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## Giant Beaver (*Castoroides*) Palaeoecology Inferred from Stable Isotopes

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Graduate Program in Geology  
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## Abstract

Stable isotope analysis was used to explore unresolved questions about the palaeoecology of the extinct Pleistocene giant beaver (*Castoroides*). The  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  of bone collagen and structural carbonate from enamel served as proxies for palaeodiet and palaeoclimate. A new baseline for freshwater and terrestrial plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was utilized in a mixing model (SIAR) to assess rodent feeding niche. SIAR analysis indicated that *Castoroides*' consumed a diet of predominantly macrophytes, making them reliant on wetland habitat for food. Based on isotopic data for potential modern analog species (*Castor* and *Ondatra*), SIAR also indicated that *Castor* and *Castoroides* occupied complementary dietary niches, allowing them to share habitat space throughout the Pleistocene. The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of *Castoroides*' ever-growing incisors also recorded seasonal fluctuations in diet and climate. Wetland loss due to climate change during the Last Glacial Maximum, and increased competition for habitat, probably factored into *Castoroides*' extinction.

## Keywords

*Castoroides*, Stable Isotope, Pleistocene, Palaeodiet, Palaeoecology, Megafauna, North America

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## Preface

— *Castoroides* – not another dam beaver!



Artistic depiction of *Castoroides*. Original artwork by Annemarie Plint (2016).

# Chapter 1

## Introduction

### 1.1 Introduction and Overview

Colloquially referred to as the “Ice Age”, the Pleistocene epoch (2.5 million to 11,000 years ago) was characterized by the global advance and retreat of continental ice sheets. During this time, ecosystems on every continent were dominated by megafauna species, and anatomically modern humans first emerged from Africa to begin their colonization of the globe. Despite the climatic instability, the epoch was a time of increased biodiversity and fostered many unique ecosystems for which there are no exact modern analogs.

These unique combinations of flora and fauna disappeared with the onset of the late Pleistocene megafauna extinctions. Within the span of a mere 70,000 years, 120 of 150 genera of existing megafauna went extinct worldwide (Barnosky et al., 2004). The ultimate cause of these extinctions remains highly contested; climate change and anthropogenic influence are currently the leading candidates (Banks and Dickman, 2007; Brook and Bowman, 2002; Cohen et al., 2015; Faith and Surovell, 2009; Grayson and Meltzer, 2002; Guthrie, 2006; Koch and Barnosky, 2006; Martin, 1973; Miller et al., 2005; Pushkina and Raia, 2008; Waters et al., 2011). Among the doomed genera of megafauna was *Castoroides*, a distant, and super-sized relative of the modern beaver.

### 1.2 Thesis Aim

Approximately the size of an American Black Bear (*Ursus Americanus*), *Castoroides* underwent a highly successful radiation across North America during the late Pleistocene. Originating in what is now the southeastern United States, it eventually colonized habitats from Florida to Alaska as climatic regimes oscillated (Cahn, 1932). Based on skeletal morphology, it is presumed to have been a semi-aquatic herbivore, much like modern beavers are today (Richards and Swinehart, 2001). However, little is known about the specifics of its diet, feeding habits, and potential impact on the wetland habitat

it occupied. Unlike other more popular genera of megafauna, comparatively little research has explored the role of *Castoroides* within Pleistocene ecosystems.

### 1.2.1 Research Questions

This thesis explores the palaeoecology of the Ice Age giant beaver (*Castoroides*) through the lens of stable isotope analysis. Stable carbon, nitrogen, and oxygen isotopes are utilized to reconstruct palaeodietary and palaeoclimatic trends that aim to answer the following research questions:

- i.* What was the diet and feeding strategy of *Castoroides*?
- ii.* Under what climatic conditions (range of temperature and humidity) did *Castoroides* thrive?

The current body of literature on *Castoroides* is relatively small. Most of what has been suggested about its diet and ecological niche is based on fossil depositional environment, skeletal anatomy, and the behaviour of living members of the Castoridae family. *Castoroides*' short limbs and bulky body would have made it poorly adapted to a life predominantly spent on land and it must have relied on access to the water for shelter from terrestrial predators (Figure 1.1). It is unknown what it ate and how it collected its food. Currently, there are two competing models of *Castoroides* diet and behaviour.

### 1.2.2 Proposed Modern Analogs

Two extant semi-aquatic rodent species have been proposed as scaled-down, potential analogs for the giant beaver: the muskrat (*Ondatra zibethicus*), and the North American beaver (*Castor canadensis*) (Anderson, 1984; Harington, 2009; Lovegrove and Mowoe, 2013; Miller et al., 2000; Richards and Swinehart, 2001). Both species co-existed with *Castoroides* in North America during the late Pleistocene, and potentially shared many morphological and behavioural traits (Harington, 1990). The biggest difference would have been size. The behaviour and ecological role of both the muskrat and the North American beaver are well documented. Using this information as a



**Figure 1.1 Complete *Castoroides ohioensis* skeleton (cast).**

framework, this thesis develops two models for *Castoroides* based on these species, and assesses them using stable isotope analysis (see Section 1.3).

The first model predicts that *Castoroides* filled an econiche similar to that of muskrat as a semi-aquatic species that primarily consumed submerged and emergent freshwater macrophytes. It would have required calm wetlands with an expansive littoral zone in order to provide sufficient macrophytes on which to graze. If similar to the muskrat, *Castoroides* would have kept shallow waterways clear of excess macrophyte growth and promoted aquatic biotic diversity. The second model predicts that *Castoroides* filled an econiche similar to that of modern beaver, as a semi-aquatic species that consumed *both* terrestrial and aquatic vegetation, and practiced tree-cutting behaviours for the purpose of lodge and dam building. The bulk of its diet would have been foliage from deciduous trees and its engineering habits would have promoted local biotic diversity and significantly impacted the hydrological patterns of the Pleistocene landscape.

### 1.3 Stable Isotopes in Palaeoecology

Stable isotope analysis of biological tissues is employed to assess the applicability of the two proposed ecological models outlined above. Stable isotopes are atoms of the

same element that possess the same number of protons, but a differing number of neutrons within their nucleus. Due to this minor difference in mass, stable isotopes of the same element have the same chemical, but slightly different physical properties. Based on the number of neutrons they possess, stable isotopes of the same element are considered either 'heavy' or 'light', and will react at differing rates (the lighter isotopes tend to react faster). This minor difference in reaction rate means they will become incorporated into biological tissues in differing quantities, typically with a greater abundance of the lighter isotope relative to the heavier isotope. This process is called isotopic fractionation.

The original relative abundances of stable isotopes can be preserved in biological tissues for thousands, if not millions of years under appropriate conditions (Barrick et al., 1996; Lécuyer et al., 2003). The original proportions of heavy and light isotopes in an organism's tissues are governed by the environmental conditions and physiological processes it experienced during its lifetime. Measuring the relative abundance (or ratio) of stable isotopes in the tissue of an extinct organism can provide insight into environmental and physiological processes from the past (i.e. climate, diet).

Stable isotope ratios are calculated as follows:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1]$$

where X represents the heavy isotope of interest, and R is the corresponding ratio of heavy to light isotope in both sample and accepted standard material (Coplen, 2011). All stable isotope results are reported in delta ( $\delta$ ) notation as ratios relative to accepted international standards. Stable carbon isotopic results are reported relative to Vienna PeeDee Belemnite (VPDB), while oxygen isotopic results may be reported relative to VPDB, or Vienna Standard Mean Ocean Water (VSMOW). Nitrogen isotopic results are reported relative to atmospheric nitrogen gas (AIR). The relative abundance of the heavier (or rarer) isotope is expressed in *per mil* (‰). In this study, the biological tissues bone and tooth collagen and bioapatite are used to examine stable carbon, nitrogen, and oxygen isotopes as proxies for palaeodiet and palaeoclimate.



### 1.3.1 Stable Carbon and Nitrogen Isotopes

Stable carbon and nitrogen isotope ratios in bone collagen are commonly used chemical tracers of nutrient flow and trophic position of organisms within a foodweb (Ambrose and DeNiro, 1986; Ambrose and DeNiro, 1989; Casey and Post, 2011; Schoeninger and DeNiro, 1984; Schoeninger 1985). As the popular expression goes, “you are what you eat, plus a few *per mil*” (DeNiro and Epstein, 1978; 1981; van der Merwe and Vogel, 1978). Predictable enrichment factors between a consumer and its diet have enabled palaeoecologists to reconstruct aspects of behaviour in extinct species that would otherwise be indiscernible from skeletal material alone (Bocherens and Drucker, 2003; Coltrain et al., 2004; Fox-Dobbs et al., 2008; Koch et al., 1998; Metcalfe et al., 2010; Schwartz-Narbonne et al., 2015; Yann and DeSantis, 2014).

Stable nitrogen isotopes are most commonly used to track consumption of protein and an animal’s relative trophic position. Due to metabolic processes, the heavier isotope  $^{15}\text{N}$  becomes more concentrated in tissues of animals situated higher up in the food chain (DeNiro and Epstein, 1981). Each consumer exhibits a ~3 to 4‰ enrichment relative to the  $\delta^{15}\text{N}$  of its diet (Bocherens and Drucker, 2003; Koch, 1998; Post, 2002; Schoeninger and DeNiro, 1984). This predictable increase in  $\delta^{15}\text{N}$  can be used to discern the number of trophic levels in a food chain, an organism’s diet (i.e. carnivore, omnivore, or herbivore), and predator-prey relationships (Bocherens, 2015; Codron et al., 2007; Palmqvist et al., 2008).

Stable carbon isotopes can also be used to track an organism’s trophic level (Bocherens and Drucker, 2003). However, they are more commonly used to reconstruct the diet of primary consumers (Hobson and Schwarcz, 1986; Kelly, 2000; Koch, 1998). The  $\delta^{13}\text{C}$  of primary producers is primarily determined by photosynthetic pathway ( $\text{C}_3$ ,  $\text{C}_4$ , CAM). The tissues of a primary consumer experiences a ~2 to 5‰ enrichment relative to the  $\delta^{13}\text{C}$  of its diet (Balasse et al., 1999; Roth and Hobson, 2000; van der Merwe, 1982). A plant’s physiology and growing environment dictate both its photosynthetic pathway and its access to carbon sources (Farquhar et al., 1989; Keeley and Sandquist, 1992; LaZerte and Szalados, 1982). Combined, these factors control the

uptake of  $^{13}\text{C}$  in both terrestrial and aquatic plant and thus overall plant  $\delta^{13}\text{C}$  composition. Plants that use the  $\text{C}_3$  photosynthetic pathway (average  $\delta^{13}\text{C}$  of  $-26\text{‰}$ ) are isotopically distinct from plants that use the  $\text{C}_4$  photosynthetic pathway (average  $\delta^{13}\text{C}$  of  $-12\text{‰}$ ) (Heaton, 1999; O'Leary, 1988; Smith and Epstein, 1971; Tieszen, 1991). In addition, the  $\delta^{13}\text{C}$  of primary producers with access to marine, terrestrial, and freshwater sources of carbon differ in relatively distinct and predictable ways. The stable carbon and nitrogen compositions of freshwater  $\text{C}_3$  plants, and how they differ from terrestrial  $\text{C}_3$  plants are discussed further in Chapter 3.

### 1.3.2 Oxygen Isotopes

Oxygen isotopic ratios of mineralized tissues are commonly used as proxies for palaeoclimate (e.g. regional temperature, humidity, degree of seasonality) (Longinelli, 1984; Stuart-Williams and Schwarcz, 1997). The oxygen isotopic composition of structural carbonate or phosphate from bone or enamel bioapatite can be used to reconstruct the  $\delta^{18}\text{O}$  of local meteoric water at the time of tissue formation, which in turn may be used to infer information about coeval climatic conditions (Bellissimo, 2013; Fricke et al., 1998; Longinelli, 1984; Sharp and Cerling, 1998). The relative abundances of oxygen isotopes ( $^{16}\text{O}$  and  $^{18}\text{O}$ ) in non-marine animal tissue are governed by the isotopic composition of its body water, which is a combination of drinking water and water content from food, both of which are predominantly influenced by meteoric (fresh) water sources. In primary consumers, the  $\delta^{18}\text{O}$  of plant water in their forage is also controlled by temperature and relative humidity (Ehleringer and Dawson, 1992; Epstein et al., 1977). In species that are obligate drinkers (which comprises most terrestrial, non-aridity adapted fauna), the  $\delta^{18}\text{O}$  of their body water is governed primarily by their drinking water, which in turn is supplied by regional precipitation (Bryant and Froelich, 1995; Dansgaard, 1964; Longinelli, 1984; Luz et al., 1984). Bioapatite forms in equilibrium with body water and incorporates its oxygen isotopic composition at the time of tissue formation. After correcting for physiological enrichment factors, this information can be used to determine the oxygen isotopic composition of regional precipitation, which in turn provides information about local environmental parameters such as temperature, humidity, latitude, and air mass source (Dansgaard, 1964). By using

a sequence of bone samples collected from a single region, but from different time periods, it is possible to track climatic variation over time.

#### 1.4 Research Design

The palaeoecology of *Castoroides* is explored here using stable carbon, nitrogen, and oxygen isotopic information. In order to accurately assess trophic position and forage preference of the giant beaver, an isotopic baseline of dietary sources must first be established. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of a wide variety of terrestrial and freshwater plants are determined and evaluated in the present study to create a baseline that best reflects the types of forage suggested in the ecological models proposed in Section 1.2.2. Forage is divided into four different categories: terrestrial trees and shrubs, as well as emergent, floating, and submerged macrophytes. These groupings were chosen to gauge *Castoroides*' level of dependence on freshwater food sources. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Castoroides* bone collagen is also measured, and corrected for enrichment due to metabolic fractionation. The results are then compared to the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the four plant categories to assess the dietary choices of the giant beaver. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Castoroides* bone collagen is also compared to that of the two proposed extant analog species (the beaver and muskrat) to test for isotopic evidence of similar patterns in forage choice. Stable Isotope Analysis in R: An Ecologist's Guide (commonly referred to as SIAR; Inger et al., 2010) produced mixing models that were then used to assess the proportion of each plant type in the diet of each rodent. Note that modern  $\delta^{13}\text{C}$  results are corrected for Suess Effect (Long et al., 2005) to render them comparable to *Castoroides* carbon isotope data.

This study examines skeletal material from three geographically and possibly temporally distinct populations of *Castoroides* in order to assess whether there was regional variability in the composition of their diet, or temporal shifts in their main source of forage during the late Pleistocene. The  $\delta^{18}\text{O}$  of bioapatite structural carbonate from *Castoroides* tooth enamel collected from several sites across North America is used to investigate the range of climatic conditions that were suitable for giant beaver

populations, as well as provide information about late Pleistocene climatic trends in North America.

## 1.5 Research Implications

It is important to determine which proposed ecological model is most applicable in order to determine the impact of *Castoroides* on the Pleistocene landscape. Modern studies demonstrate that muskrat and beaver have very different individual impacts on the ecosystems they occupy. For instance, if *Castoroides* was indeed simply a larger version of *Castor canadensis*, its positive and negative impacts on the hydrological and biotic characteristics of Ice Age North America would have been substantial (Rosell et al., 2005). It is also important to determine the level of dependence that *Castoroides* had on wetland vegetation. Since it is assumed to have preferred freshwater environments such as ponds, lakes, and swamps, the disappearance of the giant beaver raises questions about transitioning environmental conditions toward the end of the Pleistocene (McDonald and Bryson, 2010). If *Castoroides* was primarily dependent on freshwater habitats, each regional extirpation across North America may be indicative of habitat reduction after the Last Glacial Maximum.

In addition, palaeoecological studies focused on the Pleistocene in North America aim to understand the cause of the megafauna extinction event by analyzing the impact of both climate change and the introduction of anthropogenic influences (Barnosky et al., 2004; Grayson, 2007; Guthrie, 2006; Koch et al., 1998; Levy, 2011). To be most effective, studies of foodweb collapse must incorporate as many species as possible. As a widely distributed megafauna species, *Castoroides* must be better understood before the genus can be effectively incorporated into broader palaeoecology models.

## 1.6 Background Information

Information pertaining to the Pleistocene Epoch, the ecology of the rodent species incorporated in this study, and a basic introduction to macrophyte biology are provided prior to the presentation of stable isotopic results and discussion of palaeoecological implications in Chapters 3 and 4.

### 1.6.1 Setting: The late Pleistocene World

The Pleistocene Epoch spanned from approximately 2.58 million years to 11,000 years ago. Unlike the milder climate of the present (Holocene), the late Pleistocene (120,000 – 10,000 years ago) was dominated by a glacial period (Augustin et al., 2004; Johnsen et al., 2001; Shackleton, 1987). During this time, large continental ice sheets extended over much of the Northern Hemisphere and global sea level was periodically 125 meters lower than it is today. The glacial period reached its peak, also known as the Last Glacial Maximum (or LGM), around 20,000 years ago. Throughout the LGM, global temperatures were on average 5° to 7° C colder than they are today (Webb et al., 1993). A warming trend began around 14,500 years ago, followed by another brief, but intense cold snap known as the Younger Dryas, around 11,000 years ago. Following the Younger Dryas, the global climate began to warm to reach present day conditions by 4,000 years ago (Webb et al., 1993). The repeated retreat and advance of continental-scale ice sheets had a profound impact on Earth's ecosystems. The alternating warm and cool periods caused sea levels to rise and fall, and the distribution of continental vegetation belts to shift (Shuman et al., 2002).

The Pleistocene landscape on every continent was characterized by megafauna. The presence of so many large fauna created unique ecosystems for which there are no exact modern equivalents. By traditional definition, a megafauna is any terrestrial animal that weighs more than 44 kilograms (Martin, 1984; MacPhee, 1999; Stuart, 1991; Wroe et al., 2004) and the term is applicable to birds, reptiles, mammals and marsupials.

The late Pleistocene was also an important time in human evolution. Anatomically modern humans (*Homo sapiens*) first appeared in Africa between 200,000 and 100,000 years ago (Stringer, 2000). The exact date of their dispersal out of Africa is still contested, but based on archaeological evidence from southeast Asia and Australia, it occurred between 90,000 and 45,000 years ago (Kingdon, 1993; Stringer, 2000; Thorne et al., 1999). Humans went on to become one of the most successful invasive species the world has ever seen, and had colonized every continent (with the exception of Antarctica)

by at least 14,500 years ago (Dillehay et al., 2008; Goebel et al., 2008). Their role in the extinction of Pleistocene megafauna, however, is still contested.

### 1.6.2 Dietary Baseline: Freshwater Macrophyte Biology

As discussed in Section 1.4, terrestrial and freshwater plant material are used to create an isotopic dietary baseline. The creation of this baseline is imperative to the study; without it, it would be impossible to assess *Castoroides*' forage choice and overall ecological niche. Due to the relative complexity of the physiological and environmental factors that control the stable isotopic composition of primary producers in freshwater as opposed to terrestrial systems (explored in Chapter 3), basic macrophyte biology is reviewed here.

Freshwater vascular macrophytes (also known as hydrophytes) occupy the near-shore waters of lakes, ponds, and marshes. Vascular macrophytes are aquatic primary producers (categorized separately from algae, cyanobacteria, and aquatic fungi) and compose the base of any freshwater food chain. Macrophytes are an essential part of aquatic ecosystems as they provide a source of food and shelter for many organisms. They serve to disperse nutrients, as well as oxygenate and clarify the surrounding waters, making them habitable for heterotrophic life (Keddy, 2010; Reimer, 1984).

A true aquatic plant grows in association with free-standing water that has completely saturated the surrounding soil and which may be partially or completely covering the plant. Many species of macrophytes complete their entire lifecycle submerged in water, with limited or no contact with the atmosphere. True aquatic plants die shortly after being removed from the water or placed in direct sunlight (Keddy, 2010; Reimer, 1984). As a result, the leaves, stems, and roots of macrophytes differ from those of terrestrial plants and are specialized to life in the water.

Photosynthesis in aquatic macrophytes is very similar to that of terrestrial plants. Macrophytes employ the C<sub>3</sub> carbon fixation metabolic pathway, where energy is converted from sunlight to produce sugar molecules from a combination of carbon sources and water, with oxygen released as a waste product. However, the rate at which

macrophytes acquire carbon, and the sources they utilize differ from those of terrestrial plants. Some species of submerged macrophytes are capable of utilizing multiple sources of dissolved carbon (i.e.  $\text{CO}_2$ ,  $\text{HCO}_3^-$ ) in order to sustain photosynthesis in slow-moving or highly alkaline waters (Keeley and Sandquist, 1992; LaZerte and Szalados; 1982; Sculthorpe, 1967). The photosynthetic rate of underwater plants is controlled by the availability of carbon, the colour and clarity of the surrounding water, the intensity of solar radiation and the velocity of the current. Water current speed is particularly important to submerged macrophytes, since increased flow rate reduces the thickness of the boundary layer around each leaf (see Figure 3.6 in Chapter 3). A thinner boundary layer increases the supply rate of dissolved carbon to the plant (Battrup-Pedersen et al., 2013).

The leaves of submerged macrophytes in particular have adapted to underwater photosynthesis and are shaped to maximize the surface area to water volume ratio (Reimer, 1984). Submerged macrophytes that never come into contact with the atmosphere must obtain their carbon dioxide and oxygen from the water column. The larger surface area of the leaf permits more rapid exchange of gases between the plant tissue and the surrounding water. Submerged leaves also lack the waxy cuticle that floating or aerial leaves possess, as there is no need to guard against evaporation and water loss (Reimer, 1984). This makes the epidermis of submerged leaves particularly thin and pliable, which facilitates the diffusion of oxygen and other gases in and out of the plant. Specialized cells that provide the plant with structural support are also not required in submerged macrophytes. This reduction in mass allows for greater overall leaf buoyancy and increased surface area to catch sunlight (Reimer, 1984).

The roots of aquatic macrophytes serve the same purposes they do in terrestrial plants (i.e. anchorage, storage of sugars, and water/nutrient absorption). Unlike terrestrial plants, macrophyte roots share the role of nutrient and ion absorption with the stem and leaves of the plant, creating miniature aquatic pumps. This is important for circulating elements throughout the ecosystem, where ions absorbed into the plant through the roots from the substrate may later be diffused into the water column from the leaves (Reimer, 1984). Another adaptation of vascular macrophytes is the lacunar system, which is present

in submerged and floating macrophytes. This is not an organ, but rather specialized enlarged spaces or pockets between walls of tissue. These spaces serve as storage for gases such as oxygen and carbon dioxide, as well as a circulatory system used to transfer gases between different parts of the plant (Reimer, 1984).

Aquatic macrophyte growth is governed by nutrient influx and light intensity. Aquatic plants require the same nutrients as terrestrial plants and the most common cause of infertility and low productivity in wetlands is nitrogen or phosphorus deficiency (Keddy, 2010; Reimer, 1984). Nutrient deficiency limits macrophyte population growth and decreases demand for  $^{14}\text{N}$  and  $^{12}\text{C}$ . However, aquatic ecosystems are extremely diverse and there is significant variation of habitat quality within the water column at a single location. The quality of the growing conditions is also strongly influenced by water flow speed, water depth, and proximity to shore (Reimer, 1984). Local geology and topography have a profound impact on the overall productivity of a water body. The composition of the soil and the underlying bedrock determines water chemistry and the presence of dissolved carbon sources such as bicarbonate ( $\text{HCO}_3^-$ ). The size of a drainage basin controls the quantity of run-off and the influx of sediment and associated nutrients to the body of water, while the slope of the watershed also influences the degree of erosion and the amount of particulate matter entering the water (which in turn affects water clarity and access to sunlight) (Reimer, 1984).

The productivity of aquatic macrophytes in lakes and ponds can be diminished or eliminated due to habitat loss by the inevitable process of “filling-in”. Standing bodies of water are in a constant state of change due to clastic and organic detrital input, which is highly variable and dependent on location. Deep lakes can last hundreds of thousands of years, whereas shallow lakes can become completely filled or turned into marsh lands in only a few hundred years (Reimer, 1984). Other primary producers in an aquatic ecosystem can be detrimental to macrophyte growth. Certain species of cyanobacteria inhibit the germination and growth of macrophyte seedlings (Zheng et al., 2013), while eutrophication (excessively high influx of nutrients) can cause large algae blooms that produce an unprecedented quantity of allelochemicals that interfere with the growth and development of vascular plants (Keddy, 2010).



Latitude, local climate, and seasonality also impact macrophyte productivity in a water body. Latitude controls the length of the growing season, and the angle at which sunlight strikes the surface of the water. Low angles at high latitudes are more likely to reflect light from the surface of the water and deprive submerged plants of the necessary energy for photosynthesis (Reimer, 1984).

Macrophytes are divided into four categories based on the niche they occupy within the water column. Each category collects dissolved gases and nutrients from a different combination of aquatic, atmospheric, and terrestrial sources (Cloern et al., 2002; France, 1995; Keddy, 2010; Reimer, 1984) (Figure 3.1 in Chapter 3).

*i. Unattached Floating:* The bulk of the macrophyte's stem and leaves extend above the surface or float on the surface, while the roots are suspended in the upper layer of the water column. Unattached floating plants drift with the current and thrive in the open surface waters of the littoral and pelagic zones.

*ii. Floating Attached:* The macrophyte's leaves and flowers float on the surface of the water, with a stem extending down to the roots anchored in the substrate. Floating attached plants tend to occupy shallow waters with weak currents.

*iii. Submerged:* The entire macrophyte is submerged in water with its roots anchored in the substrate to prevent the plant from floating to the surface or washing on shore. The only part of the macrophyte that may come into contact with the atmosphere is the flowering stems that protrude above the water in species that rely on pollinating insects for reproduction.

*iv. Emergent:* Although still an aquatic plant, the macrophyte is primarily exposed to the atmosphere throughout its life. The base of the stem and the root system are persistently submerged, but the foliage and flowers only grow above water level. Emergent plants grow in the near-shore littoral zone, or in shallow water.

The ecological and physiological differences between the four categories of macrophytes influence their stable carbon and nitrogen isotopic composition. The

resultant relatively distinct isotopic compositions of each macrophyte category are discussed in Chapter 3.

### 1.6.3 *Castor canadensis*: Ecological Summary

The beaver is a large semi-aquatic rodent native to the Holarctic (Eurasia and North America) and *Castor* is dependent on wetland habitat and a steady supply of trees to harvest. A member of the Castoridae Family, it belongs to the only remaining extant genus of which there are two remaining species (*Castor canadensis* and *Castor fiber*). The Castoridae lineage originated during the late Eocene and previously encompassed multiple genera adapted to a wide range of habitats (Rybczynski, 2007). *Castor* first appeared during the Miocene and co-existed with *Castoroides* throughout the Pleistocene (Harington, 1986; Rybczynski, 2007).

The beaver is the second largest living rodent (second only to the capybara of South America). They are found throughout North America, from the Arctic Circle to northern Mexico. A generalist diet, and the ability to survive cold winters by storing food has allowed them to expand their range across the continent. Their morphology reflects a semi-aquatic lifestyle, with longer hind limbs than forelimbs, webbed feet, and a paddle-like tail (Reynolds, 1993). The beaver's eyes and nose are located on the top of its skull, allowing minimum bodily exposure when surfacing to breathe or to examine the surroundings above water. Adult individuals typically weigh between 15 and 30 kg, and consume an exclusively herbivorous diet that incorporates both terrestrial and freshwater vegetation. Beavers are territorial and live in family units until the young are old enough to find an undisturbed waterway to colonize.

Beavers are ecosystem engineers and are well known for their industrious nature. Their ability to shape the landscape around them to suit their needs is second only to that of humans. Their primary building material is trees that they cut down using their exceptionally sharp incisor teeth. Beavers are attracted to free-flowing water and will construct dams in order to create a pool of calm, deep water in which to live. These pools create shelter from terrestrial predators, as well as a convenient underwater refrigerator to store collected vegetation during the winter months (Slough, 1978). Dam construction

may occur within a matter of days, creating an immediate impact on surrounding and downstream hydrological patterns, not to mention local forest ecology.

The engineering abilities of the beaver can produce both negative and positive impacts on the surrounding landscape (Butler, 1995; Müller-Schwarze and Sun, 2003; Wright and Jones, 2002). Forest communities, particularly stands of deciduous trees growing in proximity to water may be entirely cleared through harvesting, or drowned due to beaver-induced flooding. Wetland habitat downstream can be greatly reduced due to diverted water flow. Locally, however, the beaver is considered a keystone species. Dam and canal construction increase both aquatic habitat area and species diversity in the immediate region, and stimulates local aquatic plant succession (Donkor and Fryxell, 2000; McGinley and Whitham, 1985; Rosell et al. 2005). Other semi-aquatic mammals, such as muskrat and otter, are known to seek shelter in actively maintained beaver ponds and lodges during the winter.

Although they exhibit highly specialized behaviours, beavers enjoy a generalist diet. This allows them to thrive in fringe habitat or under suboptimal conditions. Beavers are strategic feeders and are capable of modifying their feeding patterns to optimize their use of available food resources. Strong preference is given to fresh foliage from deciduous tree species such as willow, aspen, poplar and cottonwood (Gallant et al., 2004). Coniferous tree species are least preferred and avoided if better forage is available (Donkor and Fryxell, 2000; Roberts and Arner, 1984). The farther a beaver must travel from water (and safety) in areas of high quality forage, the more efficient and selective the animal becomes in targeting preferred tree species. In areas of low quality forage, beavers maximize overall nutrient gain and are overall less selective regardless of distance (Gallant et al., 2004). During the spring and summer, beavers feed primarily on available emergent and floating macrophytes, with a particular preference for water lily tubers which are rich in carbohydrates. Aquatic macrophytes can compose up to half of the beaver's diet, depending on region and season (Milligan and Humphries, 2010; Severud et al., 2013). Overall, beavers are adept at maximizing nutritional gain while minimizing energy expenditure and chance of predation (Gallant et al., 2004).

#### 1.6.4 *Ondatra zibethicus*: Ecological Summary

The muskrat, a small-bodied, semi-aquatic rodent native to North America, belongs to the Cricetidae Family, of which there are two extant species of ‘water-rat’ alive today: *Ondatra zibethicus* and *Neofiber alleni*. The former is the more abundant species, while the latter is found only in the southeastern United States (Florida and Georgia). Neither species is a true rat, but rather became associated by humans with the genus *Rattus* due to their shared omnivorous diet. Both *Ondatra* and *Neofiber* appear in the fossil record during the Pleistocene and shared the landscape with *Castoroides* and *Castor* (Dr. Richard Hulbert, personal comm.). Today, there are over a dozen subspecies of *Ondatra zibethicus* distributed across every state and province in North America and Northern Mexico. The only regions where the muskrat does not survive are the Arctic tundra, and west of the Cordillera in the American Southwest (Hall, 1981) due to a lack of wetland habitats with a sufficient supply of aquatic vegetation in these dry or high altitude environments.

Adult muskrats typically weigh between 0.5 to 2 kg. They are well adapted to life in the water, with a long laterally-compressed, hairless tail and webbed feet that help propel them through the water. Similar to *Castor*, they are adapted to cold water and long winters with specialized morphological traits such as compact extremities and ears, and a thick multi-layered fur coat. Like many diving mammals, muskrats have a decreased sensitivity to dissolved carbon dioxide levels in the blood stream and can stay submerged for up to a quarter of an hour (Andersen, 1966). During these dives, muskrats seek out aquatic vegetation and small freshwater animals to eat. Their ever-growing incisors are ideal for gnawing into freshwater crustacean shells or clipping stalks of aquatic vegetation. As an adaptation to their aquatic diet, the muskrat’s premaxillae are elongated, causing their incisor teeth to protrude well beyond their cheeks. Specialized lips are then closed behind the teeth allowing the muskrat to chew and collect emergent macrophyte stalks while underwater without accidentally inhaling water. A similar pattern is seen in *Castoroides* skulls, where the incisors are unusually long and pronounced.

Muskrats prefer shallow open water or marshy ecosystems, so frequently overlap with modern *Castor canadensis*, but as there is enough of a disparity in their diets, they co-exist without facing major competition. Muskrats feed primarily on the fresh shoots and roots of emergent macrophytes such as rushes, sedges, and cattails found in the shallow water of wetlands, or along the shores of lakes, ponds, and rivers (Boutin and Birkenholz, 1987; Danell, 1979). Emergent plants are not only a primary food source, but are also used in muskrat lodge construction. In addition to emergent aquatic plants, muskrats eat root tubers from floating macrophytes, fleshy submerged macrophytes such as bladderworts, and the occasional small animal such as frogs, fish, clams, and mussels (Danell, 1979; Sietman, 2003). Animal protein is a minor component of their diet (approximately 5%) and its consumption varies depending on region and forage availability. When not actively foraging for food, muskrats rest in small off-shore lodges constructed from reeds and rushes, in burrows, or on feeding platforms made of mud and piled vegetation. They construct these shelters to provide protection against the elements and from predators, and as a place to raise their young (Messier and Virgl, 1992). These lodges and burrows are multipurpose and are frequently shared by other small vertebrate species for protection or as an aid to thermal regulation.

Muskrats are also considered ecosystem engineers, although their activities impact the ecosystem in a less dramatic fashion than the beaver's. Muskrat foraging impacts wetland habitat and biodiversity by creating openings in dense stands of vegetation and clearing aquatic habitat. This alters plant community composition and allows other animals to take advantage of sunlight and open water (Messier and Virgl, 1992). This is particularly beneficial to bird and fish populations as it increases the amount of open water and available feeding area (Danell, 1979).

Muskrats, particularly juveniles, are susceptible to extreme fluctuations in water level (Errington, 1939). Food supplies become scarce during an extended drought, and protective lodges and burrows are submerged or swept away during periods of intense flooding. Environmental triggers that affect water level unexpectedly and the species' tolerance to habitat alteration are important considerations for the survival of all semi-aquatic rodents.

### 1.6.5 *Castoroides*: Palaeontological Summary

*Castoroides*, or the giant beaver, belongs to the Castoridae family. It is not a direct ancestor to the extant *Castor*. *Castoroides* was an exceptionally large, semi-aquatic rodent that once ranged across North America from Florida to Alaska (Cahn, 1932; Harington, 1978; Martin, 1969; Miller et al., 2000). It is one of the most widely distributed species represented in the North American Pleistocene fossil assemblage, with a lineage that extends into the late Miocene.

On average, *Castoroides* weighed 100 kg and reached 2.5 meters in length from nose to tail (approximately six times larger than the average *Castor*) (Reynolds, 2002). Giant beaver skeletal morphology suggests that it was best suited for life in the water. Its limbs were very short relative to body size, even in comparison to *Castor*, which would have made it awkward on land. It also possessed a long, narrow tail, and proportionally larger hind feet that were likely webbed. Akin to all rodents, giant beavers possessed a pair of continuously incisors in both upper and lower jaws. The incisors are roughly circular, reaching 15 to 20 centimeters in length, with the anterior and lateral surface of each incisor being covered in a thick coating of enamel with longitudinal ridges (Figures 1.2 and 1.3). Its cheek teeth are aradicular (or open-rooted), with four per quadrant, each folded into distinct S-shaped lophs covered in smooth enamel. The folded enamel provided a more durable grinding surface, suitable for efficient chewing of tough or gritty plant material (Barbour, 1931). *Castoroides* has highly pronounced incisors, and an extended diastema (the space between incisors and cheek teeth).



**Figure 1.2 Lateral view (above left) and anterior view (above right) of a complete *Castoroides* skull and dentition (cast).**



**Figure 1.3 Complete *Castoroides* mandible and dentition (cast).**

There are currently three giant beaver species present in the fossil record: *Castoroides leiseyorum*, *C. dilophidus*, and *C. ohioensis*. *C. leiseyorum* was indigenous to the American southeast (Florida and Georgia) during the early Pleistocene (Irvingtonian), and gave rise to *C. dilophidus* during the late Pleistocene (Hulbert et al., 2014). *C. ohioensis* represents a more successful late Pleistocene radiation of the genus and was found throughout the Mississippi drainage basin (although primarily east of the Missouri River), the Great Lakes Basin, and the Old Crow Basin.

It remains unclear why *Castoroides* does not appear in the Canadian fossil record outside of deposits in central Yukon, a single specimen from the Toronto region, and a single discovery lacking stratigraphic context from New Brunswick. As a consequence, it is unknown how or precisely when *Castoroides* populations began to expand their range west and north, and if this expansion occurred just once, or multiple times. It is presumed that *Castoroides*' range expanded into Canada during warmer, wetter interglacial periods. Although the chronology of population fluctuation is currently poorly documented, the species likely appeared in Yukon Territory and Alaska during the unusually warm Sangamonian interglacial, but did not continue to thrive in the region when temperatures began to cool. During the Last Glacial Maximum, the genus was concentrated south of the ice sheets, within the Great Lakes Basin and eastern United States (Cahn, 1932; Martin, 1969). The precise time of their extinction remains unknown, although it is estimated to have occurred between 13,000 and 11,000 years BP (Boulanger and Lyman, 2014). This approximately coincides with the arrival of *Homo sapiens* in parts of North America, however there is no evidence to date that *Castoroides* was hunted or that the two species ever co-existed in the same region. It is likely that the giant beaver became extinct before humans reached the interior of the continent.

To date, there have neither been confirmed discoveries of dam or lodge structures built by giant beavers, nor substantiated evidence of Pleistocene-era cut wood that matches the occlusal surface and angle of *Castoroides* incisors (Hillerud, 1975; Rybczynski, 2007). Their protrusion from the skull and their lack of a thin, chisel-like edge rendered giant beaver incisors ineffective tools for cutting down trees (Stirton, 1965). This has not stopped speculation that masses of branches discovered in proximity to *Castoroides* fossils in glacial sediments were built by the giant beaver (Miller et al., 2000). However, tree harvesting behaviours for the purpose of food and building material has been present in the Castoridae family since the Miocene (Rybczynski, 2006; Rybczynski, 2008). Evidence of woodcutting behaviour and lodge construction was discovered for the high Arctic castorid *Dipoides* (Rybczynski, 2008; Tedford and Harington, 2003) and indicates that tree-cutting and environmental engineering behaviours appeared as far back as the Pliocene. *Dipoides* is ancestral to both *Castoroides* and *Castor*, and it remains unknown if both genera inherited these traits.



## 1.7 Sample Material

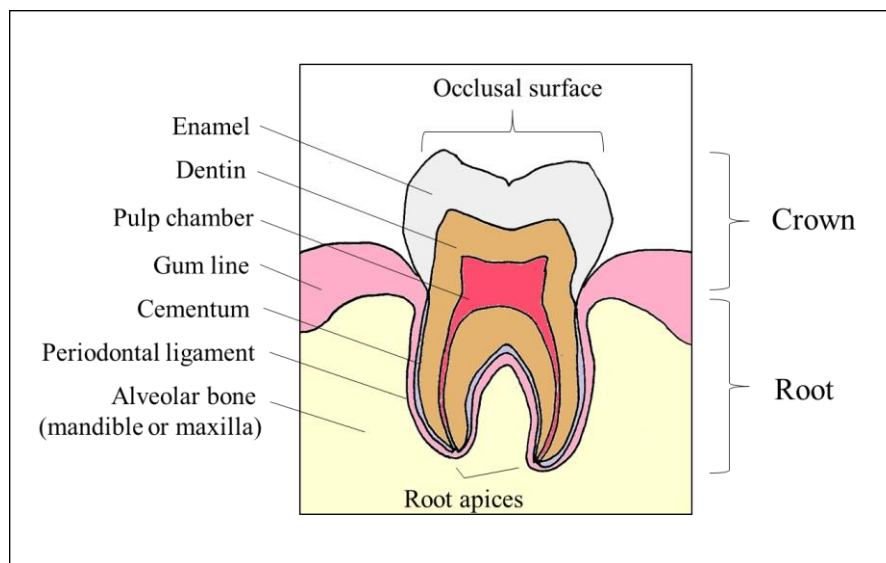
A variety of biological tissues are necessary to explore the palaeoecological questions outlined in Section 1.2.1. In order to obtain the necessary stable isotope information, both plant and skeletal material were sampled. The following section outlines relevant information concerning the skeletal and dental tissues utilized in this study.

### 1.7.1 Calcified Tissues - Bone

All vertebrate organisms possess a calcified skeleton. Bone is a living tissue composed of approximately 70% mineralized hydroxyapatite and 30% organic collagen protein (White et al., 2012). This combination provides bone with the strength and flexibility to withstand both weight-bearing and torsional forces. Bone is considered an organ within the body and is interwoven with a complex network of blood vessels and nerves. Although the primary function of the skeleton is to provide structural support, it is also a key fat and mineral storage center for the body, serves as an attachment point for muscles, and houses bone marrow. Bone marrow is the center of hematopoiesis (blood production) in the body, as well as a key component of the vertebrate immune system.

There are two types of bone: compact and spongy. The former is composed of dense cortical bone that provides each skeletal element with a protective outer shell. Spongy bone fills the interior of the cortical shell, and is composed of a mesh-work of trabeculae that mature to form trabecular, or cancellous bone (White et al., 2012). Because bone is a living tissue, it is continuously adapting to external factors such as body growth, muscle development, pathological stressors (such as injury or disease), and high-impact activities. The rate at which cellular replacement (or remodeling) occurs depends on the age of the individual, their diet, and the degree of physical activity. In large-bodied adult mammals, the replacement rate for skeletal tissues is typically several years.

In palaeoecological studies, both the mineral and organic portions of bone provide valuable stable isotopic information. The state of preservation of the bone dictates



**Figure 1.4 Simplified mammalian tooth anatomy.**

whether collagen protein or biological hydroxyapatite (bioapatite) is the best material to analyze. In palaeodietary studies, collagen is the preferred tissue as it can provide both stable carbon and nitrogen isotopic information. However, poorly preserved bone, particularly from water-logged depositional environments, has low to negligible collagen yields. In these instances, bioapatite becomes the only option to obtain isotopic data. Bioapatite however, is not impervious to post-depositional alteration, and poor preservation may also impact its original isotopic composition.

#### 1.7.2 Calcified Tissues - Teeth

Teeth are another calcified tissue whose durability and mineral composition make them ideal sample material in palaeoecological studies that employ stable isotope analysis. Teeth are composed of a different set of tissues and fulfil a very different function than the rest of the skeleton. They allow animals to harvest food, and process it in a manner that maximizes the efficacy of its digestion. Individual tooth morphology is dependent on the animal's diet. Teeth are categorized as incisors, canines, premolars, or molars depending on function. Not all mammalian species have the same number or types of teeth (see Hillson, 2005 for a thorough review).

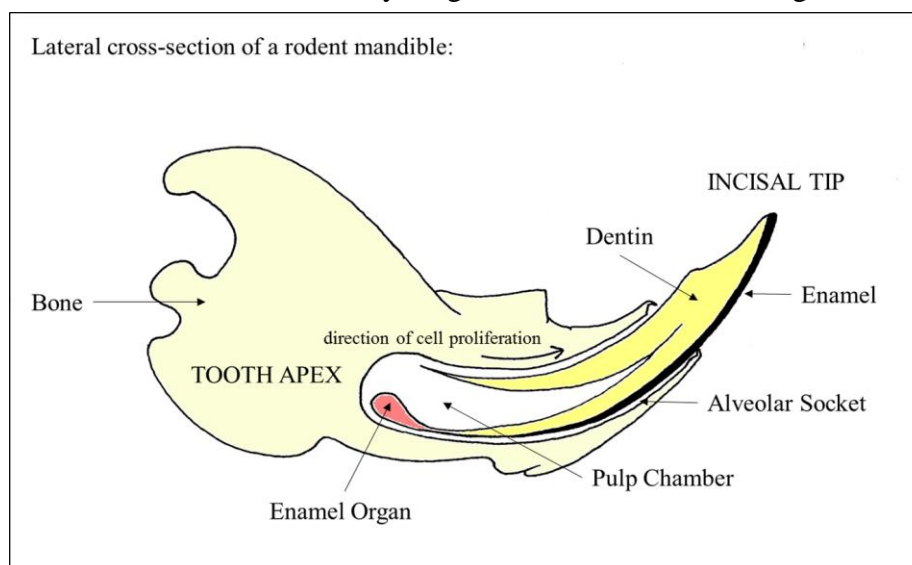
Mammalian teeth are composed of dentin, enamel, cementum, and pulp (Hillson, 2005) (Figure 1.4). The heavily mineralized dentin and enamel form durable cusps ideal for the rigors of tearing and chewing food, while roots composed of dentin and cementum anchor the teeth into the jaws. The pulp chamber is located at the core of the tooth and is supplied via the apex of the roots by blood vessels and nerves from the surrounding alveolar bone. Dentin, enamel, and cementum are each composed of differing ratios of inorganic and organic components. The degree of mineralization affects the viability of each tissue for different stable isotope analyses. Dentin is approximately 70% hydroxyapatite, and 15% collagen protein. If well-preserved, dentin can provide both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from extracted collagen, and  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  from structural carbonate from bioapatite. In contrast, enamel is the hardest tissue in the mammalian body and is composed of 97% hydroxyapatite. As a result, it is not possible to extract any organic material, and enamel only provides  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  from structural carbonate, or  $\delta^{18}\text{O}$  from phosphate.

#### 1.7.2.1 Rodent Dental Morphology

Mammalian teeth vary considerably in morphology and function depending on species (Hillson, 2005). This variability is particularly noticeable in the taxonomic order Rodentia. While most other mammals only produce two complete sets of teeth within their lifetime (diphyodont), rodents possess a pair of continuously incisors in both their upper and lower jaw. This dental adaptation is integral to their success in colonizing an incredibly diverse range of terrestrial and freshwater habitats on every continent, with the exception of Antarctica. A continuous supply of enamel and dentin allows rodents to consume virtually any food source without the risk of permanently abrading or damaging the cusps of their teeth. In many other animals, a broken tooth or the loss of enamel would result in difficulty eating, and eventual starvation.

All rodents have aradicular, or open-rooted incisors specialized for gnawing. The lack of a closed root allows specialized cells that secrete dentin and enamel (odontoblasts and ameloblasts) to continue growth throughout the animal's lifetime. A few genera of rodents also possess aradicular cheek teeth. The body of the tooth is composed of dentin with a thin layer of hard enamel on the anterior surface. The upper and lower incisors curve to meet so that as the rodent gnaws, the lower incisor continuously sharpens the upper incisor. The incisor erupts from the alveolar socket of the jaw as amelocytes and odontocytes secrete enamel and dentin cells in tandem (Figure 1.5) (Zajicek, 1976). The enamel and dentin cells mature as they are pushed from the tooth apex towards the incisal tip. Fibroblast cells secrete collagen fibers along the exterior of tooth, which act as connective tissue that secures the tooth within its socket. As the collagen fibers mature, they slowly pull the tooth from the socket, aiding tooth eruption (Zajicek, 1976).

The rate of tooth eruption is in part controlled by the degree of use. The tooth can erupt slightly faster to compensate for periods of particularly intense wear; however, the rate of tissue proliferation remains relatively constant throughout life. Tooth eruption was found to occur at a rate of approximately 0.5 mm per day in laboratory-raised rats (Zajicek, 1976), and 0.75 mm per day in wild populations of *Castor canadensis* (Stuart-Williams and Schwarcz, 1997). The continuous growth can pose health risks to the rodent if it does not have access to sufficiently tough foods, or other items to gnaw on. If a



**Figure 1.5 Anatomy of tooth eruption and growth in rodents.**

rodent does not have an outlet for chewing, its incisors continue to grow in a spiral exiting the front of the mandible, eventually rendering it impossible for the animal to eat at all. The incisors can even pierce the skull if starvation does not kill the animal first. This is not frequently a problem for wild rodents.

## 1.8 Organization of Dissertation

This thesis is divided into a further four chapters. Chapter 2 reports the results of two methodological studies that explore the effects of sample pretreatment processes on the original stable isotopic composition of skeletal and dental sample material. Chapter 3 discusses the stable carbon and nitrogen isotope variation observed for modern aquatic plants, the causes of this variation, and why stable isotopic data used for interpreting freshwater food webs should be examined on a site-specific basis. Chapter 4 discusses the palaeoecological implications of the stable isotopic data obtained from *Castoroides* skeletal remains. Chapter 5 summarizes the conclusions concerning *Castoroides* palaeoecology drawn from these data, and explores giant beaver population radiation and extinction as a means of tracking climatic regimes during the late Pleistocene.

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## Chapter 2

### Methodological Studies

#### 2.1 Introduction

Virtually all sample material utilized in stable isotope studies requires a degree of preparatory work prior to analysis. Preparatory work is typically labour and time intensive, and aims to isolate a specific constituent for analysis. It is therefore important to ensure that the process intended to isolate or purify the sample material of interest does not impact its original isotopic composition. The following chapter describes two methodological studies that pertain to the preparation of skeletal and dental material, and the potential effect of these procedures on sample  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$ .

#### 2.2 The effects of different defleshing practices on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk collagen from modern mammalian bone

##### 2.2.1 Research Aim

Stable isotope data obtained from modern faunal skeletal material is frequently used to investigate food-web dynamics and nutrient flow in ecological studies. Unlike skeletal material that is of archaeological or paleontological origin, modern faunal material typically requires an extra stage of preparatory work prior to analysis. If the animal has been recently collected, it must be fully or partially defleshed in order to access the necessary skeletal material. Removing all the soft tissue from an animal carcass can be a time and energy consuming process depending on size and state of preservation. This process can be accelerated in a number of ways. Commonly used techniques include placing the manually defleshed skeletal elements in heated water, or in a solution of water and polypeptide-chain breaking enzyme (Davis and Payne, 1992; Roberts et al., 2002). However, it is unclear if these processing techniques alter the stable isotopic composition of collagen protein, and if so, to what degree. The structural integrity of bone is dependent on the ratio of organic to inorganic material (collagen and bioapatite). Extensive boiling (over 9 hours) has been shown to substantially alter the ratio of organic to inorganic tissue, resulting in crystallinity changes in the mineral

portion of the bone (Roberts et al., 2002). Recrystallization can result in ion exchange, altering the original carbon and oxygen isotopic compositions. The majority existing literature only explores the effects of defleshing method on the isotopic composition of mineralized tissues. The hydrolyzing nature of proteolytic enzymes and heat can degrade polypeptide chains and influence the isotopic composition of organic tissues. Accordingly, the effect of water, heat, and enzyme use to the stable carbon and nitrogen isotopic composition of bulk collagen is explored here.

### 2.2.2 Methodology

A small-scale study was conducted to determine the effect of four common defleshing methods on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of bulk collagen from modern mammalian bone. The right mandible was removed from each of four adult beaver (*Castor canadensis*) carcasses, donated by local trappers, two animals (male) originating from central Ontario (B3-TP2014 and B4-TP2014) and two (sex unknown) from southern Yukon Territory (B6-TP2014 and B8-TP2014). The mandibles were manually defleshed with a knife to remove the bulk of the adhering soft tissue (Figure 2.1), and then cut into four pieces of approximately equal weight (~500 mg) (Figure 2.2). The subsamples from each mandible underwent one of four different processing methods prior to preparation for isotopic analysis. These methods were designed to mimic popular defleshing strategies commonly used to build modern comparative skeletal collections. Processing Methods B, C, and D, in particular, are designed to make connective and soft tissues easier to remove, and to make this process faster.

*Method A:* Bone subsamples underwent no further processing beyond manual defleshing to remove remaining soft tissue. Samples were air-dried at room temperature.

*Method B:* Bone subsamples were soaked in room-temperature distilled water in separate containers for four days. Remaining soft tissue was removed from the bone with a toothbrush. Samples were air-dried at room temperature.



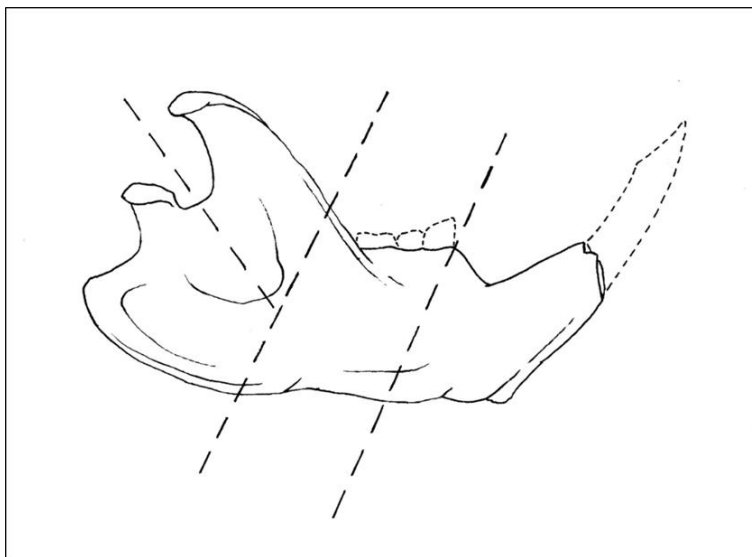
*Method C:* Bone subsamples were cooked at a constant rolling boil ( $\sim 95^{\circ}\text{C}$ ) in distilled water in separate containers for three hours. Remaining cooked soft tissue was removed from the bone with a toothbrush. Samples were air-dried at room temperature.

*Method D:* Bone subsamples were soaked in a gently heated ( $35\text{-}45^{\circ}\text{C}$ ) solution of 1:150 Protease enzyme and distilled water ( $\sim 7\text{mL}$  Protease/ $1\text{L}$   $\text{H}_2\text{O}$ ) for 16 hours. Remaining soft tissue was removed from the bone with a toothbrush and samples were air-dried at room temperature.

Each dried bone subsample ( $\sim 500$  mg) was crushed to between  $180$  and  $850$   $\mu\text{m}$ . Lipid extraction was performed using a 2:1 solution of chloroform and methanol. Collagen extraction was performed using a modified Longin (1971) method. Subsamples were demineralized in  $0.5$  M HCl at room temperature for approximately eight weeks.



**Figure 2.1 A** *Castor canadensis* mandible defleshed using Method A.



**Figure 2.2 Mandible sampling strategy. The dashed lines indicate the division of the mandible into four samples of approximately equal weight.**

Subsamples were then rinsed three times in distilled water, and soaked in a 0.1 M NaOH to remove humic acids. Collagen was gelatinized in pH 3 0.25 M HCl at 90°C for ~16 hours, and then desiccated at 90°C for ~24-36 hours. The collagen extraction procedure was completed for an additional four method duplicates. Dried collagen was crushed and ~0.38 mg was weighed into tin capsules. All samples were analyzed using a Costech Elemental Analyzer coupled to a ThermoScientific Delta Plus XL isotope ratio mass spectrometer (IRMS) operated in continuous-flow mode. Helium was used as the transport gas to carry the CO<sub>2</sub> or N<sub>2</sub> produced from sample combustion to the IRMS for isotopic analysis.

All stable carbon and nitrogen isotope results are reported in delta ( $\delta$ ) notation as ratios relative to the international standards VPDB and AIR, respectively, as follows:

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the heavy to light isotope ratios (i.e. <sup>13</sup>C/<sup>12</sup>C, <sup>15</sup>N/<sup>14</sup>N) of the same element in both the sample material and the standard (Coplen, 2011).

Data were collected over the course of two analytical sessions. International and internal laboratory standards were analyzed concurrently to create the calibration curve and to monitor instrument precision and accuracy. The carbon isotopic results were calibrated to VPDB using USGS-40 ( $\pm 0.2$  ‰ one standard deviation (SD),  $n = 8$ ; accepted  $\delta^{13}\text{C} = -26.4$  ‰) and USGS-41 ( $\pm 0.4$  ‰ SD,  $n = 9$ ; accepted  $\delta^{13}\text{C} = +37.6 \pm 0.4$  ‰) (Qi et al., 2003). The nitrogen isotopic results were calibrated to AIR using USGS-40 ( $\pm 0.1$  ‰ SD,  $n = 8$ ; accepted  $\delta^{15}\text{N} = -4.5$  ‰) and USGS-41 ( $\pm 0.1$  ‰ SD,  $n = 9$ ; accepted  $\delta^{15}\text{N} = +47.6$  ‰) (Qi et al., 2003). Keratin and IAEA-CH-6 were used to track analytical precision and accuracy. Keratin (MP Biomedicals Inc., Cat No. 90211, Lot No. 9966H) had a  $\delta^{13}\text{C}$  of  $-24.1 \pm 0.1$  ‰ ( $n = 18$ ), which compares well with its accepted  $\delta^{13}\text{C}$  of  $-24.0$  ‰, and  $\delta^{15}\text{N}$  of  $+6.36 \pm 0.1$  ‰, which compares well with its accepted  $\delta^{15}\text{N}$  of  $+6.4$  ‰. IAEA-CH-6 had a  $\delta^{13}\text{C}$  of  $-10.5 \pm 0.1$  ‰, which compares well with its accepted value of  $-10.45$  ‰. A minimum of one internal and one international standard was analyzed for every five samples. One duplicate was included for every ten samples analyzed. For six duplicated analyses, the average reproducibility was  $\pm 0.1$  ‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### 2.2.3 Results

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  obtained for the four individuals are presented in Table 2.1 and lie within the expected range for *C. canadensis*, as reported in previous studies (Katzenberg, 1989, 2006), with the exception of  $\delta^{15}\text{N}$  from B4-TP2014. The  $\delta^{15}\text{N}$  from this individual is at least a trophic level higher than expected for a purely herbivorous animal. This might be related to nitrogen enrichment that southern Ontario ecosystems experience due to anthropogenic activities such as agricultural and septic runoff, as discussed in Chapter 4.

Table 2.1  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bulk collagen results for *C. canadensis* bone subjected to differing defleshing protocols. Results shown in bold-faced font are the average of duplicate analyses. The standard deviation reported for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  encompasses the variance among samples from each individual.

Sample No.	Processing Method	$\delta^{13}\text{C}$ (‰, VPDB)	Std. Dev. $\pm$	$\delta^{15}\text{N}$ (‰, AIR)	Std. Dev. $\pm$
B3-TP2014-1	A	-23.7	0.1	+2.0	0.1
B3-TP2014-2	B	<b>-23.4</b>	0.1	<b>+2.2</b>	0.1
B3-TP2014-3	C	<b>-23.5</b>	0.1	<b>+2.0</b>	0.1
B3-TP2014-4	D	<b>-23.4</b>	0.1	<b>+2.2</b>	0.1
B4-TP2014-1	A	-23.1	0.2	+9.5	1.1
B4-TP2014-2	B	-22.9	0.2	+9.9	1.1
B4-TP2014-3	C	-23.3	0.2	+7.8	1.1
B4-TP2014-4	D	-23.3	0.2	+8.0	1.1
B6-TP2014-1	A	-23.7	0.1	+2.0	0.1
B6-TP2014-2	B	-23.3	0.1	+2.0	0.1
B6-TP2014-3	C	-23.5	0.1	+1.9	0.1
B6-TP2014-4	D	<b>-23.6</b>	0.1	<b>+2.0</b>	0.1
B8-TP2014-1	A	-23.7	0.1	+2.2	0.1
B8-TP2014-2	B	<b>-23.8</b>	0.1	<b>+2.1</b>	0.1
B8-TP2014-3	C	-23.6	0.1	+2.1	0.1
B8-TP2014-4	D	<b>-23.6</b>	0.1	<b>+2.2</b>	0.1

Each sample set was examined for method-dependent variation that might indicate alteration of the primary isotopic composition of the bone collagen. B3-TP2014, B6-TP2014, and B8-TP2014 demonstrate very low variability for  $\delta^{13}\text{C}$  ( $\pm 0.1$  ‰, 1 SD) and  $\delta^{15}\text{N}$  ( $\pm 0.1$  ‰, SD) among defleshing methods. Specimen B4-TP2014 demonstrates the most variability among methods for both  $\delta^{13}\text{C}$  ( $\pm 0.2$  ‰, SD) and  $\delta^{15}\text{N}$  ( $\pm 1.1$  ‰, SD). There is no consistent trend in the specimen group regarding the small increases and decreases in  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enrichment between processing methodologies, as illustrated in Figures 2.3 to 2.6. Overall, there is minimal isotopic variation among the subsamples taken from each individual.

Total error was calculated from method duplicates, where separate different aliquots of the same subsample are prepared separately using the same technique. This error amounted to  $\pm 0.1$  ‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The isotopic variability measured for specimens B3-TP2014, B6-TP2014, and B8-TP2014 was within error and is therefore negligible. The isotopic variation in specimen B4-TP2014 cannot be accounted for by analytical error. The two subsamples from B4-TP2014 that underwent the least invasive defleshing methods (methods A and B) have higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  relative to subsamples defleshed using more rigorous methods (C and D). A range of 0.42 ‰ for  $\delta^{13}\text{C}$  and 2.2 ‰ for  $\delta^{15}\text{N}$  indicates another source of influence on the isotopic composition of B4-TP2014.

#### 2.2.4 Discussion

A defleshing method, if effective for isotopic analyses, should have little impact on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *C. canadensis* bone collagen. The only specimen that demonstrated isotopic variability outside the range of analytical error was B4-TP2014.

Natural isotopic variability is known to occur within skeletal elements from a single individual (Leatherdale et al., 2014; Olsen et al., 2014). Documented variability is relatively small in humans. Non-pathological skeletal elements have a normal variation of  $0.0 \pm 0.1$  ‰ for  $\delta^{13}\text{C}$  and  $0.1 \pm 0.4$  ‰ for  $\delta^{15}\text{N}$  (Olsen et al., 2014). A study of human, humeral, intracortical isotopic variability found a maximum range of 2.0 ‰ for  $\delta^{13}\text{C}$  and 2.9 ‰ for  $\delta^{15}\text{N}$  within a single humerus (Leatherdale et al., 2014). This natural isotopic

variability within a single skeletal element can arise from changes in diet or temporary stressors that occur during bone growth or remodeling.

Preparatory error could also account for the isotopic variability recorded among subsamples from specimen B4-TP2014. Defleshing methods A and B are the least effective at removing other tissues from the bone (method A in particular). Incomplete defleshing and the inclusion of other soft tissues can lead to the creation of an impure collagen sample, thus changing its  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  relative to pure collagen. Blood residue and muscle fibers are composed primarily of proteins and are not removed during the collagen extraction process, which only targets humics, lipids, and mineralized tissues.

Diet-tissue isotopic fractionation and amino acid composition are different among tissue types (blood, muscle, bone collagen, bone apatite). These differences result in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  offsets between tissues (Crowley et al., Hare et al., 1991; Styring et al., 2010). There is a documented positive  $^{13}\text{C}$ -enrichment ( $\Delta^{13}\text{C}_{\text{collagen-muscle}}$ ) between bone collagen and muscle from the same individual. Crowley et al. (2010) reports on a number of terrestrial mammals, with observed  $\Delta^{13}\text{C}_{\text{collagen-muscle}}$  that range from  $+1.1 \pm 1.6 \text{‰}$  to  $+4.1 \pm 0.1 \text{‰}$  (where collagen is more  $^{13}\text{C}$ -enriched than muscle). The observed  $\Delta^{15}\text{N}_{\text{collagen-muscle}}$  is smaller, ranging from  $-0.5 \pm 0.8 \text{‰}$  to  $+0.9 \pm 0.6 \text{‰}$ , depending on species. Roth and Hobson (2000) found small  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enrichment between red fox muscle and blood cells ( $-0.5 \text{‰}$  and  $+1.0 \text{‰}$ , respectively). As a contaminant, blood proteins and muscle fibers would still contribute relatively little to the overall mass of the sample. However, small quantities could be responsible for the slightly lower  $\delta^{13}\text{C}$  and slightly higher  $\delta^{15}\text{N}$  seen in subsamples B4-TP2014-1 and -2, relative to subsamples -3 and -4. Defleshing methods C and D, by comparison, are highly effective at removing remnants of blood, muscle and other connective tissues from the bone surface and produce unaltered bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### 2.2.5 Conclusions

While this study requires further expansion beyond four individuals to verify its conclusions, the initial results strongly suggest that the four different defleshing methods

employed, in general, do not significantly alter the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of bulk collagen of modern mammalian bone. This information is helpful to biologists, zooarchaeologists, and palaeontologists looking to incorporate stable carbon and nitrogen isotopic information from modern taxa into their research design. Defleshing methods that employ water, heat, and/or proteolytic enzymes are considerably faster, and save both time and labour. These results also highlight the importance of thorough removal of all tissue from the bone surface or cavities, as residual soft tissues or fluids are more likely to contaminate bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . For these reasons, Method D (which gently heats samples in a solution of Protease enzyme) is preferable to use.

## 2.3 The effects of sample pretreatment on $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of structural carbonates from enamel bioapatite

### 2.3.1 Overview and Research Aim

This section explores the effect of pretreatment that employs dilute acetic acid and sodium hypochlorite on  $\delta^{13}\text{C}_{\text{sc}}$ ,  $\delta^{18}\text{O}_{\text{sc}}$ , and FTIR parameters of palaeontological enamel samples. The teeth incorporated into this study are from Pleistocene *Castoroides* collected from a variety of location across North America.

Palaeoecologists commonly employ the stable carbon and oxygen isotopic compositions of bioapatite structural carbonate ( $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ ) from tooth enamel as a proxy to reconstruct dietary and climatic trends from ancient ecosystems (e.g. Cerling and Harris, 1999; Clementz et al., 2006; Stuart-Williams and Schwarcz, 1997). Enamel is the hardest tissue in the mammalian body and is composed of 97 % inorganic material. This material is similar chemically to hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}^-)_2$ ), although substitutions for the phosphate or hydroxyl groups commonly occur and crystallinity is much poorer, leading to the term ‘bioapatite’ for this material. Carbonate anionic complexes ( $\text{CO}_3$ ) commonly substitute for phosphate ( $\text{PO}_4$ ), resulting in the presence of “structural carbonates” within the bioapatite enamel matrix. Bioapatite is also the primary mineral constituent of dentin and bone, although it is present in lower concentrations

(~60 to 70 %). For a succinct, yet thorough review of dental tissue morphology, growth, and composition, refer to Hillson (2005).

The highly compact nature of bioapatite crystals in the enamel layer of a tooth renders it less porous (1%) than either dentin or bone (40%) (Lee-Thorp, 2008; LeGeros, 1991; Wang and Cerling, 1994). The availability of pore space impacts the durability of calcified tissues, where higher porosity and associated permeability allows a greater volume of fluid to enter and interact with the crystal structure after death (i.e. *post-mortem* alteration, diagenesis). When such fluids (most commonly water) enter the tissue, phosphate and carbonate groups are partially or completely dissolved in solution, and new material (secondary carbonate) is precipitated and adsorbed onto the crystal surface. This process can occur multiple times *post-mortem*, facilitated by the presence of water and warm temperatures.

Dentin and bone contain a high proportion of organic material, mainly in the form of collagen fibers and short-chain polypeptides (Hillson, 2005). Collagen fibers are tightly interlaced with bioapatite crystals seeded between them (Hillson, 2005). The decomposition of collagen after death once again increases the pore space and permeability around the bioapatite crystals and makes dentin and bone more susceptible to fracture or chemical exchange with fluids. Enamel is thus more resistant to *post-mortem* mechanical abrasion and diagenetic processes than either dentin or bone. This means that teeth are more commonly preserved in the palaeontological record and are more likely to preserve original  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  obtained from diet and drinking water during an animal's lifetime (Lee-Thorp and van der Merwe, 1987; 1991). The distinctive nature of tooth crowns means they are often readily identifiable to taxon and are of high utility to palaeontologists (Hillson, 2005).

Each tissue type (enamel, dentin, cementum) within the tooth retains a slightly different suite of isotopic information (refer to Katzenberg and Saunders (2011) for a comprehensive review). Unless exposed to excessive heat or microbial activity, collagen is extremely stable and retains its original  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with collagen yields as low as 1 % (van Klinken, 1999). In situations where dentin collagen has fully decomposed,



however, structural carbonate from enamel bioapatite can still provide dietary information in the form of  $\delta^{13}\text{C}_{\text{sc}}$ . Enamel and cementum also provide  $\delta^{18}\text{O}$  from the structural carbonate and phosphate components of bioapatite. The  $\delta^{18}\text{O}$  from phosphate is less susceptible to alteration; however, bioapatite structural carbonate is less labour intensive to prepare for isotopic analysis and provides data for both carbon and oxygen isotopes.

While it is a very durable tissue, enamel is not immune to physical and chemical alteration. Bioapatite crystals can be lost, replaced, or altered *post-mortem*. Some of the most common types of alteration affecting palaeontological tooth specimens occur in the presence of groundwater (i.e. bioapatite recrystallization and isotopic exchange; loss of structural carbonate due to dissolution, addition of secondary calcite precipitated from groundwater).

Bioapatite-water interactions occur when water enters the enamel pore space. Not only can this interaction modify the bioapatite crystal morphology, but isotopic exchange can also occur. Values of  $\delta^{18}\text{O}_{\text{sc}}$  are documented to be more vulnerable to diagenetic modification than  $\delta^{13}\text{C}_{\text{sc}}$  (Wang and Cerling, 1994). Values of  $\delta^{13}\text{C}_{\text{sc}}$  are less sensitive to temperature fluctuation in the depositional environment; carbon isotopes generally undergo minimal isotopic exchange with secondary fluids (typically water) within the temperature range of 25° to 120°C, given the typically low dissolved carbon contents in water relative to bioapatite (Wang and Cerling, 1994). In well-preserved teeth with minimal enamel porosity and permeability, therefore, *post-mortem* carbon isotopic exchange is generally negligible. Oxygen isotopes, on the other hand, readily exchange with water at ambient surface temperatures (~25°C), particularly given the high ratio of oxygen in water relative to bioapatite. As a result, enamel bioapatite with long-term exposure to warm, waterlogged environments commonly does not reflect original  $\delta^{18}\text{O}_{\text{sc}}$ . This susceptibility to *post-mortem* isotopic alteration mean that palaeoecologists must take certain precautions before making conclusions based on  $\delta^{18}\text{O}_{\text{sc}}$  and, to a lesser degree,  $\delta^{13}\text{C}_{\text{sc}}$ .

Consequently, in studies when bioapatite structural carbonate from enamel is the preferred tissue for isotopic analysis, the tooth must be assessed for preservation quality. Fourier transform infrared spectroscopy (FTIR) is a commonly used tool in stable isotope science to define the mineral composition of a powdered sample material. Specific FTIR parameters can help to identify loss, alteration, and(or) addition of carbonate that may have affected original bioapatite isotopic composition. These parameters are discussed in the following section. If the tooth is well preserved with no addition or loss of material, no further special preparations are necessary prior to isotopic analysis. However, if the FTIR parameters indicate the presence of exogenous material (in the form of secondary carbonate deposits), the structural carbonate material is pretreated prior to isotopic analysis. Organic carbon from soils, bacteria, or even remnant proteins and lipids included in enamel pores, also need to be considered during the sample purification process.

Pretreatments are designed to target contaminant material (organic and mineral) that would otherwise contaminate original bioapatite  $\delta^{13}\text{C}_{\text{sc}}$  or  $\delta^{18}\text{O}_{\text{sc}}$ . Since the most common types of contaminants are already identified in the literature (secondary carbonate and residual organic material), most pretreatments involve a combination of acetic acid and sodium hypochlorite (bleach) or hydrogen peroxide to remove these phases. Controlled exposure to dilute acetic acid is intended to preferentially react with secondary carbonates that have smaller, more disorganized crystal structures, which are then removed in solution when the acid is rinsed away with Millipore water. Oxidizing solutions such as bleach and peroxides not only kill microbial organisms, but actively denature and destroy cell membranes, so that residual organic material breaks down and is more easily rinsed away.

Multiple pretreatment strategies have been developed by many investigators, but no clear consensus has been reached on the preferred approach or combination of approaches for removal of secondary carbonates and organic matter (see Snoeck and Pellegrini, 2015 for a comprehensive review). For any given study, it is likely best to conduct tests that not only determine the efficacy of the pretreatment procedure used for

removing contaminants, but also to determine if the pretreatment procedure results in changes to the original enamel (or bone)  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ .

The impact of various pretreatment protocols on enamel  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  has been described in several other studies. The results vary due to different preferred methods among laboratories, and differences in specimen *post-mortem* history. The following studies are of particular interest here as their methodologies are very similar to the pretreatment procedure employed in this study. Garvie-Lok et al. (2004) and Koch et al. (1997) documented isotopic shifts of approximately  $-1.5$  to  $-0.2$  ‰ for  $\delta^{13}\text{C}_{\text{sc}}$ , and  $+0.8$  to  $+1.0$  ‰ for  $\delta^{18}\text{O}_{\text{sc}}$  between untreated and pretreated aliquots of enamel. Garvie-Lok et al. pretreated using 0.1 M acetic acid, while Koch et al. pretreated using combined 2 % bleach and 0.1 M acetic acid. Pellegrini and Snoeck (2016) tested the impact of a wide variety of commonly used pretreatment strategies on  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  from bones and teeth. They employed a pretreatment of 2.5 % bleach and 0.1 M acetic acid (both at room temperature). They documented a shift of  $-0.5$  to  $+0.7$  ‰ for  $\delta^{13}\text{C}_{\text{sc}}$ , and  $-0.5$  to  $+1.6$  ‰ for  $\delta^{18}\text{O}_{\text{sc}}$  between untreated and pretreated aliquots of archaeological enamel. With these considerations in mind, this study was undertaken to document the isotopic shifts associated with the Laboratory for Stable Isotope Science's (LSIS) standard operating procedure for pretreatment of bioapatite structural carbonate from bone and enamel (Metcalf, 2004). FTIR parameters were measured before and after pretreatment to interpret *post-mortem* alteration to the enamel and the pretreatment's efficacy at removing carbonate contaminants, as evaluated using FTIR parameters.

FTIR was employed to measure the Crystallinity Index (CI) and carbonate-phosphate ratio (C/P) of eight enamel samples. CI describes the degree of *post-mortem* recrystallization that has occurred within the enamel bioapatite matrix. The crystal size and degree of crystal organization both increase as a result of recrystallization after the



**Figure 2.3** Fragmented *Castoroides* incisors. These fragments display the distinctive longitudinal enamel ridges.

**Image courtesy of FLMNH.**

death of an animal (Bartsiokas and Middleton, 1992; Munro et al., 2007; Olsen et al., 2008; Surovell and Stiner, 2001). Waterlogged depositional environments accelerate the rate of crystal reorganization (Stiner et al., 2001). The accepted CI for unaltered enamel ranges from 3.0 to 3.5. Values above 3.5 indicate crystal growth or substantial reorganization. The C/P ratio is indicative of the loss or addition of carbonate or phosphate material from/to the enamel matrix (Surovell and Stiner, 2001). The accepted C/P ratio of unaltered enamel is ~0.4 (Smith et al., 2007; Webb et al., 2014; Wright and Schwarcz, 1996). A lower ratio suggests *post-mortem* loss of carbonate. A C/P ratio higher than 0.5 indicates the presence of more carbonate than expected for normal bioapatite, which typically results from precipitation of secondary carbonates within the enamel matrix.

## 2.3.2 Methodology

### 2.3.2.1 Sampling Strategy

Enamel (Figure 2.3) from eight late Pleistocene *Castoroides* (giant beaver) incisors were analyzed in this study. Approximately 100 mg of enamel was removed from each tooth using a Dremel<sup>®</sup> tool fitted with a cutting wheel drill bit. The specimens were free of consolidant prior to sampling and any adhering dentin was removed using a Dremel<sup>®</sup>. Enamel samples were ground to between 45 and 63  $\mu\text{m}$  for structural carbonate analysis and FTIR. Enamel powder from each individual was further divided into two aliquots. Approximately 8 mg of sieved powder underwent no further preparatory work prior to isotopic analysis, while a further 10-20 mg of enamel powder was pretreated prior to isotopic analysis. Both the untreated and pretreated portions of the sample were analyzed using FTIR spectroscopy.

### 2.3.2.2 FTIR Methodology

Approximately 2 mg of untreated and treated aliquots of powdered enamel from each sample were combined with 200 mg of potassium bromide (KBr) powder and ground until it reached a pasty consistency. The samples were dried at 90°C for 24 to 48 hours, and then compressed in a hydraulic press at 10 tons for 10 minutes to form a solid pellet suitable for analysis. Pellets were scanned using a Bruker Vector 22 FTIR spectrometer. Background reference spectra were collected with an empty chamber prior to each sample analysis. Absorbance spectra were plotted from 400 to 2500  $\text{cm}^{-1}$ . For each absorbance spectrum collected, the baseline was corrected. Peak heights were measured at 565  $\text{cm}^{-1}$  ( $\text{PO}_4$ ), 605  $\text{cm}^{-1}$  ( $\text{PO}_4$ ), 1035  $\text{cm}^{-1}$  ( $\text{PO}_4$ ), and 1415  $\text{cm}^{-1}$  ( $\text{CO}_3$ ), and at the valley between 565 and 605  $\text{cm}^{-1}$  (approximately 590  $\text{cm}^{-1}$ ).

CI was calculated according to the following equation:

$$\text{CI} = (\text{A} + \text{B})/\text{C},$$

where (A) and (B) refer to the height of  $\nu_4$   $\text{PO}_4$  group peaks at 565  $\text{cm}^{-1}$  and 605  $\text{cm}^{-1}$ , respectively, and (C) refers to the height of valley located at 590  $\text{cm}^{-1}$  between these

peaks on a baseline-corrected spectrum (Munro, 2003; Webb et al., 2014; Weiner and Bar-Yosef, 1990; Wright and Schwarcz, 1996).

Carbonate-phosphate ratio was calculated using the following equation:

$$C/P = (X)/(Y),$$

where (X) and (Y) respectively refer to the absolute height of the CO<sub>3</sub> peak at 1415 cm<sup>-1</sup> and the PO<sub>4</sub> peak at 1035 cm<sup>-1</sup> on a baseline-corrected FTIR spectra (Munro, 2003; Smith et al., 2007; Wright and Schwarcz, 1996).

### 2.3.2.3 Pretreatment Methodology

The following pretreatment procedure was employed following Metcalfe's (2004) standard operating protocol for carbonate analysis of enamel and bone. Enamel powder was soaked in 2 % reagent-grade bleach (NaOCl) for 24 hours, rinsed five times in Millipore water, then soaked in 0.1 M acetic acid (C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>) for four hours, and then rinsed a further five times with Millipore water. Samples were promptly frozen to prevent isotopic exchange between the enamel powder and Millipore water. Once solid, the samples were placed in a freeze-drier for 24 to 48 hours until completely desiccated.

Approximately 0.9 mg of enamel powder from both pretreated and non-pretreated aliquots of sample was weighed into glass vials, and reacted with phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) under vacuum at 90°C for 20 minutes using a Micromass Multiprep autosampler. The isotopic composition of the CO<sub>2</sub> gas produced by the reaction of the sample and the acid was analyzed using a VG Optima dual-inlet IRMS. The stable isotope data were collected over a single analytical session. A series of internal and international standards were analyzed concurrently to create a calibration curve and to monitor instrument precision and accuracy. Values of δ<sup>13</sup>C were calibrated to VPDB using international standards NBS-19 (± 0.0 ‰ SD; n = 5; accepted δ<sup>13</sup>C = +1.95 ‰) and LSVEC (± 0.2 ‰ SD, n = 3; accepted δ<sup>13</sup>C = -46.6 ‰) (Coplen et al., 2006). Values of δ<sup>18</sup>O were calibrated to VSMOW using international standards NBS-18 (± 0.1 ‰ SD, n = 3; accepted δ<sup>18</sup>O = +7.20 ‰) and NBS-19 (± 0.1 ‰ SD, n = 5; accepted δ<sup>18</sup>O = +28.65

‰) (Coplen, 1996). Laboratory calcite standards WS-1 and Suprapur were used to test for accuracy of the calibration curve. WS-1  $\delta^{13}\text{C} = +0.5 \pm 0.4$  ‰ ( $n = 2$ ), which compares satisfactorily with its accepted  $\delta^{13}\text{C}$  of +0.76 ‰. WS-1  $\delta^{18}\text{O} = +26.0 \pm 0.2$  ‰, which compares well with its accepted  $\delta^{18}\text{O}$  of +26.23 ‰. Suprapur  $\delta^{13}\text{C} = -35.8$  ‰ ( $n = 1$ ), which compares well with its accepted  $\delta^{13}\text{C}$  of -35.55 ‰. Suprapur  $\delta^{18}\text{O} = +13.1$  ‰ ( $n = 1$ ), which compares well with its accepted  $\delta^{18}\text{O}$  of +13.1 ‰. Internal and international standards were analyzed every five samples. A minimum of one duplicate for every ten samples analyzed. For the five duplicate samples included, the average reproducibility was 0.04 ‰ for  $\delta^{13}\text{C}$  and 0.03 ‰ for  $\delta^{18}\text{O}$ .

### 2.3.3 Results

#### 2.3.3.1 Enamel $\delta^{13}\text{C}_{\text{sc}}$ and $\delta^{18}\text{O}_{\text{sc}}$

The stable carbon and oxygen isotope results are reported in delta ( $\delta$ ) notation, as described earlier, relative to the international standards VPDB and VSMOW, respectively. The isotopic results are presented in Table 2.2. There were statistically significant changes in  $\delta^{18}\text{O}_{\text{sc}}$  before and after pretreatment (paired t-test,  $p = 0.01$ ). Values of  $\delta^{13}\text{C}_{\text{sc}}$ , by comparison, did not vary significantly before and after pretreatment (paired t-test,  $p = 0.06$ ). The effect of pretreatment on  $\delta^{13}\text{C}_{\text{sc}}$  are discussed nonetheless, since changes in  $\delta^{13}\text{C}_{\text{sc}}$  between untreated and pretreated aliquots of some samples still exceeded the reproducibility typically obtained for replicate analysis of the identical powder.

After pretreatment,  $\delta^{18}\text{O}_{\text{sc}}$  increased on average by 1.0 ‰, while  $\delta^{13}\text{C}_{\text{sc}}$  decreased by an average of 0.4 ‰. The change between the  $\delta^{18}\text{O}_{\text{sc}}$  of untreated and pretreated samples ranged from +0.3 to +2.6 ‰. The change between the  $\delta^{13}\text{C}_{\text{sc}}$  of untreated and pretreated ranged from -1.2 to +0.1 ‰. Overall, pretreatment had a greater effect on  $\delta^{18}\text{O}_{\text{sc}}$  than  $\delta^{13}\text{C}_{\text{sc}}$ . Specimens collected from Alaska and Ohio (C15-TP2014 and C20-TP2014, respectively) demonstrated minimal alteration of either  $\delta^{13}\text{C}_{\text{sc}}$  or  $\delta^{18}\text{O}_{\text{sc}}$  after pretreatment. Specimens collected from Florida (C26-TP2014 to C33-TP2014) showed the greatest change in  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  after pretreatment.

Table 2.2  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of untreated and pretreated bioapatite structural carbonate from *Castoroides* enamel. Results shown in bold-faced font are the mean of duplicate analyses.

Sample No.	Origin	Untreated	Pretreated	Untreated	Pretreated
		$\delta^{13}\text{C}$ (‰, VPDB)	$\delta^{13}\text{C}$ (‰, VPDB)	$\delta^{18}\text{O}$ (‰, VSMOW)	$\delta^{18}\text{O}$ (‰, VSMOW)
C15-TP2014	Alaska	-14.2	-14.1	+15.2	+15.5
C20-TP2014	Ohio	-10.4	-10.7	+26.2	+26.6
C26-TP2014	Florida	-13.5	<b>-13.6</b>	+29.2	+29.8
C28-TP2014	Florida	-11.9	-12.1	+27.7	+29.6
C29-TP2014	Florida	<b>-12.7</b>	-13.6	+25.3	+26.4
C31-TP2014	Florida	-18.7	-19.9	+24.8	+25.3
C32-TP2014	Florida	-9.6	-9.8	+26.8	+29.3
C33-TP2014	Florida	<b>-10.8</b>	<b>-11.0</b>	+24.1	+25.3

### 2.3.3.2 FTIR Parameters

Overall, pretreatment had minimal effect on the CI and C/P ratio of these enamel samples (Table 2.3). Neither parameter showed statistically significant alteration after pretreatment (t-test, CI p-value = 0.8; C/P p-value = 0.1). The average CI for untreated enamel was  $3.5 \pm 0.2$ , and the average C/P ratio was  $0.15 \pm 0.0$ . After pretreatment, the average CI remained  $3.5 \pm 0.2$ , and the average C/P ratio increased slightly to  $0.18 \pm 0.0$ .

Table 2.3 FTIR parameters (CI and C/P ratio) for bioapatite structural carbonate from *Castoroides* enamel.

Sample No.	Origin	Untreated	Pretreated	Untreated	Pretreated
		FTIR CI	FTIR CI	FTIR C/P	FTIR C/P
C15-TP2014	Alaska	3.4	3.4	0.17	0.18
C20-TP2014	Ohio	3.7	3.4	0.12	0.17
C26-TP2014	Florida	3.7	3.4	0.13	0.20
C28-TP2014	Florida	3.4	3.3	0.17	0.20
C29-TP2014	Florida	3.4	3.6	0.17	0.19
C31-TP2014	Florida	3.5	3.5	0.18	0.17
C32-TP2014	Florida	3.7	3.9	0.14	0.15
C33-TP2014	Florida	3.5	3.6	0.16	0.15

The CI of all samples prior to pretreatment was 3.4 or higher. Pretreatment did not alter CI in a consistent manner. After pretreatment, the CI of some samples increased above the acceptable limit for unaltered enamel ( $>3.5$ ). In other samples, CI decreased after pretreatment. The C/P ratio for all samples did not differ significantly ( $\leq 0.2$ ) before



or after pretreatment and remained below the acceptable value of  $\sim 0.4$ . A peak at  $710\text{ cm}^{-1}$ , indicating the presence of calcite ( $\text{CaCO}_3$ ), was not observed in the FTIR spectra for any of the samples before or after pretreatment.

#### 2.3.4 Discussion

The pretreatment method employed in this study did result in some changes in the stable carbon and oxygen isotopic compositions of bioapatite structural carbonate from enamel. Pretreatment produced greater changes in  $\delta^{18}\text{O}_{\text{sc}}$  than  $\delta^{13}\text{C}_{\text{sc}}$ . This was as expected, since  $\delta^{18}\text{O}_{\text{sc}}$  is documented to be more vulnerable to diagenetic modification than  $\delta^{13}\text{C}_{\text{sc}}$ . The fluids (acetic acid, bleach, Millipore water) to which the enamel was exposed during pretreatment are effectively another avenue for possible *post-mortem* alteration prompted by fluid-bioapatite interaction. The degree of change in isotopic compositions is similar to that documented by Garvie-Lok et al. (2004), Koch et al. (1997), and Pellegrini and Snoeck (2016), amounting to a small  $<\pm 1\text{ ‰}$  shift in  $\delta^{13}\text{C}_{\text{sc}}$ , and approximately  $+1\text{ ‰}$  shift in  $\delta^{18}\text{O}_{\text{sc}}$ . These average results, however, provide only a guideline for possible isotopic differences following pretreatment of archaeological and palaeontological enamel. The  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  of enamel do not vary in a consistent manner even when all samples undergo the same pretreatment method. The preservation of the individual tooth specimen and its prior exposure(s) to diagenetic fluids dictate how it will behave. If there is sufficient sample material from each specimen, it is best to always compare the  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  of untreated and pretreated aliquots.

The FTIR parameters (CI and C/P ratios) suggest that these *Castoroides* teeth were not exceptionally well-preserved. The CI for all samples before and after pretreatment was near or above the limit of 3.5 for well-preserved enamel. High CI values indicate that the enamel bioapatite has undergone recrystallization (Wright and Schwarcz, 1996). Crystal size and degree of organization have both increased as a result of *post-mortem* processes. Any increase to CI after pretreatment is likely the result of the dilute acetic acid solution removing smaller, less organized bioapatite crystals from the enamel matrix. The low C/P ratios ( $\leq 0.2$ ) documented for all samples strongly suggest that a large portion of enamel structural carbonate was lost from the bioapatite during

*post-mortem* processes. A low C/P ratio can also hypothetically indicate the *post-mortem* addition of phosphate material. The small average increase to C/P ratio after pretreatment suggests that the method either further removed phosphate or added carbonate material.

In the context of these *Castoroides* specimens, the FTIR parameters point to structural carbonate loss in the depositional environment. All specimens, with the exception of C20-TP2014, were collected from gravel riverbeds. The majority of *Castoroides* specimens across North America have been discovered in active river deposits, or excavated from sediments indicative of past alluvial or lacustrine environments. The semi-aquatic nature of the giant beaver meant that most animals died in, or in proximity to water. Long-term deposition of the skeletal remains in waterlogged environments facilitates water-bioapatite interaction, and attendant processes such as structural carbonate dissolution or isotopic exchange between the structural carbonate and the water. The specimens from Florida, in particular, originate from depositional conditions (waterlogged environment and year-round warm temperatures) that facilitate mineral dissolution and oxygen isotopic exchange.

The pretreatment process is presumed to have removed additional structural carbonate from the enamel bioapatite, as the FTIR spectral data, CP ratios and CI values of untreated specimens did not indicate the presence of secondary calcite (CaCO<sub>3</sub>) or other secondary carbonates. C/P ratios >0.5, or CI <3.0 strongly suggest addition of secondary carbonate, neither of which was observed (Table 2.3). The dilute acetic acid used in pretreatment is intended to preferentially target the smaller, more disorganized secondary carbonate crystals adsorbed onto the enamel surface; however, if these are absent, the acid begins to dissolve the bioapatite instead, unless the acid has been buffered in some fashion to prevent this occurrence.

The combined use of bleach and dilute acetic acid had a small effect on *Castoroides* enamel  $\delta^{13}\text{C}_{\text{sc}}$ . Bleach is proven to be highly effective at removing organic material from bioapatite (Pellegrini and Snoeck, 2016), but it should only be used when necessary. The use of bleach facilitates the dissolution of atmospheric CO<sub>2</sub> and its subsequent conversion to ions that form calcium carbonate (Pellegrini and Snoeck, 2016).

This results in the precipitation of secondary carbonates on the enamel powder during pretreatment. Acetic acid is the second step in pretreatment and should remove most of these freshly adsorbed secondary carbonates, but there is no guarantee (Pellegrini and Snoeck, 2016). The utility of bleach is therefore questionable. Organics do not typically react with the phosphoric acid during isotopic analysis, and therefore do not contribute to the final  $\delta^{13}\text{C}$ . If the enamel sample is well-preserved to begin with, bleach can cause unnecessary changes to  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ . Bleach treatment is best applied to bones (higher porosity and organic content), or to specimens excavated from organic-rich, active soil layers.

### 2.3.5 Conclusions

The pretreatment methods employed in this study do result in changes to enamel  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ . The changes in isotopic composition, however, are generally minimal ( $< \pm 1$  ‰ for  $\delta^{13}\text{C}_{\text{sc}}$  and  $\sim +1$  ‰ for  $\delta^{18}\text{O}_{\text{sc}}$ ), depending on the individual tooth specimen's *post-mortem* history. This outcome is consistent with the findings of earlier studies (Garvie-Lok et al., 2004; Koch et al., 1997; Pellegrini and Snoeck, 2016), and supports the conclusion that original  $\delta^{13}\text{C}_{\text{sc}}$  is better preserved during *post-mortem* processes, diagenesis *and* chemical pretreatment. The chemical pretreatment method may be effective at removing secondary calcite; however, in its absence, enamel structural carbonate may be partially dissolved. More generally, poor sample preservation (i.e. loss of structural carbonate material) cannot be fully remedied using the pretreatment methods described in the current study.

The effects of the pretreatment methods employed in this study do not change original  $\delta^{13}\text{C}_{\text{sc}}$  enough to confound dietary interpretations based on tooth enamel structural carbonate. The  $\delta^{13}\text{C}$  of  $\text{C}_3$  terrestrial plants typically range from  $-35$  to  $-20$  ‰, while  $\delta^{13}\text{C}$  of  $\text{C}_4$  plants ranges from  $-14$  to  $-9$  ‰ (Heaton, 1999; O'Leary, 1988; Smith and Epstein, 1971; Tieszen, 1991). Hence a shift of  $\pm 1$  ‰ in  $\delta^{13}\text{C}_{\text{sc}}$  is insufficient to confuse the isotopic dietary signal of an animal consuming a predominantly  $\text{C}_3$  versus  $\text{C}_4$  diet. A shift of  $\pm 1$  ‰ however, would modify the projected proportions of forage sources

in the diet of a mixed-feeder herbivore predicted by various Bayesian isotopic mixing models such as SIAR (Inger et al., 2010).

Some researchers prefer to pretreat all bioapatite samples, regardless of preservation quality, in order to maintain the principle of identical treatment among samples and projects. We suggest that the need for pretreatment should be determined on a study-specific basis. Robust testing of untreated versus pretreated samples, including a thorough investigation of crystallinity, C/P ratios, and potential contaminants (e.g., secondary calcite) of the specimens in question is recommended. Other innovative techniques, such as various microscopies, trace element analysis, advanced infrared and X-ray methods, triple O-isotope measurements, and multiply substituted clumped isotope measurements should also be investigated by the intrepid researcher, as all of these methods have potential to provide further evidence for or against *post-mortem* and diagenetic alteration, and associated changes in structural carbonate isotopic compositions.

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## Chapter 3

# Isotopic Variability in Aquatic Plants from Yukon Territory and Ontario

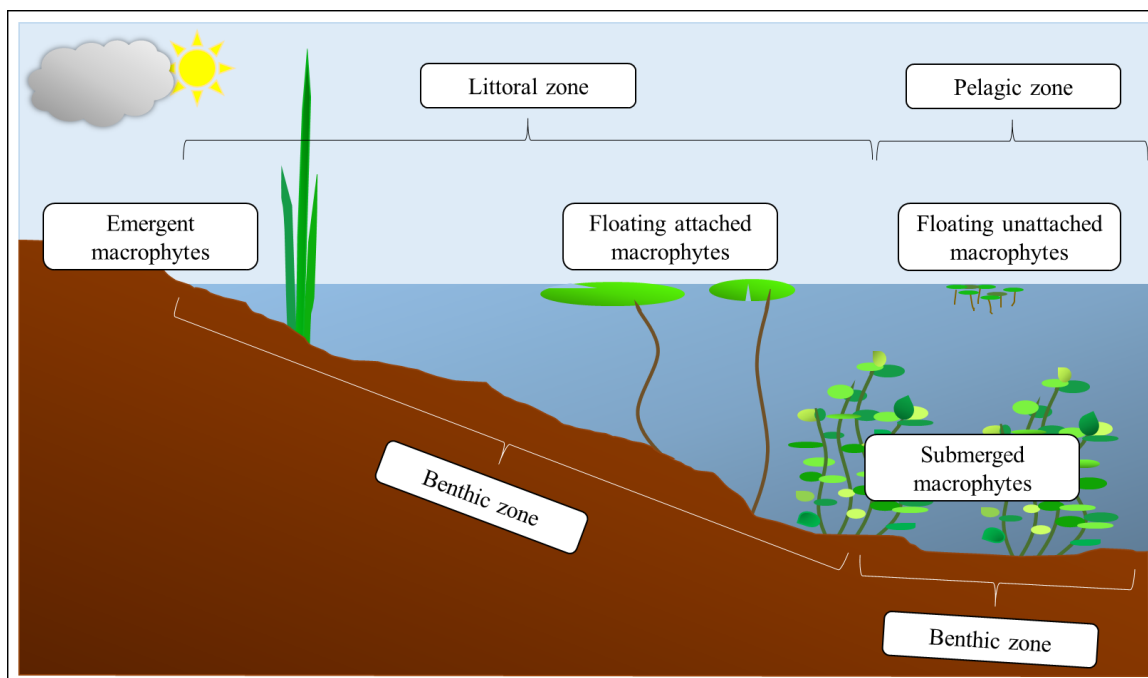
### 3.1 Chapter Overview

Stable carbon and nitrogen isotopes are used to trace nutrient flow and trophic position of organisms within a food web (DeNiro and Epstein, 1978; 1981). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of a consumer, however, cannot be interpreted without the context of a dietary baseline comprising the isotopic composition of available or known food sources. In order to assess *Castoroides*' forage preference and ecological niche, it was necessary to create a dietary baseline consisting of aquatic and terrestrial plant isotopic compositions. Freshwater plant carbon and nitrogen isotopic composition is known to vary with latitude, local geology, and local habitat conditions. In order to create an accurate baseline, plant samples were collected from multiple temperate and subarctic sites where *Castoroides* specimens had also been found.

Interpretation of giant beaver diet using the dietary baseline is discussed in Chapter 4. This chapter explores variability in plant isotopic composition: specifically why freshwater macrophytes are distinct in isotopic composition from terrestrial browse, and how macrophyte  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  vary according to habitat division and physiology.

### 3.2 Introduction

Stable carbon and nitrogen isotope dynamics in freshwater systems are more complex and variable than those of terrestrial systems, and consequently macrophytes exhibit greater isotopic variability than do terrestrial plants (Casey and Post, 2011; Cloern et al., 2002; Farquhar et al., 1989; France, 2008; Keeley and Sandquist, 1992; Kendall et al., 2001; LaZerte and Szalados, 1992; Mendonça et al., 2013; Milligan and Humphries, 2010; Osmond et al., 1981; Reynolds, 2008; Severud et al., 2013). Local environmental conditions and macrophyte physiology account for broad-scale variability, while microhabitat and differing access to bioavailable carbon and nitrogen pools account for



**Figure 3.1 Stylized profile view of freshwater macrophyte habitat division. Aquatic ecosystems are spatially divided into the littoral (near-shore), pelagic (open-water), and benthic (substrate-dwelling) zone.**

further inter- and intra-specific isotopic variation. As a result, mean macrophyte  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can differ considerably from site to site (Casey and Post, 2011; Keeley and Sandquist, 1992; Mendonça et al., 2013). To avoid confounding interpretations concerning aquatic food web dynamics, individual isotopic baselines must be established for each study (Casey and Post, 2011 and references therein). A study-specific baseline helps account for variability between wetland habitat types (i.e. lake, river, marsh), or geographic regions (i.e. latitude, altitude, biome). A baseline tailored to a particular region can also account for anthropogenic activities that change the baseline  $\delta^{15}\text{N}$  and render the data incompatible for palaeoecological investigations.

### 3.2.1 Plant Sample Collection

Aquatic plant samples were collected from small lake, pond, and river/channel habitats. Foliage samples from terrestrial trees and shrubs growing in varying proximity to the water's edge were also collected. Multiple samples of each species were taken to

best represent the vascular plant diversity found at each location. Plant samples were collected from the Great Lakes Basin (southern Ontario) region and from Yukon Territory, including regions that once comprised unglaciated Beringia. Lakes and ponds were targeted because they are the proposed habitat preferred by the genus *Castoroides*. These habitats would have provided adequate space, protection from terrestrial predators, and a sufficient supply of freshwater macrophytes (one of the hypothesized diets of the giant beaver) (Lovegrove and Mowoe, 2013; Stirton, 1965). The sample collection sites include both temperate and subarctic regions of North America that currently support modern muskrat and beaver populations, in addition to yielding fossil evidence of giant beavers.

There are many challenges to establishing a comparative dietary baseline for an extinct species that lived during an epoch where biomes and plant communities differed greatly from the present day. The lack of preservation and/or the inaccessibility of Pleistocene palaeobotanical material from the geographic regions of interest rendered it necessary to use modern plant samples for this study. The isotopic baseline presented in this chapter is not a perfect analog for Pleistocene wetlands, but it is more accurate than one created solely from the existing body of literature – without consideration of the geographic origin of the giant beaver specimens analyzed for this study.

### 3.2.2 Macrophyte Habitat Division

Macrophytes collected to create the baseline were categorized based on habitat division within a wetland, and not by phylogenetic relationship. The three macrophyte categories are submerged, emergent, and floating (Figure 3.1). Each category (submerged, emergent, floating, and terrestrial) is defined by the plant's differing level of exposure to the atmosphere, the water column, and the sediment/soil substrate (Figure 3.1). These three mediums control macrophyte access to bioavailable carbon and nitrogen pools, which has a strong impact on their overall isotopic composition. Based on these habitat divisions and preexisting literature on aquatic macrophytes (Boon and Bunn, 1994; Fry, 1991; Keeley and Sandquist, 1992; Milligan and Humphries, 2010), we expected each category to be distinct in isotopic composition. Terrestrial plant samples

comprised trees and shrubs that employ a C<sub>3</sub>-photosynthetic pathway. All terrestrial plants are included in a single category.

### 3.2.3 Sources of Isotopic Variability in Freshwater Macrophytes

Macrophyte  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are controlled by: (i) access to bioavailable carbon or nitrogen sources, (ii) the isotopic composition of each source, and (iii) the isotopic fractionation associated with access to, or uptake of each source. The following section explores the carbon and nitrogen sinks and sources present in aquatic and terrestrial habitats, as well as associated isotopic variability due to environmental conditions (see Figures 3.3 through 3.6).

#### 3.2.3.1 Bioavailable Carbon in Terrestrial and Freshwater Systems

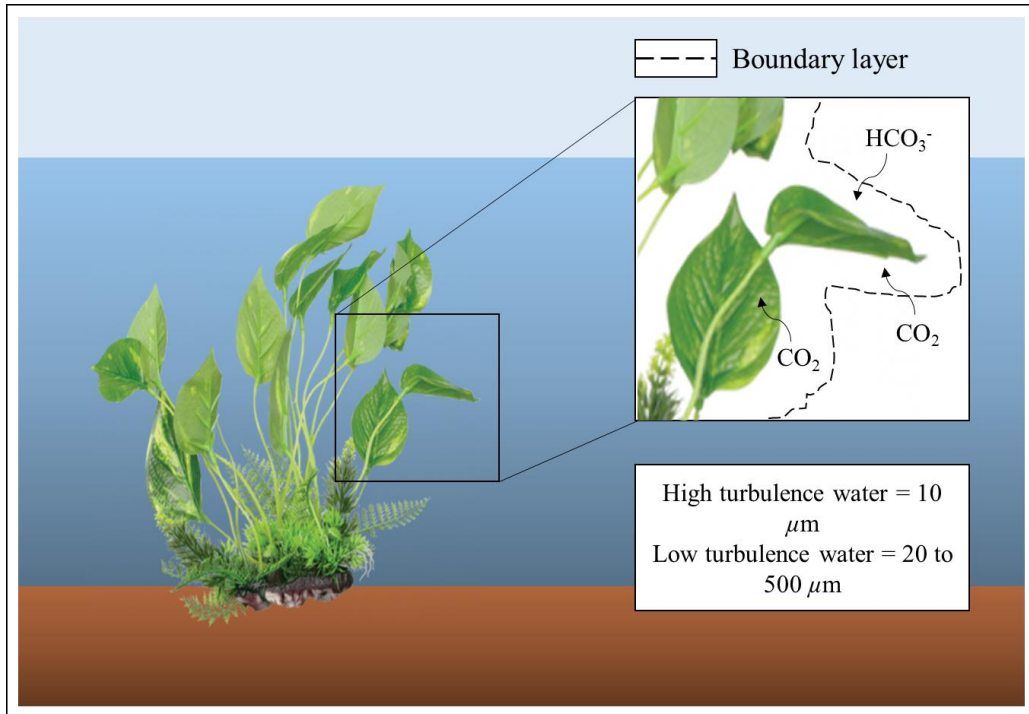
Carbon is a key component of photosynthesis and is vital to all plant growth and survival. Freshwater aquatic systems have different carbon sources and sinks than do terrestrial systems (Figures 3.3 and 3.4). A source represents a form of the element that is fully accessible to photosynthesis using organisms, whereas a sink represents an inaccessible form. In terrestrial systems, the greatest carbon source available to plants is atmospheric CO<sub>2</sub>, and to a lesser degree, respired CO<sub>2</sub> (Smith and Epstein, 1971). In aquatic systems, plants may obtain carbon from an array of sources. Emergent and floating macrophytes with aerial leaves rely primarily on atmospheric CO<sub>2</sub>. Macrophytes with leaves growing partially or completely submerged in water can access dissolved (atmospheric and respired) CO<sub>2</sub>, as well as dissolved bicarbonate (HCO<sub>3</sub><sup>-</sup>) derived from the dissolution of carbonate sediments (Allen and Spence, 1981; Keeley and Sandquist, 1992; Smith and Walker, 1980). Each of these carbon sources possesses a different average isotopic composition, and some submerged macrophytes may uptake carbon from a combination of all three. The relative abundances of the different carbon source(s) incorporated during photosynthetic activity determine the  $\delta^{13}\text{C}$  of plant tissue.

As an adaptation to a life spent completely underwater, many submerged macrophytes are opportunistic and alternate between carbon sources when necessary to sustain photosynthesis. Dissolved HCO<sub>3</sub><sup>-</sup> is inaccessible to other plant life and is only

known to be a carbon source for submerged macrophytes. Macrophyte species from multiple genera (i.e. *Myriophyllum*, *Hydrilla*, *Vallisneria*, *Potamogeton*, *Hippuris*, *Fontinalis*, and *Ranunculus*) possess the ability to uptake  $\text{HCO}_3^-$  in addition to  $\text{CO}_2$  (Allen and Spence, 1981; Keeley and Sandquist, 1992). This is advantageous in an aquatic environment where the influx of dissolved  $\text{CO}_2$  from the atmosphere is spatially and temporally inconsistent (Keeley and Sandquist, 1992; Smith and Walker, 1980). The  $\delta^{13}\text{C}$  of submerged macrophytes is determined by the presence of this physiological adaptation and by the availability of  $\text{CO}_2$  versus  $\text{HCO}_3^-$ . These carbon sources have distinct isotopic compositions. The  $\delta^{13}\text{C}$  of  $\text{HCO}_3^-$  derived from marine carbonate rock dissolution is typically  $\sim 0$  to  $+1$  ‰. In contrast, the  $\delta^{13}\text{C}$  of  $\text{CO}_2$  derived from aquatic plant respiration and from the atmosphere are  $-27$  ‰ and  $-8$  ‰, respectively (Keeley and Sandquist, 1992).

Regardless of this adaptation, all macrophytes preferentially uptake dissolved  $\text{CO}_2$  if it is accessible. Submerged species capable of utilizing  $\text{HCO}_3^-$  will do so only when photosynthetic rate and the demand for carbon exceed  $\text{CO}_2$  availability in the boundary layer (Figure 3.2). The boundary layer is the microscopic layer of still water (or still air, for terrestrial plants) that surrounds the leaf and stem tissues, separating the plant from the rest of the water column (Keeley and Sandquist, 1992; Smith and Walker, 1980). Nutrients and dissolved gases must diffuse into this layer in order to become accessible to the plant. The rate at which  $\text{CO}_2$  diffuses into the boundary layer is a product of water turbulence. Rapidly flowing waters, such as river or stream environments, typically have high diffusion rates. In comparison, the calm waters of shallow lakes or ponds have low diffusion rates.

Diffusion rate impacts the fractionation of carbon isotopes during photosynthesis. If diffusion rates are low and  $\text{CO}_2$  in the boundary layer is replenished very slowly, the plant must choose from a finite carbon pool and ceases to discriminate against  $^{13}\text{CO}_2$  in an attempt to sustain photosynthetic rate (Keeley and Sandquist, 1992). As a result, the tissues of submerged macrophytes living in slow-moving waters are relatively  $^{13}\text{C}$ -



**Figure 3.2 Depiction of the boundary layer around macrophyte foliage. The boundary layer refers to the unstirred water surrounding leaf or stem tissues. Dissolved gases and nutrients must diffuse out of the water column and through this layer before they become accessible to the plant. The thickness of the boundary layer is a factor of water turbulence. Diagram not to scale.**

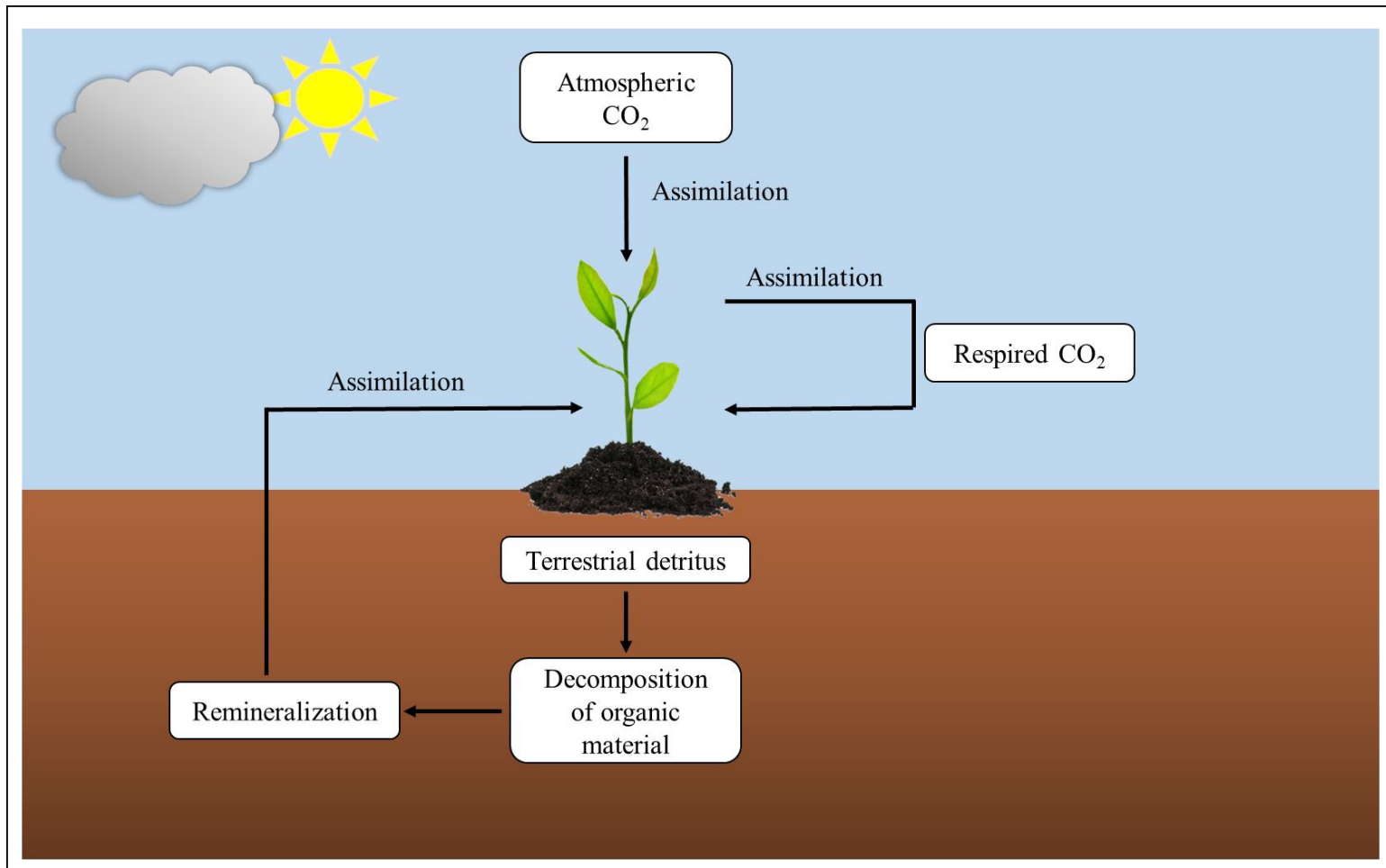
enriched (regardless of the ability to uptake  $\text{HCO}_3^-$ ) (Fogel and Cifuentes, 1993; Goericke and Fry, 1994; Smith and Walker, 1980).

The overall concentration of dissolved  $\text{CO}_2$  in the water column is determined by water temperature and pH. Cold water temperatures and low pH are associated with high concentrations of dissolved  $\text{CO}_2$  (MacLeod and Barton, 1998; Mendonça et al., 2013; Michener and Kaufman, 2007). Thus, the availability of dissolved  $\text{CO}_2$  in the boundary layer for submerged or partially submerged macrophytes is controlled by both the concentration of  $\text{CO}_2$  in the water column and the diffusion rate.

Waters with high pH, low concentration of  $\text{CO}_2$ , and a high concentration of dissolved  $\text{HCO}_3^-$  will prompt macrophytes that are physiologically capable to switch

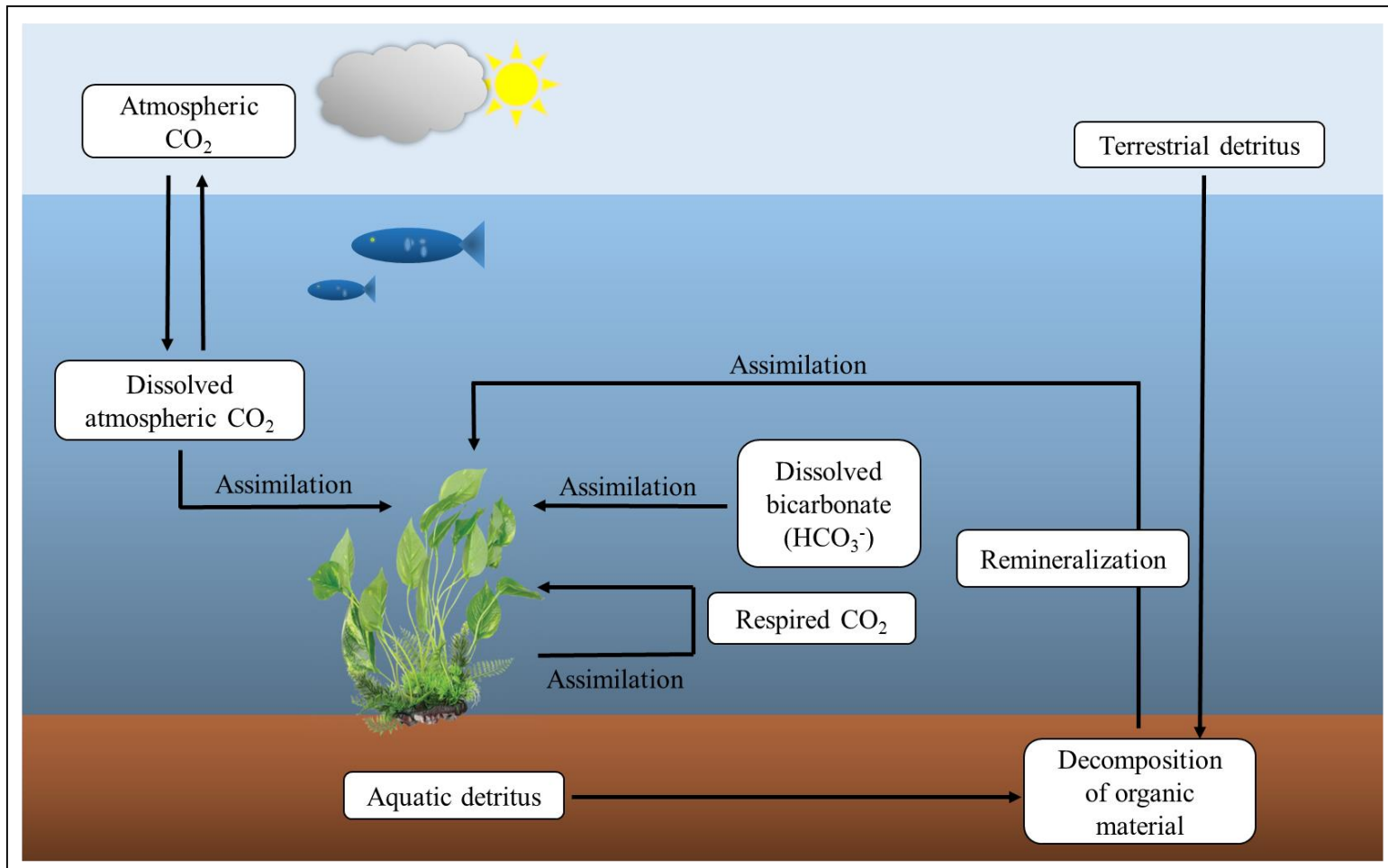
uptake pathways. The utilization of  $\text{HCO}_3^-$  instead of  $\text{CO}_2$  as a carbon source is, however, less cost-effective. Diffusion of  $\text{CO}_2$  gas into the leaf occurs by passive transport, whereas assimilation of  $\text{HCO}_3^-$  molecules requires active transport and energy output from the plant (Jones, 2005). The increased energy cost associated with  $\text{HCO}_3^-$  use results in decreased photosynthetic capacity (~10 %) (Allen and Spence, 1981). The compensation point at which  $\text{HCO}_3^-$  becomes necessary varies among plant genera. Macrophytes capable of employing both uptake pathways alternate as water conditions change throughout the day.

$\text{CO}_2$  produced from remineralized organic material may also influence the  $\delta^{13}\text{C}$  of aquatic macrophytes. Remineralization occurs when organic material decays, releasing inorganic carbon compounds back into the system (Figure 3.7). Organic material present in a wetland habitat can be either terrestrial or aquatic in origin. Terrestrial detritus possesses a lower  $\delta^{13}\text{C}$  that reflects that of terrestrial  $\text{C}_3$  plants (approximately  $-26\text{‰}$ ). The influx of terrestrial detritus into aquatic systems is greater in regions with steep surrounding topography, abundant forests, and wet climates. Leaf matter is especially common. In contrast, local detritus originating from within the aquatic habitat reflects the carbon isotopic composition of the dominant macrophyte type(s). This results in  $^{13}\text{C}$ -enriched detritus in wetlands abundant in submerged macrophytes, and  $^{13}\text{C}$ -depleted detritus (similar to a terrestrial signature) in wetlands dominated by emergent macrophytes. The dominant source of detritus (terrestrial or aquatic) impacts the  $\delta^{13}\text{C}$  of living macrophytes.



**Figure 3.3 Bioavailable carbon sources in terrestrial systems. Plant graphic from Newdesignfile.com.**





**Figure 3.4 Bioavailable carbon sources in freshwater systems. Plant graphic from Fluvalaquatics.com.**

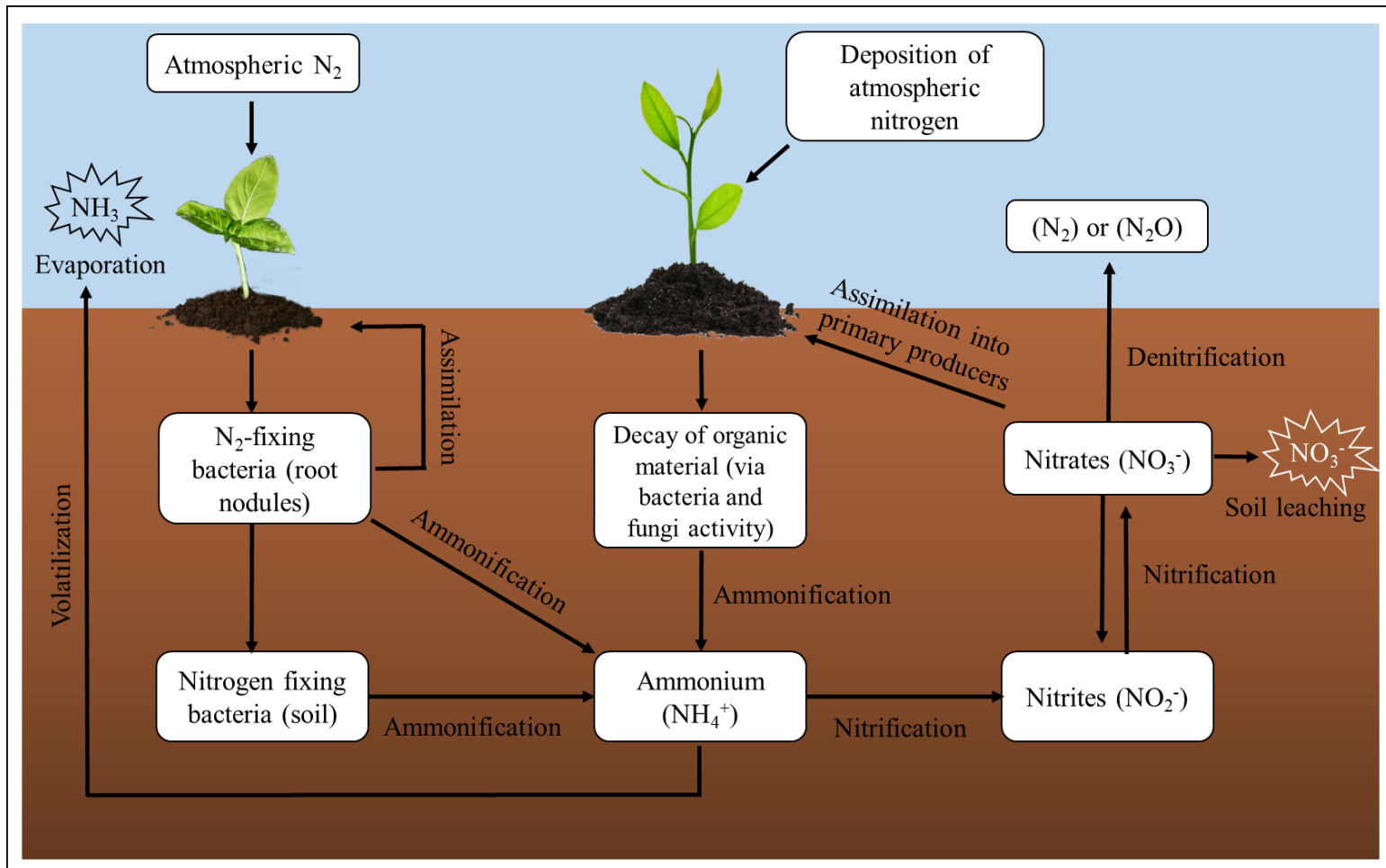


Figure 3.5 The stages of the nitrogen cycle in terrestrial systems. Plant graphic from Newdesignfile.com.

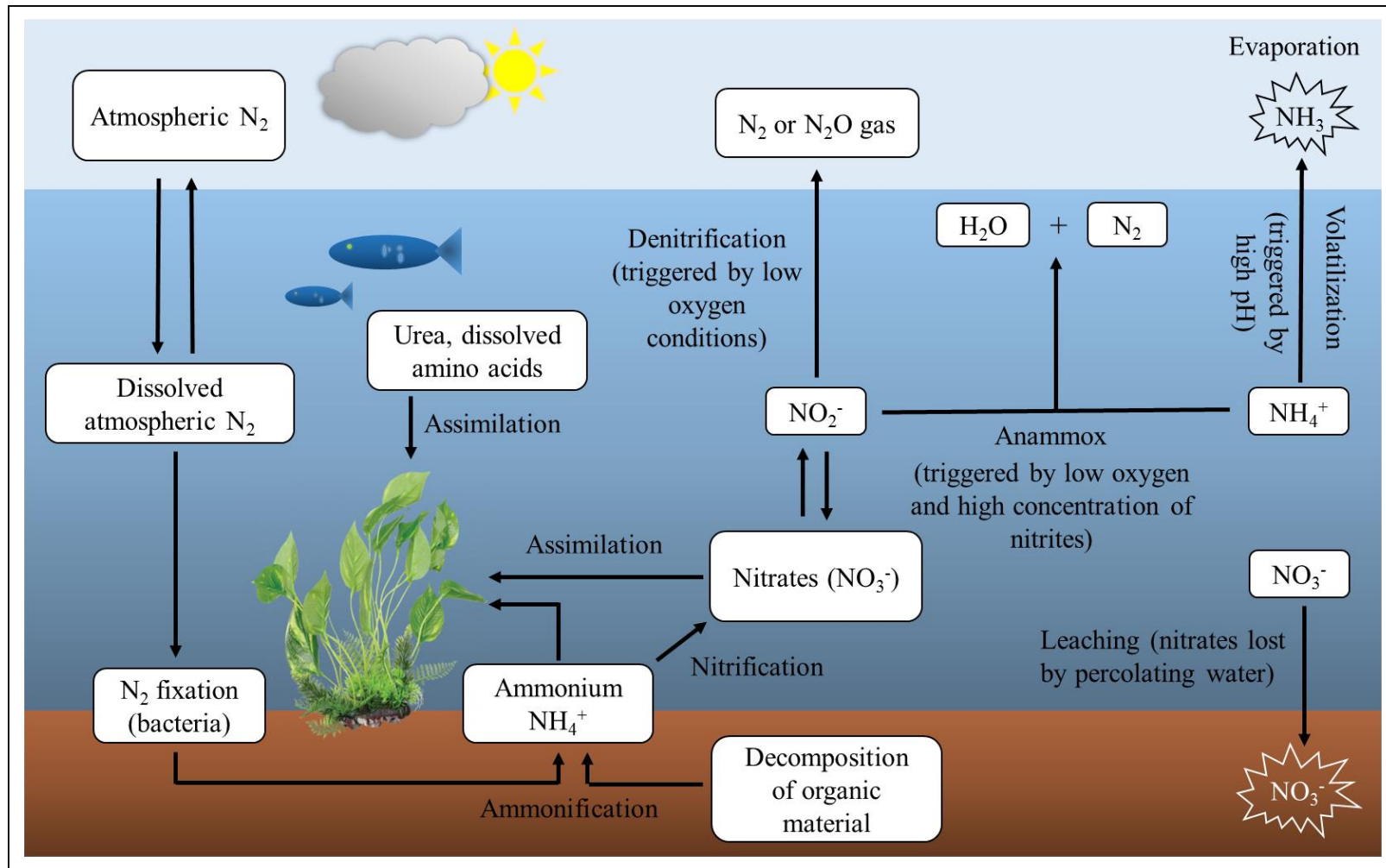


Figure 3.6 The stages of the nitrogen cycle in freshwater systems. Plant graphic from Fluvalaquatics.com.

### 3.2.3.2 Bioavailable Nitrogen in Terrestrial and Freshwater Systems

Nitrogen is essential to vascular plant growth, development, and reproduction. Despite being one of the most abundant elements on the planet, very few forms of nitrogen are bioavailable to plants. Nitrogen is an essential component of chlorophyll, structural proteins, and DNA; however, nitrogen stored in the Earth's crust and atmosphere is not directly accessible by plants. As a result, nitrogen is a limiting factor for plant growth in all environments. The vast majority of plants rely on microbial activity to supply them with usable forms of inorganic nitrogen (also referred to as "fixed" nitrogen). Bacteria are responsible for the majority of the stages of the nitrogen cycle in terrestrial and aquatic environments. The same forms of bioavailable nitrogen are used by all plant life (i.e. nitrites, nitrates, ammonia, ammonium, urea, amino acids). The specific conditions of individual aquatic and terrestrial habitats determine the prevalence of each compound, and its respective stable nitrogen isotopic composition. For a comprehensive review of the stages of the nitrogen cycle, its history, and the impact of anthropogenic activities, see Canfield et al. (2010), Kendall (1998), Sprent (1987), and Vitousek et al. (1997).

The nitrogen cycle is fueled primarily by atmospheric  $N_2$ , and is driven by bacteria and the movement of water. Whether on land or in the water, the nitrogen cycle begins with  $N_2$  fixation (Figure 3.5 and 3.6). Symbiotic rhizobia bacteria associated with plant root nodules convert  $N_2$  gas into ammonia compounds. The bacteria receive energy through this reaction, and the host plant receives bioavailable nitrogen. The rate at which nitrogen-fixing bacterial processes occur increases with temperature. In warm climates, various stages of the nitrogen cycle are accelerated in the soil or in the water column. Atmospheric  $N_2$  is currently used as the international isotopic standard, with a  $\delta^{15}N$  of 0 ‰. There is a small associated fractionation factor, where the  $\delta^{15}N$  of organic matter created using fixed atmospheric  $N_2$  lies between  $-2$  and  $+2$  ‰ (Brandes and Devol, 2002, Dähnke and Thamdrup, 2013; Delwiche and Steyn, 1970; Hoering and Ford, 1960). In addition to legumes, some genera of trees are also capable of nitrogen fixation. Nitrogen-fixing trees increase the fertility of surrounding soils by exuding a small percentage of

reactive nitrogen through their root systems (Uselman et al., 1999). Other types of symbiotic relationships have evolved to enable plants to obtain bioavailable nitrogen. Many forest plants form symbiotic relationships with fungi in the soil known as mycorrhizae. These decomposers release ammonia ( $\text{NH}_3$ ) that is depleted of  $^{15}\text{N}$  relative to the organic matter in the soil and on the forest floor (Uselman et al., 1999).

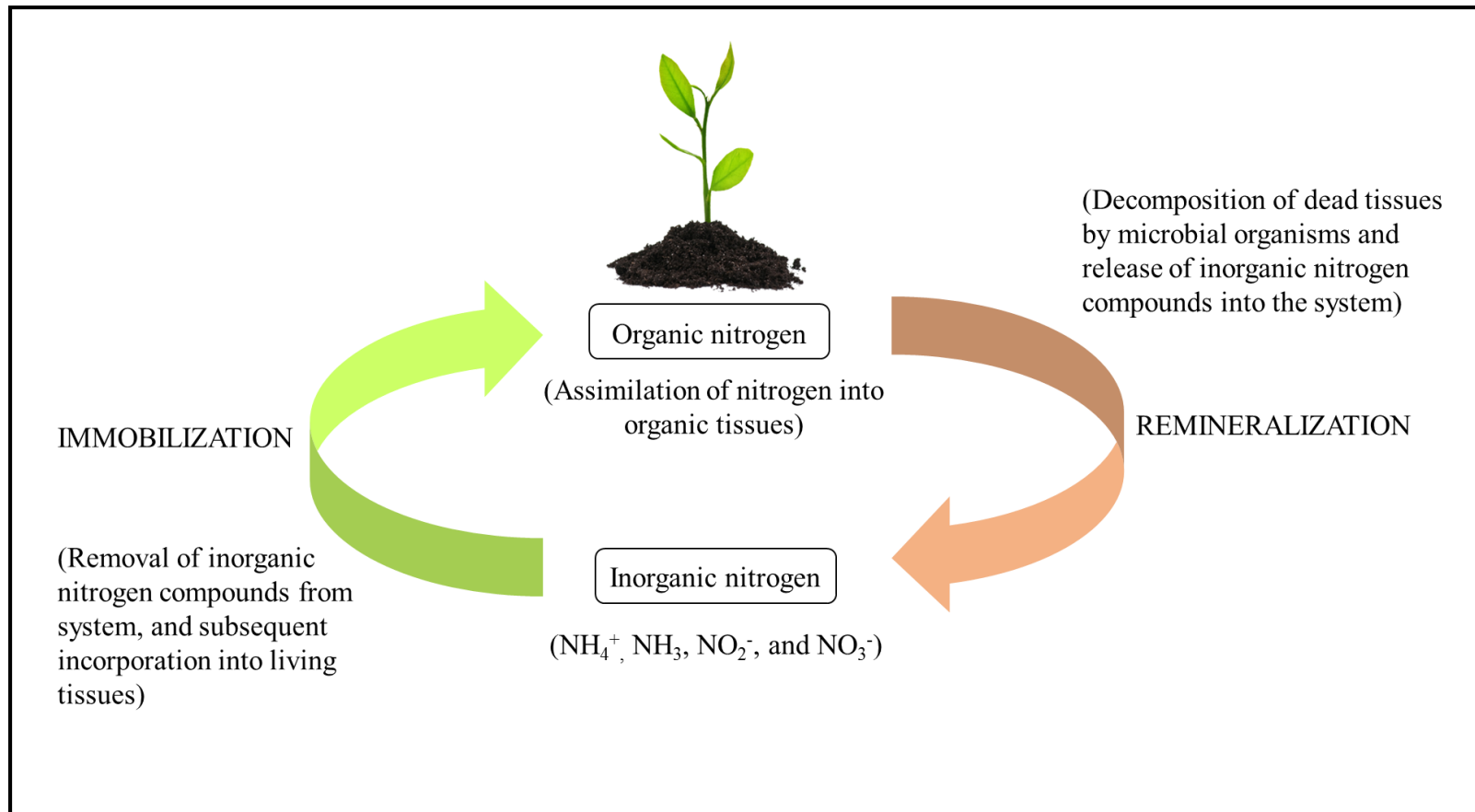
Another major stage of the nitrogen cycle is nitrification. Nitrifying bacteria (*Nitrosomonas* or *Nitrobacter*) drive the oxidation of ammonia and ammonium ( $\text{NH}_3$  and  $\text{NH}_4^+$ ) in aerobic environments, successively producing nitrites ( $\text{NO}_2^-$ ) and nitrates ( $\text{NO}_3^-$ ) (Sprent, 1987). Nitrates, like all mineral nutrients are readily dissolved and absorbed through plant root and foliage systems (Cedergreen and Madsen, 2002; Marschner, 1995). The  $\text{NH}_3$  and  $\text{NH}_4^+$  utilized in the formation of nitrates are typically formed by nitrogen-fixing bacteria, as a byproduct of organic decomposition, or atmospheric nitrogen deposition. Nitrifying bacteria preferentially use compounds containing  $^{14}\text{N}$ . As a result, the remaining pool of ammonium becomes progressively more  $^{15}\text{N}$ -enriched, while the initial product ( $\text{NO}_3^-$ ) is  $^{15}\text{N}$ -depleted relative to the reactant. The magnitude of the isotopic fractionation between the reactant ( $\text{NH}_4^+$ ) and product ( $\text{NO}_3^-$ ) is dependent on the nitrifying bacteria completing the reaction. *Nitrosomonas*-driven reactions are slow and result in an enrichment factor of 12 to 29 ‰. *Nitrobacter*-driven reactions occur very quickly and produce very little fractionation, with little to no difference between reactant and product  $\delta^{15}\text{N}$  (Kendall, 1998; Kendall et al., 2007; Sprent, 1987).

While nitrification creates a bioavailable nitrogen source, the process of denitrification removes nitrate and creates a nitrogen sink (Zumft, 1997). Nitrate is reduced by denitrifying bacteria to produce oxygen and nitrous oxide ( $\text{N}_2\text{O}$ ) or dinitrogen gas ( $\text{N}_2$ ), which are readily dissolved in water or lost to the atmosphere. Low oxygen or anoxic conditions trigger the denitrification process, as bacteria search for a source of oxygen. As a result, denitrification is common in aquatic systems, particularly in warm eutrophic lakes and ponds. Denitrifying bacteria preferentially use  $^{14}\text{NO}_3^-$ , so the remaining nitrate pool becomes progressively more  $^{15}\text{N}$ -enriched as low oxygen conditions persist (Ambrose, 1991; Kendall, 1998; Lehman et al., 2003; Shearer and Kohl, 1986). Similar to nitrification, the speed at which nitrate is converted to free

oxygen and N<sub>2</sub> gas has an impact on the degree of isotopic fractionation between reactant and product. Denitrification can produce very large isotopic fractionations (up to 30 ‰) and nitrate pools with a very high  $\delta^{15}\text{N}$  (Mariotti et al., 1981; Mengis, 1999). Slow denitrification or partial use by bacteria of the available nitrate pool results in a large isotopic fractionation, while rapid denitrification of small-scale nitrate pools typically result in little to no isotopic fractionation.

Nitrate  $\delta^{15}\text{N}$  is highly variable depending on source, speed of reaction, and biochemical driver (Cole, 2003; Lehman et al., 2003; Russell, 2015; Sprent, 1987). In southwestern Ontario, Mengis et al. (1999) found that the  $\delta^{15}\text{N}$  of dissolved nitrates collected from groundwater and riparian systems ranged from +3.8 to +48.8 ‰ (although nitrate  $\delta^{15}\text{N}$  was most commonly between +10 and +30 ‰). The concentration of dissolved nitrates decreased, while their average  $\delta^{15}\text{N}$  increased as collection sites transitioned from groundwater to riparian systems. These changes are indicative of denitrifying bacteria and photosynthetic organisms selectively removing  $^{14}\text{NO}_3^-$  as it enters stream systems. Russell (2015) analyzed the nitrogen isotopic composition of nitrates from a variety of sources (i.e. precipitation, soil, fertilizer, septic discharge) surrounding Pinery Provincial Park, Ontario, and reported that nitrate  $\delta^{15}\text{N}$  ranged from  $-1.4 \pm 4.5$  ‰ to  $+16.2 \pm 6.2$  ‰, depending on the source. Nitrate derived from human septic systems had the highest  $\delta^{15}\text{N}$ , while nitrification of  $\text{NH}_3$  and  $\text{NH}_4^+$  produced  $\text{NO}_3^-$  with low  $\delta^{15}\text{N}$  relative to the reactant. Although not directly comparable with freshwater systems, dissolved nitrates are the predominant form of reactive nitrogen in the oceans. This source has a mean  $\delta^{15}\text{N}$  of +5.0 ‰ (Sigman et al., 2000). There is a small isotopic fractionation associated with nitrate uptake by vascular plants (Sprent, 1987). Kendall (1998) describe the range as  $-2.2$  ‰ to  $+0.5$  ‰, with an average of  $-0.25$  ‰.

The decay of organic material also contributes to the nitrogen cycle. Decomposition releases inorganic nitrogen compounds (typically  $\text{NH}_4^+$ ) back into aquatic and terrestrial systems. This process is referred to as remineralization (Figure 3.7). There is only a small fractionation associated with the remineralization of organic material to



**Figure 3.7** The addition and removal of bioavailable nitrogen in an ecosystem. Plant graphic from [Newdesignfile.com](http://Newdesignfile.com).

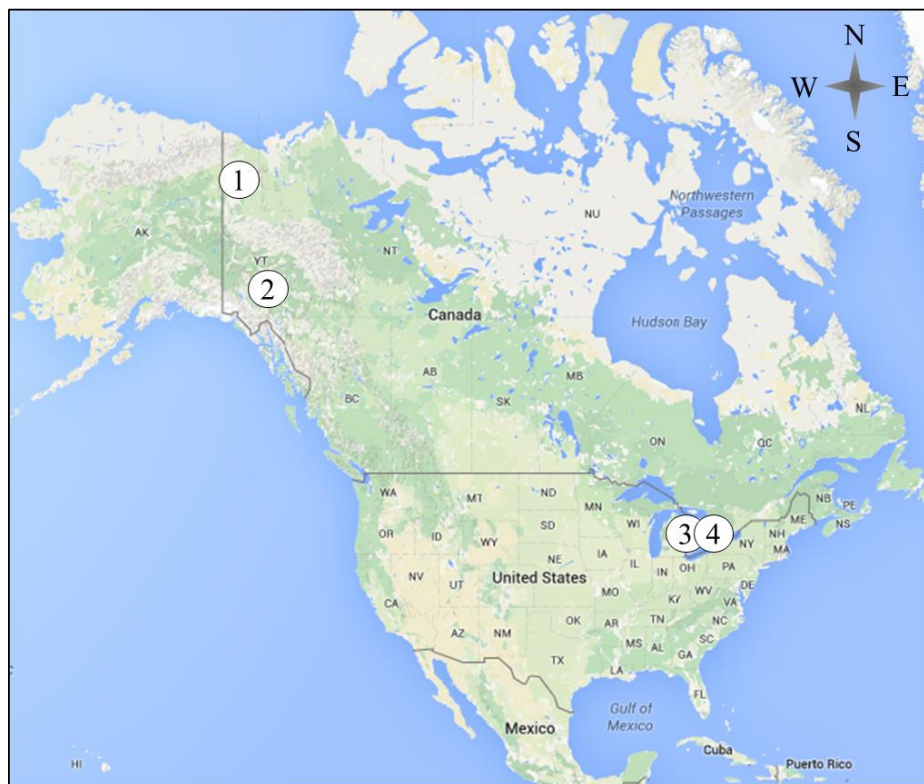
produce  $\text{NH}_4^+$  (approximately 1 ‰) (Kendall, 1998). As a result, remineralized nitrogen compounds retain a similar isotopic composition to the parent material. The primary source of remineralized nitrogen utilized by terrestrial plants comes from the decay of organic tissue (predominantly leaf litter and woody tissues). Terrestrial and aquatic sources can each contribute particulate organic nitrogen (PON) to wetland habitats. The degree of input of terrestrial PON influences the baseline  $\delta^{15}\text{N}$  of any aquatic system. Large influxes of terrestrial PON result in an increasingly lower baseline  $\delta^{15}\text{N}$ . The smaller the wetland habitat, the more pronounced the effect (Gu, 2009; Post, 2002). Urea and amino acids released from living organisms are also a source of bioavailable nitrogen as they are easily dispersed in aquatic environments, where they are absorbed via root systems. Some species of aquatic macrophytes also absorb these compounds directly through their leaves. Rapid plant growth rate increases nitrogen demands, which results in less  $^{15}\text{N}$  discrimination and a  $\delta^{15}\text{N}$  that more closely reflects that of the primary nitrogen source.

Other nitrogen sinks include the burial of particulate organic nitrogen in sediments, the leaching of soil nitrate by percolating water, and the volatilization of ammonium to ammonia, which readily evaporates from both the soil and water column. More bioavailable nitrogen is lost in systems with more open nitrogen cycles. Overall, this increases the  $\delta^{15}\text{N}$  of remaining nitrogen sources.

### 3.3 Methodology

Terrestrial and macrophyte plant samples were collected from four different Canadian localities: Old Crow Basin, Yukon Territory; Whitehorse, Yukon Territory; Pinery Provincial Park, Ontario; and London, Ontario (Figure 3.8). Plant samples were deliberately collected from similar localities as *Castoroides*, *Castor canadensis*, and *Ondatra zibethicus* samples (see Chapter 4). Plant samples from Yukon Territory and London, Ontario were collected in August 2014. Unpublished data from previously collected terrestrial plants from Pinery Provincial Park were also included to complete the baseline (Longstaffe, unpublished data). Aquatic macrophytes from Pinery





**Figure 3.8 Plant sample collection sites: 1 – Old Crow, Yukon Territory; 2 – Whitehorse, Yukon; 3 – Pinery Provincial Park, Ontario; 4 – London, Ontario. Image from Google Maps.**

Provincial Park were collected in August 2014, whereas terrestrial trees and shrubs were collected in August 2000.

All plant sample collection sites were chosen from areas with minimal perceived human impact (i.e. non-urban areas). Macrophytes from London, Ontario, were collected from a pond site on the outskirts of town, situated in recreational parkland. All other sample sites were either small lakes, channels, rivers, or ponds. Photographs of sampling sites are shown in Figures 3.9 through 3.14.

After collection, plant samples were air-dried for a minimum of five days at room temperature. Subsamples based on plant part of interest (i.e. foliage, flower, bark) were removed from the original specimens and washed in distilled water using a soft brush and fine mesh sieve. Subsamples were sonicated for approximately 60 seconds to remove adhering algae or debris. Washed subsamples were then dried overnight in a 90°C oven

before being crushed into a fine powder. Powders were stored in sealed glass vials until analysis.

Approximately  $0.38 \pm 0.02$  mg of sample powder was weighed into tin capsules for concurrent carbon and nitrogen isotopic analysis, and carbon and nitrogen abundance analysis. Concurrent analyses allowed optimum sample weights to be recalculated based on individual plant N%. Samples were reweighed and accurate nitrogen isotope data were obtained during a series of nitrogen-only isotopic analytical sessions. Analyses were performed on a Costech Elemental Analyzer coupled to a ThermoFisher Delta Plus XL isotope ratio mass spectrometer operated in continuous-flow mode, using helium as a carrier gas.

All stable isotope results are reported in delta ( $\delta$ ) notation as ratios relative to the relevant international standards (VPDB and AIR):

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the heavy to light isotope ratios (i.e.  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ) of the same element in both the sample material and the applicable standard (Coplen, 2011). The carbon and nitrogen isotopic data were collected over eight analytical sessions. International and internal laboratory standards were analyzed concurrently to create the calibration curve and to monitor accuracy and precision. A minimum of one internal and one international standard were included for every five samples. One duplicate was included for every ten samples analyzed.

The carbon isotopic results were calibrated to VPDB using USGS-40 ( $\pm 0.1$  ‰ (1 SD),  $n = 19$ ; accepted  $\delta^{13}\text{C} = -26.4$  ‰) and USGS-41 ( $\pm 0.1$  ‰ SD,  $n = 20$ ; accepted  $\delta^{13}\text{C} = +37.6$  ‰). Keratin and IAEA-CH-6 were used as internal and external standards, respectively, to track analytical precision and accuracy. Keratin (MP Biomedicals Inc., Cat No. 90211, Lot No. 9966H)  $\delta^{13}\text{C} = -24.0 \pm 0.1$  ‰ ( $n = 38$ ), which compares well with its accepted  $\delta^{13}\text{C}$  of  $-24.0$  ‰. IAEA-CH-6  $\delta^{13}\text{C} = -10.5 \pm 0.1$  ‰ ( $n = 14$ ) which compares well with its accepted  $\delta^{13}\text{C}$  of  $-10.45$  ‰.



**Figure 3.9** Shallow lake adjacent to the Porcupine River, Old Crow, Yukon Territory, Canada.



**Figure 3.10** Hidden Lakes, Whitehorse, Yukon Territory, Canada.



**Figure 3.11** McIntyre Creek, Whitehorse, Yukon Territory, Canada.



**Figure 3.12** Airport Road wetlands, Whitehorse, Yukon Territory, Canada.





**Figure 3.13 Old Ausable River Channel, Pinery Provincial Park, Ontario.**



**Figure 3.14 Weldon Park pond, London, Ontario, Canada.**

The nitrogen isotopic results from the nitrogen-only analytical sessions were calibrated to AIR using USGS-40 ( $\pm 0.1$  SD,  $n = 11$ ; accepted  $\delta^{15}\text{N} = -4.5$  ‰) and USGS-41 ( $\pm 0.2$  ‰ SD,  $n = 15$ ; accepted  $\delta^{15}\text{N} = +47.6$  ‰). NIST-1547 and keratin were used as internal standards to track analytical precision and accuracy. Keratin (MP Biomedicals Inc., Cat No. 90211, Lot No. 9966H)  $\delta^{15}\text{N} = +6.5 \pm 0.1$  ‰ ( $n = 31$ ), which compares well with its accepted value of  $+6.4$  ‰. NIST-1547  $\delta^{15}\text{N} = +2.0 \pm 0.1$  ‰ ( $n = 11$ ), which compares well with its accepted value of  $+1.98$  ‰. Analytical error for concurrent C-N and N-only sessions was  $\pm 0.2$  ‰.

### 3.4 Results

Plant isotopic results are presented in Appendix A, along with taxonomic and sample site information. Plant samples were identified to genus and species (when possible) using photographs taken in the field during collection. It was not always possible to confirm taxon from a photograph alone. In these instances, plants were categorized by their common name, or when no other information could be reliably assigned, by growing environment. Not all plant sample isotopic data presented in Appendix A (i.e. results for lichen, moss, grasses) were included in the isotopic baseline created to assess rodent feeding niche in Chapter 4. The omitted samples are not included in the Discussion.

Isotopic results are presented by plant category (submerged, emergent, floating, and terrestrial) for both northern and southern latitude sampling sites. Results for the specific plant parts (e.g. bulk foliage, woody tissues) are also listed. Bulk foliage samples consist of multiple whole leaves from a single plant. Woody tissues comprise bark, wood, or twigs from trees and shrubs. Specific plant part sampling was undertaken to better interpret herbivore diet. Bulk foliage samples, in particular, reflect the isotopic composition of the most accessible portion of the plant available to browsing herbivores, while woody tissues reflect a main macromolecular component of *Castor canadensis* diet.

Table 3.1 Mean and range  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for all plant categories from northern and southern sampling sites. Isotopic summaries are also provided for plant-part specific sampling in each category. Sampling strategy was designed to isolate known dietary preferences of beavers and muskrats (part-specific samples are listed in the Description).

<b>Plant category</b>	<b>Description</b>	<b>n</b>	$\delta^{13}\text{C}_{\text{mean}}$ (‰, VPDB)	$\delta^{13}\text{C}_{\text{range}}$ (‰, VPDB)	$\delta^{15}\text{N}_{\text{mean}}$ (‰, AIR)	$\delta^{15}\text{N}_{\text{range}}$ (‰, AIR)
<i>Northern sites</i>						
<b>Submerged macrophyte</b>	<i>all samples</i>	23	-25.5	-41.2 to -13.1	+1.6	-2.7 to +5.5
	<i>bulk foliage*, entire plant</i>	18	-26.1		+1.7	
<b>Emergent macrophyte</b>	<i>all samples</i>	19	-28.7	-35.3 to -25.6	+3.9	+0.9 to +6.6
	<i>bulk foliage*, stem</i>	16	-28.8		+4.2	
<b>Floating macrophyte</b>	<i>all samples</i>	3	-27.0	-31.6 to -23.3	+3.9	+2.7 to +5.4
	<i>entire plant</i>	2	-28.9		+4.1	
<b>Terrestrial trees and shrubs</b>	<i>all samples</i>	25	-29.1	-31.3 to -27.2	-1.6	-8.5 to +3.9
	<i>bulk foliage*</i>	17	-29.2		-1.5	
	<i>woody tissues**</i>	6	-28.6		+1.4	
	<i>conifer bulk foliage</i>	3	-28.0		-4.0	
	<i>deciduous bulk foliage</i>	14	-29.4		-1.5	

<b>Plant category</b>	<b>Description</b>	<b>n</b>	<b><math>\delta^{13}\text{C}_{\text{mean}}</math> (‰, VPDB)</b>	<b><math>\delta^{13}\text{C}_{\text{range}}</math> (‰, VPDB)</b>	<b><math>\delta^{15}\text{N}_{\text{mean}}</math> (‰, AIR)</b>	<b><math>\delta^{15}\text{N}_{\text{range}}</math> (‰, AIR)</b>
<i>Southern sites</i>						
<b>Submerged macrophyte</b>	<i>all samples</i>	12	-19.9	-29.9 to -12.9	-0.5	-3.5 to +3.1
	<i>bulk foliage*</i>	11	-20.0		-0.4	
<b>Emergent macrophyte</b>	<i>all samples</i>	10	-29.6	-31.2 to -28.2	+1.5	-0.8 to +4.3
	<i>bulk foliage*,stem</i>	8	-29.8		+1.9	
<b>Floating macrophyte</b>	<i>all samples</i>	6	-26.8	-29.9 to -24.7	+3.5	+0.1 to +7.2
	<i>bulk foliage*</i>	5	-26.7		+3.2	
<b>Terrestrial Trees and Shrubs</b>	<i>all samples</i>	48	-28.3	-30.7 to -23.9	-5.0	-10.6 to -0.8
	<i>bulk foliage*</i>	26	-28.7		-5.0	
	<i>woody tissues**</i>	26	-27.9		-5.1	
	<i>conifer bulk foliage</i>	7	-27.8		-4.6	
	<i>deciduous bulk foliage</i>	19	-29.0		-5.1	

\* Bulk foliage samples consist of multiple whole leaves from a single plant

\*\* Woody tissue samples consist of either bark, wood, or twigs from a single plant

### 3.4.1 Isotopic Summary: Northern and Southern Sites

The mean and range of carbon and nitrogen isotopic results for each plant category and for specific plant parts from both northern and southern sites are summarized in Table 3.1. Northern sampling sites from subarctic regions include Old Crow Basin and Whitehorse, Yukon Territory. Southern sampling sites from temperate regions include Pinery Provincial Park and London, Ontario.

### 3.4.2 Isotopic Trends: Macrophytes and Terrestrial Plants

Terrestrial trees and shrubs (as a category) are distinguishable in isotopic composition from macrophytes when both their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are considered. There are two particularly diagnostic differences. First, macrophytes as a collective group exhibit significantly higher mean  $\delta^{15}\text{N}$  than do terrestrial trees and shrubs. Second, submerged macrophytes are the only category out of the four to exhibit  $\delta^{13}\text{C}$  higher than  $-20\text{‰}$ .

The underlying trends in isotopic composition of each plant category are the result of local growing conditions, and differential access to bioavailable carbon and nitrogen sources. The following list outlines general trends in the data that are applicable to wetland-terrestrial isotopic dynamics on a broader geographic scale. The specific isotopic composition of individual plants species, of course, will always vary from site to site.

- Emergent macrophytes, floating macrophytes, and terrestrial trees and shrubs share a similar mean and range of  $\delta^{13}\text{C}$ .
- Submerged macrophytes exhibit the greatest range of  $\delta^{13}\text{C}$ , as well as a bimodal distribution of  $\delta^{13}\text{C}$  values. Submerged macrophytes have the highest  $\delta^{13}\text{C}$ .
- On average, floating macrophytes have higher  $\delta^{13}\text{C}$  than emergent macrophytes.
- Floating and emergent macrophytes share a similar mean and range for  $\delta^{15}\text{N}$ .
- On average, floating and emergent macrophytes have higher  $\delta^{15}\text{N}$  than submerged macrophytes.
- Macrophytes (regardless of category) have higher mean  $\delta^{15}\text{N}$  than terrestrial trees and shrubs.



- On average, coniferous tree species have higher  $\delta^{13}\text{C}$  than deciduous tree species.
- Trees and shrubs collected from southern sites have higher mean  $\delta^{13}\text{C}$  and lower mean  $\delta^{15}\text{N}$  than those from northern sites.
- Tree and shrub foliage have higher mean  $\delta^{13}\text{C}$  than cellulose tissues (regardless of latitude).

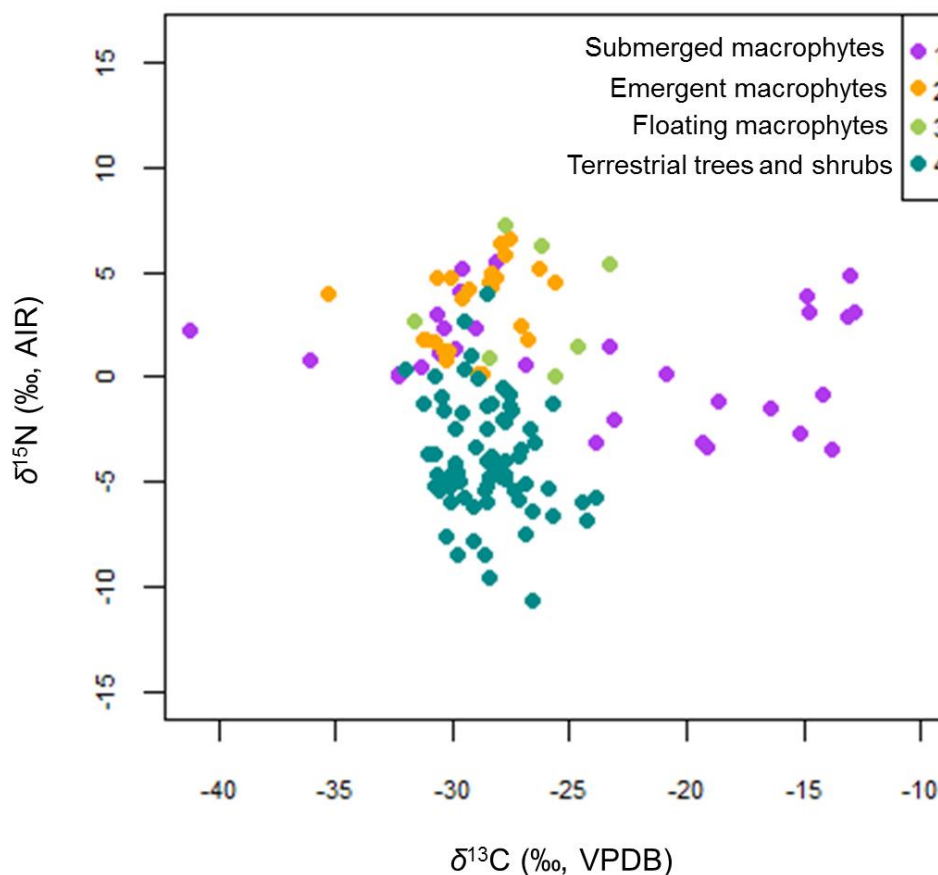
### 3.5 Discussion

The specific isotopic mean and range for each of the potential inorganic and organic carbon and nitrogen sources available to plants at each sampling site are – unfortunately – not known. Without such data, it is impossible to quantitatively define the contribution of each putative carbon and nitrogen source. Nonetheless it is still possible to discuss the physiological characteristics and environmental conditions that account for the *general* isotopic patterns obtained for each of the four plant categories. These matters are considered next.

#### 3.5.1 Statistical Modelling in R Studio

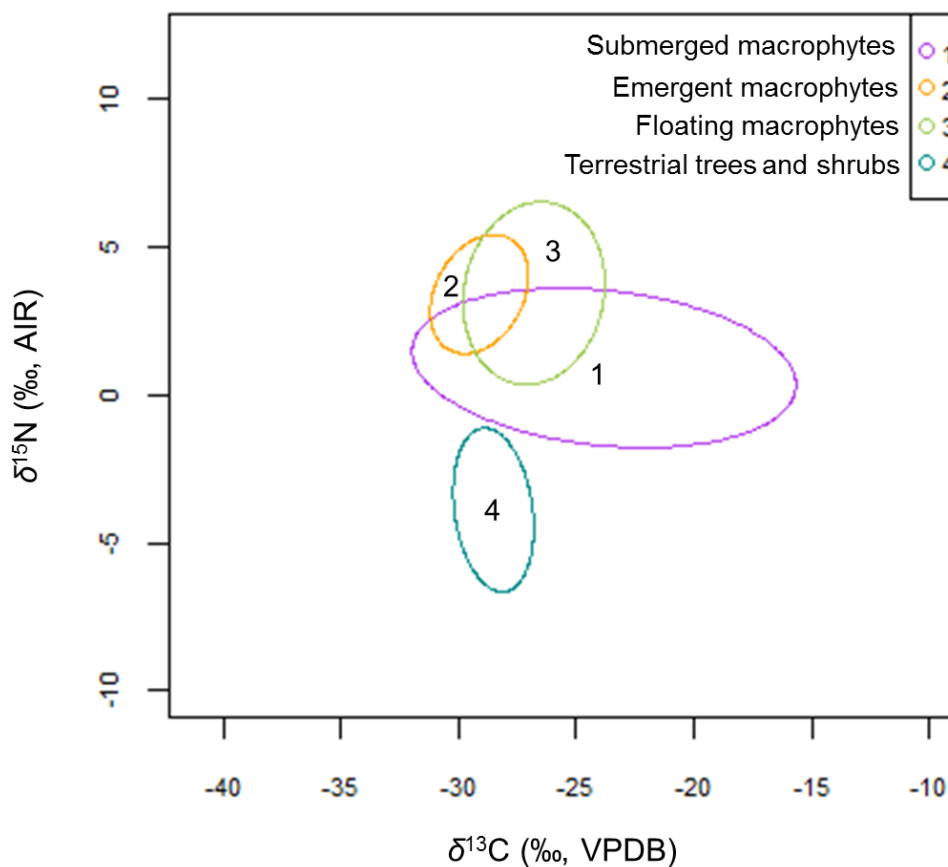
The statistical modelling package Stable Isotope Bayesian Ellipses in R (SIBER) fits ellipses to groups of data to create clear visual representation. Ellipses are presented on isotope biplots (in this case,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). The ellipses can be corrected for small sample size or coded to represent a set percentage of the data. In this instance, SIBER is used to model ecological data. Specifically, isotopic niche is of interest. Isotopic niche is defined by resource use (in this case, forage) by a consumer, as illustrated by  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Newsome et al., 2007).

The stable carbon and nitrogen isotopic data for individual plant categories are illustrated in Figure 3.15. SIBER was employed to infer and compare isotopic niche size among plant categories (Figure 3.16). Each ellipse has been corrected statistically for small sample size and incorporates 40% of the data per category. By encompassing only 40% of the data, each individual ellipse more clearly demonstrates the isotopic separation among plant categories. This separation represents different isotopic niches that translate



**Figure 3.15 Stable carbon and nitrogen isotopic data for each plant category. Plot produced using Stable Isotope Bayesian Ellipses in R (SIBER) in R Studio.**

to potential feeding niches. Convex Hulls, by comparison, enclose 100% of the data for each plant category and show the total isotopic area of each plant category. Hulls are illustrated in Figure 3.17. The Convex Hulls describe a similar pattern as the ellipses. There are limitations to both methods and neither SIBER plot represents all patterns of interest in the data. For this reason, the original scatter plot (Figure 3.15) should always be included for reference. In this case, neither the ellipses, nor the Convex Hulls capture the bimodal nature of submerged macrophyte  $\delta^{13}\text{C}$  (this is an important ecological

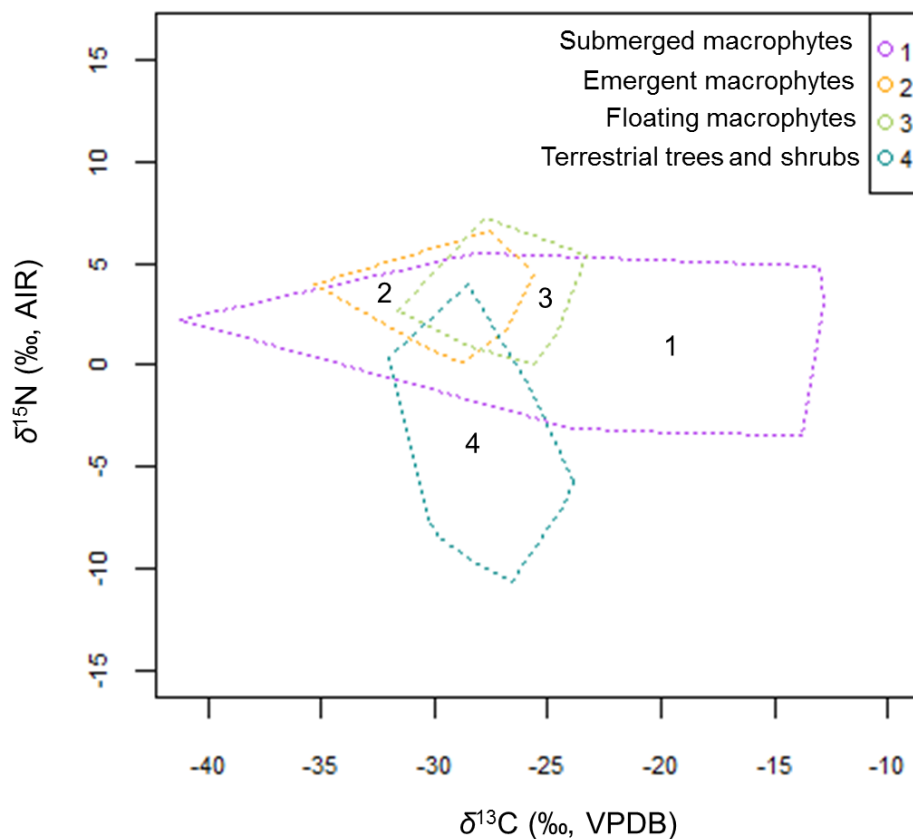


**Figure 3.16 Isotopic niche size and position inferred by Stable Isotope Bayesian Ellipses in R (SIBER). Each ellipse contains 40% of the data points/category and is corrected for small sample size.**

distinction). The ellipses do, however, clearly distinguish the shift in  $\delta^{15}\text{N}$  between emergent and floating macrophytes, submerged macrophytes, and terrestrial trees and shrubs.

### 3.5.2 Macrophyte $\delta^{13}\text{C}$

Atmospheric  $\text{CO}_2$  is the predominant carbon source for all emergent and floating macrophytes collected from both northern and southern sampling sites. Carbon sources for submerged macrophytes are more complex, as discussed earlier. All



**Figure 3.17 Isotopic area of each plant category inferred by Convex Hulls produced using Stable Isotope Bayesian Ellipses in R (SIBER) in R Studio. Each hull encloses 100% of the data/category.**

macrophyte samples fall well within their previously reported  $\delta^{13}\text{C}$  range ( $-50$  to  $-11$  ‰; Keeley and Sandquist, 1992; Osmond et al., 1981).

The range of carbon isotopic compositions obtained for submerged macrophytes ( $-41.2$  to  $-12.9$  ‰) is the result of the plants' access to multiple carbon sources in the water column. Genera of submerged macrophytes that uptake  $\text{HCO}_3^-$  by active transport when dissolved  $\text{CO}_2$  concentrations become too low are more enriched in  $^{13}\text{C}$  than those that do not. The lower  $\delta^{13}\text{C}$  of samples collected from colder climates (Yukon Territory) reflect both cooler waters and in some cases, high water turbulence (i.e. McIntyre Creek).

Both conditions increase the concentration of dissolved CO<sub>2</sub>, which favours uptake of <sup>12</sup>CO<sub>2</sub>.

Macrophyte genera *Vallisneria*, *Potamogeton*, *Myriophyllum*, and *Ranunculus* are all capable of HCO<sub>3</sub><sup>-</sup> uptake, while *Hippurus*, *Utricularia* and species of aquatic moss are not (Keeley and Sandquist 1992 and sources therein). Samples of genera capable of HCO<sub>3</sub><sup>-</sup> uptake collected both in Yukon Territory and Ontario all demonstrate a wide range of δ<sup>13</sup>C, including a trend towards enrichment in <sup>13</sup>C. *Nitella* collected at Pinery Provincial Park is a species of branched algae with a high affinity for HCO<sub>3</sub><sup>-</sup>, and is <sup>13</sup>C-enriched as a result.

Bicarbonate uptake is not the only reason for high δ<sup>13</sup>C (> -20 ‰) in submerged macrophyte samples. The majority of wetlands sampling sites in this study had low to moderate water turbulence. The slow replenishment of CO<sub>2</sub> in the boundary layer decreased discrimination against <sup>13</sup>C, thus leading to high δ<sup>13</sup>C in these samples. All sample sites were eutrophic and contained detrital organic material. The decay of <sup>13</sup>C-enriched aquatic plants and algae in wetlands also drives up the δ<sup>13</sup>C of the local carbon pool, as described earlier.

The majority of macrophyte samples were collected from dense stands of vegetation, which also increases inter-individual competition for carbon. Microhabitat conditions dictate the available carbon sources and account for the intra-specific carbon isotopic variation seen in genera such as *Myriophyllum* and *Potamogeton*. Productive conditions found at all sites (i.e. warm water temperatures, abundant access to sunlight) increase photosynthetic activity. Increased photosynthetic activity results in the more complete use of the available carbon pool in the boundary layer and less overall fractionation between the source and the plant. Sustained growth under highly productive conditions results in submerged macrophytes with <sup>13</sup>C-enriched tissues (relative to their primary carbon source), regardless of what uptake pathway they employ or the availability of CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> in the water column.

Submerged macrophyte samples with low  $\delta^{13}\text{C}$  ( $< -30$  ‰) indicate the use of respired  $\text{CO}_2$ , or the influence of remineralized carbon from decayed terrestrial organic matter. Plant respiration (instead of photosynthesis) becomes the dominant process during the latter half of the growing season in aquatic systems. This process fuels the dissolved inorganic carbon pool with highly  $^{13}\text{C}$ -depleted  $\text{CO}_2$ . All plant samples in this study were collected late in the growing season (August), although the majority of tissue formation likely occurred earlier in the season. During the late summer, respiration has a particularly pronounced effect on the  $\delta^{13}\text{C}$  of dense stands of underwater vegetation. The influx arising from release of carbon from decaying terrestrial or emergent macrophyte matter creates a localized  $^{13}\text{C}$ -depleted carbon pool that is utilized during the day by plants growing in that area (Keeley and Sandquist, 1992). All wetland sample sites (both northern and southern) were surrounded by forested environments where terrestrial organic material could easily be washed or blown into the water. In conjunction with respired  $\text{CO}_2$ , this phenomenon likely accounts for the samples demonstrating  $\delta^{13}\text{C}$  of  $-41$  to  $-30$  ‰ at Old Crow, Whitehorse, and London sites. As previously mentioned, high concentrations of dissolved  $\text{CO}_2$  and increased water turbulence at a site can also drive down the  $\delta^{13}\text{C}$  of submerged macrophytes.

### 3.5.3 Macrophyte $\delta^{15}\text{N}$

Overall, macrophytes collected for this study appear to be using dissolved nitrates, ammonium, and fixed atmospheric  $\text{N}_2$  to meet their nitrogen requirements. Emergent and floating macrophytes with aerial foliage are also subject to atmospheric deposition of nitrogen in the form of nitrates or ammonia (Duce et al., 2008). Nitrogen deposition is more pronounced at southern sampling sites that are in close proximity to highly urban or industrial centers. Macrophytes, especially those rooted in deeper water make use of urea or fecal pellets that sink and accumulate in the benthic zone (Altabet and Small, 1990; Checkley and Entzeroth, 1985; Montoya et al., 1992). In wetlands rich in mammal, fish and reptile life (i.e. sampling sites around Whitehorse and Pinery Provincial Park),  $^{15}\text{N}$ -enriched organic waste products can also contribute to an increase in plant  $\delta^{15}\text{N}$ .

The timing of sample collection also impacts plant  $\delta^{15}\text{N}$ . As previously mentioned, all plant samples were collected relatively late in the growing season. During late summer, the initial pool of available dissolved nitrate decreases as macrophyte growth increases the demand for nitrogen. The  $\delta^{15}\text{N}$  of the residual nitrate increases with prolonged preferential use of  $^{14}\text{NO}_3^-$ . This impacts the  $\delta^{15}\text{N}$  of additional growth that occurs late in the season. In addition, warmer temperatures and slow current speed present at most sites during the summer decreases the availability of dissolved oxygen in the water column. This effect is more pronounced at sites in Southwestern Ontario than Yukon Territory. Low oxygen or eutrophic conditions in the benthic zone stimulate bacterial denitrification and drive up the  $\delta^{15}\text{N}$  of available nitrate. A combination of both of these factors means that all macrophytes sampled in this study were drawing on a more  $^{15}\text{N}$ -enriched pool of nitrates, than if they had been sampled earlier in the summer. More specific physiological explanations for the  $\delta^{15}\text{N}$  of individual macrophyte taxon are provided below.

Genus *Utricularia* is a submerged macrophyte and a carnivorous plant that consumes small freshwater animals, such as protozoa, insect larvae, and worms that live suspended in the water column. A sudden influx of water transports prey into dozens of tiny traps (bladders) hanging from *Utricularia*'s stem, where prey are digested. This carnivorous habit of *Utricularia* places it at a higher trophic level than other submerged macrophytes from the same site, and it has a higher  $\delta^{15}\text{N}$  as a result.

Many species of detached floating macrophytes absorb nitrogen directly into their leaves, as well as through their root system. *Lemna*, commonly known as duckweed, is a detached floating macrophyte. Samples of *Lemna* were collected from both northern and southern sampling sites. *Lemna* differs from terrestrial plants in its capability for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake directly into its frond (Cedergreen and Madsen, 2002). *Lemna* plants uptake dissolved  $\text{NH}_4^+$  much more readily than dissolved  $\text{NO}_3^-$  (3 to 11 times faster) (Cedergreen and Madsen, 2002). The  $\delta^{15}\text{N}$  of all *Lemna* samples in this study is greater than 0 ‰, reflecting the mixture of dissolved  $\text{NH}_4^+$  and  $\text{NO}_3^-$  at each site, with a heavier weighting towards  $\text{NH}_4^+$ .

Emergent macrophyte mean  $\delta^{15}\text{N}$  is consistently higher than that of submerged macrophytes. This trend is apparent at all sampling sites. Both emergent and submerged macrophytes are rooted and have access to commonly available dissolved inorganic nitrogen sources (i.e.  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) in the soil and the water column. One possibility that could account for the difference in mean  $\delta^{15}\text{N}$  between the two plant categories is emergent macrophyte uptake of organic nitrogen. Emergent macrophyte species belonging to the family Cyperaceae (sedge), as well as other species of non-mycorrhizal reeds and rushes, can absorb dissolved free amino acids and urea directly from the soil or water column (Mozdzer, 2010; Raab et al., 1999). Amino acids are released from plant and animal proteins during the decay of organic matter. We hypothesize that uptake of these amino acids by species that have a high affinity for them can cause plant  $^{15}\text{N}$ -enrichment. Further research is necessary, however, to determine how amino acid uptake affects emergent macrophyte  $\delta^{15}\text{N}$ .

#### 3.5.4 Pinery Provincial Park Macrophyte $\delta^{15}\text{N}$

Russell (2015) and Russell et al. (2016) have reported on the availability and the isotopic composition of aquatic nitrogen sources in the Old Ausable River Channel at Pinery Provincial Park. This site-specific information is useful for crafting a more detailed understanding of macrophyte  $\delta^{15}\text{N}$ .

In the present study, all aquatic plant samples from Pinery Provincial Park were collected from the Old Ausable River Channel (OARC). This channel is fed primarily by shallow ground water derived from local precipitation. Russell et al.'s (2016) isotopic data indicate that nitrate is the most common source of bioavailable nitrogen in the OARC, and that local anthropogenic contribution (e.g. septic effluent, manure) is minimal (Russell, 2015). Nitrate in the Pinery section of the OARC is derived from a combination of atmospheric and terrestrial sources; rainwater-borne nitrate collected from the Pinery has a  $\delta^{15}\text{N}$  of  $+1.9 \pm 4.4$  ‰ and groundwater has a  $\delta^{15}\text{N}$  of  $+2.5 \pm 1.0$  ‰ (Russell, 2015; Russell et al., 2016). These compositions indicate a mixing of sources with low  $\delta^{15}\text{N}$ , which can include nitrified ammonium from precipitation, ammonia originating from agricultural fertilizer (both chemical and manure), and nitrate



originating directly from soil and rainwater. Macrophytes collected from Pinery Provincial Park have an average  $\delta^{15}\text{N}$  of +0.2 ‰. This composition also reflects uptake of nitrogen sources with low  $\delta^{15}\text{N}$  and minimal impact of septic discharge or agricultural manure run-off, both of which have high  $\delta^{15}\text{N}$ . Individual floating and emergent macrophytes with above-water foliage are also subject to atmospheric nitrogen deposition, which can result in nitrogen saturation and increased discrimination against  $^{15}\text{N}$ .

### 3.5.5 Tree and Shrub $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

All terrestrial trees and shrubs sampled in this study utilize the  $\text{C}_3$  photosynthetic pathway and use atmospheric  $\text{CO}_2$  as their primary source of carbon. The majority of samples were collected from areas with moderate to dense forest cover, with the exception of those collected from open dune environments at Pinery Provincial Park (indicated in specimen identification code as site O-A or O-B). In forested environments, recycled respired  $\text{CO}_2$  and the canopy effect can contribute to lower  $\delta^{13}\text{C}$  of their foliage (van der Merwe and Medina, 1991).

Tree and shrub mean  $\delta^{15}\text{N}$  varies with latitude. The  $\delta^{15}\text{N}$  of samples collected from southwestern Ontario are on average 3.5 ‰ lower than samples collected from Yukon Territory. This depletion of  $^{15}\text{N}$  may be a result of warmer temperatures and higher precipitation amounts in the south. Higher rainfall is inversely correlated with  $\delta^{15}\text{N}$ , and wetter ecosystems tend to produce more  $^{15}\text{N}$ -depleted plants (Ambrose, 1991; Mariotti et al., 1980; Shearer et al., 1978; Swap et al., 2004). Warmer climates are also associated with more bacterially active soils and more productive conditions. The increase in production of organic matter and the subsequent remineralization of this organic material into inorganic compounds drives down plant  $\delta^{15}\text{N}$ .

The specific ecology of Pinery Provincial Park and the climate of southwestern Ontario both contribute to the low  $\delta^{15}\text{N}$  measured for trees and shrubs growing there. The Pinery is located on the south-east shore of Lake Huron, and consists of an Oak Savannah ecosystem that overlays a succession of ancient dune ridges. Average annual precipitation and temperature are both higher than those experienced at Whitehorse or Old Crow,

Yukon Territory (Environment Canada, 2016). The sandy soils and precipitation rates of ~1000 mm per year at the Pinery facilitate leaching of nitrates from the upper layers of soil. The elevated dune ridges limit root access of smaller plants to dissolved nitrates available in regional groundwater. The dominant sources of bioavailable nitrogen to terrestrial plants in the area are atmospheric N<sub>2</sub> fixed by bacteria, NH<sub>3</sub> released by decomposers in the soil, and deposition of atmospheric nitrogen. These three processes induce low  $\delta^{15}\text{N}$  in plants. Input of decayed terrestrial organic material also perpetuates a nitrogen pool with low  $\delta^{15}\text{N}$ . The warmer temperatures in southern latitudes stimulate increased biomass productivity and accelerate bacterial processes that drive the nitrogen cycle. Increased rates of bacterial activity (i.e. nitrogen fixation, decomposition) result in more inorganic nitrogen and more opportunity to discriminate against <sup>15</sup>N during plant growth. Anthropogenic impacts on the nitrogen cycle are also minimal at the Pinery (Russell, 2015; Russell et al., 2016).

The sites from Yukon Territory experience lower average annual temperatures and precipitation rates (between 200 mm and 300 mm per year) (Environment Canada, 2016) than the Pinery. The growing season is also considerably shorter and the decomposition of organic material occurs more slowly. Bioavailable nitrogen is in shorter supply in less productive systems. Finite pools of bioavailable nitrogen are likely to be used more completely, resulting in less <sup>15</sup>N-discrimination and plant  $\delta^{15}\text{N}$  that more closely reflects source  $\delta^{15}\text{N}$ . Fixed atmospheric nitrogen and NH<sub>3</sub> released by decomposition remain the primary bioavailable nitrogen sources for northern trees and shrubs, but there is less discrimination against <sup>15</sup>N during its uptake.

Regional aridity is also linked to <sup>15</sup>N-enrichment in plants (Handley et al., 1999; Schulze et al., 1998). This is unlikely to be a major contributing factor, but mild aridity-related effects could influence plant  $\delta^{15}\text{N}$  at Old Crow and Pinery Provincial Park. Depending on precipitation and temperature conditions, aridity can impact plants at different points over the course of the growing season. In this instance, Old Crow experiences low precipitation rates over the winter, while Pinery Provincial Park experiences more arid conditions in the spring and summer (Environment Canada, 2016).

Northern tree and shrub samples exhibit some species-specific isotopic trends. Willow (*Salix*) samples from Yukon Territory were collected in close proximity to river and lake habitats. Willow foliage  $\delta^{15}\text{N}$  is higher than any other terrestrial plant species collected for this study. Willows growing along the shoreline have root-access to bioavailable nitrogen delivered from the water column into the surrounding saturated substrate. This includes  $^{15}\text{N}$ -enriched sources, primarily dissolved nitrates that have undergone denitrification, and decomposing aquatic organic material. In contrast, tree and shrub samples from the Pinery were collected from both open dune environments at minimum 50 meters from the shoreline, and from closed canopy forests situated on ancient dune successions. These plants do not have the opportunity to tap into aquatic nitrogen pools.

Alder (*Alnus*) is an unusual member of the northern forest community. Alder are nitrogen-fixing trees and can increase the nitrogen fertility of the adjacent soils (Binkley, 1983; Binkley et al., 1992). The  $\delta^{15}\text{N}$  of alder trees sampled in this study range from  $-1.5$  to  $-1.2$  ‰, as is expected for plants that received their nitrogen directly from atmospheric sources.

### 3.5.6 Conifer versus Deciduous $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

Conifer bulk foliage  $\delta^{13}\text{C}$  is consistently higher than deciduous bulk foliage at both northern and southern sampling sites. This isotopic pattern can be explained by physiological differences. The water transportation system of conifer species is less efficient than that of most temperate broadleaf species (Beerling, 1996; Beerling and Woodward, 1993; Jolly and Haxeltine, 1997; Loehle, 2007; McCarroll and Loader 2004; Robinson, 1994; Saxe et al., 1998). Hydraulic conductivity is linked to stomatal conductivity, which impacts plant discrimination against  $^{13}\text{C}$ . When water conservation is in effect in conifers, stomatal conductivity decreases. This process slows both the rate of water leaving and of  $\text{CO}_2$  entering the needle. Carbon dioxide is replenished at a slower rate, creating a more finite carbon pool. As a result, the plant must discriminate less against  $^{13}\text{C}$  in order to maintain photosynthesis. This physiological trait makes conifer species more drought-resistant and as a by-product, more  $^{13}\text{C}$ -enriched.

Conifer bulk foliage  $\delta^{15}\text{N}$  is also consistently lower than deciduous bulk foliage at northern sampling sites. This is again, likely due to physiological differences between conifer and deciduous trees. Many conifer species preferentially uptake atmospheric  $\text{NH}_3$  gas through their stomata. The concentration of inorganic nitrogen circulating in the global atmosphere has increased due to recent anthropogenic activities (i.e. agricultural fertilizers, forest fires, fossil fuel combustion).  $\text{NH}_3$  is  $^{15}\text{N}$ -depleted and acts as an atmospheric fertilizer (Kendall et al., 2007). Conifer needles uptake  $\text{NH}_3$  gas through their stomata, where it increases foliar nitrogen concentration. Abundant bioavailable nitrogen allows for a greater discrimination against  $^{15}\text{N}$  and overall, lower  $\delta^{15}\text{N}$ . Conifer and deciduous bulk foliage  $\delta^{15}\text{N}$  from southern sampling sites, however, are comparable. The low  $\delta^{15}\text{N}$  (relative to macrophytes) consistently observed in southern tree and shrub species is likely due to greater deposition of atmospheric nitrogen on aerial foliage, driven by concentrated use of agricultural fertilizer and fossil fuel combustion in southwestern Ontario. Regional concentration of  $\text{NH}_3$ , particle  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in the atmosphere due to anthropogenic activities impacts all plants with aerial foliage (Aber et al., 1989; Krupa, 2003; Perez-Soba and Van der Eerden, 1993). Terrestrial samples collected from the Pinery were also not growing in close enough proximity to wetlands to access dissolved nitrates and  $^{15}\text{N}$ -enriched aquatic organic material. In addition, many conifer species prefer well-drained, coarse grained soils. The sandy soils of the Pinery allow rapid leaching of nitrates and loss of access to this potentially  $^{15}\text{N}$ -enriched source of nitrogen.

### 3.6 Conclusions

The isotopic distinction between macrophytes, and trees and shrubs is a useful tool for ecologists aiming to untangle nutrient flow in mixed terrestrial and freshwater food webs. Access to aquatic-origin organic material (POM, urea, dissolved free amino acids), and dissolved nitrates that have undergone denitrification allow for the high level of  $^{15}\text{N}$ -enrichment seen in macrophytes, and *Salix* growing in riparian systems. Bicarbonate uptake, in addition to slow current speed and low concentrations of dissolved atmospheric  $\text{CO}_2$  account for  $\delta^{13}\text{C}$  in submerged macrophytes that are akin to those of  $\text{C}_4$  grasses. The effects of latitude are visible in both macrophyte and tree and shrub  $\delta^{13}\text{C}$  and

$\delta^{15}\text{N}$ , where warmer temperatures and annual precipitation amounts impact the degree of isotopic fractionation in the carbon and nitrogen cycle. As ever, context is crucial when constructing an isotopic dietary baseline composed of vascular plants, as there is considerable inter- and intra-specific variation between sites. A site-specific or a wetland habitat-specific dietary isotopic baseline is prerequisite to the interpretation of diets based on isotopic data from collagen or other animal tissues.

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## Chapter 4

### Stable Isotopic Palaeoecology of *Castoroides*

#### 4.1 Introduction

The palaeoecology of *Castoroides* has not been extensively explored using stable isotopic analysis. This 100 kg member of the Castoridae family once roamed the continent from Florida to Alaska (Cahn, 1932; Martin, 1969; Reynolds, 2002). It, along with 35 other genera of North American megafauna, disappeared during the late Pleistocene extinction event (Barnosky et al., 2004; Faith and Surovell, 2009). The role of the giant beaver within an ecosystem, its impact on the surrounding Pleistocene landscape, and its ecological vulnerabilities as a genus are explored in this chapter using stable carbon, nitrogen, and oxygen isotopes.

Stable carbon and nitrogen isotopes are used in palaeodietary reconstruction to trace trophic level and nutrient flow within ancient food webs. Oxygen isotopes are commonly used as a proxy in palaeoclimate studies for determining controls on local precipitation and temperature. Stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic data are used in this study to determine diet and habitat preference of the giant beaver, while oxygen ( $\delta^{18}\text{O}$ ) isotopic records are used to examine palaeoclimatic conditions in multiple regions where giant beaver populations once lived. Two pre-existing stable isotopic studies of *Castoroides* fossils (each incorporating a single specimen) suggest that the genus consumed aquatic vegetation and thrived in warm, strongly seasonal conditions in mid-latitude regions during the Sangamonian interglacial (Perkins, 2009; Stuart-Williams and Schwarz, 1997). Other studies incorporating macrophysical climate models, skeletal morphology, and sedimentology of fossil depositional environment hypothesize that the giant beaver was a relatively cold-tolerant species, preferentially consumed emergent macrophytes, and lived in ponds and shallow lakes bordered by marshlands (Harrington, 1986; Holman, 1995; Kurten and Anderson, 1980; McDonald and Bryson, 2010; Powell, 1948; Richards and Swinehart, 2001; Stirton, 1965). Whether or not *Castoroides* engineered its habitat by constructing dams or lodges remains a topic of debate (Cahn,

1932; Hay, 1912; Holman, 1975; Holman, 1995; Kurten and Anderson, 1980; McDonald, 1994; Moore, 1890; Powell, 1948; Stirton, 1965). In this chapter, palaeoecological conclusions from the literature are re-evaluated in light of the new stable isotope evidence. The chapter concludes with a discussion about palaeoenvironmental conditions that led to the extinction of the giant beaver at the end of the Pleistocene.

The habitat preference, forage choices, and ecological engineering habits of the giant beaver are also assessed through comparison with two modern semi-aquatic rodent species with well-understood ecologies: *Castor canadensis* (beaver) and *Ondatra zibethicus* (muskrat). These two species are proposed by different authors as possible modern analogs to the giant beaver (Harington, 2003; Lovegrove and Mowoe, 2013; Rybczynski, 2008; Tedford and Harington, 2003; Zazula, personal comm., 2013). The econiche of each species is summarized in Table 4.1. Stable carbon and nitrogen isotopic information for biological tissues (bone collagen and bioapatite) from all three rodent species is compared within the context of an isotopic dietary baseline constructed from modern aquatic and terrestrial plants. This comparison allows us to determine the degree of dietary overlap among species, *Castoroides*' level of dependence on aquatic habitat space, and the viability of using *C. canadensis* versus *O. zibethicus* as a modern analog in future palaeoecological models incorporating *Castoroides*.

The econiche of each rodent is then quantified using Stable Isotope Analysis in R (SIAR) mixing model capabilities. SIAR was designed specifically to interrogate isotopic data in an ecological context. Stable carbon and oxygen isotope data obtained for giant beaver ever-growing incisors are used to explore regional climatic conditions during the Pleistocene and the potential of giant beaver incisors to record isotopic information ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) about seasonal shifts in diet and climate. Three geographically separate populations of *Castoroides* are examined to determine if giant beaver diet varied by region (outlined in Table 4.2). Radiocarbon dates procured in this study provide new information about the chronology of fluctuations in the geographic range of the giant beaver.

Table 4.1 Ecological summary of *Castor canadensis* and *Ondatra zibethicus*.

Species	Habitat	Diet	Ecological Impact
<i>Castor canadensis</i>	Rivers, lakes, ponds	Generalist herbivore (bulk diet: predominantly deciduous tree foliage and cambium, rhizomes of floating macrophytes)	Dam building diverts flowing water from downstream habitats; tree harvesting can cause local deforestation; beaver ponds increase local aquatic biodiversity; lodges provide shelter to other semi-aquatic animals.
<i>Ondatra zibethicus</i>	Rivers, ponds, marshes	Predominantly herbivorous with occasional omnivorous tendencies (bulk diet: predominantly emergent macrophytes, submerged macrophytes, floating macrophytes, crustaceans, bivalves, small invertebrates)	Feeding creates shallow, open water habitats by clearing stands of vegetation; creates habitat for fish and water fowl; lodges and feeding-platforms provide thermoregulatory shelter to small semi-aquatic animals.

Table 4.2 *Castoroides* specimens utilized in this study were procured from multiple institutions. Collections were selected based on their representation of geographically separate giant beaver populations.

Sample no.	Institution	Specimen origin
C1-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C2-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C3-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C4-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C5-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C6-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C7-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C8-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C9-TP2014	Canadian Museum of Nature	Old Crow Basin, Yukon Territory
C10-TP2014	Ohio Historical Society	Clear Creek Township, Ohio
C11-TP2014	Ohio Historical Society	Williams County, Ohio
C12-TP2014	Florida Museum of Natural History	Santa Fe River, Florida
C13-TP2014	Florida Museum of Natural History	Oklawaha River, Florida
C14-TP2014	Florida Museum of Natural History	Santa Fe River, Florida
C15-TP2014	Museum of the North	Galena, Alaska
C20-TP2014	Ohio Historical Society	Williams County, Ohio
C22-TP2014	Ohio Historical Society	Harmony Township, Ohio
C26-TP2014	Florida Museum of Natural History	Peace River, Florida
C28-TP2014	Florida Museum of Natural History	Santa Fe River, Florida

C29-TP2014	Florida Museum of Natural History	Oklawaha River, Florida
C31-TP2014	Florida Museum of Natural History	Santa Fe River, Florida
C32-TP2014	Florida Museum of Natural History	Peace River, Florida
C33-TP2014	Florida Museum of Natural History	Santa Fe River, Florida

## 4.2 Materials and Methods

Unless otherwise noted, all analyses were conducted at the Laboratory for Stable Isotope Science (LSIS) at the University of Western Ontario, in London, Ontario, Canada. Ancient samples originated from a mixture of sites in Alaska, Yukon Territory, Ohio, and Florida (refer to Table 4.2). Modern rodent samples originated from multiple localities in southern Ontario and Yukon Territory (refer to Tables 4.5 and 4.6).

### 4.2.1 Radiocarbon Dating

A series of radiocarbon dates were obtained using *Castoroides* bone collagen. Ten samples were analyzed at the Keck Carbon Cycle AMS Facility at the University of California Irvine. Approximately 200 to 250 mg of bone were removed, crushed, and decalcified for 48 hours in 0.5 M hydrochloric acid (HCl). Demineralized bone was gelatinized at 60°C in 0.01 M HCl. Gelatinized collagen was ultrafiltered once at 30kDa, and a second time at 3kDa to remove exogenous organic material. Two samples (C7-TP2014 and C8-TP2014) were suspected of being coated with preparatory consolidant and were treated to remove it prior to collagen extraction. Consolidant-removal treatment consisted of two rounds of sonication for 1 hour in each of 2:1 chloroform and methanol solution, acetone, methanol, and Millipore water. A further three bone samples underwent collagen extraction and analysis at the Accelerator Mass Spectrometry Laboratory at the University of Arizona (ultrafiltration was not employed for these samples). All radiocarbon dates are reported as uncalibrated radiocarbon years before present (BP).

#### 4.2.2 Bulk Collagen

Modern muskrat and beaver carcasses and skeletons from Ontario and Yukon Territory were opportunistically collected from trappers and conservations projects. The location of origin of each animal is listed in Tables 4.5 and 4.6. These samples were prepared specifically for this study. Samples were either defleshed by hand without the use of water or proteolytic enzyme, or by using dermestid beetles. Approximately 1 to 3 g of cortical bone were removed from selected *Castoroides*, *C. canadensis*, and *O. zibethicus* skeletal elements using a Dremel<sup>®</sup> tool fitted with a circular cutting wheel drill bit. Most *Castoroides* specimens were free of museum preparatory consolidant, however, the Dremel<sup>®</sup> was used to remove the surface layer of cortical bone on ancient specimens that were suspect of being coated in consolidant (primarily specimens from Florida). Skeletal elements with a thick layer of cortical bone were preferentially sampled, and any remaining cancellous bone was removed using the Dremel<sup>®</sup> tool. Soft tissue and teeth were removed prior to bone sampling of modern specimens. In addition, one dentin sample was removed from a *Castoroides* incisor for collagen extraction. Dentin was free of consolidant and any adhering enamel was removed using the Dremel<sup>®</sup> drill. One method duplicate (replication of entire analytical process, beginning with a separate sample of bone or tooth from the same individual) was performed for every ten samples prepared for collagen extraction to determine reproducibility.

The collagen extraction procedure was virtually identical for ancient and modern bone samples. Approximately 500 mg of sample material was crushed to between 850 and 180  $\mu\text{m}$ . Lipid extraction was performed using a 2:1 solution of chloroform and methanol. Collagen extraction was performed using a modified Longin (1971) method. Samples were demineralized in 0.5 M HCl at room temperature for one to eight weeks (modern bone samples demineralized much more slowly). Samples were then rinsed three times in distilled water, and soaked in a 0.1 M sodium hydroxide (NaOH) solution to remove humic acids. Collagen was gelatinized in pH 3 0.25 M HCl at 90°C for ~16 hours, and then desiccated at 90°C for ~24-36 hours. Dried collagen was crushed and ~0.38 mg was weighed into tin capsules for analysis. All samples were analyzed using a Costech Elemental Analyzer coupled with a ThermoFisher Delta Plus XL isotope ratio



mass spectrometer (IRMS) operated in continuous-flow mode. Helium was used as the transport gas to carry the CO<sub>2</sub> or N<sub>2</sub> produced from sample combustion to IRMS for analysis.

The data were collected over two analytical sessions. International and internal laboratory standards were analyzed concurrently to create the calibration curve and to monitor instrument precision and accuracy. The carbon isotopic results were calibrated to VPDB using USGS-40 ( $\pm 0.2$  ‰ one standard deviation (SD),  $n = 8$ ; accepted  $\delta^{13}\text{C} = -26.4$  ‰) and USGS-41 ( $\pm 0.4$  ‰ SD,  $n = 9$ ; accepted  $\delta^{13}\text{C} = +37.6 \pm 0.4$  ‰). The nitrogen isotopic results were calibrated to AIR using USGS-40 ( $\pm 0.1$  ‰ SD,  $n = 8$ ; accepted  $\delta^{15}\text{N} = -4.5$  ‰) and USGS-41 ( $\pm 0.1$  ‰ SD,  $n = 9$ ; accepted  $\delta^{15}\text{N} = +47.6$  ‰). Keratin and IAEA-CH-6 were used to track analytical precision and accuracy. Keratin (MP Biomedicals Inc., Cat No. 90211, Lot No. 9966H) had a  $\delta^{13}\text{C} = -24.1 \pm 0.1$  ‰ ( $n = 18$ ), which compares well with its accepted  $\delta^{13}\text{C}$  of  $-24.0$  ‰, and  $\delta^{15}\text{N} = +6.36 \pm 0.1$  ‰, which compares well with its accepted  $\delta^{15}\text{N}$  of  $+6.4$  ‰. IAEA-CH-6 had a  $\delta^{13}\text{C}$  of  $-10.5 \pm 0.1$  ‰, which compares well with its accepted value of  $-10.4$  ‰. A minimum of one internal and one international standard was analyzed for every five samples. One methodological and one method duplicate were included for every ten samples analyzed. Reproducibility between duplicates was  $\pm 0.3$  ‰ for  $\delta^{13}\text{C}$  and  $\pm 0.1$  ‰ for  $\delta^{15}\text{N}$ .

#### 4.2.3 Structural Carbonate

Bulk samples and serial-sampled structural carbonate from *Castoroides* tooth enamel were collected and analyzed for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Serial-sampling is a process that removes successive, small portions of enamel from recorded locations along the tooth. A bulk sample, in contrast, is produced by removing a large portion of enamel from an indiscriminate location, which is then turned into a homogenized powder. All bulk samples of structural carbonate material were pretreated using the following method. Due to small sample size restrictions, serial-sampled structural carbonate was not pretreated. A bulk sample of enamel was analyzed from the serial-sampled incisor (specimen C15-TP2014) to test whether the tooth was sufficiently well preserved.

#### 4.2.3.1 Bulk Enamel Samples

Bulk samples of enamel were collected and adhering dentin removed using a Dremel® fitted with a cutting wheel drill bit. Approximately 10 to 30 mg of enamel was ground to between 63 and 45  $\mu\text{m}$  for structural carbonate analysis. A further portion was ground to <45  $\mu\text{m}$  for Fourier Transform Infrared spectroscopic analysis (FTIR). Enamel powder was then soaked in 2% reagent-grade bleach for 24 hours at room temperature, rinsed five times in Millipore water, then soaked in 0.1 M acetic acid for four hours, and rinsed a further five times. Samples were promptly frozen to prevent isotopic exchange between the enamel powder and Millipore water. Once solid, the samples were freeze-dried for 24-48 hours until completely desiccated.

#### 4.2.3.2 Serial-Sampled Enamel

Enamel from the apex portion of an incomplete upper incisor from an adult *Castoroides* was serial-sampled to test for seasonal shifts in diet and drinking water as expressed by  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . The incisor was collected by local residents from a riverbed near Galena, eastern Alaska. The incisor had been coated in a clear consolidant (likely PVA glue), which was removed from the portion to be sampled using acetone. Consolidant was clearly visible as a translucent yellow coating. The enamel surface at each sample site was viewed under a Leica S8 AP0 stereomicroscope to ensure complete removal prior to sampling. A series of four microsamples were collected using a Merchantek MicroMill Drill coupled with a Leica GZ6 microscope. The sample sites were arranged in a longitudinal pattern, from the apex towards the occlusal surface, with sites located approximately 5 mm apart. A minimum of 2 mg of enamel was collected from each site (Figure 4.1). In order for each enamel sample to represent the shortest possible unit of growth time, a very limited quantity of powder was collected from each site. The thinness of *Castoroides* enamel also posed a challenge during sampling. Pretreatment of the serial-sampled structural carbonate was not possible, since the procedure would likely result in 30% sample powder loss, leaving insufficient powder to complete the intended isotopic analyses (including duplicates). Instead, pretreatment and FTIR measurements were performed on a bulk sample obtained from an enamel flake

from the same tooth. These results were used to determine preservation quality and reliability of the serial-sample isotopic results.

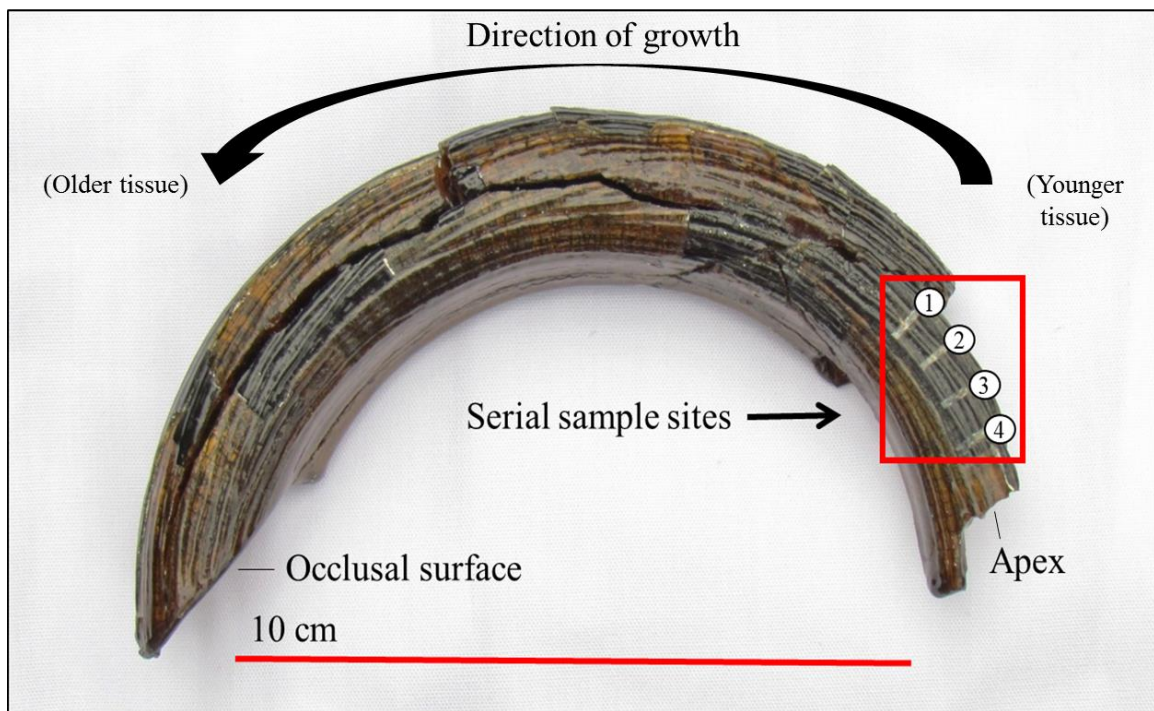
#### 4.2.3.3 Stable Isotope Analysis of Structural Carbonate

Approximately 0.9 mg of sample powder was weighed into glass vials, and reacted with phosphoric acid ( $\text{H}_3\text{PO}_4$ ) under vacuum at  $90^\circ\text{C}$  for 20 minutes using a Micromass Multiprep autosampler. The isotopic composition of the  $\text{CO}_2$  gas produced by the reaction of the sample and the acid was analyzed using a VG Optima dual-inlet IRMS. The stable isotope data were collected over a single analytical session. A series of internal and international standards were analyzed concurrently to create a calibration curve and to monitor instrument precision and accuracy. Values of  $\delta^{13}\text{C}$  were calibrated to VPDB using international standards NBS-19 ( $\pm 0.0$  ‰ SD;  $n = 5$ ; accepted  $\delta^{13}\text{C} = +1.95$  ‰) and LSVEC ( $\pm 0.2$  ‰ SD,  $n = 3$ ; accepted  $\delta^{13}\text{C} = -46.6$  ‰). Values of  $\delta^{18}\text{O}$  were calibrated to VSMOW using international standards NBS-18 ( $\pm 0.1$  ‰ SD,  $n = 3$ ; accepted  $\delta^{18}\text{O} = +7.20$  ‰) and NBS-19 ( $\pm 0.1$  ‰ SD,  $n = 5$ ; accepted  $\delta^{18}\text{O} = +28.65$  ‰). Laboratory calcite standards WS-1 and Suprapur were used to test for accuracy of the calibration curve. WS-1  $\delta^{13}\text{C} = +0.5 \pm 0.4$  ‰ ( $n = 2$ ), which compares satisfactorily with its accepted  $\delta^{13}\text{C}$  of  $+0.76$  ‰. WS-1  $\delta^{18}\text{O} = +26.0 \pm 0.2$  ‰, which compares well with its accepted  $\delta^{18}\text{O}$  of  $+26.23$  ‰. Suprapur  $\delta^{13}\text{C} = -35.8$  ‰ ( $n = 1$ ), which compares well with its accepted  $\delta^{13}\text{C}$  of  $-35.55$  ‰. Suprapur  $\delta^{18}\text{O} = +13.1$  ‰ ( $n = 1$ ), which compares well with its accepted  $\delta^{18}\text{O}$  of  $+13.1$  ‰. Internal and international standards were analyzed every five samples. A minimum of one duplicate and one method duplicate were included

for every ten samples analyzed. Sample precision was  $\pm 0.1$  ‰ for  $\delta^{13}\text{C}$ , and  $\pm 0.2$  ‰ for  $\delta^{18}\text{O}$ .

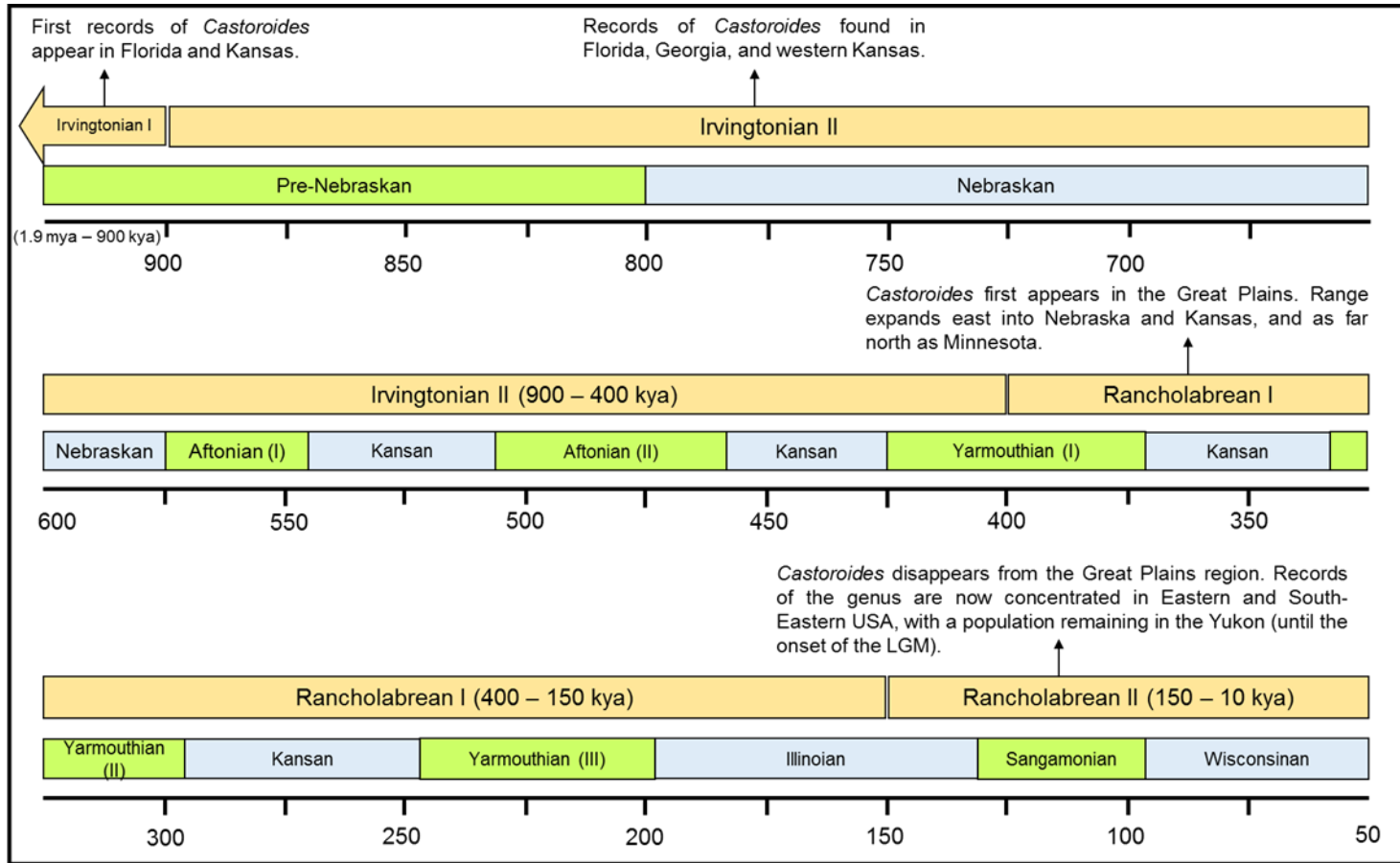
#### 4.2.3.4 Fourier transform infrared spectroscopy

As described in Chapter 2, Fourier transform infrared spectroscopy (or FTIR) was used to assess *post-mortem* alteration of the *Castoroides* bulk enamel bioapatite structure and isotopic composition. The crystallinity index (CI) and carbonate-phosphate ratio (C/P) were examined for each sample to determine the effects of pretreatment on bulk structural carbonate  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . The pros and cons of pretreatment methodologies and the implications of the CI and C/P results for this sample set are discussed in depth in Chapter 2. In short, the FTIR parameters employed indicated that the specimens were not ideally preserved and most had experienced a loss of carbonate material and undergone some oxygen isotopic exchange in the depositional environment.



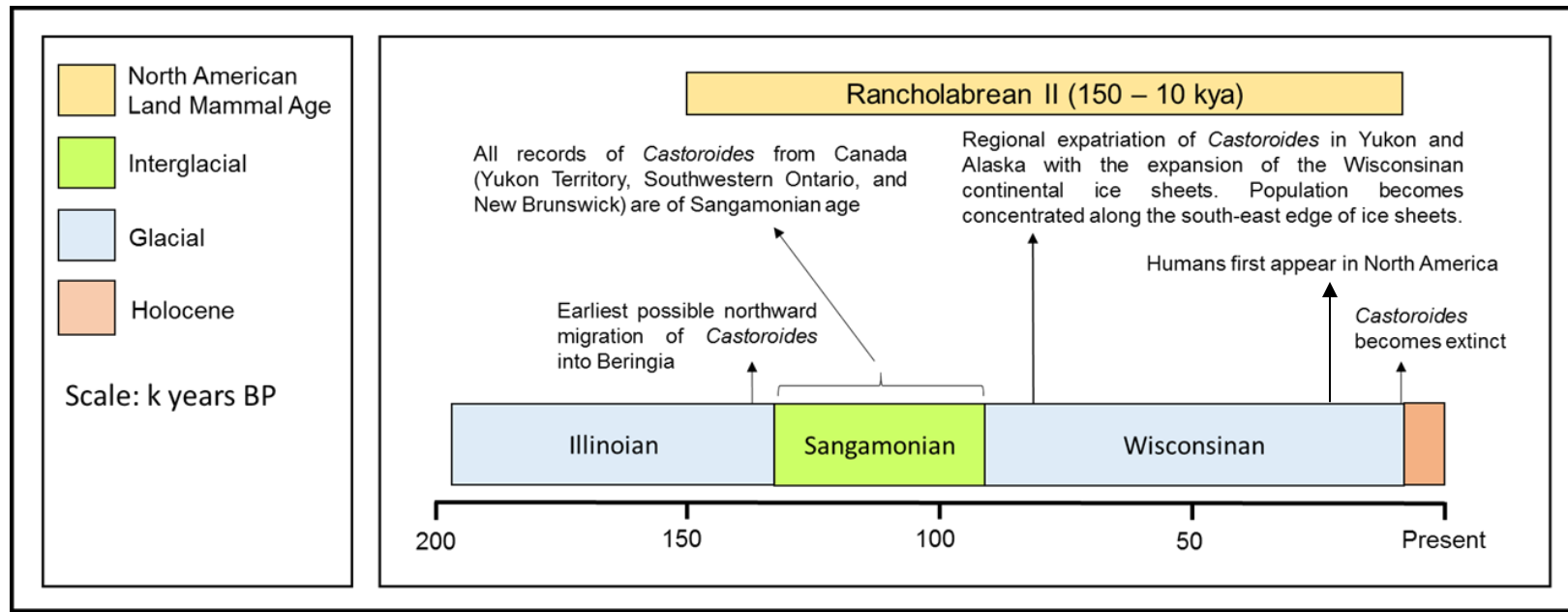
**Figure 4.1 Incisor C15-TP2014 displaying serial sample sites.**

# PLEISTOCENE NORTH AMERICA



North American Land Mammal Age  
 Interglacial  
 Glacial  
 Scale: k years BP

**Figure 4.2** Timeline of known geographic fluctuations in *Castoroides* distribution.



**Figure 4.3** Timeline of known geographic fluctuations in *Castoroides* distribution during the terminal Pleistocene. Diagrams compiled using Barbour, 1931; Boulanger and Lyman, 2014; Cahn, 1932; FAUNMAP Working Group, 1994; Harington, 1978; Harington, 2011; Hulbert et al., 2014; McDonald and Bryson, 2010; McDonald and Glotzhober, 2008; Martin, 1969; Miller et al., 2000; Morgan and White, 1995; Parmalee and Graham, 2002; Richards and Swinehart, 2001; Stirton, 1965; Stuart-Williams and Schwarcz, 1997.

### 4.3 Results

All stable isotope results are reported in delta ( $\delta$ ) notation as ratios relative to the relevant international standards (VPDB, AIR, and VSMOW) as follows:

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1],$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the heavy to light isotope ratios (i.e.  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$ ) of the same element in both the sample material and the standard (Coplen, 2011).

#### 4.3.1 Radiocarbon Dating

Radiocarbon dates obtained for *Castoroides* bone collagen are reported in Table 4.3. Specimens from three geographically separate giant beaver populations were sent for dating. Bone samples that originated from Florida were not sufficiently well preserved to procure collagen for radiocarbon dating. However, the genus is known to have a long history of occupation in southeastern North America, as is demonstrated by the presence of *Castoroides leiseyorum* (Irvingtonian age) and *Castoroides dilophidus* (Rancholabrean age) fossils in deposits from Florida and Georgia (Hulbert et al., 2014) (Figures 4.2 and 4.3).

Specimens from Ohio and Yukon Territory were better preserved and produced viable collagen for dating. Bone collagen from giant beavers that lived within the Arctic Circle yielded dates that were infinite or approaching the upper limit for radiocarbon dating (>40,000 years BP). It is unsurprising that *Castoroides* inhabited the north prior to 50,000 years ago, as giant beaver populations could only have migrated northward during warmer intervals following ice sheet retreat and the presence of meltwater-fueled wetlands across Canada. Giant beavers likely inhabited Yukon Territory and Alaska around the time of the Sangamonian interglacial (Figures 4.2 and 4.3). It is also possible that giant beaver returned periodically to the north during warm interstadials during the mid-Wisconsin. Dates from Ohio indicate that the Great Lakes Basin was home to one of the last remaining populations of giant beaver prior to their extinction. Giant beaver

(specifically *Castoroides ohioensis*) are commonly found in late Pleistocene sediments within the Great Lakes Basin and the Mississippi drainage basin, and appear to have been concentrated south of the fluctuating continental ice sheets during the Last Glacial Maximum prior to their extinction (Boulanger and Lyman, 2014; Cahn, 1932; Holman, 1995; McDonald and Bryson, 2010; McDonald and Glotzhofer, 2008; Richards and Swinehart, 2001).

#### 4.3.2 Bulk Collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

The carbon and nitrogen isotopic composition of bulk collagen ( $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$ , respectively) obtained for *Castoroides*, *C. canadensis*, and *O. zibethicus* are presented in Table 4.4 to 4.6, along with measures of collagen protein preservation quality (atomic C:N and collagen yields). *Castoroides* (n = 11)  $\delta^{13}\text{C}_{\text{col}}$  ranges from  $-21.2$  to  $-10.9$  ‰, with a mean of  $-17.6$  ‰. Values of  $\delta^{15}\text{N}_{\text{col}}$  range from  $+1.9$  to  $+7.7$  ‰, with a mean of  $+5.8$  ‰. *Castoroides* from Arctic regions (Old Crow Basin) exhibit a greater range in both  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  than *Castoroides* from temperate regions (Ohio). Northern animals have statistically higher mean  $\delta^{13}\text{C}_{\text{col}}$  ( $-16.7 \pm 4.0$  ‰) than southern animals ( $-20.1 \pm 0.5$  ‰) (t-test, p = 0.04).

The potential modern analog species, *C. canadensis* and *O. zibethicus*, demonstrate distinctive isotopic compositions relative to one another. Note that the modern species were deliberately collected from similar geographic regions as the *Castoroides* fossils. The range and distribution of muskrat  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  more closely resembles that of *Castoroides*, than the modern beaver. Modern beaver (n = 8)  $\delta^{13}\text{C}_{\text{col}}$  varies very little, ranging from  $-24.0$  to  $-23.2$  ‰, with a mean of  $-23.5$  ‰. Modern beaver  $\delta^{15}\text{N}_{\text{col}}$  is more variable, ranging from  $+1.4$  to  $+9.5$  ‰, with a mean of  $+4.5$  ‰. The variation in  $\delta^{15}\text{N}_{\text{col}}$  of beaver samples collected for this study, however, is likely misrepresentative of modern beavers living in the wild, and is not strictly comparable to ancient species. The diet of *C. canadensis* living in agricultural regions such as southern and eastern Ontario can easily be heavily influenced by natural and synthetic fertilizer products, as well as septic discharge whose  $\delta^{15}\text{N}$  may range from  $-2.0$  to  $+20.0$  ‰ (Aravena et al., 1993 and references therein; Russell, 2015). Any increase in



the  $\delta^{15}\text{N}$  of the local baseline is transferred through the food chain via the plants grown in the contaminated soil. Agricultural and septic runoff easily collects and spreads throughout drainage basins occupied by beavers, where it influences both macrophytes, and terrestrial plants growing peripherally to the contaminated wetland habitat. We propose that beavers trapped and collected from New Liskeard and Inverhuron, Ontario, were living and feeding in drainage catchments heavily impacted by localized anthropogenic activities.

*O. zibethicus* exhibited significant isotopic overlap with *Castoroides*. Muskrat ( $n = 12$ )  $\delta^{13}\text{C}_{\text{col}}$  ranged from  $-22.7$  to  $-10.7$  ‰, with a mean of  $-15.3$  ‰. Muskrat  $\delta^{15}\text{N}_{\text{col}}$  ranged from  $+2.2$  to  $+6.6$  ‰, with a mean of  $+5.1$  ‰. Nearly all muskrat samples were collected from regions with no agricultural industry, and therefore  $\delta^{15}\text{N}_{\text{col}}$  is highly unlikely to be compromised in a manner similar to the beavers collected from New Liskeard and Inverhuron.

### 4.3.3 Structural Carbonate

#### 4.3.3.1 Fourier transform infrared spectroscopy

FTIR was performed on the pretreated structural carbonate samples (Table 4.7). In the interests of maintaining consistent sample preparatory conditions within L.S.I.S., only the isotopic results of pretreated samples are discussed in this Chapter. A peak at  $710\text{ cm}^{-1}$  was not observed in the FTIR spectra for any of the enamel samples analyzed. A peak at this position indicates the presence of calcite (secondary carbonate precipitated from the burial environment). The accepted CI values for unaltered enamel are 3.0 to 3.5. Values higher than this are indicative of bioapatite recrystallization or growth in crystal size. The accepted C/P ratio of unaltered enamel is  $\sim 0.4$  (Smith et al., 2007; Webb et al., 2014; Wright and Schwarcz, 1996). A lower ratio suggests that carbonate material has been lost during burial. A ratio higher than 0.5 suggests that secondary carbonates have been included in the sample and are a source of contamination. Several *Castoroides* specimens from Florida exhibited CI values higher than the accepted range (3.6 to 3.9), while those from Ohio and Alaska did not (3.4). All specimens have a C/P ratio lower than the accepted value. Pretreatment of structural carbonate material is intended to

improve FTIR parameters by removing contaminants. The changes to CI and C/P after the pretreatment, however, are not statistically significant (t-test, CI p-value = 0.76; C/P p-value = 0.06) (FTIR results for both untreated and pretreated samples included in methodological study in Chapter 2). The FTIR parameters indicate carbonate loss or exchange within the bioapatite matrix (not the inclusion of secondary carbonate); as such, pretreatment is of limited utility here. Nonetheless, the FTIR parameters do indicate that the *Castoroides* tooth specimens utilized in this study were not ideally preserved.

#### 4.3.3.2 Bulk Enamel Sample $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

The stable carbon and oxygen isotopic compositions of bulk pretreated structural carbonate ( $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ ) from *Castoroides* enamel are presented in Table 4.7. *Castoroides*  $\delta^{13}\text{C}_{\text{sc}}$  (n = 8) ranges from  $-19.9$  to  $-9.8$  ‰ (VPDB), with a mean of  $-13.1$  ‰. The  $\delta^{18}\text{O}_{\text{sc}}$  ranges from  $+15.5$  to  $+29.8$  ‰ (VSMOW), with a mean of  $+26.0$  ‰.

#### 4.3.3.3 Serial-Sampled Enamel $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$

The  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  of untreated serial-sampled enamel from specimen C15-TP2014 are presented in Table 4.8. The  $\delta^{13}\text{C}_{\text{sc}}$  ranges from  $-14.1$  to  $-11.9$  ‰, while  $\delta^{18}\text{O}_{\text{sc}}$  ranges from  $+12.8$  to  $+14.3$  ‰. The  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  both became lower with decreasing age of enamel formation (meaning the most recently formed enamel possesses the most  $^{13}\text{C}$ - and  $^{18}\text{O}$ -depleted isotopic signatures) (see Figure 4.1).

Table 4.3.  $^{14}\text{C}$  age (BP) obtained from *Castoroides* bone bulk collagen. Date for C10-TP2014 from McDonald and Glotzhober, 2008.

<b>Sample no.</b>	<b>Sample origin</b>	<b><math>^{14}\text{C}</math> age (BP)</b>	<b><math>\pm</math></b>	<b>Lab code</b>
C3-TP2014	Old Crow Basin, Yukon Territory	>44,600		UCIAMS151528
C4-TP2014	Old Crow Basin, Yukon Territory	>43,700		UCIAMS151529
C6-TP2014	Old Crow Basin, Yukon Territory	44,600	2600	UCIAMS151530
C8-TP2014	Old Crow Basin, Yukon Territory	>42,200		UCIAMS151531
C10-TP2014	Clear Creek Township, Ohio	12,040	35	UCLAMS11219
C11-TP2014	Williams County, Ohio	11,961	80	AA105557
C22-TP2014	Harmony Township, Ohio	11,168	53	AA105559

Table 4.4 *Castoroides*  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$ . Values in bold indicate mean and 1 SD where multiple analyses were performed for the same specimen.

Sample no.	Sample origin	$\delta^{13}\text{C}_{\text{col}}$ (‰, VPDB)	$\delta^{15}\text{N}_{\text{col}}$ (‰, AIR)	Collagen Yield (%)	C:N
C1-TP2014	Old Crow Basin, YT	-21.2	+6.3	1.4	3.4
C3-TP2014	Old Crow Basin, YT	<b>-19.1 ± 0.3</b>	<b>+1.9 ± 0.1</b>	1.4	<b>3.4</b>
C4-TP2014	Old Crow Basin, YT	<b>-10.7 ± 0.2</b>	<b>+5.7 ± 0.1</b>	3.6	<b>3.3</b>
C5-TP2014	Old Crow Basin, YT	-18.5	+7.7	Data not provided*	3.4
C6-TP2014	Old Crow Basin, YT	-16.0	+6.0	1.1	3.2
C7-TP2014	Old Crow Basin, YT	-14.0	+7.4	Data not provided*	3.5
C8-TP2014	Old Crow Basin, YT	<b>-12.4 ± 0.2</b>	<b>+6.2 ± 0.0</b>	2.4	<b>3.3</b>
C9-TP2014	Old Crow Basin, YT	-21.2	+6.8	Data not provided*	3.5
C10-TP2014	Clear Creek Township, Ohio	<b>-20.2 ± 0.1</b>	<b>+5.6 ± 0.1</b>	3.1	<b>3.1</b>
C11-TP2014	Williams County, Ohio	<b>-20.6 ± 0.1</b>	<b>+4.5 ± 0.1</b>	11.2	<b>3.3</b>
C22-TP2014	Harmony Township, Ohio	-19.5	+5.4	3.8	3.2
C2-TP2014	Old Crow Basin, YT	n/a	n/a	0.39	n/a
C12-TP2014	Santa Fe River, Florida	n/a	n/a	0.06	n/a
C13-TP2014	Oklawaha River, Florida	n/a	n/a	0.02	n/a
C14-TP2014	Santa Fe River, Florida	n/a	n/a	0.06	n/a

\*Collagen extraction performed at the Keck Carbon Cycle AMS Facility at the University of California, Irvine for radiocarbon dating purposes. Remainder of collagen powder returned and analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at LSIS.

Table 4.5 *Castor canadensis*  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$ .

Sample no.	Sample origin	$\delta^{13}\text{C}_{\text{col}}$ (‰, VPDB)	$\delta^{15}\text{N}_{\text{col}}$ (‰, AIR)
B1-TP2013	Inverhuron, Ontario	-23.6	+7.1
B2-TP2013-A	Dawson City, YT	-24.0	+3.4
B3-TP2014-1	New Liskeard, Ontario	-23.7	+2.0
B4-TP2014-1	New Liskeard, Ontario	-23.1	+9.5
B5-TP2014	New Liskeard, Ontario	-23.2	+8.0
B6-TP2014-1	Whitehorse, YT	-23.7	+2.0
B7-TP2014	Whitehorse, YT	-23.3	+1.4
B8-TP2014-1	Whitehorse, YT	-23.7	+2.2

Table 4.6 *Ondatra zibethicus*  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$ . Values in bold indicate mean and 1 SD when multiple analyses were performed for the same specimen.

Sample no.	Sample origin	$\delta^{13}\text{C}_{\text{col}}$ (‰, VPDB)	$\delta^{15}\text{N}_{\text{col}}$ (‰, AIR)
M1-TP2013	London, Ontario	-22.7	+5.0
M3-TP2014	Old Crow Flats, YT	-13.6	+5.3
M4-TP2014	Old Crow Flats, YT	-14.2	+2.2
M5-TP2014	Old Crow Flats, YT	-17.5	+3.8
M6-TP2014	Old Crow Flats, YT	-22.0	+5.9
M7-TP2014	Old Crow Flats, YT	-15.1	+6.1
M8-TP2014	Old Crow Flats, YT	-14.6	+3.9
M9-TP2014	Old Crow Flats, YT	-14.3	+6.6
M10-TP2014	Old Crow Flats, YT	-15.0	+5.3
M11-TP2014	Old Crow Flats, YT	-12.6	+5.7
M13-TP2014	Old Crow Flats, YT	<b>-10.7 ± 0.1</b>	<b>+6.0 ± 0.0</b>
M14-TP2014	Old Crow Flats, YT	-11.7	+4.7

Table 4.7  $\delta^{13}\text{C}_{\text{sc}}$ ,  $\delta^{18}\text{O}_{\text{sc}}$ , and FTIR parameters of pretreated structural carbonate from *Castoroides* enamel. The equations used to calculate  $\delta^{18}\text{O}_{\text{p}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  are outlined in Section 4.4.4.

Sample no.	Sample origin	$\delta^{13}\text{C}_{\text{sc}}$ (‰, VPDB)	$\delta^{18}\text{O}_{\text{sc}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{p}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{mw}}$ (‰, VSMOW)	FTIR CI	FTIR C/P
C15-TP2014	Galena, Alaska	-14.1	+15.5	+6.9	-22.2	3.4	0.18
C20-TP2014	Williams County, Ohio	-10.7	+26.6	+17.7	-6.9	3.4	0.17
C26-TP2014	Peace River, Florida	-13.6	+29.8	+20.9	-2.4	3.4	0.20
C28-TP2014	Santa Fe River, Florida	-12.1	+29.6	+20.7	-2.7	3.3	0.20
C29-TP2014	Oklawaha River, Florida	-13.6	+26.3	+17.5	-7.2	3.6	0.19
C31-TP2014	Santa Fe River, Florida	-19.9	+25.3	+16.5	-8.6	3.6	0.17
C32-TP2014	Peace River, Florida	-9.8	+29.3	+20.4	-3.1	3.9	0.15
C33-TP2014	Santa Fe River, Florida	-11.0	+25.3	+16.5	-8.6	3.6	0.15

Table 4.8  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  of untreated structural carbonate from serial-sampled *Castoroides* enamel.

The equations used to calculate  $\delta^{18}\text{O}_{\text{p}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  are outlined in Section 4.4.4. Tooth specimen originated from Galena, Alaska.

Sample no.	$\delta^{13}\text{C}_{\text{sc}}$ (‰, VPDB)	$\delta^{18}\text{O}_{\text{sc}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{p}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{mw}}$ (‰, VSMOW)
C15-TP2014-1	-11.9	+14.3	+5.7	-23.8
C15-TP2014-2	-13.4	+13.0	+4.4	-25.7
C15-TP2014-3	-13.9	+12.8	+4.2	-25.9
C15-TP2014-4	-14.1	+12.8	+4.2	-25.9

## 4.4 Discussion

### 4.4.1 Bone Collagen: Suess Effect Correction

The Suess Effect must be taken into account when employing stable carbon isotopic analysis as a tool for palaeodietary reconstruction (Long et al., 2005). The  $\delta^{13}\text{C}$  of animal and plant tissues collected at different points in time must be adjusted so that they are isotopically comparable. Recent anthropogenic activity, primarily the widespread combustion of fossil fuels, has changed the average  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  (Long et al., 2005). The onset of the Industrial Revolution marks the rapid addition of large quantities of  $^{12}\text{CO}_2$  to Earth's atmosphere, which has resulted in the lowering of atmospheric  $\text{CO}_2$   $\delta^{13}\text{C}$  by approximately  $-2.1$  ‰ (as of 2014) over the last two centuries (Scripps  $\text{CO}_2$  Program, 2015).

Atmospheric  $\text{CO}_2$  is the dominant source of carbon to the majority of photosynthetic organisms on Earth. As a result,  $\delta^{13}\text{C}$  of primary producers growing today reflect the current isotopic composition of atmospheric  $\text{CO}_2$ . In studies where isotopic data from modern plants are incorporated into dietary baselines for identifying forage preferences of ancient herbivores, a correction factor must be applied so that the palaeodietary interpretations are made relative to a common baseline.

In this study, all modern *C. canadensis*, *O. zibethicus*, and plant samples were collected in 2014. The *Castoroides* specimens from which bulk collagen was obtained lived during the late Pleistocene. The  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$  in 2014 and in the late Pleistocene were  $-8.6$  ‰ and  $-6.5$  ‰, respectively (Schmitt et al., 2012; Scripps  $\text{CO}_2$  Program, 2014). Accordingly, a correction factor of  $2.1$  ‰ was applied to *C. canadensis* and *O. zibethicus*  $\delta^{13}\text{C}_{\text{col}}$ , as well as plant  $\delta^{13}\text{C}$  to ensure comparability with *Castoroides*  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{13}\text{C}_{\text{sc}}$  in the statistically-based dietary mixing model.

### 4.4.2 Collagen-Diet Isotopic Discrimination

Isotopic discrimination between rodent collagen and diet for both carbon and nitrogen isotopes must be considered before forage preference can be determined. There

is a direct relationship between the isotopic composition of an animal and its diet. Isotopic fractionation, or the preferential use of heavy versus light isotopes of a given element, occurs as macronutrients from the diet are incorporated into consumer tissues. As a result, the tissue of a consumer becomes more enriched in heavy isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) relative to its diet. An animal's position in the food chain also affects the degree of fractionation between bodily tissues and diet. For example, herbivores exhibit a greater enrichment in  $^{13}\text{C}$  from diet to tissue than do omnivores and carnivores. The exact degree of  $^{13}\text{C}$  or  $^{15}\text{N}$  enrichment depends on species, diet, and tissue type.

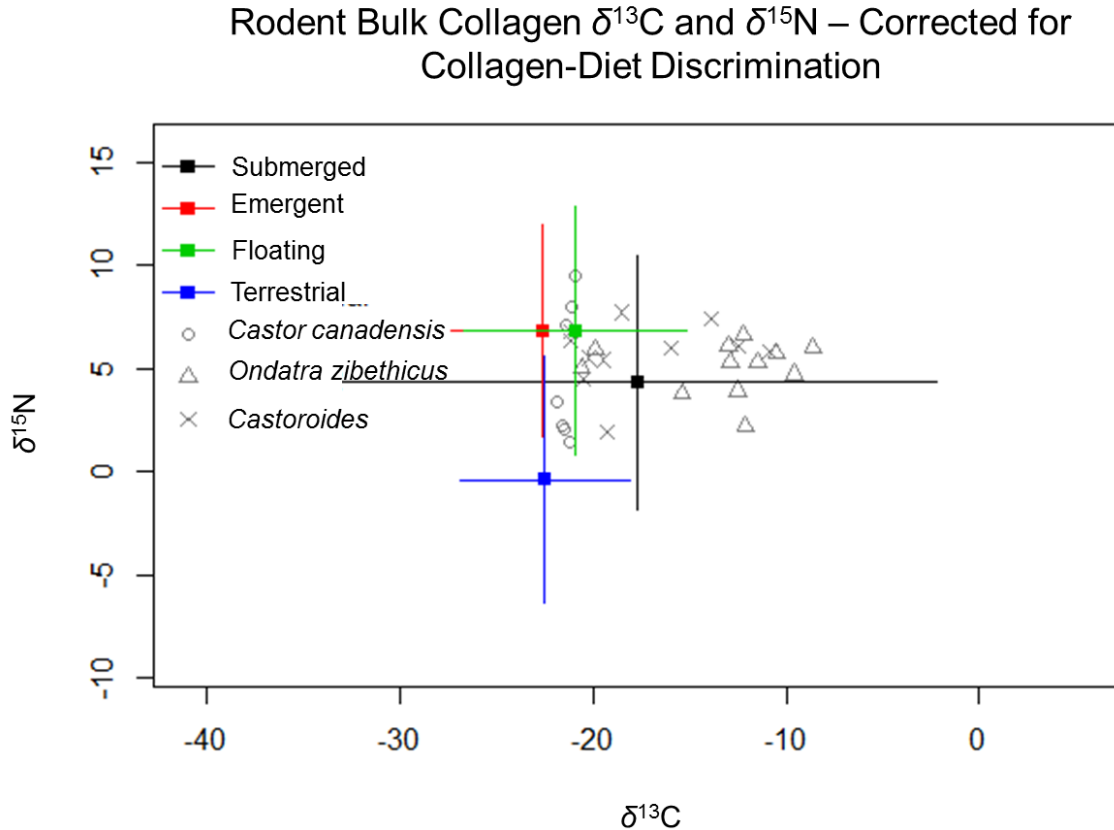
In large-bodied herbivores, bone collagen is typically procured from ~75% protein and ~25% energy (lipids and carbohydrates) macronutrients (Fernandes et al., 2012; Morrison et al., 2000). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of collagen is closely correlated with that of dietary protein (Ambrose, 2000). The percentage of protein in the diet does not impact the  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  of bone collagen, so long as the animal is not suffering from chronic protein deficiency (Ambrose, 2000; Ambrose and Norr, 1993; Froehle et al., 2010; Jim et al., 2004; Krueger and Sullivan, 1984; Loftus and Sealy, 2012). Based on prior studies of small rodents being fed a diet ~20 % protein and comprised of entirely  $\text{C}_3$  foodstuffs,  $\Delta^{13}\text{C}_{\text{collagen-diet}}$  was found to be between +3.7 and +5.0 ‰ (average +4.2 ‰) (Ambrose and Norr, 1993; Froehle et al., 2010; Jim et al., 2004; Tieszen and Fagre, 1993). Combined  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  datasets for rodent bone collagen from controlled feeding studies are scarce; however, the most widely accepted  $\Delta^{15}\text{N}_{\text{collagen-diet}}$  is +3.0 ‰ (Ambrose, 2002). This applies to mammals not suffering from aridity or water-stress (Ambrose, 1991). Rodent bone  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  are therefore predicted to be higher than diet by +4.2 ‰ and +3.0 ‰, respectively (Ambrose, 1991; 2000; 2002; DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Froehle et al., 2010; Kelly, 2000; Schoeninger and DeNiro, 1984). The SIAR mixing model used here incorporated these respective dietary discrimination factors for all three rodent species. SIAR also incorporated the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of each plant category included in the dietary baseline presented in Chapter 3. A dietary composition was then calculated using the SIAR mixing model for each rodent species, as is discussed next.



#### 4.4.3 Statistical Modelling in R

Following Suess Effect and dietary discrimination isotopic corrections, the  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  of *Castoroides*, *Castor canadensis*, and *Ondatra zibethicus* bone collagen are directly comparable to the dietary isotopic baseline (Figure 4.4). The dietary baseline has been constructed using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  obtained for modern aquatic and terrestrial plant samples, as described and itemized in Chapter 3. The plant samples originate from a mixture of North American subarctic and mid-latitude locations that were intentionally similar to the sites where both modern and ancient rodent samples were collected.

The rodent feeding niche was determined using a Bayesian statistical mixing model in R Studio, with code developed specifically for food web analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of primary producers and consumers (Stable Isotope Analysis in R). The mixing model was used to determine which sources of forage contributed to the diet of each rodent, and in what proportions. The outcomes of the modelling are presented in Figures 4.5 to 4.7.



**Figure 4.4 SIAR mixing model incorporating the dietary baseline and rodent bulk bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Corrected for Suess Effect and Collagen-Diet Discrimination.**

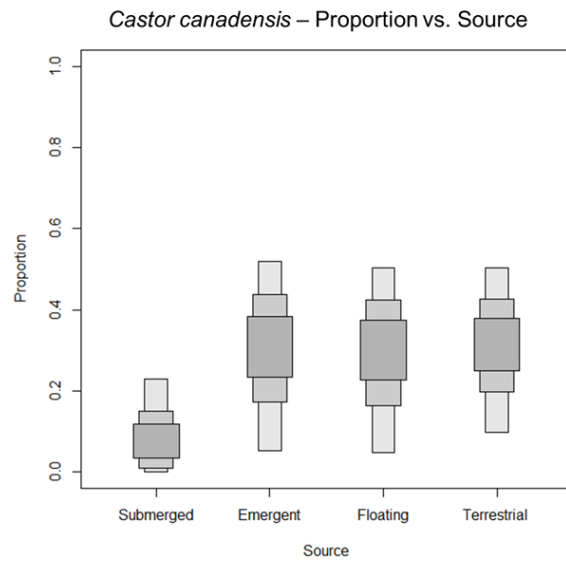
The Proportion versus Source boxplot for *Castor canadensis* (Figure 4.5) indicates that the bulk of the species' diet was composed of terrestrial browse, with approximately equal quantities of emergent and floating macrophytes, and a very small proportion of submerged macrophytes. This correlates relatively well with what is known about modern beaver diet (extensive consumption of tree foliage and floating macrophyte rhizomes).

The data incorporated in this study are not strictly representative of wild beaver diet. As previously discussed, the  $\delta^{15}\text{N}_{\text{col}}$  from three specimens (B1-TP2013, B4-TP2014-1, and B5-TP2014) are seemingly a trophic level too high for what is known to be a purely herbivorous animal. The  $\delta^{15}\text{N}$  of North American beaver from archaeological sites

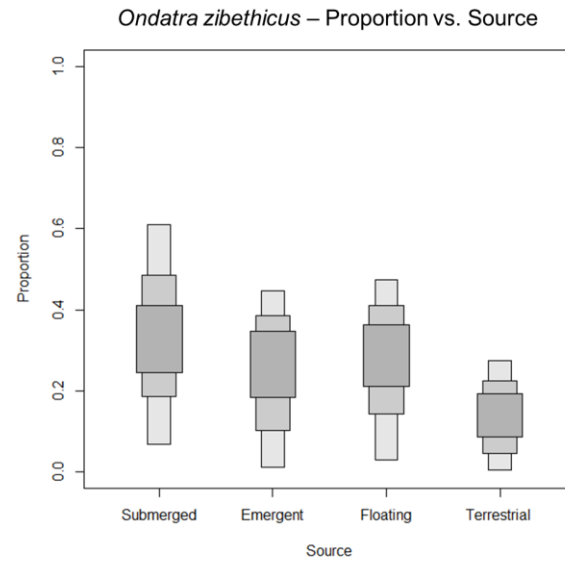
and from regions with very limited anthropogenic impact fall within the range of +3.4 to +5.2 ‰ (Katzenberg, 2006; Milligan, 2008). The unexpectedly high  $\delta^{15}\text{N}$  from animals from New Liskeard and Inverhuron represent individuals living in wetlands where the baseline  $\delta^{15}\text{N}$  of local aquatic plants and shoreline tree species (i.e. *Salix*) are highly  $^{15}\text{N}$ -enriched as a result of septic or fertilizer contaminants. If these three samples were removed, the projected proportion of emergent and floating macrophytes would decrease for the *Castor canadensis* category. Unfortunately, the current sample size is too small to test this point rigorously.

The Proportion versus Source boxplot for *Ondatra zibethicus* (Figure 4.6) indicates a diet composed of approximately equal proportions of each macrophyte type, with a lower proportion of terrestrial material. Further observation of live animals in the wild is necessary to confirm this point, but it appears that floating and submerged macrophytes are more integral to muskrat diet than previously supposed. In current literature, emergent macrophytes are typically emphasized as the primary source of forage (i.e. Campbell and MacArthur, 1996; Danell, 1979). Terrestrial plants likely contribute less than a quarter to dietary forage. Given their small size, however, muskrats are not consuming tree foliage, but grasses, forbs, and shrubs growing in proximity to the water's edge. This analysis must also be interpreted with caution as up to 5% of muskrat diet may be obtained from animal protein in the form of small vertebrates and invertebrates. The scope of the present project did not include potential omnivorous sources; hence, it is not known how minor degrees of omnivory impact the isotopic composition of muskrat bone collagen.

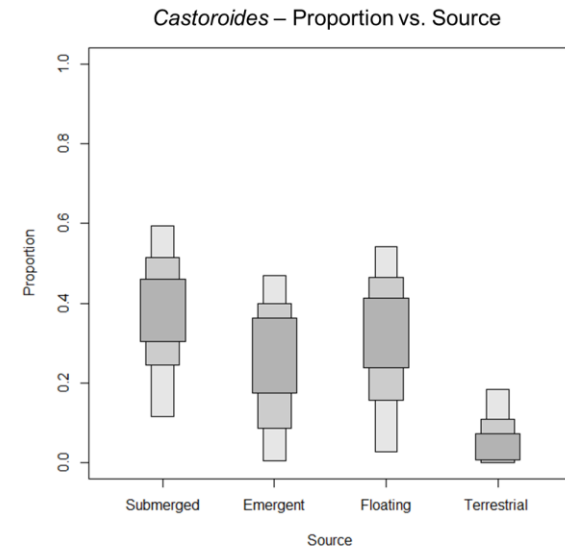
The Proportion versus Source boxplot for *Castoroides* (Figure 4.7) indicates that macrophytes were its predominant source of forage. Submerged and floating macrophytes probably contributed the most to giant beaver diet, while terrestrial forage probably contributed the least. This pattern is the opposite to that of *Castor canadensis*, suggesting that the giant beaver and its smaller cousin had complementary forage preferences and were not in direct competition for the same food resources. Overall, macrophytes appear to contribute a greater proportion to the diet of the giant beaver than they do to the diet of the modern muskrat or beaver.



**Figure 4.5 SIAR Proportion versus Source boxplot for *Castor canadensis*. Corrected for Suess Effect and Collagen-Diet Discrimination.**



**Figure 4.6 SIAR Proportion versus Source boxplot for *Ondatra zibethicus*. Corrected for Suess Effect and Collagen-Diet Discrimination.**



**Figure 4.7 SIAR Proportion versus Source boxplot for *Castoroides*. Corrected for Suess Effect and Collagen-Diet Discrimination.**

#### 4.4.4 Oxygen Isotopes as a Palaeoclimate Proxy

The oxygen isotopic composition of bulk structural carbonate ( $\delta^{18}\text{O}_{\text{sc}}$ ) was measured for *Castoroides* tooth enamel to help discern climatic conditions present when the animals were alive. The oxygen isotopic composition of bioapatite from ancient mammalian bones and teeth is a well-established proxy for palaeoclimate (Barrick et al., 1996; Koch et al., 1989; Lee-Thorp et al., 2003; Metcalfe et al., 2011; Palmqvist et al., 2008; Yann and DeSantis, 2014, to list a few examples). Structural carbonate and phosphate both contribute to the mineral portion (bioapatite) of bones and teeth. The oxygen isotopic composition of herbivore bioapatite phosphate ( $\delta^{18}\text{O}_{\text{p}}$ ) and structural carbonate ( $\delta^{18}\text{O}_{\text{sc}}$ ) are strongly correlated to the  $\delta^{18}\text{O}$  of ingested water (Longinelli, 1984). Here, bulk  $\delta^{18}\text{O}_{\text{sc}}$  of *Castoroides* tooth enamel is used to calculate the  $\delta^{18}\text{O}$  of local precipitation from sites across Pleistocene North America (Table 4.2).

Mammals are 65 to 70% water by body weight. Species that are obligate drinkers acquire the majority of their water from liquid surface water fueled by local precipitation. Rayleigh distillation processes, in addition to atmospheric moisture residence times, and rain fall type (see Aggarwall, 2012, 2016) control the fractionation of both oxygen and hydrogen isotopes in water vapour and subsequent rainfall (Dansgaard, 1953, 1954). The fractionation associated with Rayleigh distillation creates a  $\delta^{18}\text{O}_{\text{mw}}$  gradient between equatorial and polar regions (Dansgaard, 1964). As a result,  $\delta^{18}\text{O}_{\text{mw}}$  becomes lower with increasing latitude, altitude and increasing distance inland (continentality). As a result, meteoric water collected from continental interiors, mountainous regions or high latitudes will be more depleted of  $^{18}\text{O}$  than meteoric water in coastal regions, lowlands or equatorial regions. For example,  $\delta^{18}\text{O}_{\text{mw}}$  commonly becomes lower by  $\sim 0.6\text{‰}$  per increasing degree of latitude (IAEA, 2001), with this effect becoming even more pronounced at very high latitudes. In concert with geographic and topographic effects, climate (especially seasonality) also plays a role in determining  $\delta^{18}\text{O}_{\text{mw}}$ . Surface temperature, humidity, and air mass source all influence the oxygen isotopic composition of seasonal precipitation. In short, obtaining  $\delta^{18}\text{O}_{\text{mw}}$  for ancient ecosystems from isotopic

analysis of bioapatite can provide a wealth of information about climatic conditions in the past.

Herbivore bioapatite phosphate precipitates in oxygen isotopic equilibrium with blood water, which is predominantly sourced from ingested drinking water, and to a lesser degree, from leaf water in forage (Longinelli, 1984; Longinelli and Padalino, 1980). The oxygen isotopic composition of drinking water is closely related to that of local meteoric water in most cases (Daux et al., 2008; White et al., 2007). Mammals weighing more than 1 kg have thermoregulatory abilities and maintain their body temperature at or near 37°C. The fractionation factor between bioapatite  $\delta^{18}\text{O}$  and blood water  $\delta^{18}\text{O}$  is known for this temperature (Bryant and Froelich, 1995; Luz and Kolodny, 1985; Stuart-Williams and Schwarcz, 1997). Hence, if  $\delta^{18}\text{O}_p$  is known, it is possible to calculate  $\delta^{18}\text{O}_{mw}$  for the region from which the sample material originated.

In this study,  $\delta^{18}\text{O}_{sc}$  was acquired from *Castoroides* enamel. In order to determine  $\delta^{18}\text{O}_{dw}$  for the various fossil collection sites,  $\delta^{18}\text{O}_{sc}$  was first recalculated to its equivalent as  $\delta^{18}\text{O}_p$ . This was accomplished using the generally accepted oxygen isotope relationship between structural carbonate and phosphate for medium and large terrestrial herbivores (>1 kg), for the same element of the same individual (Bryant and Froelich, 1995; Iacumin et al., 1996; Metcalfe et al., (2011):

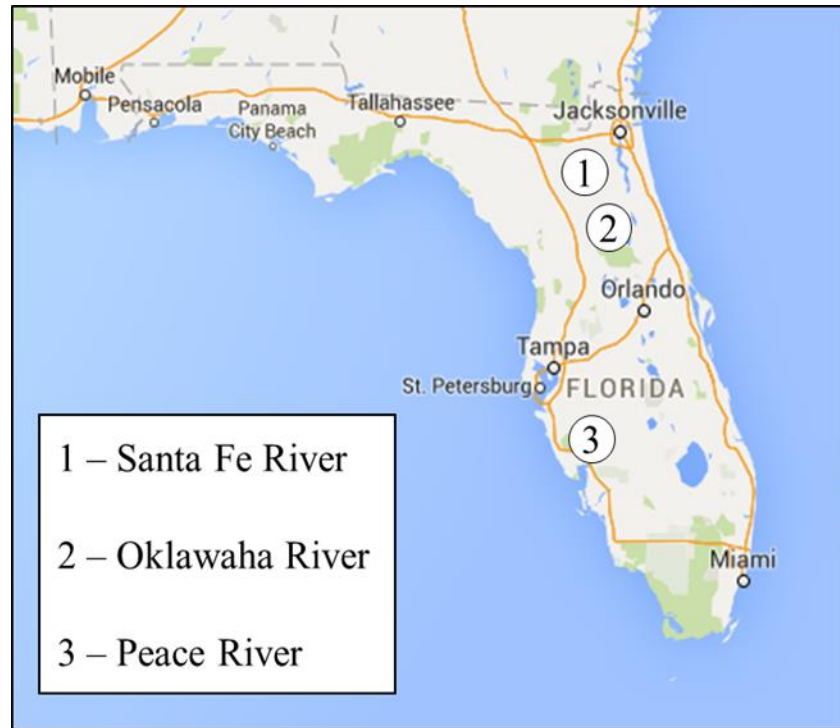
$$\delta^{18}\text{O}_p = 0.98\delta^{18}\text{O}_{sc} - 8.32 \quad (\text{Equation 4.1})$$

Average  $\delta^{18}\text{O}_{mw}$  was then calculated from  $\delta^{18}\text{O}_p$  following Huertas et al. (1995):

$$\delta^{18}\text{O}_p = 0.71\delta^{18}\text{O}_{mw} + 22.60 \quad (\text{Equation 4.2})$$

The results of these calculations for each individual *Castoroides* are listed in Table 4.7. The  $\delta^{18}\text{O}_{mw}$  derived from the *Castoroides* enamel samples is only representative of precipitation over the span of a few months, and not average annual precipitation. With this caveat, Pleistocene  $\delta^{18}\text{O}_{mw}$  from each site can now be compared to modern isotopic compositions at the sites where the *Castoroides* remains were found (Figure 4.8).





**Figure 4.9** *Castoroides* specimen localities from Florida.

**Image obtained from Google Maps, 2016.**

individual lived during transitional conditions, and after the ice sheets had made a full retreat from the Great Lakes region.

Results for the Florida samples, whose locations are illustrated in Figure 4.9, were more variable. Three specimens have calculated  $\delta^{18}\text{O}_{\text{mw}}$  ( $-3.1$  to  $-2.4$  ‰) that are within the present-day average annual range for precipitation. The calculated  $\delta^{18}\text{O}_{\text{mw}}$  for the three remaining samples ( $-8.6$  to  $-7.2$  ‰) are suggestive of cooler conditions. Two hypotheses could account for this discrepancy in  $\delta^{18}\text{O}_{\text{mw}}$  observed for specimens from Florida. First, these animals (C29-TP2014, C31-TP2014, and C33-TP2014) may have lived during a different time period and under a different climatic regime than the other Florida specimens (C26-TP2014, C28-TP2014, and C32-TP2014). Episodes of more  $^{18}\text{O}$ -depleted precipitation in Florida could be the product of glacial advance and translate to lower  $\delta^{18}\text{O}_{\text{sc}}$ . The compression of atmospheric circulation cells at the equator due to the expansion of large ice sheets south of the Great Lakes could create a steeper climatic gradient between latitudes. A steeper climatic gradient would produce lower  $\delta^{18}\text{O}_{\text{mw}}$  at



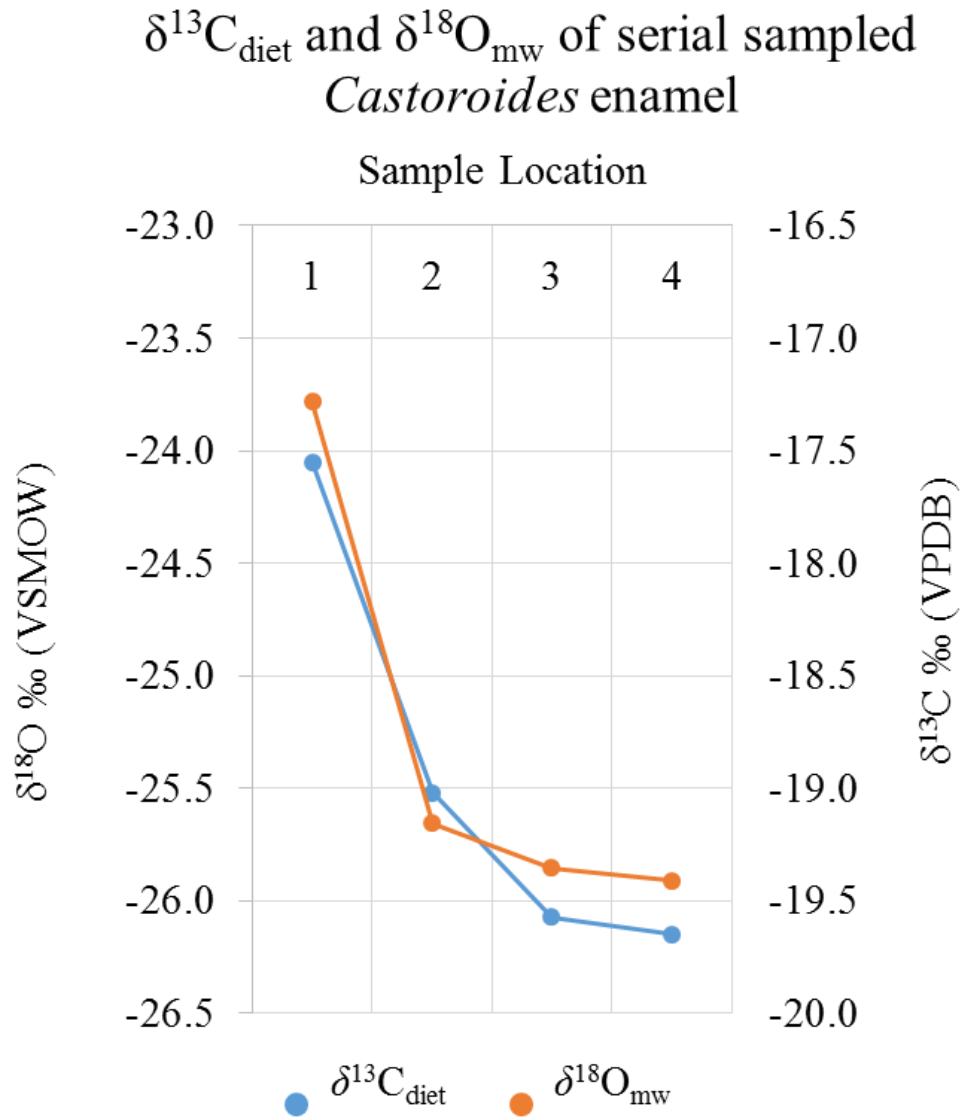
lower latitudinal regions. Second, oxygen-isotopic exchange has occurred between present-day surface water and the enamel structural carbonate matrix. Isotopic exchange is facilitated by warm temperatures and the presence of water. The second hypothesis is more likely, considering all specimens were collected from water-logged river deposits in subtropical/equatorial climate zones. The FTIR results also indicate the possibility of *post-mortem* diagenetic changes for these samples. Oxygen isotopic analyses of the phosphate component of giant beaver enamel is necessary to further evaluate either hypothesis.

The oxygen isotopic results from Florida giant beavers are inconclusive. The calculated  $\delta^{18}\text{O}_{\text{mw}}$  for Alaska and Ohio specimens suggest, however, that these giant beavers lived under interglacial conditions.

#### 4.4.5 Carbon Isotope Discrimination between Bioapatite and Collagen

An offset in  $\delta^{13}\text{C}$  exists between mineralized and proteinaceous tissue carbon isotope values (Ambrose and Norr, 1993; Bellissimo, 2013; Crowley et al., 2010; Jim et al., 2004; Luz and Kolodny, 1985; Warinner and Tuross, 2009; Webb et al., 2014). The magnitude of this offset varies with individual species diet and physiology. An accurate value for this difference is needed for *Castoroides* to render  $\delta^{13}\text{C}_{\text{sc}}$  comparable to  $\delta^{13}\text{C}_{\text{col}}$  for further discussion of palaeodiet.

In large bodied herbivores consuming a  $\text{C}_3$  diet, the average  $\delta^{13}\text{C}$  offset between bone bioapatite and bone collagen is approximately +7 ‰ (Crowley et al., 2010; Hare et al., 1991; Jim et al., 2004; Kellner and Schoeninger, 2007; Nelson et al., 1986; Sullivan and Krueger, 1981). In addition, there is an intra-individual  $\delta^{13}\text{C}$  offset between bone bioapatite and enamel bioapatite. These two tissues mineralize at different rates and thus reflect different temporal periods of dietary intake. Studies of humans and pigs found a



**Figure 4.10** Seasonal trends in  $\delta^{13}\text{C}_{\text{diet}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  from serial-sampled *Castoroides incisor* (C15-TP2014). The original  $\delta^{13}\text{C}_{\text{sc}}$  values were corrected for carbon isotopic discrimination between bone structural carbonate and bone collagen. The  $\delta^{13}\text{C}_{\text{col}}$  values were then corrected for dietary enrichment.

+2 to +5 ‰ offset between bone structural carbonate and enamel structural carbonate (Warinner and Tuross, 2009; Webb et al., 2014). According to both tissue offsets, enamel structural carbonate is expected to be more enriched in  $^{13}\text{C}$  than bone collagen. The

expected offset between *Castoroides*  $\delta^{13}\text{C}_{\text{sc}}$  and *Castoroides*  $\delta^{13}\text{C}_{\text{col}}$  is therefore expected to be between +9 and +13 ‰. A paired sample set from a single individual (C20-TP2014) has an offset of +9.8 ‰ ( $\delta^{13}\text{C}_{\text{sc}} = -10.7$  ‰ and  $\delta^{13}\text{C}_{\text{col}} = -20.5$  ‰), within the expected range. This offset is applied to  $\delta^{13}\text{C}_{\text{sc}}$  from the serial-sampled *Castoroides* incisor from Alaska to render it comparable with  $\delta^{13}\text{C}_{\text{col}}$  presented elsewhere in this chapter (Figure 4.10).

#### 4.4.6 Seasonal Variation Recorded by *Castoroides* Incisor

The original  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  values obtained from the serial-sampled *Castoroides* incisor were converted to  $\delta^{13}\text{C}_{\text{diet}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  in order to best reflect surrounding environmental conditions.  $\delta^{13}\text{C}_{\text{sc}}$  was corrected for carbon isotopic discrimination between bone structural carbonate and bone collagen. The resultant  $\delta^{13}\text{C}_{\text{col}}$  values were then corrected by +4.2 ‰ to render them equivalent to diet. Both  $\delta^{13}\text{C}_{\text{diet}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  show a change with growth direction (Figure 4.10; Table 4.8), which is interpreted in terms of seasonal variation in diet and climatic conditions.

The  $\delta^{13}\text{C}_{\text{diet}}$  and  $\delta^{18}\text{O}_{\text{mw}}$  of specimen C15-TP2014 decrease as tooth growth progressed (Figure 4.10). If the incremental growth rate of giant beaver incisors is assumed to be similar to that of *Castor canadensis*, these four serial samples represent approximately two months of growth (Rinaldi and Cole, 2004). The steady depletion of  $^{18}\text{O}$  and  $^{13}\text{C}$  likely represents a seasonal transition in the  $\delta^{18}\text{O}$  of local meteoric water and the changing availability of forage types or forage nutritional quality. Possible avenues of interpretation include the weather becoming colder, and the animal transitioning to a diet that contains less submerged macrophytes. A diet containing more floating and emergent macrophytes would account for lower  $\delta^{13}\text{C}_{\text{diet}}$  in a C3-dominated ecosystem. Future studies should employ higher resolution (5mm or less) sampling along the length of an entire *Castoroides* incisor to determine the amplitude of seasonal variation during the Pleistocene and how it compares to present day conditions. The oxygen isotopic composition is expected to fluctuate in a sinusoidal pattern in regions that experience strong seasonal changes in temperature and precipitation quantity or type (e.g. temperate mid-latitude locations). The stable carbon isotopic composition is also expected to

fluctuate over the length of an entire tooth, particularly if there is a temporally defined growing season and if *Castoroides* relied on fat reserves to survive the winter months. High resolution serial sampling of an entire tooth would provide a more complete understanding about the magnitude of seasonality and dietary variability experienced by *Castoroides* across North America. Incrementally growing giant beaver dentin might also be viable for serial sampling, particularly for obtaining  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  pertaining to seasonal dietary changes.

#### 4.5 Palaeoecology of *Castoroides*

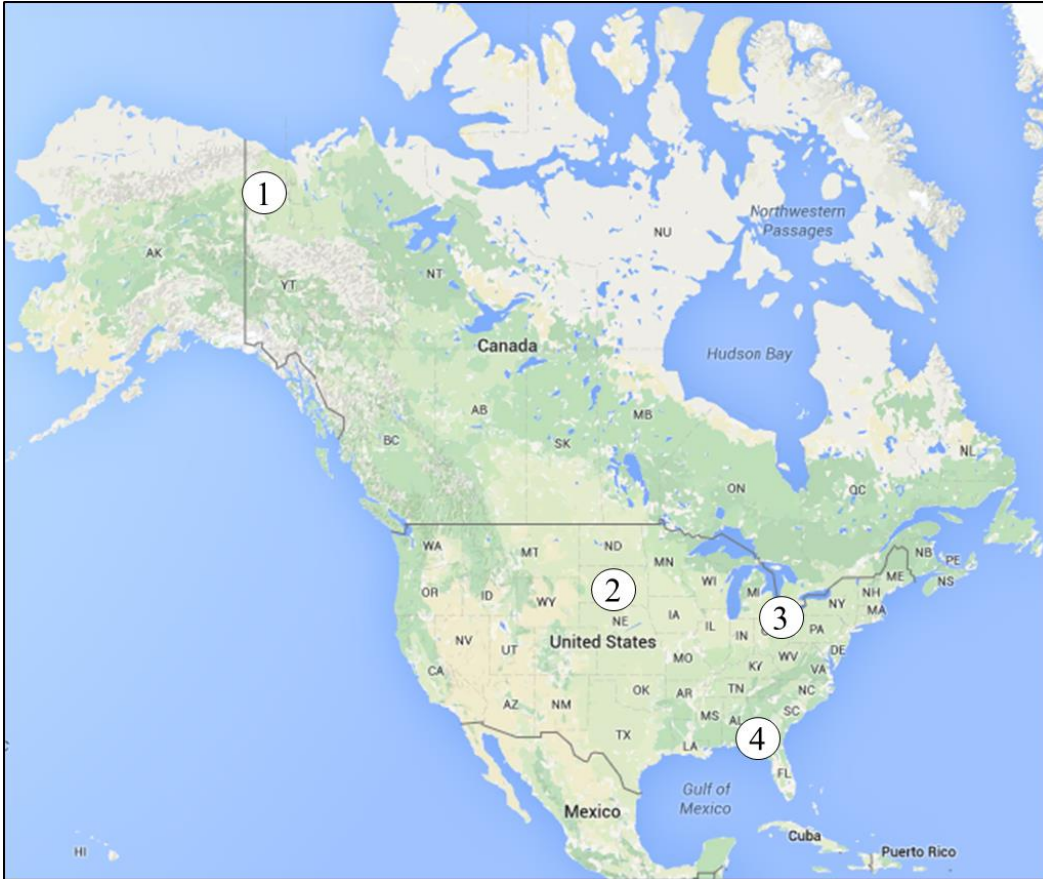
*Castoroides* bone  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  support the hypothesis that giant beavers were semi-aquatic rodents that grazed predominantly on macrophytes. A strong preference for submerged macrophytes made *Castoroides* highly dependent on wetland habitat for sustenance. The information obtained from tooth enamel  $\delta^{18}\text{O}_{\text{sc}}$  generally tentatively suggests that giant beavers survived in northern regions only when warmer conditions were present. The Proportion versus Source boxplots generated in SIAR strongly suggest that *C. canadensis* and *Castoroides* occupied complementary dietary niches, and had ecologies that were different enough to allow the two genera to co-exist in wetland habitats across North America for the majority of the Pleistocene. This interpretation of complementary dietary niches should be further explored by analyzing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of bulk bone collagen of Pleistocene *Castor* specimens. This is a necessary comparison to ensure that this pattern is not simply the product of differences in the carbon and nitrogen cycle during the Pleistocene versus the present day. While neither *C. canadensis* nor *O. zibethicus* constitute a perfect analog species, the muskrat is the closest modern semi-aquatic rodent that could be used to describe the diet and ecological impact of the giant beaver.

*Castoroides* does not appear to have been an ecological engineer capable of modifying the landscape for purposeful creation of suitable aquatic habitat space. There is no evidence of dams, lodges, or underwater food caches constructed by giant beavers from the Pleistocene sedimentological record. If the giant beaver *was* actively cutting down trees for food, the stable isotopic composition of its bone collagen would reflect

terrestrial browse, and not submerged macrophytes. This conclusion is further supported by skeletal morphology. The morphology of giant beaver incisors is not well-adapted to the cutting down of trees, lacking the chisel-like edge that *Castor* incisors possess (Rinaldi et al., 2009). Comparative analyses of brain size and surface texture from interior brain-case casts also suggests that *Castor* was capable of more complex social behaviour than *Castoroides* (Holman, 1995).

Based on body size and the reproductive patterns of modern large-bodied rodents, it is likely that *Castoroides* was K-selected. By definition, K-selected species reproduce slowly. They produce relatively low numbers of offspring per gestational cycle and invest in extended parental care of the young. Assuming a stable ecosystem, K-selected species maintain a stable population over time. Large rodents from the present day also display K-selected reproductive strategies. The largest rodent alive today is the capybara. This semi-aquatic rodent can reach up to 70 kg and has a gestation period of approximately five months. They wean their young after four months. Modern beaver also invest significant periods of time rearing their off-spring and produce a single litter per year. The young stay within the family unit for up to two years before leaving to establish a new colony. Based on this comparison, it is likely that *Castoroides* also had a long gestation period, produced fewer off-spring, and had a more constrained breeding season.

The dispersal of different giant beaver species across North America demonstrates that they were reasonably adaptable animals. Temperature range tolerance and dietary composition must have varied with latitude and species. *C. ohioensis* was evidently capable of surviving highly seasonal fluctuations in temperature and light level. The poor preservation of bone collagen in fossils from the southeastern USA, however, has limited further investigation of dietary differences between northern and southern species.



**Figure 4.11 Regions of interest when studying trends that link environmental conditions to *Castoroides* population success. Image obtained from Google Maps, 2016.**

#### 4.5.1 Palaeoecological Implications: Combining Lines of Evidence

This new isotopic information regarding giant beaver diet and climatic preferences allows the genus to be better assessed within the context of changing environmental conditions and biome shifts during the Pleistocene. In a world of transitioning environmental conditions, the adaptability of a species is put to the test. *Castoroides* had several factors that limited its adaptability. A slow reproductive cycle, greater space and caloric requirements, and competition with *Castor* for habitat space were highly limiting on a landscape shaped by glacial cycles and biome shifts.

Two primary factors likely limited the spread of *Castoroides* populations: aridity and plant community (specifically, the presence of open, cool-adapted forest in lowland regions). A lack of associated dates for many *Castoroides* fossils prior to the Sangamonian, however, makes it difficult to conclude whether the giant beavers thrived best in transitional conditions, or during full interglacial times.

The environmental conditions associated with the presence of giant beaver populations throughout the Pleistocene are reviewed in the following section. This review is intended to discern patterns of environmental conditions that disrupted giant beaver ecology and resulted in regional extirpation of the genus. This review emphasizes the incorporation of new ecological information supported by stable isotopic data presented in this chapter (this includes *Castoroides*' reliance on wetland habitat and its preference for warmer, wetter climatic conditions). The four regions of interest are Yukon Territory and Alaska, Central North America (Great Plains), Eastern North America (Great Lakes Basin region), and Southeastern North America (Florida, Georgia, and the Coastal Plains) (Figure 4.). Each region has substantial fossil evidence that *Castoroides* once lived there.

#### Yukon Territory and Alaska

The Castoridae family has been documented in the Canadian Arctic for nearly 2 million years (Figure 4.2; 4.3). *Castoroides* and *Castor* were present in unglaciated lowland regions of Yukon Territory and Alaska throughout the Rancholabrean II North American Land Mammal Age. *Castoroides* and *Castor* fossils of late Illinoian age were found in lake bed deposits from the Old Crow region (Harington, 2011). The remains were found in association with aquatic and cold-adapted animals (e.g. fish, lemmings, Arctic fox, mammoth, horse, camel, caribou, muskoxen, grouse, ducks, and geese) (Harington, 2011). This indicates that *Castoroides* was reasonably well-adapted to cold climate regimes prior to the onset of full interglacial conditions, and was capable of surviving the dark Arctic winters. It also indicates that substantial wetland habitat existed in areas of the mammoth-steppe. The late-Illinoian was a transitional time, where melting ice-sheets likely fueled wetland habitat in lowland areas of northern Yukon Territory and

adjacent Northwest Territories. The lake-bed faunal assemblage from Old Crow also demonstrates that interglacial and glacial faunas mixed during transitional times.

Giant beaver remains are most abundant in the north in sediments from the Sangamonian interglacial. *Castoroides* fossils present in faunal assemblages from the Old Crow region date from approximately 130,000 to 80,000 years BP (Harington, 2011). In addition to the giant beaver, the assemblage was dominated by other species dependent on wetland habitat, such as beaver, muskrat, fish and water fowl. Although not directly associated with any giant beaver specimens, radiocarbon-dated mastodon remains from the north strongly suggest that an open spruce forest was present in Yukon Territory as far north as Herschel Island during the Sangamonian (Harington, 2011; Zazula et al., 2014).

*Castoroides* remains from deposits in northern Yukon Territory were dated as the early and mid-Wisconsinan (Harington, 2011). However, it is unknown if the giant beaver was capable of surviving full glacial conditions, and may have undergone periodic regional extirpation. Northern giant beaver populations of mid-Wisconsinan age are probably linked to warm interstadial phases that occurred during this time. *Castoroides* became regionally extinct in the Arctic during the mid- to late Wisconsinan, at which point forests were absent north of 55° latitude, and unglaciated terrain was dominated by graminoid and forb plant communities (Zazula et al., 2003). By the end of the Last Glacial Maximum (LGM), tundra conditions became widespread in northern Yukon Territory and Alaska (Strong and Hills, 2005).

#### Central North America

The first records of *Castoroides* in the Great Plains region appear in Kansas during the Irvingtonian I NALMA. Populations became well-established across Kansas, Nebraska, and Oklahoma during the Irvingtonian II and Rancholabrean I NALMA (Cahn, 1932; FAUNMAP Working Group, 1994). After the onset of the Illinoian glaciation, giant beaver fossils disappeared from the Great Plains record and became concentrated east of the Mississippi River (Cahn, 1932; FAUNMAP Working Group, 1994). The onset of cold, arid conditions associated with the growth of continental ice sheets likely



resulted in a loss of favourable climate and wetland habitat. Unfortunately, few of the *Castoroides* remains from central North America have associated dates. Therefore it is currently impossible to link population fluctuations to specific regional changes in environmental conditions. However, palaeobotany studies by Jackson et al. (2000) and Jackson and Weng (1999) suggest that late Pleistocene plant communities from the continental interior lack modern analogs. This regional loss of habitat or associated ecosystem would have limited *Castoroides* populations from returning to central North America.

These no-analog communities are partly a result of the extinction of various tree species during the Pleistocene-Holocene transition (now-extinct *Picea* species were likely a major component of the vegetation in both central and southeastern North America during the late Pleistocene) (Jackson and Weng, 1999; Jackson, 2000). By 14,000 BP, these no-analog plant communities were lost, and grassland dominated the continental interior, with Aspen Parkland bordering Boreal forest along the southern margin of the retreating ice sheets (Strong and Hills, 2005). The climatic conditions during the onset of the early Holocene encouraged the expansion of grass and herb communities, while Boreal forest in Minnesota was replaced by the northward-advance of warm-adapted oak savannah ecosystems (Jackson et al., 2000; Strong and Hills, 2005). In this region, increasing aridity and the replacement of forests by grasslands appear to have resulted in the disappearance of *Castoroides*.

#### Eastern North America

Eastern North America comprises the Great Lakes Basin and the states immediately to the south of the Great Lakes. The Eastern seaboard is also included in this category, although very few giant beaver remains have been found in this region (Miller et al., 2000). Eastern North America has a particularly high concentration of giant beaver fossils, although most are of Sangamonian or Wisconsinan age (Boulienger and Lyman, 2014; Cahn, 1932; McDonald and Bryson, 2010; McDonald and Glotzhober, 2008). The successive advance and retreat of the Laurentide ice sheet obliterated the majority of sediments deposited in this region during prior intervals (Holman, 1995). Fortunately, the

deposits from the most recent glacial interval are exceptionally well-preserved and have yielded abundant faunal and floral remains for palaeoecological analysis. The excellent degree of preservation in this region allows for a stronger correlation of changing environmental conditions to late Pleistocene giant beaver population fluctuations.

*Castoroides* fossils of Sangamonian age are recorded from deposits in southwestern Ontario, Ohio, Illinois, and Indiana (Cahn, 1932). Outside of Yukon Territory, giant beaver remains are rarely found within Canada. However, the Don Formation and Scarborough Formation in the Toronto region have both yielded a few specimens from the last interglacial (Coleman, 1933; Eyles and Clark, 1988; Miller et al., 2000). These deposits correlate to a time of higher lake level (and a greater abundance of wetland habitat) in the Lake Ontario basin (Holman, 1995).

During the Wisconsin glacial, giant beaver fossils are more commonly associated with warmer interstadial times, when the retreat of continental ice sheets left the Great Lakes region flooded with meltwater. When plotted on a map, giant beaver fossils from Eastern North America tend to skirt the southern edge of the fluctuating ice sheets (Cahn, 1932; FAUNMAP Working Group, 1994). The cool, moist climate produced at temperate latitudes by the melting ice appears to have provided ideal habitat space. A study of the climatic parameters associated with late Pleistocene giant beaver remains from the Great Lakes region concluded that the genus was cold-tolerant (McDonald and Bryson, 2010). The study also determined that the giant beaver thrived under different seasonal conditions than are present in the region today (i.e. colder average winter and summer temperatures; highest annual precipitation occurring in late summer-fall instead of early spring) (McDonald and Bryson, 2010). Stuart-Williams and Schwarz (1997) also concluded that giant beavers that lived in the Great Lakes region during the Sangamonian experienced greater seasonal fluctuations in temperature and rainfall than are typical of the region at present.

The mid-Wisconsinan experienced three successive periods of cooling between 55,000 and 22,500 BP, each becoming progressively more extreme as the LGM was approached (Berti, 1975). From the beginning of the first interstadial (55,000 BP) to the

onset of the LGM (24,000 BP), the Great Lakes region transitioned from a warm, mixed forest environment to mixed Boreal forest and tundra conditions (Berti, 1975; Jackson et al., 2000). Giant beaver populations appear to be present throughout the mid-Wisconsinan, although transitional environmental conditions between peak glacial and interglacial times may have been more favourable to the species.

Open oak savannah ecosystems were common south of the Great Lakes during the height of the first interstadial, before gradual replacement during the final Wisconsinan interstadial by cool-adapted forest communities (i.e. spruce, pine, birch, willow, alder, larch, and cold-adapted shrubs). The transition between each interstadial and stadial saw the presence of an open forest environment dominated by cold-adapted, Boreal-like plant communities (Berti, 1975). There is evidence of extensive lacustrine environments in the Lake Erie and Lake Ontario basins during the mid-Wisconsinan (Berti, 1975). Varieties of submerged, emergent, and floating macrophytes that are common to the region today have all been documented from sediment cores (Berti, 1975). Species of sedge and water lily indicate the presence of shallow, relatively calm waters.

At the height of the LGM cold period, ice sheets extended into central Ohio and tundra-forest ecosystems developed peripheral to the ice (Berti, 1975; Loehle, 2007). Despite the full glacial conditions, precipitation rates increased over Eastern North America, fueling lakes and sustaining forests. The ice sheets facilitated the split of the westerly jet stream and diverted a higher proportion of storms to the region (COHMAP, 1988). Further south of the ice, cool and moist adapted forest communities dominated from Indiana to Tennessee, and along much of the Eastern seaboard (Jackson et al., 1997; Jackson et al., 2000). Based on LGM pollen-profiles, Indiana, Illinois, Missouri, Kentucky, and Tennessee were dominated by forest communities with no modern analog (Bernabo and Webb, 1976). At the height of the LGM, *Castoroides* fossils only appear east of the Mississippi river and south of ice sheets. As the ice retreated, *Castoroides*' geographic range shrank to occupy the southern margin of the Great Lakes basin. This may be indicative that the only suitable habitat remaining at temperate latitudes existed in the wake of the melting ice. Fossil remains are relatively common in post-glaciation deposits from Indiana, Illinois, Ohio, and New York (Boulanger and Lyman, 2014;

FAUNMAP Working Group, 1994; McDonald and Glotzhofer, 2008; Richards and Swinehart, 2001). *Castoroides* fossils from Indiana dating to 11,240 BP were found in association with shallow lake sediments containing abundant emergent and submerged macrophyte macrofossils (Richards and Swinehart, 2001). Prior to the lake in-filling, the wetland was surrounded by a Boreal forest community dominated by spruce and larch (Richards and Swinehart, 2001).

The giant beaver disappeared from Eastern North America shortly before the Pleistocene-Holocene transition. With the disappearance of this population, the genus became globally extinct. The vegetation of Eastern North America continued to change with the onset of the Holocene warm period. Cold-adapted biomes shifted north to their present latitudes, and mixed deciduous and coniferous forests dominated the landscape around the Great Lakes region. Immediately south of the lakes, a diverse range of deciduous trees became established (Jacobson et al., 1987).

Anthropogenic factors may have also played a role. Although there is currently no direct evidence, humans cannot be ruled out as a contributing factor in the extirpation of the giant beaver in the Great Lakes region. The only known instance of direct temporal and spatial overlap between humans and *Castoroides* was discovered in New York State. A *Castoroides* specimen dating to  $10,150 \pm 50$  BP (late Pleistocene post-glacial times) indicates that megafauna populations may have overlapped with Paleoindian culture for up to a thousand years (Boulianger and Lyman, 2014). However, there is at present no zooarchaeological evidence that humans butchered, hunted, or otherwise utilized the giant beaver as a resource.

#### Southeastern North America

Southeastern North America comprises Florida, Georgia, and a portion of the American Coastal Plains (North and South Carolina, Virginia). There is a fairly continuous record of *Castoroides* in this region throughout the late Pleistocene, with the earliest instance appearing in the Irvingtonian I NALMA (Martin, 1969, 1975; Morgan and White, 1995; Parmalee and Graham, 2002). This region is home to the oldest known form of the genus, *C. leiseyorum*, which is assumed to have given rise to both *C.*

*ohioensis* and *C. dilophidus* (Hulbert et al., 2014). *C. ohioensis* was comparably more successful as a species, and radiated north to occupy the American Midwest, the Great Lakes Region, and Beringia. *C. dilophidus* appears confined to the Southeast, with the highest concentration of fossil remains dating to the Rancholabrean NALMA (FAUNMAP Working Group, 1994). *Castoroides* remains from the Wisconsin Glaciation have been found in Florida, South Carolina, and even Virginia (FAUNMAP Working Group, 1994). Virtually all specimens from Florida have been collected from river systems (FAUNMAP Working Group, 1994; Hulbert, 2014 personal comm.), and many lack precise contextual information. An insecure faunal chronology, combined with scarce pollen and plant macrofossil records for the region prior to the Wisconsin Glaciation, limit our ability to discern patterns over time. However, it is still possible to examine factors that may have impacted giant beaver populations in the Southeast during the last glaciation and during the Pleistocene-Holocene transition period.

South-central Florida offers some of the oldest palynological records for southeastern North America. Between 44,300 to 33,300 BP, vegetation in this transitional zone between ecotones was composed of herbs, oak, and some pine (Watts, 1980). During the mid-Wisconsin, tropical vegetation occurred in southern Florida, and with more mesic warm-adapted mixed forests to the north. *Castoroides* fossils are rarely found south of Tampa, FL (Hulbert, 2014 personal comm.). This is due to a continuous lack of suitable habitat in the southern tip of Florida during both interglacial and glacial times. *Castoroides* apparently could not thrive in tropical vegetation that existed there during warm intervals, or in the prairie conditions that were widespread in the southern tip of Florida during more arid glacial times. Highly seasonal rainfall during glacial times flooded saplings and forests failed to thrive (Hulbert, 2014 personal comm.). Megafauna commonly associated with *Castoroides* (i.e. mastodon, giant ground sloth) are also absent from the fossil record of this region. Instead, grazing fauna (i.e. horse, bison) are predominant (Hulbert, 2014 personal comm.). It should be noted that peninsular Florida expanded geographically as sea level dropped during glacial advances. By approximately 23,000 BP, the climate across the Southeast became colder and drier (documented by the loss of forested swamps in northern Georgia) (Watts, 1980).

During late Wisconsin full-glacial conditions, the vegetation of the Coastal Plain and Georgia was fairly homogenous, with pollen assemblages dominated by pine, along with some spruce, broad-leaved trees (oak and ironwood), and prairie herb species (Watts, 1980). Fossil sand dunes are also common from this region during the late Wisconsin, indicating a dry, windy climate (Watts, 1980). The presence of spruce trees in southeastern North America varied with latitude during the LGM and spruce was largely absent in northern peninsular Florida. Here, LGM plant communities were instead composed mainly of pine, with some broad-leaved tree species and prairie herbs (Watts, 1980). Pine was predominant in southern Florida forests during late glacial times (~16,000 BP) (Watts and Hansen, 1994; Watts and Hanson, 1988). Pine gave way to oak forests and mixed scrub-prairie around 14,000 BP, which lasted until the mid-Holocene. By ~6000 BP, the climatic conditions that favoured pine forests and swamps returned (Watts and Hansen, 1994; Watts and Hanson, 1988). Northern Florida underwent a similar transition with the onset of warmer, drier conditions at the end of the Pleistocene. Between 17,500 and 14,500 BP, broad-leaved mesic forests were predominant in northern Florida. These forests were replaced by pine and herb communities, and lasted until 11,500 BP (Watts and Hanson, 1988). Oak forests and scrub developed during the early Holocene, to eventually be replaced during the late Holocene by pine forests and swamps (Watts and Hanson, 1988). It is currently hypothesized that the presence of swamps and/or deciduous trees indicate bouts of wetter conditions over the past 17,000 years (Watts and Hanson, 1988). The loss of swamps and the appearance of interspersed scrub and prairie conditions indicate increased regional aridity in the region. Presumably, these conditions were not beneficial to sustaining wetland habitat. Water table height, temperature, and precipitation rates for the region all fluctuated during the transition between the LGM and the late Holocene. The appropriate combination resulted in the repeated loss of wetland habitat in Florida.

Overall, the combination of open-conifer woodland and warm-adapted mixed forests in both Florida and the Coastal Plain were entirely replaced by warm-adapted mixed forest during the early to mid-Holocene (Jackson et al., 2000). Pleistocene vegetation communities from southeastern North America (the Florida peninsula in particular) lack modern analogs, and the Holocene warm-adapted forests are composed of

a different combination of plant species. The plant communities that previously supported Pleistocene megafauna disappeared.

Approximately half of the *Castoroides* specimens discovered in northern Florida date stratigraphically to the Wisconsin Glaciation (FAUNMAP Working Group, 1994). The remainder are categorized only as Rancholabrean age. While we lack more specific time constraints, we do know that suitable habitat existed (at least periodically) in the Southeast during glacial times. However, according to Watts and Hanson (1988), Florida underwent multiple periods of increased aridity, and conditions that favoured swampland did not return until the mid- to late Holocene. This period of several thousand years was likely sufficient to result in a regional extirpation of giant beaver populations in southeastern North America.

Temperature maps compiled of the North Atlantic for 18,000 BP indicate that the West Atlantic waters off the coast of the Florida peninsula were 3°C colder than present (Watts, 1980). This relatively small difference in water surface temperature had a significant impact on terrestrial climate. At the height of the glacial advance, the summer climate of Florida and the Coastal Plain was very different from that of today. Average July temperatures were colder by about 12°C, and surface conditions on the Plain were drier and windier (Watts, 1980). These rapidly shifting climatic conditions affected the distribution of plant species, the composition of the plant communities, and the rate at which plant species could migrate or recolonize. The loss of specialized plant community likely limited megafauna distribution.

Towards the Pleistocene-Holocene transition, the spruce and pine forests of the Coastal Plain were replaced by mesic deciduous forest (i.e. oak, elm, ironwood hickory, beech). However, this biome did not survive more than a few thousand years, and was gradually replaced by modern floras. Pine forests returned, while swamps and bogs became more abundant because of increased rainfall and an elevated water table during the mid-Holocene (Koch et al., 1998; Watts, 1980; Watts and Hanson, 1988). Overall, the combination of open conifer woodland, warm-mixed forest, and a small proportion of cool-mixed forest in southeastern North America during the LGM was entirely replaced

during the early Holocene by warm-mixed forest (Jackson et al., 2000). In addition, conifer forests (mainly pine) became widespread in the southeast and along the Gulf of Mexico during the late Holocene (Jacobson et al., 1987).

Despite the limited information, certain environmental conditions correlate with the presence of the giant beaver in the southeast. The majority of Wisconsin-age *Castoroides* fossils originate from Georgia and northern peninsular Florida. During much of the mid-Wisconsin, the region supported forested swamps and warm-adapted mixed forests. During the LGM, conditions became cooler and drier. Scrub and prairie conditions became present in parts of Georgia, the Coastal Plain, and in southern Florida during both glacial and post-glacial times. The resultant loss of forest and wetland biomes had a negative impact on the giant beaver, likely isolating remaining populations in pockets of suitable habitat in northern peninsular Florida. While this may be a preservation bias, *Castoroides* fossils of all ages from the Southeast are concentrated along the west coast of peninsular Florida. Again, this emphasizes aridity as a controlling factor for giant beaver survival. While Georgia and the Coastal Plains became increasingly drier and windier towards the end of the Pleistocene, the ocean currents surrounding the peninsula ensured a humid climate capable of supporting some wetland habitat, even during full-glacial conditions. Despite this, post-glacial/early Holocene temperatures in the region may have surpassed the tolerance range of the giant beaver. Strongly seasonal precipitation and the disappearance of plant communities unique to the Pleistocene also likely contributed to the decline of *Castoroides* and associated fauna in peninsular Florida (Jackson and Weng, 1999; Jackson et al., 2000).

#### 4.6 Conclusions

*Castoroides* skeletal and dental material provided a wealth of stable isotopic information regarding the palaeoecology of this Ice Age mega-rodent. *Castoroides* incisor enamel retained palaeoclimate proxy information in the form of  $\delta^{18}\text{Osc}$ . Unfortunately, majority of the tooth specimens employed in this study had lost structural carbonate material *post-mortem* and were too poorly preserved to make conclusive statements about climatic conditions during the Pleistocene. While better preserved



samples are necessary to confirm this, the results suggest that *Castoroides* survived at different latitudes across North America when warm, non-glacial conditions existed. The serial-sampled incisor was sufficiently well-preserved and proved that giant beaver teeth can record seasonal changes.

*Castoroides* bone collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  indicate the genus consumed a diet composed predominantly of macrophytes. SIAR mixing models that employed a dietary baseline composed of different plant types indicate that submerged macrophytes were the preferred source of forage. None of the isotopic results support the hypothesis that *Castoroides* cut down trees or had ecological engineering habits akin to *Castor canadensis*. SIAR Proportion versus Source boxplots strongly support an ecological model where *Castor* and *Castoroides* co-existed without competing for food resources.

Without apparent refugia after the LGM, it is possible that the genus dwindled to an isolated population located in the lowlands south of the Great Lakes, where profound climate change (and possibly human influence) resulted in their extinction. The role that loss of preferred habitat and increased competition for space played in the extinction of this genus are explored more fully in Chapter 5, which concludes this thesis.

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## Chapter 5

### General Discussion and Conclusions

#### 5.1 Chapter Overview

This thesis explored the palaeoecology of the extinct Ice Age giant beaver (*Castoroides*) through the lens of stable isotope analyses. This chapter summarizes and expands on the main conclusions from Chapters 2, 3, and 4, and suggests avenues of future research that would expand our understanding of the giant beaver.

#### 5.2 Methodological Developments

Chapter 2 presented two methodological studies designed to explore the effects of sample preparation on the isotopic compositions of collagen and bioapatite from modern beaver and giant beaver, respectively. The first study examined possible effects of four defleshing techniques. The work was deemed necessary because, although defleshing by hand is very slow, this approach is commonly preferred by isotopists who do not have access to dermestid beetles. Exposure to heat and(or) a proteolytic enzyme during defleshing has been suspected of altering the original carbon and nitrogen isotopic compositions of bone collagen. It is promising, therefore, that in three out of four cases, bulk collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  demonstrated negligible differences among the defleshing methods tested (A: manual defleshing; B: soaking in room-temperature distilled water for four days; C: immersion in distilled water at a constant rolling boil for three hours; and D: soaking in a gently heated (35-45°C) solution of Protease enzyme and distilled water for 16 hours). This study needs to be expanded to more samples before these approaches can be incorporated into any standard operating protocol; however, this is a promising initial step towards reducing the preparation time of modern skeletal material for stable isotopic analysis.

The second study examined effects on the carbon and oxygen isotopic compositions of enamel bioapatite structural carbonate ( $\delta^{13}\text{C}_{\text{sc}}$ ,  $\delta^{18}\text{O}_{\text{sc}}$ ) arising from techniques used to remove residual organic matter and secondary carbonate precipitated in the sample after death. A standard pretreatment was applied to Pleistocene giant beaver

teeth. Pretreatment involved 2 % bleach and 0.1 M (unbuffered) acetic acid at room temperature and did change the enamel  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ . The effect was small, however, and it remains unclear how much of the change in isotopic composition results from successful removal of secondary carbonates, and how much is due to precipitation of fresh carbonates prompted by dissolution of atmospheric  $\text{CO}_2$  in the bleach solution (Pellegrini and Snoeck, 2016). The eight *Castoroides* teeth used in this experiment appeared to have lost structural carbonate during *post-mortem* processes and Fourier Transform Infrared spectroscopy (FTIR) tests did not indicate the presence of secondary carbonates (classic signs include a calcite peak at  $710\text{ cm}^{-1}$  on the spectra; high C/P ratio; low CI). It is possible that while the pretreatment did remove secondary carbonates precipitated in the depositional environment, it also contributed new carbonate formation that contaminated the  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  of “clean” pretreated samples. The bleach step in the Laboratory for Stable Isotope Science (LSIS) standard operating protocol for carbonate analysis should be reconsidered; alternately, well-preserved enamel samples should not be pretreated.

### 5.3 Macrophyte $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Isotopic Dietary Baselines

Chapter 3 expands the existing body of literature documenting the variability and complexity of plant carbon and nitrogen isotopic compositions in freshwater systems (Casey and Post, 2011; Keeley and Sandquist, 1992; Mendonça et al., 2013).

Macrophytes are isotopically distinguishable from trees and shrubs when both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are measured, and these data can be successfully incorporated into stable isotope mixing models that are used to characterize herbivore diet. The high degree of variability in macrophyte  $\delta^{13}\text{C}$  ( $-50$  to  $-10$  ‰), however, encompasses the entire range of carbon isotope signatures for both  $\text{C}_3$  and  $\text{C}_4$  plants on land (Keeley and Sandquist, 1992).

Incorporation of macrophytes into the conventional  $\text{C}_3$ - $\text{C}_4$  model for defining palaeodiet in non-marine herbivores might highly confound otherwise more straightforward dietary interpretations. Submerged macrophytes with high  $\delta^{13}\text{C}$  appear, from an isotopic perspective, very similar to  $\text{C}_4$  grasses. The  $\delta^{13}\text{C}$  of submerged, emergent, and floating macrophytes is highly variable and overlaps with the  $\delta^{13}\text{C}$  of terrestrial  $\text{C}_3$  trees and

shrubs. Some confusion, however, can be mitigated by using a regionally specific isotopic foodweb baseline, which incorporates paired  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data sets for the vegetation.

Classic biology texts historically assumed macrophyte herbivory to be an insignificant part of even the aquatic food chain (Cummins and Klug, 1979; Hutchinson, 1981; Polunin, 1984; Mann, 1988). This perspective has since shifted, however, and macrophyte  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are becoming better documented in freshwater foodweb studies (i.e. Fry, 1991; Milligan et al., 2010). Overall, the inclusion of isotopic data for macrophytes should be considered more commonly for stable isotope studies that explore human and faunal palaeodiet. This consideration is especially important in palaeontology when understanding of an extinct animal's ecology can remain highly speculative. While *Castoroides* possesses several skeletal traits that indicate a semi-aquatic lifestyle, skeletal morphology is not necessarily a definite indicator of aquatic resource or habitat use. *Alces alces* is a good example of a morphologically terrestrial animal that still derives an important portion of its summer diet from grazing on macrophytes in shallow wetlands (Bump et al., 2009; Tischler, 2004). That macrophyte isotopic data are not as universally transferrable among regions (as is commonly the case for terrestrial environments) makes it particularly challenging for researchers to acquire a suitable macrophyte foodweb baseline. It would be worse, however, to ignore aquatic plants as a possible forage choice. Such an ill-advised approach could severely confound dietary interpretations obtained using stable isotope mixing models.

#### 5.4 *Castoroides* Palaeoecology and Implications for Extinction

Chapter 4 compares the  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  of modern *Castor* (beaver) and *Ondatra* (muskrat) with that of *Castoroides*. From this isotopic perspective, muskrat collagen, and hence diet, provides the closest modern equivalent to the extinct giant beaver (mean *Castoroides*  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}_{\text{col}}$  are  $-17.6\text{‰}$  and  $+5.8\text{‰}$ , respectively; mean *Ondatra zibethicus*  $\delta^{13}\text{C}_{\text{col}}$  (Suess Effect corrected) and  $\delta^{15}\text{N}_{\text{col}}$  are  $-13.2\text{‰}$  and  $+5.1\text{‰}$ , respectively). The Stable Isotope Analysis in R mixing model indicates that macrophytes were *Castoroides*' preferred forage. Floating and submerged macrophytes

likely contributed the most to giant beaver diet, while terrestrial plants contributed the least. These SIAR results imply that *Castoroides* was reliant on wetland habitat for food.

The  $\delta^{13}\text{C}_{\text{sc}}$  of serially sampled *Castoroides* incisor enamel demonstrates that giant beaver diet varied seasonally, although the precise dietary changes that prompted these isotopic shifts have not yet been identified. Results of Fourier transform infrared spectroscopy (FTIR) suggested that the *Castoroides* enamel used for  $\delta^{18}\text{O}_{\text{sc}}$  measurements, and from which, estimates of regional meteoric water ( $\delta^{18}\text{O}_{\text{mw}}$ ) were derived was not particularly well-preserved. Hence palaeoclimate interpretations of these data are tentative; initial results nonetheless suggest that *Castoroides* thrived best under warmer, non-glacial conditions.

Despite an incomplete palaeobotanical and palaeoclimatic record, it is possible to discern patterns of environmental conditions that favoured the survival of the giant beaver. The appearance of giant beaver remains across North America coincides with conditions that supported ample swamps and shallow lakes. These wetlands were fueled by precipitation or glacial meltwaters, and were often surrounded by open conifer- or mixed-forest.

Sediment infilling, loss of glacial meltwater, decreased regional precipitation, and increased temperature all contributed (in various combinations) to wetland habitat loss across North America during the terminal Pleistocene. Vegetation biomes were dramatically rearranged at the end of the late Pleistocene, and many plant communities disappeared entirely. Between the LGM and the early Holocene, several biomes shifted north several degrees of latitude (i.e. tundra; Taiga/Boreal forest; cool-adapted mixed forest; warm-adapted forest), forming thick West-East bands across the continent (Jackson et al., 2000). This disrupted the former, more mosaic-like distribution of plant communities that existed in eastern and southeastern North America during the late Pleistocene (Jackson et al., 2000). The extinction and regional extirpation of various North American tree species (particularly pine and spruce), the lack of modern analogs for many Pleistocene plant communities, and the rise in abundance of many deciduous

tree species after the LGM transformed the landscape into one that continued to support the modern beaver (*Castor*), but not *Castoroides*.

Pleistocene megafauna in North America had previously endured several continental-scale glaciations. Two factors made the LGM particularly disruptive, however, especially to species with already fragmented populations. First, biomes transitioned very rapidly between the onset of LGM, and the early Holocene. Fossil pollen analyses for 700 sites across north and eastern North America documented plant communities and biome shifts during the late Wisconsin (Williams et al., 2004). That study demonstrated that vegetation distribution and composition were relatively stable during glacial times, and during the mid-late Holocene, but transitioned relatively rapidly during the late-glacial and early Holocene (between 16,000-8,000 BP) (Williams et al., 2004).

Second, global atmospheric CO<sub>2</sub> concentrations during the LGM were the lowest they had been for approximately half a million years, measuring just 45% of pre-industrial Holocene concentrations (Barnola et al., 1987). Atmospheric CO<sub>2</sub> concentration fluctuated in tandem with ice sheet growth throughout the Pleistocene (Sigman and Boyle, 2000). Besides its obvious greenhouse effects, atmospheric CO<sub>2</sub> has a direct physiological impact on plants and influences their response to climate (Loehle, 2007). In particular, low CO<sub>2</sub> decreases plant water-use efficiency, regardless of water availability. Low atmospheric CO<sub>2</sub> concentration throughout much of the past 400,000 years favoured more open vegetation communities and plants with increased drought tolerance. Faunal communities no doubt adapted to the influence of prolonged low CO<sub>2</sub> conditions on plant communities. Atmospheric conditions during glacial cycles gave conifers a distinct advantage over deciduous trees, as many conifer species are drought-tolerant and better adapted to maximizing carbon-use efficiency (Beerling, 1996; Beerling and Woodward, 1993; Jolly and Haxeltine, 1997; Robinson, 1994; Saxe et al., 1998). The atmospheric CO<sub>2</sub> concentrations of the Holocene were a stark contrast to those of the LGM. Broad-leaved deciduous trees took the advantage, becoming increasingly abundant in temperate regions. Many previously confined deciduous species greatly expanded their geographic ranges for the first time. The disappearance of open

forests predominantly composed of conifers had opposing impacts on *Castoroides* and *Castor*. *Castoroides* populations disappeared while *Castor* continued to thrive, likely because of an increase in its preferred forage (softwood deciduous trees).

The loss of preferred habitat conditions and increased competition for space, in combination with a third factor, likely drove giant beavers to extinction. The third factor is that *Castoroides* populations would have been relatively easy to geographically and genetically isolate. Their skeletal morphology, and their tie to regions that could continuously support wetlands, would suggest they were not capable of travelling far from water. Regions of North America where *Castoroides* fossils are now concentrated (i.e. Old Crow Basin, Great Lakes Basin, Georgia-Florida Coastal Plain, western Kentucky-Tennessee) are lowlands surrounded by either ocean or mountainous/high-elevation regions. The lowland corridors between regions could have disappeared during warmer, more arid times. These geographic boundaries could have isolated regional populations of a species with a slow reproductive rate, contributing to decreased genetic diversity and increased population sensitivity to stressors.

## 5.5 Future Work

This section suggests avenues for future work to further explore giant beaver palaeoecology.

First, the serial samples taken from the partial *Castoroides* incisor C15-TP2014 successfully demonstrated the potential for recording changes in  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$  over time. It also demonstrated that powdered enamel samples as small as 1 mg can successfully yield stable carbon and oxygen isotopic data. Complete incisors from geographically separate populations of *Castoroides* should be identified in museums collections, and obtained for sampling. A series of enamel samples spaced every 5 cm from apex to occlusal surface will provide a more complete and detailed regional record of the mean and magnitude of variation in *Castoroides*  $\delta^{13}\text{C}_{\text{sc}}$  and  $\delta^{18}\text{O}_{\text{sc}}$ . These isotopic data, in turn, will provide insight into dietary and climatic variation during the animal's lifetime on an annual and likely seasonal timescale.



The number of dentinal bands observed in each *Castoroides* incisor should also be counted. Rinaldi and Cole (2004) determined that the banding is equivalent to modern beaver circadian rhythm banding, where the pairing of light-dark coloured bands represents a 24 hour cycle of dentin growth. The isotopic results should then be described in the context of band number. This would provide an excellent tool for palaeontologists to determine the precise number of weeks represented in a single tooth, and to tie isotopic shifts to discrete units of time.

Second, future *in-situ* discoveries of *Castoroides* specimens from Pleistocene sediments should be accompanied by detailed, site-specific environmental analysis. The semi-aquatic nature of *Castoroides* means that *in-situ* discoveries are possible. Ancient shallow lakes and marshes can be preserved by gradual filling with sediment. Ancient river beds can be preserved due to shifts in their channels and the creation of ox-bow lakes. Environmental analysis should include the collection and identification of plant macrofossils, pollen, and any other faunal remains (vertebrate or invertebrate) discovered in the surrounding strata. The sedimentological processes that created the depositional environment should also be described. There are few published studies that provide detailed documentation of the depositional environment of *in-situ* *Castoroides* specimens (i.e. Richards and Swinehart, 2001). If possible, radiocarbon dates should also be obtained to determine if the *Castoroides* remains and the surrounding wetland are contemporary. Collectively, such information would help to provide a more comprehensive understanding of the ecology of wetlands inhabited by giant beavers.

## 5.6 References

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## Appendices

Appendix A. Stable carbon and nitrogen isotopic results for freshwater macrophyte and terrestrial plant samples.

Sample ID	Taxon	Origin	Category	Tissue type	$\delta^{13}\text{C}$ (‰, VPDB)	$\delta^{15}\text{N}$ (‰, AIR)
TP005	<i>Utricularia sp.</i>	Old Crow Basin	submerged	bulk foliage	-29.74	+4.12
TP007	<i>Potamogeton</i>	Old Crow Basin	submerged	bulk foliage	-15.20	-2.70
TP008	Moss	Old Crow Basin	submerged	bulk foliage	-31.36	+0.53
TP010	<i>Utricularia sp.</i>	Old Crow Basin	submerged	bulk foliage	-29.61	+5.13
TP011	<i>Potamogeton</i>	Old Crow Basin	submerged	entire plant	-20.89	+0.12
TP012A	<i>Myriophyllum</i>	Old Crow Basin	submerged	bulk foliage	-14.17	-0.86
TP012B	<i>Myriophyllum</i>	Old Crow Basin	submerged	roots	-16.16	-3.69
TP016	<i>Hippurus</i>	Old Crow Basin	submerged	bulk foliage	-30.63	+3.02
TP017A	<i>Hippurus</i>	Old Crow Basin	submerged	bulk foliage	-32.35	+0.14
TP017B	<i>Hippurus</i>	Old Crow Basin	submerged	roots	-28.61	+3.20
TP026A	Pondweed	Old Crow Basin	submerged	bulk foliage	-30.60	+1.16
TP026B	Pondweed	Old Crow Basin	submerged	roots	-30.36	+0.95
TP030	Pondweed	Old Crow Basin	submerged	entire plant	-26.91	+0.63
TP031	<i>Hippurus</i>	Old Crow Basin	submerged	bulk foliage	-32.29	+0.07
TP032	<i>Hippurus</i>	Old Crow Basin	submerged	bulk foliage	-30.40	+2.29
TP034	Moss	Old Crow Basin	submerged	bulk foliage	-36.11	+0.82
TP035	<i>Potamogeton</i>	Old Crow Basin	submerged	bulk foliage	-23.15	-2.04
TP051A	<i>Myriophyllum sp.</i>	Whitehorse	submerged	bulk foliage	-13.05	+4.86
TP051B	<i>Myriophyllum sp.</i>	Whitehorse	submerged	roots	-16.18	+4.04
TP052A	<i>Potamogeton sp.</i>	Whitehorse	submerged	bulk foliage	-14.84	+3.83
TP052B	<i>Potamogeton sp.</i>	Whitehorse	submerged	bulk foliage	-14.80	+3.14

TP062	<i>Vallisneria</i>	Whitehorse	submerged	bulk foliage	-28.12	+5.52
TP089	<i>Myriophyllum sp.</i>	Whitehorse	submerged	bulk foliage	-41.17	+2.22
TP091A	<i>Myriophyllum</i>	Pinery Provincial Park	submerged	bulk foliage	-18.63	-1.15
TP091B	<i>Myriophyllum</i>	Pinery Provincial Park	submerged	roots	-18.94	-1.75
TP093	<i>Utricularia sp.</i>	Pinery Provincial Park	submerged	bulk foliage	-28.97	+2.36
TP095	<i>Nitella sp. (algae)</i>	Pinery Provincial Park	submerged	bulk foliage	-19.17	-3.32
TP099	<i>Myriophyllum</i>	Pinery Provincial Park	submerged	bulk foliage	-23.92	-3.06
TP101A	<i>Potamogeton sp.</i>	Pinery Provincial Park	submerged	bulk foliage	-13.09	+2.84
TP101B	<i>Potamogeton sp.</i>	Pinery Provincial Park	submerged	bulk foliage	-12.89	+3.12
TP107	?	Pinery Provincial Park	submerged	bulk foliage	-13.86	-3.47
TP108	<i>Myriophyllum</i>	Pinery Provincial Park	submerged	bulk foliage	-19.33	-3.13
TP110	<i>Potamogeton sp.</i>	Pinery Provincial Park	submerged	bulk foliage	-16.44	-1.49
TP116	?	London	submerged	bulk foliage	-23.35	+1.44
TP002	<i>Menyanthes trifoliata</i>	Old Crow Basin	emergent	bulk foliage	-30.39	+1.29
TP013A	<i>Carex</i>	Old Crow Basin	emergent	bulk foliage	-30.25	+0.87
TP013B	<i>Carex</i>	Old Crow Basin	emergent	roots	-29.27	+2.25
TP015	<i>Equisetum fluviatile</i>	Old Crow Basin	emergent	bulk foliage	-27.12	+2.49
TP025A	<i>Menyanthes trifoliata</i>	Old Crow Basin	emergent	bulk foliage	-26.83	+1.82
TP025B	<i>Menyanthes trifoliata</i>	Old Crow Basin	emergent	roots	-26.39	+1.38
TP027	<i>Equisetum pratense</i>	Old Crow Basin	emergent	bulk foliage	-29.56	+3.73
TP053A	<i>Carex sp.</i>	Whitehorse	emergent	base of stem	-27.75	+5.87
TP053B	<i>Carex sp.</i>	Whitehorse	emergent	bulk leaves/seeds	-27.94	+6.42
TP056	Grass	Whitehorse	emergent	bulk foliage	-30.65	+4.75
TP057	<i>Equisetum</i>	Whitehorse	emergent	bulk foliage	-27.53	+6.56
TP059A	<i>Sparganium sp.</i>	Whitehorse	emergent	bulk foliage/seeds	-28.18	+4.79
TP059B	<i>Sparganium sp.</i>	Whitehorse	emergent	roots	-28.50	+3.56

TP060A	<i>Carex sp.</i>	Whitehorse	emergent	bulk foliage & seeds	-30.10	+4.77
TP060B	<i>Carex sp.</i>	Whitehorse	emergent	base of stem	-28.34	+4.97
TP061	<i>Sparganium sp.</i>	Whitehorse	emergent	bulk foliage	-28.48	+4.57
TP074A	<i>Carex sp.</i>	Whitehorse	emergent	bulk foliage	-26.30	+5.14
TP074B	<i>Carex sp.</i>	Whitehorse	emergent	base of stem	-25.58	+4.55
TP088	<i>Hippurus</i>	Whitehorse	emergent	bulk foliage	-35.33	+4.01
TP096A	<i>Bidens</i>	Pinery Provincial Park	emergent	bulk foliage	-30.17	+1.28
TP096B	<i>Bidens</i>	Pinery Provincial Park	emergent	roots	-29.21	+0.08
TP097A	Grass	Pinery Provincial Park	emergent	bulk foliage	-28.94	+0.16
TP097B	Grass	Pinery Provincial Park	emergent	base of stem	-28.76	+0.14
TP097C	Grass	Pinery Provincial Park	emergent	roots	-28.15	-0.75
TP098	Grass	Pinery Provincial Park	emergent	bulk foliage	-29.34	+4.20
TP112	<i>Typha</i>	London	emergent	base of stem	-31.23	+1.77
TP113	<i>Typha</i>	London	emergent	bulk cattail	-28.29	+4.34
TP114	<i>Sagittaria rigida</i>	London	emergent	bulk foliage	-30.74	+1.65
TP117A	<i>Typha</i>	London	emergent	bulk foliage	-31.14	+1.81
TP003B	<i>Calla palustris</i>	Old Crow Basin	floating	roots	-26.20	+3.43
TP018	<i>Lemna</i>	Old Crow Basin	floating	entire plant	-31.62	+2.70
TP050	<i>Lemna trisulca</i>	Whitehorse	floating	entire plant	-23.28	+5.44
TP094	<i>Lemna sp.</i>	Pinery Provincial Park	floating	entire plant	-28.42	+0.96
TP102	<i>Potamogeton sp.</i>	Pinery Provincial Park	floating	bulk foliage	-25.66	+0.09
TP103	<i>Nuphar variegata</i>	Pinery Provincial Park	floating	bulk flower	-24.70	+1.49
TP104	<i>Utricularia macrorhiza</i>	Pinery Provincial Park	floating	bulk foliage	-29.85	+1.34
TP111A	<i>Sagittaria rigida</i>	London	floating	bulk foliage	-28.26	+7.20
TP111B	<i>Sagittaria rigida</i>	London	floating	roots	-27.75	+5.24

TP118	<i>Nuphar</i>	London	floating	bulk foliage	-26.18	+6.25
TP028A	<i>Vaccinium vitis-idaea</i>	Old Crow Basin	terrestrial	bulk foliage	-29.55	-1.64
TP028B	<i>Vaccinium vitis-idaea</i>	Old Crow Basin	terrestrial	berries	-29.26	-2.18
TP043A	<i>Picea glauca</i>	Old Crow Basin	terrestrial	bulk foliage	-28.67	-8.47
TP043B	<i>Picea glauca</i>	Old Crow Basin	terrestrial	wood	-24.28	-6.82
TP045A	<i>Alnus viridis subspecies crispa</i>	Old Crow Basin	terrestrial	bulk foliage	-28.55	-1.41
TP046A	<i>Salix sp.</i>	Old Crow Basin	terrestrial	bulk foliage	-29.52	+2.62
TP046B	<i>Salix sp.</i>	Old Crow Basin	terrestrial	wood	-29.23	+1.06
TP047	<i>Salix sp.</i>	Old Crow Basin	terrestrial	bulk foliage	-28.90	-0.04
TP048A	<i>Alnus viridis subspecies crispa</i>	Old Crow Basin	terrestrial	bulk foliage	-28.34	-1.22
TP048B	<i>Alnus viridis subspecies crispa</i>	Old Crow Basin	terrestrial	wood	-27.51	-1.54
TP049	<i>Betula neolaskana</i>	Old Crow Basin	terrestrial	bulk foliage	-29.77	-5.01
TP063A	<i>Salix sp.</i>	Whitehorse	terrestrial	wood	-30.46	-0.88
TP063B	<i>Salix sp.</i>	Whitehorse	terrestrial	bark	-32.00	+0.33
TP064A	<i>Salix sp.</i>	Whitehorse	terrestrial	bulk foliage	-29.53	+0.42
TP064B	<i>Salix sp.</i>	Whitehorse	terrestrial	wood	-27.85	-0.53
TP065A	<i>Viburnum edule</i>	Whitehorse	terrestrial	bulk foliage	-31.07	-3.67
TP065B	<i>Viburnum edule</i>	Whitehorse	terrestrial	berries	-30.88	-3.96
TP066	<i>Shepherdia canadensis</i>	Whitehorse	terrestrial	bulk foliage	-30.80	+0.07
TP068	<i>Populus tremuloides</i>	Whitehorse	terrestrial	bulk foliage	-31.26	-1.29
TP071	<i>Picea glauca</i>	Whitehorse	terrestrial	bulk foliage	-27.52	-1.35
TP077	<i>Salix sp.</i>	Whitehorse	terrestrial	bulk foliage	-28.50	+3.94
TP081	<i>Betula glandulosa</i>	Whitehorse	terrestrial	bulk foliage	-30.34	-1.53
TP082	<i>Rhododendron groenlandicum</i>	Whitehorse	terrestrial	bulk foliage	-28.53	-2.46

TP087	<i>Pinus contorta</i>	Whitehorse	terrestrial	bulk foliage	-27.74	-2.09
TP119A	<i>Betula</i>	London	terrestrial	bulk foliage	-27.58	-0.81
TP119B	<i>Betula</i>	London	terrestrial	wood	-25.70	-1.24
TP029	Moss	Old Crow Basin	terrestrial	bulk foliage	-33.15	-1.15
TP036A	Lichen	Old Crow Basin	terrestrial	entire plant	-24.82	-3.99
TP036B	Lichen	Old Crow Basin	terrestrial	entire plant	-24.83	-4.02
TP039	<i>Peltigera leucophlebia</i>	Old Crow Basin	terrestrial	entire plant	-33.94	-0.52
TP086	<i>Peltigera leucophlebia</i>	Whitehorse	terrestrial	entire plant	-35.09	-0.23
*VEGTO-BAUG1/00#1A	<i>Juniperus virginiana</i>	Pinery Provincial Park	terrestrial	bulk foliage	-27.05	-3.48
*VEGTO-BAUG1/00#1B	<i>Juniperus virginiana</i>	Pinery Provincial Park	terrestrial	bulk twig	-26.50	-3.06
*VEGSO-BAUG1/00#1A	<i>Populus balsamifera</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.28	-4.21
*VEGSO-BAUG1/00#1B	<i>Populus balsamifera</i>	Pinery Provincial Park	terrestrial	bulk twig	-26.63	-6.36
*VEGSO-BAUG1/00#2A	<i>Juniperus communis</i>	Pinery Provincial Park	terrestrial	bulk foliage	-25.92	-5.25
*VEGSO-BAUG1/00#2B	<i>Juniperus communis</i>	Pinery Provincial Park	terrestrial	bulk twig	-23.93	-5.76
*VEGSO-BAUG1/00#3A	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk foliage	-27.82	-2.04
*VEGSO-BAUG1/00#3B	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk twig	-26.70	-2.44
*VEGSO-AAUG3/00#1A	<i>Juniperus communis</i>	Pinery Provincial Park	terrestrial	bulk foliage	-25.69	-6.60
*VEGSO-AAUG3/00#1B	<i>Juniperus communis</i>	Pinery Provincial Park	terrestrial	bulk twig	-24.44	-5.97
*VEGSO-AAUG3/00#2A	<i>Populus balsamifera</i>	Pinery Provincial Park	terrestrial	bulk foliage	-26.86	-7.49
*VEGSO-AAUG3/00#3A	<i>Arctostaphylos uva ursi</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.47	-9.54
*VEGSO-AAUG3/00#3B	<i>Arctostaphylos uva ursi</i>	Pinery Provincial Park	terrestrial	bulk twig	-26.64	-10.61
*VEGT1AUG11/00#1A	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.45	-4.69
*VEGT1AUG11/00#1B	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk twig	-28.37	-3.78
*VEGT1AUG11/00#2A	<i>Juniperus virginiana</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.49	-5.13
*VEGT1AUG11/00#2B	<i>Juniperus virginiana</i>	Pinery Provincial Park	terrestrial	bulk twig	-27.19	-3.78
*VEGT1AUG11/00#3A	<i>Quercus velutina</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.49	-5.89



*VEGT1AUG11/00#3B	<i>Quercus velutina</i>	Pinery Provincial Park	terrestrial	bulk twig	-26.91	-5.11
*VEGT1AUG11/00#4A	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk foliage	-30.74	-3.70
*VEGT1AUG11/00#4B	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk twig	-30.08	-5.22
*VEGT1AUG11/00#5A	<i>Quercus prinoides</i>	Pinery Provincial Park	terrestrial	bulk foliage	-28.51	-3.96
*VEGT1AUG11/00#5B	<i>Quercus prinoides</i>	Pinery Provincial Park	terrestrial	bulk twig	-28.09	-4.26
*VEGS1AUG3/00#2A	<i>Symphoricarpos duham</i>	Pinery Provincial Park	terrestrial	bulk foliage	-30.25	-7.55
*VEGS1AUG3/00#2B	<i>Symphoricarpos duham</i>	Pinery Provincial Park	terrestrial	bulk twig	-29.82	-8.46
*VEGT2AUG16/00#1A	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.01	-3.27
*VEGT2AUG16/00#1B	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk twig	-27.72	-3.95
*VEGT2AUG16/00#2A	<i>Quercus velutina</i>	Pinery Provincial Park	terrestrial	bulk foliage	-27.75	-4.63
*VEGT2AUG16/00#2B	<i>Quercus velutina</i>	Pinery Provincial Park	terrestrial	bulk twig	-27.8	-4.9
*VEGT2AUG16/00#4A	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.47	-5.73
*VEGT2AUG16/00#4B	<i>Prunus serotina</i>	Pinery Provincial Park	terrestrial	bulk twig	-28.66	-5.42
*VEGT2AUG16/00#5A	<i>Prunus virginiana</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.89	-2.45
*VEGT2AUG16/00#5B	<i>Prunus virginiana</i>	Pinery Provincial Park	terrestrial	bulk twig	-29.86	-4.09
*VEGS2AUG16/00#1A	<i>Symphoricarpos duham</i>	Pinery Provincial Park	terrestrial	bulk foliage	-30.71	-5.16
*VEGT5AUG21/00#1A	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk foliage	-27.94	-4.73
*VEGT5AUG21/00#1B	<i>Pinus strobus</i>	Pinery Provincial Park	terrestrial	bulk twig	-27.19	-5.83
*VEGT5AUG21/00#3A	<i>Quercus alba</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.65	-4.97
*VEGT5AUG21/00#3B	<i>Quercus alba</i>	Pinery Provincial Park	terrestrial	bulk twig	-30.10	-5.94
*VEGS5AUG21/00/#1A	<i>Rhus aromatica</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.16	-7.76
*VEGS5AUG21/00#2A	<i>Vaccinium</i>	Pinery Provincial Park	terrestrial	bulk foliage	-30.65	-4.65
*VEGS5AUG21/00#2B	<i>Vaccinium</i>	Pinery Provincial Park	terrestrial	bulk twig	-30.06	-4.60
*VEGS5AUG21/00#3A	<i>Rubus</i>	Pinery Provincial Park	terrestrial	bulk foliage	-30.58	-5.06
*VEGS5AUG21/00#3B	<i>Rubus</i>	Pinery Provincial Park	terrestrial	bulk twig	-30.52	-5.42
*VEGS5AUG21/00#6A	<i>Prunus virginiana</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.09	-6.17
*VEGS.5AUG03/00#2A	<i>Populus balsamifera</i>	Pinery Provincial Park	terrestrial	bulk foliage	-29.79	-4.51

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*VEGS.5AUG03/00#2B	<i>Populus balsamifera</i>	Pinery Provincial Park	terrestrial	bulk twig	-27.37	-5.42
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\* From Longstaffe (unpublished)

## Curriculum Vitae

**Name:** Tessa Plint

**Post-secondary Education and Degrees:** The University of Western Ontario  
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2013-2016 M.Sc. Earth Sciences

The University of Western Ontario  
London, Ontario, Canada  
2008-2012 B.A. Honors Specialization in Anthropology

**Honours and Awards:** Alexander Graham Bell  
Canada Graduate Scholarship-Master's  
2014-2015

Arcangelo Rea Family Foundation  
Graduate Scholarship  
2014

Northern Training Grant (Northern Scientific Training Program)  
Research Grant  
2014

Western Science Undergraduate Pre-Thesis Award  
Undergraduate Scholarship  
2013

**Related Work Experience:** Research Technician (LSIS-AFAR)  
Department of Biology  
The University of Western Ontario  
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Teaching Assistant  
Department of Earth Sciences  
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**Relevant Publications:**

Plint, T., Longstaffe, F. J., Hodgetts, L., Hodgins, G., Rybczynski, N., Southon, J., and Zazula, G., 2016. Stable isotopic investigations of giant beaver diet and

econiche – Not another dam beaver. *Geological Association of Canada-Mineralogical Association of Canada*, Whitehorse, Yukon Territory, Canada (Conference presentation).

Plint, T., and Longstaffe, F. J., 2016. Giant beaver palaeoecology inferred from stable isotopes. *Canadian Society for Vertebrate Paleontology*, Mississauga, Ontario, Canada (Conference presentation).

Plint, T., Longstaffe, F. J., and Hodgetts, L., 2014. Stable Isotope Analysis of *Castoroides*: Unraveling the Ecology of the Ice Age Giant Beaver. *Advances in Earth Science Research Conference*. Ottawa, Ontario, Canada (Conference presentation).

Plint, T., Longstaffe, F. J., Zazula, G., Yansa, C., McDonald, H. G., Rybczynski, N., and MacPhee, R., 2013. Stable Isotope Analysis of *Castoroides*: Unraveling the Ecology of the Ice Age Giant Beaver. *Canadian Quaternary Association*, Edmonton, Alberta, Canada (Conference poster).