# THERMOREGULATION IN LATE CRETACEOUS MARINE REPTILES OF MANITOBA

by

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ABSTRACT

The thermoregulation of mosasaurs and plesiosaurs have been debated within the field of paleontology for many years, specifically whether these marine reptiles were ectothermic or endothermic. Some degree of endothermy has been proposed for both mosasaurs and plesiosaurs based on histological, morphological, and geochemical methods. Geochemical studies have suggested elevated body temperature in mosasaurs and plesiosaurs compared to ambient seawater temperatures, but the topic remains understudied. For this study, bulk oxygen isotope paleothermometry was used to calculate the body temperatures for Late Cretaceous mosasaurs and plesiosaurs from the Pembina Member of the Pierre Shale in south-central Manitoba. Body temperatures were also calculated for coeval fish and birds (*Hesperornis*) from the Pembina Member which were used as ectothermic and endothermic 'end members', respectively. Before temperatures were calculated, the preservation of 25 fossils specimens were assessed using scanning electron microscope analysis, relative total rare earth element concentrations, principal component analysis of bulk element concentrations, and the difference between the $\delta^{18}O$ in the phosphate and carbonate fractions. Using bulk oxygen isotope palaeothermometry, body temperatures were calculated to be 36.4 ± 1.1°C SD for mosasaurs, 42.8 ± 0.3°C for plesiosaurs, 28.1°C for fish and 41.1°C for *Hesperornis*. Statistical analysis could not be conducted due to the small sample size, but the body temperatures of Manitoba mosasaurs did fall between coeval ectothermic fish and endothermic birds and were consistent with those calculated from other localities. Plesiosaur specimens also possessed elevated body temperatures but exceeded those calculated for both fish and birds as well as those previously determined for plesiosaurs from different localities. The elevated temperatures calculated for the Manitoba plesiosaur specimens may reflect differences in body temperatures between genera or may be the result of diagenetic alteration. The body temperatures calculated for the marine reptiles in this study are elevated compared to ambient seawater temperatures and thus, can be taken as evidence of endothermy in Late Cretaceous Manitoba mosasaurs and plesiosaurs.

DEDICATION

To parents Kurt and Jackie, sister Emma, brother-in-law Aaron, nephew Rohan, and my husband Colin.

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# CHAPTER 1: LITERATURE REVIEW

Marine reptiles, like mosasaurs and plesiosaurs, dominated the oceanic landscapes during the Cretaceous period and were a prominent component of the ecosystem at the time (Nicholls and Russell, 1990). They were especially abundant in the Western Interior Seaway (WIS), a large shallow inland sea that stretched across North America from the Arctic Ocean to the Gulf of Mexico (Hayes *et al.,* 1993). As they are not closely related, mosasaurs and plesiosaurs were both secondarily adapted aquatic carnivorous reptiles that convergently evolved to inhabit similar niches. Fossil discoveries of both clades in North and South America, Europe, Asia, Australia, and Antarctica further indicate that they exploited environments ranging from tropical to polar (Ross, 2009; Ketchum and Benson, 2010). However, key aspects of their biology are unknown, such as whether these marine reptiles were able to regulate their body temperature (especially at extreme latitudes) owing to potential differences in ambient seawater temperatures across their ranges. Scientists have thus attempted to address whether mosasaurs and plesiosaurs were endothermic, ectothermic, or gigantothermic, but consensus has not been met (Bernard *et al.,* 2010; Harrell *et al.,* 2016).

The thermal biology of extinct animals has long been debated within the scientific community, especially the metabolic status of large extinct reptiles (Bernard *et al.,* 2010). There are various analytical techniques that can be employed to gain insight into the thermal biology of extinct species including histological, morphological, and geochemical analyses. In this study, bulk oxygen isotope analysis, a geochemical technique, was employed to gain insight into the thermoregulatory strategy of extinct marine reptiles collected from Late Cretaceous (Campanian) deposits within a mid-latitude region of the WIS. Bulk oxygen isotopic analysis allows the internal body temperatures to be calculated directly from skeletal elements using the relationship between oxygen isotopes and temperature. Urey (1947) first theorized bulk oxygen isotope palaeothermometry through the equilibrium constant and the oxygen isotopic exchange between a carbonate mineral and the water in which it formed. He then applied this new technique to belemnite fossils (Urey *et al.,* 1951), which was further developed and refined by McCrea (1950), Epstein *et al.* (1953), and Emiliani (1955). Since then, bulk oxygen isotope analysis has been used extensively within paleoceanography (Pucéat *et al.,* 2003; Friedrich *et al.,* 2012),

paleoecology (Clementz and Koch, 2001), and paleoenvironmental (Knudson, 2009) studies. In recent years, bulk oxygen isotope paleothermometry has been employed to deduce the metabolic status for a variety of dinosaurs (Barrick *et al.*, 1996; Fricke and Rogers, 2000; Amiot *et al.*, 2006) and large macropredatory sharks (Ferrón, 2017). However, very few studies include or concentrate on marine reptiles and even fewer still focus on specimens from the WIS. Bernard *et al*. (2010) and Harrell *et al*. (2016) used this method to infer the relative intensity of metabolic rates amongst marine reptiles such as plesiosaurs, ichthyosaurs, and mosasaurs. Both studies support the hypothesis that mosasaurs and plesiosaurs were able to maintain higher core body temperatures relative to coeval ectothermic fish and therefore were likely to be endothermic. For the present study, body temperatures were calculated through the $\delta^{18}O$ in ~81 million-year old mosasaur and plesiosaur skeletal elements from the WIS in Southwestern Manitoba. The estimated body temperatures from the marine reptiles were compared to coeval ectothermic fish and the endothermic aquatic bird (*Hesperornis*) as thermoregulatory "end members", in order to explore possible thermoregulatory systems for mosasaurs and plesiosaurs.

Since there is a general lack of consensus regarding the thermoregulation of Cretaceous-age marine reptiles like mosasaurs and plesiosaurs, the ecological implications of their metabolic status are also unclear: i.e. its effect on feeding, locomotion, evolution, and diversification of mosasaurs and plesiosaurs (Harrell *et al.*, 2016). The further implications and the impact of mosasaur and plesiosaur thermal biology on the greater paleoecology of the WIS are also discussed in this thesis.

Little work comparing the body temperature of marine reptiles from different latitudes has been conducted (Bernard *et al.*, 2010; Harrell *et al.*, 2016). The mosasaur results from this analysis were compared to those derived from a southwestern Alabama study to see how the body temperatures change with the temperature of the ambient water (Harrell *et al.*, 2016). Mosasaur and plesiosaur specimens were also compared to those from other localities to see if the results in this study are comparable (Bernard *et al.*, 2010). Variables that could contribute to the results obtained were explored such as seasonality and temporal differences.

The accuracy of paleothermometry is dependent on the original composition remaining unaltered, as diagenetically overwritten specimens would result in inaccurate body temperature values. So, multiple methods were used to assess the level of diagenetic alteration of each

specimen, namely, identifying outliers of bulk element concentrations prone to diagenetic alteration and assessing the relative levels of total rare earth elements (REEs).

The $\delta^{13}C$ values were also obtained in this study and were used to infer a dietary niche for all animal groups. Similar to $\delta^{18}O$, carbon isotopes get incorporated into bioapatite when the tooth is forming but primarily reflect diet instead of formation temperature (Leuzinger *et al.,* 2022). The $\delta^{13}C$ values varies depending on the source (i.e. latitude, depth and distance from shore) of an aquatic animal's food source (Goericke and Fry, 1994). Therefore, a comparison with previous research from fossilized material was used to assess if these animal groups had a deep water offshore or shallow water near shore dietary niche (Leuzinger *et al.,* 2022).

## 1.1 MARINE REPTILES

Marine reptiles are vertebrate animals which have secondarily adapted to aquatic environments over evolutionary time from terrestrial ancestors. The expansion of reptiles into marine habitats is thought to have been driven by previous extinctions and increased competition in their original habitats; in addition, the proclivity of the organism to adapt to new aquatic environments and the degree of enrichment in near shore environments was also a significant factor (Vermeij and Motani, 2018). Marine colonization occurred independently several times through multiple lineages of reptiles, which resulted in diverse morphologies and ecologies (Motani, 2009). Though extant marine reptiles are rare today (e.g. sea turtles and crocodiles), they were more abundant and diverse in the Mesozoic era and included ichthyosaurs, plesiosaurs, and mosasaurs.

Mosasaurs were a type of squamate (lizards, snakes, and amphisbaenians) marine reptile (Reeder *et al.,* 2015; Harrell *et al.,* 2016). They evolved rapidly in the early Late Cretaceous from small semi-aquatic varanoid-like lizards and lived until the K-Pg extinction (Caldwell and Paci, 2007; Reeder *et al.,* 2015). Basal mosasauroids such as *Dallasaurus* were small in size with modified tails used for aquatic locomotion and possessed plesiopedal limbs (Polcyn and Bell, 2005). Though physically functional in terrestrial environments, these basal mosasauroids likely lived most of their lives in aquatic environments (Caldwell and Palci, 2007). More derived (fully aquatic) mosasaurs are characterized by long, streamlined bodies, specialized hypocercal tails for axial locomotion, and hydropedal paddle-like limbs. Mosasaurs ranged in size from the small *Dallasaurus* measuring around 1 m in length to the huge *Mosasaursus* which could have reached

up to 17 m (Polcyn and Bell, 2005; Grigoriev, 2014). Mosasaur fossils have been found on every continent, suggesting that they lived in a variety of habitats from near shore to deep ocean environments (Kiernan, 2002). Though some mosasaurs like *Globidens* exhibited feeding specializations (i.e., durophagy), mosasaurs were generalized feeders with prey type depending on their body size (Massare, 1987). Smaller mosasaurs mainly hunted smaller prey such as fish and cephalopods while larger mosasaurs hunted larger prey such as large fish or other marine reptiles.

Plesiosaurs were a clade of marine reptiles which evolved from basal aquatic sauropterygians that first appeared in the Late Triassic period (O'Keefe, 2002; O'Keefe *et al*., 2019). They diversified throughout the Jurassic Period until finally going extinct during the K-Pg extinction event (O'Keefe, 2002; Wintrich *et al.,* 2017b; O'Keefe *et al*., 2019). Plesiosaurs are characterized by large broad bodies, short tails, and large ridged flippers with well ossified wrists and ankles used for appendicular locomotion (Montani, 2009). The overall body plan of the plesiosaur clade varied substantively creating two distinct end members – the plesiosauromorphs and the pliosauromorphs. The plesiosauromorphs are characterized by elongated necks and small heads relative to their body size. Pliosauromorphs, on the other hand, have relatively large heads when compared to their body size but very short necks. Overall body sizes ranged from the 2 m long *Tatenectes* to the 15 m long *Elasmosaurus* (O'Keefe and Street, 2009; Sachs *et al.,* 2013). Like mosasaurs, plesiosaurs have been discovered on every continent, implying a wide range in habitats from near shore to open ocean. Due to the vastly different bauplans, plesiosaurs' predation style varied. While short-necked plesiosaurs would have been pursuit predators, their long-neck counterparts relied little on swimming to catch prey (Motani, 2009). Plesiosaurs mainly consumed soft-bodied fish and cephalopods, though larger pliosaurs might have preyed upon other marine reptiles (Massare, 1987).

## 1.2 THERMOREGULATION

There are numerous ways in which the thermophysiology of an animal presents itself in nature and so there are multiple terms that can be defined to characterize the thermoregulation of an organism. It is important to note that these terms are not mutually exclusive and are used in tandem to best describe a particular organism. Identifying the temperature range in which an organism can optimally function and the extremes it can withstand is one of the most common

ways to describe thermoregulation. Poikilotherms are organisms that are unable to generate meaningful heat from metabolic processes, and thus their body temperature tends to, but not exclusively, be highly variable with fluctuating ambient temperatures (Whittow, 1973; Romanovsky, 2018). The fluctuating body temperature of poikilotherms closely tracks with ambient temperatures and can be daily or seasonally cyclical (Cubo, 2021). Homeotherms are organisms that have (relatively) consistent body temperatures that are less likely to fluctuate with ambient temperatures. Optimal energy output for a homeotherm is achieved when the organism's core body temperature is within a narrow, species-specific range (Cubo, 2021). Though the optimal temperature range of homeotherms are defined as narrow, body temperature can still fluctuate (Hetem *et al.,* 2014). Gigantotherms – animals able to maintain body temperatures above ambient arising primarily from their large size (low surface area to volume ratio) – also possess relatively consistent core body temperatures in relation to surrounding environments but achieve this passively due to their large size and low body surface area to volume ratio. Gigantothermic thermoregulation has been proposed for sauropod dinosaurs (Grady *et al.,* 2014) and modern leatherback turtles (Paladino *et al.,* 1990). Heterotherms are organisms that may exhibit components of both poikilothermic and homeothermic systems. Some heterothermic animals can switch between these two systems temporarily. A variety of bats, rodents, shrews, and primates show temporal heterothermy through either daily or seasonal torpor (McKechnie and Mzilikazi, 2011; Stawski *et al.,* 2014; Romanosky, 2018). Organisms may also express regional heterothermy, where certain portions of the body, usually the extremities, will deviate from core body temperatures. This type of thermoregulation is seen in a variety of homeothermic animals and is particularly prominent in those that live in polar habitats like emperor penguins and arctic reindeer, wolves, and seals (Ponganis *et al.,* 2003; Blix, 2016).

Another way to describe thermoregulation in an organism is to classify the way they maintain their body temperatures. Ectotherms are organisms that depend on behavioural mechanisms to keep their body temperatures within their livable range (Seebacher, 2009). Behavioural mechanisms to maintain body temperature include basking, changes in posture, energy intake (digestion), physical activity, and physical location which could be innate and/or learned behaviour (Golovanov, 2006; Terrien *et al*., 2011). By contrast, endotherms regulate their body temperature mainly through internal physio-biochemical mechanisms for thermogenesis, i.e., the metabolic production of heat within the body. This non-mechanical form

of thermogenesis is the principal process in which endotherms produce the heat through their metabolism necessary to maintain core body temperature (Romanosky, 2018). For instance, some modern mammal groups have thermogenic brown adipose tissue (BAT) that contributes significantly to facultative thermogenesis (Oelkrug *et al.,* 2015). The mitochondria within this tissue allows protons to move through activated uncoupling proteins within the inner mitochondrial membrane, thereby directly releasing heat generated through substrate catabolism and hence drive production of the energy storage molecule adenosine triphosphate (ATP). Endotherms are generally characterized by higher metabolic rates as well as more rapid bone growth than ectotherms (Liebe and Hurum, 2012; Krahl *et al.,* 2013; Wintrich *et al.,* 2017a; Fleischle *et al.,* 2018). Mesothermic animals such as sea turtles and likely many dinosaurs (Grady *et al.,* 2014; Okutama *et al.,* 2021) are a 'middle ground' between endothermy and ectothermy where endogenic heat produced is retained though behavioral mechanisms which are heavily relied on to maintain their body temperatures (Paladino *et al.,* 1990; Bernal *et al.,* 2001; Geiser *et al.,* 2017).

Body temperature regulation is potentially challenging for many organisms, but it presents a unique set of challenges for aquatic animals. Heat, which is the energy within an atom that contributes to its molecular motion, can be transferred in four different ways: radiation, evaporation, conduction, and convection (Castellini, 2009; Favilla and Costa, 2020). Convection is where heat is dissipated away from the body through the movement of external fluids (e.g. air or water). This heat transfer can be created by temperature differences, currents/wind, or physical movement of the organism (Davis, 2019). Convection is the most common type of heat loss in aquatic animals, though these species can exhibit both radiative (the transfer of heat directly from one object to another through the emission/absorption of energy waves) and evaporative (heat loss when liquid on the body phase transitions to vapor) heat loss when at the water surface (Davis, 2019). When on land, semi-aquatic mammals also must contend with conduction (heat loss through direct contact with another body or object) (Davis, 2019).

The rate at which heat is lost from a body through convection depends in large part on the thermal gradient—the ability of a medium to transfer heat through temperature differences separated by a specific distance. In animals, the rate at which heat will move from the warmer medium to the colder will depend on some factors: the thickness and thermal conductivity (speed of heat transfer through a given tissue/substrate) of their insulation layers (e.g. blubber, skin, fur,

feathers) and surrounding medium (i.e. water or air), the temperature gradient between themselves and the medium, and the body surface area to volume ratio. Due to this relationship, an organism will lose more heat if the medium in which they are surrounded has a high thermal conductivity (Davis, 2019). Water has a thermal conductivity that is ~25x greater than that of air and therefore aquatic animals are expected to be more likely to lose heat at a faster rate compared to their terrestrial counterparts (Davis, 2019).

The substantially greater thermal conductivity of water makes maintaining core body temperatures more difficult for aquatic animals. Accordingly, extant aquatic poikilothermic ectotherms, like sea snakes or marine iguanas, possess body temperatures only slightly above ambient temperatures and are typically limited to inhabiting warm tropical or subtropical waters not prone to low temperatures (Bertolero *et al.,* 2009; Heatwole *et al.,* 2012). Behavioural mechanisms are also employed to minimize the difference between optimal body and ambient temperatures such as basking at the water's surface and navigating to preferred microhabitats (Buttemer and Dawson, 1993; Heatwole *et al.,* 2012). Heat loss in water is a particular issue for aquatic homeothermic endotherms that need to keep body temperatures within a specific range independent of seawater temperatures (Davis, 2019). There are two ways in which a homeothermic endotherm can combat heat loss: through trapping endogenic heat via insulation and/or through increasing internal heat production. In modern aquatic endotherms (birds and mammals), maximizing insulation is a key strategy to maintain body heat. For example, the thick blubber layer of cetaceans (whales and dolphins) and sirenians (sea cows) acts as a thermal insulator, a buffer between core body temperature and the surrounding water. This modified adipose tissue is a subcutaneous insulator located underneath the hypodermis and is supported by collagen and elastic fibres (Davis, 2019). The thickness, composition, lipid content and conductivity of blubber varies depending on the species, age, sex, seasonal conditions, and body size of the individual (Davis, 2019). Rates of heat loss through the blubber can rapidly be controlled through vasoconstriction causing a restriction of blood flow through dermal tissues via arteriovenous anastomoses. This vasoconstriction can be reversed whereby blood flow is preferentially directed towards dermal layer to induce heat loss if the individual is overheating (Davis, 2019). Evidence of blubber in Mesozoic marine reptiles is scarce but Lindgren *et al.,* (2018) did observe a condensed glossy black tissue layered on top of a fibrous mat in the Jurassic ichthyosaur *Stenopterygius* similar to modern cetacean blubber. Fur may also be used as

insulation to help maintain the body temperature of aquatic mammals. In pinnipeds and sea otters, a thick water-proof fur covers the majority of the body and is used for insulation. Fur can minimize heat loss in three different ways: 1) by reducing direct contact of the surface epidermal layer with surrounding water and air, 2) by reducing heat radiation from skin through an air layer, and 3) by slowing down heat transfer via its low thermal conductivity. The hairs need to be waterproof to be effective and have elongated cuticular scales on the underhairs to trap pockets of air against the body surface. These pockets of air, which have low thermal conductivity, would then increase the animal's thermal insulation. This dense fur has two lengths, the long thick solitary hairs which provide an increased length of the thermal gradient from the body surface to the water and the finer shorter underhairs that are densely packed and primarily used as insulation (Irving, 1973; Davis, 2019). Hair density of fur on seals can be up to 17x the thickness of terrestrial mammal fur but this comes at a cost of hydrodynamic resistance and therefore the length of the solitary hairs is tempered to reduce drag (Davis, 2019). Fur is a more static insulator than blubber but can be slightly altered using thermal windows – parts of the body less covered in fur or completely naked like flippers – to help regulate body temperature. Blood flow is rerouted via autonomic vasomotor controls to these windows that are prone to heat dissipation in order to promote heat loss (Davis, 2019). However, it is unlikely that these marine reptiles would have had fur.

An aquatic endotherm must balance the inherently greater rate of heat loss associated with living in water with their own endogenous heat production (Davis, 2019). Marine endotherms such as seals, whales, and otters generate heat at twice the rate of similar-sized terrestrial counterparts; this excludes Sirenia such as Florida manatees, which are slow-moving herbivores (Irving, 1973; Davis, 2019). Accordingly, high resting metabolic rates are seen in many marine mammals except for Sirenia whose slow herbivorous lifestyles may reduce the need for a fast metabolism and a small livable thermal range restricts extant species to tropical waters (Irving, 1973; Davis, 2019). Cetaceans, pinnipeds, and sea otters independently adapted to aquatic environments and therefore the unique challenges of living in water presumably drove the increase in metabolic rates in all three families (Davis, 2019). It is thought that in extant aquatic mammals the elevated thermogenesis and resting metabolic rates may have been adapted to compensate for a higher rate of heat loss in water (Davis, 2019). Although metabolic rates are higher in marine animals than their terrestrial counterparts, endogenic heat is produced in a

similar way. The majority of endogenic heat in vertebrate animals is produced by skeletal muscle thermogenesis through muscle contraction (either by activity or shivering) and mitochondrial proton leakage. During muscle contractions or shivering, ATP is quickly broken down into adenosine diphosphate (ADP) by multiple different processes (Periasamy *et al.*, 2017), thereby requiring high rates of heat generating substrate catabolism to maintain cellular ATP levels. Since this form of heat generation requires muscle movement and can exhaust the muscle, non-shivering thermogenesis from proton leakage in the mitochondria is also beneficial to endotherms (Periasamy *et al.,* 2017; Wright, 2021). This process involves the movement of protons ($H^+$) through the inner mitochondrial membrane, which dissipates the proton gradient (proton motive force) between the intermembrane space and mitochondrial matrix without the use of F0/F1 ATP-synthase (Divakaruni and Brand, 2011). By bypassing the F0/F1 ATP-synthase and traveling through proton leak pathways, the proton is not used in the process of synthesizing ATP with the preceding heat generated by substrate catabolism being released into the body (Mozo *et al.,* 2005); ergo, increased rates of substrate catabolism are required to generate an equivalent amount of ATP. The skeletal muscle leak capacity, i.e. the ability for protons to move through the mitochondrial membrane, is positively related to the basal metabolic rates (BMR) of marine endotherms (Wright, 2021). This is especially prominent in sea otters, which possess elevated BMRs and muscle leak capacities suggesting a strong dependence on this type of thermogenesis (Wright, 2021). In some marine mammals (seals and otters), heat generation can also be achieved through the uncoupling proteins in BAT. BAT is found in some pinnipeds (but not cetaceans or sirenians) and functions the same as in terrestrial mammals (Gaudry *et al.,* 2017). Within the mitochondrial-rich adipose cells of BAT, heat is directly released into the body due to the movement of protons through uncoupling protein UCP1. This integral protein allows the movement of $H^+$ through the inner mitochondrial membrane instead of the proton motive force being harnessed to synthesize ATP (Mozo *et al.*, 2005), thereby resulting in an increase in mitochondrial substrate catabolism to maintain cellular ATP levels. Another suggested mechanism of mammalian non-shivering thermogenesis involves sarcolipin within skeletal muscles (Bal *et al.,* 2012). Briefly, during a muscle contraction, $Ca^{2+}$ is released from the sarcoplasmic reticulum into the cytoplasm, and then returned to the sarcoplasmic reticulum via the $Ca^{2+}$-ATPase (SERCA). Sarcolipin is bound to the $Ca^{2+}$-ATPase and has been proposed to induce $Ca^{2+}$ slippage into the cytosolic side rather than back into the sarcoplasmic

reticulum following muscle contraction (Bal *et al.,* 2012). The energy of some ATP molecules through hydrolysis to ADP is given off without $Ca^{2+}$ transfer, thus requiring elevated rates of (heat generating) substrate catabolism to maintain cellular ATP homeostasis (Bal *et al.,* 2012). However, the role that sarcolipin plays in SERCA non-shivering thermogenesis in mammals and other vertebrates, and the degree of endogenic heat produced by this mechanism is debated (Campbell and Dicke, 2018; Bal *et al.,* 2018).

In modern aquatic mammals, whole body endothermy was inherited from their terrestrial ancestors and then further adapted independently to meet the demands of aquatic environments (Davis, 2019). When assessing the thermoregulatory status of marine reptiles via comparison with modern marine mammals it is important to remember that the endothermy of the latter predated their secondary entrance to aquatic environments. Modern aquatic mammals may thus not be the best comparators since there is no evidence of inherited endothermy in mosasaurs or plesiosaurs. Therefore, aquatic organisms whose thermoregulation was not inherited from terrestrial ancestors such as regionally endothermic fish or sea turtles should also be considered as they evolved at least partial endothermy independently while inhabiting aquatic environments (Legendre and Davesne, 2020).

## 1.3 THERMOREGULATION IN PALEONTOLOGY AND MODERN MARINE ANALOGS

Assessing the thermal biology of extinct animals poses unique challenges since it cannot be directly measured. Inferring this parameter is also challenging as thermoregulation is complex, and is implemented mostly through multiple soft tissue organs (Newman, 2011). Since these systems are seldom preserved in the fossil record, indirect evidence, usually from skeletal elements that are more likely to be preserved (e.g. teeth, bones), must be used to estimate metabolic rate, bone growth rate, and activity levels. The thermoregulation of an extinct animal can be inferred using these pieces of evidence and can be approached in a variety of ways. Histological analyses use the presence, absence, or frequency of specific structures within bone tissue to characterize bone growth rates and metabolic rates (Legendre *et al*., 2016; Olivier *et al.,* 2017; Fleischle *et al*., 2018). Features such as high vascularization and secondary osteons as well as the presence of exterior epiphyseal pits and woven bone textures are more common in endothermic organisms whereas features such as arrested growth lines are seen in animals with slower bone growth rates as in ectotherms (Cubo *et al.,* 2008). Using modern species with

similar features as reference, the thermoregulation of extinct animals can be predicted. Similarly, morphological studies focus on the overall form and characteristics of skeletal elements to gain insight into the thermophysiology of extinct animals. For instance, the presence of nasal turbinates, large thoracic cavities, hair or hair-like filaments and blubber are taken as evidence of high metabolic rates since these are seen in modern endothermic animals (Seymour *et al.,* 2012; Ganse *et al.,* 2011; Porter and Witmer, 2020; Lindgren *et al.,* 2014). Geochemical analyses, like those employed here (see Chapter 2), use the geochemical composition of skeletal elements to deduce body temperatures (Bernard *et al.*, 2010; Harrell *et al*., 2016; Ferrón, 2017). The resulting body temperatures are calculated using temperature dependent relationships between the isotopic concentrations of $^{18}O$ and the temperatures in which the skeletal elements are formed. The calculated body temperatures can then be compared with coeval or modern ectothermic and endothermic 'end members' to assess the relative degree of endothermy.

Unfortunately, mosasaurs and plesiosaurs have no direct descendants and there are no large carnivorous marine reptiles with a large ecological range living today. Therefore, multiple types of extant animals have been used as modern analogs to infer their thermal status. Saltwater crocodiles have been used for this purpose as they are the largest carnivorous reptile living today (Seebacher *et al.,* 1999; Mulder, 2001; Krahl and Witzel, 2021). Modern crocodiles are ectotherms but demonstrate a degree of homeothermy, showing reduced fluctuations in daily body temperature range compared to ambient temperatures (Seebacher *et al.,* 1999). Crocodiles rely little on metabolic processes and instead primarily use behavioural means to keep body temperatures within an optimal range (Seebacher *et al.,* 1999). These behavioural mechanisms include basking or avoiding direct sunlight and increasing or decreasing physical activities, as well as adjusting their surface area to thermoregulate (Seebacher *et al.,* 1999). The body temperature of saltwater crocodiles varies between 15-33°C depending on the season and whether they are on land or in the water (Grigg *et al.*, 1998). Core temperatures can also fluctuate on a daily cycle, the amplitude of which is inversely proportional to the individual's size and is largely independent of ambient temperature. Glanville and Seebacher (2006) noted that crocodiles who were acclimated to a 20°C environment had maximum and mean body temperatures significantly lower than those who were acclimated to 30°C but followed similar daily cycles. These cold-acclimated crocodiles still performed swimming tasks optimally and had critical sustained swimming speed similar to the warm-acclimated crocodiles. Saltwater

crocodiles also maintain blood pressure between 6.2-7.9 kPa consistent with the range for ectothermic animals (Axelsson *et al.,* 1997; Altimiras *et al.,* 1998). Despite overwhelming evidence of ectothermy in modern-day crocodiles, stem archosaurs are thought to have been endothermic (Seymour *et al.,* 2004). Basal archosaurs, the clade that includes both the Crurotarsi (crocodile lineage) and Ornithodira (dinosaur-avian lineage) evolved in the Late Permian (Seymour *et al.,* 2004). These early archosaurs such as the proterosuchids and erythrosuchids might have been endothermic due to their upright posture, highly vascularized bone tissue, and low predator to high prey ratios (Bakker, 1986). Throughout the Triassic, the general body size and shape of stem archosaurs trended towards the aquatic lifestyle of the more derived eusuchians (Seymour *et al.,* 2004). The upright limb postures of basal crocodylomorphs changed indicating a shift towards aquatic locomotion. Extant crocodiles also have four chambered hearts, a condition associated with endothermy, high metabolic rates, and high blood flow, but still maintain ectothermic thermoregulation. It is thought that this condition was a remnant from endothermic crocodilians in the Mesozoic but was lost when the lineage went back to exploiting aquatic habitats (Seymour *et al.,* 2004). This indicates that evolution of thermoregulation is not directly linear and endothermy is not beneficial to all organisms even in aquatic environments.

Leatherback turtles may also provide insight into the thermoregulation of extinct Mesozoic reptiles (Paladino *et al.,* 1990). They, like mosasaurs and plesiosaurs, have a wide global range and are open-water pelagic swimmers (Bostrom *et al.,* 2010). Like other reptiles, leatherback turtles depend on behavioural mechanisms such as adjusting activity levels to maintain body temperatures. However, they also rely on specific morphological specializations such as countercurrent heat exchangers in extremities and peripheral layers of adipose tissue that aid in the regulation of body temperatures (Bostrom *et al.,* 2010). The core body temperatures of leatherback turtles can be sustained above ambient temperatures. For example, when swimming in water of 7°C, they can maintain core body temperatures around 27.5°C (Paladino *et al.,* 1990). In addition, their mass-specific resting metabolic rates when nesting are elevated compared to lizards and other sea turtles and fall closer to the metabolic rates of mammals (Paladino *et al.,* 1990). Mathematical scaling indicates that these elevated rates are related in large part to their size (i.e. gigantothermy), where larger leatherback turtles can maintain wider temperature gradients between themselves and ambient water (Paladino *et al.,* 1990). This gradient is achieved through minimal blood flow from the core to the exterior surface and in the process

maintains a thermal gradient of 10-20°C. As with endotherms, blood flow to peripheral areas can increase when heat dissipation is required to avoid overheating. Because of the relationship between size and heat retention, leatherback turtles have traditionally been defined as gigantothermic (Paladino *et al.*, 1990). However, this interpretation has been challenged in recent years. Leatherback turtles do possess some insulation to help retain body heat (Davenport *et al.*, 2009). Deposits of adipose tissue around the neck and head regions as well as deep set cranial blood vessels are thought to protect the endocranium and salt glands from heat loss (Davenport *et al.*, 2009). Bostrom *et al.* (2010) found that, though endogenic heat production via muscle contraction was inefficient, it did increase as ambient temperature decreased. For these reasons, leatherback turtles have also been defined as endothermic (Davenport *et al.*, 2009; Bostrom *et al.*, 2010).

Fossil fish within the Pembina assemblage of the WIS are predominantly identified as aulopiforms (lizardfish) like cimolichthyids and enchodontids as well as ichthyodectiforms like *Xiphactinus* (Nicholls and Russell, 1990). Though these Cretaceous fish are extinct, living aulopiforms like dragon snapper (*Latropiscis purpurissatus*) can be used to gain insight into their biology (Newbrey and Konishi, 2015). Extant aulopiforms have predominantly been classified as poikilothermic ectotherms. This means that they employ behavioural mechanisms like increasing/decreasing activity levels and changing physical locations as the principal method for thermoregulation, with their body temperatures fluctuating with ambient seawater temperature (Nordahl *et al.*, 2018; Haesemeyer, 2020). Indeed, modern fish generally have a species-specific preferred temperature called a final preference temperature, a temperature range in which an individual functions optimally (Golovanov, 2006). These temperature ranges are affected by the ecology, seasonality, ontogenetic stage, and other internal and external factors (Golovanov, 2014). Though fish can survive outside these temperatures, individuals will often manage their body temperatures through navigation strategies which are a key component in the thermoregulation in modern fish. Individuals will either laterally or vertically pursue these preferred temperatures within their habitat to maintain optimal metabolic function (Haesemeyer, 2020). Seeking out preferential temperatures has been observed in wild Atlantic cod (Rose and Leggett, 1988), trout (Goyer *et al.*, 2014), and salmon (Booker *et al.*, 2008; Byron and Burke, 2014). Thermal navigational strategies have also been observed in laboratory studies. Here, fish thermoregulated by swimming in specific parts of a tank divided into temperature zones, thus

showing that these genera can navigate through temperature gradients to locate their preferred temperatures (Neill *et al.*, 1972; Neill and Magnuson, 1974). When outside these preferred temperatures, fish will often increase or decrease swim speeds and bouts (Haesemeyer, 2020). Though fish are likely to want to stay within preferred temperatures, there are times in which they will purposely leave. Fish will choose water with elevated temperatures for inducing fever or digesting food and choose lower temperatures when in hypoxic conditions (Haesemeyer, 2020). This thermoregulatory behaviour closely interacts with schooling, feeding, and anti-predation behaviours (Golovanov, 2006; Golovanov *et al.*, 2014).

Though most fish are described as ectothermic, a small proportion (~30 species) of large active fish including tuna, lamnid sharks, and billfishes can maintain elevated body temperatures and have been described as regionally endothermic with some endogenic heat production (Stevens, 1972; Dickson and Graham, 2004). These large pelagic swimmers migrate extensively and also move vertically throughout the water column, encountering large variations in water temperature (Golovanov, 2014). Regional endothermy in these fish presumably evolved for improved aerobic activities (Golovanov, 2014; Harding *et al.*, 2021). In regional endothermic fish, blood vessels are organized into countercurrent heat exchangers to keep heat within the core tissue and/or specific organs (Dickson and Graham 2004; Golovanov, 2014). Metabolic heat production varies among each group, with red myotomal muscle endothermy, visceral endothermy, and cranial endothermy all being employed (Dickson and Graham, 2004). Fish with red muscle endothermy have myotomal muscles that are continuously contracting (thereby producing heat) due to their sustained locomotion, which is then entrapped to elevate red muscle temperatures (Dickson and Graham, 2004). Compared to ectothermic fish, where the myotomal muscles are external, those of regionally endothermic fish are situated internally and therefore heat loss is further reduced by the overlying/surrounding muscles (Dickson and Graham, 2004). This form of thermogenesis is found in some tunas, such as *Auxis, Euthynnus, Allothunnus* and *Thunnus,* as well as the thresher shark. Visceral endothermy is the entrapment of heat that is normally produced through the digestion, absorption, and protein synthesis associated with feeding (Dickson and Graham, 2004). The organs heated through this process differ depending on the type of endothermic fish, for example, the pyloric caeca in tunas and spiral valve intestine in lamnid sharks. Visceral endothermy can also include the heating of the overall core specifically in deep diving tunas like *Thunnus alalunga, T. thynnus, T. maccoyii, T. orientalis,*

and *T. onggol* (Dickson and Graham, 2004). Cranial endothermy is heat produced within the body that is then directed to the cranial organs such as the brain and eyes. The source of heat production for cranial endothermy is still not fully understood in tunas and lamnid sharks but is thought to be generated by warm (continuously contracting) myotomal muscles and then transferred to the cranium through the frontoparietal skull fenestrae (Dickson and Graham, 2004; Legendre and Davesne, 2020). In swordfish, a highly specialized heater tissue maintains the ocular muscles and brain 10$^{o}$C–15$^{o}$C above ambient seawater temperatures (Fritsches *et al.*, 2005). Facultative thermogenesis occurs in cranial endothermic billfishes and mackerel via a cranial heater tissue. This modified, non-contractile muscle tissue is adjacent to the eye and has a high (70% by volume) mitochondrial content (Block, 1987). Thermogenesis occurs within heater tissue primarily via the futile cycling of $Ca^{2+}$ between the sarcoplasmic reticulum and cytosol. Briefly, upon stimulation $Ca^{2+}$ is released into the cytoplasm via dihydropyridine-mediated ryanodine receptors. As this specialized muscle tissue lacks $Ca^{2+}$ binding proteins, the $Ca^{2+}$ is returned to the sarcoplasm via the SERCA pump, increasing cellular ATP hydrolysis. Unlike in other tissues the ryanodine receptor isoform in fish heater tissues is slow to close, thereby prolonging the futile cycling of $Ca^{2+}$ (Morrissette *et al.,* 2003). Influx-efflux pathways in the mitochondria further increase ATP utilization, thereby also promoting elevated mitochondrial substrate catabolism and hence thermogenesis. It is important to note that these endothermic fish will also use behavioural and physiological mechanisms to maintain body temperatures, such as navigational strategies and adjusting blood flow to either increase or decrease heat retention (Dickson and Graham, 2004).

Although the extinct Cretaceous bird *Hesperornis* does not have any living descendants, penguins are often used as a modern comparator since ancestral members of both lineages evolved into flightless semi-aquatic diving birds (Wilson and Chin, 2014). Like all extant birds, penguins are endothermic, maintaining their body temperature through metabolic thermogenesis. However, unlike mammals, birds do not have BAT and rely on skeletal muscles for thermogenesis (Mozo *et al.*, 2005; Walter and Seebacher, 2009). Birds are thought to utilize both shivering and non-shivering skeletal muscle thermogenesis in order to maintain body temperatures, however the reliance on one or the other depends on the muscle group and age of the individual (Stager and Cheviron, 2020). Recent studies suggest that penguins rely heavily on muscle contraction either via shivering or locomotion to generate endogenic heat and the

capacity of skeletal muscle thermogenesis in penguins is consistent regardless of their sea acclimation temperatures (Roussel *et al.,* 2020). Skeletal non-shivering thermogenesis in birds is presumably similar to that seen in mammals, though this process is not completely understood and it is unlikely that sarcolipin plays a similar thermogenic role to that suggested for mammals (Mozo *et al.,* 2005; Campbell and Dicke, 2018; Stager and Cheviron, 2020; Griggs *et al.,* 2022). Non-shivering thermogenesis through uncoupling proteins (avUCP) has also been suggested for king penguins and some other modern birds (Talbot *et al.,* 2003). This gene is thought to be an ortholog to mammalian UCP3 but may function similar to mammalian UCP1 since this latter gene, along with UCP2, is absent in modern birds (Newman *et al.,* 2013). The avUCP protein may thus, like mammalian UCP1, allow the flow of hydrogen ions from the proton-rich mitochondrial intermembrane space to the proton-depleted mitochondrial matrix without synthesizing ATP, thereby increasing metabolic heat production (Mozo *et al.,* 2005). However, this hypothesis has been disputed in recent years as the avUCP gene does not seem to possess meaningful thermogenic capabilities as once thought (Gaudry *et al.,* 2019). To prevent endogenic heat from escaping the body, marine birds make use of morphological adaptations such as insulating layers of fat and densely packed water-proof feathers (Grémillet *et al.,* 2001; William *et al.,* 2015). Additionally, birds including penguins employ countercurrent heat exchangers to help control core body temperature, and also use vasoconstriction and vasodilation to regulate blood flow to high thermal conductance areas (Loudon *et al.,* 2012; Fitzpatrick *et al.,* 2015). For instance, King penguins maintain vasoconstriction in high conductive areas like the flippers and feet after diving bouts to minimize heat loss (Lewden *et al.,* 2020). Penguins also rely on behavioural processes to maintain body temperatures, such as huddling and changing their posture to maintain temperature, but these activities are used only when they are on land (Simeone *et al.,* 2004; William *et al.,* 2015; Zagrai and Hassanalian 2020).

## 1.4 PREVIOUS RESEARCH ON THE THERMOREGULATION OF EXTINCT MESOZOIC REPTILES

Modern endotherms are associated with increased aerobic efficiency, physical stamina, and growth rates, allowing them to exploit habitats or niches not conducive for ectotherms (Hedrick and Hillman, 2016). The production of metabolically derived endogenic heat allows endothermic animals some independence from the surrounding environment (Stevens, 1972).

The ability to maintain core body temperatures grants an advantage to many different types of organisms, especially those in extreme environments (Polymeropoulos *et al.,* 2018). However, these benefits come with a cost, as the increased growth rates and muscle activity by endotherms require food consumption rates that are approximately 4-8 times higher than their similar-sized ectothermic counterparts (Geiser *et al.,* 2017). Ectothermy is relatively 'low cost', with the behavioral mechanisms used to maintain body temperatures requiring less energy (Polymeropoulos *et al.,* 2018). So how did so many clades evolve endothermic systems?

Three hypotheses have been proposed to explain the driving force behind the evolution of endothermy in vertebrate animals (Grigg *et al.,* 2004; Polymeropoulos *et al.,* 2018). First, animals that possess elevated body temperatures have thermoregulatory advantages to exploit specific environments, which then promotes higher metabolic rates. For example, Crompton *et al.* (1978) argued that early mammals were likely homeothermic to capitalize on nocturnal niches with wide temperature ranges. Multiple lineages of mammals could have thus acquired higher metabolic rates independently allowing for diurnal activity. Second, increased physical activity lead to an increase in metabolic rate, which in turn resulted in higher body temperatures (Heinrich, 1977). Animals with high activity rates would have a higher body temperature from the resulting endogenic heat. High core body temperatures would have been particularly important for large highly active animals, which would have then biologically restructured their metabolic enzymes to function more efficiently at these higher, more stable temperatures (Heinrich, 1977). Third, increased body temperature may be beneficial for growth in early life stages and reduces infant mortality. Higher heat generation capacities and metabolic rates would produce higher growth rates which, in turn, would be beneficial for incubation and juvenile feeding (Koteja, 2004). However, the evolution of endothermy might have components of all three hypotheses (Polymeropoulos *et al.,* 2018).

The evolution of endothermy within the superclass Tetrapoda is complex, evolving independently in modern birds and mammals. True endothermy in modern birds is thought to have evolved in a step-wise fashion soon after the Permian-Triassic extinction event and continued throughout the Mesozoic within the avian branch of Dinosauria (Lovegrove, 2016; Benton, 2021). However, there is evidence that some other diapsid lineages, such as mosasaur, plesiosaurs and ichthyosaurs, also evolved endothermic characteristics and thus their metabolic status has been debated. Therefore, to characterize endothermy in marine reptiles, it is important

to consider other extinct diapsids. Limb position and bone growth rates indicative of endothermy are seen in early diapsids like *Erythrosuchus* in the late Permian (Gross, 1934; Bakker, 1971; Benton 2021). Some Cretaceous non-avian dinosaurs have also been described as possessing characteristics of modern birds, including feather-like filaments in non-avian theropods (Norell and Xu, 2005) and convoluted nasal turbinates in pachycephalosaurs (Bourke *et al.*, 2014). Unidirectional air flow has also been proposed for some pterosaurs (Claessens *et al.,* 2009) and is observed in several modern reptiles such as crocodiles and squamates (Cieri and Farmer, 2016). Though again, there is some debate whether these features are associated with endogenic heat production itself (Cieri and Farmer, 2016).

Among marine reptiles, early sauropterygians such as nothosaurs and placodonts were thought to have been poikilothermic ectotherms (Surmik and Pelc, 2012). However, Bernard *et al.* (2010) found that the $\delta\,^{18}O_p$ values in tooth enamel from derived plesiosaurs (18.2 to 20.1‰) are generally lower compared to coeval marine ectotherms (19.6 – 21.7‰), suggesting a degree of endothermy. The (cranial) body temperatures derived from this data for plesiosaurs is 26 ± 2°C; of note, these derived temperatures are indicative of the temperatures from surrounding tissue and not the whole body. When looking at the histology of plesiosaur cortical bone, a similar pattern of increasing endothermic characteristics with more derived genera emerges. Slower growth rates for early plesiosaurs like *Nothetosaurus* from the Upper Triassic are observed through the prevalence of lamellar zonal bone tissue (Krahl *et al.,* 2013). Faster growth and (likely) higher metabolic rates have been suggested for more derived plesiosaurs such as *Pistosaurus* and *Plesiosaurus* through the presence of fibrolamellar bone tissue (Krahl *et al.*, 2013). In addition to fibrolamellar bone tissue, the Late Triassic plesiosaur *Rhaeticosaurus* also possessed high vascularization, secondary osteons, and exterior epiphysis pits, which are indicators of increased metabolic rates (Wintrich *et al.,* 2017a). These features are also seen in the Late Jurassic plesiosaurs *Djupedalia* and *Colymbosaurus* (Liebe and Hurum, 2012) and the Late Cretaceous *Dolichorhynchops* (O'Keefe *et al.,* 2018). However, it is important to remember that this type of analysis is qualitative and cannot quantify metabolic or growth rates to fully infer thermoregulatory systems of these extinct animals (Sander and Wintrich, 2021). Derived sauropterygians, like pistosauroids, show wider blood vessel diameters in osteological thin sections when compared to similar sections from stem plesiosaurs (Fleischle *et al.*, 2019). This indicates that pistosauroids possessed enlarged red blood cells both in volume and diameter.

Quantitative histological analyses also support an endothermic thermophysiology for plesiosaurs (Fleischle *et al.*, 2018). Using phylogenetic eigenvector maps with vascular bone density as the predictor variable, Fleischle *et al.* (2018) suggested that the resting metabolic and bone growth rates of sauropterygians and some eosauropterygians were close to the range of modern birds. In looking at the gross osteo-morphology of plesiosaurs, almost all have a unique series of paired canals within the ventral side of their vertebral centra; it is thought that these foramina would have housed intersegmental arteries that passed through the centra and would connect the spinal artery to the vertebral artery (Wintrich *et al.*, 2017b). The increased oxygen supply associated with the intersegmental arteries could potentially support the higher needs for endothermic thermoregulation (Wintrich *et al.*, 2017b).

For ichthyosaurs (another independently derived marine reptile lineage), the $\delta^{18}O_p$ values measured from bulk tooth samples are between 18.6 to 19.7‰ which corresponds to a body temperature of 26 ± 2°C (Bernard *et al.*, 2010). The $\delta^{18}O_p$ of coeval ectothermic fish from those localities measured between 18.2 – 21.2‰. Soft tissue analysis of the Jurassic ichthyosaur *Stenopterygius* has indicated that these marine reptiles might have possessed blubber, a tissue closely associated with marine endotherms (Lindgren *et al.*, 2018). This subcutaneous layer consists of highly condensed glossy black tissue layered on top of a fibrous mat. The biogeopolymers of this substance were similar in abundance to the lipid-rich tissue of cetaceans. The fibrous mat is also structurally similar to the collagen and elastane fibers seen in cetacean blubber when subjected to similar pressure and temperatures associated with fossilization. Due to these similarities, the purpose of blubber in ichthyosaurs was speculated to be comparable to modern cetaceans, and was likely primarily used for insulation. This layer might have aided ichthyosaurs to retain body heat and help maintain a consistently elevated body temperature (Lindgren *et al.*, 2018). The thermophysiology of ichthyosaurs has also been described through histological studies. Early ichthyosaurs like *Mixosaurus* show more ectothermic-like bone growth with parallel-fibered or lamellar–zonal tissue (Talevi and Fernández, 2012). However, high bone vascularization is observed in many ichthyosaurs suggesting higher growth rates than ectothermic reptiles (Housseaye *et al.*, 2014). Additionally, the presence of woven-bone texture in more derived ichthyosaurs like *Omphalosaurus, Stenopterygius*, and *Ichthyosaurus* has also provided evidence in support of high metabolic rates for these marine reptiles (De Buffrenil and

Mazin, 1990). This suggests a trend towards higher bone growth rates and metabolic rates in this clade towards the Jurassic.

Like other Mesozoic marine reptiles, mosasaurs have been described to be at least partially endothermic in various studies (Bernard *et al.*, 2010; Harrell *et al.*, 2016). Geochemical analysis of $\delta^{18}O_p$ values for mosasaur teeth from a variety of localities was found to be between 18.1 – 19.9‰, with corresponding values for marine ectotherms being between 18.4 – 21.1‰ (Bernard *et al*., 2010). Bernard also calculated body temperatures of these mosasaurs to be 30 ± 2°C, which are slightly higher than those calculated for plesiosaurs and ichthyosaurs. Harrell *et al.* (2016) also employed this geochemical technique to estimate body temperatures for three different genera of mosasaurs from southern Alabama (*Clidastes*, *Platecarpus*, and *Tylosaurus*), arriving at a body temperature range of 33.1 to 36.3°C. These body temperatures did not correlate to the mean body lengths of each genus, which suggested that body temperatures of mosasaurs are not directly tied to body size and therefore it is unlikely that mosasaurs were gigantothermic (Harrell *et al.*, 2016). The gross osteo-histology of mosasaurs tracks closely with modern ectothermic varanid lizards, a close extant relative of these marine reptiles, with prominent arrested lines of growth in bones (Pellegrini, 2007). However, the interline spacing, which represents the amount of bone growth within a year, suggests that bone growth rates of mosasaurs may have been even higher than in extant varanid lizards indicating elevated metabolic rates (Pellegrini, 2007). Other histological features associated with endothermy, including the presence of unusual fibrolamellar bone with large and randomly shaped osteocyte lacunae, have also been observed in mosasaurs, suggesting that early genera such as *Dallasaurus* possessed higher metabolic rates when compared to extant turtles but less than derived plesiosaurs and ichthyosaurs (Houssaye *et al.*, 2013). In modern varanid lizards unidirectional air flow is present, which is a defining morphological feature found in modern birds (Cieri *et al.*, 2014; Cieri and Farmer, 2016). Since unidirectional air flow has been found within the modern relatives of the squamate and archosaur lineage, it is thought to have been inherited from a common ancestor, in which case, mosasaurs may have also possessed this trait. Unidirectional air flow has traditionally been associated with endothermy as it was thought to increase aerobic efficiency. However, this trait has been discovered in ectotherms like modern crocodiles and squamates and thus, the validity of this association has been questioned (Cieri and Farmer,

2016). Therefore, unidirectional air flow in squamates should not be taken as direct evidence of endothermy in mosasaurs.

Through these methods, paleontologists have been able to infer some level of endothermy in these mosasaurs, plesiosaurs, and ichthyosaur. However, the more research is needed to fully understand the thermoregulation of these Mesozoic marine reptiles.

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# CHAPTER 2: THERMOREGULATION IN MARINE REPTILES OF MANITOBA

## 2.1 ABSTRACT

Mosasaurs and plesiosaurs were both large carnivorous marine reptiles that thrived in a range of aquatic environments during the Mesozoic era. Previous research has suggested that these apex predators might have had some degree of endothermy, but more research is needed to fully understand their thermoregulatory strategies. In this chapter, the body temperatures of mosasaurs and plesiosaurs from the Late Cretaceous Pembina Member of the Pierre Shale of Southwestern Manitoba were calculated using bulk oxygen isotope paleothermometry. A thermoregulatory status was then inferred through a comparison to those calculated from coeval ectothermic fish and endothermic bird specimens. The preservation of each specimen (eleven mosasaurs, three plesiosaurs, six fish and five birds) was first assessed so that only those with the best preservation were included in the paleothermometry analysis. This was achieved through multiple methods including scanning electron microscopy, relative total rare earth element concentrations, principal component analysis of bulk element concentrations, and the difference between the $\delta^{18}O$ in the phosphate and carbonate fractions. Six specimens were deemed well-preserved (two mosasaur specimens, two plesiosaur specimens, one fish specimen and one bird specimen) using these methods. The measured $\delta^{18}O$ of bulk tooth bioapatite from each of the well-preserved specimens was measured using oxygen isotope ratio mass spectrometry, which was then used to calculate their body temperatures relative to ectothermic fish and endothermic bird (*Hesperornis*) specimens from the same member. The average mosasaur body temperatures of 36.4°C ± 1.1°C (SD) fell between the ectothermic fish specimen (28.1°C) and endothermic bird specimen (41.1°C), but the average plesiosaur body temperature of 42.8°C ± 0.3°C exceeded all other animal groups. The body temperatures of Manitoba mosasaurs are consistent with those previously calculated from southern Alabama. By contrast, the body temperatures of plesiosaurs were much higher compared to previously published values which may have arisen from various factors. However, the elevated temperatures in both marine reptile lineages does infer some degree of endothermy in Late Cretaceous mosasaurs and plesiosaurs of the northern portion of the Western Interior Seaway. This would imply that these marine reptiles would have

had higher bone growth rates, higher metabolic rates, and higher predation frequency compared to their ectothermic relatives.

## 2.2 INTRODUCTION

The term 'marine reptile' is used to describe a variety of reptile groups (e.g. sea turtles, crocodiles, sea snakes, mosasaurs, plesiosaurs, ichthyosaurus) that evolved secondary adaptations for aquatic environments but which are not necessarily closely related to one another. For example, mosasaurs and plesiosaurs independently evolved aquatic habits during the Mesozoic and dominated aquatic environments until their demise at the K-Pg extinction event. These two marine reptile groups were a large part of the ecology of the Western Interior Seaway (WIS), particularly mosasaurs in the northern portion (Nicholls and Russell, 1990). Plesiosaurs like *Trinacromerum* hunted small fish and other soft bodied organisms while mosasaurs, like *Tylosaurus* and *Platecarpus,* hunted large fish, sea turtles and even other marine reptiles (Massare, 1987). As avid predators in the WIS, the thermoregulation of mosasaurs and plesiosaurs would have greatly affected the ecological dynamic of the seaway since endothermic systems are associated with higher energy levels, growth rates, and predation frequency (Hedrick and Hillman, 2016; Geiser *et al.,* 2017).

Various approaches have been used to study the thermoregulation of extinct reptiles. For example, histological evidence such as fibrolamellar bone tissue, high tissue vascularization, secondary osteons, and/or exterior epiphysis pits, all of which are indicative of high bone growth rates amongst some genera of plesiosaurs (Liebe and Hurum, 2012; Krahl *et al.,* 2013; Wintrich *et al.,* 2017a; Fleischle *et al.,* 2018). Histological analyses have also suggested that mosasaurs had more rapid bone growth rates compared to modern ectothermic varanid lizards (Pellegrini, 2007). Early mosasaurs possessed endothermic bone characteristics such as fibrolamellar bone with large and randomly shaped osteocyte lacunae (Houssaye *et al.* 2013). The osteo-morphology of plesiosaur vertebrae also suggests some level of endothermy as they have a unique set of paired channels of intersegmental arteries that has been suggested to increase oxygen transport to the head (Wintrich *et al.,* 2017b).

Isotopic paleothermometry is a geochemical method used to infer thermoregulatory status of extinct animals though remains understudied in both plesiosaurs and mosasaurs. Using this methodology, Bernard *et al*. (2010) found that the body temperatures of mosasaurs and

plesiosaurs from various localities were consistently elevated compared to seawater temperatures. Harrell *et al.* (2016) similarly concluded some level of endothermy for three Late Cretaceous mosasaur genera from southern Alabama, *Clidastes*, *Platecarpus,* and *Tylosaurus,* which exhibited temperatures ranging between 33.1 to 36.3°C, that are higher than coeval ectothermic fish (28.3°C) but lower than the endothermic avian *Icthyornis* (38.6°C). This study aims to estimate the thermoregulatory status of Late Cretaceous mosasaurs and plesiosaurs from Manitoba using stable isotopic paleothermometry to assess their degree of endothermy relative to more southerly distributed specimens (Bernard *et al*. 2010; Harrell *et al*. 2016). Similar body temperatures between specimens from different latitudes would then suggest that elevated body temperatures persisted in these Mesozoic marine reptiles across these portions of the WIS.

Before a body temperature can be calculated through isotopic analysis, it is important to assess the level of diagenetic alteration within a specimen since the accuracy of paleothermometry is dependent on the original tooth isotope concentration remaining intact (Harrell *et al.,* 2016). Diagenetic alteration often occurs during the fossilization process but can be difficult to quantify. In this study, multiple processes were used to assess the level of diagenetic alteration, with only the well-preserved specimens being used for downstream body temperature analysis.

## 2.3 GEOLOGICAL SETTING

The WIS was a large inland sea that covered the majority of central North America during the Campanian epoch of the Late Cretaceous Period approximately 83–72 million years ago (Mya). The seaway formed during the late Albian (~100 Mya) when rising global sea levels flooded the lowlands of North America and emptied ~66 Mya. The boundaries of the WIS fluctuated as multiple transgressive-regressive cycles occurred from the Early Cretaceous to the Late Cretaceous (McNeil and Caldwell, 1981). At its largest, it extended from the modern-day Arctic Ocean to the Gulf of Mexico and covered parts of Alberta, Saskatchewan, and Manitoba. The climate varied throughout the seaway, ranging from sub-tropical at lower paleo-latitudes to polar at higher latitudes (Hay *et al.,* 1993). Water temperatures calculated via clumped isotope analysis on late Campanian to late Maastrichtian mollusc shells indicate a decreasing seawater temperature towards more northern latitudes, ranging from 10-20°C at a paleolatitude of 35°N to 5-15°C at a paleolatitude of 50°N (Petersen *et al.,* 2016). Similar temperatures at northern paleolatitudes, 10-12°C, were obtained through bulk oxygen isotope analysis of late

Maastrichtian ammonite and belemnite fossils from the Pierre Shale in South Dakota (Cochran *et al.,* 2003). However, other studies have calculated higher seawater temperatures for this part of the WIS. Clumped isotope data indicated average water temperatures ranging from 21.5°C (SE = 3.2°C) to 29.3°C (SE = 1.9°C) from ~73.5 Mya fossils found in the Fox Hills Formation of South Dakota (Dennis *et al.,* 2013). Sea temperatures calculated from belemnites in middle to late Cenomanian deposits in Manitoba are also quite variable, ranging between 13-28°C (Prokoph and Karbashewski, 2006). The variable seawater temperatures calculated for the northern portion of the WIS might reflect increased seasonality typically associated with more northern latitudes.

Cretaceous deposits in Manitoba are concentrated along the Manitoba Escarpment which runs NW-SE along the western side of the province. In this study, I used fossil specimens collected from the Pembina Member of the Pierre Shale (McNeil and Caldwell, 1981). These beds were deposited in the Campanian approximately 80-83 Mya during the regressive stage of the Niobrara cycle (McNeil and Caldwell, 1981). Of the seven abundance-related acme zones described for the WIS, the Pembina Member has been placed into the *Hesperornis-Latoplatecarpus* zone and correlates with the Sharon Springs Member of South Dakota (Kilmury and Brink, 2022). The Pembina Member is characterized by massive non-calcareous black-grey shale but gradually changes into dark brown waxy shale with less organic material. Seams of beige bentonite interbed the layer of shale throughout the member (McNeil and Caldwell, 1981). Siderite concretions are present and gypsum commonly occurs either as singular crystals or in aggregates. The Pembina Member has been interpreted as a quiet water environment deposited below the wave base under anoxic conditions (Nicholls and Russell, 1990).

The Pembina member is fossiliferous though preservation quality is variable. Campanian fossils from Manitoba are part of the Pembina Assemblage (Nicholls and Russell, 1990) which extends into Saskatchewan and North Dakota. This assemblage is characterized by the high concentration but low generic diversity of mosasaurs and marine bird fossils compared to southern portion of the WIS. Of the mosasaurs, *Platecarpus* is the most common, but *Clidastes* and *Tylosaurus* are also present in Manitoba. Avian *Hesperornis* fossils are abundant within the Pembina Member and are the dominant marine bird in the assemblage though a few *Ichthyornis* specimens are known. Plesiosaurs are also represented in the assemblage, with *Trinacromerum* being the most common genus though *Elasmosaurus* specimens are also present. Turtles like *Toxochelys* and *Protostega* and sharks like *Squalicorax* are found but form a very small

proportion of the assemblage. Fish fossils are abundant in Manitoba Cretaceous strata and multiple genera groups co-occur within the Pembina member: *Enchodus-Ichthyodectes-Xiphactinus*, *Enchodus-Gillicus-Ichthyodectes-Xiphactinus*, and *Cimolichthys-Enchodus-Ichthyodectes-Xiphactinus* (Kilmury and Brink, 2022). Invertebrate fossils are surprisingly sparse in the Pembina Member with the exception of teuthids (Nicholls and Russell, 1990). Compared to other Campanian fossil assemblages at lower paleo-latitudes, the Pembina Member shows an overall decrease in diversity. The change in faunal diversity has mainly been attributed to temperature differences (Nicholls and Russell, 1990). As one moves northward, marine animals reliant on higher water temperatures become less common whereas animals like mosasaurs, plesiosaurs, and marine birds make up a larger component of the ecosystem.

## 2.4 METHODOLOGY

### 2.4.1 SAMPLES

Twenty-five tooth and bone specimens housed in the Canadian Fossil Discovery Centre (CFDC) were examined in this study (Table 1).

These fossils were recovered from documented localities within the Pembina Member of the Pierre Shale and dated to the Late Cretaceous (80-83 Mya; McNeil and Caldwell, 1981). Two mosasaurs were identified as *Platecarpus* (M.80.29.14 and M.83.06.18) and one *Tylosaurus* (M.74.06.06), with the rest being indeterminate. Two plesiosaurs were identified as *Trinacromerum* (P.04.01.15 and P.81.01.16) and one indeterminate. Two fish specimens were identified as *Enchodus* (F.06.01.15 and F.06.01.23) and the rest were indeterminate. All bird specimens were identified as *Hesperornis*. Specimens were visually assessed and chosen based on the apparent quality of physical preservation (i.e. only the best quality specimens were selected). Mosasaur, plesiosaur, and fish teeth were preferentially chosen since bioapatite is less likely to be diagenetically altered. No *Hesperornis* teeth were available and so cortical bone samples from the tibiotarsus, femur or vertebrae were chosen instead.

Table 1: Specimen list including the Canadian Fossil Discovery Centre (CFDC) specimen name, the animal group, genera (if known), and fossil material used.

| CFDC specimen name | Used for $\delta^{18}O_p$ analysis | Animal group | Identification | Material |
|---|---|---|---|---|
| M.80.29.14 | No | Mosasaur | *Platecarpus* | Tooth bioapatite |
| M.82.00.17 | No | Mosasaur | Indeterminate | Tooth bioapatite |
| M.07.03.23 | No | Mosasaur | Indeterminate | Tooth bioapatite |
| M.83.06.18 | No | Mosasaur | *Platecarpus* | Tooth bioapatite |
| M.01.02.04 | Yes | Mosasaur | Indeterminate | Tooth bioapatite |
| M.99.03.XX | No | Mosasaur | Indeterminate | Tooth bioapatite |
| M.81.02.16 | No | Mosasaur | Indeterminate | Tooth bioapatite |
| M.2010.02.15 | Yes | Mosasaur | Indeterminate | Tooth bioapatite |
| M.74.06.06 | No | Mosasaur | *Tylosaurus* | Tooth bioapatite |
| M.78.01.07 | Yes | Mosasaur | Indeterminate | Tooth bioapatite |
| M.06.01.05 | No | Mosasaur | Indeterminate | Tooth bioapatite |
| P.04.01.15 | Yes | Plesiosaur | *Trinacromerum* | Tooth bioapatite |
| P.81.01.16 | Yes | Plesiosaur | *Trinacromerum* | Tooth bioapatite |
| P.06.03.15 | Yes | Plesiosaur | Indeterminate | Tooth bioapatite |
| F.06.01.15 | Yes | Fish | *Enchodus* | Tooth bioapatite |
| F.81.08.16 | No | Fish | Indeterminate | Tooth bioapatite |
| F.06.01.23 | Yes | Fish | *Enchodus* | Tooth bioapatite |
| F.78.01.07 | No | Fish | Indeterminate | Tooth bioapatite |
| F.08.01.13 | Yes | Fish | Indeterminate | Tooth bioapatite |
| F.06.02.15 | No | Fish | Indeterminate | Tooth bioapatite |
| B.07.02.15 | No | Bird | *Hesperornis* | Tibiotarsus |
| B.03.03.05 | Yes | Bird | *Hesperornis* | Femur |
| B.78.01.07 | No | Bird | *Hesperornis* | Tibiotarsus |
| B.83.01.18 | Yes | Bird | *Hesperornis* | Unidentified |
| B.06.02.15 | Yes | Bird | *Hesperornis* | Vertebra |

The mosasaur, plesiosaur, and fish teeth and bird cortical bone fossils were processed in the University of Manitoba Palaeontology Lab in the Department of Earth Sciences. Excess matrix obscuring the fossils was first removed using hand tools. Specimens were then washed in an ultrasonic cleaner for five minutes with acetone and then distilled water. Dried samples were embedded in an epoxy resin (Epoxicure Expoxi Resin 20-8130-032 and Expoxicure Epoxi Hardener 02-8232-008 at a 5/1 ratio) and cut transversely using an Isomet 1000 diamond sectioning saw (Buehler, Lake Bluff, IL, USA) at 200 rpm speed. The cut side of each half was sanded using a Buehler Handimet II roll grinder with 600 grit then processed with a Buehler Metaserv 2000 polisher using 0.3 μm grit.

### 2.4.2 SCANNING ELECTRON MICROSCOPE

Prior to scanning electron microscope (SEM) analysis, samples were observed under a standard Nikon SMZ-18 Zoom Stereo Microscope to identify areas for imaging. Specimens were first polished and etched with 5% hydrochloric acid for 15-120 seconds to reveal microscopic details. A Philips XL 30 SEM was then used to analyze the microanatomy of the tooth enamel and dentine from the mosasaur, plesiosaur, and fish specimens and cortical bone of the *Hesperornis* specimens. SEM analysis was conducted using a voltage of 15.00 HV and a window diameter of 11.0 WD.

### 2.4.3 LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

Using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) the concentrations of Mg, Na, Sr, S, Fe, and Mn were measured, as well as total rare earth elements (REEs). LA-ICP-MS measurements were conducted using a Thermo/Finnigan Element 2 equipped with a laser ablation system (New Wave Research UP-213). Surface enamel and dentine samples were each ablated twice with a 30 μm beam diameter with a fluence of 9.51 J/cm$^2$ for 80 seconds for a total of four spots measured per sample. The laser ablation system had a wavelength of 213 nm and a pulse duration time of 4 ns. Spot analysis was also conducted twice on the fibrolamellar cortical bone of the *Hesperornis* specimens with the same beam size and analysis time. The software program Iolite 3.7 was used for data reduction with an internal $Ca^{2+}$ standard of 36%, which is consistent with $Ca^{2+}$ values from previous studies (Owocki and Madzia, 2020). This standard is the average $Ca^{2+}$ concentration of specimens M.80.29.14,

P.04.01.15, F.06.01.15 and B.07.02.15—which were chosen based on their apparent physical preservation—as measured on a Cameca SX-100 Microprobe (Fitchburg, WI, USA).

### 2.4.4 ISOTOPE RATIO MASS SPECTROMETRY

Carbon and oxygen isotope analyses were conducted via isotope ratio mass spectrometry (IRMS) at the Manitoba Isotope Research Facility at the University of Manitoba to obtain $\delta^{13}C$ and $\delta^{18}O_c$ values for all specimens. A tungsten pick was used to extract 5-50 mg of powdered samples for $\delta^{13}C$ and $\delta^{18}O_c$ analysis. The NBS-18 and IAEA 603 standards were used at the beginning, middle and end of each run (Ishimura *et al.,* 2008). One calibrated internal calcite standard, CHI, was analyzed with specimens and yielded $\delta^{13}C_{VPDB}$ = -7.97 ± 0.08‰; $\delta^{18}O_{VPDB}$ = -11.60 ± 0.05‰ with the lab reporting an error of $\delta^{13}C_{VPDB}$ = 0.09‰ and $\delta^{18}O_{VPDB}$ = 0.007‰ assuming 100% calcite (VPDB =Vienna Pee Dee Belemnite). However, only the specimens (M.78.01.07, M.01.02.04, M.2010.02.15, P.04.01.15, P.81.01.16, P.06.03.15, F.06.01.15, F.06.01.23, F.78.01.07, B.03.03.05, B.78.01.07, B.06.02.15)—based on their SEM, relative REEs and PCA preservation classifications—were used for the $\delta^{18}O_p$ analysis. For the $\delta^{18}O_p$ analysis, the simplified silver phosphate extraction method was used (Shabaga *et al.,* 2018). A tungsten pick was first used to extract 5-50 mg of powdered sample from each specimen. Samples were then pre-treated and titrated as described in Shabaga *et al.* (2018). Briefly, the precipitated $Ag_3PO_4$ crystals were analyzed using a Delta VPlus isotope ratio mass spectrometer (ThermoFisher, Waltham, MA, USA) to obtain $\delta^{18}O_p$ values for each sample. Calibration was performed at the beginning, middle and end of run by analyzing two silver phosphate standards (Acros $Ag_3PO_4$, $\delta^{18}O_{VSMOW}$ = 12.0 ± 0.4‰; B2207 $Ag_3PO_4$, $\delta^{18}O_{VSMOW}$ = 21.7 ± 0.3‰; VSMOW=Vienna Standard Mean Ocean Water). The lab reported an external precision of 0.2‰ assuming 100% pure $Ag_3PO_4$ crystals. Tooth formation temperatures (a proxy for body temperature, Tb) were calculated from the isotopic concentration of the phosphate using the Pucéat *et al.* (2010) equation:

$$Tb(°C) = 118.7 - 4.22[(\delta^{18}O_p + (22.6 - \delta^{18}O_{NBS120c})) - \delta^{18}O_w]$$

The standard NBS120c ($\delta^{18}O$ = 22.6‰) was used in the calculation. Due to the uncertainty of mean $\delta^{18}O$ seawater values ($\delta^{18}O_w$) during the Cretaceous, four different $\delta^{18}O_w$ values were initially used to calculate body temperatures, -1.37, -0.36, 0.00, 0.23‰ (Pucéat *et al.,* 2010). However, only body temperatures derived using -1.37‰ were interpreted as this is the

closest estimate, calculated from Late Cretaceous marine turtle bones from Southern Dakota, for Manitoba $\delta^{18}O$ seawater values (Coulson, 2008).

### 2.4.5 PRESERVATION ASSESSMENT

The preservation of each specimen was assessed using three methods. First, the physical preservation of each specimen was investigated through SEM analysis. Scanning electron microscopy (SEM) was employed to assess the relative degree of physical alteration that occurred during or after fossilization. The physical preservation of the crystalline units is a marker of alteration as high degrees of diagenesis will reshape bioapatite microstructures (Casella *et al.,* 2018). Both thermal and hydrothermal alteration of bioapatite can fundamentally modify the organization of crystallites and in some cases, lead to recrystallization which would not reflect the primary geochemical composition (Casella *et al.,* 2018). Additionally, the presence of physical alteration such as borings and inclusions could jeopardise the geochemical integrity of these fossils (Hollund *et al.,* 2013; Hollund *et al.,* 2014). Specimens with frequent signs of physical alteration (poor crystallite preservation, exterior weathering, borings, fractures and infills) were classified as poorly preserved. Specimens with a moderate amount of physical alteration were deemed moderately preserved, and those with little physical alterations were deemed well preserved.

Second, the LA-ICP-MS data was used in two ways to assess the level of chemical preservation for each specimen. During the process of fossilization, biogenic apatite is likely to recrystalize and, in doing so, can easily incorporate external elements into its crystalline structure (Patrick *et al.,* 2007). These elements can be absorbed from surrounding sediments or fluids into the bioapatite during early diagenesis (Patrick *et al.,* 2007). The relative levels of total REEs were calculated for each specimen as the concentration of total REEs will increase with diagenetic alteration compared to modern bioapatite (~20 ppm). Specimens with relative total REEs concentrations higher than 1500 ppm were assessed as poorly preserved, specimens with concentrations between 300 – 1500 ppm were assessed as moderately preserved, and specimens with less than 300 ppm were assessed as well preserved. Bulk element concentrations in tooth bioapatite can also change with diagenetic alteration but can be affected by other factors. Because of this, principal component analysis was conducted using PAST 4.03 software

(Hammer, 2001) to assess which specimens are most similar in bulk element concentration to the other specimens within the same animal group. This statistical analysis transforms a large data set of variables, in this case bulk element concentrations, into smaller sets called components (PC 1, PC 2 etc.). Each component is composed of a combination of these variable (i.e., bulk element concentrations) and their proportions are called the loading scores. Through the loading scores, principal component scores can be calculated for each specimen. When plotted on a PCA graph, specimens with similar principal component scores will plot closely and be more similar in bulk element concentrations than those that plot farther away from each other. Therefore, this plot can identify those specimens that have a variance greater than most of the specimen set. Those with z-scores, calculated from the principal component scores, over 1 were interpreted to be poorly preserved. Those that had z-scores between 0.5-1 were deemed moderately preserved and those with z scores less than 0.5 were deemed well preserved. The specimen set was then narrowed down to those that had better preservation based on the SEM and LA-ICP-MS data (M.78.01.07, M.01.02.04, M.2010.02.15, P.04.01.15, P.81.01.16, P.06.03.15, F.06.01.15, F.06.01.23, F.78.01.07, B.03.03.05, B.78.01.07, B.06.02.15) and were used for the $\delta^{18}O_p$ analysis.

A Wilcoxon t-test was also conducted on the LA-ICP-MS data using the software JASP (Love *et al.,* 2019) to compare bulk element and total REE concentrations in the enamel versus dentine for each animal group. Due to the structural differences in enamel and dentine, diagenetic alteration will preferentially occur within the softer dentine (Nielsen-Marsh and Hedges, 2000; Hedges, 2002; Turner-Walker, 2008; Keenan, 2016; Chen *et al.,* 2015; Malferrari *et al.,* 2019). However, at higher levels of diagenesis, chemical alteration can also occur in the enamel and elemental concentrations can be homologous (Leuzinger *et al.,* 2022). Therefore, by comparing each element concentration in the dentine vs. enamel, a general level of diagenetic alteration for each animal group can be inferred.

In modern animal bioapatite, the $\delta^{18}O_p$ value is related to the $\delta^{18}O_c$ value and can be expressed as $\delta^{18}O_p + 1 = 0.9787 (\delta^{18}O_c + 1) + 0.0142$ (Iacumin *et al.,* 2022). This relationship will alter during diagenetic processes and so can be an additional way to indicate diagenetic alteration in fossil specimens, with specimens lacking the expected relationship between $\delta^{18}O_p$ and $\delta^{18}O_c$ providing evidence of diagenetic alteration. The residual standard deviation, which is the difference between expected $\delta^{18}O_p$ value predicted by Iacumin's regression line and the

measured $\delta^{18}O_p$, was used to assess the level of preservation for each specimen with those that have greater difference interpreted to have higher levels of diagenetic alteration. Specimens with residual standard deviation more than 1 were determined as poorly preserved, those between 0.5-1 were deemed moderately preserved and those with less than 0.5 were deemed as well preserved. A final preservation assessment was given to each specimen using a combination of the SEM, relative REEs, PCA of bulk elements and residual standard deviation from the Iacumin *et al.,* (2022) regression line. The total REEs concentration and the residual standard deviation from the Iacumin *et al.* (2022) regression line were weighted more than the other methods in the final preservation assessment as the former two were quantitative. All values presented are mean ± 1 standard deviation (SD) where applicable.

## 2.5 RESULTS

### 2.5.1 ASSESSMENT OF DIAGENETIC ALTERATION OF FOSSILS FROM THE PEMBINA MEMBER OF THE PIERRE SHALE OF MANITOBA

Defined columnar units that measured up to 8 μm wide were evident within the enamel of all mosasaur specimens (Fig. 1). In each unit, crystallites branched off to the edge of the adjacent units from an indistinct central line that runs through the middle of each column. The columnar units are aligned perpendicular to the enamel dentine junction (EDJ). Boundaries between units became less distinct closer to the outer edge of the enamel. In some samples the enamel was partially separated from the EDJ revealing the tangential view of enamel basal surface. The semi-circular bases of the columnar units were observable from this vantage point.

The mosasaur enamel observed in this study is consistent with descriptions in previous research (Owocki and Madzia, 2020). The enamel thickness of the 11 mosasaur tooth samples varied due to extensive weathering in certain areas on each tooth. Mosasaur enamel, measured perpendicular from the EDJ to the outer enamel surface, varied from 140 μm thick to, on occasion, being completely absent (Table 2). Although evidence of exterior weathering was evident, the physical order of the crystallites was intact for most areas in each tooth if enamel was present.

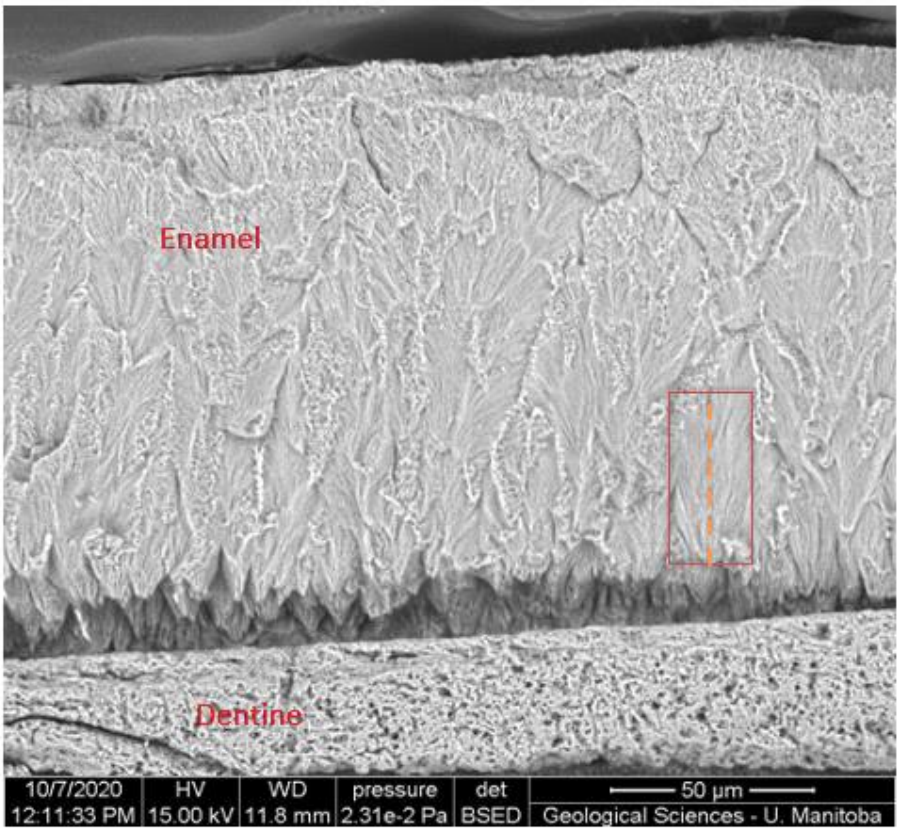


Figure 1: SEM image of a tooth section from mosasaur specimen M.80.29.14, with the enamel and dentine layers labelled. An example of a columnar unit is outlined in red with the midline represented by a dashed orange line.

Table 2: Summary of enamel characteristics for each tooth specimen including enamel thickness and visible physical alterations. All specimens were grouped into one of three preservation categories: well, moderate and poor (see text for details).

| Specimen number | Enamel thickness (µm) | Crystallite preservation | Physical alterations | Preservation classification |
|---|---|---|---|---|
| M.80.29.14 | 17-94 | Well | Slightly weathered and fractures infilled with sediment | Well |
| M.82.00.17 | 98-140 | Well | Slightly weathered and fractures | Well |
| M.07.03.23 | 0-20 | Moderate | Highly weathered and fractures | Moderate |
| M.83.06.18 | 0-46 | Moderate | Highly weathered and fractures infilled with sediment | Poor |

| | | | | |
|---|---|---|---|---|
| M.01.02.04 | 0-25 | Moderate | Highly weathered, fractures, and possible borings | Moderate |
| M.99.03.XX | 30-118 | Well | Slightly weathered | Well |
| M.81.02.16 | 0-15 | Poor | Highly weathered and fractures | Poor |
| M.2010.02.15 | 30-105 | Well | Slight weathering and fractures | Well |
| M.74.06.06 | 0-44 | Poor | Highly weathered and fractures infilled with sediment | Poor |
| M.78.01.07 | 32-92 | Moderate | Moderately weathered and fractures infilled with sediment | Moderate |
| M.06.01.05 | 0-47 | Poor | Highly weathered and fractures infilled with sediment | Well |
| P.04.01.15 | 52-106 | Well | Slightly weathered | Well |
| P.81.01.16 | 20-68 | Well | Moderately weathered and fractures | Moderate |
| P.06.03.15 | 0-18 | Poor | Highly weathered and fractures infilled with sediment | Poor |
| F.06.01.15 | 0-75 | Moderate | Highly weathered, fractures, and possible borings | Moderate |
| F.81.08.16 | 0-32 | Poor | Highly weathered, fractures, and possible borings | Poor |
| F.06.01.23 | 0-25 | Poor | Highly weathered | Moderate |
| F.78.01.07 | 0-22 | Poor | Moderate weathering and fractures | Moderate |
| F.08.01.13 | 0-12 | Poor | Highly weathered and fractures infilled with sediment | Poor |
| F.06.02.15 | 0-17 | Poor | Highly weathered and fractures | Poor |
| B.07.02.15 | - | N/A | Highly weathered and fractures | Poor |
| B.03.03.05 | - | N/A | Highly weathered and fractures infilled with sediment | Poor |

| | | | | |
|---|---|---|---|---|
| B.78.01.07 | - | N/A | Slightly weathered and fractures | Moderate |
| B.83.01.18 | - | N/A | Slightly weathered and fractures | Moderate |
| B.06.01.15 | - | N/A | Highly weathered and fractures | Poor |

Defined columnar units that measured up 11 μm wide were also observed in all three plesiosaur specimens (Fig. 2). Columnar units were aligned perpendicular to the EDJ with each unit consisting of crystallites that branch off from the central line to the edge of the adjacent units. Towards the outer edge of the enamel the boundaries of each unit become less distinct. A tangential view of the enamel basal surface was present in some specimens where the enamel separated from the dentine; from this view, the semi-circular bases of the columnar units were visible (Fig. 3).

The columnar units are only partially consistent with descriptions of enamel from the plesiosaur *Polycotylus* (Testin, 2011). For example, *Polycotylus* crystallites run parallel to each other (Testin, 2011), though this was not observed in any of the specimens in this study. This could be due to species-specific differences although this seems unlikely as *Polycotylus* and *Trinacromerum* are closely related and a part of the same family (Adams, 1997). The enamel thickness of the examined plesiosaur teeth varied due to extensive weathering in certain areas on each tooth. Measured perpendicular from the EDJ to the outer enamel surface, plesiosaur enamel was completely absent in some areas or up to 106 μm thick in other areas. In one plesiosaur specimen (P.04.01.15), possible borings up to 75 μm deep into the enamel surface were observed (Fig. 3). Irregular fractures were also present in all specimens but highly variable in size, ranging from 15 to 350 μm long. These fractures were often infilled with external material such as shale and gypsum and generally propagated from the exterior surface and meandered towards the interior, although were sometimes observed to run sub-parallel to EDJ (Fig. 3). Although there was evidence of exterior weathering for all examined specimens, the physical order of the crystallites remained intact for most areas in each tooth if enamel was present.

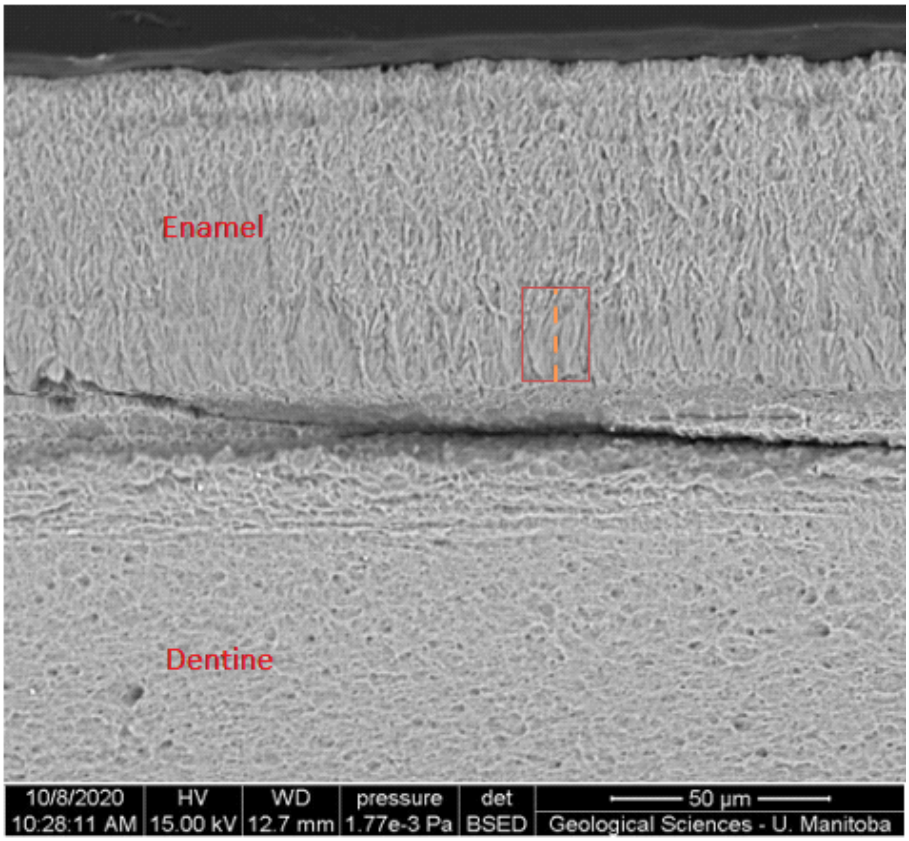

Figure 2: SEM image of a tooth section from plesiosaur specimen P.04.01.15, with the enamel and dentine layers labelled. An example of a columnar unit is outlined in red with the midline represented by a dashed orange line.

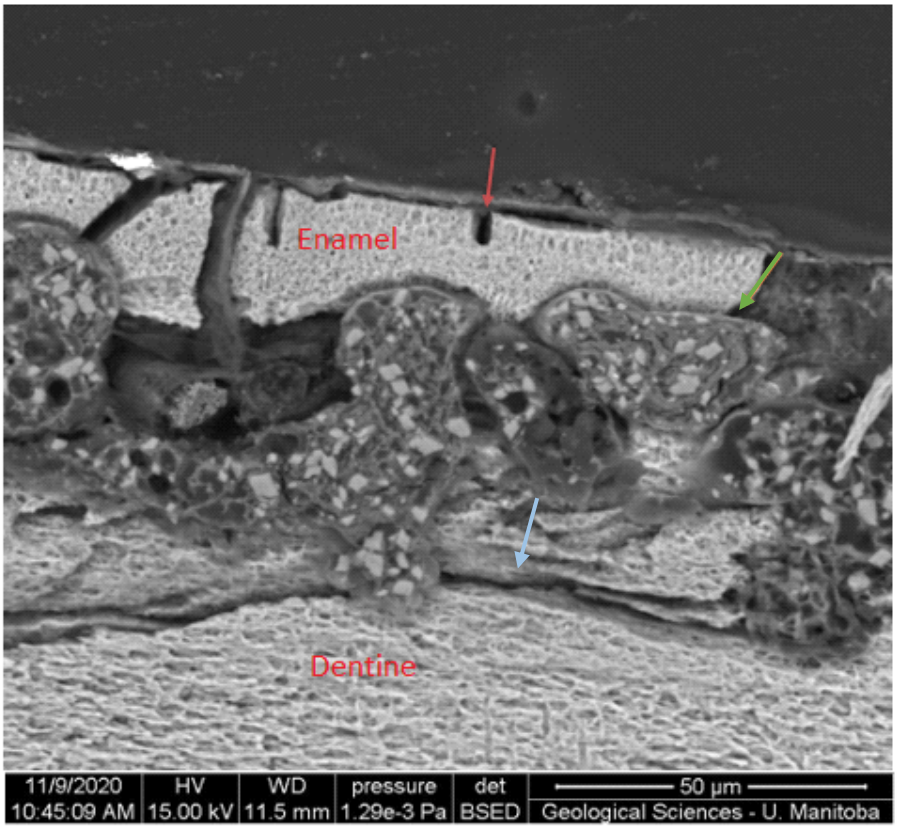


Figure 3: SEM image of a tooth section from plesiosaur specimen P.04.01.15 illustrating post-depositional borings (red arrow), fractures (blue arrow) and infills (green arrow).

The six fish specimens examined in this study possessed much thinner enamel than the mosasaur and plesiosaur samples (Fig. 4). Due to the lack of higher magnification on the SEM that was used, microstructures were difficult to observe. From images collected, thin discontinuous columnar units which measured from 2 to 5 µm wide were present in the fish enamel. In each unit, crystallites branched off to the edge of the adjacent units from an indistinct central line that runs through the middle of each column. The columnar units are aligned perpendicular to the EDJ.

The enamel thickness in fish samples varied from 0 to 20 µm thick due to extensive weathering in certain areas on each tooth. Crystalline units became more visible towards the outer enamel surface. Borings (~5 µm deep) were observed on the outer enamel surface of one of the fish specimens (F.08.01.13). The physical order of the crystallites was intact for most areas in each tooth when enamel was present however, due to the limited magnification of the SEM used, these crystallites are difficult to observe.

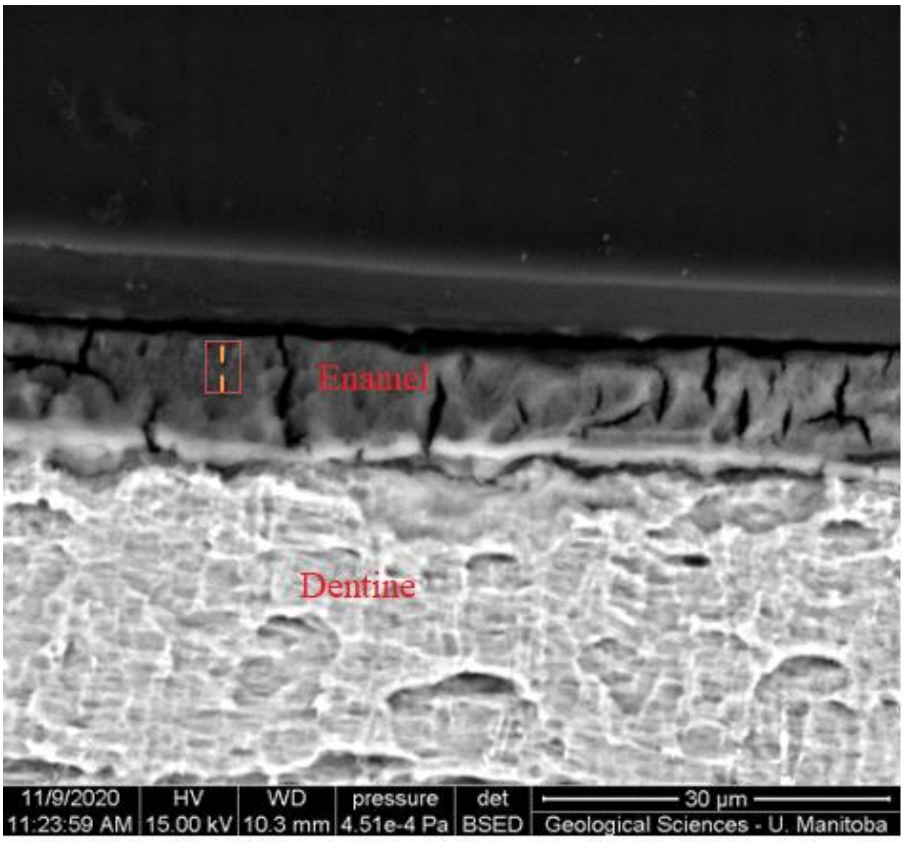


Figure 4: SEM image of a tooth section of fish specimen F.06.01.15, with the enamel and dentine layers labelled. An example of a columnar unit is outlined in red with the midline represented by a dashed orange line.

Due to the lack of *Hesperornis* specimens with dental elements, cortical bones were used for SEM analyses (Fig. 5). The outer circumference layer of the *Hesperornis* samples was characterized by well-organized collagen planes with vascular canals and was typically weathered on the exterior surface. Fibrolamellar cortex, characterized by a woven bone texture with embedded osteocytes, was present in all five specimens and well defined. It is from this layer that most of the LA-ICP-MS spots were taken. The above description of these layers of bone is consistent with previous research (Wilson and Chin, 2014), suggesting little physical alteration within these specimens.

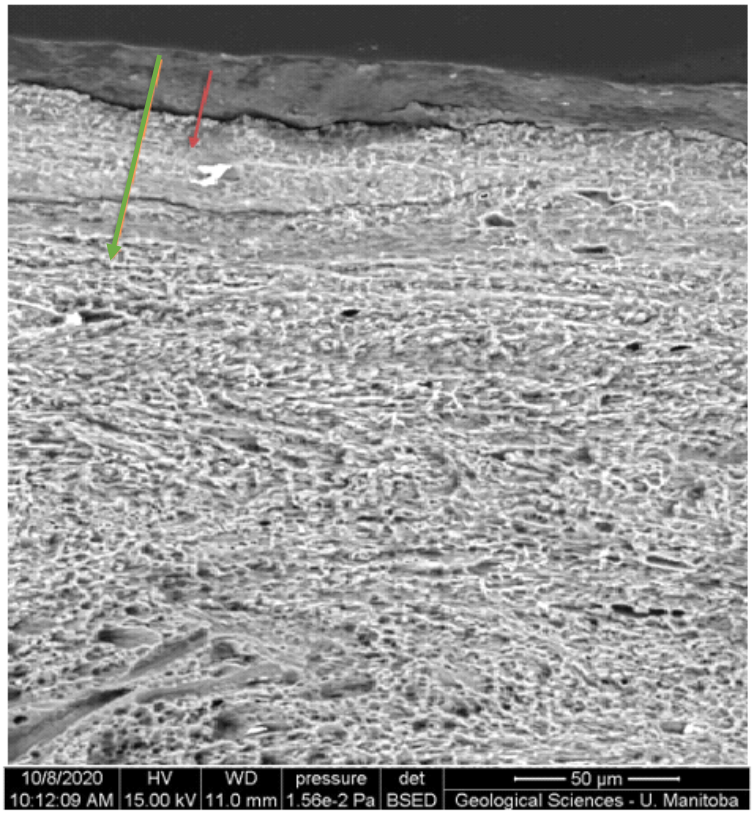

Figure 5: SEM image of a cortical bone section from *Hesperornis* specimen B.07.02.15. The outer cortical layer (red arrow) and fibrolamellar cortical bone layer (green arrow) are denoted.

The full list of bulk elements and total REEs (‰) concentrations for each specimen obtained through LA-ICP-MS analysis can be found in Appendix A. Due to the nature of REE absorption into fossilized material, concentrations above those found in modern teeth (~20 ppm) are thought to indicate diagenesis (Harrell and Perez-Huerta, 2015). Therefore, the relative total REE concentrations within fossilized material was used to assess diagenetic alteration for each specimen relative to the others within specimens. In this study, specimens with less than 300 ppm relative total REEs were considered the least diagenetically altered and determined to be well preserved. Those above 1500 ppm were considered the most diagenetically altered and were designated as poorly preserved. Specimens that had total relative REEs between 300 – 1500 ppm were determined to be moderately preserved (Table 3).

Principal component analysis was also conducted on the bulk element concentrations measured from the LA-ICP-MS analysis. A PCA score plot was used to identify clusters of bulk element concentration data with samples that plot closely together interpreted to be more similar

in component variations to each other than the rest of the samples (Fig. 6). The loadings and principal component scores can be found in Appendix A. Not enough enamel was present on M.06.01.05 and P.06.03.15 to accurately measure bulk element concentrations so just dentine was measured for those specimens. From these PCA score plots, specimens with z-scores greater than 1 were defined as poorly preserved, specimens with z-scores between 0.5-1 were defined as moderately preserved and those that had z-score less than 0.5 were defined as well preserved.

A Wilcoxon paired t-test was used to assess disparities between enamel and dentine bulk element and total REEs concentrations for mosasaurs, plesiosaur, and fish (a paired T-test was not possible for bird specimens since cortical bone was analyzed for *Hesperornis*). High levels of diagenetic alteration are expected to equalize differences in bulk element concentrations and total REEs in the enamel and dentine resulting in statistically insignificant differences between the two (Dauphin and Williams, 2007). In plesiosaurs, no statistical differences between each of the bulk element concentrations ($z=1.342$, $p=0.500$) and total REEs ($z=0.447$, $p=1.00$) were observed in dentine and enamel (See Appendix A for all statistical information) implying that these plesiosaur teeth had diagenetic alteration high enough to affect both layers of tooth material. In mosasaurs, the Mg ($z=-1.478$, $p=0.160$), S ($z=1.070$, $p=0.322$), Fe ($z=0.459$, $p=0.695$), Sr ($z=1.784$, $p=0.084$) and total REE ($z=-0.255$, $p=0.846$) concentrations were not statistically different between the mosasaur dentine and enamel. However, Na ($z=2.701$, $p=0.004$) concentrations were elevated in enamel compared to dentine, and Mn ($z=2.497$, $p=0.010$) concentrations were elevated in dentine compared to enamel. In fish, Na ($z=-2.201$, $p=0.031$), S ($z=-1.363$, $p=0.219$), Fe ($z=0.105$, $p=1.000$) and Sr ($z=-1.153$, $p=0.313$) were not statistically different between dentine and enamel, however the Mg ($z=-2.201$, $p=0.031$), Mn ($z=-2.201$, $p=0.031$) and total REE ($z=-2.201$, $p=0.031$) had statistically higher concentrations within the enamel compared to dentine. Therefore, it is unclear to what extent diagenetic alteration affected the concentrations of these bulk elements in both tooth materials.

Using the three analysis, SEM, relative REEs concentrations and PCA score plot outliers (summarized in Table 3), the specimen set was narrowed down to 13 specimens for $\delta^{18}O_p$ analysis (Table 4). During this process, the relative REEs concentrations assessment was given more weight since this analysis is quantitative and REEs concentrations are closely associated with diagenetic alteration. Specimens were narrowed down as those who were deemed poorly preserved were unlikely to give reliable $\delta^{18}O_p$ values. Note that specimen M.80.29.14 was

designated within this group but due to its small size, did not have enough powdered sample to proceed to the $\delta^{18}O_p$ analysis.

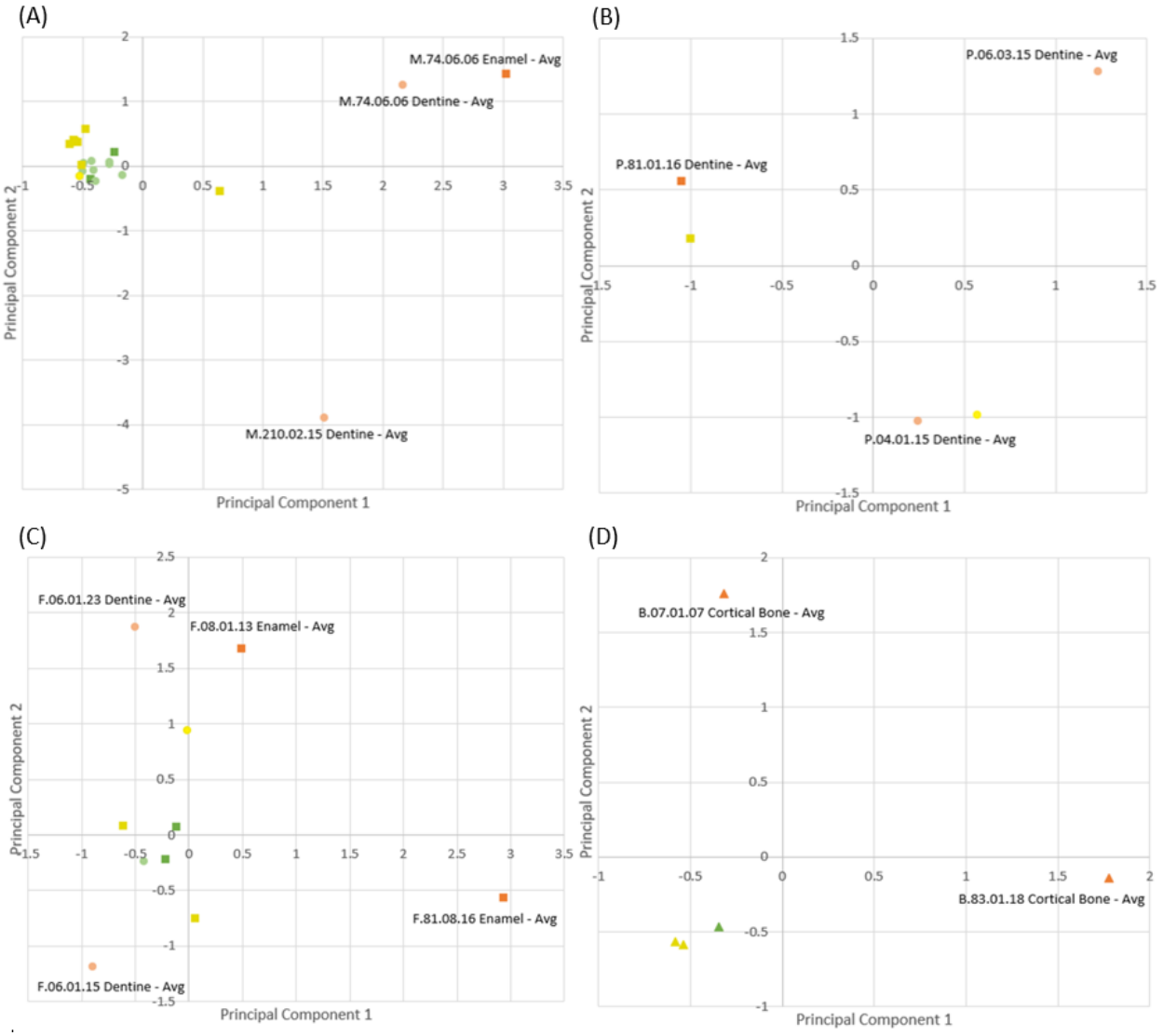


Figure 6: Principal component score plot of bulk elements for (A) 11 mosasaur specimens, (B) 3 plesiosaur specimens, (C) 6 fish specimens and (D) five bird specimens. The LA-ICP-MS data from the enamel (squares) and dentine (circles) were measured separately for mosasaur, plesiosaurs and fish. Cortical bone tissue was measured for the bird specimens (triangle). The preservation assessment derived from the bulk element data is indicated by colour, poorly preserved (orange), moderately preserved (yellow) and well preserved (green). Poorly preserved specimens are also labeled with their specimen name.

Table 3: The final diagenetic assessment of each specimen from the SEM, relative total REEs concentrations (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu), PCA score plot outliers and residual standard deviation from Iacumin *et al.,* 2022 regression line.

| Specimen number | SEM preservation | Relative total REEs | PCA score plot | Residual standard deviation from Iacumin *et al.,* 2022 | **Final preservation classification** |
|---|---|---|---|---|---|
| M.80.29.14 | Well | Well | Moderate | N/A | **Well** |
| M.82.00.17 | Well | Poor | Moderate | N/A | **Poor** |
| M.07.03.23 | Moderate | Poor | Moderate | N/A | **Poor** |
| M.83.06.18 | Poor | Poor | Well | N/A | **Poor** |
| M.01.02.04 | Moderate | Moderate | Moderate | Well | **Well** |
| M.99.03.XX | Well | Poor | Moderate | N/A | **Poor** |
| M.81.02.16 | Poor | Poor | Well | N/A | **Poor** |
| M.2010.02.15 | Well | Well | Poor | Poor | **Moderate** |
| M.74.06.06 | Poor | Poor | Poor | N/A | **Poor** |
| M.78.01.07 | Moderate | Well | Moderate | Well | **Well** |
| M.06.01.05 | Well | Poor | Well | N/A | **Poor** |
| P.04.01.15 | Well | Well | Poor | Moderate | **Well** |
| P.81.01.16 | Moderate | Well | Poor | Well | **Well** |
| P.06.03.15 | Poor | Well | Poor | Poor | **Moderate** |
| F.06.01.15 | Moderate | Moderate | Poor | Poor | **Moderate** |
| F.81.08.16 | Poor | Poor | Poor | N/A | **Poor** |
| F.06.01.23 | Moderate | Well | Poor | Poor | **Moderate** |
| F.78.01.07 | Moderate | Well | Well | Moderate | **Well** |
| F.08.01.13 | Poor | Poor | Poor | N/A | **Poor** |
| F.06.02.15 | Poor | Poor | Moderate | N/A | **Poor** |
| B.07.02.15 | Poor | Poor | Poor | N/A | **Poor** |
| B.03.03.05 | Poor | Well | Well | Moderate | **Well** |
| B.78.01.07 | Moderate | Well | Moderate | Poor | **Moderate** |
| B.83.01.18 | Moderate | Poor | Poor | N/A | **Poor** |
| B.06.01.15 | Poor | Well | Moderate | Poor | **Moderate** |

$\delta^{18}O_c$ data was obtained from all specimens but since diagenetic alteration can only be assessed through the relationship between $\delta^{18}O_p$ vs. $\delta^{18}O_c$, only specimens employed for the

$\delta^{18}O_p$ analysis were used for this assessment. Since the linear relationship between $\delta^{18}O_p$ vs. $\delta^{18}O_c$ in modern unaltered tooth bioapatite is known, any deviation from this pattern can be taken as evidence of diagenetic alteration (Iacumin *et al*., 2022). Most of the Manitoban specimens plotted near or slightly above the regression line (Fig. 7), though others markedly deviated from the regression line. Moreover, the specimens in this study do not have a statistically significant correlation between the $\delta^{18}O_p$ and $\delta^{18}O_c$ concentration ($r=0.069$; $p=0.832$) as expected for modern tooth bioapatite further signifying diagenetic alteration is present in the dataset (Fig.10).

The residual standard deviation of each specimen from the Iacumin *et al.* (2022) regression line was used to further narrow down the specimen set for body temperatures calculations (Table 3). Specimens with deviation greater than 1 were determined as poorly preserved, those between 0.5-1 were deemed moderately preserved and specimens with deviation less than 0.5 were determined as well preserved. This method was used in combination with the previous analyses (SEM, PCA, and total REEs analysis) to determine a final preservation classification of each specimen. Again, the quantitative analyses (total REEs concentrations and residual standard deviation) were given more weight in determining the final preservation classification. The final preservation classification narrowed down the 'well preserved' specimen set to the following: M.78.01.07, M.01.02.04, P.04.01.15, P.81.01.16, F.78.01.07 and B.03.03.05, which were the specimens used to assess the thermoregulation in the discussion section of this thesis.

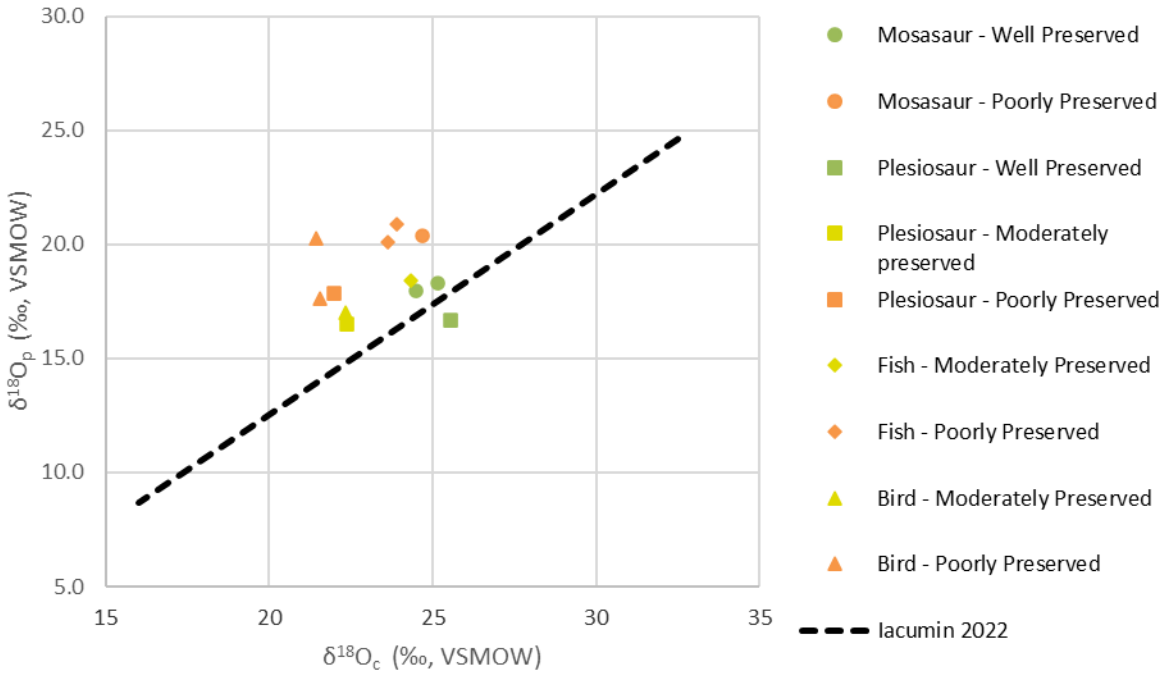


Figure 7: Plot of $\delta^{18}O_p$ versus $\delta^{18}O_c$ concentration for mosasaur, plesiosaur, bird, and fish specimens analyzed in this study colour coded by preservation classification from residual standard deviation (see Table 3). The regression line of Iacumin *et al.* (2022) illustrates the linear relationship between $\delta^{18}O_p$ and $\delta^{18}O_c$ in modern bioapatite. VSMOW = Vienna Standard Mean Ocean Water

### 2.5.2 TEMPERATURE CALCULATIONS AND DIETARY NICHES OF LATE CRETACEOUS MOSASAURS AND PLESIOSAURS

The $\delta^{18}O_p$ values and resulting body temperature calculations for the well preserved and moderately preserved specimen are compiled in Table 4. Using the body temperatures of the fish specimen (F.78.01.07) as a proxy, the seawater temperatures of the Western Interior Seaway at 50° paleolatitude was calculated to be 28.1°C. Average body temperatures for the two well-preserved mosasaurs were calculated to be 36.4°C ±1.1°C. The average body temperatures for the well-preserved plesiosaurs (42.8°C ± 0.3°C) were slightly higher than that of the well-preserved *Hesperornis* specimen (41.1°C) (Table 4). The distribution of body temperatures is illustrated in Figure 8. This indicates that the mosasaurs and plesiosaurs do have elevated body temperatures compared to coeval ectothermic fish and endothermic birds. Due to the

preservation classification determined in Table 3, the specimen set is too small to statistically test for differences and therefore the elevated temperature cannot be statistically confirmed.

Table 4: The $\delta^{18}O_p$ for each specimen denoted as moderate or well-preserved in Table 3 with body temperatures calculated via Puceat *et al.,* (2010) equation with corresponding seawater ($\delta^{18}O_w$) compositions (-1.37, -0.36, 0.00, and 0.23‰).

| | | | Temperature °C ($\delta^{18}O_w$) | | | |
|---|---|---|---|---|---|---|
| **Specimens** | **Final Preservation Classification** | **$\delta^{18}O_p$ (‰)** | **-1.37** | **-0.36** | **0.00** | **0.23** |
| M.01.02.04 | Well | 18.3 | 35.6 | 39.8 | 41.4 | 42.3 |
| M.2010.02.15 | Moderate | 20.4 | 26.8 | 31.1 | 32.6 | 33.6 |
| M.78.01.07 | Well | 18.0 | 37.2 | 41.4 | 42.9 | 43.9 |
| P.04.01.15 | Well | 16.5 | 43.3 | 47.5 | 49.0 | 50.0 |
| P.81.01.06 | Well | 16.7 | 42.6 | 46.8 | 48.4 | 49.3 |
| P.06.03.15 | Moderate | 17.8 | 37.7 | 41.9 | 43.5 | 44.4 |
| F.06.01.15 | Moderate | 20.9 | 24.8 | 29.1 | 30.6 | 31.5 |
| F.06.01.23 | Moderate | 18.4 | 35.1 | 39.4 | 40.9 | 41.9 |
| F.78.01.07 | Well | 20.1 | 28.1 | 32.4 | 33.9 | 34.9 |
| B.03.03.05 | Well | 17.0 | 41.1 | 45.3 | 46.9 | 47.8 |
| B.78.01.07 | Moderate | 17.7 | 38.4 | 42.7 | 44.2 | 45.2 |
| B.06.02.15 | Moderate | 20.3 | 27.3 | 31.6 | 33.1 | 34.1 |

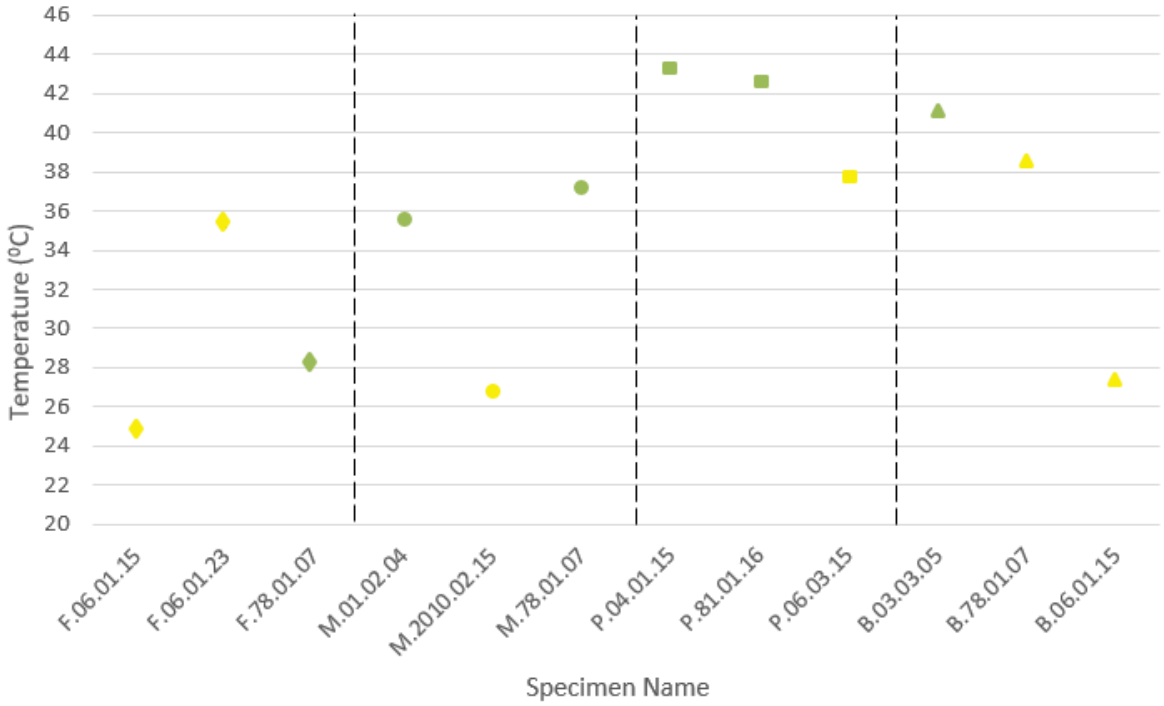


Figure 8: The distribution of calculated temperatures of individual fish (diamonds), mosasaur (circles), plesiosaur (squares) and bird (triangles) samples derived from $\delta^{18}O_p$ concentrations using $\delta^{18}O_w$ -1.37 values. Well preserved specimens (green) and moderate preserved specimens (yellow).

Through the IRMS analysis, the average $\delta^{13}C$ value measured from tooth bioapatite was -4.64 ± 2.42‰ for the mosasaur specimens, -5.04 ± 2.38‰ for the plesiosaurs specimens and -1.42 ± 0.40‰ for the fish specimens. The average $\delta^{13}C$ measured from *Hesperornis* cortical bone was -5.96 ± 0.50‰ (Figure 9). Analysis of $\delta^{13}C$ values for all specimens did pass Levene's test of homogeneity of variance (p=0.095) and a one-way ANOVA test further revealed a statistical difference between the four animal groups (F(3, 22)=7.3, p=0.001). The Tukey's HSD post-hoc test indicated that there was a statistically significant difference in $\delta^{13}C$ values between fish and birds (p=0.001, 95% CI= (-7.450, -1.648)), fish and mosasaurs (p=0.006, 95% CI= (0.894, 5.998)), and fish and plesiosaurs (p=0.042, 95% CI=(0.101, 7.208).

The Manitoba mosasaur $\delta^{13}C$ data was compared to values measured from other localities using a Welches ANOVA (F(4,53)=2.775 p=0.036) since the Levene's test of variance was significant (p=0.004). A Games-Howell post-hoc test revealed that there is a statistical difference in $\delta^{13}C$ values from mosasaurs between Manitoba and Antarctica (p=0.011; 95% CI=(-6.661, -0.662)) and Manitoba and the Netherlands (p=0.005; 95% CI=(1.297, 7.835)) (Giltaij *et al.,*

2021; Leuzinger *et al.,* 2022). The Levene's test of variance was found to be not significant for the plesiosaur data (p=0.232) and a one way ANOVA found that there was a statistically significant difference in $\delta^{13}C$ values (F(1,11)=5.211 p=0.043) between Manitoba plesiosaurs and Antarctica plesiosaurs (Giltaij *et al.,* 2021; Leuzinger *et al.,* 2022). The range of $\delta^{13}C$ values from the Manitoba specimens is consistent those interpreted to have an off-shore foraging niche (Fig. 10). An off-shore foraging niche also likely for the *Hesperornis* specimens in this study as they have statistically similar $\delta^{13}C$ values to those of mosasaurs and plesiosaurs whereas the fish likely inhabited near shore niches due to their more positive $\delta^{13}C$ values (Leuzinger *et al.,* 2022).

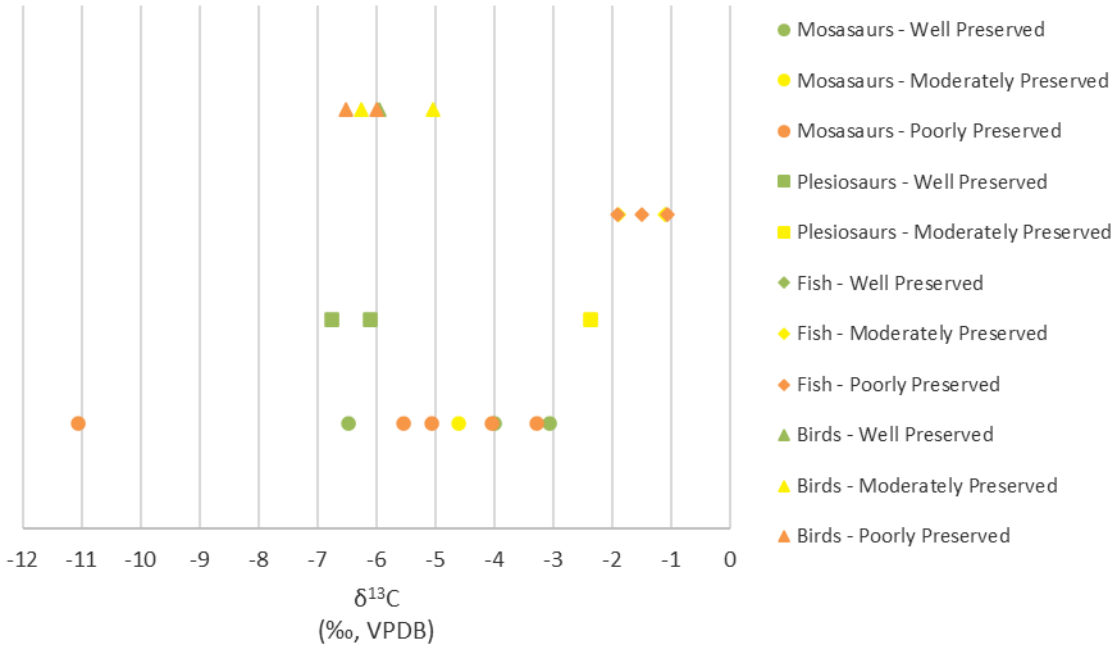


Figure 9: Tooth $\delta^{13}C$ of mosasaurs (circles), plesiosaurs (squares), birds (triangle) and fish (diamonds) are colour coded to indicate to indicate the final preservation designated in table 3. No clear pattern between the preservation and $\delta^{13}C$ was observed within each group. VPDB = Vienna Pee Dee Belemnite.

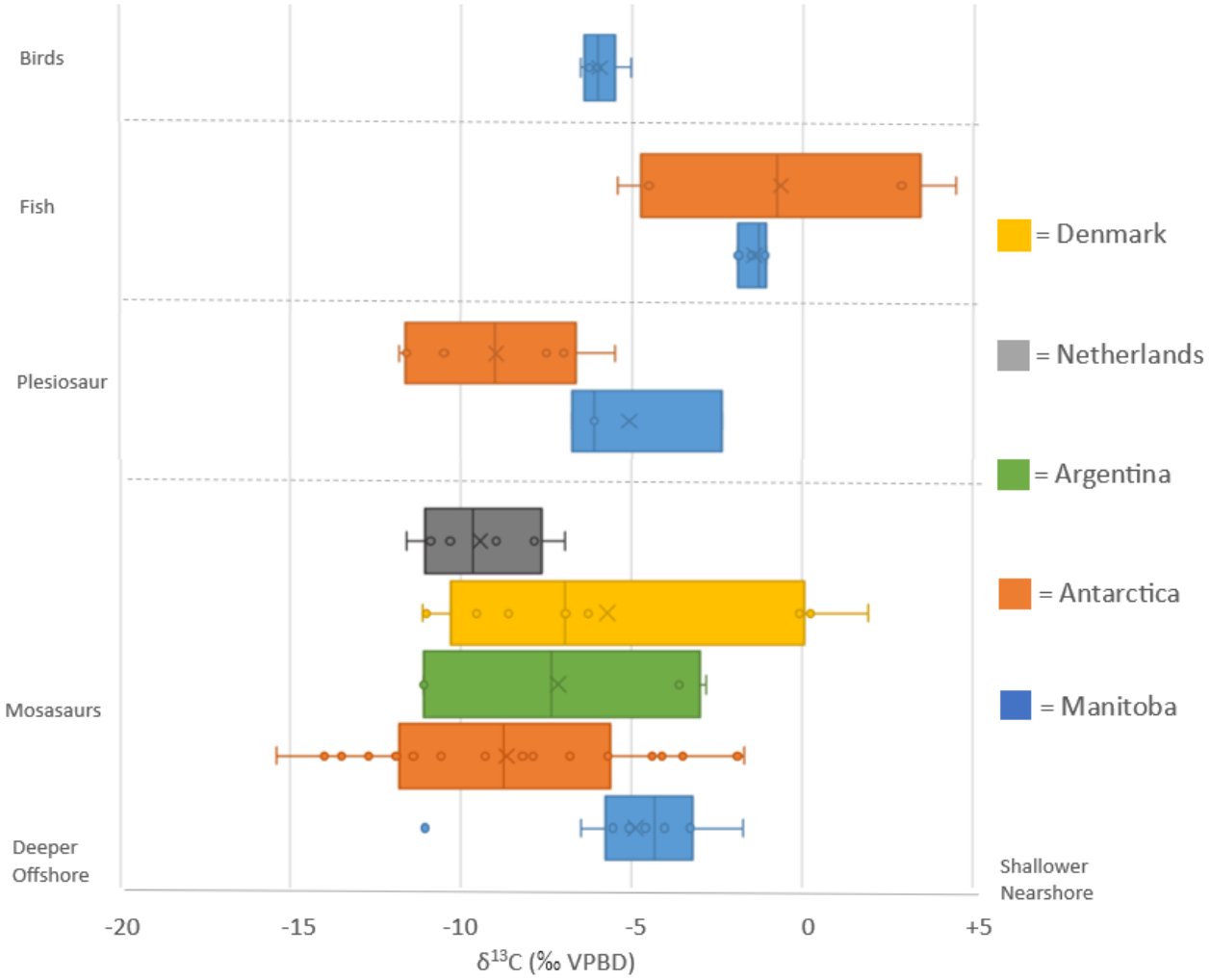


Figure 10: Plot of $\delta^{13}C$ values collected from fossil specimens (circles) in this study (blue) relative to those from Antarctica (orange), Argentina (green), Denmark (yellow) and the Netherlands (grey) (Giltaij *et al.,* 2021; Leuzinger *et al.,* 2022). Specimens that plot towards the right are interpreted to have a near-shore niche whereas those that plot more towards the left are interpreted to have an offshore niche (Leuzinger *et al.,* 2022). VPDB = Vienna Pee Dee Belemnite.

## 2.6. DISCUSSION

### 2.6.1 INTERPRETATION OF $\delta^{18}O_p$ DATA AND CALCULATED BODY TEMPERATURES

The body temperatures of mosasaur (36.4 ± 1.1°C) and plesiosaur (42.8 ± 0.3°C) determined in this study are elevated compared to the ectothermic fish sample (28.1°C) but, due to the small sample size, could not be statistically tested. However, the body temperatures of the

Manitoba mosasaurs calculated in this study do fall within the range of those calculated for the Alabama *Clidastes* (33.1°C), *Platecarpus* (36.5°C), and *Tylosaurus* (34.3°C) specimens. By contrast, the body temperatures (27.5°C) for the *Platecarpus* specimens from South Dakota (Harrell *et al.,* 2016) calculated with the same -1.37‰ $\delta^{18}O_w$ assumption are well below those of the Manitoba mosasaurs. However, Harrell *et al*. (2016) noted that these specimens did not produce sufficient $Ag_3PO_4$ crystals during IRMS preparation, probably due to the poor preservation of the samples, and therefore may not reflect accurate body temperatures. The $\delta^{18}O_p$ values of the mosasaur specimens in this study are also comparable to those obtained from specimens in localities like Asen and Ullstrop, Sweden (19.0 and 19.1‰ respectively) as well as those from South Dakota (18.5‰) Kansas (18.1‰), Morocco (19.9‰) and Israel (18.8 and 18.3‰) (Bernard *et al.,* 2010). The observation that temperatures of mosasaur specimens are relatively consistent across paleolatitudes both within and outside of the Western Interior Seaway suggest some degree endothermy.

By contrast, the $\delta^{18}O_p$ of the Manitoba plesiosaur specimens (16.5-16.7‰) were lower (and thus had higher body temperatures) compared with those of Swedish specimens (19.6 and 20.1‰) as well as others from Morocco (19.8‰), United Kingdom (19.2 and 20.0‰), France (18.8, 19.1 and 19.3‰), Netherlands (19.6‰) and Australia (18.2‰) (Bernard *et al.,* 2010). The body temperatures for plesiosaurs calculated in this study even exceed those calculated from *Hesperornis* (41.1°C), which was almost certainly endothermic. This discrepancy could be caused by many factors including temperature differences between genera or diagenetic alteration in the Manitoba specimens. The average body temperatures for *Hesperornis* calculated in this study are similar to those calculated for *Ichthyornis* in Alabama (38.6°C) and are also consistent with those measured in modern penguins (37.5 °C) (Prinzlinger *et al.,* 1991). Body temperatures of fish from this study (28.1°C) and thus seawater temperatures, are only slightly higher than the range of values (13 to 28°C) previously determined from Manitoba (Prokoph and Karbashewski, 2006) but are consistent with seawater temperatures, 21.5 ± 3.2°C to 29.3 ± 1.9°C, calculated from South Dakota (Dennis *et al.,* 2013). Harrell *et al.* (2016) found that seawater temperatures of Late Cretaceous Alabama (27.2 °C) was slightly cooler compared to those calculated for South Dakota and Manitoba, despite being at a lower paleolatitude.

To further assess the thermoregulatory status of these marine reptiles the mean body temperature of each specimen was compared using the Bernard *et al.* (2010) model (Figure 11).

The two mosasaur body temperatures plot within the endothermic regression lines. However, the plesiosaur and bird specimens in this study lie above the maximal expected endothermic range. This could be due to several factors such as diagenetic alteration inflating body temperatures and the different species used in this study and those used to create the endothermic regression line. It is also important to note that the X axis in this graph is based on one fish specimen (F.78.01.07) and therefore the left-shifted position of the Manitoba specimens might not be accurate.

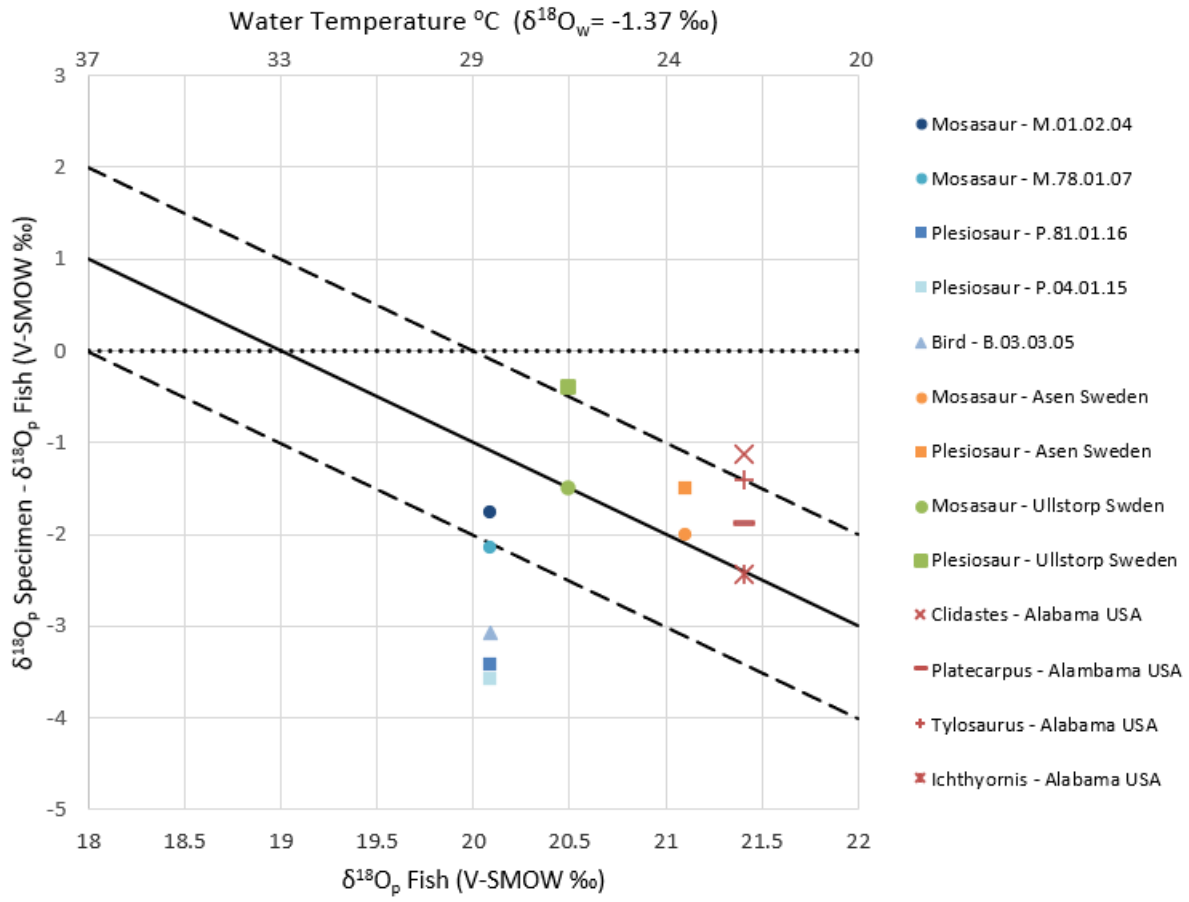


Figure 11: $\delta^{18}O_p$ of fish (a proxy for seawater temperature; X-axis) versus the difference between the mean $\delta^{18}O_p$ value ($\delta^{18}O_p$ specimen) obtained for mosasaurs (circles), plesiosaurs (squares), and bird specimens (triangles) and $\delta^{18}O_p$ value obtained for fish. Mean $\delta^{18}O_p$ from Bernard *et al.* (2010) (green) and Harrell *et al.* (2016) are also plotted for comparison. The ectothermic regression line (dotted line) represents body temperatures that do not differ from ambient seawater. Specimens that plot along the average (solid) and minimum/maximum (dashed lines) endothermic regression lines (Bernard *et al.,* 2010) have body temperatures above that of ectothermic organisms (i.e., ambient seawater). V-SMOW, Vienna standard mean ocean water.

### 2.6.2 DFFICULTIES ASSOCIATED WITH CALCULATING BODY TEMPERATURES

Diagenetic alteration is difficult to quantify and has been assessed through multiple processes in this study. Since the concentration of total REEs is absorbed into tooth bioapatite primarily through diagenetic alteration, this method has been used to assess the preservation of fossils previously (Harrell and Perez-Huerta, 2015). It has been documented that certain bulk element concentrations (Na, Mg, Mn S, Fe and Sr) can also be altered during diagenesis and that those concentrations in the enamel and dentine will become homogenous (Dauphin and Williams, 2007). The Wilcoxon t-test of the LA-ICP-MS data indicated that all bulk element concentrations and total REEs Manitoba plesiosaur enamel and dentine were statistically similar and that some bulk elements were statistically similar between the mosasaur and fish enamel and dentine implying some degree of alteration within each animal group. However, there are other factors like diet that can affect the bulk concentrations in tooth bioapatite and the relationship between diagenetic alteration and these bulk element concentration in modern and fossilized bioapatite is unclear (Dauphin and Williams, 2007). Therefore, the preservation assessment of each specimen using the PCA score plots might be influenced through factors other than preservation. Therefore, even though multiple methods were used to assess preservation of the specimens, there is still a chance that, even the well-preserved specimens have some degree of diagenetic alteration.

The paleothermometry equation outlined by Puceat *et al.* (2010) requires the isotopic composition of seawater to be assumed, since it is rarely preserved in the geological record. Although there are various ways to estimate this composition, a level of uncertainty is still inherent (Ghosh *et al*., 2006). The oxygen phosphate concentration in marine turtle bones has been used to used to assess the isotopic composition of the Western Interior Seaway (Coulson *et al.,* 2008; Harrell *et al.,* 2016). This area of research currently remains understudied in Manitoba and therefore I employed seawater isotopic composition values determined by Coulson (2008) from Campanian marine turtle bones found in South Dakota for the body temperature calculations. However, the isotopic composition of seawater within the Western Interior Seaway varied both temporally and spatially, with isotopic composition tending to be more negative with open water environments (He *et al.,* 2005; Coulson *et al.,* 2008). Although the seawater composition values from South Dakota are likely not an exact match for those of Manitoba, it was assumed for this study that they are similar. If in the future Coulson's study is replicated for

Manitoba and the seawater composition values are found to be significantly different, then the body temperatures from this study would have to be recalculated.

Isotopic fractionation, the partitioning of heavy and light isotopes, is a natural phenomenon that should be considered when using paleothermometry (Kolondy *et al.,* 1983; Luz *et al.,* 1985). When a tooth grows, the oxygen isotopes are exchanged between the phosphate fraction of the bioapatite and internal body water, and hence not with the seawater directly (Bryant *et al.,* 1996). The isotopic composition of body water within an organism correlates with temperature and isotopic composition of ingested water (Kolondy *et al.,* 1983; Luz *et al.,* 1985). Therefore, oxygen isotopic composition of body water in marine animals is typically consistent with seawater composition since water ingested is primarily from oceanic sources, as in modern fish (Kolondy *et al.*, 1983). However, the body water in certain fish can vary due to migration to other oceanic habitats and/or freshwater/brackish environments (Harrell *et al.,* 2016). In modern semi-aquatic animals, ingested meteoric waters can alter body water composition compared to the ambient composition (Harrell *et al.,* 2016). The isotopic variation between meteoric water and seawater is largely associated with the phase transition during evaporation (Koch, 2007). Through the vapor phase of evaporation, isotopic composition of meteoric water is depleted in heavier isotopes compared to seawater (Koch, 2007). While terrestrial animals are expected to have body-water enriched in lighter isotopes, marine animals are considered to be purely oceanic organisms and, in this study, have been treated as such. However, recent research has challenged that assumption for mosasaurs. Through consecutive sampling of *Clidastes* and *Platecarpus* teeth of southern Western Interior Seaway specimens, dental bioapatite shows evidence of some freshwater intake through periodic negative spikes (1-4‰) in $\delta^{18}O_p$, indicating that mosasaur might have regularly consumed freshwater (Taylor *et al.,* 2021). If the negative spikes present in a mosasaur tooth are included within the sample, then this isotopic variation could lead to inflated body temperatures. Migration could also be the cause for the high body temperature in the Manitoba plesiosaur specimens. Although intraspecific tooth sampling on plesiosaurs like the Taylor *et al.* (2021) has not been conducted yet, there is evidence that some plesiosaurs did migrate and could live in freshwater environments (Vandermark *et al.,* 2006; Gao *et al.,* 2019). More research is needed to fully understand the effects of freshwater intake in extinct marine reptile body water. Of the animals used in this study, the avian specimens are the most likely to have their body water affected by ingested meteoric water. *Hesperornis*, the only Cretaceous bird

used in this study, was a flightless diving bird and was semi-aquatic (Reynaud, 2006). Although *Hesperornis* specimens are typically found in open marine deposits, especially in the Western Interior Seaway, they have also been found in freshwater and estuary deposits in other localities which could indicate that they might have lived in environments with more meteoric water intake (Rees and Lindgren, 2005). It is currently unknown how much time *Hesperornis* would have spent on land as their bauplan made it difficult for terrestrial locomotion (Bell and Chiappe, 2022). Although it is unclear how much meteoric water would have affected body water, the body temperatures for the bird specimens calculated in this study fall within the temperature range for modern shore birds and penguins (38.5°C to 40.9°C) (Prinzniger *et al.,* 1991).

Variations in calculated body temperatures might also occur due to factors including seasonality, temporal differences, and migration. Even if preservation of tooth material is pristine and not diagenetically altered, oxygen isotopic concentrations only reflect the temperature when the bioapatite mineralized (Fick, 2007; Surmik and Pelc, 2012). For example, body temperatures of modern (and hence extinct) ectothermic species may change on a seasonal basis (Surmik and Pelc, 2012). To fully assess the seasonal differences within an individual, either intra tooth sampling should be implemented or, like in this study, multiple individuals should be sampled (Surmik and Pelc, 2012). Although specimens were chosen from only the Pembina member of the Pierre Shale, this period still represents approximately three million years (Nicholls and Russell, 1990). Within this time period, many variables such as seawater isotopic composition and temperature might have changed. Migration could also have affected the resulting body temperature estimates. There is evidence that both mosasaurs and plesiosaurs migrated as well as the flightless bird *Hesperornis* which is suggested to have swum north during breeding seasons (Feduccia, 1996; Berthold, 2001; Jagt, 2005; Taylor *et al*., 2021; Vavrek *et al.,* 2014). Because of this, it remains difficult to determine if the teeth and bones sampled in this study represent the Western Interior Seaway in Manitoba, where they were collected, or if they represent a wider range due to animal movement.

### 2.6.3 THERMOREGULATION IN MARINE REPTILES

Although endothermy has previously been proposed in plesiosaurs and mosasaurs, the potential physiological mechanism(s) of thermogenesis have not been explored in detail. If marine reptiles were gigantotherms, as proposed by Motani (2010), then the elevated body

temperatures calculated for these marine reptiles would be a result of their large size (resulting in a relatively low body surface area to body volume ratio) and not through facultative, metabolically driven thermogenic processes. If, however, these Mesozoic marine reptiles were either regional or whole-body endotherms, then they presumably were capable of some type of thermogenesis. It is unlikely that marine reptiles produced endogenic heat through brown adipose tissue, as this thermogenic tissue is exclusively found in mammals, and particularly important to those of small size (Mezentseva *et al.,* 2008; Newman, 2011; Gaudry *et al.,* 2017; Griggs *et al.,* 2022).

If extinct marine reptiles were regionally endothermic, they might have generated heat through red muscle thermogenesis, similar to that seen in modern fish and lamnid sharks (Bernal *et al.,* 2003). In these latter groups, the heat generated through internal myotomal muscles during swimming is entrapped in the core through counter-current heat exchangers (Legendre and Davesne, 2020). Myotome muscles are found within all living vertebrates and, therefore, it is likely that mosasaurs and plesiosaurs would have also possessed this muscle type (Lewandowski *et al.,* 2020). A thermogenic role of myotomal muscles might have evolved in mosasaurs and plesiosaurs under similar selection pressures as regionally endothermic fish, namely to facilitate increased swimming performance (Harding *et al.,* 2021). Owing to a lack of fossil preservation, there is currently no direct evidence that myotomal muscles in mosasaurs and plesiosaurs were used solely for motor function or contributed to the thermogenic capacities of these groups. However, when comparing regionally endothermic fish and Mesozoic marine reptiles it is important to note that members of each of these groups are classified as constant cruisers, meaning that they continually swim within the water column. Accordingly, it is plausible that red myotomal muscle endothermy evolved independently in both modern regionally endothermic fish and these extinct marine reptile lineages (Bernard *et al.,* 2010; Legendre and Davesne, 2020).

When assessing the thermoregulatory status of marine reptiles, or any organism, using palaeothermometry on tooth bioapatite, it is important to note that the body temperatures obtained only reflect those of the tissue in which they were deposited. Therefore, any inferences on endogenic heat production can only be made to this region of the body. As such, the elevated body temperatures within marine reptiles in this study, and others like this, can only be taken at best as evidence of elevated temperatures within cranial tissue. Cranial endothermy is a form of

thermogenesis typically seen in some modern fish (tuna, billfish, opah) and thresher sharks in which the temperature of cranial tissues, particularly ocular muscles, are consistently elevated (Stevens, 1972; Dickson and Graham, 2004; Legendre and Davesne, 2020). Providing and maintaining this heat, however, can be an issue since ocular muscles have a high rate of heat loss particularly in aquatic environments (Ninomiya and Yoshida, 2007). A variety of processes in cranial endotherms are used to retain heat within the eyes but typically include counter-current heat exchangers within the cranium close to the eye muscles (Block, 1987). Keeping ocular muscles warm is pivotal for open water-pelagic swimmers like tunas, opahs, and some sharks as keeping the thermal levels consistently elevated protects the central nervous system against rapid temperature change. A warm retina is thought to improve visual sensitivity and temporal resolution thus increasing their ability to detect rapid movement (Block and Carey, 1985; Fritsches *et al.,* 2005). Thus, this type of endothermy would theoretically be useful to visual predators like mosasaurs and plesiosaurs (Yamashita *et al.,* 2015). Visual predation in both mosasaurs and plesiosaurs is aided by the presence of a sclerotic ring, a series of bones within the eye socket which can overlap with each other forming a ring (Franz-Odendaal, 2020). Reptiles have sclerotic ring to support the eye and or help with visual accommodation i.e. the physical adjustments within the eye to see at different distances. In aquatic reptiles, sclerotic rings are present to aid in lens manipulation due to the negation of cornea refractive power in water (Franz-Odendaal, 2020).

Both cranial and red muscle endothermy in fish adds additional challenges as heat loss occurs from the gills, with (cooled) oxygenated blood then traveling directly to the heart and the rest of the body (Steven and Sutterlin, 1976). Interestingly, non-endothermic hammerhead sharks have recently been proposed to minimize heat loss to the water when diving in cold deep water by closing their gill slits (Royer *et al.,* 2023), though it is unknown if endothermic fish utilize a similar heat conservation tactic. Regardless, since marine reptiles were air breathers, this form of heat loss would not have been an issue and frequent trips to the surface would have meant less time in cooler deep waters.

The body plan of an animal can have a large effect on thermoregulation, as specific size, surface area and insulation can greatly impact heat retention (Favilla and Costa, 2020). Since heat loss through convection is largely affected by body size, surface area, and shape (Favilla and Costa, 2020), these factors of marine reptiles should be taken into account when assessing their

possible thermoregulatory strategies. A small surface-area-to-body-size ratio is more efficient at retaining heat and so a large body size can help offset these factors. This is seen in modern day blue whales and emperor penguins (Favilla and Costa, 2020) as well as in modern gigantotherms like leatherback turtles and crocodiles. In modern crocodiles for example, their large size along with behavioural thermoregulation allows them to maintain relatively stable and slightly elevated body temperatures without the use of endothermy (Seebacher *et al.,* 1999). Seebacher argues using this logic that a 10,000 kg 'dinosaur' could have theoretically maintained a body temperature of 31°C in winter without the use of additional endogenic heat production. However, crocodiles are semi-aquatic and can, when too cold, escape the high heat capacity of water by relocating to terrestrial environments.

Skin pigmentation patterns are another factor that might have helped these marine reptiles maintain elevated body temperature. For example, a dark dorsal integumental colouration is reconstructed for mosasaurs based on the high concentrations of eumelanin associated with fossilized melanosomes (Lindgren *et al.,* 2014). This colouration potentially aided mosasaurs with thermoregulation and the exploitation of polar habitats as the darker colouration on dorsal side would have absorbed more heat when at the waters surface (Lindgren *et al.,* 2014).

Thermoregulation can also have a great effect on the organism's distribution into different environments with endothermy thought to increase the ability to exploit cooler polar habitats (Harrell *et al.,* 2016). Harrell *et al.* (2016) argued that the occurrence of Mesozoic marine reptiles like mosasaurs and plesiosaurs in polar habitats (Chin *et al.,* 2008; Ross 2009; Ketchum and Benson, 2010) is consistent with the ability to maintain elevated body temperatures in colder waters. Previous studies have argued that because of this, an endothermic system is likely for marine reptiles (Harrell *et al.,* 2016). Although some modern endotherms can exploit polar oceanic environments well, these environments are not exclusive to full body endotherms. Moreover, the temperatures calculated for polar regions in the Late Cretaceous would have been much warmer than they are today, for instance Cretaceous marine deposits from Devon Island calculated an average surface temperature of 15°C (Chin *et al.,* 2008). Therefore, when discussing the distribution of Late Cretaceous marine reptiles and their thermoregulatory status, it is important to remember these factors when using distribution to infer the thermoregulation of mosasaurs and plesiosaurs.

### 2.6.4 INTERPRETATION OF $\delta^{13}C$ DATA

Previous research has indicated that the $\delta^{13}C$ value in marine animals primarily reflects the source of their diet (Leuzinger *et al.,* 2022). Dissolved organic and inorganic carbon concentrations vary depending on latitude, water depth and distance from shore (Goericke and Fry, 1994). Aquatic animals that rely on food sources within specific areas in the ocean would have prey $\delta^{13}C$ values incorporated into their tooth bioapatite (Leuzinger *et al.,* 2022). Therefore, marine animals who kept to deeper water and farther from shore are expected to have more negative $\delta^{13}C$ values compared to those that stayed close to the shallow waters of the coastline (Leuzinger *et al.,* 2022). In modern marine mammals, the diet and habitat partitioning are reflected in tooth enamel and dentine $\delta^{13}C$ values (Leuzinger *et al.,* 2022). Off-shore pinnipeds in California have $\delta^{13}C$ values between -14 ‰ and -11 ‰ whereas near shore cetaceans have $\delta^{13}C$ values between -11‰ and -9‰ (Leuzinger *et al.,* 2022). In this study, the exploitation of open water environments for foraging have been corroborated for mosasaurs, plesiosaurs, and *Hesperornis* based the $\delta^{13}C$ values. $\delta^{13}C$ values for the Manitoba mosasaur and plesiosaur specimens are consistent with $\delta^{13}C$ values from previous studies and therefore can also be described to have an offshore marine niche (Giltaij *et al.,* 2021; Leuzinger *et al.,* 2022). The more positive $\delta^{13}C$ values in fish specimens is also consistent with previous research and has been interpreted to have a shallower near shore niche (Leuzinger *et al.,* 2022). However, other factors can cause these $\delta^{13}C$ values to positively skew results. Results can be skewed by the incorporation of dissolved inorganic carbon (DIC), which has a $\delta^{13}C$ values between 0 – 2.5‰, into either the enamel or dentine. Incorporation of DIC and other diagenetic alteration is more likely to occur with dentine and therefore, the $\delta^{13}C$ values within these tooth materials differ, with dentine consistently measuring more positive than enamel. Indeed, the difference between average enamel and dentine was 5.3‰ for mosasaurs, 5.1‰ for plesiosaurs and 8.3‰ for fish (Leuzinger *et al.,* 2022). Therefore, the bulk samples, like the ones in this study, are more likely to skew positively. Additionally, modern mammals that forage in estuaries a have $\delta^{13}C$ values around -8 ‰ (Leuzinger *et al*., 2022). If mosasaurs and plesiosaurs did migrate to estuaries periodically, these specimens might also skew positively due to freshwater intake. Other factors like diving might also affect the $\delta^{13}C$ values in marine reptile bioapatite. Isotopic values skew

more negatively when an animal holds their breath and so diving animals, like mosasaurs, plesiosaurs and *Hesperornis*, tend to have lower $\delta^{13}C$ values in their tooth bioapatite (Schulp *et al.,* 2013). However, the extent of isotopic fractionation when breath holding and the impact of diving on marine reptile tooth bioapatite is still unknown (Schulp *et al.,* 2013, Leuzinger *et al.,* 2022).

## 2.7 CONCLUSIONS

Marine reptiles, particularly mosasaurs, were a large part of the Campanian aquatic ecosystem of Manitoba. Therefore, their lifestyle must have contributed to the paleoecology within the Western Interior Seaway (Bennet and Ruben, 1979). The findings of this study suggest elevated body temperatures in mosasaurs and plesiosaurs compared to coeval ectothermic fish. These apparent elevated body temperatures can be taken as evidence of some type of endothermy. The elevated body temperatures calculated for mosasaurs are consistent with previous studies (Bernard *et al.,* 2010; Harrell *et al.,* 2016) and are also consistent with the endothermic reptile regression line given by Bernard (2010). However, the two plesiosaur body temperatures were so elevated that they exceed those from the known endotherm, *Hesperornis*, and therefore might represent diagenetic alteration. Due to the nature of paleothermometry, the body temperatures calculated for the marine reptiles should only be taken as cranial temperatures since only teeth were used as samples. Although full body endothermy is possible in mosasaurs and plesiosaurs, the results from this study can only infer regional endothermy.

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# CHAPTER 3: CONCLUSION

The concept of large carnivorous reptiles, like mosasaurs and plesiosaurs, dominating the Cretaceous seas is difficult to comprehend since there are no analogs or direct descendants of these groups living today (Nicholls and Russell, 1990). Because of this, many questions regarding their physiology and ethology remain unanswered. The thermoregulation of marine reptiles, and other extinct diapsids, remains a topic of debate amongst paleontologists as there are many factors to consider (Motani, 2010; Bernard *et al.,* 2010; Harrell *et al.,* 2016). An ectothermic thermoregulatory status was assumed in the past but recent research regarding their morphology, histology and distribution all suggests an endothermic-like system for both mosasaurs (Pellegrini, 2007; Bernard *et al.,* 2010; Houssaye *et al.,* 2013; Harrell *et al.,* 2016) and plesiosaurs (Bernard *et al.,* 2010; Krahl *et al.,* 2013; Wintrich *et al.,* 2017a; Wintrich *et al.,* 2017b; Fleischle *et al.,* 2018).

In this study, the thermoregulatory status was inferred for Late Cretaceous mosasaurs and plesiosaurs through bulk isotope paleothermometry. This method uses the temperature dependant relationship between the isotopic concentration in forming bioapatite and surrounding water (Urey, 1947). Since the accuracy of paleothermometry is dependent on the preservation of the original isotopic composition in fossil material, the degree of diagenetic alteration needs to be determined first (Harrell *et al.,* 2016). In this study, the diagenetic alteration of each specimen was assessed through SEM analysis, relative total REE concentrations, PCA of bulk element concentrations, and the differences between $\delta\,^{18}O_p$ and $\delta\,^{18}O_c$. Only the specimens with the designated classification of well preserved were used to assess body temperatures.

The average body temperatures for mosasaurs were found to be elevated compared to coeval ectothermic fish but did not exceed endothermic bird values although these differences could not be statistically demonstrated. Mosasaur body temperatures are also consistent with previous research, which found elevated temperatures for these marine reptiles (Bernard *et al.,* 2010; Harrell *et al.,* 2016). However, the average plesiosaur body temperatures were elevated compared to coeval ectothermic fish and mosasaurs as well as previously published values for members of this lineage (Bernard *et al.,* 2010). This could reflect a large natural difference in

body temperature between mosasaurs and plesiosaurs though may reflect a degree of diagenetic alteration.

Paleothermometry has limits and there are multiple factors that can affect results which might lead to unreliable calculated body temperatures. Diagenetic alteration during or after the fossilization process can alter the physical and chemical structure of bioapatite, rewriting the original isotopic concentration when the tooth formed (Kohn *et al.,* 1999; Nielsen-Marsh and Hedge, 2000; Hedges, 2002; Turner-Walker, 2008; Keenan, 2016; Chen *et al.,* 2015; Malferrari *et al.,* 2019). Calculations may also be inaccurate due to uncertainties in estimated isotopic concentrations of ambient seawater as well as isotopic fractionation between the ambient seawater and body water (Kolondy *et al.*, 1983; Bryant *et al.,* 1996; Ghosh *et al*., 2006; Coulson *et al.,* 2008; Taylor *et al.,* 2021). Other factors such as temporality, seasonality, and migration might influence isotopic compositions but are difficult to constrain (Feduccia, 1996; Berthold, 2001; Jagt, 2005; Surmik and Pelc, 2012; Vavrek *et al.,* 2014; Taylor *et al*., 2021). Though this thesis has tried to account for these factors through its study design, they should be taken into consideration when assessing the thermoregulatory status of mosasaurs and plesiosaurs.

Mosasaurs and plesiosaurs, were an integral part of the ecology of northern portion of the WIS, with larger genera like *Platecarpus* and *Tylosaurus* being the top predators (Nicholls and Russell, 1990). Therefore, their thermoregulation would have had a large effect on the ecology of WIS as it can affect the predation frequency of an organism. A full or regionally endothermic thermoregulatory systems would likely increase metabolic rate and therefore require higher food intake rates (Bennet and Rubin, 1979). If mosasaurs and plesiosaurs were regional or full body endotherms, predation frequency would likely be higher compared to ectothermic predators. It is well known that the northern portion of the WIS is characterized by relatively high abundance, but low generic diversity of marine reptiles, specifically mosasaurs (Nicholls and Russell 1990; Kilmury and Brink, 2022). An endothermic system for mosasaurs and plesiosaurs would then show that the WIS would have been rich enough to sustain a large population of these predators.

Since only tooth bioapatite was used, however, one can only confidently infer that the cranium exhibited an elevated temperature. Therefore, elevated body temperatures of Late Cretaceous mosasaurs and plesiosaurs from the northern portion of the Western Interior Seaway

can only be taken as evidence of regional endothermy. One can speculate regarding full body endothermy within these marine reptiles, but more research is needed.

Several areas need additional research to gain clarity on the thermoregulatory and metabolic status of extinct Mesozoic marine reptiles. A continuation of bulk oxygen isotope paleothermoetry should be conducted in various localities to further confirm the elevated body temperatures for Mesozoic marine reptiles over a range of environment/ambient temperatures (Bernard *et al.,* 2010; Harrell *et al.,* 2016). A study following methodology similar to Coulson (2008) to measure the isotopic composition in Manitoba Cretaceous seawater is also needed to reduce uncertainties in temperature calculations. Depending on the preservation of the fossils, clumped isotope paleothermometry could also be used to determine if the body temperatures of these marine reptiles can be confirmed using different methods (Kluge *et al.,* 2015). To study full body endothermy in marine reptiles, body temperature calculated from cortical bone could be used to compare against values from tooth bioapatite. Harrell *et al.* (2016) measured the isotopic composition in cortical bone and tooth bioapatite from one mosasaur which produced similar body temperature estimates; however more research is needed to confirm the efficacy of this method. Continued research is also needed to fully understand the mechanisms and the repercussions of thermoregulatory systems in Mesozoic marine reptiles like mosasaurs and plesiosaurs. Understanding the thermoregulation could shed light onto many characteristics of these marine reptiles, such as likely feeding habits and locomotion. This knowledge would allow us to better understand the evolution of endothermy with the diapsid lineage.

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APPENDIIX A

## 1.1.0 LA ICP-MS VALUES

### 1.1.1 MOSASAURS

Table 1: Bulk element concentrations and total REEs concentrations for mosasaur specimens measured through LA-ICP-MS.

| Specimen Name | Na (ppm) | Na Int2SE | Mg (ppm) | Mg Int2SE | S (ppm) | S Int2SE |
|---|---|---|---|---|---|---|
| M.80.29.14-bEnamel1 | 12150 | 590 | 1450 | 98 | 667 | 67 |
| M.80.29.14-bEnamel2 | 12120 | 390 | 1592 | 72 | 772 | 71 |
| M.80.29.14-bDentine1 | 7130 | 210 | 1441 | 29 | 5800 | 160 |
| M.80.29.14-bDentine2 | 6910 | 200 | 1383 | 44 | 5950 | 170 |
| M.82.00.17-bEnamel1 | 11900 | 610 | 1103 | 60 | 2760 | 150 |
| M.82.00.17-bEnamel2 | 12590 | 730 | 1026 | 48 | 2790 | 130 |
| M.82.00.17-bDentine1 | 10770 | 270 | 795 | 14 | 9960 | 390 |
| M.82.00.17-bDentine2 | 10760 | 240 | 769 | 14 | 12230 | 270 |
| M.07.03.23-bEnamel1 | 0 | 0 | 1706 | 85 | 4220 | 650 |
| M.07.03.23-bEnamel2 | 13170 | 660 | 2030 | 240 | 4090 | 460 |
| M.07.03.23-bDentine1 | 6450 | 180 | 1511 | 43 | 10320 | 790 |
| M.07.03.23-bDentine2 | 6420 | 160 | 1389 | 43 | 8700 | 320 |
| M.83.06.18-bEnamel1 | 15490 | 680 | 1180 | 120 | 6680 | 270 |
| M.83.06.18-bEnamel2 | 13010 | 620 | 1570 | 230 | 1.61E+04 | 2.00E+03 |
| M.83.06.18-bDentine1 | 7950 | 200 | 1130 | 20 | 12220 | 450 |
| M.83.06.18-bDentine2 | 8150 | 260 | 1181 | 30 | 12680 | 600 |
| M.01.02.04-bEnamel1 | 9.40E+03 | 1.20E+03 | 1928 | 95 | 7.44E+04 | 4.00E+03 |
| M.01.02.04-bEnamel2 | 1.15E+04 | 2.20E+03 | 2310 | 190 | 3.38E+04 | 8.40E+03 |
| M.01.02.04-bDentine1 | 4900 | 81 | 1368 | 24 | 5800 | 190 |
| M.01.02.04-bDentine2 | 4954 | 88 | 1379 | 22 | 5769 | 92 |
| M.99.03.XX-bEnamel1 | 1.39E+04 | 1.60E+03 | 1485 | 69 | 4260 | 370 |
| M.99.03.XX - bEnamel2 | 15970 | 720 | 1390 | 83 | 4530 | 190 |
| M.99.03.XX - bDentine1 | 9380 | 210 | 1447 | 35 | 9600 | 360 |
| M.99.03.XX - bDentine2 | 13430 | 350 | 1351 | 29 | 15330 | 490 |
| M.99.03.XX - bDentine3 | 13720 | 340 | 1473 | 30 | 16740 | 640 |
| M.81.02.16-bEnamel1 | 4520 | 270 | 1320 | 130 | 9060 | 720 |
| M.81.02.16-bEnamel2 | 5270 | 330 | 903 | 60 | 8770 | 500 |
| M.81.02.16-bDentine1 | 4880 | 190 | 856 | 39 | 12600 | 560 |

| | | | | | | |
|---|---|---|---|---|---|---|
| M.81.02.16-bDentine2 | 4950 | 170 | 879 | 42 | 10450 | 370 |
| M.2010.02.15-bEnamel1 | 12500 | 630 | 1307 | 90 | 2350 | 160 |
| M.2010.02.15-bEnamel2 | 12120 | 480 | 1168 | 41 | 2320 | 150 |
| M.2010.02.15-bDentine1 | 7260 | 160 | 1326 | 23 | 4.69E+04 | 1.40E+03 |
| M.2010.02.15-bDentine2 | 7140 | 220 | 1312 | 40 | 8.29E+04 | 6.80E+03 |
| M.74.06.06-aEnamel1 | 5.85E+04 | 2.70E+03 | 1220 | 130 | 1.21E+05 | 6.50E+03 |
| M.74.06.06-aEnamel2 | 5.53E+04 | 2.40E+03 | 1084 | 52 | 1.12E+05 | 5.60E+03 |
| M.74.06.06-aDentine1 | 4.46E+04 | 1.80E+03 | 1174 | 45 | 8.76E+04 | 4.80E+03 |
| M.74.06.06-aDentine2 | 4.42E+04 | 2.00E+03 | 1148 | 36 | 9.40E+04 | 6.90E+03 |
| M.78.01.07-bEnamel1 | 12780 | 610 | 708 | 47 | 2400 | 140 |
| M.78.01.07-bEnamel2 | 12480 | 340 | 673 | 25 | 1952 | 69 |
| M.78.01.07-bDentine1 | 9620 | 290 | 1381 | 30 | 8860 | 190 |
| M.78.01.07-bDentine2 | 8760 | 260 | 1431 | 23 | 8990 | 220 |
| M.06.01.05-bulk1 | 5870 | 270 | 1443 | 31 | 5840 | 140 |
| M.06.01.05-bulk2 | 6150 | 180 | 1476 | 54 | 5800 | 130 |

| Specimen Name | Mn (ppm) | Mn Int2SE | Fe (ppm) | Fe Int2SE | Sr (ppm) | Sr Int2SE | Total REE (ppm) |
|---|---|---|---|---|---|---|---|
| M.80.29.14-bEnamel1 | 563 | 69 | 1530 | 120 | 1117 | 61 | 4.251 |
| M.80.29.14-bEnamel2 | 2050 | 640 | 1990 | 130 | 1060 | 34 | 75.128 |
| M.80.29.14-bDentine1 | 2220 | 120 | 4140 | 140 | 2213 | 41 | 197.532 |
| M.80.29.14-bDentine2 | 2037 | 70 | 4020 | 110 | 2222 | 47 | 193.611 |
| M.82.00.17-bEnamel1 | 1718 | 73 | 2430 | 100 | 1521 | 50 | 1531.49 |
| M.82.00.17-bEnamel2 | 1797 | 86 | 2460 | 100 | 1552 | 61 | 1485.87 |
| M.82.00.17-bDentine1 | 4632 | 84 | 8700 | 520 | 2781 | 94 | 4657.75 |
| M.82.00.17-bDentine2 | 4787 | 70 | 10560 | 420 | 3580 | 380 | 4365.96 |
| M.07.03.23-bEnamel1 | 3390 | 220 | 4150 | 340 | 1350 | 180 | 4998.7 |
| M.07.03.23-bEnamel2 | 2700 | 150 | 5.70E+03 | 1.20E+03 | 1119 | 39 | 5564.2 |
| M.07.03.23-bDentine1 | 4050 | 110 | 4900 | 150 | 3180 | 330 | 2364.07 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| M.07.03.23-bDentine2 | 4109 | 80 | 4860 | 130 | 2460 | 110 | 1744.97 |
| M.83.06.18-bEnamel1 | 2810 | 120 | 6160 | 400 | 1092 | 37 | 4426.9 |
| M.83.06.18-bEnamel2 | 3440 | 210 | 1.43E+04 | 1.30E+03 | 1730 | 110 | 7108.7 |
| M.83.06.18-bDentine1 | 5140 | 65 | 8010 | 180 | 2722 | 94 | 7392.4 |
| M.83.06.18-bDentine2 | 5120 | 78 | 8110 | 170 | 2590 | 100 | 7561.85 |
| M.01.02.04-bEnamel1 | 645 | 65 | 8740 | 660 | 1161 | 78 | 38.36 |
| M.01.02.04-bEnamel2 | 766 | 95 | 2.12E+04 | 3.10E+03 | 1270 | 210 | 122.93 |
| M.01.02.04-bDentine1 | 1395 | 22 | 3402 | 77 | 1960 | 27 | 869.59 |
| M.01.02.04-bDentine2 | 1434 | 25 | 3512 | 50 | 1949 | 30 | 945.71 |
| M.99.03.XX-bEnamel1 | 962 | 42 | 3220 | 320 | 1433 | 94 | 3357.48 |
| M.99.03.XX-bEnamel2 | 1014 | 91 | 3410 | 180 | 1477 | 52 | 3390.22 |
| M.99.03.XX-bDentine1 | 3114 | 63 | 6160 | 380 | 2530 | 130 | 1830.32 |
| M.99.03.XX-bDentine2 | 2247 | 53 | 14370 | 850 | 3540 | 280 | 5542.07 |
| M.99.03.XX-bDentine3 | 2295 | 55 | 2.01E+04 | 2.00E+03 | 2760 | 180 | 5570.82 |
| M.81.02.16-bEnamel1 | 1921 | 80 | 6.90E+03 | 1.10E+03 | 2319 | 91 | 2635.2 |
| M.81.02.16-bEnamel2 | 1510 | 100 | 3580 | 610 | 2280 | 140 | 1560.3 |
| M.81.02.16-bDentine1 | 1399 | 53 | 5830 | 480 | 2590 | 100 | 105.988 |
| M.81.02.16-bDentine2 | 1526 | 57 | 3830 | 260 | 2424 | 83 | 100.786 |
| M.2010.02.15-bEnamel1 | 1326 | 77 | 1700 | 140 | 1573 | 79 | 323.09 |
| M.2010.02.15-bEnamel2 | 1261 | 53 | 1380 | 66 | 1617 | 88 | 261.23 |
| M.2010.02.15-bDentine1 | 2903 | 40 | 4.91E+04 | 1.60E+03 | 2321 | 38 | 51.529 |
| M.2010.02.15-bDentine2 | 2940 | 80 | 8.99E+04 | 7.70E+03 | 2294 | 40 | 22.198 |
| M.74.06.06-aEnamel1 | 6980 | 270 | 6.28E+04 | 2.30E+03 | 20210 | 720 | 30659.1 |
| M.74.06.06-aEnamel2 | 7200 | 320 | 6.27E+04 | 2.40E+03 | 20610 | 910 | 31162.9 |
| M.74.06.06-aDentine1 | 6850 | 140 | 4.43E+04 | 1.10E+03 | 14370 | 360 | 24670.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| M.74.06.06-aDentine2 | 6910 | 160 | 4.70E+04 | 1.20E+03 | 15340 | 440 | 25746.8 |
| M.78.01.07-bEnamel1 | 325 | 23 | 1388 | 56 | 1395 | 78 | 163.99 |
| M.78.01.07-bEnamel2 | 284.9 | 6.4 | 1341 | 36 | 1312 | 33 | 50.078 |
| M.78.01.07-bDentine1 | 961 | 21 | 3880 | 110 | 2343 | 64 | 19.412 |
| M.78.01.07-bDentine2 | 947 | 18 | 3842 | 76 | 2560 | 66 | 41.783 |
| M.06.01.05-bulk1 | 2870 | 240 | 3960 | 160 | 2794 | 67 | 4654.85 |
| M.06.01.05-bulk2 | 4370 | 650 | 3890 | 140 | 2929 | 69 | 4191.78 |

### 1.1.2 PLESIOSAURS

Table 2: Bulk element concentrations and total REEs concentrations for plesiosaur specimens measured through LA-ICP-MS.

| Specimen Name | Na (ppm) | Na Int2SE | Mg (ppm) | Mg Int2SE | S (ppm) | S Int2SE |
|---|---|---|---|---|---|---|
| P.04.01.15-bEnamel1 | 10950 | 520 | 476 | 20 | 639 | 36 |
| P.04.01.15-bEnamel2 | 11900 | 460 | 529 | 20 | 678 | 41 |
| P.04.01.15-bDentine1 | 6820 | 170 | 1045 | 27 | 3489 | 81 |
| P.04.01.15-bDentine2 | 6820 | 120 | 1037 | 26 | 3453 | 76 |
| P.81.01.16-bEnamel1 | 12100 | 380 | 698 | 28 | 840 | 65 |
| P.81.01.16-bEnamel2 | 12220 | 440 | 726 | 26 | 795 | 63 |
| P.81.01.16-bDentine1 | 6030 | 200 | 1303 | 40 | 4990 | 150 |
| P.81.01.16-bDentine2 | 5970 | 200 | 1267 | 47 | 4520 | 160 |
| P.06.03.15-bulk1 | 7510 | 260 | 1103 | 29 | 8860 | 240 |
| P.06.03.15-bulk2 | 7730 | 330 | 1162 | 66 | 9570 | 360 |

| Specimen Name | Mn (ppm) | Mn Int2SE | Fe (ppm) | Fe Int2SE | Sr (ppm) | Sr Int2SE | Total REE (ppm) |
|---|---|---|---|---|---|---|---|
| P.04.01.15-bEnamel1 | 337 | 15 | 1261 | 47 | 1356 | 49 | 1.828 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P.04.01.15-bEnamel2 | 381 | 13 | 1360 | 45 | 1387 | 51 | 0.2632 |
| P.04.01.15-bDentine1 | 981 | 25 | 3305 | 68 | 2378 | 48 | 25.431 |
| P.04.01.15-bDentine2 | 999 | 19 | 3256 | 61 | 2492 | 40 | 8.242 |
| P.81.01.16-bEnamel1 | 365 | 13 | 1350 | 62 | 1573 | 60 | 0.17 |
| P.81.01.16-bEnamel2 | 361 | 16 | 1314 | 50 | 1566 | 47 | 0.384 |
| P.81.01.16-bDentine1 | 1003 | 29 | 3310 | 120 | 2334 | 49 | 0.167 |
| P.81.01.16-bDentine2 | 981 | 31 | 3339 | 94 | 2328 | 70 | 0.2183 |
| P.06.03.15-bulk1 | 1321 | 75 | 3940 | 130 | 2866 | 58 | 286.56 |
| P.06.03.15-bulk2 | 2340 | 210 | 4520 | 320 | 2816 | 56 | 306.83 |

### 1.1.3 FISH

Table 3: Bulk element concentrations and total REEs concentrations for fish specimens measured through LA ICP-MS

| Specimen Name | Na (ppm) | Na Int2SE | Mg (ppm) | Mg Int2SE | S (ppm) | S Int2SE |
|---|---|---|---|---|---|---|
| F.06.01.15-bEnamel1 | 5420 | 460 | 1750 | 200 | 10650 | 740 |
| F.06.01.15-bEnamel2 | 6400 | 350 | 1021 | 95 | 8350 | 570 |
| F.06.01.15-bDentine1 | 6460 | 120 | 1086 | 25 | 5380 | 290 |
| F.06.01.15-bDentine2 | 4.10E+03 | 1.20E+03 | 770 | 220 | 3.70E+03 | 1.20E+03 |
| F.81.08.16-bEnamel1 | 11200 | 560 | 1362 | 55 | 15340 | 780 |
| F.81.08.16-bEnamel2 | 12140 | 780 | 1330 | 59 | 14140 | 860 |
| F.81.08.16-bDentine1 | 8330 | 180 | 1132 | 39 | 12920 | 370 |
| F.81.08.16-bDentine2 | 9040 | 220 | 1133 | 26 | 13910 | 550 |
| F.06.01.23-aEnamel1 | 6150 | 200 | 1327 | 45 | 8620 | 610 |
| F.06.01.23-aEnamel2 | 6050 | 220 | 1276 | 57 | 6590 | 490 |

| | | | | | | |
|---|---|---|---|---|---|---|
| F.06.01.23-aDentine1 | 6050 | 140 | 1162 | 19 | 11820 | 410 |
| F.06.01.23-aDentine2 | 5870 | 150 | 1166 | 30 | 9910 | 290 |
| F.78.01.07-aEnamel1 | 7890 | 480 | 1506 | 74 | 9790 | 580 |
| F.78.01.07-aEnamel2 | 8880 | 510 | 1520 | 110 | 10360 | 590 |
| F.78.01.07-aDentine1 | 8350 | 210 | 1237 | 32 | 9630 | 230 |
| F.78.01.07-aDentine2 | 8450 | 240 | 1189 | 28 | 9310 | 250 |
| F.08.01.13-aEnamel1 | 8520 | 800 | 5000 | 940 | 2.08E+04 | 4.30E+03 |
| F.08.01.13-aEnamel2 | 6240 | 310 | 721 | 35 | 9060 | 470 |
| F.08.01.13-aDentine1 | 5880 | 170 | 697 | 24 | 8710 | 150 |
| F.08.01.13-aDentine2 | 5850 | 170 | 704 | 27 | 9030 | 370 |
| F.06.02.15-bEnamel1 | 7050 | 360 | 1213 | 88 | 8390 | 340 |
| F.06.02.15-bEnamel2 | 7780 | 390 | 1026 | 50 | 8430 | 540 |
| F.06.02.15-bDentine1 | 6400 | 220 | 911 | 22 | 6610 | 550 |
| F.06.02.15-bDentine2 | 6500 | 170 | 923 | 40 | 6730 | 260 |
| F.06.02.15-bDentine3 | 6880 | 190 | 1029 | 27 | 4550 | 100 |
| F.06.02.15-bDentine4 | 6830 | 150 | 1015 | 22 | 4300 | 110 |

| Specimen Name | Mn (ppm) | Mn Int2SE | Fe (ppm) | Fe Int2SE | Sr (ppm) | Sr Int2SE | Total REE (ppm) |
|---|---|---|---|---|---|---|---|
| F.06.01.15-bEnamel1 | 2570 | 130 | 7670 | 590 | 2950 | 300 | 5139.3 |
| F.06.01.15-bEnamel2 | 1714 | 93 | 5000 | 300 | 2290 | 140 | 186.71 |
| F.06.01.15-bDentine1 | 949 | 23 | 4590 | 190 | 3056 | 68 | 2.476 |
| F.06.01.15-bDentine2 | 680 | 200 | 3.30E+03 | 1.00E+03 | 2110 | 600 | 2.939 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| F.81.08.16-bEnamel1 | 7410 | 310 | 4490 | 580 | 3270 | 130 | 17766.6 |
| F.81.08.16-bEnamel2 | 7050 | 210 | 3890 | 390 | 3260 | 100 | 18278.7 |
| F.81.08.16-bDentine1 | 3040 | 290 | 6080 | 300 | 2924 | 63 | 9.403 |
| F.81.08.16-bDentine2 | 4099 | 94 | 6610 | 450 | 2977 | 48 | 380.25 |
| F.06.01.23-aEnamel1 | 1275 | 50 | 8150 | 700 | 3110 | 130 | 229.76 |
| F.06.01.23-aEnamel2 | 1306 | 40 | 6180 | 540 | 3008 | 83 | 373.17 |
| F.06.01.23-aDentine1 | 1115 | 19 | 13650 | 590 | 2974 | 50 | 4.425 |
| F.06.01.23-aDentine2 | 1079 | 17 | 11180 | 430 | 2978 | 36 | 5.142 |
| F.78.01.07-aEnamel1 | 1470 | 230 | 4040 | 290 | 3070 | 110 | 1168.7 |
| F.78.01.07-aEnamel2 | 1410 | 130 | 3710 | 140 | 3000 | 120 | 728.77 |
| F.78.01.07-aDentine1 | 1092 | 30 | 3930 | 140 | 2967 | 59 | 7.591 |
| F.78.01.07-aDentine2 | 1064 | 34 | 4090 | 260 | 2988 | 71 | 9.664 |
| F.08.01.13-aEnamel1 | 2460 | 330 | 1.42E+04 | 2.20E+03 | 5980 | 990 | 4342 |
| F.08.01.13-aEnamel2 | 1441 | 44 | 3860 | 150 | 2330 | 100 | 3711.33 |
| F.08.01.13-aDentine1 | 1605 | 24 | 3548 | 56 | 2356 | 41 | 3285.39 |
| F.08.01.13-aDentine2 | 1533 | 27 | 3540 | 130 | 2383 | 91 | 3457.44 |
| F.06.02.15-bEnamel1 | 5840 | 840 | 4210 | 390 | 2480 | 120 | 2993.9 |
| F.06.02.15-bEnamel2 | 4400 | 380 | 3680 | 170 | 2520 | 120 | 2566 |
| F.06.02.15-bDentine1 | 953 | 19 | 5910 | 600 | 2836 | 96 | 60.254 |
| F.06.02.15-bDentine2 | 979 | 39 | 5640 | 640 | 3000 | 110 | 176.2 |
| F.06.02.15-bDentine3 | 1557 | 64 | 2997 | 77 | 2929 | 66 | 841.11 |
| F.06.02.15-bDentine4 | 1747 | 33 | 2864 | 69 | 2831 | 51 | 907.83 |

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### 1.1.4 BIRDS

Table 4: Bulk element concentrations and total REEs concentrations for bird specimens measured through LA-ICP-MS.

| Specimen Name | Na (ppm) | Na Int2SE | Mg (ppm) | Mg Int2SE | S (ppm) | S Int2SE |
|---|---|---|---|---|---|---|
| B.07.02.15-bulk1 | 6860 | 230 | 653 | 25 | 8660 | 190 |
| B.07.02.15-bulk2 | 6030 | 120 | 573.7 | 6.9 | 7170 | 180 |
| B.03.03.05-bulk1 | 8530 | 200 | 1279 | 23 | 9920 | 300 |
| B.03.03.05-bulk2 | 7860 | 140 | 1282 | 27 | 8970 | 150 |
| B.78.01.07-bulk1 | 7760 | 140 | 1227 | 31 | 4878 | 93 |
| B.78.01.07-bulk2 | 7580 | 170 | 1241 | 30 | 5230 | 110 |
| B.83.01.18-bulk1 | 12670 | 300 | 1012 | 42 | 4.60E+04 | 1.20E+03 |
| B.83.01.18-bulk2 | 11340 | 380 | 1029 | 27 | 35050 | 830 |
| B.06.02.15-bulk1 | 7350 | 110 | 1384 | 24 | 6810 | 160 |
| B.06.02.15-bulk2 | 6900 | 140 | 1248 | 21 | 5190 | 99 |

| Specimen Name | Mn (ppm) | Mn Int2SE | Fe (ppm) | Fe Int2SE | Sr (ppm) | Sr Int2SE | Total REE (ppm) |
|---|---|---|---|---|---|---|---|
| B.07.02.15-bulk1 | 2058 | 60 | 4370 | 130 | 1957 | 31 | 10173.88 |
| B.07.02.15-bulk2 | 2008 | 33 | 3054 | 72 | 2193 | 63 | 9752.69 |
| B.03.03.05-bulk1 | 4530 | 130 | 4000 | 220 | 2226 | 72 | 1919.5 |
| B.03.03.05-bulk2 | 3861 | 57 | 3330 | 130 | 2237 | 44 | 811.92 |
| B.78.01.07-bulk1 | 897 | 17 | 3159 | 81 | 2391 | 49 | 9.525 |
| B.78.01.07-bulk2 | 859 | 16 | 3067 | 51 | 2472 | 49 | 11.519 |
| B.83.01.18-bulk1 | 6360 | 140 | 3.50E+04 | 4.90E+03 | 4420 | 290 | 12851.9 |
| B.83.01.18-bulk2 | 5840 | 150 | 17620 | 780 | 3590 | 150 | 11776.2 |
| B.06.02.15-bulk1 | 985 | 13 | 3660 | 150 | 2485 | 37 | 10.315 |
| B.06.02.15-bulk2 | 826 | 14 | 2942 | 40 | 2456 | 27 | 11.322 |

## 1.2.0 PRINCIPAL COMPONENTS

### 1.2.1 MOSASAURS

Table 5: The Eigenvalues and variance for each principal components of the mosasaur specimens.

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 1.55E+09 | 91.603 |
| 2 | 1.03E+08 | 6.0998 |
| 3 | 2.98E+07 | 1.7663 |

Table 6: The loading scores of each bulk element and total REEs for each principal component in the mosasaur specimens.

| Element | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| Na (ppm) | 0.27675 | 0.644 | 0.40869 |
| Mg (ppm) | -6.31E-05 | -0.0043 | -0.02715 |
| S (ppm) | 0.8112 | 0.037394 | -0.58142 |
| Mn (ppm) | 0.032841 | 0.044465 | 0.10247 |
| Fe (ppm) | 0.4736 | -0.62643 | 0.6161 |
| Sr (ppm) | 0.10645 | 0.19636 | 0.11794 |
| Total REE | 0.16928 | 0.38846 | 0.30033 |

Table 7: The Principal Component scores for each mosasaur specimens (enamel and dentine averaged separately).

| Specimen | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| M.80.29.14 Enamel - Avg | -0.60053 | 0.33486 | 0.46893 |
| M.80.29.14 Dentine - Avg | -0.49782 | -0.08252 | -0.15215 |
| M.82.00.17 Enamel - Avg | -0.54119 | 0.3742 | 0.43679 |
| M.82.00.17 Dentine - Avg | -0.27359 | 0.027213 | 0.5083 |
| M.07.03.23 Enamel - Avg | -0.50619 | 0.010864 | 0.3672 |
| M.07.03.23 Dentine - Avg | -0.40605 | -0.06429 | -0.34103 |
| M.83.06.18 Enamel - Avg | -0.23646 | 0.21908 | 0.8036 |
| M.83.06.18 Dentine - Avg | -0.27196 | 0.061873 | 0.14148 |
| M.01.02.04 Enamel - Avg | 0.64792 | -0.38973 | -3.8598 |
| M.01.02.04 Dentine - Avg | -0.52016 | -0.15817 | -0.34918 |
| M.99.03.XX Enamel - Avg | -0.4712 | 0.56348 | 0.64832 |
| M.99.03.XX Dentine - Avg | -0.16216 | -0.13635 | 0.69823 |
| M.81.02.16 Enamel - Avg | -0.42803 | -0.20507 | -0.40386 |
| M.81.02.16 Dentine - Avg | -0.38721 | -0.24221 | -0.83528 |
| M.2010.02.15 Enamel - Avg | -0.56617 | 0.38503 | 0.31118 |
| M.2010.02.15 Dentine - Avg | 1.5092 | -3.893 | 0.96639 |
| M.74.06.06 Enamel - Avg | 3.0296 | 1.4189 | 0.58782 |
| M.74.06.06 Dentine - Avg | 2.1637 | 1.2595 | 0.032386 |
| M.78.01.07 Enamel - Avg | -0.57159 | 0.39972 | 0.30103 |
| M.78.01.07 Dentine - Avg | -0.42332 | 0.073023 | -0.36523 |
| M.06.01.05 Enamel - Avg | -0.48674 | 0.043567 | 0.034818 |

### 1.2.2 PLESIOSAUR

Table 8: The Eigenvalues and variance for each principal components of the plesiosaur specimens.

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 1.96E+07 | 86.204 |
| 2 | 3.07E+06 | 13.531 |
| 3 | 45446.8 | 0.20026 |
| 4 | 14707.6 | 0.064809 |

Table 9: The loading scores of each bulk element and total REEs for each principal component in the plesiosaur specimens.

| Element | PC 1 | PC 2 |
|---|---|---|
| Na (ppm) | -0.54702 | 0.80249 |
| Mg (ppm) | 0.064095 | -0.05739 |
| S (ppm) | 0.75752 | 0.58393 |
| Mn (ppm) | 0.1314 | 0.086999 |
| Fe (ppm) | 0.29422 | -0.03809 |
| Sr (ppm) | 0.13623 | -0.00719 |
| Total REE | 0.020838 | 0.051612 |

Table 10: The Principal Component scores for each plesiosaur specimens (enamel and dentine averaged separately).

| Specimen | PC 1 | PC 2 |
|---|---|---|
| P.04.01.15 Enamel - Avg | -0.99692 | 0.1768 |
| P.04.01.15 Dentine - Avg | 0.24472 | -1.0279 |
| P.81.01.16 Enamel - Avg | -1.0499 | 0.55841 |
| P.81.01.16 Dentine - Avg | 0.56929 | -0.98447 |
| P.06.03.15 Dentine - Avg | 1.2328 | 1.2772 |

### 1.2.3 FISH

Table 11: The Eigenvalues and variance for each principal components of the fish specimens.

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 3.45E+07 | 67.642 |
| 2 | 1.11E+07 | 21.794 |
| 3 | 3.50E+06 | 6.8706 |
| 4 | 1.12E+06 | 2.2045 |
| 5 | 545326 | 1.0702 |

| | | |
|---|---|---|
| 6 | 204496 | 0.40133 |
| 7 | 9112.47 | 0.017883 |

Table 12: The loading scores of each bulk element and total REEs for each principal component in the fish specimens.

| Element | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 |
|---|---|---|---|---|---|
| Na (ppm) | 0.24888 | -0.0075 | 0.43048 | 0.13803 | 0.8444 |
| Mg (ppm) | 0.023633 | 0.094927 | 0.032068 | -0.1732 | -0.04057 |
| S (ppm) | 0.39396 | 0.62743 | 0.51825 | -0.20601 | -0.34353 |
| Mn (ppm) | 0.28581 | -0.07958 | 0.1122 | 0.88132 | -0.26321 |
| Fe (ppm) | -0.05521 | 0.73057 | -0.5733 | 0.247 | 0.26536 |
| Sr (ppm) | 0.028026 | 0.084298 | 0.022004 | -0.15047 | 0.16567 |
| Total REE | 0.83473 | -0.22383 | -0.45092 | -0.2194 | 0.013627 |

Table 13: The Principal Component scores for each fish specimens (enamel and dentine averaged separately).

| Specimen | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 |
|---|---|---|---|---|---|
| F.06.01.15 Enamel - Avg | -0.11014 | 0.071031 | -0.58831 | -0.13772 | -1.2331 |
| F.06.01.15 Dentine - Avg | -0.89209 | -1.189 | -0.82152 | -0.28582 | -0.06398 |
| F.81.08.16 Enamel - Avg | 2.9403 | -0.56358 | -0.54413 | 0.060265 | 0.76156 |
| F.81.08.16 Dentine - Avg | -0.01076 | 0.93694 | 1.8114 | 1.1574 | -0.34397 |
| F.06.01.23 Enamel - Avg | -0.61251 | 0.08344 | -0.802 | 0.18098 | 0.527 |
| F.06.01.23 Dentine - Avg | -0.50161 | 1.867 | -1.4833 | 0.68734 | 0.78931 |
| F.78.01.07 Enamel - Avg | -0.21891 | -0.21955 | 1.2722 | -0.80909 | 0.75018 |
| F.78.01.07 Dentine - Avg | -0.41292 | -0.24211 | 1.2658 | -0.7073 | 1.2126 |
| F.08.01.13 Enamel - Avg | 0.48904 | 1.6747 | 0.13135 | -1.2742 | -0.75308 |
| F.08.01.13 Dentine - Avg | -0.0592 | -0.71911 | -0.13776 | -1.1472 | -1.7956 |
| F.06.02.15 Enamel - Avg | 0.062961 | -0.75113 | 0.33276 | 2.2258 | -0.93554 |
| F.06.02.15 Dentine - Avg | -0.6742 | -0.94876 | -0.43652 | 0.049486 | 1.0847 |

1.2.4 BIRDS

Table 14: The Eigenvalues and variance for each principal components of the bird specimens.

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 3.59E+08 | 95.261 |
| 2 | 1.58E+07 | 4.1816 |
| 3 | 2.02E+06 | 0.53739 |
| 4 | 73633.9 | 0.019548 |

Table 15: The loading scores of each bulk element and total REEs for each principal component in the bird specimens

| Element | PC 1 | PC 2 |
|---|---|---|
| Na (ppm) | 0.10829 | -0.16869 |
| Mg (ppm) | -0.0032 | -0.07135 |
| S (ppm) | 0.79327 | -0.15553 |
| Mn (ppm) | 0.10244 | -0.01602 |
| Fe (ppm) | 0.53764 | -0.15847 |
| Sr (ppm) | 0.03902 | -0.04875 |
| Total REE | 0.24067 | 0.9563 |

Table 16: The Principal Component scores for each bird specimen.

| Specimen | PC 1 | PC 2 |
|---|---|---|
| B.07.02.15 Cortical Bone - Avg | -0.31765 | 1.7596 |
| B.03.03.05 Cortical Bone - Avg | -0.34224 | -0.46717 |
| B.78.01.07 Cortical Bone - Avg | -0.57952 | -0.5655 |
| B.83.01.18 Cortical Bone - Avg | 1.7769 | -0.13799 |
| B.06.02.15 Cortical Bone - Avg | -0.53747 | -0.58891 |

1.3.0 WILCOXON T-TEST

Table 17: Wilcoxon signed rank paired t-test to compare the bulk element concentrations in mosasaur dentine versus enamel.

| **Measure 1** | | **Measure 2** | **W** | **z** | **df** | **p** |
|---|---|---|---|---|---|---|
| Na-D | - | Na-E | 1.000 | -2.701 | | 0.004 |
| Mg-D | - | Mg-E | 13.000 | -1.478 | | 0.160 |
| S-D | - | S-E | 38.000 | 1.070 | | 0.322 |
| Mn-D | - | Mn-E | 52.000 | 2.497 | | 0.010 |
| Fe-D | - | Fe-E | 32.000 | 0.459 | | 0.695 |
| Sr-D | - | Sr-E | 45.000 | 1.784 | | 0.084 |
| REE-D | - | REE-E | 25.000 | -0.255 | | 0.846 |

| Measure 1 | Measure 2 | W | z | df | p |
|---|---|---|---|---|---|

*Note.* Wilcoxon signed-rank test.

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Table 18: Wilcoxon signed rank paired t-test to compare the bulk element concentrations in plesiosaur dentine versus enamel.

| Measure 1 | Measure 2 | W | z | df | p |
|---|---|---|---|---|---|
| Na-D | - Na-E | 0.000 | -1.342 | | 0.500 |
| Mg-D | - | 3.000 | 1.342 | | 0.500 |
| S-D | - Mg-E | 3.000 | 1.342 | | 0.500 |
| Mn-D | - Mn-E | 3.000 | 1.342 | | 0.500 |
| Fe-D | - Fe-E | 3.000 | 1.342 | | 0.500 |
| Sr-D | - Sr-E | 3.000 | 1.342 | | 0.500 |
| REE-D | - REE-E | 2.000 | 0.447 | | 1.000 |

*Note.* Wilcoxon signed-rank test.

Table 19: Wilcoxon signed rank paired t-test to compare the bulk element concentrations in fish dentine versus enamel.

| Measure 1 | Measure 2 | W | z | df | p |
|---|---|---|---|---|---|
| Na-D | - Na-E | 1.000 | -1.992 | | 0.063 |
| Mg-D | - Mg-E | 0.000 | -2.201 | | 0.031 |
| S-D | - S-E | 4.000 | -1.363 | | 0.219 |
| Mn-D | - Mn-E | 0.000 | -2.201 | | 0.031 |
| Fe-D | - Fe-E | 11.000 | 0.105 | | 1.000 |
| Sr-D | - Sr-E | 5.000 | -1.153 | | 0.313 |
| REE-D | - REE-E | 0.000 | -2.201 | | 0.031 |

*Note.* Wilcoxon signed-rank test.

## 1.4.0 ONE WAY ANOVA ON $\delta^{13}C$

Table 20: A one way ANOVA of the $\delta^{13}C$ data to determine any difference between animal groups.

**ANOVA -** $\delta^{13}C$

| Cases | Sum of Squares | df | Mean Square | F | p | $\eta^2$ | $\eta^2_p$ |
|---|---|---|---|---|---|---|---|
| Animal | 71.725 | 3 | 23.908 | 7.300 | 0.001 | 0.499 | 0.499 |
| Residuals | 72.048 | 22 | 3.275 | | | | |

*Note.* Type III Sum of Squares

Table 21: The post-hoc comparison of the $\delta^{13}C$ data which illustrates which animal groups are statistically different from each other.

**Post Hoc Comparisons - Animal**

| | | | 95% CI for Mean Difference | | | | |
|---|---|---|---|---|---|---|---|
| | | Mean Difference | Lower | Upper | SE | t | $p_{tukey}$ |
| Bird | Fish | -4.549 | -7.450 | -1.648 | 1.045 | -4.354 | 0.001** |
| | Mosasaur | -1.104 | -3.654 | 1.446 | 0.918 | -1.202 | 0.632 |
| | Plesiosaur | -0.895 | -4.448 | 2.659 | 1.280 | -0.699 | 0.896 |
| Fish | Mosasaur | 3.445 | 0.894 | 5.995 | 0.918 | 3.751 | 0.006** |
| | Plesiosaur | 3.654 | 0.101 | 7.208 | 1.280 | 2.856 | 0.042* |
| Mosasaur | Plesiosaur | 0.209 | -3.064 | 3.482 | 1.179 | 0.178 | 0.998 |

* p < .05, ** p < .01

Table 22: A one way ANOVA of the $\delta^{13}C$ data to determine any difference between the Manitoba plesiosaurs and other studies.

**ANOVA - $\delta^{13}C$ (VPDB)**

| Homogeneity Correction | Cases | Sum of Squares | df | Mean Square | F | p | $\eta^2$ | $\eta^2_p$ |
|---|---|---|---|---|---|---|---|---|
| None | Place | 156.733 | 4.000 | 39.183 | 2.775 | 0.036 | 0.173 | 0.173 |
| | Residuals | 748.415 | 53.000 | 14.121 | | | | |
| Welch | Place | 156.733 | 4.000 | 39.183 | 4.992 | 0.011 | 0.173 | 0.173 |
| | Residuals | 748.415 | 13.595 | 55.052 | | | | |

*Note.* Type III Sum of Squares

Table 23: The post-hoc comparison of the $\delta^{13}C$ data which illustrates which localities are statistically different from each other.

**Games-Howell Post Hoc Comparisons - Place**

| | | 95% CI for Mean Difference | | | | | |
|---|---|---|---|---|---|---|---|
| Comparison | Mean Difference | Lower | Upper | SE | t | df | $p_{tukey}$ |
| Ant - Arg | -1.375 | -12.615 | 9.865 | 2.400 | -0.573 | 3.638 | 0.972 |
| Ant - Dmk | -2.811 | -8.786 | 3.165 | 1.851 | -1.519 | 11.103 | 0.572 |
| Ant - Mb | -3.661 | -6.661 | -0.662 | 1.032 | -3.547 | 29.156 | 0.011* |
| Ant - Nth | 0.905 | -2.269 | 4.079 | 1.040 | 0.870 | 16.511 | 0.904 |
| Arg - Dmk | -1.436 | -11.859 | 8.988 | 2.850 | -0.504 | 6.493 | 0.984 |
| Arg - Mb | -2.286 | -13.537 | 8.965 | 2.400 | -0.952 | 3.632 | 0.863 |
| Arg - Nth | 2.280 | -8.973 | 13.533 | 2.403 | 0.949 | 3.639 | 0.864 |
| Dmk - Mb | -0.851 | -6.844 | 5.142 | 1.851 | -0.460 | 10.926 | 0.990 |
| Dmk - Nth | 3.716 | -2.313 | 9.744 | 1.855 | 2.003 | 10.709 | 0.327 |
| Mb - Nth | 4.566 | 1.297 | 7.835 | 1.041 | 4.388 | 13.202 | 0.005** |

* p < .05, ** p < .01

Table 24: A one way ANOVA of the $\delta^{13}C$ data to determine any difference between the Manitoba plesiosaurs and those from Antarctica.

**ANOVA - $\delta^{13}C$ (VPDB)**

| Cases | Sum of Squares | df | Mean Square | F | p | $\eta^2$ | $\eta^2_p$ |
|---|---|---|---|---|---|---|---|
| Place | 40.258 | 1 | 40.258 | 5.211 | 0.043 | 0.321 | 0.321 |
| Residuals | 84.985 | 11 | 7.726 | | | | |

*Note.* Type III Sum of Squares