Stable Isotope Analysis of a Platecarpus tympaniticus (Squamata, Mosasauridae) with Actinocamax sternbergi (Mollusca, Belemnoidea) reveals possible endothermic thermoregulation

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STABLE ISOTOPE ANALYSIS OF A *PLATECARPUS TYPANITICUS*
(SQUAMATA, MOSASAURIDAE) WITH *ACTINOCAMAX STERNBERGI*
(MOLLUSCA, BELEMNOIDEA) REVEALS POSSIBLE ENDOTHERMIC THERMOREGULATION

A Thesis Presented to the Graduate Faculty of Fort Hays State University in Partial Fulfillment of the Requirements for the Degree of Master of Science by

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Date 04/26/2022 Approved

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GRADUATE COMMITTEE APPROVAL

The graduate committee of Mitchell W. Lukens approves this thesis as meeting partial fulfillment of the requirements for the Degree of Master of Science.

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ABSTRACT

Mosasaurs, ancient marine reptiles, dominated the late Cretaceous oceans. However, their ecological success is a contentious topic. Were they ectothermic, like their modern relatives the varanid lizards? Or endothermic like extant marine mammals? Stable isotopes can reveal temperature and physiological variances within skeletons, but do not differentiate between body temperature and ambient environmental temperature. A rare mosasaur specimen from the Smoky Hill Chalk of a partial, articulated *Platecarpus tympaniticus* with stomach contents of belemnites provides a possible direct temperature contrast between predator and prey. The belemnites, related to modern coleoids, are identified as *Actinocamax sternbergi*. These animals possessed body temperatures equivalent to the ambient seawater. Stable oxygen isotope ratio values, reported as $\delta^{18}$O values, preserve temperature signature proxies during bioapatite and calcite precipitation. Utilizing previous stable isotopic studies, these values can be interpreted to paleotemperatures through equations describing known phosphate and carbonate ion deposition. Contrasting temperatures between the mosasaur and belemnites would indicate an elevated thermophysiology of the mosasaur compared to its environment.

Bone, chalk, and belemnite rostrum samples were collected as powdered samples by drilling into visually undamaged zones. Bone samples underwent two different treatment methods to isolate phosphate and structural carbonate ions for analysis. For the phosphates, bone powder was dissolved in nitric acid to isolate and precipitate the phosphate ions as silver phosphate for analysis. Silver phosphate samples underwent pyrolysis and the resulting carbon monoxide gas analyzed in a mass spectrometer for $\delta^{18}$O values.

For structural carbonate, bone powder was soaked in a triammonium citrate solution to dissolve secondary calcite formed during the fossilization process, leaving the structural carbonate ion within the bone behind. These treated samples, along with untreated samples,
chalk, and belemnite rostrum powder samples were reacted in orthophosphoric acid to release carbon dioxide for analysis in a gas-chromatograph mass spectrometer to give $\delta^{13}C$ and $\delta^{18}O$ values.

Bone phosphate values (n=26) ($\delta^{18}O_P$) ranged from 14.83 to 21.12‰ with a mean value of 18.30 ± 1.53‰ (1σ). Untreated bone carbonate values (n=6) ($\delta^{13}C_{cc}$, $\delta^{18}O_{cc}$) ranged from -4.9 to -0.8‰ with a mean value of -2.3 ± 1.6‰; and -9.5 to -7.6‰ with a mean value of -8.5 ± 0.8‰ respectively. Treated bone carbonate values (n=6) ($\delta^{13}C_c$, $\delta^{18}O_c$) ranged from -5.7 to -0.7‰ with a mean value of -3.0 ± 1.9‰; and -9.2 to -7.3‰ with a mean value of -8.1 ± 0.8‰ respectively. Chalk sample values (n=4) ($\delta^{13}C_M$, $\delta^{18}O_M$) ranged from 2.1 to 2.3‰ with a mean value of 2.2 ± 0.08‰; and -6.5 to -6.4‰ with a mean value of -6.4 ± 0.04‰ respectively. Belemnite sample values (n=3) ($\delta^{13}C_{BEL}$, $\delta^{18}O_{BEL}$) ranged from 0.4 to 0.5‰ with a mean of 0.4 ± 0.06‰; and -3.4 to -3.0‰ with a mean value of -3.3 ± 0.25‰.

Bone powder samples revealed diagenetic alteration of the structural carbonate. Secondary alteration, however, did not impact oxygen isotope values from the bone phosphate, belemnite rostrum carbonate, or the chalk. Scanning electron microscope and energy dispersion system images confirm the isotopic integrity of the phosphates and carbonates for analysis. Intra- and inter-variation of the bone phosphate values within each bone and across the skeleton suggest temperature fluctuations of the entire body or changes in body water oxygen isotopic composition reflecting migration into different bodies of water throughout the animal’s life.

Direct comparison of the calculated temperatures from the belemnites (15.0°C), chalk (22.1°C), and the mosasaur (36.2 – 37.5°C) provides evidence that this mosasaur possessed an elevated thermophysiology relative to its prey and environment. Variable bone phosphate oxygen isotope values across the appendicular and axial skeleton infer this mosasaur exhibited
heterothermic endothermy, a hot body core with varying temperatures in the flippers. The interpreted temperatures of the chalk and belemnites suggest the presence of a thermocline in the Western Interior Seaway. Diving to feed on belemnites is an inferred behavior from the isotope-derived colder temperatures preserved in the rostrums and avascular necrosis associated to decompression-sickness syndrome in other Platecarpus specimens.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ABSTRACT</strong></td>
<td>i</td>
</tr>
<tr>
<td></td>
<td><strong>ACKNOWLEDGEMENTS</strong></td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td><strong>TABLE OF CONTENTS</strong></td>
<td>v</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF TABLES</strong></td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF FIGURES</strong></td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF APPENDICES</strong></td>
<td>x</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF ABBREVIATIONS</strong></td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF SYMBOLS</strong></td>
<td>xii</td>
</tr>
<tr>
<td>1</td>
<td><strong>CHAPTER 1: STABLE ISOTOPE BASICS</strong></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Isotopes, Delta Notation, and Standards</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Carbonate Isotope Signatures</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Temperature Equations</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td><strong>CHAPTER 2: BELEMNITES</strong></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>INTRODUCTION</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>METHODS</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>RESULTS</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>DISCUSSION AND CONCLUSIONS</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Evidence for Digestion</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Importance of the Belemnites</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td><strong>CHAPTER 3: MOSASAUR THERMOPHYSIOLOGY AND CONCURRENT SEAWAY TEMPERATURES</strong></td>
<td>16</td>
</tr>
</tbody>
</table>
INTRODUCTION .........................................................................................................................16

BACKGROUND .....................................................................................................................21

Geologic Setting ..................................................................................................................21

  Stratigraphic Position ........................................................................................................21

Mosasaur Belle .....................................................................................................................25

Mosasaur Taxonomic Identification ......................................................................................27

Coccolithophores ..................................................................................................................31

Isotopic Water Values for Western Interior Seaway ...............................................................32

Seawater Temperatures .......................................................................................................33

METHODS ...............................................................................................................................35

  Sample Collection .............................................................................................................35

  Pre-Treatment of Bone Samples .........................................................................................37

  Bone Phosphate Preparation and Analysis .........................................................................37

  Carbonate Preparation and Analysis ..................................................................................39

RESULTS ...................................................................................................................................41

  Bone Phosphate Values ......................................................................................................41

  Bone Carbonate Values ......................................................................................................43

  Belemnite and Chalk Carbonate Values .............................................................................43

DISCUSSION ............................................................................................................................46

  Diagenesis ..........................................................................................................................46

    Bone Carbonate ...............................................................................................................47

    Bone Phosphates .............................................................................................................48
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>List of diagnostic characters of <em>Actinocamax sternbergi</em></td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Measurements from FHSM IP-705 compared to KGM0016</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>List of skeletal characteristics of <em>Platecarpus tympaniticus</em></td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Bone phosphate oxygen isotope values</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>Carbonate carbon and oxygen isotope values</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>Calculated temperatures from mean $\delta^{18}O_p$ values of each bone</td>
<td>59</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Known $\delta^{13}C$ and $\delta^{18}O$ value ranges of carbonates</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>General body reconstruction of a belemnite</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Dorsal views of <em>Actinocamax sternbergi</em> holotype (FHSM IP-705 and in-situ, isolated rostrum preserved with Belle)</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Kansas County Map relative to reconstructed Western Interior Seaway</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>Stratigraphy of Belle’s locality</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>KGM0016: Mosasaur Belle</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Mosasaur Belle’s belemnites</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>Bones sampled for study labeled on a representative mosasaur skeletal drawing</td>
<td>36</td>
</tr>
<tr>
<td>9</td>
<td>Cross-plot of $\delta^{13}C$ and $\delta^{18}O$ values from sampled carbonates</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>Pictures exhibiting preservation of Belle’s skeleton</td>
<td>47</td>
</tr>
<tr>
<td>11</td>
<td>Cross-plot of bone carbonate $\delta^{13}C$ and $\delta^{18}O$ values</td>
<td>48</td>
</tr>
<tr>
<td>12</td>
<td>Dot plot of bone carbonate and phosphate values</td>
<td>49</td>
</tr>
<tr>
<td>13</td>
<td>Cross-plot of $\delta^{18}O_C$ and $\delta^{18}O_P$ values</td>
<td>49</td>
</tr>
<tr>
<td>14</td>
<td>Box and whisker plots of $\delta^{18}O_P$ values from different <em>Platecarpus</em> specimens</td>
<td>51</td>
</tr>
<tr>
<td>15</td>
<td>Temperature variance (°C) of sampled bones relative to mean temperature</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>Box and whisker plots of $\delta^{18}O_P$ values from each bone</td>
<td>61</td>
</tr>
<tr>
<td>Page</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Bar chart comparing calculated temperatures of belemnites, chalk and Mosasaur Belle</td>
<td>62</td>
</tr>
<tr>
<td>18</td>
<td>SEM and EDS images of chalk matrix</td>
<td>75</td>
</tr>
<tr>
<td>11</td>
<td>SEM and EDS image of bone with embedded chalk exhibiting celestine and organics</td>
<td>76</td>
</tr>
<tr>
<td>12</td>
<td>EDS images of embedded chalk in bone fissure</td>
<td>78</td>
</tr>
<tr>
<td>21</td>
<td>X-ray Diffraction graphs of cortical and cancellous bone powder samples</td>
<td>79</td>
</tr>
<tr>
<td>22</td>
<td>X-ray Diffraction graph of SMB006B bone powder sample</td>
<td>79</td>
</tr>
<tr>
<td>23</td>
<td>SEM and EDS images of silver phosphate precipitates</td>
<td>81</td>
</tr>
<tr>
<td>24</td>
<td>Photograph of silver phosphate precipitates under microscope</td>
<td>83</td>
</tr>
<tr>
<td>25</td>
<td>SEM images of coccoliths</td>
<td>84</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Appendix Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sample Collection Methods</td>
<td>106</td>
</tr>
<tr>
<td>B</td>
<td>Mosasaur Belle Bone and Mineral Sample Collection Summary</td>
<td>112</td>
</tr>
<tr>
<td>C</td>
<td>Considerations for Organics</td>
<td>113</td>
</tr>
<tr>
<td>D</td>
<td>Comparison of structural carbonate treatment techniques</td>
<td>115</td>
</tr>
<tr>
<td>E</td>
<td>Reagent preparations</td>
<td>117</td>
</tr>
<tr>
<td>F</td>
<td>Bone carbonate preparation</td>
<td>120</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belle</td>
<td>KGM0016 Mosasaur Belle, <em>Platecarpus tympaniticus</em></td>
</tr>
<tr>
<td>EDS</td>
<td>Energy Dispersive System</td>
</tr>
<tr>
<td>FHSM</td>
<td>Fort Hays State University’s Sternberg Museum of Natural History</td>
</tr>
<tr>
<td>FHSU</td>
<td>Fort Hays State University</td>
</tr>
<tr>
<td>KGM</td>
<td>Keystone Gallery Museum</td>
</tr>
<tr>
<td>IP</td>
<td>Invertebrate Paleontology Collection FHSM</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>VP</td>
<td>Vertebrate Paleontology Collection FHSM</td>
</tr>
<tr>
<td>VPDB</td>
<td>Vienna-Pee Dee Belemnite</td>
</tr>
<tr>
<td>V-SMOW</td>
<td>Vienna-Standard Mean Ocean Water</td>
</tr>
<tr>
<td>WIS</td>
<td>Western Interior Seaway</td>
</tr>
</tbody>
</table>
# LIST OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta )</td>
<td>Delta notation for stable isotope measurements</td>
</tr>
<tr>
<td>( \delta^{18}O_P )</td>
<td>Delta value of oxygen in bone phosphate</td>
</tr>
<tr>
<td>( \delta^{18}O_C )</td>
<td>Delta value of oxygen in bone carbonate</td>
</tr>
<tr>
<td>( \delta^{18}O_{CC} )</td>
<td>Delta value of oxygen of coexisting calcite cements and bone carbonate</td>
</tr>
<tr>
<td>( \delta^{18}O_{BEL} )</td>
<td>Delta value of oxygen in belemnites</td>
</tr>
<tr>
<td>( \delta^{18}O_M )</td>
<td>Delta value of oxygen in chalk matrix</td>
</tr>
<tr>
<td>( \delta^{13}C_C )</td>
<td>Delta value of carbon in bone carbonate</td>
</tr>
<tr>
<td>( \delta^{13}C_{CC} )</td>
<td>Delta value of carbon of coexisting calcite cements and bone carbonate</td>
</tr>
<tr>
<td>( \delta^{13}C_{BEL} )</td>
<td>Delta value of carbon in belemnites</td>
</tr>
<tr>
<td>( \delta^{13}C_M )</td>
<td>Delta value of carbon in chalk matrix</td>
</tr>
<tr>
<td>( \delta^{18}O_w )</td>
<td>Delta value of oxygen in environmental water</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>Standard deviation</td>
</tr>
</tbody>
</table>
CHAPTER 1: STABLE ISOTOPE BASICS

Isotopes, Delta Notation, and Standards

The stable isotopic ratios in natural compounds are expressed using the “delta” notation, defined as follows:

\[ \delta_{\text{SAMPLE/STD}} (\text{‰}) = \left( \frac{R_{\text{SAMPLE}}}{R_{\text{STD}}} - 1 \right) \times 1000 \]

where \( R_{\text{SAMPLE}} \) represents the value of a sample substance and \( R_{\text{STD}} \) represents a known reference standard value for comparison. This equation refers to the relative difference, in parts per thousand (‰) [permil], of the isotopic ratios of a sample and of a reference standard. Oxygen isotope values are reported as \( \delta^{18}\text{O} \) as a measure of the ratio of stable isotopes \(^{18}\text{O}:/^{16}\text{O}. \) The samples are compared to a lab standard, derived from comparison to an international standard of Vienna-Standard Mean Ocean Water (V-SMOW) (Brand et al. 2014):

\[ \text{V-SMOW } \delta^{18}\text{O} = 0.00 \text{ ‰} \]

Carbon isotope values are reported as \( \delta^{13}\text{C} \) as a measure of the ratio of stable isotopes \(^{13}\text{C}:/^{12}\text{C}. \) Carbon and oxygen isotope samples are compared to the NBS-19 (expressed relative to VPDB [Vienna PeeDee Belemnite]) calcite standard:

\[ \text{VPDB } ^{13}\text{C}/^{12}\text{C} = 1.95 \text{ ‰} \]

\[ \text{VPDB } ^{18}\text{O}/^{16}\text{O} = -2.20 \text{ ‰} \]

Oxygen isotope values in V-SMOW units can be converted to VPDB values using the following conversion equation (Brand et al. 2014).

\[ \delta^{18}\text{O}_{\text{‰}} \text{ VPDB} = 0.97001 \times \delta^{18}\text{O}_{\text{‰}} \text{ VSMOW} - 29.99 \text{‰} \]
Carbonate-based minerals display a variety of $\delta^{13}C$ and $\delta^{18}O$ values correlating to different precipitation and depositional environments of carbonate (CO$_3^{2-}$) (Figure 1). Nelson and Smith (1996) plotted these values on a bivariate plot, modified from Figure 1 of Hudson (1977) to demonstrate these relationships, creating a reference of expected values for carbonate studies. Hudson (1977) reported chalks retain their original carbon isotopic composition, and oxygen isotopic compositions changing very little. Their values range from $\delta^{13}C$ values of 0 to 5‰ VPDB, and $\delta^{18}O$ values of -0.5 to -4.0‰ V-SMOW (Hudson 1977).
Temperature Equations

Multiple authors (Epstein et al. 1953; O’Neil et al. 1969; Anderson and Arthur 1983; Longinelli 1965; Pucéat et al. 2010; Lécuyer et al. 2013) have determined different paleothermometry equations to mathematically recreate physiological; seawater; and mineral deposition temperatures. These equations account for different variances in deposition of carbonate or phosphate. Under equilibrium conditions, the $\delta^{18}O$ of carbonate and phosphatic minerals depends on the temperature of precipitation, which includes biological deposition, and the $\delta^{18}O$ of the ambient water (Koch 1998; Lee-Thorp 2002; Coulson et al. 2011; Grossman 2012; Harrell et al. 2016).

To calculate temperature based on calcite isotopic equilibrium with water, the following equation from O’Neil et al. (1969) has been used.

$$10^3 \ln \alpha = (2.78 \times 10^6)/T^2 - 2.89$$

where $\alpha$ is the fractionation factor equal to:

$$\alpha_{cc-H_2O} = \frac{^{18}R_{cc}}{^{18}R_{H_2O}} = \frac{n_{cc}(^{18}O)/n_{cc}(^{16}O)}{n_{H_2O}(^{18}O)/n_{H_2O}(^{16}O)} = \frac{\delta^{18}O_{cc} + 1000}{\delta^{18}O_{H_2O} + 1000}$$

where $\delta^{18}O_{cc}$ is the $\delta^{18}O$ value of the measured calcite and $\delta^{18}O_{H_2O}$ is the $\delta^{18}O$ value of the precipitating water, both relative to VPDB. $T$ (temperature) is calculated in Kelvin. For the purposes of this thesis, $\delta^{18}O_{cc}$ will be defined as either $\delta^{18}O_{BEL}$ or $\delta^{18}O_{M}$ for the belemnites or the coccolithophore (chalk) matrix (See List of Symbols).

For carbonates, this relationship assumes isotopic equilibrium between calcite (CaCO$_3$) based shells and the ambient water. Many different factors can affect this equilibrium. Partitioning of $^{18}O$ and $^{16}O$ during calcite precipitation changes directly as a function of temperature (Grossman 2012).
A second equation determined by Anderson and Arthur (1983), modified from Epstein et al. (1953), calculated calcite precipitation temperatures relative to V-SMOW.

\[ T^\circ C = 16.0 - 4.14(\delta c - \delta w) + 0.13(\delta c - \delta w)^2 \]

where \( \delta c \) is the \( \delta^{18}O \) value of the measured calcite and \( \delta w \) is the \( \delta^{18}O \) value of the precipitating water, both relative to V-SMOW. The calculated difference in temperature is less than 1\(^\circ\)F between the two equations. Therefore, for this study, the Anderson and Arthur (1983) equation will be utilized for the belemnites’ calculated temperatures due to the direct relationship to mollusks. Coccolithophores too are assumed to grow their tests in isotopic equilibrium with the seawater.

For phosphates, bone and enamel are the primary medium studied. Bone \((Ca_{8.3}(PO_{4})_{4.3}(CO_{3})_{3x}(HPO_{4})_{y}(OH)_{0.3})\) is a complex, interwoven lattice of crystalline hydroxy-carbonate-apatite and organic tissue (Vallet-Regí and Naverrette 2015). Carbonate \((CO_{3}^{2-})\), biphosphate \((HPO_{4}^{2-})\), and phosphate \((PO_{4}^{3-})\) ions interchange within the lattice network depending on osteoblast formation or osteocyte breakdown of bone (Vallet-Regí and Naverrette 2015). Oxygen atoms are a major component of each of these anions. A natural material’s oxygen isotopic composition is dependent on two variables: the material formation temperature and the oxygen isotopic composition of the water from which the material precipitates (Coulson et al. 2008). Oxygen naturally occurs as three stable isotopes: \(^{16}O\), \(^{17}O\), and \(^{18}O\). Less energy is required to incorporate a lighter isotope than a heavier one. The more energy available (the higher the ambient temperature), the greater preference for lighter atomic masses. Therefore, bone records its formation temperature.

Longinelli (1965) derived the first phosphate temperature equation:

\[ T(\circ C) = 111.4 - 4.3(\delta p - \delta w) \]
where $\delta_p$ is equal to the $\delta^{18}O$ value from a phosphate sample and $\delta_w$ is the $\delta^{18}O$ value from the metabolic (body) water of the vertebrate, fish in this study. For this thesis, $\delta_p$ will be defined as $\delta^{18}O_p$ and $\delta_w$ is defined as either $\delta^{18}O_w$, for environmental water, and $\delta^{18}O_{bw}$, for body water (See List of Symbols).

Multiple studies (Longinelli and Nutti 1973; Kolodny et al. 1983; Kolodny and Raab 1988) further refined the paleotemperature equation from the phosphates of fish bone and teeth and other vertebrates.

Puceát et al. (2010) further refined the paleotemperature equation, below, by studying the bone phosphate of aqua-cultured fish raised at a controlled temperature and water monitored for its oxygen isotope signature.

$$T(\circ C) = 118.7 - 4.22[(\delta^{18}O_{PO4} + (22.6 - \delta^{18}O_{STD})) - \delta^{18}O_w]$$

$\delta^{18}O_{PO4} [=\delta^{18}O_p]$ is the oxygen isotope value of the fish bone and enamel phosphate. $\delta^{18}O_{STD}$ is the oxygen isotope value of the standard NIST 120c included with the bone phosphate reactions to check for chemistry errors and precision of the mass spectrometer. The standard is used in this equation as a correction. $\delta^{18}O_w$ is the oxygen isotope value of the environmental water.

Lecuyer et al. (2013) published the most current refinement of the equation to reflect the accuracy and precision of newer mass spectrometers and computer capabilities.

$$T(\circ C) = 117.4 - 4.5 * (\delta^{18}O_p - \delta^{18}O_w)$$

$\delta^{18}O_p$ is the oxygen isotope value from bone and enamel, and $\delta^{18}O_w$ is the value for the environmental water. This equation is calculated from the deposition of phosphate in equilibrium with calcite in brachiopods, ammonites, and fish. One consideration for this equation is that it does not allow for vital effects in vertebrates. A vital effect value ($\%$) will be added to the $\delta^{18}O_w$ value to compensate for body water fractionation.
INTRODUCTION

Belemnites are an extinct group of Mesozoic Era, coleoid cephalopods related to extant squids. Belemnites possessed a soft-tissue outer body that enclosed a hard, internal rostrum. Hoffman and Stevens (2020) summarized the possible purposes of the rostrum: 1) a supporting structure or muscle attachment site for the posterior fins (Figure 2); 2) a hydrostatic device, like a sepiid’s (cuttlefish) cuttlebone; 3) protection of the phragmocone; 4) compensation for positive buoyancy; 5) or a counterweight to the soft body.

Composed of low-Mg calcite, these rostrums precipitated proportionately to the temperature and isotopic signatures of the environmental water (Dutton 2007; Weirzbowski 2019; Hoffman and Stevens 2020). As poikilotherms, this relationship preserves the ambient water temperature in which these animals lived. This form of calcite is recognized as being resistant to alteration, becoming a candidate for chemical and biological studies (Spaeth et al. 1971; Stevens and Clayton 1971; Wierzbowski 2009; Hoffman and Stevens 2020). Typically, the rostrums are the only preserved remains of these animals (Figures 2, 3, and 7).

Figure 2: Generalized reconstruction of an extinct belemnite. The rostrums found with Belle are the only remnants of these squid-related cephalopods. Figure 7 from Hoffman and Stevens, 2020.
Belemnite fossils in the Niobrara Chalk are uncommon to rare (Everhart 2017) This rarity is confirmed from conversations with two of the leading paleontologists studying the Niobrara Chalk based on their field observations (M. Everhart personal communication, 2020-2022; A. Maltese, personal communication, 2022). Invertebrate fossils, outside of the common inoceramid clams, are rare in the Niobrara Chalk possibly from preservation bias due to the dissolution of aragonite and calcite back into the seawater (Everhart 2017). Another possible taphonomic bias for cephalopods could be from body fluids containing high levels of ammonia, changing their pH during decomposition to preclude them from fossilization (Clements et al. 2017). Four belemnite species, all described in the genus Actinocamax, are known from the Smoky Hills Member of the Niobrara Chalk (Jeletzky 1961; Miller 1957).

Compared to the Western Interior Seaway, belemnites are well-known from other epicontinental seaway deposits across the world. In North America, the Sundance Formation preserves the sediments of the Sundance Sea, an inland sea that extended from northwestern Canada to Wyoming during the middle Jurassic period (McMullen et al. 2014). In Europe, belemnite species are utilized as biostratigraphic markers throughout Jurassic and Cretaceous beds (Neige et al. 2021; Mitchell 2005). Belemnite rostrums are so plentiful, large assemblages are referred to as “battlefields” from their resemblance to bullets (Doyle and MacDonald 1993; Hoffman and Stevens, 2020). These assemblages have allowed for studying their ontogenetic growth stages, spatial ecologies relative to the interpreted lithofacies; and temperatures of the waters they inhabited (Wierzbowski, 2009; Hoffman and Stevens, 2020).

In stable isotope geochemistry, the belemnite, Belemnitella americana, from the Peedee Formation of North and South Carolina is used as the main standard of comparison in determining the isotopic composition of carbon and oxygen (Hoefs 2018). The belemnite
rostrums provided accurate, precise, and consistent measurements to create a reference standard for isotope ratios, denoted as PDB. This standard is referred to as VPDB (Vienna Peedee Belemnite) as the original material was depleted. Belemnite rostrums are composed of low-Mg calcite. Due to their crystalline structure, they are more resistant to diagenetic alteration (Spaeth et al. 1971; Wierzbowski 2009; Hoffman et al. 2021) and are assumed to form in isotopic equilibrium with seawater and seawater temperature. Therefore, these animals became an ideal candidate to recreate paleotemperatures of the epicontinental seaways these animals lived in.

Belemnites are interpreted to have lived in shallow, offshore coastal settings, or epipelagic zones. This determination is based on (1) belemnite depositional facies, (2) modeled mechanical limitations of the rostrums, (3) isotopic data, and (4) comparisons to modern coleoids (Hoffman and Stevens 2020).

Belemnites are commonly found in lagoonal and shallow water marine facies deposits. However, they become quite sparse in deeper water settings interpreted as mesopelagic and bathypelagic (Immenhauser 2009). This contrast is apparent in the number of rostrums collected from deep water chalks and shales than in limestones and shallow, silt-rich coastal deposits (A. Maltese, personal communication, 2022; personal observation). These depositional environments differed in water depth ranging from 50 to 200 meters.

*Sepia officinalus*, the common cuttlefish, is one of the few modern coleoids that possesses an internal calcified structure, the cuttlebone. The cuttlebone can withstand pressures at 200 meters (Denton 1974). Modeling the cuttlebone structure to belemnite rostrums, belemnites are estimated to inhabit waters ranging in depth from 1-250 meters (Hoffman and Stevens 2020).
Isotopic data from multiple belemnite species and studies (Spaeth et al. 1971; Stevens and Clayton 1971; Wierzbowski and Joachimski 2009; Armendáriz et al. 2012) provides a range of $\delta^{13}$C and $\delta^{18}$O values. These values are dependent on the bicarbonate ions (HCO$_3^-$) in the environmental water. Belemnites are assumed to deposit their rostrums in equilibrium with the ambient conditions, assumed to relate directly to bottom water temperature signals (Wierzbowski 2013; Wilmsen and Niebuhr 2017).

Modern coleoids occur in all marine habitats but are restricted to salinity concentrations of 27-37 practical salinity units (psu) and high oxygenation (Jereb and Roper 2005). Depleted dissolved oxygen zones occur at >500 m depths in the modern oceans and assumed to have occurred in Mesozoic oceans (Takashima et al. 2006). Sepiids live at temperatures between 10-30 $^\circ$C (Jereb and Roper 2005). Benthic squids migrate from deeper waters to the surface at night to feed on prey, using the warmer temperatures for increased metabolisms for hunting (Watanabe et al. 2006; Rosa and Siebel 2010; Judkins and Vecchione 2020). These interpretations infer belemnites lived in 1-200 m of water depth, salinity between 27 and 37 psu, and temperatures that ranged from 10 to 30°C (Hoffman and Stevens 2020).
METHODS

Fort Hays State University’s Sternberg Museum (FHSM) houses 26 specimens of belemnite rostrums from the Niobrara Formation, representing four species under the genus *Actinocamax* (Miller 1957; Jeletzky 1961). Jeletzky (1961) calculated two ratios: maximum dorso-ventral diameter to overall length and maximum lateral diameter to overall length, to identify each species. Belemnite rostrums also exhibit shapes in longitudinal- and cross-section, correlating to each species. Lateral grooves in apical (posterior) and alveolar (anterior) views distinguish species further (Figure 2) (Hoffman and Stevens, 2020).

Each holotype specimens’ anterior end was photographed with a Canon® T6 Rebel digital camera with an 18-55 mm Canon® lens, an 18-135 mm Canon® lens, a Macro lens, and a Cross-Polarized-Light filter. Lateral and dorsal lateral ovate shapes along with any surface ornamentation were documented. Rostrums were measured for length and diameter with Pittsburgh® 12 in. digital calipers to build a database of measurement ratios to their described genus and species. Jeletzky’s (1961) description of each holotype provided measurements and information for identification.

An in-situ, isolated belemnite rostrum on the left, lateral side of Belle’s dorsal vertebra 10 was selected for comparison to these holotypes. Only the ventral and lateral surfaces are exposed for investigation. Measurements of the rostrum’s maximum length and posterior, middle, and anterior diameters were recorded for comparison. Direct comparison of photos and measurements of the holotype specimens with Belle’s belemnites determined the species identification.
RESULTS

Following the description of FHSM IP-705 by Jeletzky (1961), a general description of Belle’s associated belemnites is given.

SYSTEMATIC PALEONTOLOGY

CEPHALOPODA Cuvier, 1797

BELEMNITIDA Zittel, 1895

BELEMNITELLIDAE Cobban, 1983

ACTINOCAMAX Miller, 1823

The belemnite rostrums are identified belonging to the genus and species *cf. Actinocamax sternbergi* (Jeletzky 1961). They are identified through both morphological characteristics and length and diameter measurements of the species (Mutterlose 1983; Doyle 1990; Schlegelmilch 1998; Hoffman and Stevens 2020) Macroscopic photography reveals surface granulation, a compressed cross-section, and a hastate, ortholineate longitudinal section indicative of *A. sternbergi* (Figure 3). The overall length of the isolated rostrum is 61.35 mm. Measured lateral and dorsoventral diameters at the anterior end are 6.54 mm and 7.40 mm respectively, and 7.50 mm in lateral diameter at the midpoint. Due to the rostrum’s embedment, the posterior end could not be measured, nor a dorsoventral diameter measurement taken at the midpoint. The overall length to maximum dorsolateral diameter is calculated to 0.12 compared to Jeletzky’s (1961) ratio of 0.16. Measurements are compared in Table 2. The surface granulation, 0.5-1 millimeter in size and randomly distributed is only attributed to two species, *A. sternbergi* and *A. groenlandicus*. Table 1 provides a detailed overview of each characteristic.

Jeletzky (1961) makes a direct note that *A. sternbergi* is possibly a subspecies of *A. groenlandicus*, a belemnite species that is documented in European deposits. Doyle and Bennett
(1995) concluded that *A. groenlandicus* lived during the earlier Santonian stage of the late Cretaceous period.

<table>
<thead>
<tr>
<th>Rostrum Characteristic</th>
<th>Match</th>
<th>Obscured</th>
<th>Different</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guard large and moderately stout, slightly lanceolate in ventral aspect</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>entire surface covered with closely spaced, small granules, which generally show no definite arrangement</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>the alveolar end flatly truncated but with a protruding low, rounded mound in the middle</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>a small, rounded hole with the protoconch at its bottom at the top of the mound</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>gaping ventral fissure runs across the ventral side of the alveolar end beginning only about 0.5 mm above the protoconch, extending ~ 8 mm below the rim of the alveolar end on the ventral side of the guard</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>double dorso-lateral furrows are well defined, shallow, flat-bottomed, and unusually wide (0.8-1.2 mm), separated from each other by well defined, unusually wide (0.9-1.1 mm) interspaces</td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. List of characteristics from the diagnosis of *Actinocamax sternbergi* (Jeletzky, 1961). The specimen’s associated belemnites share 3 of the 7 defined characters of *A. sternbergi*. Match = feature is present; obscured = feature obscured by matrix; different = does not match diagnosis.
<table>
<thead>
<tr>
<th>Specimen # &amp; Species</th>
<th>Length (mm)</th>
<th>Anterior, Lateral Diameter (mm)</th>
<th>Anterior, Vertical Diameter (mm)</th>
<th>Middle, Lateral Diameter (mm)</th>
<th>Posterior, Lateral Diameter (mm)</th>
<th>Length: Middle, Lateral Diameter Ratio</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP-705 <em>A. sternbergi</em></td>
<td>88.98</td>
<td>11.22</td>
<td>12.19</td>
<td>13.89</td>
<td>4.89</td>
<td>0.16</td>
<td>tip pinches in laterally; converging furrows on dorsal surface; single deep furrow on ventral surface; surface projections</td>
</tr>
<tr>
<td>KGM0016</td>
<td>61.35</td>
<td>6.54</td>
<td>7.4</td>
<td>7.5</td>
<td>-</td>
<td>0.12</td>
<td>disassociated from main group; surface projections; pitted surface; short, deep ventral furrow</td>
</tr>
</tbody>
</table>

Table 2. Measurements (mm) of FHSM IP-705 compared to isolated rostrum with KGM0016.

Figure 3 Top: Dorsal view of holotype of *Actinocamax sternbergi*, FHSM IP-705. Bottom: In-situ, isolated belemnite rostrum positioned next to vertebral column. This rostrum exhibits both surface projections, a decorative and functional feature for tissue attachment, and pitting from acid dissolution from digestion. Comparing the two rostrums, the specimen’s belemnites compare favorably with *A. sternbergi*. 
DISCUSSION AND CONCLUSIONS

Evidence for Digestion of Belemnites

A fundamental component of studying KGM0016 is the assumption that these animals lived concurrently, and the belemnites became Belle’s last meal. The rostrums are aligned pointing posteriorly within the cavity, suggesting that the belemnites were ingested headfirst. The rostrums could then be forced together into a bolus within the stomach or in the proximal small intestine. Small, random pitting (<1mm in diameter) is present on the belemnite rostrum cluster and the isolated rostrum. Formation of these pits from seawater, groundwater, or diagenetic alteration from high pH, carbonate-rich solutions is unlikely as the calcite-rich chalk becomes a buffer to changes in pH. Dissolution from stomach acids is a more plausible cause. Belemnite specimens identified as gut contents in a hybodont shark, plesiosaurs, pliosaurids, ichthyosaurs, and the metriorhychid crocodile, Metriorhynchus, also exhibit these pitting features associated with stomach acid digestion (Hoffman and Stevens 2020). Tooth marks on the rostrums are not present. However, further preparation of the cluster may reveal them.

The probability of these belemnites being preserved in a tight cluster from another cause is unlikely. While modern cephalopods are known to feed on decomposing animals on the ocean floor, their simultaneous deaths would be unlikely. If scavenging was occurring on Belle, other animals’ body and trace fossils would also be preserved with Belle. No evidence of predation or scavenging is expressed on Belle or her associated belemnites. However, scavenging and predation cannot be ruled out on the missing tail, posterior paddles, and posterior body cavity. Deep ocean currents are also not powerful enough to cause imbrication and stacking of the rostrums into a tight, piled bundle against Belle.
An important observation to note is the body’s position before burial. Belle’s current position, dorsal side up, is not the original orientation. The body’s original orientation was ventral side up: the body sank to the ocean floor belly-up. The current presentation is due to the jacketing process of Belle, requiring Belle to be flipped over for transportation. The belly-up orientation means the internal organs could shift from within the body cavity to outside next to Belle. This movement could explain how the individual rostrum became dislodged from the cluster and preserved underneath the vertebral column. This interpretation explains how the cluster became preserved close to Belle’s lower jaw. Therefore, the belemnites are interpreted to be contemporary to Belle as her last meal and preserved stomach contents.

Importance of the Belemnites

Belemnites are rare in the Smoky Hill Chalk. Actinocamax sternbergi is only known from one rostrum. The holotype is housed in the Sternberg Museum’s Collections (FHSM). Actinocamax is the only known, described genus of belemnite from the Niobrara Chalk. At FHSM, the holotypes of the three other species of Actinocamax (A. laevigatus, A. manitobensis, A. walkeri) are also housed. Of these species, the collection is only comprised of 24 rostrums (A. Baumgartner, personal communication, 2020-2022; Collective Access Online Portal). One belemnite rostrum from the Niobrara Chalk is at the Rocky Mountain Dinosaur Research Center in Woodland Park, Colorado (A. Maltese, personal communication, 2022). Two rostrums attributed to Actinocamax from the Niobrara Chalk are housed at the KU Biodiversity Institute & Natural History Museum (Specify 6 Collections Search). Six rostrums are housed at the Keystone Gallery (C. Bonner, personal communication, 2020-2022). A search of various other institutions collections found no other known Actinocamax rostrums from the Niobrara Chalk. Belle is adding a minimum of 13 rostrums to the 34 known belemnites collected.
CHAPTER 3: MOSASAUR THERMOPHYSIOLOGY
AND CONCURRENT SEAWAY TEMPERATURES

INTRODUCTION

Mosasaurs, ancient marine reptiles, dominated the Late Cretaceous epoch oceans. Their fossils have been found on every continent (Russell 1957; Martin et al. 2002; Konishi et al. 2016). Before the end of the Mesozoic era, these animals evolved into a wide array of species: the massive, macrophagous Mosasaurus sp. and Prognathodon sp.; the durophagous Globidens sp. and Carinodens sp., eating hard-shelled invertebrates (Massare 1987; Martin 2007); and even Plotosaurus sp. and Galvialimimus evolving fusiform body shapes (Lindgren et al. 2009; Strong et al. 2020). However, their ecological dominance has always presented a question. What physiological characteristics enabled them to radiate across the world’s oceans? One characteristic could be thermoregulation.

Thermoregulation is an animal’s physiological ability to maintain their core body temperature. Most animals fall into three physiologies: poikilothermic, ectothermic, or endothermic. Poikilothermy is the pattern of an animal’s internal body temperature fluctuating relative to its ambient environment’s temperature (IUPS Thermal Commission 2001; Guschina and Harwood 2006). Poikilothermic animals, such as aquatic invertebrates, fish, and amphibians, function over a wider range of temperatures. However, their biological processes are dependent on specific temperature ranges for chemical reactions to occur (Cavalier-Smith 1991).

Ectothermy is the pattern of animals’ temperature regulation in which body temperature depends mainly on the behaviorally controlled exchange of heat with the environment (IUPS Thermal Commission 2001; Ivanov 2006). Relative to poikilotherms, most reptiles are ectothermic, requiring and seeking an external heat source to warm and maintain their bodies at
an optimum biological temperature range (Harlow et al. 2010). Temperature maintenance is usually achieved by basking in sunlight, varying their exposure through shade and indirect light. Extant marine reptiles like hard-shelled sea turtles, sea snakes, sea kraits, and marine iguanas rely on basking on land or at the sea surface to regain body heat (Favilla and Costa 2020).

In contrast, endothermy is an animal’s pattern of temperature regulation in which the body temperature depends on a high and controlled rate of heat production primarily from metabolic processes (IUPS Thermal Commission 2001; Ivanov 2006). Mammals maintain elevated body temperatures through high metabolic rates releasing large quantities of heat and energy (Ivanov 2006). Within endothermy, animals can be classified as being homeothermic or heterothermic. Homeothermy is the condition of endothermic thermoregulation that maintains a stable internal body temperature regardless of outside influences (McNab 1978), maintaining core temperatures within ± 2°C (Scholander, 1955). Heterothermy is an endothermic condition with a self-regulating body temperature but allows the surrounding environment to affect it (McNab 1978). Some animals, such as bats and birds, exhibit this physiology to combat cold weather and reduce their metabolism during hibernation (Hut et al. 2002; Tøein et al. 2011). Warm-blooded animals remain active at all times of the day and endure harsher temperatures in higher latitude environments.

What thermoregulation strategy did mosasaurus use? Observed rapid postnatal bone growth in ichthyosaurs inferred a rapid ontogenetic development that might have been related to a “high, ‘endothermic-like’ metabolic rate” (de Buffrénil and Mazin 1990). Mosasaurus have also been inferred to be ectothermic from their skeletal similarities to modern varanid lizards and snakes (Massare 1994). However, modern reptilian metabolisms possibly could not be compatible with many marine Mesozoic reptiles (Motani 2002). Their large body sizes would
have allowed them to regulate body temperature to some extent. Histological studies of a juvenile *Clidastes sp.*, a small mosasaur, revealed a high metabolism during ontogenetic growth, suggesting an endothermic physiology (Houssaye and Tafforeau 2012; Houssaye 2013). As skeletal form and histology provide some insight, what about a mosasaur’s lifestyle as a marine reptile?

Marine, air-breathing vertebrates contend with significant thermoregulatory challenges due to the high thermal conductivity of water (Favilla and Costa 2020). Heat transfers from a source to its environment 25 times faster in water than in air. An air-breathing, aquatic animal faces not only the changes in temperature across its habitat, but also temperature changes in minutes to seconds as it dives to access food or return to the surface for air (Favilla and Costa 2020). To retain heat, marine mammals rely on a thick layer of blubber, insulating their bodies from rapid heat loss. Most modern marine reptiles are reliant on external heat sources to maintain their body temperature. An important exception in this group is the leatherback turtle (*Dermochelys coriacea*). These turtles are unique as the only modern, large marine reptiles to have an elevated body temperature. Leatherback turtles maintain their body temperature about 8°C above the ambient temperature of the seawater (James and Mrosovsky 2004). Most reptiles become lethargic in cold temperatures. Their elevated body temperature is attributed to three factors: an insulating oily layer and blubber, high physical activity generating heat, and countercurrent heat exchange (James and Mrosovsky 2004; Davenport et al. 2015). These turtles instead migrate long distances from the poles to the equator, preferring to feed on jellyfish in temperate, cold waters of about 15-17°C (James and Mrosovsky 2004). The potential for elevated thermoregulation in other, extinct reptiles therefore could be a possibility.
Mosasaur fossils are found in polar regions (Martin et al. 2002). Their presence could infer their body temperature adaptation to these climates, like the physiology of a leatherback turtle. Sea surface temperatures in the northern Tethys Ocean are estimated at \(~20^\circ C\) (Jenkyns et al. 2004). Mosasaur body temperatures could have been like leatherback turtles or even warmer. Blubber, or fat, deposits are unknown in mosasaur to provide heat insulation.

One method to analyze extinct animals for their preserved body temperature signatures is geochemical stable isotope analysis. Utilizing this method, some studies focus on the preserved concentration ratios of $^{18}O$ to $^{16}O$ atoms (See Chapter 1) within tooth enamel and bone bioapatite to reveal elevated thermoregulation of body temperature in multiple extinct species (Kolodny et al. 1983; Kolodny and Raab 1988; Barrick and Showers 1994; Barrick and Showers 1996; Barrick 1998; Koch et al. 2007; Harrell et al. 2016). Specific temperature signatures are captured in the preferential deposition of lighter oxygen isotope-bearing phosphate ions correlating to elevated temperature (Longinelli 1965; Kolodny et al. 1983; Coulson et al. 2008).

Multiple studies applied these methods to explore the body temperatures of marine reptiles. Bernard et al. (2010) examined ichthyosaurs, plesiosaurs, and mosasaur from 16 different localities across the globe. Their study compared tooth enamel values from each group to coexisting fish found within the same formations. A direct temperature comparison of the marine reptiles to the ambient water temperature preserved in the poikilothermic fish revealed contrasting values. Ichthyosaurs and plesiosaurs exhibited a mean temperature of $26 \pm 2^\circ C$. Mosasaur exhibited a higher, mean body temperature of $30 \pm 2^\circ C$. The fish expressed temperatures from a temperate $12 \pm 2^\circ C$ to a quite warm $36 \pm 2^\circ C$. The coeval fish may not have come from the same layers, suggesting that unknown amounts of time occurred in between deposition of the fossils.
Motani (2010) concurred with Bernard et al.’s (2010) study, however noting “some fish might survive in waters that warm, (but) they would not be able to grow under such stressful conditions.”

Harrell et al. (2016) compared the cortical bone and tooth enamel phosphate-oxygen isotope signatures from three different mosasaur genera within the Western Interior Seaway of North America to the bone and tooth enamel phosphate signatures of coeval fish and birds. The mosasours, *Clidastes, Platecarpus*, and *Tylosaurus*, exhibited average body temperatures of 33.1°C, 36.3°C, and 34.3°C respectively. None of the specimens in the study occurred at the same stratigraphic horizon, nor the same locality from the mosasours’ collection sites.

These studies lack directly associated poikilothermic or ectothermic organisms for strongly contrasting temperatures to distinguish an elevated thermophysiology within mosasours. A semi-complete mosasour fossil with gut contents of belemnites (See Chapter 2) from western Kansas will attempt to resolve this problem as their coexistence presents an opportunity to directly compare organisms from the same moment in time. Through isotopic analysis of bone, belemnites, and chalk, an elevated thermophysiology in mosasours could be revealed by comparing the body temperature of a mosasour to its coeval, final meal and the coccolithophores living in the seawater.
BACKGROUND

Geologic Setting

During the Late Cretaceous epoch, the North American tectonic plate moved west, overriding the Farallon plate. This movement created mountain uplift and basin subsidence known as the Sevier orogeny. During these tectonic events, a continent-wide foreland basin was created, extending from Mexico to Canada. This basin flooded with ocean water as subsidence occurred from sediment infilling from the rising Rocky Mountains. At its maximum extent, this flooded basin connected the northern Tethys Ocean with the Gulf of Mexico, creating an epicontinental seaway known as the Western Interior Seaway (WIS) (Figure 4) (Coulson et al. 2016). In western Kansas, the WIS is preserved as a series of marine, transgressive-regressive units of sandstone, shale, limestone, and chalk (Decelles 2004; Yonkee and Weil 2015). The Niobrara Formation consists of two members, the Fort Hays Limestone and the Smoky Hill Chalk (Meek and Hayden 1862; Hattin 1982). The Smoky Hill Chalk, specifically, is about 600 feet thick in Kansas and is built primarily of the calcite tests of brown algae, coccolithophores, that flourished in the surface waters (Everhart 2017; Hattin 1982). These chalk beds also preserve the fauna and flora that flourished in the WIS, including the top predators, mosasaurs.

Stratigraphic Position

Geologic context for this specimen creates a temporal background for interpretation. To discern when Belle lived, the original fossil discovery site needed to be relocated. Using personal notes and memory, Mr. Bonner relocated the quarry in a locality he referred to as the “Valley of the Mosasaur.” Prior to arrival, a desktop review of geologic maps, aerial photography, and a county map using the Public Land Survey System (PLSS) map coordinates from Mr. Bonner
Figure 4: A county map of the state of Kansas depicting the Niobara Chalk’s extent. This map is an exploded view in relation to a reconstruction of the Western Interior Seaway during the late Santonian stage of the late Cretaceous period. The red star designates Belle’s locality in south-central Logan County. Modified Kansas county map from Kansas Geological Survey (Google Images); modified Western Interior Seaway 84 MA Santonian (Late) map from DeepTimeMaps.com.

provided an approximate stratigraphic placement in the upper chalk of the Niobrara Formation. A GPS location using the onX: Hunt application, a smartphone application, was taken at the quarry’s locality (onX: Hunt, 2021). At the locality, by cross-referencing the original localities of Don Hattin’s geologic mapping of the Niobrara Formation in western Kansas (Hattin, 1982; Bennett, 2000), a pedestrian survey of the outcrops located distinctive marker beds. These marker beds consist of laterally and regionally continuous intervals of bentonite layers with distinct thicknesses and iterating layers of bentonite and chalk. (Hattin, 1982; Bennett, 2000). Reference units of known stratigraphic distance above or below the marker unit also provide additional features to further identify these units. A Jacob’s staff was used to measure the distance from the top of one of these reference units to the base of the quarry.
The specimen’s quarry lies 139.15 meters above the base of the basal contact of the Niobrara Formation and the underlying Carlile Shale. Within the stratigraphic section, the specimen’s quarry is 6.95 meters above Hattin’s (1982) Marker Unit 15. Marker Unit 15 is a unit of three distinct beds. The bottom bed is a 5-10 cm thick layer of cream-white chalk. The middle bed is a 30-50 cm thick bed of granular chalk. The top bed is a series of thinly laminated, darker color chalk overlain by a ferruginous, 5 cm thick bentonite seam, denoting the top of the unit. Within the eroded drainage, this unit is distinguished by the cream-white band of chalk, capping low-relief exposures. At the quarry site (Figure 5), two, thin ferruginous bentonite layers separated by 13-15 cm of chalk are a direct reference aid 1.6-1.8 meters above Marker Unit 15. This unit dates Belle to the Santonian stage of the Late Cretaceous epoch.
Figure 5: Top-Left: Modified, generalized stratigraphic column of the Niobrara Chalk with Belle’s locality marked in blue. Top-Right: Profile view of Belle’s locality, denoted by blue arrow, showing two bentonite seams, denoted by blue arrows, as a reference unit 1.6 to 1.8 meters above Marker Unit 15. Photo looking north. Bottom: Overview of “Valley of the Mosasaur” with red arrows denoting eroded, cream white chalk layer marking the base of Marker Unit 15.
Mosasaur Belle

Chuck Bonner discovered a small, coiled mosasaur, KGM0016 (Figure 6), in the late 1990s on private property in south-central Logan County, Kansas, USA (Figure 4). This mosasaur is composed of a partially complete, semi-articulated skeleton with a complete skull, partial, articulated vertebral column, and dissociated front paddles. While preparing the specimen for display, Mr. Bonner uncovered what appeared to be this mosasaur’s last meal: belemnites in the form of a tightly packed cluster of rostrums within the animal’s stomach (Figure 7). Recognizing the fossils, he affectionately renamed the mosasaur “Mosasaur Belle,” or simply Belle, as a nod to its last meal. Finding a semi-articulated, associated specimen is rare. Finding a fossil with its last meal is even rarer, making Belle a truly remarkable fossil.

Mosasaurs

Mosasaurs are an extinct group of large, marine reptiles that lived during the Late Cretaceous epoch. Adapted to a completely aquatic lifestyle, these animals became the apex predators of the ocean (Everhart 2017). The closest modern relatives to mosasaurs are thought to be snakes (Caldwell 1999; Everhart 2017) and varanid lizards, such as water monitors and Komodo dragons. Exploiting their heritage, mosasaurs adapted their kinetic jaws and pterygoidal teeth (Russell 1967) to eat anything within their ecosystems that could fit in their mouths (Massare 1987; Everhart 2017). These animals evolved into a wide range of sizes, with unique dentition to eat both hard and soft prey items (Massare 1987; Konishi et al. 2011; Everhart 2017).

Multiple mosasaur specimens are known with associated gut contents. These last meals ranged from sea turtles, fish, plesiosaurs, sharks, bivalves, and a smaller mosasaur (Konishi et al. 2014). Only one mosasaur specimen, a *Plioplatecarpus marshi*, is known to have two belemnites as associated gut contents (Russell 1967; Massare 1987; Konishi et al. 2014).
Figure 6: Full relief view of specimen KGM0016, Mosasaur Belle. The blue circle denotes this mosasaur’s last meal: belemnites, squid-like cephalopods. Scale bar = 50 cm.

Figure 7: In-situ, belemnite cluster within Belle. Note the belemnite rostrums are all oriented with the posterior ends in the same direction.
Mosasaur Taxonomic Identification

Following the osteological description of LACM 128319, a Platecarpus tympaniticus, and the emended diagnosis of the genus and species by Konishi et al. (2010), a general osteological description for Belle is given.

SYSTEMATIC PALEONTOLOGY

REPTILIA Linnaeus, 1758
SQUAMATA Oppel, 1811
MOSASAURIDAE Gervais, 1852
RUSSELLOSAURINA Polcyn and Bell, 2005
PLIOPLATECARPINAE Dollo, 1884

PLATECARPUS Cope, 1869a

Referred Material-KGM0016, discovered in south-central Logan County, Kansas, United States.

KGM0016 shares 17 of 33 defined characters with the emended diagnosis of P. tympaniticus. Table 3 provides a detailed overview of each characteristic. The other 16 described characters are either obscured with matrix and further preparation of Belle is required to reveal them; or the features were not preserved with Belle due to fossilization or erosion of the exposed skeleton prior to discovery. After diagnosing these characteristics, KGM0016 is designated to the genus and species cf. Platecarpus tympaniticus.

Belle does not exhibit 16 of the defined characteristics of Platecarpus tympaniticus from either being obscured or missing from weathering and erosion. Accounting for this discrepancy, other known mosasaurs at that geologic time include Clidastes sp., Ectenosaurus sp., and Tylosaurus sp. (Carpenter 2008). Each genus, including Platecarpus, is distinguished by tooth
count, number of vertebrae, size and shape of the paddles, the quadrate’s morphology, and overall size (Russell 1967). However, Belle’s incomplete preparation and overall partial skeleton prohibits direct comparisons. Belle’s vertebrae do not resemble those of *Clidastes* *sp.* *Clidastes* *sp.* vertebrae are remarkably like python vertebrae (Everhart 2017), making this condition a defining character of the genus. Belle’s vertebrae do exhibit the procoelus vertebral form known in squamates but compare more favorably with the dorsal vertebrae of *Platecarpus*.

*Ectenosaurus* is described with a long, narrow snout (Russell 1967; Everhart 2017). Belle’s snout is overall shorter in length and the palate is wider than that of *Ectenosaurus*. Additionally, Belle’s quadrate in lateral aspect compares more favorably with *Platecarpus*’ “circular-form” than the “question-mark” form of *Tylosaurus*. *Platecarpus* specimens have been the most common mosasaurs collected from the Niobrara Chalk (Everhart 2017). For these reasons, Belle is recognized as a *Platecarpus tympaniticus*. This study also recognizes the position of Konishi et al. (2010) that *P. tympaniticus* is the only species recognized for the genus *Platecarpus*
<table>
<thead>
<tr>
<th>Skeletal Characteristic</th>
<th>Match</th>
<th>Obscured</th>
<th>Not Preserved</th>
</tr>
</thead>
<tbody>
<tr>
<td>no predental rostrum on premaxilla</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>first pair of premaxillary teeth procumbent</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>prefrontal posteriorly contacting postorbitofrontal above orbit</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>frontal supraorbital border thin to slightly thickened lateral to prefrontal- postorbitofrontal contact</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>frontal median dorsal keel present</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>pair of broadly shallow parasagittal excavations extending in parallel with the keel on dorsal side</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>supraorbital bulge distinct</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>parietal foramen anterior border occurring within one foramen length from frontal- parietal suture</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>parietal table triangular, wider than long</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>parietal table lateral borders posteriorly converging with weak convexity</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>parietal crest typically obtuse angled</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>quadratocephalic condyle anterior border straight</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>same condyle with round, obtuse anteromedial corner</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>stapedial pit oval with straight lateral borders</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>medial vertical ridge broadly rounded</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>quadrato shaft with or without slight posteroventral eminence with straight posterior border</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>mandibular condyle transversely wide, teardrop shaped in outline with its apex pointing anteromedially</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>suprastapedial process distally terminating in rounded expansion</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>basal tubera moderately well developed but widely separated from each other</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>coronoid process moderately developed</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>Characteristic</td>
<td>Match</td>
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<tr>
<td>-------------------------------------------------------------------------------</td>
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<td></td>
<td></td>
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<tr>
<td>coronoid posterior border posteriorly descending at about 45 degrees from</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>horizontal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>retroarticular process rounded</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eighth intercentrum (hypapophyseal peduncle) absent on seventh cervical</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>centrum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>zygapophyses functional throughout pre-pygal series</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 to 23? dorsal vertebrae</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>five to six pygal vertebrae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>approximately 85 post-pygal caudal vertebrae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>scapula and coracoid sub-equal in size</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pectoral crest distinct but narrow</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>radius hatched-shaped to nearly semi-circular</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>carpals typically four</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tarsals three</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paddle broad, base of fifth digit divaricate from the others at 60 degrees or</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>greater angle</td>
<td></td>
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</tbody>
</table>

Table 3. List of skeletal characteristics of *Platecarpus tympanicus* as defined by Konishi et al (Konishi et al., 2010). KGM0016, “Belle”, shares 17 of 33 characteristics with *P. tympanicus*. **Match** = feature is present; **Obscured** = feature obscured by matrix; **Not Preserved** = Bone/element/trait not preserved with specimen.
Coccolithophores

The main building blocks of the Niobrara Chalk are the coccoliths, the calcite tests of coccolithophores. These single-celled nano-plankton provided the base of the food chain in the Western Interior Seaway (Hattin 1982; Everhart 2017). Many different genera and species are preserved in the chalk (Hattin 1982; Dr. Watkins, personal communication, 2021). Chalk is also composed of foraminifera, diatoms, and other phytoplankton that lived, died, and sank to the bottom of the seaway. Each species of coccolithophore exhibits its own vital effect, or fractionation, of carbonate because biomineralization of the test occurs intracellularly (Candelier et al. 2013; Hermoso et al. 2015). Chalk’s utility to preserve sea surface temperature has previously been explored (Liu 2009; Candelier et al. 2013; Hermoso et al. 2015; Thibault et al. 2016). Diverging values of the carbon and oxygen isotope signals demonstrate the variability of these phytoplankton. Thibault et al. (2016) utilized a scanning electron microscope (SEM) to count the coccolithophores preserved in the Boreal Chalk of the North Sea, counting individuals per species to estimate population proportions at the time of burial. This process is time consuming, and impractical without access to an SEM. These studies utilized the bulk carbonate temperature equation from Anderson and Arthur (1983).

Candelier et al. (2013) studied the coccolithophore species Calcidiscus spp. for its oxygen isotope fractionation in seawater relative to temperature, isotopic water values, and light conditions. Plotting their data determined a linear relationship between $^{18}$O fractionation (precipitation) and temperature of calcification through the following equation.

$$T \, (^{\circ}C) = -5.83 \times (\delta^{18}O_{Calcidiscus} - \delta^{18}O_{medium}) + 4.83$$

where $\delta^{18}O_{Calcidiscus}$ is the $^{18}$O value of the carbonate from Calcidiscus tests and $\delta^{18}O_{medium}$ is the $^{18}$O value of the environmental water (expressed as $\delta^{18}O_w$ in this thesis).
This study assumed a monospecific culture to attempt to recreate paleotemperatures. An approximately ~1‰ vital effect is seen in this species. Assuming the oxygen isotope values from the multiple species of the Niobrara Chalk are homogenized, this equation becomes a useful tool to estimate seawater surface temperature of the Western Interior Seaway during Belle’s life. This equation will be compared to Anderson and Arthur’s (1983) for sea surface temperature estimation.

Isotopic Water Values for Western Interior Seaway

Modern day seawater is relatively homogenous with a $\delta^{18}O_w$ value of 0.0 ± 0.5‰ (Muehlenbachs 1998). This value is influenced at the poles and the equator by meltwater input and evaporation respectively. The isotopic values of ocean water fluctuate due to the indirect effects of temperature producing fractionation of isotopic water values through evaporation, precipitation, and storage as snow and ice. As ice accumulates due to decreased global temperatures, the $\delta^{18}O_w$ value will increase due to ice storing large amounts of $^{16}O$. This preference is due to the isotopically light atmospheric moisture condensing and freezing into snow, enriching glaciers and ice caps with $^{16}O$ (Grossman 2012; Koch 1998). The combined effect is $\delta^{18}O$ increases with decreasing temperature. Low, depleted $\delta^{18}O$ values in fossil carbonates therefore indicate warmer paleotemperatures (Rohling, 2013). During the Late Cretaceous period, a value of -1.0‰ is assumed as the mean, global seawater $\delta^{18}O_w$ value with the planet assumed being ice free, enriching the oceans with $^{16}O$ (Peterson et al. 2016). However, this value does not compensate for the enrichment of isotopically light meteoric water in ocean water across a latitudinal gradient from the equator to the poles, resulting in a more depleted $\delta^{18}O$ value as distance increases from the equator (Rohling, 2013).
Several workers (Bernard et al. 2010; Harrell et al. 2016; Séon et al. 2020, 2022) utilize this value for estimating body temperatures assuming the animals’ bone deposition occurred in these environmental waters.

For the WIS, water composition was directly influenced by large inputs of enriched $^{16}$O freshwater from streams flowing across Laramidia and Appalachia (Dettman and Lohmann 2000; Fricke et al. 2010; Coulson et al. 2011). The WIS experienced multiple transgression-regression events that either disconnected or connected the seaway across North America, changing circulation pathways of the seaway and weather patterns (Coulson et al. 2011; Peterson et al. 2016). Therefore, determining the water values for specific locations within the seaway for accurate paleotemperature recreation has proven to be difficult.

Coulson et al. (2011) utilized the relationship of δ$^{18}$O$_{p}$ values of bone hydroxyapatite from aquatic turtles where the turtle’s body water remains in isotopic equilibrium with their environmental water. This research expounded on the original research of Barrick et al. (1999) and Matson and Fox (2008). Analyzing sea turtle fossils with coeval fish and shark fossils from western Kansas and the Mississippi embayment, water values of -3.45 ± 0.26‰ for the Niobrara Seaway over western Kansas and -1.37 ± 0.13‰ for the Mississippi Embayment are calculated. These δ$^{18}$O$_{w}$ values enable a higher resolution of 2.45‰ from an estimated value of -1‰ to better calculate body temperatures from phosphates. This thesis assumes the value of -3.45‰ as the δ$^{18}$O$_{w}$ used for Belle’s body temperature calculations.

Seawater Temperatures

Without any modern equivalents of an epicontinental seaway, estimating seawater temperatures of the WIS is an ongoing area of study. Tourtelot and Rye (1969) noted that most paleotemperature studies of ancient oceans utilized belemnites as the main proxy to calculate
temperatures based on their known resilience to secondary alteration. However, the WIS does not preserve belemnites in abundance. Belemnites, however, are interpreted to preserve a bottom water signature rather than a surface or average water temperature (Hoffman and Stevens, 2020).

Coulson et al. (2011) examined coeval, poikilothermic fish from western Kansas for the Niobrara Sea. Using their $\delta^{18}O_w$ value of $-3.45\%$ for the environmental water and applying Puceat et al.’s (2010) temperature equation, their study calculated a seawater surface temperature of 23.4 to 23.7°C (74.1 to 74.7°F). The fish analyzed were chosen to represent taxa interpreted from the upper water column, thereby a surface water temperature.

Inoceramid clams are candidates for preserving temperatures of the seaway. Walliser et al. (2018) tested large Platyceramus platinus clams from Monument Rocks, Kansas. These clams are found at Marker Unit 10 within the Niobrara Chalk. The clams yielded carbonate isotopic signatures that track varying temperatures throughout their lives as they constantly grew on the seafloor. Their shells exhibit temperature fluxes between 12.5 ± 3.0 and 25.5 ± 1.1°C, with a mean temperature of 17.0 ± 4.1°C. These values concur with the water circulation model of Coulson et al. (2011), where Kansas is a gyre of water.
METHODS

Sample Collection

A common method for collecting bone and enamel samples is using a small diamond rotary bit to grind off small aliquots of bone and tooth enamel (Barrick and Showers 1994; Coulson et al. 2009; Harrell et al. 2016). Adapted from the methods of Harrell et al. (2016), a Dremel® Rotary Tool with a 1/8-inch diamond-tipped burr drilled singular boreholes into the surfaces of a partial rib shaft section, a manus phalange, radius, coracoid, and two dorsal vertebrae, D3 and a float dorsal vertebra (Figure 8). The float vertebra was originally misidentified as a pygal/sacral vertebra but reclassified after examining the bone’s morphology and relative position as associated float to the skeleton. These bones, minus the in-situ dorsal, are separated from the main articulated skeleton, allowing for ease of sample collection. The in-situ articulated dorsal vertebra is located approximately above where the heart would have been in life.

Each bone and belemnite received a mechanical scrubbing with a toothbrush and deionized water to remove any surficial matrix still present on the surface. To look for variances of isotopic values, each bone was sequentially sampled every one to two millimeters (mm) in depth relative to the center of the respective bone. Each bone was measured with calipers for the diameter and divided by two to calculate the center at each selected borehole site. 25 aliquots of approximately 20 mg samples of bone powder were collected onto glassine weighing paper and transferred to screwcap, glass vials for each depth increment. To sample the tooth, a cylindrical diamond burr bit abraded the enamel from the surface of the tooth into an agate mortar and was ground with a pestle. The produced powder was transferred with glassine weighing paper to a glass vial as a single, bulk sample.
A partially broken belemnite rostrum on the edge of the main cluster was chosen for carbonate samples for analysis. Two small boreholes were drilled into the mid-posterior section of the broken rostrum solidum, collecting two bulk samples of approximately 10 mg each. Three broken fragments from the fractured rostrum cavum of one of the rostrums were also collected. These pieces were later ground with a mortar and pestle as a single, bulk sample of approximately 10 mg and transferred to a glass vial.

Four samples of chalk matrix were also collected to provide oxygen isotope analyses of these carbonates, looking for secondary alteration and possible surface temperature values from preserved coccolithophores. These samples were taken from (1) immediately adjacent to the sampled dorsal vertebra, D3; (2) adjacent to the belemnite rostrum cluster; and (3) a borehole to the left of the vertebral column into the chalk matrix sampling material at 5 mm and 10 mm in depth.

For detailed sampling procedures, see Appendix A.

For a summary of phosphate sample depths and masses, see Appendix B.

Figure 8: Representative mosasaur skeletal drawing showing bone sample collection sites. Six bones, one in-situ and five associated float bones, were selected for sampling purposes. Vertebra number 11, dorsal vertebra three, should yield the strongest temperature signature for core body temperature. [https://www.skeletaldrawing.com/non-dinosaurs/tylosaurus](https://www.skeletaldrawing.com/non-dinosaurs/tylosaurus)
Pre-Treatment of Bone Samples

After discovering high concentrations of diagenetic calcite in the bone powders (see Chapter 4), each sample underwent a treatment to remove the excess calcite for phosphate analysis. Following the same pre-treatment methods for analyzing bone carbonate from Silverman et al. (1954) and Coulson (2009), five mg aliquots of bone and enamel were placed in microcentrifuge tubes and soaked in two milliliters (ml) of an 8.1±0.1 pH triammonium citrate solution for 36 hours at room temperature, ~25°C. During soaking, each tube was periodically vortex-mixed to ensure complete surface contact with the solution. After soaking, each sample was rinsed four times using ultra-pure, deionized water. All samples were dried in a drying oven overnight at 50°C.

See Appendix E for preparation of triammonium citrate.

Bone Phosphate Preparation and Analysis

Following a modified method of O’Neill et al. (1993) and Vennemann (2002) prepared by the University of Kansas, each bone apatite sample selected for stable isotope analysis underwent a Ag₃PO₄ (silver phosphate) precipitation reaction to isolate the phosphate ions (PO₄³⁻) from the collected bone powder. Sample preparation took place at the University of Arkansas-Fayetteville, following their guide to silver phosphate extraction. 300-500 microgram (µg) aliquots from 25 bone powder samples, 1 tooth enamel powder sample, and three NIST-120c Florida Phosphate Rock powder samples were massed on a Mettler Toledo XPE26 DeltaRange® Microbalance and placed into microcentrifuge tubes. Each tube received 100 microliters (µL) of 0.5 M HNO₃ (nitric acid) to completely dissolve the bone samples, and the vials were closed. After soaking overnight for a total of 16 hours, some of the bone samples had not completely
dissolved. To ensure complete dissolution, each sample was mixed on a Vortex Genie and heated in a 50 °C oven for two and a half more hours.

75 μL of 0.5 M KOH (potassium hydroxide) was then added to each vial to both increase pH and decrease solubility of CaF₂ (calcium difluoride). Each vial then received 200 μL of 0.17 M KF (potassium fluoride) to precipitate out the Ca²⁺ cations as CaF₂. The vials were vortexed to ensure complete solution mixing, leaving the vials open for one to two hours. The vials were then centrifuged down in a swing-bucket centrifuge at 3500 rpm for 5 minutes, and the supernatant transferred to a second, low-bind microcentrifuge tube. 250 μL of silver ammine solution (0.2 M AgNO₃, 0.35 M NH₄NO₃, 0.74 M NH₄OH) was then pipetted into each vial, which began the precipitation of yellow, Ag₃PO₄ crystals. Next, the vials were placed, with their caps open to allow for some evaporation, into a 50°C oven for 20-24 hours.

After the heat treatment, the vials were centrifuged for five minutes at 3500 rpm to pelletize the silver phosphate crystals. Under a microscope, each vial was pipetted to only remove the remaining fluid, leaving only the crystals behind. Each vial then received one milliliter (ml) of deionized/distilled water and agitated on the Vortex Genie for a rinse. Next, the vials were spun down in the centrifuge. These rinses were repeated 4 more times. Samples were then dried out completely overnight in a 50°C oven. Each sample was tapped out onto glassine weighing paper, massed on a microbalance, and transferred into 3.5 x 5.0 mm silver capsules.

The capsules were then loaded into a zero-headspace Thermofisher Triplus™ RSH autosampler attached to a Finnigan® MA TC/EA (thermo-combustion/elemental analyzer) operated at 1400°C. The resulting CO was measured in-line by a Thermofisher Delta V™ Plus gas chromatograph-IRMS. Results were normalized to internal values (returned values): BTC (14.7 ± 1.5‰, n=5), NBS 127 (8.4 ± 0.1‰, n=3), USGS 80 (13.1 ±0.0‰, n=4), USGS 81 (34.7
± 0.9‰, n=4) reported in standard δ¹⁸O notation relative to V-SMOW (Vienna Standard Mean Ocean Water). As a quality control standard, rather than a lab calibration standard, NIST 120c, Florida Phosphate Rock, was also analyzed and produced an average value of 23.2 ± 0.3‰, n=3. Lab reported external precision ~0.3‰.

See Chapter 4 for a comparison of bone phosphate preparation methods.

See Appendix E for instructions to prepare silver ammine solution.

Carbonate Preparation and Analysis

Bone carbonate isolation for analysis utilized the methods developed by Silverman et al. (1952) and modified from Lee-Thorp (2002). Five mg aliquots from each bone sample were placed into two ml microcentrifuge tubes. Each sample soaked in one milliliter of 5% sodium hypochlorite, after mixing in a Vortex Genie, for 24 hours to oxidize and dissolve any organics present. After soaking, each sample was rinsed four times with one milliliter of deionized water using a Vortex Genie, and then centrifuged in an Eppendorf™ Minispin™ Microcentrifuge for five minutes at 10,000 rpm. After rinsing, each aliquot received two milliliters of 0.5 M, 8.1±0.1 pH triammonium citrate (C₆H₁₇N₃O₇) solution for a 36-hour soak. This step dissolved any secondary, diagenetic calcite deposited in the bone during fossilization. Then, each sample received one milliliter of deionized water for rinsing and centrifuged at 10,000 rpm for five minutes. Each rinsing was repeated four times. Each sample then was dried overnight and weighed to measure mass loss for how much calcite was dissolved.

A second set of five mg bone powder samples from the same bones and depths were also analyzed for bulk carbonate (secondary calcite and structural carbonate) values to create a baseline for comparison of untreated versus treated bone.
The University of Alabama Stable Isotope Lab analyzed the carbonate samples of bone, matrix, and belemnite. Seven samples of 0.05-0.1 mg amounts of chalk and belemnite; six samples of 0.1-0.2 mg of untreated bone powder; and six samples of 0.5-1.0 mg of treated bone powder were analyzed. Each set reacted with phosphoric acid at 50 °C in a ThermoScientific™ GasBench II for varying times: 2 hours for the chalk and belemnites, and from 30 to 51+ hours for the bone samples. The produced CO₂ gas travelled directly into a ThermoScientific™ Delta Plus mass spectrometer. Results were normalized to internal values (returned values): IAEA-603 ($\delta^{13}$C: -0.3 ± 0.05‰; $\delta^{18}$O: -4.2 ± 0.09‰) reported in standard $\delta^{18}$O notation relative to V-PDB (Vienna-Pee Dee Belemnite).

See Appendix F for a comparison of carbonate preparation techniques.
RESULTS

Bone Phosphate Values

$\delta^{18}O_P$ data from the phosphates ($\text{PO}_4^{3-}$) is summarized in Table 4. Samples SMB004B and SMB006B did not produce sufficient silver phosphate crystals for analysis. Samples SMB001A and SMB002C reported excessive peak counts. Sample SMB006F reported a minimal peak count. These values are included with the final data set and included into the calculated values as their values are within one standard deviation (1σ) of the mean of each element. $\delta^{18}O_P$ values range from a maximum value of 21.12‰ and a minimum value of 14.83‰. The mean value of the data is 18.30 ± 1.53‰. SMB004D’s value of 14.83‰ is an outlier within the data set. When removed, the mean value of the coracoid increases to 17.28‰ and decreases the standard deviation to ±1.25‰. Using Lécuyer et al.’s (2013) temperature equation for phosphate precipitation, two temperature ranges are calculated. An assumed environmental water value of -3.45‰ is used. If Belle had a $\delta^{18}O_w$ body water value of +4‰ enriched above the environmental water, comparable to modern ectothermic turtles (Séon et al., 2019), the calculated average temperature range would be 35.7 – 39.4°C. If Belle had a $\delta^{18}O_w$ value of +3.7‰ above the environmental water, comparable to crocodiles (Amiot et al. 2007), the calculated average temperature range would be 34.4 – 37.94°C. Each bone exhibits an average intra-bone oxygen isotope variability of 2.30 ± 0.43‰.
<table>
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<tr>
<th>Sample (SMB###A)</th>
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<th>± 1σ</th>
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<td>Phalange</td>
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Table 4. Bone and enamel phosphate oxygen isotope data from TC/EA. δ¹⁸O values are expressed relative to standard V-SMOW (‰).
Bone Carbonate Values

$\delta^{13}C$ and $\delta^{18}O$ values from the carbonates (CO$_3^{2-}$) are summarized in Table 5. SMB 001A-R, SMB003A, SMB003A-R each reported a low number of number of peaks below the 1000 mV necessary threshold for accurate data. These data values are included with the final data set and included into the calculated values. Untreated bone sample values of $\delta^{13}C_{cc}$ ranged from $-4.9\%$ to $-0.8\%$ with a mean of $-2.3 \pm 1.6\%$ (1σ), and $\delta^{18}O_{cc}$ ranged from $-9.5\%$ to $-7.6\%$ with a mean of $-8.5 \pm 0.8\%$. Treated bone sample values of $\delta^{13}C_{Cc}$ ranged from $-5.7\%$ to $-0.7\%$ with a mean of $-3.0 \pm 1.9\%$, and $\delta^{18}O_{Cc}$ ranged from $-9.2\%$ to $-7.3\%$ with a mean of $-8.1 \pm 0.8\%$.

Belemnite and Chalk Matrix Carbonate Values

$\delta^{13}C$ and $\delta^{18}O$ values are summarized in Table 5. Belemnite $\delta^{13}C_{BEL}$ values ranged from 0.4‰ to 0.5‰ with a mean of 0.4 ± 0.06‰ (1σ) and $\delta^{18}O_{BEL}$ values ranged from -3.4‰ to -3.0‰ with a mean of -3.3 ± 0.25‰. Applying the Arthur and Anderson (1983) paleothermometer equation to the belemnites, an average temperature of 15.2°C (59.4°F) is calculated.

Chalk matrix $\delta^{13}C_{M}$ values ranged from 2.1‰ to 2.3‰ with a mean value of 2.2 ± 0.08‰ (1σ) and $\delta^{18}O_{M}$ values ranged from -6.5‰ to -6.4‰ with a mean of -6.4 ± 0.04‰. Utilizing the Candelier et al. (2013) equation, the chalk yields a temperature of 22.1°C (71.7°F). Applying the Arthur and Anderson (1983) paleothermometer equation, a temperature of 29.4°C is calculated.

All carbonate values are plotted on a $\delta^{13}C$ and $\delta^{18}O$ cross-plot to show distinct data sets (Figure 9).
<table>
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<th>Sample (SMB00#A)</th>
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Table 5: Bone carbonate, secondary calcite, belemnite, and matrix carbon and oxygen isotope data from GCMS. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are expressed relative to standard V-PDB (‰). -R distinguishes bone treated with triammonium citrate for secondary calcite.
Figure 9: Cross-plot of $\delta^{13}$C and $\delta^{18}$O isotopic values for the bone bulk, treated bone, belemnite, and matrix samples. Each set of data distinctively plot differently from each other, indicating diagenesis might not have altered their signals. The bone carbonate values plot linearly, suggesting the original structural carbonate may be altered or simply replaced.
DISCUSSION

Diagenesis

Re-examining Belle’s skeleton, large portions are partially degraded, ranging from well-preserved bone to friable, powdery zones (Figure 10A, B). This degradation could be from several sources. One of these sources could be plant roots that are known to extract minerals and nutrients, specifically phosphorus, from vertebrate fossils (Noto 2009; Francischini et al. 2019). Some of Belle’s bones are interwoven with fine rootlets (Figure 10A). However, bone sampling sites chosen for this study did not coincide with these degraded zones of bone.

Freeze-thaw groundwater interactions could be a second source. Frost wedging is a common, physical weathering action that breaks down rock. Water expands and contracts in volume during phase changes from a liquid to ice, creating an outward, exerted force on the surrounding rock. Belle’s degradation could be attributed to this weathering. However, only small, random sectors of the specimen are affected, compared to a complete horizon effect.

A third possible explanation for the bone degradation is “gypsum rot.” Gypsum, CaSO₄, is a common evaporite mineral deposited from evaporating mineral-rich water. Gypsum can also form as a by-product of pyrite oxidation and hydrolysis. This interaction produces sulfuric acid (H₂SO₄) and will react with calcium-based substances, such as calcite. Belle’s right dentary and teeth are coated in gypsum. The underlying bone is extremely brittle and friable. Gypsum can readily dissolve into solution, creating sulfuric acid when interacting with water (Odin et al., 2019). This acid production could explain the bone’s condition. However, pyrite was not found in association with Belle (See Chapter 4). The rest of the skeleton does not exhibit severe friability.
Figure 10: A) Isolated phalange from Belle exhibiting “gypsum rot” and plant rootlets leaching phosphorus. B) Close-up view of vertebral column with disarticulated rib. Friable, degraded zones are visible. C) Sampled phalange showing good bone preservation. D) Right lateral view of vertebral column showing borehole sampling dorsal vertebrae three. Each bone sampled for analysis did not exhibit degradation. Scale bar is 10 centimeters.

**Bone Carbonate**

The bone structural carbonate proved to be unviable. Comparing the $\delta^{13}C$ and $\delta^{18}O$ values between the treated and untreated samples, a strong relationship is observed between both sets of values plotted against each other on a cross plot (Figure 11). Both sets exhibit similar values and fit a close linear trend. This connection indicates that the structural carbonate is completely replaced or altered. The carbon and oxygen isotope values fit within the range of precipitated meteoric calcites (Figure 1). Aufort et al. (2019) reported structural carbonate is readily incorporated into secondary calcite deposited within bone during experiments of varying aqueous environments. Importantly, these values are completely different than both the
belemnites and chalk carbonate values (Figure 9). This contrast suggests that the meteoric water
did not alter either the rostrums or the coccoliths. Their values fall within known values of other
belemnites and chalk values (Thibault 2016; Hoffman and Stevens 2020).

![Figure 1: Cross-plot of δ¹³C and δ¹⁸O comparing carbon and oxygen isotope values from untreated and treated bone values. Six samples of bone powder were analyzed for their structural carbonate and bulk calcite values preserved within the bone tissue. A strong relationship between the two is exhibited, implying the structural carbonates are altered.](image1.png)

**Bone Phosphates**

The oxygen isotope values of the bone phosphates are the most integral component of
this research. Their preservation integrity is therefore critical. Direct comparing the bone
phosphate values against the carbonate values reveals a distinct separation between the data
arrays (Figure 12). A second test is a direct comparison of phosphate oxygen isotope values
against their corresponding treated and untreated structural and bulk carbonate oxygen isotope
values (Figure 13). Both comparisons (bulk carbonate vs. phosphates: n = 6, $R^2 = 0.13$; structural
carbonate vs. phosphates: n = 6, $R^2 = 0.52$) express low correlation of phosphate values
Figure 12: Dot plot comparing carbonate values against phosphate values from bone powder samples. A distinct contrast is exhibited in the ranges of each data set. A ~4‰ offset is exhibited between the means of the carbonates and the phosphates.

Figure 13: Cross plot of δ¹⁸Oₚ (bone phosphate) against δ¹⁸Oₐ (bone structural carbonate) and δ¹⁸O₈ (bulk, secondary calcite) oxygen isotope values. The weak relationship of the phosphate values to both analyzed carbonates within the same bone powder sample are indicated through the visual spread of the data, and the low correlation of the two data sets.
being influenced by carbonate values. A Pearson product-moment correlation of each pairing yields -0.89 for untreated, bulk carbonate versus phosphate values and -0.88 for treated, structural carbonate versus phosphate values. These values indicate that the secondary calcites are not influencing the phosphate values.

The specimen’s $\delta^{18}O_P$ values were compared to $\delta^{18}O_P$ values from three other Platecarpus tympaniticus specimens: FHSM VP 322, FHSM VP 2077, FHSM VP 15533 (I. Trevathan, personal communication, unpublished data, 2022). All three specimens were collected from the Smoky Hill Chalk member of the Niobrara Chalk, east of Belle’s location in different counties and different stratigraphic positions. Bone sampling of each specimen followed the same methods used in this study. Silver phosphate precipitation followed the methods of Coulson (2009). Bone phosphate isotopic values were selected from cervical, dorsal, and caudal vertebrae, assuming the phosphate values of the bones would reflect a narrower temperature range of the axial skeleton.

KGM0016 provided 10 signatures with a mean of 18.59‰; FHSM 2077 provided 9 signatures with a mean of 15.63‰; FHSM 15553 provide 10 signatures with a mean of 14.41‰; and FHSM 322 provided 9 signatures with a mean of 16.99‰.

To test for statistical differences between each sample, a parametric ANOVA test, performed in R, compared these samples. The significance level for this test was 0.05. A Shapiro-Wilks test determined each data set to be normally distributed [FHSM 322: p = 0.56; FHSM 2077: p = 0.38; FHSM 15533: p = 0.83; KGM0016: p = 0.87]. A Levene’s test determined equal variances between the samples (df = 3, F-value = 0.71, Pr (>F) = 0.55) [Figure 14]. For this parametric test, the following assumptions were met: the data consist of random and independent samples; equal variances; and normal distributions. The test determined that the
samples were significantly different from each other (F=23.49, df=3, p<0.001). A Tukey’s test determined that a statistical difference is present between (1) KGM0016 and FHSM VP 2077 (p<0.001); (2) KGM0016 and FHSM VP 15553 (p<0.001); (3) FHSM VP 15553 and FHSM VP 322 (p<0.001); (4) KGM0016 and FHSM VP 322 (p=0.026)

No significant statistical difference is present between (5) FHSM VP 2077 and FHSM VP 15553 (p=0.124); (6) and FHSM VP 2077 and FHSM VP 322 (p=0.083). These ambiguous results suggest that Belle’s phosphate values are not similar to FHSM VP 322, FHSM VP 2077, or FHSM VP 15553. This disconnection could be attributed to different environmental water conditions; burial at different times; inadequate silver phosphate precipitation for FHSM VP 322, 2077, and 15553; or diagenetic alteration. The important takeaway of this data is the equal variances of the phosphate signatures from each specimen, signifying the relatedness of Platecarpus sp. specimens through time.
A fundamental question of bone phosphate’s usability is being unaltered from diagenesis due to heat, pressure, recrystallization, or groundwater fluid interactions during fossilization. Multiple studies refute bone and enamel phosphate’s viability to preserve true, original biological signals (Kolodny et al. 1996; Sharp et al. 2000; Trueman et al. 2003; Trueman et al. 2008) arguing the decomposition of collagen, groundwater interactions, and recrystallization of the hydroxyapatite from dahlite to francolite could change the stable isotopic signatures of the bone. For these reasons, tooth enamel is preferred to both cancellous and cortical bone because of its constrained porosity and permeability, thereby being more resistant to diagenetic alteration. Kral et al. (2021) investigated cortical bone’s resistance to aqueous dissolution and determined that cortical bone is not readily susceptible to dissolution, even at a micrometer diameter scale of harversian canals.

The phosphate signature from Belle’s tooth enamel, 18.7‰, is within the range of Belle’s bone phosphate values, and very close to Belle’s mean value of 18.3‰. If the tooth’s value varied greatly from Belle’s data range, diagenesis could be a possible factor to consider in interpreting these values. However, the tight association is an indicator that the skeleton’s isotopic integrity is still intact.

Belle’s phosphate values, therefore, are viable signatures of original bone deposition and temperature based on the negative relationship of the bone carbonate to the phosphates, the equal variance of Belle’s data to other specimens, Belle’s skeletal preservation, and the isotopic value similarity across Belle.

*Outlier Bone Phosphate Values*

The lowest value of SMB004D (4D), 14.83‰, and the highest value of SMB006C (6C), 21.12‰, of the δ₁⁸O_p values skew the data towards hotter and colder temperatures than the rest
of the data. 4D is from cancellous tissue, whereas 6C is from the contact of cortical and cancellous bone. Cancellous bone is more porous and permeable than cortical bone, making these tissues more susceptible to alteration. However, the other peripheral bone phosphate values would also reflect strong variations. The peripheral values instead are, on average, within $\pm 1.3\%$ of each other. Both values could represent improper sample precipitation as silver phosphate; partially dissolved samples bone samples, artificially fractionating the oxygen isotope ratio before analysis; or mass spectrometer errors.

**Belemnites**

*Carbonates*

Strong precision is exhibited by the belemnite carbonate data ($n=3$, $\mu(\delta^{13}C_{BEL}) = 0.4 \pm 0.06\% \ (1\sigma)$, $\mu(\delta^{18}O_{BEL}) = -3.3 \pm 0.25\% \ (1\sigma)$). However, only one rostrum was sampled for this study. Samples were drilled from the right lateral surface of the rostrum solidum (Figure 2) to target the best calcite for analysis. Choosing this zone attempted to avoid organic and aragonite zones within the rostrum. However, checks for diagenetic alteration were not performed on the rostrum carbonate. Thin-sectioned samples can reveal natural breaks within the material and distinguish recrystallized or replaced zones within the carbonate. Cathodoluminescence of thin-sectioned samples can also reveal zones of altered carbonate from the fluorescence of calcite and magnesite, demonstrating interchanges of calcium, magnesium, manganese, and strontium. These altered zones can exhibit different oxygen isotope values from the original calcite. Micro-milling under a microscope is a method to precisely sample unaltered zones to minimize false signatures and avoid these zones.

A study by Hoffman et al. (2021) revealed two distinct phases of low-Mg calcite deposited during rostrum growth of *Megateuthis*, an exceptionally large belemnite. The phases,
CP1 and CP2, express a systematic 2‰ offset, equating to ~8°C contrast. Hoffman et al. (2021) encourages sampling of CP1 for accurate calcite precipitation temperatures coeval with seawater temperature. For their bulk *Megateuthis* samples of both CP1 and CP2 mixed together, their samples exhibited about a +1.2‰ offset, biasing temperature estimates to colder temperatures by ~5°C. No studies are known for *Actinocamax* to determine calcite composition.

If the +1.2‰ offset is applied to the mean of the belemnite values, the resulting value - 4.5‰ renders a temperature of 20.3°C (68.6°F). This temperature concurs with claims of the Western Interior Seaway lacking a thermocline or a weak thermal gradient and fits within the 10-30°C predicted ecological temperature range of belemnites (Hoffman and Stevens 2020). However, this disagrees with isotopic studies of ocean circulations for the Western Interior Seaway during the late Santonian from oxygen isotope studies of clams, turtles, and foraminifera (Fisher and Arthur 2002; Coulson 2011; Peterson et al. 2016; Walliser et al. 2018). Further investigations of different belemnite species at different geologic times are needed to supplement this offset.

Tourtelot and Rye (1969) analyzed one rostrum of *Belemnitella bulbosa* from the Pierre Shale of Mobridge, South Dakota. The rostrum yielded an average value of δ¹³C of ~ +1.2‰ and δ¹⁸O of ~ -2.0‰, with a calculated temperature range, using the Epstein and Mayeda (1953) equation, between 17-18°C from the average oxygen isotope value. This single rostrum provides a reference point for belemnites within the Western Interior Seaway. Belle’s *Actinocamax sternbergi* does not match but compares favorably to this value. However, the Pierre Shale’s deposition occurred after the Niobrara Chalk and would exhibit different water isotopic values. The oxygen isotope signatures of the belemnite carbonate samples are therefore considered to be unaltered for the purpose of recreating their ambient environmental temperature.
Temperatures

The oxygen isotope values from the belemnite carbonate samples yield a calculated temperature of 15.2 ± 1.0°C (59.4 ± 1.8°F) from Anderson and Arthur (1983) and 14.8 ± 1.1°C (58.7 ± 1.9°F) from O’Neil (1969). Both equations use the -3.45‰ value for the environmental water value (Coulson et al. 2011). These values compare favorably with Hoffman and Stevens (2020) predictions for temperature range, 10-30°C, of belemnites. Coupled with the interpreted temperatures of clams and modern habits of coleoids, the ~15°C temperature corroborates to a bottom water signal within the WIS.

Chalk

Carbonates

Stable isotope analysis of the chalk checked for diagenetic alteration of Belle and the belemnites. Analysis of the carbon and oxygen isotope values revealed a separation of the matrix values from both the bone and belemnite carbonate isotopic values. This separateness indicated each data set represents independent signals.

Strong precision is exhibited by the chalk carbonate data \([n = 4, \mu(\delta^{13}C_M) = 2.2 \pm 0.08\%, \mu(\delta^{18}O_M) = -6.4 \pm 0.04\%]\). Four different aliquots at different locations across the specimen were tested to examine for variation (See Methods, Sample Collection). The absence of variances indicates that the matrix is not altered, preserving the original carbonate values of the coccoliths. Known carbon and oxygen values of carbonates (Figure 1) compare favorably with the chalk values. Isotopic values from other chalk formations also compare favorably (Jenkyns et al. 1994; Liu 2009; Hermoso et al. 2015; Thibault 2016) using respective environmental water values.

SEM images of embedded chalk in Belle’s bone fragments reveal well-preserved coccoliths. Further examination of the images determined a lack of calcite overgrowths on the
coccoliths. Calcite overgrowths are indicative of dissolution and re-precipitation of calcite. These crystals can alter or skew carbon and oxygen isotope data from their original values, giving false values. The lack of these crystals fortifies the probability that isotopic signatures of the chalk are original and unaltered.

These well-preserved coccoliths (see Chapter 4) prompt an interpretation for seawater surface temperature of the WIS. At least five genera are identified in the chalk around Belle. Each modern species of coccolithophore fractionates carbonate differently, each expressing a different vital effect (Hermoso et al. 2015; Thibault et al. 2016). Each known species in the WIS has not been studied for their stable isotopic values. To compensate for these discrepancies, a bulk carbonate value is assumed to reflect a homogeneous mixture of the species (Thibault et al. 2016).

Temperatures

Applying the Candelier et al. (2013) equation for *Calcidiscus* coccoliths, the mean δ¹⁸O₉ value yields a seawater surface temperature (SST) of 22.1°C (71.7°F). The Anderson and Arthur (1983) bulk calcite equation yields an SST of 29.4°C (84.9°F). 22.1°C compares favorably to the SST from Coulson et al. (2011) of 23.4 – 23.7°C from coeval fish. It is also within the range of temperatures from *Platyceramus* clams, 12.5 ± 3.0°C to 25.5 ± 1.1°C collected from Monument Rocks, close to Belle’s locality (Walliser et al. 2018). 29.4°C could be an overestimation from not accounting for the different vital effects of mollusks. 22.1°C is therefore chosen as the more favorable interpreted temperature.
Body Water

An important consideration for calculating phosphate deposition temperature in vertebrates is their known vital effect to fractionate ingested water (Barrick et al. 1999; Coulson 2009; Séon et al. 2022). This ingested water then becomes incorporated into the animal’s body water. Body water is the water contained within the tissues, bones, blood, and other fluids within an animal’s body (Edelman and Leibman 1959). For terrestrial organisms, body water is a homogeneous mixture of water from drinking, metabolic breakdown of food, and the water within food (Amiot 2007). Excretion and respiration also change the body water volumes and compositions of an organism. Air-breathing, marine vertebrates derive their body water just from their ingested food, primarily fish and invertebrates (Yoshida and Miyazaki 1991; Amiot et al. 2008; Bernard et al. 2010; Séon et al. 2020). The fish and invertebrates are in osmotic and isotopic equilibrium with their body water from the environmental water (Kolodny et al. 1983). Therefore, the body water of the air-breathing, marine vertebrates reflects both the environmental water and their own biological fractionation of oxygen isotopes from cellular processes. Phosphate oxygen isotope values are therefore directly linked to the body water’s isotopic signature and the temperature of the animal.

Exploring this relationship, multiple studies have calculated the biological fractionations of terrestrial and marine taxa (Amiot et al. 2007, Clauzel et al. 2020). Specifically, bone phosphate fractionation equations are known for two reptiles.

For turtles (Barrick et al. 1999):

$$\delta^{18}O_w = 1.01 \times \delta^{18}O_p - 22.23$$
And for crocodiles (Amiot et al. 2007):

\[ \delta^{18}\text{O}_W = 0.82*\delta^{18}\text{O}_P - 19.13 \]

where \( \delta^{18}\text{O}_P \) is the phosphate oxygen isotope value and \( \delta^{18}\text{O}_W \) is the oxygen isotope value for the environmental water. Amiot et al. (2007) reported that there is no significant statistical difference between the crocodile and turtle values. No corroborating, directly measured body water values are known for crocodiles, only turtles (Barrick et al. 1995).

Both turtles and crocodiles are distantly related to mosasaurs in the reptile clade (Russell 1967). Oxygen isotope values from the body water of terrestrial or semi-aquatic varanid lizards is not known. However, their body water is assumed to fluctuate from multiple sources of ingested and metabolic water depending on each species lifestyle.

For this study, both turtle, +4‰, and crocodilian body water values, +3.7‰, will be applied with Belle’s phosphate values to calculate a range of estimated body temperatures. These vital effects values are derived from direct comparison of environmental water to body water in turtles (Barrick et al. 1999) and the offset of 0.3‰ from the slopes of the crocodile and turtle bone phosphate value equations using arbitrary values of 0‰ and 1‰.

**Temperatures**

Lécuyer et al.’s (2013) phosphate temperature equation, assuming an environmental water value of -3.45‰, yields a mean temperature of 37.5 ± 6.9°C using a turtle body water value. A mean temperature range of 36.2 ± 6.9°C is calculated from the crocodilian body water value. Removing the outlier phosphate values of 14.8‰ and 21.1‰, two different ranges of temperatures are produced. 29.2 – 44.9°C is produced from a turtle body water value. 27.8 – 43.5°C from a crocodile body water value.
Table 6: Calculated mean temperatures from each sampled bone relative to turtle (+4.0‰) and crocodile (+3.7‰) body water values.

<table>
<thead>
<tr>
<th>Skeletal Element</th>
<th>Mean δ¹⁸O‰ (VSMOW)</th>
<th>T(°C) from Turtle Body Water (+4.0‰)</th>
<th>T(°C) from Crocodile Body Water (+3.7‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tooth</td>
<td>18.66</td>
<td>35.9</td>
<td>34.6</td>
</tr>
<tr>
<td>Phalange</td>
<td>18.19</td>
<td>38.0</td>
<td>36.7</td>
</tr>
<tr>
<td>Rib Fragment</td>
<td>20.16</td>
<td>29.2</td>
<td>27.8</td>
</tr>
<tr>
<td>Radius</td>
<td>17.35</td>
<td>41.8</td>
<td>40.5</td>
</tr>
<tr>
<td>Coracoid</td>
<td>16.67</td>
<td>44.9</td>
<td>43.5</td>
</tr>
<tr>
<td>Dorsal Vertebra</td>
<td>18.63</td>
<td>36.0</td>
<td>34.7</td>
</tr>
<tr>
<td>Dorsal D3</td>
<td>18.55</td>
<td>36.4</td>
<td>35.1</td>
</tr>
</tbody>
</table>

Variation of Phosphate Values

Different ranges of phosphate values are present within each bone. Each bone expresses a mean standard deviation of 1.28 ± 0.41‰ from each bone’s mean phosphate value, equivalent to a temperature fluctuation of ~5.1°C from the Lecuyer et al. (2013) equation. Phosphate values can profoundly vary across a skeleton due to different bone deposition not only based on temperature and body water composition, but also due to different biological processes (Barrick and Showers 1995; Barrick 1998).

The rib fragment, 20.2‰, features the strongest difference from the mean phosphate value, 18.3‰, of -1.9‰. This disparity causes the rib fragment to appear hotter than the rest of the skeleton with a 7.4°C decrease from the mean body temperature (Figure 15). Tissue thickness is thin over the rib cage of varanid lizards (Cieri 2018). The bone’s proximity to the ambient environment could reflect temperature diversions from cooling. However, this rib fragment was collected as float material below Belle’s locality. The bone could be altered from weathering and
giving false “cold” values. Visually, the bone fragment is slightly sun-bleached, but otherwise intact. Further research into this strong signal is required.

The coracoid, 17.3‰ (excluding the 14.8‰ value), and the radius, 17.4‰, both express positive changes of +1.0‰ and +0.9‰ respectively. These differences make the bones appear colder than the rest of the skeleton with a calculated temperature difference of ~4℃ (Figure 15) Appendicular bones are known to have higher temperature fluctuations than mean core body temperatures (Barrick 1995, 1998; Coulson et al. 2008; Sémon et al. 2022) from the large fluctuations of temperature from physical activity and distance from the body core.

One intra-bone variation trend is discernible in each bone sampled. An average depletion of -1.7‰ is discerned between the outer most layers of bone (0-2 mm) and the next sampled zones (2-4 mm). This offset could represent migration of Belle from different isotopic waters.

Two discernible intervariable trends within the skeleton are visible (Figure 16): 1) decreasing values transitioning from distal to proximal across the appendicular skeletal elements;

Figure 15: Calculated temperature (℃) differences from mean body temperature, 36.2℃, from phosphate values. The coracoid exhibits the coldest temperature changes from the high phosphate value variability. The rib fragment exhibits the hottest temperature changes, possibly from temperature fluctuations of water depth or alteration from weathering. “Warmer” and “Colder” arrows indicate actual temperatures.
Figure 16: $\delta^{18}O_p$ variance across sampled skeletal elements. Phosphate values become more depleted as distance from the body core decreases.

and 2) decreasing values transitioning from lateral to medial from the rib fragment to dorsal three, D3, across the body cavity. Both trends can be interpreted as natural temperature fluctuation from temperature increasing proximally towards the body core (Seebacher and Franklin 2005). These trends are like those found in North Atlantic dolphins studied for their bone phosphate values (Séon et al. 2022). Their study provided bone phosphate data to demonstrate regional heterothermy, or hotter temperature zones, within extant marine vertebrates. The dolphins exhibited regional heterothermy with their skulls and vertebrae being warmer than the appendicular skeleton. The vertebrae of Belle express this trend as well.

**Elevated Thermophysiology**

Juxtaposing the calculated mean temperatures of the belemnites (15°C), chalk (22.1°C), and Belle (36.2 – 37.5°C) provides strong evidence that Belle possessed an elevated body temperature above the ambient water temperature (Figure 17). These organisms’ concurrence adds strength to these values not being influenced by different water values at different successions of the WIS. Sampling the chalk at three different locations across the specimen
provides proof of the calculated sea surface temperature during Belle’s life. The direct predator-prey relationship provides a strong contrast of at least 21°C between the belemnites’ ambient environment temperature against Belle’s calculated body temperature. The contrast between the sea surface temperature also indicates Belle maintained a body temperature at least 14°C greater than the upper water column. This elevation therefore implies a form of endothermy to maintain a mean core body temperature of 36.2°C.

Figure 17: Comparison of calculated temperatures from δ18O values from carbonates of the belemnites and coccoliths, and phosphates from the mosasaur’s bone and tooth. The strong contrast between the three temperatures strongly suggests that Mosasaur Belle possessed an elevated thermophysiology compared to her prey and the ambient seawater. Images: Google Images; Platecarpus outline modified from Konishi et al. 2011.

A leatherback turtle maintains a temperature ~8°C above the ambient water temperature. Belle’s calculated temperature suggests a maintained temperature well above this plateau. This high maintenance could be an adaptation to the lack of fat or blubber to insulate Belle from heat loss.
The phosphate oxygen isotope values’ variability could suggest two different physiologies. If Belle is migrating within the WIS, the body water values will reflect more negatively or more positively relative to the environmental water surrounding Belle. These values would influence the bone phosphate ions deposited, enriching or depleting, the phosphate oxygen isotope values relative to the environment. Assuming Belle’s body temperature is constant, Belle’s thermophysiology could be homeothermic.

Intra-bone variation, however, suggests temperatures did fluctuate to some degree. Modern cetaceans and pinnipeds exhibit varying temperatures in their limbs from heat loss due to water interactions, diving behaviors, and migration (Yoshida and Miyazaki 1991; Favilla and Costa 2020; Séon et al. 2022). These animals are described as heterothermic endotherms, where temperature can vary across the skeleton, but a constant deep core temperature is maintained.

If Belle is living in the same ocean waters throughout its life, the body water value would remain close to the same value. For the phosphate values to change, drastic temperature shifts would occur within the body. These temperatures fluxes could reflect Belle moving into colder waters to the north in the WIS. Other unknown biologic oxygen fractionations could account for these differences as well. Alternatively, these phosphate values could also be indicative of diving behavior.

**Ecological Interactions**

Massare (1987) inferred the diets of several marine reptile clades from their tooth morphology. She deduced that some mosasaurs fed on fish and cephalopods (squids, ammonites, and belemnites). Belle provides direct evidence as the possible first occurrence of this behavior. There are two possible scenarios of this feeding behavior. (1) The belemnites could have been ingested at night when the belemnites would migrate from the epipelagic zone of the WIS to the
surface. (2) Belle could have dived down into the epipelagic zone to feed on the belemnites. If Belle dove, its possible regional heterothermy would have enabled her to remain active at colder temperatures, and produce heat in her brain, optic nerves, and eyes for feeding. Rothschild and Martin (1987; 2005) discovered evidence for diving behavior by analyzing different mosasaurs for bone tissue damage. Each *Platecarpus* they examined exhibited avascular necrosis, decompression syndrome-related (“The Bends”), pathologies. These pathologies occur in the vertebrae and proximal ends of humeri and femora consist of areas of subsided and degraded bone from bone tissue death (Rothschild and Storrs 2003) from rapid ascension to the surface and degassing of dissolved gasses from blood and bodily fluids. This evidence infers *Platecarpus* spent long periods at depth in the water column, a possible sign of feeding behavior. The belemnites’ temperature suggests they lived in cooler, bottom water, implying the presence of a thermocline.

This predator-prey interaction, however, suggests that this mosasaur died very quickly after ingesting these belemnites. Mosasaur coprolites only contain fragments of bone with no pieces of shell (Everhart 2017; Hunt and Lucas 2018). This disparity infers the strength of mosasaur’s stomach acid to break down ingested bone and shell. Finding only minor pitting on the belemnite rostrums suggests incomplete digestion. The rostrums orientation and tight cluster advocates the belemnites were forced together into a bolus within the stomach. This bolus of 13 rostrums may have created an impaction within the stomach or upper small intestine, preventing the animal from eating. A rupture of the stomach or intestinal lining could have occurred too.

*Evidence of a Thermocline*

The contrasting temperatures of the chalk and the belemnites prompts a possible interpretation for a thermocline within the water column of the WIS. A thermocline is an oceanic
water layer where temperature decreases rapidly with increasing depth (He et al. 2005; Peterson et al. 2016). A widespread, permanent thermocline is found in the modern oceans at about 200 meters to 1000 meters. Shallower, seasonal thermoclines can form during the summer months from increased solar heating and biological productivity. Thermoclines are also associated with pycnoclines, rapidly changing density layers of water forming from changing temperature and salinity (He et al. 2005). However, Belle’s seven values of belemnite and chalk are insufficient to create a statistically significant study to strongly suggest the presence of a thermocline. Further sampling of belemnites and chalk could help answer this question.
CONCLUSIONS

An elevated thermophysiology is a biological factor that enabled mosasaurs to become the apex predators in the world’s oceans of the late Cretaceous period. However, previous studies of mosasaur body temperature did not contrast concurrent organisms. Stable isotope analysis of Mosasaur Belle, a *Platecarpus tympaniticus*, unveils a snapshot of the Western Interior Seaway during the late Santonian stage. Belle could be the first mosasaur fossil associated with belemnites as stomach contents, confirming tooth morphology research into mosasaur diet. This predator-prey interaction presented an opportunity to compare the body temperature of a mosasaur to its prey from the same moment in time.

The belemnite rostrums are identified to the species *Actinocamax sternbergi* from rostrum measurements and surficial features. Belle’s 13 rostrums add a significant new population to the rare belemnite species of the Western Interior Seaway. Carbon and oxygen isotope analysis of one of the rostrums reports viable preservation of the authigenic, biologic carbonates. The belemnites express a temperature of 15.0°C, agreeing with comparisons to modern coleoids and other belemnite studies.

Investigating the chalk matrix for diagenetic alteration presented unexpected opportunities to study more than isotopic viability. High-precision values initially inferred a lack of diagenetic alteration. Scanning electron microscope images of the chalk displayed very well-preserved coccolithophores, whose unaltered tests demonstrated their preservation and potential for recreating the water temperature in which they grew. Oxygen isotope values from bulk chalk samples provided a mean seawater surface temperature of 22.1°C, corroborating studies of poikilothermic fish for an estimated temperature of the surface waters of the Western Interior Seaway.
Stable isotope analysis of Belle’s bones yielded a study of both fossilization and physiology. Isolating and evaluating the bone structural carbonate proved to be unviable from secondary calcite replacement. Significant permineralization of the bone during fossilization prompted a series of isotopic integrity checks. Statistical tests and comparisons strongly suggest that the bone is not altered and is viable for stable isotope analysis. Both turtle and crocodilian body water isotopic compositions were considered to infer mosasaur physiology for bone deposition. Temperatures calculated from the phosphates reveals a body temperature of 36.2°C – 37.5°C, with varying temperatures across the body suggesting heterothermic endothermy.

Directly comparing the three temperatures, an elevated thermophysiology is present in Mosasaur Belle. This physiology would have enabled higher activity in different water temperatures and aided in probable diving behavior to hunt belemnites in the depths of the Western Interior Seaway.
FUTURE WORK

Multiple questions remain unanswered from this research. How does Belle compare to other *Platecarpus sp.* specimens? Sample collection and analysis from other specimens would yield more data and provide comparisons of temperature for the species.

An ecological study of Belle compared to other mosasaurs may provide more insights into how these animals lived and interacted with their environment. Modern squid migrate from deeper waters to the surface each night to feed. The isotopic data of the belemnites preserved within Belle reflects their rostrums forming in cooler waters, that may reflect the deeper waters of the Western Interior Seaway. Two known traits of *Platecarpus* may hint at where Belle fed on these animals. One trait of *Platecarpus sp.* are their very large orbits, with robust sclerotic rings. Large orbits are associated with large eyes designed to see in low-light conditions. Robust sclerotic rings imply resistance to high pressure environments. Multiple penguin species also possess robust sclerotic rings and associated with deep diving to their prey.

A second trait seen in *Platecarpus sp.* and other mosasaurs’ vertebrae is avascular necrosis. Avascular necrosis, or osteonecrosis, is a bone disease resulting from the temporary or permanent loss of blood supply to bone. The resulting bone is severely friable and damaged. These dead tissues can occur from decompression sickness, or “the bends,” from dissolved gasses in blood and tissues undergoing rapid dissolution and gas bubble formation, causing ruptured blood capillaries, bruising, and irreversible damage to tissues. Many mosasaur specimens exhibit avascular necrosis in their bones, with *Platecarpus* specimens exhibiting the most tissue damage (Rothschild and Martin, 2005). These animals have all been attributed to be diving down to feed on prey, or ambush behavior with rapid ascension. An examination of Belle’s vertebrae may reveal avascular necrosis and indicate diving behaviors.
The belemnites also may yield more insights into their own ecology. 34 belemnite rostrums from the Niobrara Chalk are in museum collections around the world. One specimen of *Actinocamax sternbergi* is known. Belle tentatively adds 13 rostrums to this species, with the potential for more after preparation. A detailed study of this population sample would provide population information unknown to belemnites within the WIS. These animals may provide further isotopic insight into changing temperatures and salinities as the seaway changed through transgression and regression events.

Belle’s belemnites are smaller than the holotype specimen. Without a large population of specimens to reference, making ontogenetic size distinctions for this species is not suggested. A detailed study of the cluster could reveal more about the preserved population sample.

Some of the bone phosphate values exhibit unique values, skewing the temperature towards hot and cold readings. Further research of weathered and altered bone may reveal how bone phosphate changes through diagenesis.

The contrasting temperatures of the chalk and the belemnites prompts a possible interpretation for a thermocline within the water column of the Western Interior Seaway. A thermocline is an oceanic water layer where temperature decreases rapidly with increasing depth. A widespread, permanent thermocline is found in the modern oceans at about 200 meters to 1000 meters. Shallower, seasonal thermoclines can form during the summer months from increased solar heating and biological productivity. Thermoclines are also associated with pycnoclines, rapidly changing density layers of water forming from changing temperature and salinity. However, Belle’s seven values are insufficient to create a statistically significant study to strongly suggest the presence of a thermocline. Further sampling of Belle’s belemnites and chalk could help answer this question.
CHAPTER 4: CHEMISTRY AND BONE PRESERVATION

This isotopic study required multiple chemistry issues to be overcome with creative solutions from advisors and colleagues. Multiple phosphate isolation methods were considered for this project. However, the main constraint was the amount of bone that would be available for analysis. In the original proposal, a collection of 200 milligrams (mg) from the specimen was requested and accepted, limiting the methodologies that could be used.

The first suggested method uses 20 mg of bone sample (Coulson, 2009). This method is modified from the procedure of Dettman et al. (2001) and Kohn et al. (2002), both of which are based on O’Neil (1994). 20 mg guaranteed easily weighable amounts with the available mass scale. However, many of the samples only contained 20 mg. Consuming all the sample for one attempt became impractical. The second suggested method from Harrell et al. (2016) utilized 300-500 micrograms (µg) of bone or enamel for phosphate extraction. This method is modified from O’Neil (1994) and Vennemann (2002). The minimal amount of bone needed is ideal for repeatability. However, a proper microbalance with ±0.1 µg accuracy was unavailable at FHSU for massing the necessary masses. A third method is the Shabaga et al. (2018) silver phosphate precipitation method. Their method only required 5 mg of sample. This mass amount provided an ideal combination of mass measurement for the capabilities of FHSU’s available microbalance and future repeatability, which later proved invaluable.

Other methods are known for silver phosphate extraction. However, these chemistry procedures are complex, requiring strong acids and uncommon reagents for these reactions. Reagent availability and cost limited which techniques could be utilized. Most of the chemicals for the Coulson and Shabaga methods were already available from previous researchers at FHSU.
For the first attempt, the Shabaga et al. (2018) method was chosen. For the full method, please refer to Shabaga et al. (2018). The precipitated \( \text{Ag}_3\text{PO}_4 \) unfortunately yielded isotopic values on average 5‰ lower than previous studies of mosasaur bone phosphates. The precipitated crystals also exhibited a creamy, yellow color and texture compared to the expected euhedral, dark golden yellow crystals. These differences prompted an evaluation of the chemistry.

The bone samples were dissolved in 2 M HF acid, one of the strongest acids commercially available. However, complete dissolution may not have occurred due to either not enough soaking time or not enough phosphate was preserved in the bone for the reaction to occur completely. The HF may also have degraded with time and off-gassing while in storage.

Next, the silver ammine solution’s reagents (\( \text{AgNO}_3 \) (s), \( \text{NH}_4\text{NO}_3 \) (s), \( \text{NH}_4\text{OH} \) (aq)) possibly were not well sealed while stored. The salts are known to be hygroscopic and could have absorbed moisture from the air. The \( \text{NH}_4\text{OH} \) solution could have slightly evaporated, changing the overall concentration. Ammonia readily off-gasses from solution. These reagents can also breakdown naturally over time, losing their potency. An important detail omitted from this method’s instructions is the photosensitivity of the silver ammine solution. The solution can break down within a matter of days when exposed to any light source.

These chemistry methods also require pure water to mix solutions and rinse precipitates. The water needs to be devoid of any dissolved solids, bacterium, or any other suspended matter. Total Dissolved Solid (TDS) testing of the water within the Werth College building revealed very high dissolved solids. Five liters of water from a Millipore Milli-Q 3 Part Water Filter were used during the first attempt. The filters, however, had not been replaced in several months. The TDS sensor detected 1-2 ppm TDS within this water, unacceptable for isotopic measurements.
Centrifugation using a fixed-angle centrifuge created complications in unnecessarily losing sample between rinses and supernatant transfers. A phenomenon known as “creep” can occur in centrifuge tubes. If the incorrect amount of time and inappropriate rotating speed is applied to samples, the pelletized material can “creep” up the tube’s wall. When the supernatant in the tube was decanted or pipetted out, the fine material would wash out or return to suspension, being lost in the process. Samples were re-centrifuged to pelletize the material, but the “creep” continued. For these small quantities, a swing bucket centrifuge became a better tool to pelletize the material, and not have “creep” occur.

These problems and concerns were discussed with Mr. Erik Pollock, the lab director of the Stable Isotope Laboratory at the University of Arkansas – Fayetteville (UAF). Understanding the issues, he extended an invitation to use their chemicals, water, and facility to redo the bone samples.

Over a five-day period, Ms. Danielle Oberg supervised the second run of samples, following the modified methods from O’Neil (1994) and Vennemann (2002), henceforth referred to as the Arkansas method. For the full method, please refer to the Methods Section of Chapter 3. This method produced the expected euhedral, golden yellow crystals needed for analysis. However, some of these samples also precipitated a dark grey/black coating and euhedral, cuboidal crystals. These crystals were initially identified as silver sulfide (Ag₂S), prompting concerns sulfur could damage the mass spectrometer.

The presence of sulfur anions could have been indicative of pyrite or other sulfides or sulfates contaminating Belle. Pyrite is known to oxidize and undergo hydrolysis to form sulfuric acid (Larkin, 2011; Tacker, 2020). Sulfuric acid is one of the main acids used to be break down phosphate minerals in the production of phosphorus for fertilizers in agriculture (Tro, 2013). For
fossils, this reaction can be seen as “pyrite’s disease,” where a fossil can breakdown, from the inside out, and turn into dust (Larkin, 2011; Tacker, 2020). This possible breakdown could have explained why the samples did not undergo proper precipitation. The bone may have already been destroyed.

Upon a recommendation from a colleague, three bone samples underwent SEM/EDS examination and XRD analyses to assay the bone’s mineralogy with Dr. James Murowchick at the University of Missouri - Kansas City. A Tescan Vega 3 LMU variable pressure scanning electron microscope (SEM) equipped with a Brukar Quantax Energy-Dispersive Spectroscopy (EDS) system scanned and photographed three bone fragments. These scans examined for the contaminants based on visible crystal structures with SEM imaging, and correlation of identified atoms to known minerals. The SEM/EDS examination determined barite (BaSO₄) and celestite (SrSO₄) crystals embedded within the chalk matrix as the only sources of sulfur in the form of sulfate (SO₄²⁻) (Figures 18, 19, 20). Celestine and barite are known to precipitate out of seawater in the presence of decaying phytoplankton, coccolithophores and diatoms (Ganeshram et al., 2003). Their sulfate (SO₄²⁻) anions created concern that their oxygen atoms could skew the oxygen isotope ratios of the samples. However, both sulfate minerals are inconsequential to this study. Neither barite nor celestite was detected in the final silver phosphate precipitates in the SEM and EDS images to potentially be combusted in the TC/EA for the bone phosphate mass spectrometer analysis. Barite and celestite are also insoluble in orthophosphoric acid, which is used as the reagent to create carbon dioxide gas for carbonate isotopic analysis.

Bone samples were also analyzed for apatite purity and large concentrations of sulfate or sulfide minerals. Mineral presence is determined through x-ray diffraction of mineral volume presence greater than 5%. A Rigaku Miniflex automated powder x-ray diffractor (XRD)
analyzed three, ~5 mg aliquots of cortical and cancellous bone powder. The x-ray beam was aimed at the sample, cycling through 0.05° angle increments from 5° to 60°. Each sample was analyzed for 1 hour and 13 minutes each. Strong signatures of fluorapatite (Ca$_5$(PO$_4$)$_3$F), calcium carbonate (CaCO$_3$), fluorite (CaF$_2$), and carbonatehydroxylapatite – Ca$_5$(PO$_4$)$_3$(CO$_3$)(OH) are present in the bone. Anticipated sulfides [pyrite (FeS$_2$) or marcasite (FeS$_2$)] or sulfates [gypsum (CaSO$_4$)] are not present. Sample name MosaBelle1 (Figure 21) analyzed powdered cancellous bone. Sample name MosaBelle2 (Figure 21) analyzed powdered cortical bone. The third analysis scanned an aliquot of SMB006F (Figure 22), cancellous bone from Belle’s in-situ, dorsal vertebra D3. This sample was chosen specifically for the high concentration of precipitated dark, euhedral crystals from the second silver phosphate precipitation attempt.

The XRD results showed a high concentration of calcite by mass preserved within the bone tissues, with the largest concentrations within the cancellous bone. The XRD data correlates with the observed mass loss during treatment of the bone powder samples for bone carbonate analysis. Dissolution of secondary calcite from the bone samples caused an average of 56% mass loss of each sample through the soaking process. This loss suggests that, by mass, not enough overall fluorapatite (Ca$_5$(PO$_4$)$_3$F) could have been available to completely precipitate silver phosphate in the first two attempts. Both the Shabaga et al. (2018) and the Arkansas method rely on the hydrofluoric (HF) or nitric acid (HNO$_3$) reacting with any carbonates and removing them as carbon dioxide (CO$_2$) gas. The excessive calcite in these samples possibly reacted with the acid and reduced the overall amount of reagent available to break down the bone samples. The Arkansas method also relies on using the same amount of sample necessary to produce a viable stable isotope signal. If a 500 µg single bulk sample is only 250 µg of fluorapatite, that 250 µg does not meet the necessary 300 µg threshold.
Figure 18: A) SEM and B) EDS images of chalk matrix on bone fragment. These images distinguish whole and partial coccoliths, zones of authigenic clays, and associated electrically stimulated barite and celestine crystals from the barium, strontium, and sulfur atoms. BSE = backscattered electrons, Ba = barium, Sr = strontium, S = sulfur.
Figure 19: A) SEM image of chalk matrix embedded in bone fragment. Three investigation sites for atomic analysis are selected for an overall survey of mineralogy, summarized in B, C, and D. B) EDS point analysis of electronically stimulated crystal, indicating the presence of strontium, barite, and sulfur associated as celestite. Barium and strontium atoms interchange during crystal formation depending on the availability of either cation. C) Unknown organic zone with large carbon signature. D) Selected site of bone tissue for EDS analysis revealing strong signatures of hydroxyapatite. Trace elements of iron, titanium, and silicon are expressed, but deposited during permineralization.
Figure 20: EDS image of surficial fissure in bone fragment identifying the atomic structures of celestite (SrSO₄), bone apatite (Ca₅(PO₄)₃(CO₃)), coccoliths (CaCO₃), and possible authigenic clays (Si-rich zones). BSE = backscattered electrons, Si = silicon, P = phosphorus, Ca = calcium, Fe = iron, Sr = strontium, S = sulfur.
Figure 21: XRD powder diffractogram comparing a sample of cancellous bone (MosaBelle1) to cortical bone (MosaBelle2). Peak positions indicate the presence of bone apatite and calcite. Graph produced on MDI Jade software.

Figure 22: XRD analysis of SMB006F, powdered cancellous bone from dorsal vertebra, D3, from Belle. Peak positions indicate the presence of bone apatite and calcite. No distinctive peaks show the presence of sulfur. Graph produced on MDI Jade software.
Another question still needed to be answered. What were the dark grey/black crystals that precipitated with the silver phosphate crystals? A second SEM/EDS scan of the second attempt’s darker precipitates revealed the dark grey/black crystals to be euhedral crystals of pure silver (Ag(s)). No sulfur or sulfate anions were detected. The excess silver crystals indicate an incomplete reaction of the silver ammine solution with the aqueous phosphate anions in the final supernatant solution in each tube. This finding confirms the lack of phosphate available for complete reactions to occur.
Figure 23: SEM image and EDS measurement of sample SMB005A’s silver phosphate (Ag₃PO₄) precipitate. Euhedral crystals of Ag₃PO₄ and silver (Ag) were the only precipitates formed, with no other contaminants. Top: SEM image of precipitate formed from SMB005A. Bottom: EDS analysis of SMB005A1 (blue) and SMB005A2 (cyan) points displaying only silver (Ag), phosphorus (P), and oxygen (O) present in the precipitate. The carbon (C) signature is detected from the carbon tape used as an adhesive for samples and a neutral background for EDS analysis.
For a final, third attempt, each sample received a bone carbonate pre-treatment soaking to dissolve the excess calcite. These samples were allowed to soak for 48 hours in triammonium citrate. After soaking, each sample was dried, massed, repackaged into labeled microcentrifuge tubes, and shipped to ARK. Ms. Oberg then reran the samples through the Arkansas method to produce high-quality, dark, golden-yellow silver phosphate crystal. Dark blue, silver crystals also precipitated with the silver phosphate in this final attempt. The crystals could be a byproduct of triammonium citrate used to remove the excess calcite in each bone powder sample. However, water rinsing of the samples at multiple stages of the chemistry would dissolve any residual triammonium citrate. Barite and celestite, identified in the SEM/EDS screenings (Figures 18, 19, 20), are also possible identities. Both minerals are not present in large quantities in possibly intermixed matrix. These minerals also possess high-density implying that centrifugation would remove any present crystals in multiple possible steps in the chemistry. Despite these extra crystals, the decision was made to run these samples through the mass spectrometer. These samples yielded good stable isotopic oxygen values. No samples remained for additional analyses to determine the unidentified crystal’s chemical makeup.
Figure 24: Euhedral, well-formed golden yellow silver phosphate crystals intermixed with silver to blue-colored euhedral crystals. These secondary crystals precipitated alongside the silver phosphate. Photographed under a microscope at 6x magnification. Photo courtesy of Danielle Oberg.
Coccolithophore Identification

The coccoliths, revealed by the SEM/EDS scan, are preserved well-enough for identification. Dr. David Watkins, Professor Emeritus-University of Nebraska-Lincoln, identified each species based on the structure of the elements in the rims of each test, specific to each genus and family (D. Watkins, personal communication, 2021). The following species are identified. 

*Prediscosphaera intercisa*, *Biscutum constans*, *Prediscosphaera columnata*, *Broinsonia signata*, *Ahmuellerella octoradiata*, and *cf. Watznaueria barnesiae*.

Figure 25: SEM images of coccolithophores. A) *Biscutum constans*; B) *Prediscosphaera columnata* (round) and *Broinsonia signata* (ovate); C) *Prediscosphaera intercisa*; D) *Ahmuellerella octoradiata*. Specimens were not coated with conductive materials. Scale bar = 5 micrometers.
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Checked April 2022.


APPENDIX A

SAMPLE COLLECTION METHODS

Adopted from Coulson (2009), Harrell et al. (2016), and Shabaga et al. (2019)

Materials

- Dremel® 3000 Rotary Tool with Adjustable Speed Foot Pedal
- Dremel® Rotary Tool Workstation for Woodworking and Jewelry Making
- Warrior® Diamond Point Rotary Bit Set
- Warrior® Carbide Rotary Micro Bit Set
- Impact resistant glasses
- Stand light
- Clear 1 ml pipettes (20)
- Electron Microscopy Sciences Glassine Weighing Paper (500)
- 1 ml glass vials (150)
- Micro Sharpie pen
- 1 M hydrochloric acid
- Deionized water (2 Liters – 4, 500 ml bottles)
- Kimberly-Clark Professional™ Kimtech Science™ Kimwipes™
- Latex gloves
- Stainless steel spatula
- Agate mortar and pestle

Methods

Vial Labeling: SMB000, SMB001, SMB002, SMB003, SMB004, SMB005, SMB006

Phosphates – Bones, Tooth

Bone Sampling Method

Phalange, radius, coracoid, rib fragment, and pygal sampled from the outside of the bone to the measured midpoint of the shaft. Mid-pointed calculated from caliper measurement of total thickness of bone, divided by 2.

1. Don Latex gloves to handle tools and vials
2. Clean diamond rotary bit
   a. Dip bit into 1 M Hydrochloric acid
   b. Rinse bit in deionized water
c. Wipe and dry bit with Kimwipe

3. Mount rotary drill in drill press

4. After folding weighing paper, place weighing paper on drilling plane below drill

5. Place phalange/radius/coracoid/rib fragment/pygal on weighing paper

6. Without starting drill, bring drill bit into contact with surface to establish zero distance mark. Reference scale bar from depth marker to begin marking 1 mm increments

7. Drill 1 mm into bone surface to produce powder.

8. Collect powder in weighing paper, and transfer sample to glass vial.

9. Clean diamond rotary bit
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   c. Wipe and dry bit with Kimwipe

10. Replace weighing paper with fresh sheet

11. Sample sequentially each millimeter to center of bone, cleaning bit in same manner between coring

12. Repeat Steps 1 through 9 for podial, radius, coracoid, rib fragment or pygal

Bone Sampling Method: Vertebra

As Belle is in-situ in the plaster block, sample collection is difficult at best. To accommodate for this, a stainless steel spatula was used to dig out produced powder.

1. If necessary, clean the diamond rotary bit
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   a. Dip bit into 1 M hydrochloric acid

Tooth Enamel Sampling Method

To sample the singular tooth provided to the study, a Dremel abraded the enamel off of the tooth as a whole sample into an agate mortar and pestle.

   a. Dip bit into 1 M hydrochloric acid
b. Rinse bit in deionized water

c. Wipe and dry bit with Kimwipe

2. Setup the work area by placing tooth into an agate mortar as a catchment for the resulting powder

3. Using a Dremel® with a cylindrical diamond burr, abrade the surface of the tooth with alternating pressure and speed to remove just the enamel.
   a. Periodic inspection of the surface will ensure complete removal of enamel and avoidance of scouring into the underlying dentine.

4. After removing the enamel, crush any larger enamel fragments still present in the mortar with a pestle.

5. Package crushed powder into glass vial.

Carbonates – Rostrum Carbonate and Chalk Matrix

Sample Numbers: SMB007, SMB008, SMB009, SMB010

Belemnite Sampling Method

   As per the owner’s request, the damaged belemnite rostrum within the main mass of rostrums was chosen for sampling. Three holes were drilled into the thickest part of the rostrum at the widest diameter of the lanceolate shape. The borehole’s ended at approximately the siphuncle within the rostrum.

   1. If necessary, clean the diamond rotary bit
      a. Dip bit into 1 M hydrochloric acid
b. Rinse bit in deionized water
c. Wipe and dry bit with Kimwipe

2. Position fossil for easiest access to lateral edge near thickest part of lanceolate form.

3. Holding drill perpendicular to rostrum surface, drill 1 mm towards center of rostrum.

4. Due to the position of the borehole, and its closeness to the matrix surface, remove produced carbonate powder from borehole with 1 ml pipette and transfer to microcentrifuge tube.

5. Clean rotary bit.
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   c. Wipe and dry bit with Kimwipe

6. Continue drilling into belemnite, sampling incrementally every 1 mm, until reaching center.

7. After each sample, clean rotary bit.
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   c. Wipe and dry bit with Kimwipe

Matrix Sampling Method

Matrix samples were taken adjacent to sampled vertebra, sampled belemnite, and in proximity to the skull. These samples will be tested for their stable oxygen isotope ratios for diagenetic alteration of phosphate and carbonate signals. In addition, the coccoliths may reveal surface water temperature.
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   c. Wipe and dry bit with Kimwipe

2. Drill a borehole perpendicular into matrix surface, sampling every 1-5 mm, targeting individual depositional layers.

3. Using 1 ml pipette, transfer produced powders and cuttings into numbered microcentrifuge tube.

4. Clean micro carbide drill bit after each incremental depth sample
   a. Dip bit into 1 M hydrochloric acid
   b. Rinse bit in deionized water
   c. Wipe and dry bit with Kimwipe
Mosasaur Belle Bone and Mineral Sample Collection Summary (SMB: Sternberg Mosasaur Belle)  

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APPENDIX C
Considerations for Organics

Preparation of both the phosphate and carbonate samples suggests treatment for organics. Organics are defined as the presence of organic, carbon-bearing compounds or molecules preserved within the fossilized tissues or enveloping matrix. These residues can add carbon and oxygen into the produced carbon dioxide for carbonate analysis or combusted with the silver phosphate, skewing the produced carbon dioxide. A soak in 5% sodium hypochlorite for 24 hours oxidizes and dissolves organics into solution. This organic treatment is commonly used for archaeological studies as the bone still contains proteins, lipids, and collagen (Lee-Thorp and van der Merwe 1991; Lee-Thorp et al. 1997; Lee-Thorp 2002; Garvie-Lok et al. 2004, Lee-Thorp 2008). This organic treatment is also a step in both the silver phosphate preparation (O’Neill 1994; Vennemann 2002) and secondary calcite dissolution for bone carbonate treatment (Silverman et al. 1954). Fossilized bones and teeth are considered to be devoid of original or altered organic material. Multiple studies, however refute this statement, even demonstrating the preservation of bone cells, tissue structure, or the presence of modern microbial communities living within the bone (Schweitzer et al. 2005; Saitta et al. 2018; Saitta et al. 2019). Studying the EDS images (Figures 18, 19, and 20), one organic residue of unknown composition is present within the chalk matrix. Extrapolating this one example to across the specimen, organics are present, but their minute amount is negligible to impact stable isotope analysis.

Bone powder samples for phosphate analysis were not treated with a 5% sodium hypochlorite solution. Samples for carbonate analysis for bulk calcite analysis were not treated. Samples for bone carbonate were treated with the solution. Each samples’ δ¹³C value varies
between the bulk sample versus the treated sample. However, organics cannot necessarily be attributed to this difference as calcite dissolution would change the overall isotopic composition.

Future work would involve running a third set of bone powder samples treating a bulk sample to look for direct changes from the treatment of organics.
APPENDIX D

Comparison of Structural Carbonate Treatment Techniques

Apatite carbonate analysis of $\delta^{13}$C and $\delta^{18}$O is a useful tool to analyze dietary, temperature, and migrational patterns preserved in modern and fossilized bone tissue. As discussed earlier, bone apatite’s primary components are calcium, phosphate, and carbonate. The carbonate anions interchange with hydroxide and fluorine ions within the apatite crystal lattice. The carbonate component (CO$_3^{2-}$) preserves carbon and oxygen atoms useful in physiological analyses (Lee-Thorp 1997). During fossilization, calcite is a common mineral that permineralizes bone, filling the osteons and voids left behind during organic decay. These secondary calcites, containing their own $\delta^{13}$C and $\delta^{18}$O signatures, can misconstrue bone carbonates’ isotopic signatures during analysis. Multiple methods for the dissolution of this diagenetic calcite are known in preparation for stable isotope analysis. Aqueous acetic acid (CH$_3$COOH), acetic acid with a sodium acetate buffer, and aqueous triammonium citrate (C$_6$H$_{17}$N$_3$O$_7$) are the known techniques in sample preparation. Each technique utilizes a soak in solution between 24 to 36 hours to dissolve the diagenetic calcite, leaving behind the carbonate component of the bone tissue.

Lee-Thorp (1991, 1997, 2002) used 0.1 M and 1.0 M solutions of acetic acid to treat lightly permineralized archaeological bone (<10,000 years old). These studies relied heavily on removing organics and then isolating the bone carbonate to directly study inorganic carbon intake from the environment both people and animals lived in.

Garvie-Lok et al. (2004) compared the use of varying molarities of acetic acid for dissolution. Their results suggested that a soaking time of four hours with either 0.1 M or 1.0 M acetic acid caused dissolution of calcite with little to some fluctuations of $\delta^{18}$O values. However,
both Garvie-Lok et al. (2004) and Lee-Thorp (1997) noted prolonged soaking increased the chance for brushite to form. Brushite (CaHPO$_4$$\cdot$2H$_2$O) is an altered form of calcium phosphate formed in the presence of sulfates, calcite, clay, and low pH solutions. The interchange of both calcium cations and phosphate ions during the possibly re-precipitation created concerns of liberating bone carbonate anions into solution, possibly fractionating the oxygen isotope signatures away from their original values.

Demény et al. (2019) recognized the need for standardization of this process. No standard procedure is established within bioarchaeological research, and therefore paleontology. Their results indicated that structural carbonate from bone should be included in interpretations. And, tooth enamel could be interpreted as long as 102% phosphoric acid at 70°C was used to produce viable carbon dioxide gas for analysis.

Silverman et al.’s (1954) method instead relies directly on the known solubility of calcite at a specific pH, temperature, and dissolution in triammonium citrate. Each bone powder sample is soaked in an 8.1±0.1 pH solution at room temperature to dissolve spar calcite. In treating for bone carbonate using for this method, the spar calcite is assumed to be loosely deposited within the open pore spaces of the bone, compared to the bone carbonate that is attached into the bone crystal structure. The bone’s atomic bonds, therefore, are stronger than the spar calcite. The saturation of the solution with calcite is also factored in to decrease solubility through the soaking time to preclude the bone carbonate from being dissolved into solution as well. The fact that Belle is also preserved in chalk, composed of calcite-based coccolithophores, was also considered.

The Silverman et al. method was therefore chosen to treat the bone samples to analyze structural carbonate.
APPENDIX E

REAGENT PREPARATIONS

BASIC SOLUTION PREPARATION

\[ M_1 \times V_1 = M_2 \times V_2 \]

- \( M_1 \) = Molarity of Stock Solution
- \( V_1 \) = Volume (L) of stock solution
- \( M_2 \) = Desired Molarity
- \( V_2 \) = Desired Volume

0.5 M, 8.1 pH TRIAMMONIUM CITRATE SOLUTION

\( \text{C}_6\text{H}_{17}\text{N}_3\text{O}_7 \): 243.22 g/mol

To prepare a 100 ml solution:

\[ M = \frac{mol}{L}; \quad 0.5 \, M = \frac{x \, mol}{L}; \quad x = 0.5 \, \text{mol} \]

0.5 mol = 0.5 mol * 243.22 g/mol = 121.61 grams

1 L \rightarrow 100 ml = 10\% \text{ of volume} \rightarrow 12.16 \text{ grams needed for a 100 ml solution}

Triammonium citrate is shipped and stored as a salt.

*Make sure to add acid/base to water, not water to acid/base.

For a pH of 8.1:

Measure initial pH of citrate solution with pH meter – Vernier pH Sensor PH-13TA using Logger Pro on Windows 10

Triammonium citrate: initial pH = 7.6±0.1

Add ~7 drops of NH\(_4\)OH (Assay 20-22\%) to increase pH to 8.1

SILVER AMMINE SOLUTION (0.2 M AgNO\(_3\), 0.35 M NH\(_4\)NO\(_3\), 0.74 M NH\(_4\)OH)

Molar Masses (g/mol):
AgNO₃ (silver nitrate): 169.88 g/mol

NH₄NO₃ (ammonium nitrate): 80.052 g/mol

NH₄OH (ammonium hydroxide): 35.05 g/mol

Specific Gravity: 0.91 at 20°C (68°F)

For a 100 ml solution:

\[ M = \frac{mol}{L} \]

\[ 0.2 \ M = \frac{x \ mol}{L} \]
\[ x = 0.2 \text{ mol} \]

0.2 mol AgNO₃ (salt): 0.2 mol * 169.88 g/mol = 33.976 grams

33.976 grams * 10% = 3.3976 grams ~ 3.4 grams

0.35 mol NH₄NO₃ (salt): 0.35 mol * 80.052 g/mol = 28.0182 grams

28.0182 grams * 10% = 2.80182 grams ~ 2.8 grams

0.74 mol NH₄OH (aq): 0.74 mol * 35.05 g/mol = 25.937 grams

25.937 grams * 10% = 2.5937 grams ~ 2.6 grams

As ammonium hydroxide is typically available as an aqueous solution (created from the dissolution of ammonia (NH₃) gas into water), the necessary volume of liquid is needed to correlate to the needed mass. Most aqueous chemicals are shipped with an assay of chemical content. To convert assay to molarity:

\[ Molarity = \frac{\% Assay \times Specific \ Gravity \times 10}{Molar \ Mass} \]

For ammonium hydroxide:

28% ammonia (NH₃) is equal to approximately 56.6% ammonium hydroxide.

Assay ~ Purity

Specific Gravity @ Specific temperature
Molar Mass of acid, base

\[ M = \frac{56.6\% \times 0.90 \text{ g/ml} \times 10}{35.05 \text{ g/mol}} \]

\[ M = 14.534 \text{ M} \]

So, for 0.74 mol NH\(_4\)OH, use:

\[ 2.6 \text{ grams} \times 0.90 \text{ g/ml} = 2.34 \text{ ml} \]

For the proper amount of 14.534 M solution to use:

\[ M_1V_1 = M_2V_2 : (14.534 \text{ M})(x \text{ L}) = (0.74 \text{ M})(0.00234 \text{ L}) \]

\[ x \text{ L} = 1.19 \times 10^{-4} \times 1000 \text{ ml/L} = 0.12 \text{ ml of NH}_4\text{OH @ 28\% Assay} \]

Add 0.12 ml of NH\(_4\)OH, 3.4 grams AgNO\(_3\), and 2.8 grams of NH\(_4\)NO\(_3\) to 99.88 ml of water for final solution.

The silver ammine solution is extremely photosensitive. Exposure to light will quickly degrade the solution. Keep the bottle wrapped in aluminum foil or opaque paper and store in a dark cabinet.
APPENDIX F

BONE CARBONATE PREPARATION

Using the method from Silverman et al. (1952) and modified from Lee-Thorp (2002):

Materials

- 0.5 M 8.1±0.1 pH triammonium citrate (C₆H₁₇N₃O₇)
- 5% sodium hypochlorite (NaOCl)
- 2 ml microcentrifuge tube
- Deionized water
- Bone samples
- Eppendorf™ Minispin™ Microcentrifuge
- Vortex Genie Mixer
- 100 ml beaker

Procedure

1. Measure 5 mg of bone powder into a 2 ml microcentrifuge tube.

2. Add 2 ml of 5% sodium hypochlorite to the tube and let the sample soak for 24 hours.
   
   This step oxidizes and liberates any organic content present within the samples.

3. After soaking, centrifuge the sample at 10,000 rpm for 5 minutes and decant the supernatant solution.

4. Add 1 ml of deionized water to the tube. Thoroughly mix the contents to put the organics treated bone into suspension using a Vortex Genie mixer. Then centrifuge vial at 10,000 rpm for 5 minutes, decanting solution from sample into waste beaker.
   
   - A swing bucket centrifuge is preferred to a fixed angle rotor to pelletize the material effectively at the tube tip.

5. Repeat step 4 three more times for four total rinses.

6. Add 2 ml of 0.5 M, 8.1±0.1 pH triammonium citrate to centrifuge tube with sample. Mix sample into suspension with Vortex Genie.

7. Soak sample for 36 hours.
8. After soaking, centrifuge the sample at 10,000 rpm for 5 minutes and decant the waste solution.

9. Add 1 ml of deionized water to the tube. Thoroughly mix the contents to put the treated bone into suspension, preferably using a Vortex mixer. Then centrifuge vial at 10,000 rpm for 5 minutes, decanting solution from sample into waste beaker.

10. Repeat step 4 three more times for four total rinses.

11. Dry each sample completely overnight at 50°C.
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