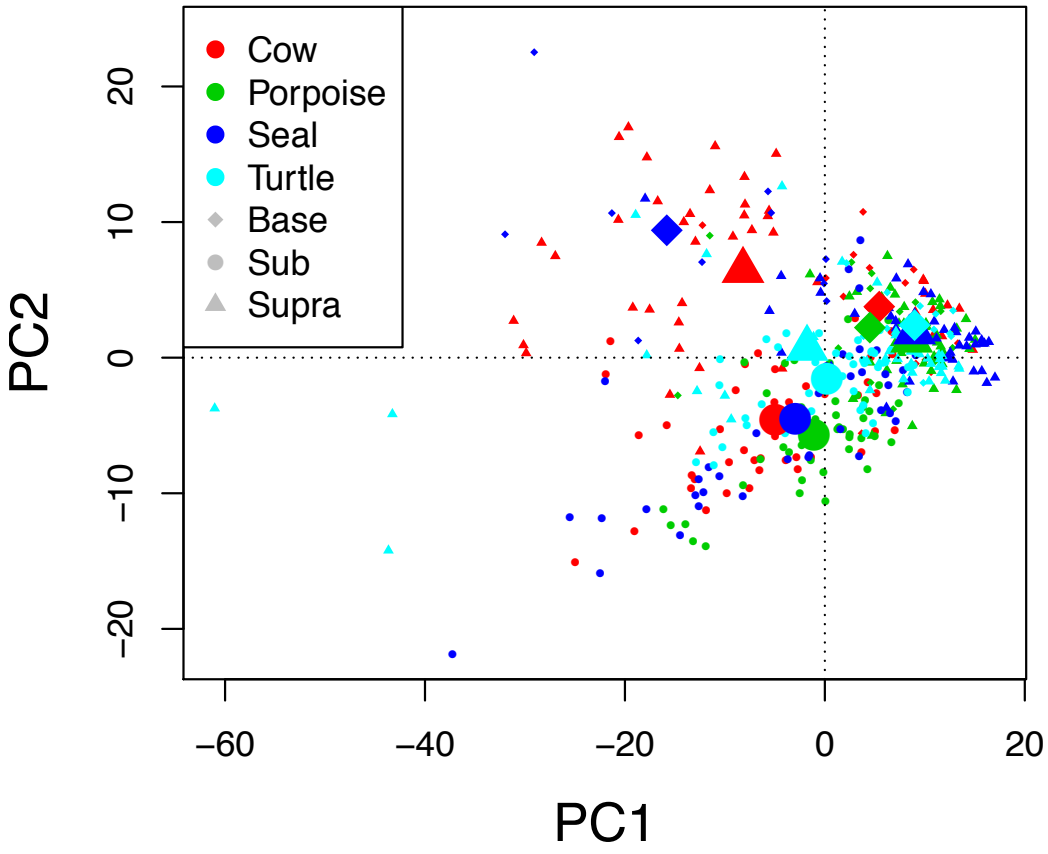
A 12: Elemental concentration for supratidal porpoise specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time



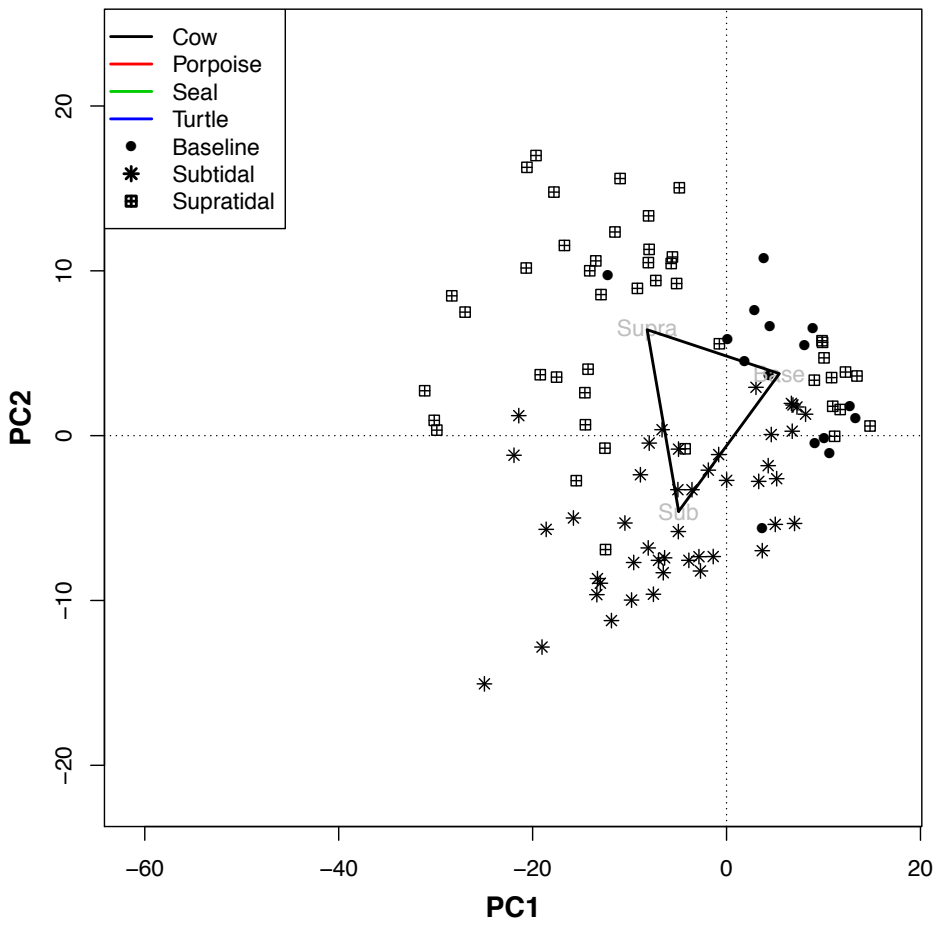
A 13: Elemental concentration for supratidal seal specimens depicting Potassium, Sulfur, Iodine, and Zirconium changes over time



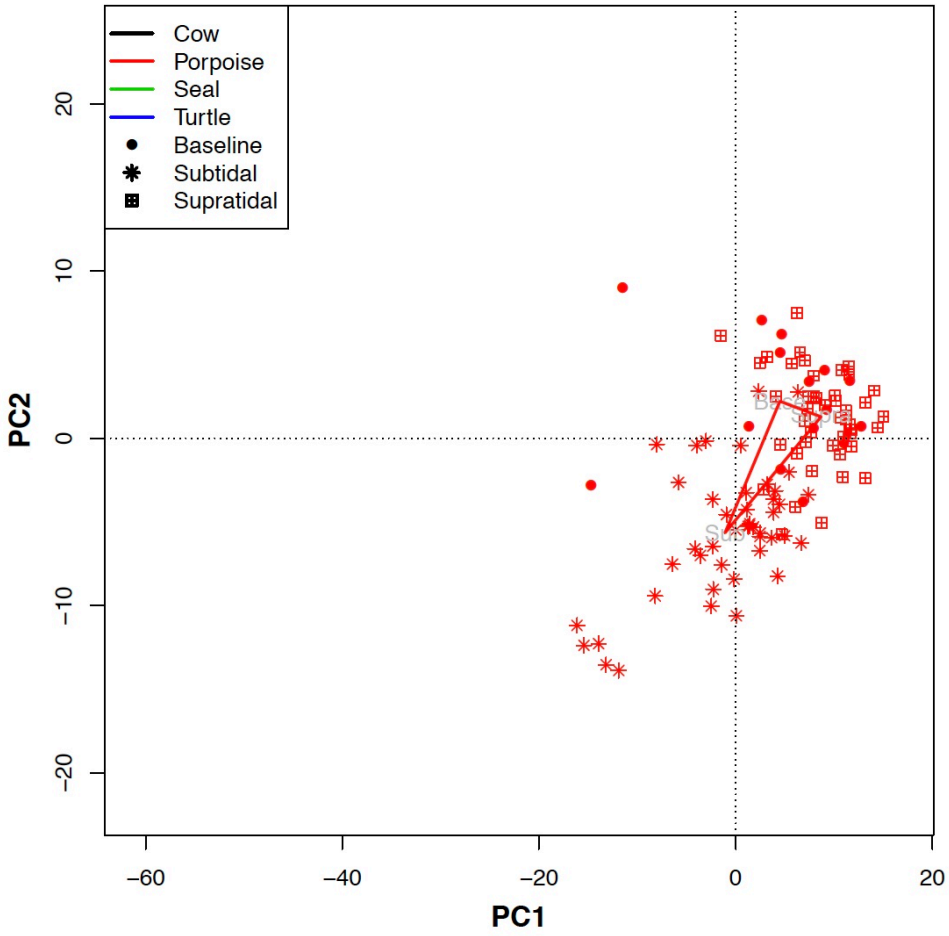
A 14: Elemental concentration percentages for supratidal turtle specimen depicting Potassium, Sulfur, Iodine, and Zirconium changes over time



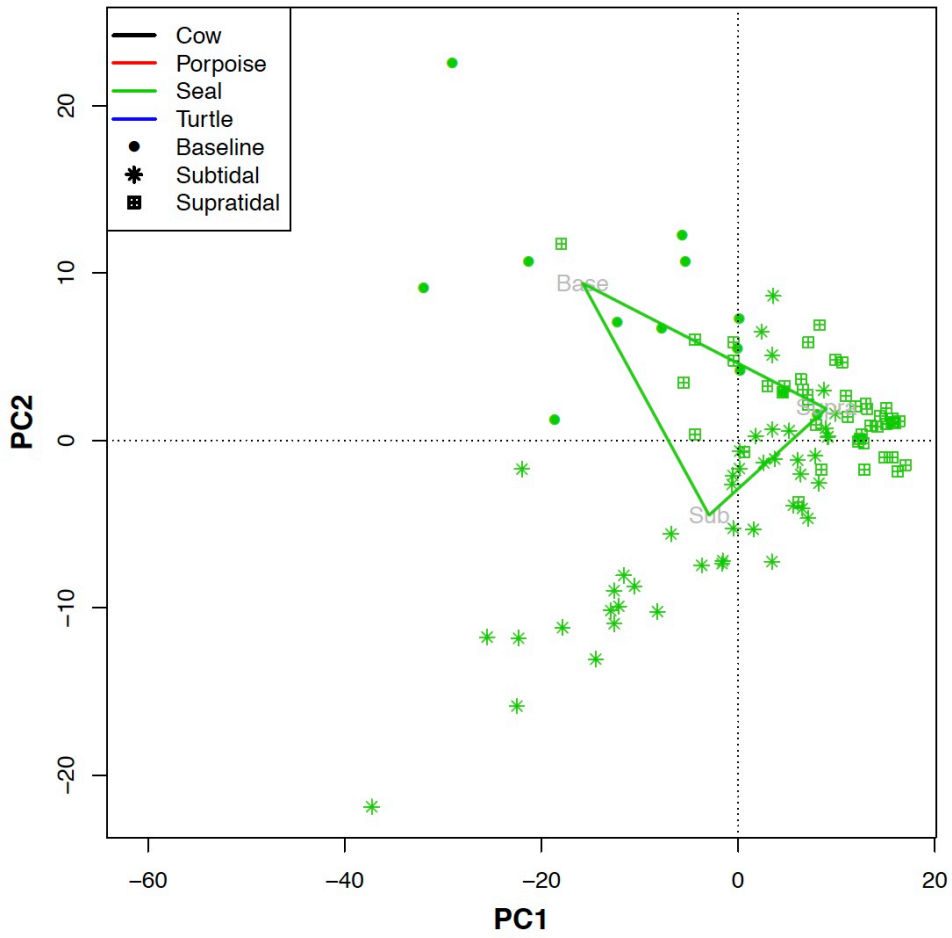
A 15: Principal Component Bi-plot for all specimens. The shape sizes are relative to the time series they are representing (smaller shapes depict baseline data and largest shapes depict final exposure series)



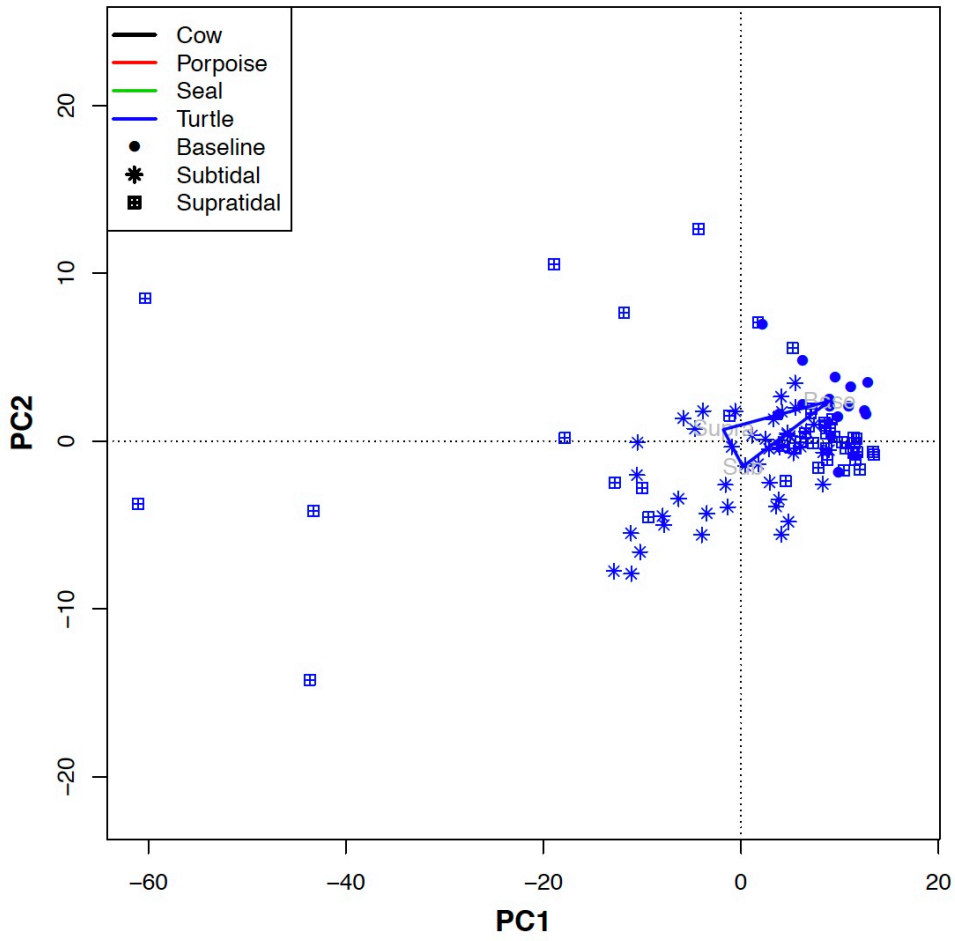
A 16: Principal Component plot of cow specimen including baseline, subtidal, and supratidal data



A 17: Principal component plot for porpoise specimen including baseline, subtidal, and supratidal data



A 18: Principal component plot for seal specimens including baseline, subtidal, and supratidal data



A 19: Principal component plot for turtle specimen including baseline, subtidal, and supratidal data

Footnotes

[1]As the study progressed, scavengers or rodents began to chew through the mesh dive bags through the bottom of the traps. To avoid bones from being scavenged or lost, the bags were suspended from the top of the trap away from where the scavengers could reach from the bottom.

[2] Detectability Limit for EDS – minimum % concentration with ability to see an element peak is 0.1% to 1%, depending on energy of X-ray line and composition of sample. Concentration Relative Error is depicted in the table below:

Concentration of Element	Relative Error %
> 10%	+/- 2 – 10%
1 – 10%	+/- 3 – 25%
< 1%	+/ - 100%

Source: Bruker AXS Inc. 2018

To reflect the precision of results reported by EDS software, all decimals are reported to the hundredths place. Due to rounding, the numerical sum of each row or column may not be exactly 1.00.

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