INCISOR INTEGRITY OF NORTH AMERICAN MOOSE (*ALCES ALCES*) AND POSSIBLE EFFECTS ON POPULATION AGE STRUCTURE

Michael Jarrod Clough

A thesis submitted to
Saint Mary's University, Halifax, Nova Scotia
in partial fulfillment of the requirements for
the Degree of Masters of Science in Applied Science

Halifax, Nova Scotia

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ISBN: 978-0-494-35758-3
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ISBN: 978-0-494-35758-3

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July 12, 2007

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INCISOR INTEGRITY OF NORTH AMERICAN MOOSE (*ALCES ALCES*) AND POSSIBLE EFFECTS ON POPULATION AGE STRUCTURE

By Michael Jarrod Clough, July 2007

ABSTRACT

For mammals, foraging efficiency and survivorship should be a function of tooth condition. There is evidence the teeth of moose from Cape Breton Island have abnormally high levels of tooth breakage. To address this issue, the objectives of this thesis were to: 1) collect, and quantitatively characterise tooth integrity from several North American moose populations; 2) relate trends in population tooth integrity to population age structure; and 3) determine whether chemistry of teeth affects tooth integrity. It was hypothesised that for moose: (i) decreased cropping efficiency, resulting from a loss of tooth integrity, would compromise energy budgets; therefore negatively affect survivorship; (ii) tooth integrity is influenced by the incorporation of elements into the hydroxyapatite crystal lattice during mineralization.

A total of 3602 individual moose incisors (I1s) were collected from 7 North American jurisdictions from the 2004 and 2005 hunting seasons: New Brunswick (NB), New Hampshire (NH), Ontario (ON), Cape Breton Island (CBI) and Vermont (VT), Newfoundland (NL) and Yukon (YK). Each I1 was characterised in terms of damage, breakage, cracking, wear and incisal depth as an indicator of tooth integrity. A total of 475 incisors from were selected for chemical analysis. Incisor integrity decreased with age at twice the rate for CBI moose relative to NB, NH, ON and VT. However, there was no relationship between loss of incisor integrity and survivorship. A Canonical Analysis of Discriminance demonstrated tooth condition may be influenced by mineral concentration within the enamel.

ACKNKOWLEDGEMENTS

Firstly I would like to thank Hugh Broders for providing me the opportunity to pursue this project academically, and for also providing many useful insights and contributions to project design and data analyses.

Completion of this project would not have been possible without the contributions made by the following area biologists/managers whom contributed samples, data and more importantly their time: Barry Adams, Cedric Alexander, Neil Dawson, Adrian McGinn, Tony Nette, Rob Otto, Kirby Punt, Kristine Rines, Dwayne Sabine and Rick Ward.

I would like to thank Dr. Alexander (Sandy) Grist and Dr Marcos Zentilli for providing lab space at Dalhousie University Fission Track Lab and for the time afforded to ensure I had quality polished sections for analysis. Also, I would like to thank Mike Tubrett for all his help on chemical data acquisition and allowing me to 'takeover' the probe lab for a week.

I'd like to thank my wife, Aminda, for her support and encouragement throughout the programme. Although I was fortunate enough not to have to spend a great deal of time away from her, many long hours were spent working on this degree rather than with her, and she was very understanding. Thank you.

I would like to thank the following organisations for funding support; Canadian Wildlife Federation, Cape Breton Highlands National Park, Natural Sciences and Engineering Research Council Canada Discovery Grant to HGB, Nova Scotia Department of Natural Resources and Saint Mary's University.

STATEMENT OF ORIGINALITY

The work presented throughout this thesis is original and principally the work of the M.Sc. candidate, except where literature has been cited in the customary manner. I played the primary role in literature reviews, project proposals, lab work, data collection, analysis of data and writing up the research in thesis/publication format. My supervisor played a major role in securing the funds required for this project.

For chapter 3, Dr. Alexander (Sandy) Grist (Dalhousie University) provided the technical expertise for polished section preparation and Mike Tubrett (Memorial University of Newfoundland) provided technical expertise in the chemical analysis of teeth using Laser Ablation Inductively Coupled Plasma-Mass Spectrometry.

My supervisor and committee members offered many comments and suggestions throughout the project, but the interpretations and writing of this thesis are considered the work of the M.Sc. candidate. At this point, I acknowledge the support offered by my supervisor, Dr. Hugh Broders, for assisting in project design and also editorship throughout the entire project.

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CHAPTER 1:

Incisor integrity of North American moose (*Alces alces*) and possible effects on population dynamics: introduction

Dentition varies among species, due in large part to variation in diet. Carnivores and herbivores have developed highly specialised dentition, with omnivores exhibiting a mixture of the two. Carnivores have developed specialised canine and carnassial teeth, important for capturing, killing and tearing flesh from prey (Orr 1961; Romer 1962; Hildebrand 1988). Herbivores have developed specialised incisors and molar teeth, used for cropping and chewing vegetation.

Organisms must have developed teeth of sufficient integrity to withstand the daily wear and tear associated with mastication (Van Valkenburgh 1988; Fenton et al. 1998). Tooth attrition has been observed in many species, including ungulates (Peterson et al. 1982; Young and Marty 1986; Smith 1992; Hindelang and Peterson 1993; Kierdorf et al. 1993; Hindelang and Peterson 1994; Clough et al. 2006; Loe et al. 2006), primates (Bibby and Losee 1970; Shearer 1983; Oliveira et al. 2006), felids (Van Valkenburgh 1988; Stander 1997; Patterson et al. 2003), canids (Gipson et al. 2000) and chiropteras (Fenton et al. 1998). Severe attrition is possibly related to diet, with carnivores having a higher probability of being affected due to a high intake of bone (Van Valkenburgh 1988), with severe attrition of canine teeth in carnivore species resulting in decreased predation efficiency (Van Valkenburgh 1988; Patterson et al. 2003). Others have suggested that severe attrition is of little consequence to individual survival. This view assumes that natural selection would lead to teeth that are vital for survival be strengthened to meet demands of the lifestyle (Vila et al. 1993; Fenton et al. 1998). Therefore, if severe attrition does occur, those teeth affected must be of little consequence to survival so individuals are still able to maintain a satisfactory level of fitness through dietary intake.

Environmental factors, particularly major and trace elements, have been the focus of research on tooth condition within humans. It has been demonstrated that severe dental disease may be geographically isolated (Bibby and Losee 1970; Curzon and Cutress 1983; Driessens and Woltgens 1986; Brown et al. 2004). Distribution of the major and trace elements vary spatially, and therefore uptake of elements within the tissues of an organism will also vary spatially, and is generally a reflection of local environmental conditions (Maisironi 2000). The local environment will be influenced by conditions such as fluctuations in local geochemistry (bedrock geology), climatic conditions (rainfall and temperature), local biological conditions (soil pH and/or moisture retention) and anthropogenic conditions, such as those associated with industry (Pilgrim and Hughes 1994; Devkota and Schmidt 2000; Adriano 2001; Kabata-Pendias 2004; Telmer et al. 2004). Therefore, local physical environments will affect the structural integrity of teeth (Bibby and Losee 1970; Curzon and Cutress 1983)

Enamel (hydroxyapatite) is the most important component of the tooth for structural integrity, forming a protective layer around less mineralised components, bearing the brunt of mastication and withstanding the effects of acid dissolution (Cutress 1983a; Bhaskar 1991). Strength and solubility of enamel is influenced by the concentration of major and trace elements, both within the enamel matrix and at the surface (Zimmerman 1976; Curzon 1983b). During enamel mineralization, elements are incorporated within the crystal structure of hydroxyapatite that are reflective of elements present within tissue fluids at the time of tooth development (Cutress 1983a). Due to the semi-permeable nature of hydroxyapatite, the enamel surface (outer 30-50μm) can incorporate major and trace elements from the oral environment (Dreizen 1976; Lazzari 1976; Cutress 1983a). Elements with a high affinity for calcium (i.e. the 'bone-seeking' elements such as fluorine, strontium or lead) accumulate at the enamel surface, whereby a tendency towards chemical equilibrium between the enamel surface and oral environment is thought to occur (Dreizen 1976; Lazzari 1976; Cutress 1983b).

Moose (*Alces alces*) belong to the Family Cervidae, are found exclusively in the Northern Hemisphere and occur in a variety of habitats such as montane forests, mixed deciduous hardwood forests, and boreal forests (Bubenik 1997; Karns 1997; Renecker and Schwartz 1997). They are non-migratory and have distinct areas in which they inhabit over their lifetime, known as home range (Cederlund and Okarma 1988; Lepitch and Gilbert 1989; Hundertmark 1997). The home range for moose represents a familiar area where they can meet their daily life requirements such as eating, resting or escaping from predators (Hundertmark 1997). Moose are generalist browsers, consuming a wide variety of plant species and plant parts (Renecker and Schwartz 1997). The type of vegetation a moose ingests is dependant on seasons and geographical location. Woody vegetation from deciduous trees, shrubs and some conifers is a major component of their winter diet , and it is this woody vegetation which is the common link between different ecoregions in which moose inhabit (Renecker and Schwartz 1997; Schwartz and Renecker 1997). This is especially true in winter months, when highly nutritive lush green vegetation is not available (Schwartz and Renecker 1997). Moose will primarily eat twigs in the winter and stripped leaves, newly emerging buds and aquatic plants in the spring/summer (Renecker and Schwartz 1997).

Incisors are important for moose to meet their daily requirements. A moose can spend up to a maximum of 7-10hrs/day foraging (Renecker and Schwartz 1997). Moose crop their food by placing the woody vegetation between the incisors (lower mandible) and the upper prehensile lip. Attrition of moose incisors has been documented only within a few North American moose populations, with the most severe documented within Alaska, U.S.A and Cape Breton, Canada (Peterson et al. 1982; Young and Marty 1986; Smith 1992; Hindelang and Peterson 1993; 1994; Clough et al. 2006). It seems likely that attrition of incisors could decrease cropping efficiency, which may have a negative effect on individual survivorship, therefore affecting population age structure (Fig 1.1).

The goal of this project was to: (i) quantitatively assess the validity of the conceptual model (Fig 1.1) by quantifying the structural integrity of moose incisors for a number of populations and compare these data to population age structure (ii) quantify chemical composition of the enamel of incisors from several North American moose populations to determine whether a mineral deficiency can account for severe attrition. Specifically, the following hypotheses and predictions are tested in this thesis:

Chapter 2, Hypothesis III: Decreased cropping efficiency compromises budgets results in a loss of incisor integrity and longevity of individuals will be negatively affected. Prediction: a decrease in incisor integrity (i.e., increased attrition) will negatively affect survivorship within populations.

Chapter 3, Hypothesis I: Structural integrity of teeth is influenced by the incorporation of major and trace elements into the hydroxyapatite crystal lattice during mineralization. Prediction: Moose populations exhibiting severe attrition will display excessive/deficient concentrations of various major and/or trace elements.

Both chapters are written as standalone manuscripts, formatted with the intention of peer reviewed publication. Some repetition is therefore inevitable. Finally, chapter 4 attempts to tie together the information from both these chapters with discussions and general conclusions.

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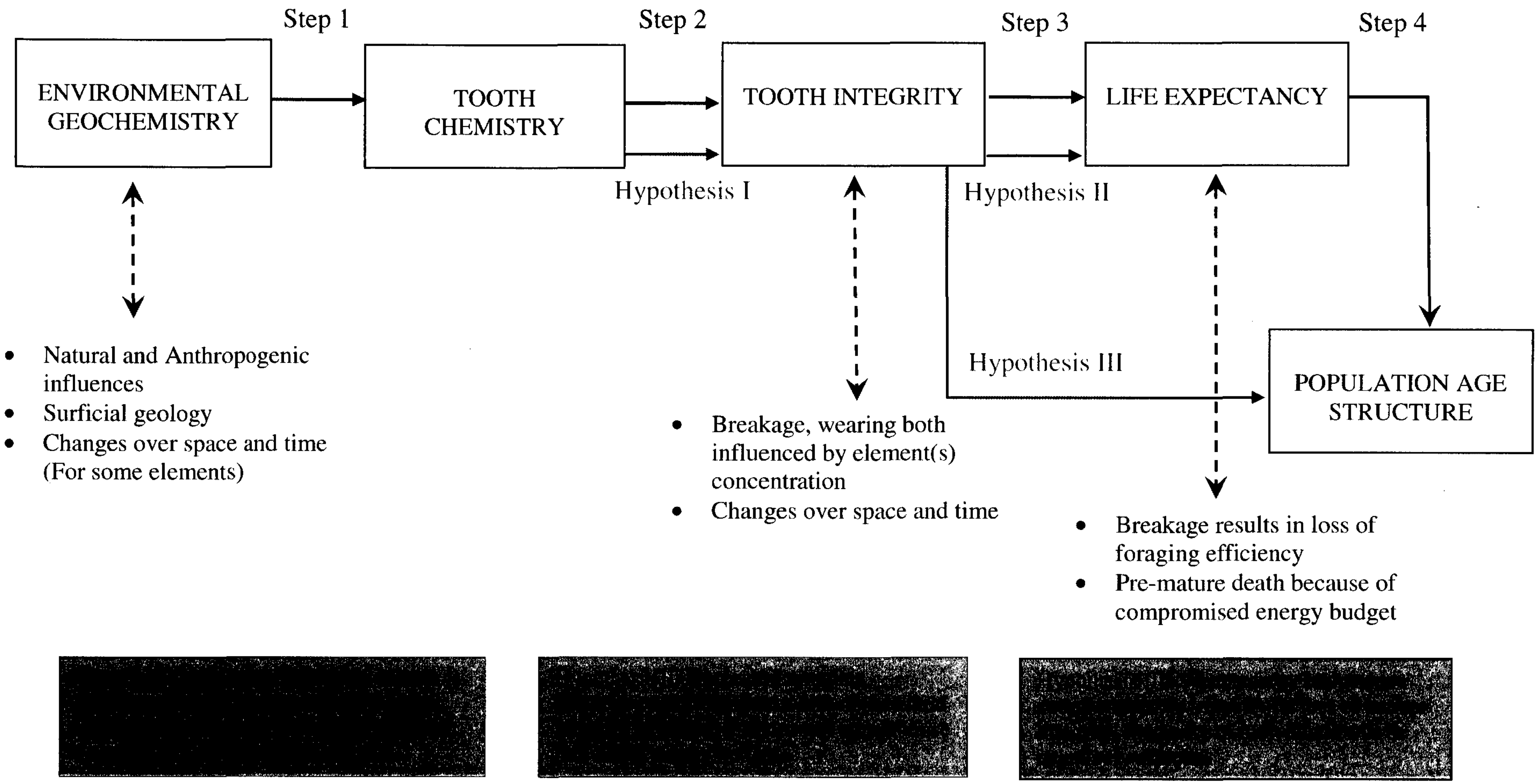

Figure 1.1. The above conceptual model is a proposition to explain how changes in moose population structure may result from geochemistry via the effects on tooth 'integrity'. Each step in the model is linked to another by a hypothesized cause and effect. The goal of this study is to test predictions of these specific hypotheses to assess the validity of the conceptual model.

CHAPTER 2:

Characterisation of incisor condition in North American moose populations and its affects on population age structure

Abstract: It has been hypothesised that animals have evolved teeth suited for wear and tear associated with their lifestyle. Therefore, mammals with similar life history traits should have similar dentition. Individuals whose teeth are structurally compromised are thought to be at a disadvantage in terms of food acquisition and ultimately survival, but some teeth may be less critical than others. This study assessed the impacts of tooth condition (wearing, breakage, etc) on population age structure of moose (*Alces alces*) from seven North American jurisdictions: Cape Breton Island, New Brunswick, New Hampshire, Newfoundland, Ontario, Yukon and Vermont. Frequency of tooth breakage is highest for Newfoundland and Cape Breton moose and even within these jurisdictions there is spatial variation in the frequency of breakage. Wearing occurs in all populations, but is 2 times higher in New Hampshire, Vermont, New Brunswick and Ontario. Incisal depth was used to measure the integrity of incisors (i.e., cumulative effects of breaking and wearing). Although a normal process, the rate of loss of incisor integrity with age was twice as fast for Cape Breton moose relative to New Brunswick, New Hampshire, Ontario and Vermont. However, there was no relationship between loss of incisor integrity and survivorship.

Introduction

Mammals rely on their teeth to perform essential daily tasks such as the capture and mastication of food and defence. Life history traits, most notably diet, dictate dentition characteristics for different species (Orr 1961; Romer 1962; Hildebrand 1988). For example, carnivores will often have prominent canine teeth and molars and premolars that are modified for shearing meat, with other cheek teeth having been reduced, or in some cases completely absent (Orr 1961; Romer 1962; Hildebrand 1988). In herbivores, incisors have developed into blade-like structures efficient at pinching and cropping vegetation (Orr 1961; Hildebrand 1988). Canine teeth are reduced, and in most cases have developed to resemble incisors in form and function (Orr 1961). Cheek teeth are important for grinding and shredding cropped food (Orr 1961; Romer 1962; Hildebrand 1988). Omnivores generally have a dentition that is a mixture of tooth types from carnivores and herbivores (Hildebrand 1988).

Specific tooth types are adapted to particular functions (Hildebrand 1988; Van Valkenburgh 1988). For example, ruminants may spend as much as 7-10h/day chewing food (Hildebrand 1988; Renecker and Schwartz 1997) that is usually coarse and may contain mixtures of grit exacerbating wear (Peterson et al. 1982; Young and Marty 1986; Hildebrand 1988). To deal with heavy wear and tear, ruminants have evolved hypsodont dentition, whereby the cheek teeth develop deep within the jaw of young animals, and the crown is very high (Hildebrand 1988). As wearing progresses, the roots progressively rise from the jaw (with the void being replaced by bone) to expose more of the crown (Romer 1962; Hildebrand 1988).

If tooth function is critical for survival in mammals, there should be strong selection pressure on teeth to withstand normal wear and tear (Van Valkenburgh 1988; Fenton et al. 1998; Patterson et al. 2003). Individuals displaying severely blunted, or in extreme cases broken teeth, may be inferior predators (Van Valkenburgh 1988; Patterson et al. 2003). Similarly, foraging and chewing efficiency in herbivores may be inhibited if structural integrity of teeth are compromised (Young and Marty 1986; Hindelang and Peterson 1994). Alternatively, it is possible other mechanisms may be responsible for tooth condition within wild populations. Spatial variation in tooth integrity may be related to local environmental conditions, i.e., major and trace element availability (Bibby and Losee 1970; Curzon and Cutress 1983) or occasional, unpredictable high stresses (e.g., falls) unrelated to selection (Van Valkenburgh 1988).

Moose (*Alces alces*) belong to Family *Cervidae* and are found exclusively in the Northern Hemisphere, occurring in a variety of habitats such as montane forests, mixed deciduous hardwood forests, and boreal forests (Bubenik 1997; Karns 1997; Renecker and Schwartz 1997). They are a generalist browser, with woody vegetation comprising a large component of their diet (Renecker and Schwartz 1997). Incisor teeth are important for moose to meet their daily nutritive requirements. Food is placed between the incisors and upper lip and cropped from the source. Attrition of incisors has been documented in some moose populations (Peterson et al. 1982; Young and Marty 1986), with the most severe forms documented in the Alaska and Cape Breton populations (Smith 1992; Clough et al. 2006). Smith (1992) observed a 58% incidence of incisor breakage in 270 mandibles from Alaskan moose, and concluded that this high frequency may be a result of high moose densities. At the time of the study, moose density on the Seward Peninsula was reported at 8 moose/km^2 (Grauvogel, 1984 in Smith 1992). Moose have since showed a gradual decline in numbers on the Seward Peninsula (Persons 2004).

When moose occur at high densities for long periods of time food resources are heavily exploited (Basquill and Thompson 1997; Moen et al. 1998; Persson et al.

2000; Edenius et al. 2002) and browse quantity and quality decreases (Renecker and Schwartz 1997). Moose require a wide variety of plants and plant parts within their diet to meet daily energy and nutritive requirements (Oldemeyer 1974; Ohlson and Staaland 2001). Where heavy browsing of vegetation occurs it is also likely that moose increase consumption to satisfy daily requirements and there may be less variety as preferred food supplies wane. These changes may also increase the risk of moose having toxic or deficient mineral nutrition (Ohlson and Staaland 2001). When nutrition is compromised, there is a possibility that the structural integrity of teeth may be compromised (Bibby and Losee 1970; Curzon and Cutress 1983; Clough et al. 2006). Such problems may further be compounded by moose foraging frozen vegetation, or having to browse vegetation that has larger diameter (which will be harder) due to a lack of new growth vegetation as a result of over foraging which may increase wear and tear.

The consequence that incisor attrition has on individuals affected is unknown, but it seems likely that fitness could be compromised as a result of reduced cropping efficiency (Peterson et al. 1982; Young and Marty 1986; Smith 1992; Clough et al. 2006). The purpose of this study was to characterize the physical condition of incisors from several moose populations across North America and relate trends in incisor condition to population age structures. It is predicted that jurisdictions with low tooth integrity among older cohorts will display lower survivorship with age.

Methods

A total of 3602 individual moose incisors (I1s) were collected from 7 North American jurisdictions from the 2005 hunting season: New Brunswick (NB), New Hampshire (NH), Ontario (ON), Cape Breton Island (CBI) and Vermont (VT), Newfoundland (NL) and Yukon (YK). For each tooth we also received an estimate of its age (determined in all cases by cementum annuli dating) and the specific moose management unit (MMU) of origin, with the exception of NL, for which age data were unavailable at the time of this study. Each I1 was characterised in terms of damage, breakage, cracking, wear and incisal depth as an indicator of tooth integrity (Table 2.1). Quantification of incisor condition was carried out blindly (i.e., origin and age was unknown) by a single observer (MC) to eliminate both inter-jurisdictional and inter-individual bias. Average moose density observed for the 2005 hunting season, presented as moose/km^2, was received for each jurisdiction. Jurisdiction MMUs were arbitrarily grouped where appropriate for ease of reference (e.g., VT was divided into northern, central and southern).

Incisal depth was used as an index of tooth integrity. For each population mean incisal depth was calculated for individual cohorts. For inter-population comparisons of incisal depth (i.e., tooth integrity) and age, simple linear regression (SLR) was used to calculate the slope and its associated standard error (SE). Using hunter killed data from CBI, NB, NH, ON, and VT static survivorship curves were derived to assess the impacts of tooth integrity on population age structure (Begon et al. 1996). Hunting strategies within these jurisdictions allow for any sex/age harvesting and populations are subjected to minimal predatory pressure with wolves being absent in all populations except ON (Tony Nette, Dwayne Sabine, Kristine Rines, Cedric Alexander and Neil Dawson, personal communication). Two major assumptions are 1) hunting biases that may exist are similar among jurisdictions and; 2) age specific mortality threats are the same in each jurisdiction (Begon et al. 1996).

Results

Cracking was observed within all populations, with an average incidence of 0.85 (Table 2.2). Newfoundland and CBI moose display a higher incidence of breakage relative to the other jurisdictions, but within these populations the incidence of breakage is highly variable (Table 2.2). Cape Breton Island moose North of Cape Breton Highlands National Park (Fig 2.1) had a 5 times increase in the incidence of breakage compared to moose from southwest CBI and NL moose from the Northern and Avalon regions had 1.5-3 times higher incidence of breakage relative to NB, NH, ON, and VT.

All populations displayed an increase in both the mean and variation of incisor depth with age (Fig 2.2). The slope of the relationships between incisor depth and age for NB, NH, ON, and VT ranges between 0.21 and 0.30 ΔTooth condition/ΔAge. Within CBI spatial variation is observed for the slope of the relationships between incisor depth and age, with a loss of tooth integrity with age increasing along a south to north gradient (Table 2.3). The loss of tooth integrity with age occurs at rate between 0.5-2 times the magnitude on CBI relative to the other jurisdictions (Table 2.3). Further, the SE range of the slope of MMU1 within CBI (0.606 $\pm$ 0.071) does not include the SE range of slope measures from MMUs 2, 3 or 4 south of Cape Breton Highlands National Park indicating a significant difference between the means (0.425 $\pm$ 0.097; 0.390 $\pm$ 0.110; 0.334 $\pm$ 0.130 respectively).

Moose densities are 3.25-13 times greater within the CBI population relative to NB, NH, ON and VT, and 11.5-46.5 times greater within the NL population relative to NB, NH, ON and VT (Table 2.4). Static survivorship curves for each of the five jurisdictions suggests tooth condition has no impact on age structure among populations (Fig 2.3).

Discussion

The positive relationship between attrition and age was as expected. Continual use of teeth and associated wearing is well documented for many species (Cutress

1983a; Hindelang and Peterson 1993; Stander 1997; Hewison et al. 1999; Gipson et al. 2000; Christianson et al. 2005; Oliveira et al. 2006). However the exceptionally high breakage frequencies within NL and CBI suggest that some other factor(s) is/are at play within these populations. These factors may include behaviour, local environment, diet or some combination of these.

There is a correlation between high breakage incidence and high moose densities demonstrated within CBI and NL. The correlation is further supported within CBI, where an increasing trend in the incidence of breakage is observed from south to north. Moose densities in the lightly harvested area north of the park are high and similar to densities within the park. South of the park, where hunting pressure is higher, moose densities are slightly lower. South west of the higher elevations density continues to decrease (Fig 2.1-Tony Nette, personal communication).

With a correlation between high moose densities and higher incidence of breakage, the data support the contention that moose density affects forage quality and growth, which may lead to problems of tooth integrity. The specific functional mechanism could be either through increased attrition caused by the structure/composition of the browse itself and/or by causing a less-than-optimal tooth chemistry, therefore affecting tooth integrity and its ability to withstand 'normal' wear and tear.

Heavy wear has been observed in several moose populations. Peterson et. al. (1982) reported differences in tooth wear between moose inhabiting the Kenai Peninsula in Alaska (AK), USA and Isle Royale National Park, USA. Heavy wear was observed in 2-3 year old animals in the AK population, comparable to wear in 15 year old Isle Royale moose (Peterson et al. 1982). Young and Marty (1986) observed excessive wear of incisors within a population of moose from Manitoba, Canada, relative to other moose populations within the province. High silica content within the food supply was thought to be the mechanism resulting in high wear within the Manitoba moose, whereas ingestion of soils was thought to be the cause within Alaska.

Moose are generalist browsers, but they will limit themselves to preferred foods when they are available (Renecker and Schwartz 1997; Moen et al. 1998; Persson et al. 2005). Although slight variation in diet occurs among populations woody vegetation is a major component of moose diet and (Peek 1974; Persson et al. 2005). Aquatic vegetation is considered a succulent food for moose, and are preferentially browsed when available (Peek 1974). Bottom sediments and abrasive particles suspended in the water are generally ingested when feeding on aquatic plants (Peterson et al. 1982; Young and Marty 1986). Furthermore, high silica content associated with certain food types (such as ground vegetation) has been suggested as acting as an abrasive agent on teeth (Peterson et al. 1982; Young and Marty 1986). Behavioural differences among populations, dictated by local environment, may be a plausible hypothesis to explain differences in incidence of tooth wear. Moose in areas of high wear may feed more exclusively on aquatic plants and ground vegetation, and therefore ingest more soil and grit. Both NH and VT which likely experience similar environmental conditions display similarities in tooth wear and breakage.

Many studies have observed correlations between local geo-environmental conditions and tooth condition, with severe forms of dental disease being geographically isolated (Bibby and Losee 1970; Curzon 1983a; Maisironi 2000). The fact that the severest form of attrition (i.e., breakage and increased incisal depth) is restricted to CBI, NL and AK warrants further investigation to consider whether local environment may be exerting a negative affect on tooth condition. Furthermore, CBI moose currently comprises the largest and most stable population within the province of Nova Scotia (NS) (Pulsifer and Nette 1995). It is the only population within the province that allows for an annual harvest. Approximately 300 licenses are issued, with over 12000 applications, annually (NSDNR 2005). Aboriginal people also rely on the moose for sustenance, and moose are a major attraction for the many thousands of tourists visiting the Cape Breton Highland National Park annually. These factors result in CBI moose being a high profile population for the province of NS. Therefore, based on the evidence from AK, which suggest tooth breakage may be an early sign of moose having reached/exceeded carrying capacity, wildlife authorities should closely monitor the populations that exhibit high tooth breakage.

There is no evidence that severe attrition has any effect on the age structure of the CBI population. The reason is unclear, but moose are able to maintain required energy levels through dietary intake. Our data do not support the hypothesis that jurisdictions with low tooth integrity among older cohorts will display lower survivorship as age increases. However, our data may support the contention that tooth condition among moose populations may be a function of density. Alternatively, because severe attrition is geographically isolated, it could also be argued that local environmental factors may be exerting an effect on tooth integrity. To further support this hypothesis, it is suggested that analysis of tooth chemical composition be carried out in order to determine if excessive/deficient mineral concentrations can further support the breakage hypothesis.

Acknowledgments

I would like to thank the following area biologists/managers for contributing samples, data and more importantly their time to making this project possible:

Barry Adams, Cedric Alexander, Neil Dawson, Adrian McGinn, Tony Nette, Rob Otto, Kirby Punt, Kristine Rines, Dwayne Sabine and Rick Ward. I would also like to thank by Dr. Brian McLaren, Dr. Marc Lamoureux and Dr. Marcos Zentilli for providing comments and suggestions, which significantly improved this document. I would also like to thank Jason Roche and Carmen Cranley for their assistance and acknowledge funding support from Nova Scotia Department of Natural Resources, Cape Breton Highlands National Park, Saint Mary's University, Canadian Wildlife Federation, and a Natural Sciences and Engineering Research Council Canada Discovery Grant to HGB.

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Table 2.1. Characteristics used to quantify tooth condition of moose teeth.

Damaged	Damage resulting from post mortem factors, identified by clean breakage with no staining or wear and/or fresh cracks. Damaged teeth were not used in population assessments of tooth condition.
Broken	Identified by characteristic rounding and brown staining which indicates breakage occurred during the lifetime of theindividual. Individual teeth were scored as either being broken or not.
Wearing	Indicated by either a brown stain or the wearing down to dentine on the incisal surface. Individual teeth were scored as either being worn or not.
Cracked	Cracks that were identifiable on the surface of the teeth that terminated at the incisal surface were recorded for each tooth. Individual teeth were scored as cracking present or not present.
Incisal Depth	Incisal depth was used as a measure of the loss of tooth integrity (i.e., volume lost or deterioration). Using a standard distance of 2 mm from the incisal edge, the depth of the incisor (from front to back) was measured at 1/3, 1/2 and 2/3 distance from one side of the tooth to the other. The sum of the three was used for analysis. Teeth that are broken and worn will have higher values for this measure and would be expected to have lower cropping efficiency (i.e., less tooth integrity).

Table 2.2. Incidence of cracking (C), wearing (W) and breakage (B) for all age classes for 7 jurisdictions

Jurisdiction	MMU	n	%C	%W	%B
Cape Breton Island					
North of Park	1	182	0.77	0.07	0.34
Immediately South of Park	2	118	0.88	0.00	0.25
Central CBI	3	58	0.90	0.07	0.14
SouthWest CBI	4	44	0.86	0.02	0.07
New Brunswick					
Northwest	1-4, 6, 10, 11	344	0.90	0.11	0.02
Northeast	5, 7, 8	299	0.83	0.17	0.03
Central	12-14, 17	195	0.88	0.23	0.01
Southwest	15, 16, 20, 21	309	0.87	0.24	0.01
Southeast	18, 19, 22-25	221	0.91	0.14	0.02
New Hampshire	A-E	82	0.87	0.30	0.04
Newfoundland					
Northern	1-5, 14, 40, 45	52	0.58	0.08	0.69
Western	5-11	29	0.86	0.03	0.21
Central	13, 15-22, 24-27, 41	45	0.93	0.18	0.31
Eastern	28-30, 42, 34, 47	33	0.88	0.18	0.21
Avalon Peninsula	31-36, 44	45	0.87	0.42	0.64
Ontario	1, 11-15, 48, 51, 55, 56	136	0.82	0.40	0.01
Vermont					
Northern	C, D, E, G, H	445	0.81	0.29	0.06
Central	I, J	24	0.83	0.17	0.04
Southern	L, M, O, P, Q	32	0.81	0.34	0.06
Yukon	2-5, 7-10	42	0.69	0.12	0.02

Table 2.3. Regression results from incisal depth vs age in moose from 5 North American jurisdictions. Also shown are the results for individual MMUs for CBI.

	B_0	B_{AGE}	B_{AGE} SE	R^2	N	P-Value
New Brunswick	5.52	0.244	0.01	36.0	1366	<0.001
New Hampshire	6.38	0.217	0.035	32.8	81	<0.001
Ontario	5.45	0.3	0.031	50.1	97	<0.001
Vermont	5.6	0.217	0.014	36.4	493	<0.001
Cape Breton Island (Total)	5.12	0.512	0.047	36.7	210	<0.001
Cape Breton Island MMU1	4.95	0.606	0.071	46.2	87	<0.001
Cape Breton Island MMU2	5.29	0.425	0.097	23.8	63	<0.001
Cape Breton Island MMU3	5.50	0.390	0.110	29.4	35	<0.001
Cape Breton Island MMU4	5.57	0.334	0.130	20.4	25	<0.001

Table 2.4. Average moose density for each jurisdiction, presented as moose/km^2 as provided by wildlife officials from each jurisdiction.

Jurisdiction	Density
Cape Breton Island	2.00
New Brunswick	0.32
New Hampshire	0.46
Newfoundland	7.00
Ontario	0.15-0.40
Vermont	0.61

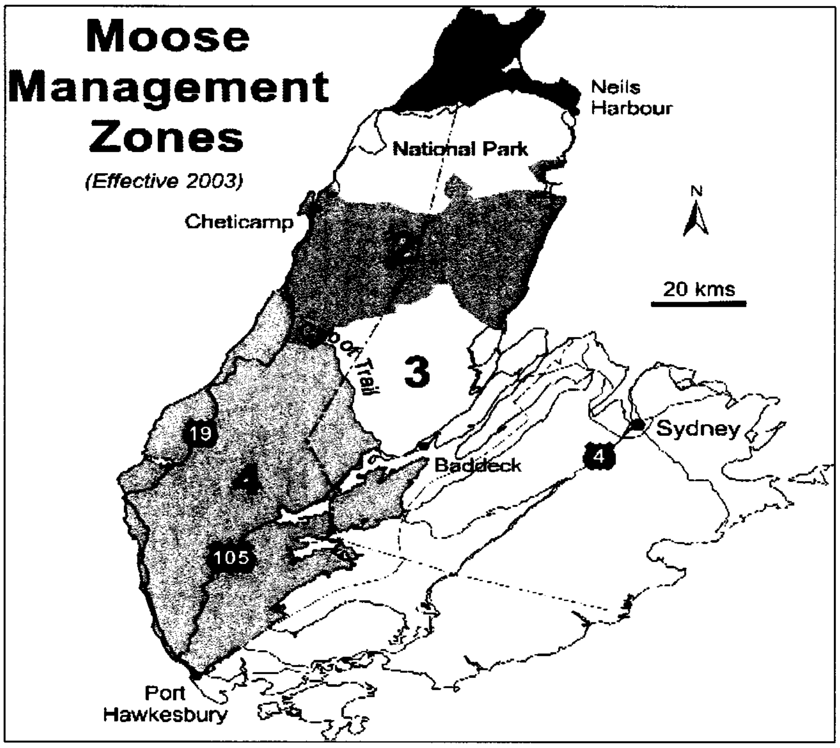

Figure 2.1. Map of Cape Breton Island illustrating the Moose Management Units where hunting is permitted. Moose do not inhabit areas southeast of units 3 & 4. Reprinted with permission from the Nova Scotia Department of Natural Resources-Wildlife Division.

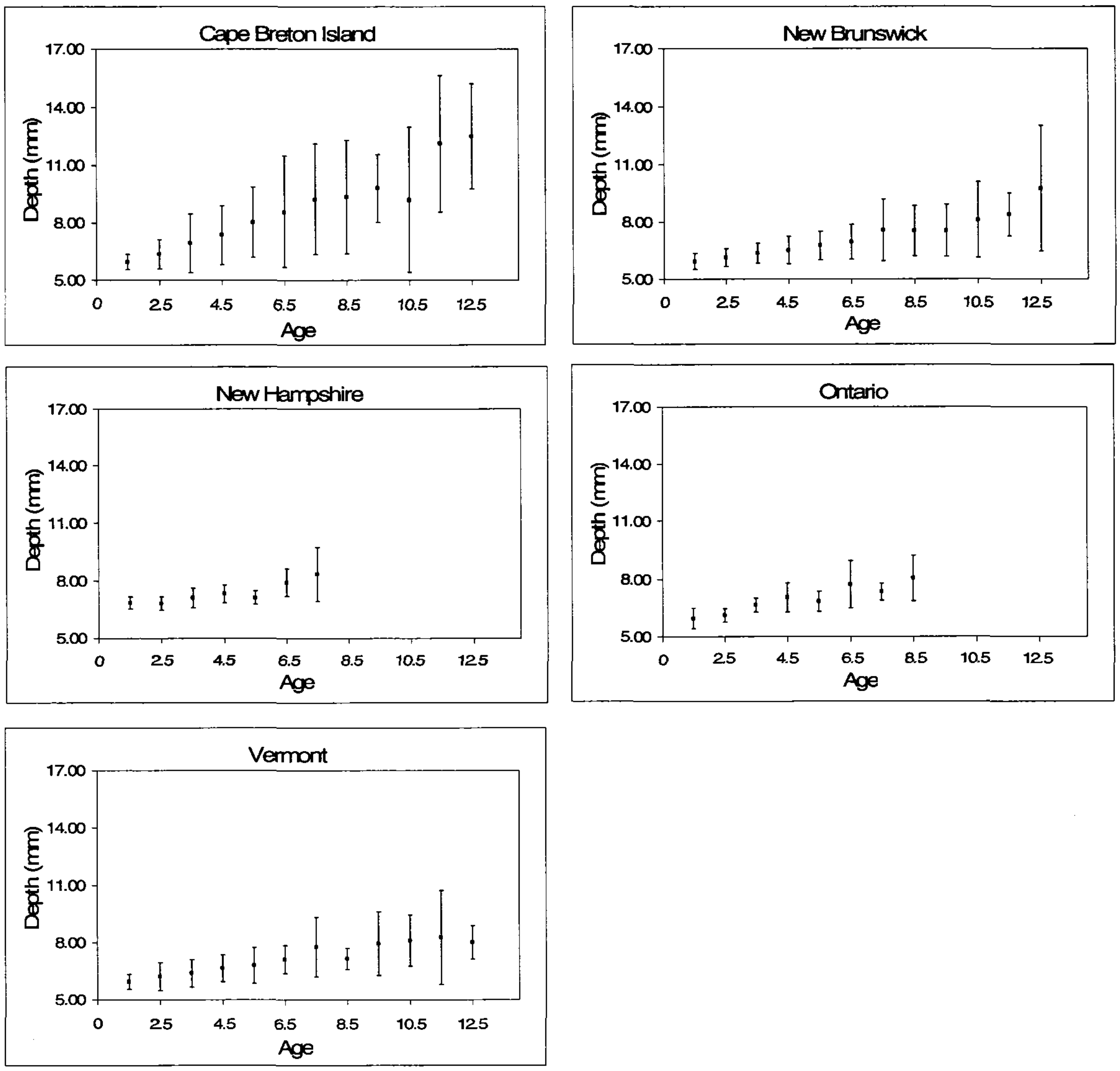

Figure 2.2. Mean incisal depth (+/- SD) of incisor teeth of moose from cohorts for 5 jurisdictions. NL and YK data are not included due to lack of age data and sample size. For NH we only received age data up to 7.5 years, and ON up to 8.5 years. Results for the regression are presented in Table 2.3.

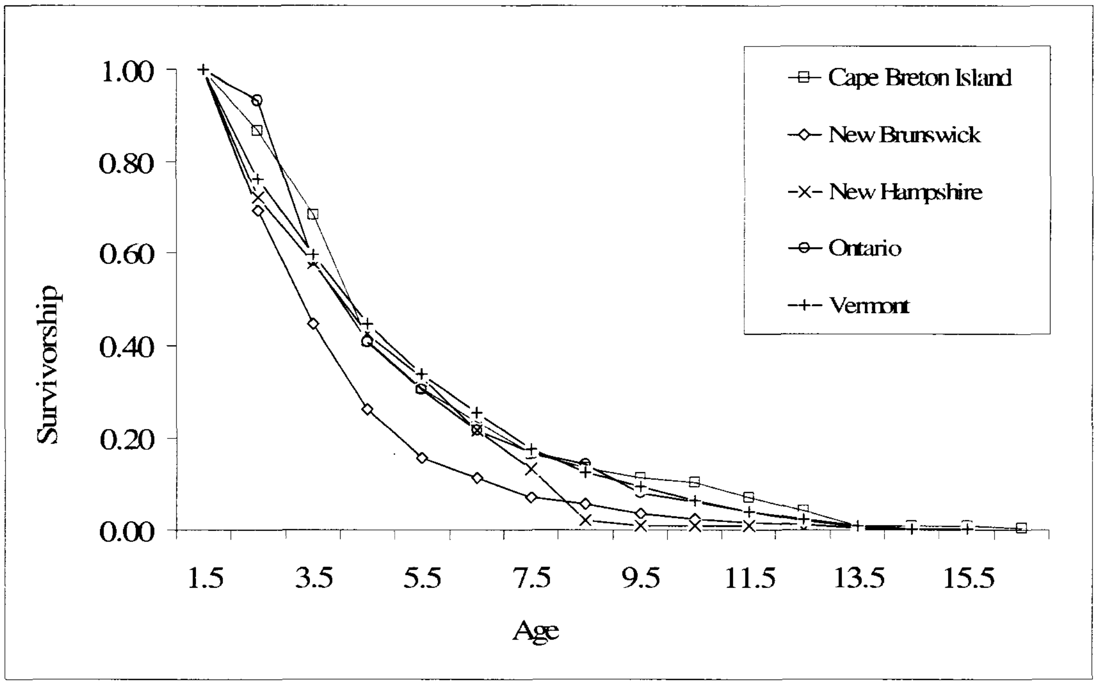

Figure 2.3 Static survivorship curves for moose populations plotted from hunter killed age data for 5 jurisdictions for the 2005 hunting season. Newfoundland and Yukon are not included due to lack of age data and sample size respectively. Values were derived by dividing the number of individuals remaining at each age class (i.e., number of individuals of an age class and older) with the total number of individuals within a jurisdiction.

CHAPTER 3:

Characterisation of incisor condition in North American moose populations and its affects on population age structure

Abstract: Tooth enamel functions as a resistant layer that protects less mineralised components of teeth and bears the brunt of mastication. If the enamel becomes cracked or broken, the less mineralised components will be compromised, and the integrity of the tooth will decrease. The structural integrity of enamel is thought to be influenced by the incorporation of major and trace elements during tooth mineralization. High breakage frequency of moose (*Alces alces)* incisors has been observed in Newfoundland and Cape Breton. This study compared concentration of 20 elements in the enamel of moose incisors from 5 North America moose jurisdictions including New Brunswick, New Hampshire, Newfoundland, Cape Breton Island and Vermont. Results of a Canonical Analysis of Discriminance suggested that incisor breakage within Cape Breton Island and Newfoundland moose may result from a mineral imbalance. Specifically, lead concentration is negatively related to incisor integrity ($P<0.10$), and rubidium is positively associated with incisor integrity ($P<0.10$). Rubidium is a normal constituent of enamel, yet has no known physiologic function. However, lead has been implicated in several studies of human dental disease. High lead ingestion results in elements, such as calcium, being displaced from their binding sites in proteins and, accordingly, may interfere with the process of calcification (i.e., of bones and/or teeth). Higher lead concentration within the enamel where high breakage incidence occurs is likely the result of atmospheric deposition from anthropogenic sources. High moose density appears to exacerbate the problem.

Introduction

In mammals, it has been demonstrated that at least 25 elements are essential for normal growth, development and daily metabolic activities (Underwood 1971; 1977; Curzon and Cutress 1983; Underwood and Suttle 1999; Bogden and Klevay 2000; Maisironi 2000). An imbalanced diet (excessive or deficient quantities of certain elements) may result in fluctuations of elements in the body above or below tolerable/required limits (Underwood and Suttle 1999). Nutritional disorders may arise, which may lead to abnormal growth and development, and possibly higher than expected rates of mortality (Underwood 1971; 1977; Curzon and Cutress 1983; Underwood and Suttle 1999; Bogden and Klevay 2000; Maisironi 2000).

Bioavailability of elements varies spatially and temporally due to fluctuations in geochemical (bedrock geology), climatic (rainfall and temperature), biological (hyper-accumulating/excluding plants), physical (soil pH, soil moisture retention, element-element interaction) and anthropogenic conditions such as industrial emissions (Pilgrim and Hughes 1994; Devkota and Schmidt 2000; Adriano 2001; Kabata-Pendias 2004; Simonetti et al. 2004; Telmer et al. 2004). Elements can enter animal food chains through plant, soil and water ingestion (Curzon 1983b; McLaughlin et al. 1999; Devkota and Schmidt 2000). Concentration of elements within an organism may also be regulated through homeostatic mechanisms (Kostial 1986; Underwood and Suttle 1999; Dolphin et al. 2005). Disorders resulting from excessive/deficient element concentration are often a result of local geo-environmental factors (Bibby and Losee 1970; Underwood 1971; Curzon and Cutress 1983; Maisironi 2000; Brown et al. 2004; Chandrajith et al. 2005; Howe et al. 2005; Farmer et al. 2006; Ljung et al. 2006).

Enamel (hydroxyapatite) is an important component of teeth. It functions as a resistant layer surrounding the less mineralised dentin, bearing the brunt of mastication (Sharaway and Yeager 1991). It is the hardest substance in the mammalian body, is very similar to apatite found in nature, and is 96% mineralised (Lazzari 1976; Simpson 1976; Melfi 1988; Simmelink 1994). The mineral apatite has a Mohs hardness of 5, considerably higher than calcite (Mohs hardness 3), a major constituent of shell and bone (Klein et al. 1999). If the enamel layer is cracked or broken, the less mineralised components of the tooth (dentin and pulp) will dissolve due to the acidic nature of the oral cavity, eventually decreasing the integrity of the tooth (Lazzari 1976).

Structural integrity of enamel is thought to be influenced by the concentrations of major and trace elements incorporated into the crystal structure during mineralization (Zimmerman 1976). Once elements are incorporated into the enamel matrix, no physiologic process will decrease the element concentration (Cutress 1983a; Melfi 1988). Content of enamel reflects the concentration of the elements within tissues and fluids at the time of tooth development (Cutress 1983a). However, due to the semi-permeable nature of hydroxyapatite, it is thought that changes may occur at the outer (outer 30-50μm) enamel surface. Elements with a high affinity for calcium (i.e., the 'bone-seeking' elements such as. fluoride, strontium or lead) may accumulate at the enamel surface during the lifetime of an individual, increasing with age (Dreizen 1976; Lazzari 1976; Cutress 1983b), whereby a tendency towards chemical equilibrium between the enamel surface and oral environment is thought to occur (Driessens 1982).

Moose (*Alces alces*) belong to the *cervidae* family and are found exclusively in the Northern Hemisphere, occurring in a variety of environments such as montane forests, mixed deciduous hardwood forests, and boreal forests (Bubenik 1997; Karns 1997; Renecker and Schwartz 1997). They are generalist browsers and diets vary spatially (Peek 1974; Basquill and Thompson 1997; Moen et al. 1998; Persson et al. 2000; Edenius et al. 2002). However, woody vegetation from deciduous trees, shrubs and some conifers are common foods for all moose (Renecker and Schwartz 1997; Schwartz and Renecker 1997).

Recent evidence suggests that moose populations of Cape Breton Island (*A. a andersoni*) and the island of Newfoundland (*A. a. americana*) are incurring higher incidence of breakage relative to other North American moose populations (Chapter 2). Spatial variation of breakage was also observed within CBI, with the incidence of breakage increasing along a south-north gradient within the region. Alaska moose (*A. a. gigas*) inhabiting the Seward Peninsula have the only other reported occurrence of excessively high incisor breakage among North American moose populations (Smith 1992).

Moose were extirpated from CBI at the turn of the 20th century, and the current population base was founded by 18 Alberta moose (*Alces alces andersoni*) which were introduced in 1947 and 1948 (Pulsifer and Nette 1995). Two moose (one male and one female) were first introduced to the island of NL in 1878 from Nova Scotia (*Alces alces americana*), followed by another introduction (2 males and 2 females) from NB (*Alces alces americana*) in 1904 (Broders et al. 1999). Due to the apparent optimal conditions (e.g., low predation, sufficient food and shelter), moose inhabiting CBI and the island of NL have increased in numbers since their respective introductions. Moose densities on CBI are approximately 2 moose/km^2. Highest densities occur north of Cape Breton Highlands National Park, and a decreasing trend is observed on a North to South gradient south of the park (See figure 2.3) (Tony Nette, personal communication). Newfoundland moose have been observed at densities up to 7 moose/km^2 (Barry Adams, personal communication).

Moose are non-migratory, having a distinct home range where they can meet their daily life requirements (Cederlund and Okarma 1988; Lepitch and Gilbert 1989; Hundertmark 1997). Local environmental conditions may vary but individuals of a given area will be subject to relatively similar environmental conditions from year to year, and should display similar element concentrations over time (Devkota and Schmidt 2000; Adriano 2001; Kabata-Pendias 2004). Furthermore, if elements accumulate at the enamel surface over an individual's lifetime (Lazzari 1976; Driessens 1982; Cutress 1983b), then it would be expected that teeth of the youngest individuals within a population will display lower concentrations of these elements at the surface relative to older individuals.

There is very little information available about tooth development in deer species (Bubenik 1997). It is therefore difficult to accurately account for the elemental deposition during tooth mineralization in moose, unlike in humans where mineralization occurs at a known rate (Johnsen 1994; Kang et al. 2004; Dolphin et al. 2005). However, most deer species will have fully erupted permanent incisors at approximately 4-6 months of age (Rue 1997). In humans, the process occurs over 4-6 years (Johnsen 1994). Mineralization of permanent incisors for moose will likely begin *in utero*. Therefore, it is assumed that element concentration of moose teeth will be influenced by both placental exchange (which is regulated in utero) and ingestion of mother's milk (Dolphin et al. 2005).

The main goal of this study was to characterize elemental composition of sectioned incisors. Specifically, the objectives were to: 1) Determine whether there are significant differences between surface and inner element concentrations of enamel for moose; 2)

Test the hypothesis that structural integrity of teeth is a function of the major and trace elements incorporated within hydroxyapatite crystal lattice during mineralization.

Methods

Tooth collection and preparation

A total of 3602 individual moose incisor (I1s) from the 2005 hunting seasons were collected from 7 North American jurisdictions: New Brunswick (NB), New Hampshire (NH), Ontario (ON), Cape Breton Island (CBI) and Vermont (VT), Newfoundland (NL) and Yukon (YK). For each tooth we also received an estimate of its age (determined in all cases by cementum annuli dating) and specific moose management unit (MMU) of origin, with the exception of NL, for which age data was unavailable at the time of this study. A total of 475 incisors from 5 jurisdictions (CBI, NB, NL, NH and VT) were selected for chemical analysis. To minimize bias, samples were randomly chosen from each age cohort, from each jurisdiction. In some cases, adjacent MMUs for some jurisdictions were grouped in order to increase the sample size. Teeth were sectioned (longitudinally) using a dremel ™ tool with a standard 1 1/2" reinforced cut off wheel.

Teeth were set in epoxy on standard 4.5x2.7 cm glass slides, and polished to optical quality using a Struers Planopol-V ™ rotary automatic polisher, using Mecaprex ™ self-adhesive polishing discs, and Struers ™ DP-Suspension diamond paste with grain sizes from 9 micrometers to 0.05 micrometers (Fig 3.1).

Chemical Analysis

Concentrations of 20 elements expressed as ^{11}B, $^{23}Na_2O$, ^{26}MgO, $^{29}SiO_2$, ^{44}CaO, ^{51}V, ^{55}MnO, ^{59}Co, ^{63}Cu, ^{64}Zn, ^{69}Ga, ^{75}As, ^{85}Rb, ^{88}Sr, ^{111}Cd, ^{118}Sn, ^{137}Ba, ^{208}Pb, ^{232}Th and ^{238}U were determined at Memorial University of Newfoundland (MUN) Inco

Innovation Centre using laser ablation inductively coupled plasma-mass spectrometry (LAM ICP-MS). Elements were chosen based on known essentiality and toxicity within mammals (Underwood 1977; Curzon and Cutress 1983; Underwood and Suttle 1999; Bogden and Klevay 2000; Adriano 2001), and also instrument capability (Mike tubrett, personal communication).

The analytical system was a Finnigan ELEMENT XR, a high resolution double focusing magnetic sector inductively coupled plasma mass spectrometer (HR-ICPMS) coupled to a GEOLAS 193 nm excimer LASER system. A helium flow rate of 1.25 L/min was used to carry ablated material to the ICP, with an additional argon make up gas added after the ablation cell. The LASER beam was rastered over the enamel to produce a rim to core profile, commencing at the enamel surface and ending at the dentine-enamel junction. Laser energy was approximately 5 J/cm^2; a laser repetition rate of 10 Hz produced a ≈ 40 μm diameter spot on the sample. Time resolved intensity data were acquired by peak-jumping in a combination of pulse-counting, analog and Faraday modes, depending on signal strength, with one point measured per peak.

Calcium oxide (CaO) was the internal standard used to control for differences in ablation yields and matrix effects between the unknown incisors and the calibration materials (NIST 612 glasses). The CaO concentrations of the unknowns were assumed to be homogeneous at $\approx 49.1\%$, as determined by ICP-MS bulk analysis (Clough et al. 2006). Approximately 30 seconds of gas background data were collected prior to each 60 sec ablation of both standards and unknowns.

The methodology employed an analytical sequence of two analyses of the NIST 612 standard and one of Durango apatite, followed by analyses of up to 14 unknown incisors, closing with a repetition of the same standards in reverse order. Having a similar matrix to hydroxyapatite, Durango apatite were treated as an unknown and data was acquired to monitor the accuracy and precision (Appendix 1). The error for this method when measuring homogeneous materials is estimated to be better than 4% relative based on the reproducibility of results for various reference materials measured from day to day over several months in the MUN laboratory. Data were acquired over a continuous period of one week. Average limits of detection for the elements analysed are detailed within Appendix 2.

Data were reduced using MUNs in-house CONVERT and LAMTRACE spreadsheet programs, which employ procedures described by Longerich et al. (1996). LAMTRACE allows selection of representative signal intervals, background subtraction, and internal standard correction for ablation yield differences, instrument sensitivity drift during the analytical session, and perform calculations converting count rates into concentrations by reference to the standards.

Data analysis

Outer and inner enamel was classified from analysis of graphs depicting variation in element concentration from the enamel surface to the dentine-enamel junction. From the surface to a depth of 100μm was classified "outer matrix" (Fig 3.2) and from 100μm depth to the dentine-enamel junction was classified as "inner matrix" (Fig 3.2). Element concentration along the inner matrix was relatively homogenous. Mean and standard deviations of element concentrations in the outer and inner matrices were calculated for individual age classes for populations with sufficient sample sizes for analysis (i.e., CBI, NB and VT). To determine whether there is a difference ($P<0.0025$ after Bonferonni correction) in mean concentration of the elements between the inner and outer matrices of enamel, two-sample t-tests were conducted using individual age classes for CBI, NB and VT (Appendix 3, 4, 5).

Forward stepwise Canonical Analysis of Discriminance (CAD) (McGarigal et al. 2000) was used to determine whether there was any variation in element profiles among moose from various jurisdictions (F-to-enter=0.05). If jurisdictions with similar elemental profiles have similar tooth integrity profiles, then elements correlated with canonical axes might explain changes in tooth integrity. To satisfy the assumptions of normality, univariate data were transformed using square root where appropriate. The concentration of the 20 elements, and also age, were the variables included in the CAD model. The concentration of the elements for the inner matrix was considered for the CAD on the assumption that elements incorporated in this region occurred during mineralization, which are believed to influence the strength of the hydroxyapatite (Zimmerman 1976).

The same procedure (forward stepwise CAD with F-to-enter=0.05) was used to determine whether a suite of elements could explain differences in tooth integrity among the 4 MMUS of CBI which showed variation in breakage frequency within the region (See chapter 2).

Simple linear regression (SLR) was conducted for the elements that were found to be significant in the CAD analysis. A total of 14 elements were individually tested (MgO, Sr, B, Na_2O, SiO_2, MnO, Co, Cu, Zn, Ga, Rb, Sn, Ba and Pb) while controlling for age. Newfoundland was not included due to missing age data. The regression equation for the model was: Tooth Integrity=$\beta_0+\beta_{age}+\beta_{element}\pm$Error.

Results

Zinc is the only element that consistently differs (P>0.0025 after Bonferonni correction) in concentration between the inner and outer matrices (Appendix 3, 4, 5). This was the case for all age classes; with the exception of 7.5 and 10.5 year old NB moose, 7.5-10.5 year old CBI moose and 9.5 year old VT moose. Zn for the outer matrix was on one to two orders of magnitude higher in concentration than the inner matrices for Cape Breton Island, New Brunswick and Vermont (Table 3.1).

Age was not an important variable in the CAD analysis (F=1.80). Therefore, the analysis was conducted again without the variable age and the NL chemical data was incorporated. Fourteen of the 20 elements were significantly correlated with the CAD axes of elemental profiles of moose from the different jurisdictions (Table 3.2). A MANOVA test, using Wilks Lambda, shows significant differences among the multivariate means of element concentration among the jurisdictions (Wilks lambda=0.115). The classification matrix shows that overall, 78% of the incisors were classified correctly to jurisdictions (Jackknifed matrix=76%). The canonical scores plot show that CBI and NL are slightly differentiated from NB, NH and VT along canonical axis 1 (Fig 3.3).

The CAD for the 4 MMUs of Cape Breton shows that 6 elements discriminate among the MMUs (Table 3.5). The Wilks lambda (0.096) shows significant differences among the multivariate means of element concentration among the MMUs. Overall, 84% of the incisors were classified correctly to MMUs (Jackknifed matrix=76%). The canonical scores plot shows that MMU 1 and 2 are well differentiated from MMU 3 and 4 (Fig 3.4).

For the SLR of the 14 elements that discriminated between the jurisdictions, lead and Rb are the only elements that have a significant ($P<0.10$) relationship with breakage (Table 3.3). Due to the management/conservation implications of this work $\alpha=0.10$ was used to minimize Type I errors.

Discussion

Element concentration does not differ significantly ($P>0.0025$ after Bonferonni correction) between the inner and outer matrices of enamel among cohorts of individual jurisdictions, with the exception of Zn. Zinc is consistently higher in concentration within the outer matrix by 2-5 orders of magnitude for all age classes within all jurisdictions. There are some discrepancies within the jurisdictions for older cohorts (i.e., 7.5 and 10.5 year old NB moose, 7.5-10.5 year old CBI moose and 9.5 year old VT moose show no significant differences ($P>0.0025$ after Bonferonni correction) in Zn concentration between the inner and outer matrix). However the mean concentration is still 2-4 times higher for the outer matrix compared to the inner matrix for these cohorts, and the discrepancy is likely associated with small sample size. Zinc is considered a bone-seeking element (Jarup 2002), and therefore its concentration would be expected to increase with age at the surface (Dreizen 1976; Lazzari 1976; Cutress 1983b). The data suggest otherwise, with Zn concentration remaining relatively constant among all cohorts for all jurisdictions.

No other elements displayed a significant difference ($P>0.0025$ after Bonferonni correction) between the inner and outer matrices, including the bone seeking elements Pb, Cd, and the alkaline earth metals (Cutress 1983b; Henriksen et al. 2002). Therefore, elemental differences observed at the enamel surface for Zn during this study cannot be related to bone-seeking elements penetrating the enamel surface with age, and some other mechanism must be involved.

If element intake during tooth mineralization is derived from placental exchange, followed by ingestion of mother's milk, a shift in concentration may be visible within the enamel matrix distinguishing these two events. This mechanism would be similar to a phenomenon observed in human primary teeth, where shifts in concentration have been demonstrated on opposing sides of the neo-natal line of teeth (Dolphin et al. 2005). The neo-natal line distinguishes element uptake during placental exchange (which is regulated *in utero*) and uptake via mothers milk (Dolphin et al. 2005). Support for this mechanism is demonstrated within the Zn data, where the concentration is consistently higher within the outer matrix relative to the inner matrix among the cohorts for all jurisdictions.

For both CAD analyses, based on the classification and jackknife matrices having little variation, the estimation of means and dispersions are reliable and sample size is sufficient (McGarigal et al. 2000). Although not well differentiated, jurisdictions with similar breakage incidence group together on the y axis and are associated with the elements highly correlated with of Canonical axis 1. The data support the prediction that areas with high breakage incidence will exhibit excessive/deficient concentrations of certain major and/or trace elements. The CAD analysis of individual MMUs for CBI further supports this prediction. Breakage trends for these MMUs show a relative increase from South to North (Chapter 2). The data illustrates that areas of higher breakage incidence (MMU 1 and 2) are well differentiated in elemental profiles from areas of lower breakage incidence (MMU 3 and 4) by elements correlated with canonical (1).

Areas with high breakage incidence exhibited higher concentrations of MgO, Sr, Na_2O, SiO_2, MnO, Zn, Ga and Pb, and lower concentrations of B, Co, Cu, Rb, Sn and Ba within the enamel. Bivariate regressions of incisal depth against each element and age suggested that Pb and Rb are the only elements exerting a significant ($P<0.10$) effect. $Beta_{Age}$ was ≈ 0.30 for all models, suggesting the influence age has on tooth condition is constant.

Element bioavailability is a complex process. Individual uptake can be affected by numerous factors including: individual homeostatic mechanisms; differences in plant uptake which varies by species; and finally climatic, geochemical, physical, and anthropogenic conditions (Frieden 1984; Rajagopalan 1984; Schrauzer 1984; Kostial 1986; Nielsen 1986; Quaterman 1986 ; Underwood and Suttle 1999; Devkota and Schmidt 2000; Adriano 2001; Kabata-Pendias 2004; Dolphin et al. 2005). It is also difficult to quantify daily requirements without species specific information, and little information exists for element requirements for moose (Schwartz and Renecker 1997; Underwood and Suttle 1999).

Many studies have demonstrated a negative relationship between Pb concentrations and dental disease (Stack 1983). High concentrations of Pb within tissues and fluids of an organism can displace other elements (Ca for example) from their binding sites in proteins and interfere with function, such as calcification of bones and/or teeth (Reichlmayr-Lais and Kirchgessner 1984). Rubidium is a normal constituent of enamel, yet has no known physiologic function (Curzon 1983a; Cutress 1983a).

Lead is released into the environment from chemical weathering of rocks and municipal/industrial discharge. However, the major source of absorbable Pb in the environment originates by atmospheric deposition from anthropogenic sources, such as the burning of fossil fuels and smelting of metal ores (Pilgrim and Hughes 1994; Mihaljevic 1999; Simonetti et al. 2004; Telmer et al. 2004; Bonham-Carter et al. 2006).

Lead particles released into the atmosphere can stay aloft for up to 64 hours, and may travel up to 1600km from the source (Pilgrim and Hughes 1994). Long range transport has been well documented (Pilgrim and Hughes 1994; Simonetti et al. 2004; Telmer et al. 2004; Shotyk et al. 2005). Ice cores in the Artic have been shown to contain high concentrations of industrial Pb originating from North American and Eurasian sources (Shotyk et al. 2005). Lead emissions from a smelter close to the Quebec/Ontario border were shown to be deposited in areas of the entire northeast region of North America (Simonetti et al. 2004).

Within CBI and NL, prevailing winds are predominantly from the south/southwest in the summer, and west/northwest in the winter (Phillips 1990). Directly east of NL and northeast of CBI is the Belledune smelter in northeastern New Brunswick, which releases up to 20 tons of Pb into the atmosphere annually (Pilgrim and Hughes 1994). It is possible that high Pb concentrations in CBI and NL are the result of long range transportation of Pb from anthropogenic sources, such as the Belledune smelter

Atmospheric deposition of Pb results in soils (and snow pack in winter) containing appreciable amounts of absorbable Pb (Pilgrim and Hughes 1994; Underwood and Suttle 1999; Telmer et al. 2004; Shotyk et al. 2005). Tree or plant uptake of this element is poor (Underwood and Suttle 1999). However, grasses have been shown to accumulate high quantities of Pb (Pilgrim and Hughes 1994). Therefore, Pb in the diet may originate via ingestion of soil, grasses or water for moose.

The south-north gradient of breakage incidence within CBI is negatively correlated with population density (which is higher in the north). It seems plausible the relationship observed with element excess/deficiency on tooth condition is being exacerbated by high moose density. Support for this is further demonstrated within the NL and Alaska populations, where breakage incidence appears to correlate with high moose densities also. The mechanism is unclear, however moose populations occurring at high densities commonly over utilise resources, reducing browse quality and quantity (Basquill and Thompson 1997; Renecker and Schwartz 1997; Moen et al. 1998; Persson et al. 2000; Edenius et al. 2002). Over utilisation of browse may result in the exclusion of preferred food from canopy, giving way to less palatable/nutritious food (Basquill and Thompson 1997). As a result, moose occurring at high densities may be altering their foraging behaviour, increasing the consumption of ground vegetation, such as grasses, which may contain a high content of soil, grit (silica) and ultimately Pb.

The high SiO_2 concentration within enamel of moose with high breakage incidence further supports this argument. In grazing livestock, the abrasive effects of substantial intakes of silicate-rich particles found within grasses has been associated with excessive tooth wear (Underwood and Suttle 1999), while in moose high silica content and soil ingestion associated with certain food types (such as ground vegetation) has been suggested as acting as an abrasive agent on teeth causing unusual wear (Peterson et al. 1982; Young and Marty 1986).

The density correlation is important. High Pb concentrations close to the Belledunde smelter are well documented (Pilgrim and Hughes 1994). Moose inhabit the surrounding areas of the smelter. Unfortunately chemical data was not collected on moose incisors from this area. However, the incidence of breakage for moose incisors in the northeast area is 0.03, which is low relative to CBI and NL (See chapter 2). Densities are also much lower in NB (0.32 moose/km^2; Dwayne Sabine, personal communication) relative to CBI or NL, adding further support that densities may exacerbate the problem of excessive/deficient mineral concentration of enamel and ultimately incisor breakage.

The data support the hypothesis for an excessive/deficient mineral concentration of enamel resulting in compromised structural integrity of moose teeth. It is possible that the excessive/deficient enamel concentration is being caused or exacerbated by both high moose densities. The breakage occurring along the North-South gradient of CBI should be examined further. Further understanding of how density may affect diet is important, and any study must be complemented by NL and NB teeth, in particular moose surrounding the Belledune smelter. Investigation of natural "background" levels of Pb (Shotyk et al. 2005) in CBI and NL, and investigation of Pb isotopes may shed light as to the source of the Pb, which may support/refute the argument for an anthropogenic source of Pb.

ACKNOWLEDGEMENTS

I would like to thank the following area biologists/managers for contributing samples, data and more importantly their time to making this project possible: Dwayne Sabine, Neil Dawson, Kirby Punt, Barry Adams, Rob Otto, Tony Nette, Adrian McGinn, Cedric Alexander, Kristine Rines and Rick Ward. Alexander (Sandy) Grist trained MC in sample preparation techniques in the Fission Track Research Laboratory of Dalhousie University. I would also like to thank by Dr. Brian McLaren, Dr. Marc Lamoureux and Dr. Marcos Zentilli for providing comments and suggestions, which significantly improved this document. We would like to acknowledge the following for funding support; Nova Scotia Department of Natural Resources, Cape Breton Highlands National Park, Saint Mary's University, Canadian Wildlife Federation, and a Natural Sciences and Engineering Research Council Canada Discovery Grant to HGB.

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Table 3.1. Mean and SD of Zn concentrations for the outer and inner matrices of enamel for 3 different jurisdictions, CBI, NB and VT. Two sample T-test were conducted to test significant differences (P<0.0025 after Bonferonni correction) among the means for inner and outer matrices

	Cape Breton						New Brunswick						Vermont					
	Outer			Inner			Outer			Inner			Outer			Inner		
Age	n	Mean	SD	Mean	SD	P-value	n	Mean	SD	Mean	SD	P-value	n	Mean	SD	Mean	SD	P-value
1.5	18	98.183	40.37	18.14	5.569	P<0.001	15	79.61	40.73	18.51	5.333	P<0.001	14	130.04	55.01	25.23	8.525	P<0.001
2.5	25	103.36	24.29	19.5	4.821	P<0.001	17	90.37	38.66	19.01	6.032	P<0.001	12	116	33.3	20.4	4.073	P<0.001
3.5	29	105.56	29.91	22.37	6.056	P<0.001	17	74.6	32.99	18.8	4.824	P<0.001	11	107.78	31.44	21.47	10.07	P<0.001
4.5	26	105.67	29.35	22.13	5.997	P<0.001	17	63.89	37.35	17.15	3.677	P<0.001	16	96.087	24.46	19.43	6.567	P<0.001
5.5	11	112.17	44.03	25.43	10.56	P<0.001	16	56.09	33.67	16.48	2.865	P<0.001	10	70.957	35.59	15.92	6.303	P<0.001
6.5	12	92.896	29.87	22.64	3.476	P<0.001	15	61.66	29.86	16.92	3.329	P<0.001	15	93.929	37.99	19.45	4.463	P<0.001
7.5	4	109.09	32.95	23.25	4.684	0.0128	5	56.68	34.89	19.3	2.045	0.0454	15	87.727	35	18.93	7.114	P<0.001
8.5	5	77.271	29.83	20.48	5.237	0.0181	9	65.9	48.54	21.67	9.833	P<0.001	12	81.959	20.47	19.52	5.51	P<0.001
9.5	5	26.75	3.881	18.81	2.581	0.0067	11	58.91	41.27	15.67	6.096	P<0.001	9	80.449	35.89	17.39	5.549	0.0068
10.5	6	48.957	27.94	21.2	3.39	0.0591	3	54.32	43.11	20.17	11.61	0.0174	10	89.445	54.45	21.99	7.667	P<0.001

Table 3.2. Canonical scores plot illustrating the discriminatory power each element has for distinguishing jurisdiction/MMU. Values that approach zero have minimal discriminatory power

	All Jurisdictions		Cape Breton Island	
	Canonical 1	Canonical 2	Canonical 1	Canonical 2
Constant	0.591	0.492	-6.718	-116.738
MgO	0.26	0.14	0.358	-0.117
CaO	-	-	0.001	0.524
Sr	0.61	0.107	-	-
B	-0.155	0.276	-	-
Na_2O	0.792	-0.582	-	-
SiO_2	0.197	-0.094	-0.3	0.132
MnO	0.105	-0.137	-	-
Co	-0.226	0.328	-	-
Cu	-0.185	-0.458	-	-
Zn	0.073	0.512	-	-
Ga	0.307	0.286	-	-
Rb	-0.997	0.253	0.225	0.432
Sn	-0.341	0.505	0.829	0.392
Ba	-1.001	-1.027	-	-
Pb	0.227	0.081	-0.875	0.641

Table 3.3. Regression results for incisal depth vs age and element concentration in moose from 4 North American jurisdictions.

	Constant	$Beta_{Age}$	$Beta_{Age\ SE}$	P-Value	$Beta_{Element}$	$Beta_{Element\ SE}$	P-Value
MgO	5.66	0.303	0.024	< 0.001	-0.381	0.882	0.666
Sr	5.33	0.294	0.024	< 0.001	0.001	0.001	0.233
B	5.18	0.291	0.024	< 0.001	0.165	0.102	0.109
Na2O	5.30	0.295	0.024	< 0.001	0.430	0.507	0.397
SiO_2	5.49	0.294	0.024	< 0.001	0.425	1.003	0.672
MnO	5.65	0.297	0.024	< 0.001	-3.288	2.627	0.211
Co	5.52	0.295	0.024	< 0.001	0.022	0.057	0.697
Cu	5.70	0.295	0.024	< 0.001	-1.160	1.180	0.324
Zn	5.20	0.296	0.024	< 0.001	0.077	0.072	0.290
Ga	5.59	0.294	0.024	< 0.001	-0.012	0.061	0.846
Rb	5.87	0.298	0.024	< 0.001	-0.410	0.235	0.080
Sn	5.44	0.294	0.024	< 0.001	0.115	0.125	0.359
Ba	5.63	0.294	0.024	< 0.001	-0.007	0.022	0.744
Pb	5.20	0.292	0.023	< 0.001	0.453	0.118	< 0.001

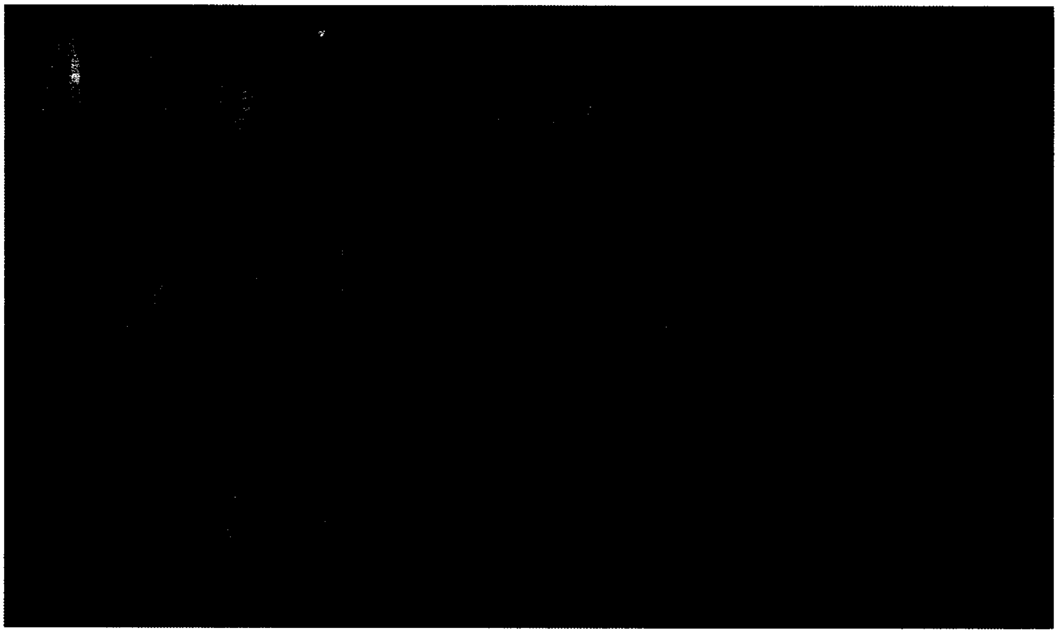

Figure 3.1. Polished incisor sections for LA-ICP-MS analysis, set in epoxy on standard 27 x 46mm glass slide.

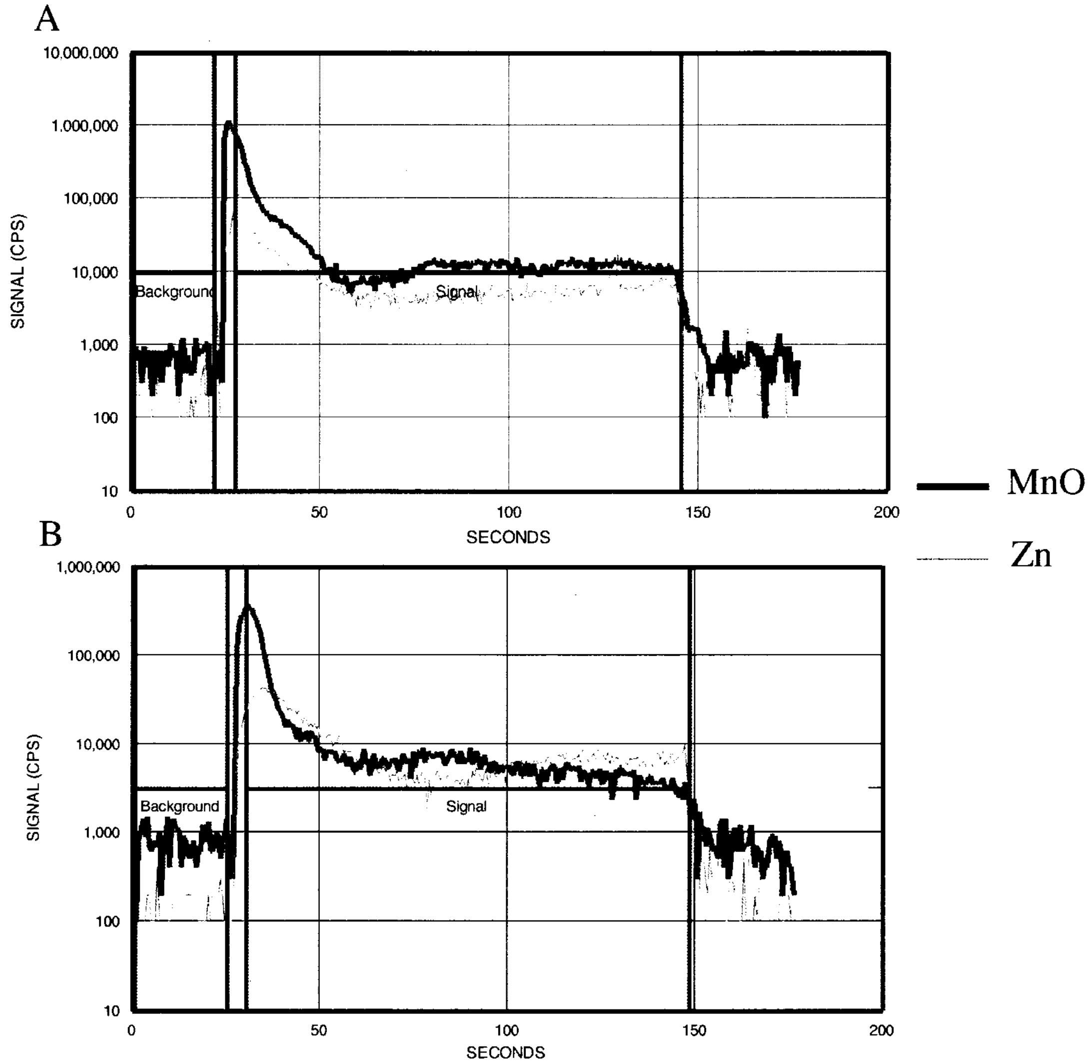

Figure 3.2 Count per second data from LAM-ICP-MS. The graphs shown do not represent absolute concentrations, but rather signal intensity over time which is converted to ppm or wt% using the program LAMTRACE. Beam movement was 5μm/sec (50 seconds=250μm) (a) Shows the profile of a 10.5 year old male moose incisor from Vermont (MMU E2). Concentration spikes at the beginning of the run (~35sec), and levels off ~100μm from the enamel surface (~55sec) (b) Shows the profile of a 1.5 year old male moose incisor from Vermont (MMU D2). Notice the profile is almost identical to the 10.5 year old moose. Vertical red lines indicate the start (enamel surface) and finish (dentine-enamel junction) of the analysis.

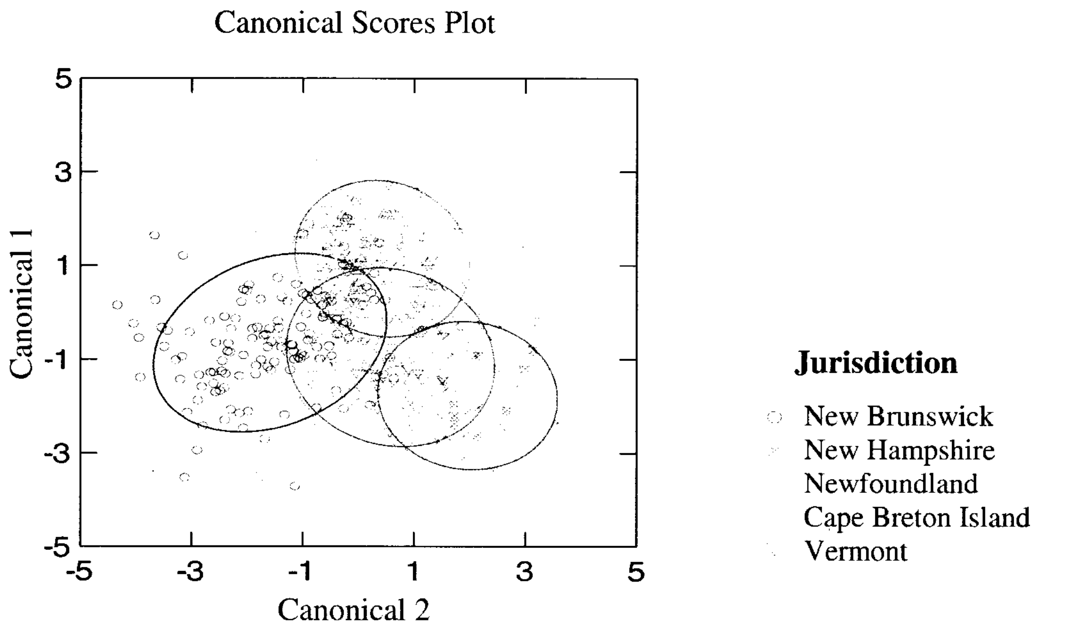

Figure 3.3. Canonical scores plot of moose from 5 jurisdictions depicting areas of high breakage frequency grouping closely based on the elements associated with canonical axis 1. Areas with low breakage frequency group closely on the x axis (Canonical 1), with differentiation occurring the y axis (Canonical 2).

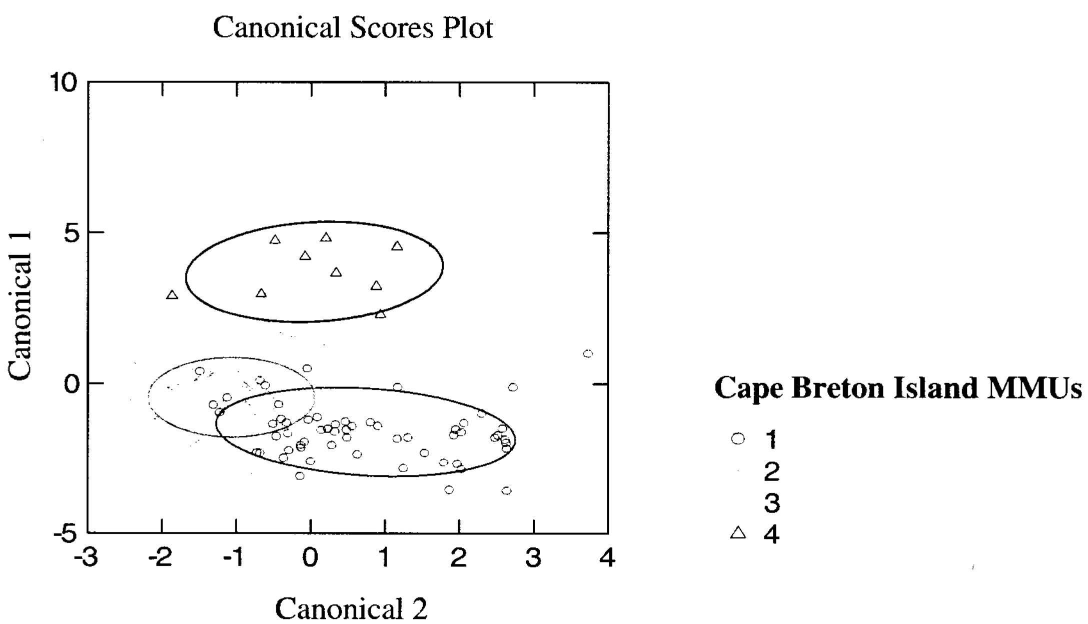

Figure. 3.4. Canonical scores plot for CBI moose depicting areas of high breakage frequency grouping closely, and areas of lower breakage frequency grouping closely, based on the elements associated with canonical 1. MMU 1 is north of Cape Breton Highlands N.P. and MMU 2 is immediately south of the park. MMU 3 and 4 are south of MMU 2, respectively.

CHAPTER 4:

Incisor integrity of North American moose (*Alces alces*) and possible effects on population dynamics: synthesis

Wear and tear of teeth is expected to occur over the lifetime of an individual. However, incisor deterioration among Cape Breton Island (CBI) moose appears abnormally high relative to New Brunswick (NB), New Hampshire (NH), Vermont (VT) and Ontario (ON). The data presented in Chapter 2 suggest that 'normal' deterioration of moose incisors with age occurs at a rate consistent with a slope of 0.2-0.3 ΔTooth condition/ΔAge. Within the CBI moose population, the slope of the line is approximately 0.54 ΔTooth condition/ΔAge. It has been suggested that cropping efficiency may be compromised when incisor integrity is compromised (Young and Marty 1986; Hindelang and Peterson 1994). Therefore, it was predicted that a decrease in incisor integrity (i.e., increased attrition) would negatively affect survivorship within moose populations.

Static survivorship curves were derived for each jurisdiction from hunter killed data to test whether jurisdictions with low tooth integrity among older cohorts display lower survivorship as age increases. There was no correlation between a decrease in incisor integrity and survivorship, suggesting moose are able to maintain sufficient food intake to meet daily energy requirements regardless of incisor condition. However, moose densities are higher in CBI and NL (see Table 2.4), and appears to be correlated with breakage incidence. Adding support is the increasing incidence of tooth breakage along a south-north gradient of within CBI. The correlation is further supported by the findings of Smith (1992), who suggested that breakage may be a result of high moose densities. However, it was not known how high moose densities cause the rapid deterioration of incisors observed during this study.

One possibility is that an excess/deficient diet of various elements might affect tooth condition. Moose inhabiting areas of higher breakage incidence exhibited higher concentrations of MgO, Sr, Na_2O, SiO_2, MnO, Zn, Ga and Pb, and lower concentrations of B, Co, Cu, Rb, Sn and Ba within the enamel. Bivariate regressions of incisal depth against each element and age suggested that Pb is the only element exerting a significant ($P<0.001$) effect (negative). Lead toxicity has been shown to displace elements (Ca for example) from binding sites, interfering with function, such as bone and teeth calcification (Reichlmayr-Lais and Kirchgessner 1984). Therefore, there is support for the hypothesis that mineral concentrations within hydroxyapatite will exert an effect on structural integrity of moose teeth.

The source of the lead within CBI and NL is most likely a result of atmospheric deposition from anthropogenic sources. The long range transport of Pb from anthropogenic emissions has been well documented, with particles travelling up to 1600km. A potential source of Pb locally is the Belledune lead smelter in northeastern New Brunswick, which is directly northeast and east of CBI and NL respectively. Prevailing winds in both CBI and NL could potentially carry Pb particles into these regions (Phillips 1990). However, investigation of natural "background" levels of Pb (Shotyk et al. 2005) in CBI and NL, and investigation of Pb isotopes may shed light as to the source of the Pb, which may support/refute the argument for an anthropogenic source of Pb.

It is plausible that breakage incidence is positively correlated with moose densities, which has been demonstrated along the south-north gradient of breakage incidence within CBI, and the relatively high densities observed within NL and Alaska. A possible mechanism is that moose at higher densities are over utilising resources, which may reduce browse quality and quantity (Basquill and Thompson 1997; Renecker and Schwartz 1997; Moen et al. 1998; Persson et al. 2000; Edenius et al. 2002). As a result moose may be altering their foraging behaviour increasing the consumption of ground vegetation, such as grasses, which may contain a higher content Pb, and even soil and grit (silica). The high SiO_2 concentration within enamel of moose with high breakage incidence further supports this argument. Increased ingestion of Silica rich soil and grasses has been demonstrated to act as an abrasive agent, resulting in excessive tooth wear for grazing livestock and moose (Peterson et al. 1982; Young and Marty 1986; Underwood and Suttle 1999).

Moose inhabit areas surrounding the Belledune smelter, but exhibit a lower breakage incidence (0.03), relative to CBI and NL (see chapter 2). Densities are also much lower in NB (0.32 moose/km^2; Dwayne Sabine, personal communication) relative to CBI or NL, adding further support that densities may exacerbate the problem of excessive/deficient element intake and ultimately incisor breakage.

With a positive correlation between breakage incidence and density demonstrated in Chapter 2, and support for an excess/deficient intake of elements resulting in compromised tooth integrity, it is plausible that excessive/deficient element concentrations are being exacerbated by high moose densities. It has been demonstrated that moose populations occurring at high densities commonly over utilise food resources, reducing browse quality and quantity (Basquill and Thompson 1997; Renecker and Schwartz 1997; Moen et al. 1998; Persson et al. 2000; Edenius et al. 2002). It is possible moose inhabiting areas of high density are altering their foraging behaviour due to changing food resources, increasing the consumption of plants containing a higher content of soil and grit (SiO_2 and Pb).

In spite of no correlation between tooth condition and moose population age structure, populations exhibiting high incidence of breakage must continue to be monitored. Higher breakage incidence negatively correlates with both breakage incidence and element excess/deficiency. Evidence from the Alaska moose populations, declining since the documentation of breakage, suggests that tooth breakage may be a possible warning sign that the population has exceeded the carrying capacity.

The breakage occurring along the North-South gradient of CBI should be examined further. Further understanding of how density may affect diet is important, and any study must be complemented by NL and NB teeth, in particular moose surrounding the Belledune smelter Investigation of natural "background" levels of Pb (Shotyk et al. 2005) in CBI and NL, and investigation of Pb isotopes may shed light as to the source of the Pb, which may support/refute the argument for an anthropogenic source of Pb.

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Appendix A: Average concentrations observed within Durango apatite during the course of this study. Mean, SD, Min, Max and relative standard deviation are presented.

	Durango				
	Mean	**SD**	**Minimum**	**Maximum**	**RSD**
^{11}B (ppm)	13.05	1.93	9.45	17	29.95%
^{23}Na^{2}O (wt%)	0.2288	0.0272	0.176	0.304	9.38%
^{26}MgO (wt%)	0.0271	0.0036	0.0081	0.0313	12.68%
^{29}SiO^{2} (wt%)	0.4478	0.0324	0.31	0.512	7.18%
^{44}CaO (wt%)	55.07	0.6800	50.7	56.3	3.06%
^{51}V (ppm)	56.74	2.479	52.5	65	3.27%
^{55}MnO (wt%)	0.0128	0.0003	0.0122	0.0141	2.25%
^{59}Co (ppm)	4.114	2.040	1.09	9.93	133.95%
^{63}Cu (ppm)	0.9357	0.3058	0.431	1.76	222.39%
^{64}Zn (ppm)	5.249	1.063	2.35	8.43	30.48%
^{69}Ga (ppm)	2.803	2.538	0.803	9.41	143.29%
^{75}As (ppm)	1191.4	50.8141	1030	1310	4.66%
^{85}Rb (ppm)	0.2427	0.1211	0.0882	0.68	56.54%
^{88}Sr (ppm)	512.9	7.7363	486	539	1.28%
^{111}Cd (ppm)	0.5494	0.3208	0.174	1.85	355.39%
^{118}Sn (ppm)	1.0765	0.7283	0.307	2.89	44.62%
^{137}Ba (ppm)	1.8708	0.2333	1.25	2.4	13.49%
^{208}Pb (ppm)	0.8619	0.0790	0.742	1.11	7.40%
^{232}Th(ppm)	266.4	14.92	229	301	5.62%
^{238}U (ppm)	12.42	0.2967	11.7	12.9	1.45%

Appendix B: Average run detection limit for the elements throughout the course of data collection. Also presented is the average relative standard deviation observed during the course of this study

Run Detection for unknowns						
	n	Mean	SD	Minimum	Maximum	RSD
^{11}B (ppm)	48	0.4577	0.6785	0.1660	5.1240	19.50%
$^{23}Na^2O$ (wt%)	48	0.0001	0.0005	0	0.0030	27.18%
^{26}MgO (wt%)	48	0.0003	0.0005	0	0.0020	20.74%
$^{29}SiO^2$ (wt%)	48	0.0045	0.0062	0.0010	0.0460	21.80%
^{44}CaO (wt%)	48	0.0044	0.0035	0.0010	0.0180	16.36%
^{51}V (ppm)	48	0.0315	0.0263	0.0170	0.2090	19.19%
^{55}MnO (wt%)	48	0	0	0	0	18.17%
^{59}Co (ppm)	48	0.7171	0.7052	0.2530	4.3290	36.01%
^{63}Cu (ppm)	48	0.2538	0.3687	0.1050	2.7890	24.36%
^{64}Zn (ppm)	48	0.3550	0.2296	0.0950	1.2920	20.25%
^{69}Ga (ppm)	48	0.1495	0.2035	0.0450	1.4820	8.387%
^{75}As (ppm)	48	0.1528	0.0556	0.0160	0.3700	22.38%
^{85}Rb (ppm)	48	0.0372	0.0415	0.0110	0.3090	22.27%
^{88}Sr (ppm)	48	0.1392	0.0830	0.0300	0.4490	17.26%
^{111}Cd (ppm)	48	0.1635	0.3708	0.0410	2.7430	22.86%
^{118}Sn (ppm)	48	0.0828	0.1364	0.0320	1.0160	143.2%
^{137}Ba (ppm)	48	0.2495	0.2570	0.0440	1.1680	19.91%
^{208}Pb (ppm)	48	0.0224	0.0410	0.0050	0.2950	21.59%
^{232}Th(ppm)	48	0.0037	0.0020	0.0010	0.0080	19.34%
^{238}U (ppm)	48	0.0030	0.0035	0	0.0210	20.61%

Appendix C: Mean and SD concentrations for the outer and inner matrices of enamel of the 20 elements for NB. Two sample T-test were conducted to test significant differences ($P<0.0025$ after bonferonni correction) between the means.

		^{11}B (ppm)					^{23}Na^{2}O (wt%)					^{26}MgO (wt%)					^{29}SiO^{2} (wt%)					^{44}CaO (wt%)				
		Outer		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	15	7.373	2.374	4.659	1.182	0.0005	0.689	0.105	0.803	0.131	0.0011	0.308	0.052	0.326	0.067	0.1857	0.026	0.016	0.028	0.017	0.3266	48.858	0.656	49.017	0.43	0.1979
2.5	17	12.08	20.135	4.993	2.329	0.0748	0.689	0.085	0.795	0.128	0.3188	0.321	0.055	0.33	0.061	0.0105	0.039	0.048	0.026	0.015	0.1767	49.288	0.664	48.943	0.368	0.5069
3.5	17	6.697	2.792	4.349	1.186	P<0.001	0.719	0.116	0.81	0.099	0.0000	0.323	0.063	0.329	0.056	0.8253	0.028	0.016	0.026	0.014	0.3175	49.523	1.565	48.955	0.431	0.2087
4.5	17	7.571	3.724	4.887	1.77	0.0003	0.667	0.059	0.756	0.041	0.0003	0.316	0.048	0.312	0.04	0.3775	0.032	0.014	0.031	0.013	0.3771	49.107	0.397	49.104	0.205	0.5717
5.5	16	10.688	9.896	6.111	5.319	0.2238	0.663	0.059	0.764	0.054	P<0.001	0.341	0.048	0.339	0.047	0.6574	0.02	0.013	0.018	0.01	0.1263	49.091	0.411	49.07	0.188	0.6926
6.5	15	7.027	3.765	4.546	1.185	0.0276	0.691	0.069	0.79	0.056	P<0.001	0.338	0.057	0.345	0.08	0.8706	0.027	0.009	0.021	0.012	0.4985	49.058	0.563	49.113	0.257	0.1490
7.5	5	6.452	1.533	6.545	4.401	0.5034	0.708	0.063	0.78	0.013	0.0067	0.317	0.039	0.317	0.037	0.1732	0.036	0.014	0.032	0.013	0.5335	49.016	0.302	49.19	0.286	0.6252
8.5	9	9.826	3.965	6.246	1.336	0.0616	0.671	0.054	0.753	0.043	0.0010	0.303	0.046	0.353	0.112	0.6103	0.034	0.016	0.028	0.015	0.6323	49.133	0.273	49.02	0.241	0.0607
9.5	11	7.244	2.464	4.197	1.101	0.3618	0.669	0.047	0.76	0.021	0.0188	0.329	0.054	0.329	0.057	0.3322	0.029	0.017	0.027	0.015	0.8150	49.264	0.317	49.058	0.18	0.3667
10.5	3	6.378	0.328	4.347	1.363	0.7446	0.665	0.026	0.743	0.019	0.0156	0.411	0.069	0.381	0.078	0.1611	0.029	0.008	0.028	0.008	0.2951	49.434	0.237	49.053	0.43	0.9640

		^{51}V (ppm)					^{55}MnO (wt%)					^{59}Co (ppm)					^{63}Cu (ppm)					^{61}Zn (ppm)				
		Outer		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*			*Outer*		*Inner*		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	15	0.051	0.048	0.061	0.042	0.3317	0.003	0.003	0.002	0.002	0.0181	9.968	20.512	15.305	25.599	0.1358	1.019	0.933	1.606	0.897	0.8970	79.61	40.729	18.508	5.333	P<0.001
2.5	17	0.029	0.039	0.042	0.043	0.1871	0.01	0.017	0.003	0.005	0.0056	3.237	6.426	8.137	12.762	0.3233	1.77	1.19	1.98	0.95	0.4715	90.367	38.655	19.008	6.032	P<0.001
3.5	17	0.047	0.045	0.051	0.046	0.7978	0.005	0.008	0.002	0.002	0.0014	4.144	12.2	8.145	18.776	0.2221	1.634	1.31	1.751	1.022	0.3669	74.6	32.987	18.795	4.824	P<0.001
4.5	17	0.053	0.078	0.064	0.041	0.3378	0.005	0.004	0.002	0.002	0.0052	1.326	2.385	1.942	2.955	0.6279	2.437	1.485	2.281	1.612	0.7125	63.888	37.347	17.151	3.677	P<0.001
5.5	16	0.032	0.042	0.068	0.04	0.4199	0.006	0.008	0.003	0.003	0.0491	4.43	8.263	5.689	10.467	0.1674	1.102	0.998	1.426	0.989	0.0049	56.092	33.667	16.475	2.865	P<0.001
6.5	15	0.049	0.039	0.046	0.032	0.8569	0.003	0.004	0.001	0.001	0.0785	2.018	5.822	2.349	4.618	0.2392	1.705	0.932	1.738	0.762	0.1749	61.66	29.864	16.923	3.329	P<0.001
7.5	5	0.076	0.056	0.067	0.036	0.3540	0.003	0.002	0.001	0	0.1778	0.978	0.399	1.721	1.107	0.2426	3.208	1.311	2.788	0.584	0.1755	56.683	34.89	19.301	2.045	0.0454
8.5	9	0.06	0.1	0.027	0.032	0.9649	0.004	0.003	0.002	0.002	0.6988	0.841	1.4	4.099	6.677	0.3743	1.674	1.154	2.031	1.248	0.5595	65.9	48.537	21.672	9.833	P<0.001
9.5	11	0.049	0.05	0.049	0.064	0.5328	0.004	0.003	0.002	0.002	0.3720	0.734	0.782	2.185	3.648	0.1789	1.676	1.153	1.813	1.09	0.1529	58.911	41.268	15.67	6.096	P<0.001
10.5	3	0.03	0.052	0.042	0.039	0.2040	0.011	0.015	0.005	0.006	0.4438	0.215	0.373	0.387	0.67	0.7531	1.739	0.63	2.491	1.87	0.2108	54.322	43.111	20.174	11.612	0.0174

		^{69}Ga (ppm)					^{75}As (ppm)					^{85}Rb (ppm)					^{88}Sr (ppm)					^{111}Cd (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	15	10.686	7.225	11.435	7.276	0.1958	0.471	0.408	0.581	0.425	0.1539	0.58	0.205	0.63	0.283	0.0026	234.96	87.039	246.57	89.555	0.0018	0.008	0.029	0.048	0.161	0.4054
2.5	17	11.733	8.851	11.45	7.824	0.6697	0.552	0.344	0.573	0.239	0.6712	0.721	0.309	0.766	0.43	0.1606	217.15	80.971	232.26	82.684	0.8532	0.033	0.075	0.125	0.362	0.3382
3.5	17	12.593	6.454	11.843	5.23	0.0001	0.48	0.364	0.53	0.361	0.3464	0.65	0.217	0.662	0.279	P<0.001	261.22	99.04	266.3	97.781	0.0025	0.157	0.252	0.144	0.225	0.6216
4.5	17	14.555	8.421	14.675	8.302	0.0006	1.057	1.555	0.552	0.205	0.3347	0.566	0.176	0.582	0.192	0.0004	206.45	57.509	220.17	61.194	0.3376	0.075	0.127	0.073	0.085	0.0567
5.5	16	11.918	9.474	10.836	9.241	0.2073	0.548	0.265	0.756	0.725	0.1535	0.676	0.173	0.691	0.174	0.2313	225.46	74.038	236.82	74.833	0.0124	0.168	0.4	0.115	0.228	0.7747
6.5	15	10.255	6.538	10.299	7.361	0.0477	0.741	0.263	0.919	0.745	0.0141	0.698	0.244	0.678	0.22	0.0011	201.74	61.899	213.43	66.823	0.0787	0.071	0.168	0	0	0.1249
7.5	5	10.941	7.657	11.557	9.514	0.1883	0.874	0.331	3.805	7.105	0.6480	0.735	0.297	0.706	0.284	0.2958	212.49	78.977	234.01	101.54	0.2887	0.057	0.127	0.023	0.052	0.4153
8.5	9	11.573	8.867	12.118	8.032	0.0415	0.692	0.7	0.561	0.308	0.7663	0.605	0.263	0.605	0.208	0.0207	215.64	73.182	235.47	78.996	0.9069	0.154	0.388	0.095	0.193	0.3506
9.5	11	9.914	5.016	10.144	4.682	0.2890	0.932	1.025	0.525	0.294	0.0275	0.711	0.261	0.709	0.254	0.1620	201.84	104.88	215.55	112.13	0.2002	0.02	0.065	0.03	0.072	0.1293
10.5	3	13.537	6.42	14.732	9.007	0.2043	0.33	0.427	0.284	0.263	0.1892	0.425	0.246	0.426	0.24	0.1915	286.2	172.47	293.49	187.32	0.3191	0.72	0.955	0	0	1.0000

		^{118}Sn (ppm)					^{137}Ba (ppm)					^{208}Pb (ppm)					^{232}Th(ppm)					^{238}U (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	15	0.682	0.297	0.616	0.21	P<0.001	175.8	101.34	174.78	98.919	0.0311	0.375	0.585	0.411	0.515	0.0005	0	0	0.002	0.007	0.7899	0	0.001	0.001	0.003	0.3120
2.5	17	0.648	0.331	0.563	0.273	0.0212	167.01	96.252	158.5	86.07	0.0514	0.943	1.177	0.726	0.882	0.1833	0	0	0	0	0.1391	0.004	0.01	0.001	0.005	0.3123
3.5	17	0.626	0.244	0.584	0.234	P<0.001	197.29	79.566	184.64	73.713	0.0009	0.849	1.043	0.726	0.873	0.0354	0	0	0	0	1.0000	0.003	0.01	0.006	0.015	0.1167
4.5	17	0.727	0.252	0.69	0.185	P<0.001	183.94	85.77	185.4	85.845	0.0003	0.687	0.817	0.634	0.67	0.0171	0	0	0	0.002	0.3343	0	0.001	0.001	0.003	0.3301
5.5	16	0.67	0.231	0.634	0.243	P<0.001	188.28	108.03	177.94	96.158	0.0054	0.608	0.929	0.597	0.785	0.0670	0	0	0.002	0.005	0.3310	0.01	0.027	0.013	0.03	0.8901
6.5	15	0.75	0.343	0.698	0.352	0.0233	136.24	72.856	139.88	77.979	0.0757	0.57	0.744	0.493	0.62	0.1408	0	0.002	0	0.001	0.4241	0.002	0.01	0.005	0.008	0.3398
7.5	5	0.746	0.239	0.714	0.268	0.8528	143.29	87.833	148	105.06	0.1941	0.443	0.221	0.407	0.186	0.4134	0	0	0	0	1.0000	0.001	0.002	0	0	0.3739
8.5	9	0.634	0.178	0.605	0.204	0.5836	160.34	90.6	168.86	91.157	0.0723	0.692	1.059	0.537	0.658	0.6102	0.001	0.004	0.001	0.003	0.3506	0.001	0.002	0.003	0.007	0.3506
9.5	11	0.612	0.25	0.6	0.136	0.2641	140.31	71.078	138.6	68.92	0.0319	1.02	1.471	0.896	1.257	0.2928	0	0	0	0	1.0000	0.003	0.008	0.002	0.005	0.2081
10.5	3	0.798	0.262	0.66	0.086	0.0204	211.71	53.012	216.56	94.84	0.1147	1.589	1.745	1.091	1	0.2302	0	0	0	0	1.0000	0	0	0	0	0.4226

Appendix D: Mean and SD concentrations for the outer and inner matrices of enamel of the 20 elements for CBI. Two sample T-test were conducted to test significant differences ($P<0.0025$ after bonferonni correction) between the means.

		^{11}B (ppm)					$^{23}Na^2O$ (wt%)					^{26}MgO (wt%)					$^{29}SIO^2$ (wt%)					^{44}CaO (wt%)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	18	13.563	6.280	5.948	1.77	0.0848	0.64	0.034	0.752	0.039	0.0231	0.433	0.125	0.415	0.092	0.0000	0.032	0.021	0.026	0.015	0.1622	49.1	0.38	49.122	0.208	0.6541
2.5	25	9.215	5.011	5.593	3.402	0.3971	0.652	0.03	0.769	0.033	0.0675	0.445	0.28	0.401	0.132	0.1317	0.028	0.013	0.025	0.011	0.7791	49.061	0.365	49.122	0.269	0.5506
3.5	29	11.602	6.608	5.594	2.006	0.4021	0.673	0.096	0.782	0.076	0.0000	0.487	0.483	0.379	0.123	0.2459	0.028	0.014	0.025	0.012	0.1704	49.19	0.729	49.171	0.279	0.0016
4.5	26	10.029	6.198	5.175	2.152	0.1808	0.666	0.06	0.78	0.069	0.0011	0.404	0.101	0.411	0.126	0.0389	0.029	0.013	0.025	0.01	0.7510	49.005	0.526	49.152	0.235	0.0006
5.5	11	9.502	4.445	5.037	1.725	0.0003	0.664	0.058	0.765	0.035	0.0000	0.428	0.111	0.374	0.056	0.0418	0.039	0.013	0.048	0.034	0.3125	49.16	0.336	49.056	0.198	0.6867
6.5	12	7.847	3.891	4.547	1.406	0.0030	0.702	0.104	0.813	0.084	0.0001	0.361	0.127	0.361	0.106	0.5068	0.028	0.019	0.03	0.021	0.6940	49.401	0.8	49.194	0.271	0.5180
7.5	4	14.742	4.944	6.624	2.461	0.4500	0.637	0.047	0.74	0.028	0.0057	0.347	0.077	0.398	0.113	0.2177	0.039	0.022	0.033	0.009	0.4931	49.287	0.213	49.206	0.126	0.9222
8.5	5	15.400	7.846	8.003	3.274	0.4587	0.654	0.036	0.751	0.042	0.0174	0.346	0.069	0.355	0.064	0.0481	0.029	0.006	0.037	0.008	0.7565	49.166	0.365	49.163	0.205	0.2565
9.5	5	9.867	5.018	5.253	2.707	0.5759	0.673	0.04	0.772	0.023	0.9907	0.365	0.092	0.339	0.044	0.6871	0.136	0.236	0.029	0.009	0.2994	49.075	0.379	49.066	0.119	0.5149
10.5	6	10.743	7.726	4.965	1.826	0.7042	0.655	0.068	0.769	0.055	0.0832	0.351	0.043	0.377	0.059	0.2351	0.028	0.011	0.029	0.009	0.7139	48.992	0.465	49.308	0.254	0.1251

		^{51}V (ppm)					^{55}MnO (wt%)					^{59}Co (ppm)					^{63}Cu (ppm)					^{61}Zn (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	18	0.028	0.043	0.041	0.041	0.3794	0.003	0.001	0.001	0	0.0313	1.983	2.545	5.357	8.434	0.0343	1.014	1.104	1.188	1.069	0.0638	98.183	40.371	18.14	5.569	P<0.001
2.5	25	0.051	0.065	0.052	0.048	0.7971	0.002	0.002	0.001	0.001	0.0036	2.493	4.027	3	2.806	0.0017	1.446	1.039	1.58	0.867	0.0974	103.36	24.287	19.495	4.821	P<0.001
3.5	29	0.047	0.059	0.05	0.034	0.0559	0.004	0.005	0.001	0.001	0.0154	2.385	4.621	6.118	14.407	0.1488	1.922	1.212	2.016	1.083	0.0046	105.56	29.911	22.367	6.056	P<0.001
4.5	26	0.041	0.069	0.054	0.036	0.4976	0.002	0.002	0.001	0.002	0.0683	3.439	8.915	7.648	15.279	0.3439	1.554	1.06	1.718	0.912	P<0.001	105.67	29.351	22.128	5.997	P<0.001
5.5	11	0.026	0.032	0.043	0.041	0.4857	0.003	0.004	0.001	0.001	0.0640	0.516	0.742	1.995	1.539	0.0064	1.969	1.231	2.351	1.508	0.6784	112.17	44.025	25.432	10.556	P<0.001
6.5	12	0.06	0.108	0.084	0.052	0.0046	0.002	0.002	0.001	0.001	0.0591	1.906	3.104	4.592	9.107	0.1815	1.876	0.835	2.244	0.635	0.0665	92.896	29.866	22.639	3.476	P<0.001
7.5	4	0	0	0.021	0.024	0.2776	0.003	0.003	0.003	0.005	0.9364	0	0	6.561	9.235	0.2816	1.001	0.69	1.856	0.689	0.0311	109.09	32.952	23.246	4.684	0.0128
8.5	5	0.025	0.035	0.033	0.056	0.3825	0.002	0.002	0	0.001	0.0288	0.392	0.876	0.574	1.284	0.5055	1.57	1.052	1.441	0.843	0.0288	77.271	29.831	20.477	5.237	0.0181
9.5	5	0.084	0.097	0.051	0.027	0.3763	0.013	0.025	0.001	0.001	0.5003	1.577	2.159	1.554	2.599	0.3648	1.281	0.232	1.538	0.299	0.0007	26.75	3.8814	18.814	2.581	0.0067
10.5	6	0.011	0.027	0.042	0.065	0.9627	0.003	0.003	0.001	0.001	0.0408	1.347	2.895	8.278	11.98	0.1595	1.891	0.797	1.774	0.821	0.0094	48.957	27.945	21.204	3.39	0.0591

		^{118}Sn (ppm)					^{137}Ba (ppm)					^{208}Pb (ppm)					^{232}Th(ppm)					^{238}U (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	18	1.644	0.947	1.215	0.54	0.0003	71.853	27.232	66.727	25.908	0.0059	1.401	1.688	1.037	1.535	0.0888	0.004	0.016	0.001	0.002	0.1653	0.022	0.058	0.007	0.013	0.7367
2.5	25	0.969	0.734	0.855	0.465	0.0320	75.248	24.192	73.949	23.391	0.0181	1.255	1.892	0.946	1.397	1.0000	0.006	0.02	0.002	0.007	0.1801	0.002	0.005	0.006	0.013	0.9374
3.5	29	0.796	0.704	0.642	0.297	P<0.001	78.26	24.803	76.456	26.611	P<0.001	2.053	2.525	1.35	1.658	0.0792	0.004	0.016	0.002	0.009	0.3322	0.021	0.052	0.006	0.016	0.8954
4.5	26	0.747	0.446	0.68	0.337	0.4314	78.707	41.857	75.726	35.036	P<0.001	2.305	2.628	1.722	1.937	0.0003	0.003	0.013	0.002	0.006	0.1893	0.006	0.022	0.011	0.03	0.8906
5.5	11	1.067	1.21	0.643	0.35	0.0438	84.708	35.201	79.311	31.086	0.0009	0.984	0.886	0.739	0.598	0.0167	0	0	0	0	1.0000	0.008	0.023	0.002	0.004	0.7778
6.5	12	0.508	0.156	0.528	0.171	P<0.001	79.384	28.676	73.54	25.168	0.0002	1.65	2.702	1.214	1.501	0.0607	0	0	0.002	0.005	0.2706	0.001	0.002	0.001	0.003	0.4386
7.5	4	1.112	0.371	1.068	0.419	0.1187	99.882	35.471	103.88	51.805	0.0639	3.534	3.625	2.933	3.008	0.1875	0	0	0.008	0.014	0.3381	0.001	0.003	0.002	0.002	0.1817
8.5	5	0.789	0.206	0.568	0.175	0.7380	79.808	17.814	74.809	18.996	0.0624	2.574	4.731	1.341	2.121	0.3536	0	0	0.003	0.006	0.3739	0.003	0.004	0	0	1.0000
9.5	5	0.734	0.905	0.689	0.571	0.6614	71.818	37.286	57.154	16.918	0.0085	1.159	0.357	0.964	0.541	0.0938	0.03	0.064	0.004	0.009	0.3739	0.003	0.004	0.008	0.012	0.2198
10.5	6	0.722	0.393	0.753	0.351	0.4723	84.44	30.129	95.712	50.858	0.0526	2.26	2.74	1.512	1.625	0.1694	0.001	0.003	0.008	0.015	0.2628	0.001	0.003	0	0	0.3632

		^{69}Ga (ppm)					^{75}As (ppm)					^{85}Rb (ppm)					^{88}Sr (ppm)					^{111}Cd (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	18	3.673	3.126	3.895	2.947	0.8860	0.4	0.381	0.396	0.26	0.6898	0.548	0.195	0.569	0.19	0.0021	204.87	54.509	211.33	53.225	0.0107	0.055	0.114	0.04	0.07	0.8739
2.5	25	5.131	2.21	5.111	2.257	0.4435	0.542	0.43	0.558	0.297	0.9303	0.551	0.22	0.547	0.197	0.9306	193.1	41.293	206.55	44.957	0.2134	0.092	0.204	0.089	0.176	0.0808
3.5	29	15.42	8.85	5.522	2.822	0.0000	0.52	0.361	0.508	0.21	0.9232	0.477	0.246	0.46	0.201	0.3814	200.2	36.082	217.41	44.784	0.0761	0.153	0.352	0.143	0.198	0.1166
4.5	26	5.43	2.884	5.171	2.973	0.1670	0.545	0.492	0.505	0.244	0.4827	0.559	0.198	0.566	0.195	0.0267	209.75	59.706	220.18	66.019	0.8952	0.09	0.135	0.087	0.111	0.7067
5.5	11	6.144	2.77	5.794	2.967	0.0005	0.866	1.022	0.5	0.28	0.2478	0.514	0.198	0.458	0.11	0.0578	202.27	48.583	216.26	47.267	0.6627	0.352	0.945	0.116	0.14	0.0574
6.5	12	6.381	2.217	5.9	2.169	0.0002	0.787	0.494	0.683	0.234	0.2389	0.642	0.383	0.619	0.346	0.6105	201.38	46.013	210.67	52.294	0.5084	0.034	0.081	0.095	0.137	0.6110
7.5	4	3.223	2.734	4.374	2.437	0.0276	0.353	0.309	0.463	0.2	0.0878	0.472	0.167	0.47	0.119	0.0212	241.79	69.661	263.76	84.574	0.7650	0	0	0.075	0.151	0.4876
8.5	5	5.523	1.838	5.434	1.532	0.1098	0.574	0.215	0.501	0.212	0.0934	0.498	0.19	0.458	0.138	0.4430	211.34	24.829	221.81	27.783	0.0693	0.087	0.195	0.063	0.09	0.5668
9.5	5	4.612	2.481	3.759	1.831	0.0366	0.517	0.37	0.515	0.33	0.2735	0.613	0.435	0.618	0.375	0.6473	195.78	51.033	198.27	46.75	0.0218	0.112	0.172	0	0	0.1843
10.5	6	6.167	1.61	6.783	2.301	0.0405	0.573	0.309	0.566	0.228	0.8754	0.496	0.172	0.491	0.146	0.3100	221.71	43.179	261.2	75.809	0.4830	0.024	0.059	0.035	0.086	0.3632

Appendix E: Mean and SD concentrations for the outer and inner matrices of enamel of the 20 elements for VT. Two sample T-test were conducted to test significant differences ($P<0.0025$ after bonferonni correction) between the means.

		^{11}B (ppm)					$^{23}Na^2O$ (wt%)					^{26}MgO (wt%)					$^{29}SiO^2$ (wt%)					^{44}CaO (wt%)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	14	6.349	2.51	5.334	1.499	0.0198	0.7	0.112	0.841	0.14	0.0011	0.318	0.102	0.348	0.063	0.1082	0.029	0.007	0.03	0.013	0.7674	49.17	0.394	49.171	0.194	0.5682
2.5	12	6.099	1.991	5.147	1.854	0.0029	0.665	0.101	0.804	0.111	0.0396	0.311	0.056	0.328	0.054	0.0024	0.029	0.006	0.027	0.006	0.2929	49.254	0.403	49.138	0.239	0.9369
3.5	11	6.651	2.584	5.897	1.655	0.0038	0.667	0.049	0.788	0.047	0.0026	0.325	0.057	0.343	0.045	0.4489	0.034	0.01	0.031	0.011	0.3996	49.201	0.37	49.209	0.252	0.2290
4.5	16	6.602	2.892	5.325	1.246	0.0065	0.692	0.109	0.803	0.089	0.0000	0.361	0.052	0.362	0.047	0.0035	0.028	0.012	0.028	0.012	0.0028	49.084	0.478	49.152	0.218	0.0472
5.5	10	5.214	2.255	7.448	7.991	0.6056	0.638	0.187	0.752	0.209	0.2038	0.318	0.099	0.312	0.095	0.2870	0.029	0.014	0.025	0.015	0.1658	45.436	11.803	45.557	11.861	0.3339
6.5	15	6.963	2.275	6.099	1.542	0.0468	0.68	0.095	0.805	0.117	0.0011	0.351	0.076	0.371	0.055	0.1334	0.025	0.009	0.028	0.011	0.3847	49.55	1.51	49.15	0.114	0.2950
7.5	15	7.265	2.254	7.609	4.049	0.5383	0.678	0.092	0.807	0.118	0.0004	0.363	0.073	0.379	0.059	0.8612	0.027	0.009	0.028	0.016	0.2126	49.174	0.262	49.117	0.228	0.7542
8.5	12	6.648	1.618	6.076	1.327	0.0987	0.647	0.062	0.755	0.071	0.0001	0.332	0.072	0.34	0.057	0.6300	0.031	0.008	0.027	0.007	0.0778	49.237	0.355	49.05	0.222	0.2487
9.5	9	6.982	1.555	6.759	1.415	0.3459	0.648	0.053	0.764	0.051	0.1142	0.333	0.027	0.37	0.071	0.7854	0.018	0.014	0.022	0.008	0.9535	49.214	0.398	49.208	0.347	0.3556
10.5	10	7.41	1.842	6.991	1.67	0.0085	0.633	0.053	0.736	0.042	0.0013	0.358	0.069	0.368	0.034	0.3117	0.054	0.082	0.024	0.008	0.3356	48.932	0.331	49.291	0.317	0.2527

		^{51}V (ppm)					^{55}MnO (wt%)					^{59}Co (ppm)					^{63}Cu (ppm)					^{51}Zn (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	14	0.037	0.055	0.073	0.042	0.0320	0.01	0.024	0.001	0	0.0805	20.607	51.512	25.043	59.369	0.2016	2.437	2.349	2.808	1.936	0.0384	130.04	55.007	25.225	8.525	P<0.001
2.5	12	0.048	0.054	0.063	0.051	0.5313	0.006	0.008	0.001	0.001	0.0418	2.706	5.088	10.209	10.609	0.5339	1.63	0.919	1.92	1.038	0.3827	116	33.298	20.401	4.073	P<0.001
3.5	11	0.012	0.039	0.038	0.03	0.8522	0.005	0.006	0.001	0.001	0.1502	0.828	1.842	6.712	12.176	0.5549	1.55	2.468	1.821	1.922	0.3507	107.78	31.435	21.468	10.069	P<0.001
4.5	16	0.022	0.034	0.05	0.059	0.4837	0.004	0.005	0.001	0.001	0.1512	3.558	9.364	6.922	14.9	0.1515	1.563	1.436	2.047	1.691	0.7811	96.087	24.463	19.434	6.567	P<0.001
5.5	10	0.084	0.227	0.046	0.051	0.1950	0.004	0.005	0.002	0.002	0.7952	4.021	6.511	8.487	12.428	0.0816	0.87	0.911	1.177	1.044	0.0334	70.957	35.591	15.915	6.303	P<0.001
6.5	15	0.03	0.058	0.03	0.044	0.0498	0.006	0.008	0.001	0.001	0.0252	1.677	2.131	5.457	6.984	0.0648	1.277	0.819	1.749	1.012	0.9053	93.929	37.989	19.454	4.463	P<0.001
7.5	15	0.033	0.06	0.052	0.049	0.6175	0.008	0.008	0.001	0.001	0.0283	1.935	3.071	4.734	4.591	0.0085	1.405	1.141	1.765	1.28	0.5364	87.727	35.003	18.928	7.114	P<0.001
8.5	12	0.057	0.057	0.082	0.074	0.7828	0.016	0.028	0.004	0.006	0.1666	5.474	14.269	14.544	22.939	0.0580	1.735	2.235	2.458	1.722	0.2974	81.959	20.468	19.515	5.51	P<0.001
9.5	9	0.035	0.042	0.036	0.045	0.7828	0.009	0.008	0.002	0.003	0.5782	1.013	1.439	9.418	17.798	0.3585	1.416	1.555	2.206	1.574	0.6210	80.449	35.887	17.393	5.549	0.0068
10.5	10	0.159	0.363	0.067	0.076	0.2750	0.01	0.011	0.001	0.001	0.1524	3.088	2.984	5.599	4.312	0.0154	2.365	1.954	2.816	1.795	0.0479	89.445	54.451	21.989	7.667	P<0.001

		^{69}Ga (ppm)					^{75}As (ppm)					^{85}Rb (ppm)					^{88}Sr (ppm)					^{111}Cd (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	14	9.289	6.407	6.921	3.404	0.0263	0.333	0.307	0.479	0.188	0.2324	0.892	0.205	0.767	0.18	0.0026	167.93	43.275	173.52	50.271	0.6900	0.083	0.216	0.075	0.127	0.4021
2.5	12	5.375	3.572	6.571	2.286	0.0667	0.449	0.309	0.54	0.232	0.1899	0.75	0.315	0.728	0.254	0.0666	196.81	46.932	209.49	52.66	0.6813	0.015	0.053	0.18	0.217	0.9694
3.5	11	4.59	2.125	4.637	2.048	0.6319	0.422	0.394	0.931	1.481	0.3677	0.9	0.204	0.877	0.203	0.0002	158.92	28.024	167.13	22.915	0.0112	0.014	0.047	0.119	0.27	0.5493
4.5	16	6.137	3.312	5.878	3.361	0.2693	0.351	0.227	0.27	0.216	0.1600	1.159	0.355	1.063	0.291	P<0.001	168.25	31.432	181.76	34.156	0.5560	0.252	0.711	0.153	0.223	0.3627
5.5	10	6.217	4.592	6.216	3.559	0.5891	0.545	0.446	0.601	0.363	0.9940	0.881	0.321	0.79	0.322	0.7706	171.3	67.793	176	65.601	0.1152	0.093	0.154	0.175	0.269	0.0996
6.5	15	5.602	2.801	6.311	3.744	0.7811	0.361	0.375	1.515	4.215	0.5553	1.286	0.493	1.14	0.468	0.0002	169.3	37.199	179.23	47.94	0.0592	0.075	0.188	0.113	0.152	0.7125
7.5	15	7.478	2.86	6.961	2.435	0.0243	0.487	0.336	0.378	0.276	0.1222	1.247	0.478	1.155	0.449	0.0001	187.82	43.173	202.39	42.871	0.3250	0.077	0.176	0.055	0.118	0.9769
8.5	12	5.633	3.449	4.905	2.772	0.9015	0.57	0.377	0.58	0.304	0.2209	1.073	0.336	0.968	0.286	0.0000	172.41	58.648	180.48	59.338	0.7829	0.14	0.369	0.096	0.202	0.4396
9.5	9	6.189	2.671	7.138	3.322	0.3753	0.472	0.352	0.491	0.255	0.9453	1.11	0.365	1.011	0.335	0.0122	177.89	47.816	188.52	45.386	0.3986	0.117	0.173	0.053	0.106	0.3435
10.5	10	5.752	2.947	6.709	3.662	0.7662	1.243	2.78	0.525	0.36	0.5999	2.772	4.72	1.069	0.371	0.0081	192.91	36.336	207.04	44.27	0.4404	0.117	0.308	0.062	0.131	0.3552

		^{118}Sn (ppm)					^{137}Ba (ppm)					^{208}Pb (ppm)					^{232}Th(ppm)					^{238}U (ppm)				
		Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner			Outer		Inner		
	n	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value	Mean	SD	Mean	SD	P-value
1.5	14	2.102	1.704	1.5	1.072	0.0705	108.1	50.007	93.893	27.1	0.1559	1.376	1.855	0.837	0.895	0.0003	0	0	0.002	0.006	0.5026	0.007	0.024	0.002	0.005	0.2137
2.5	12	1.726	1.552	1.238	0.902	0.1203	98.181	30.632	92.693	29.459	0.5561	1.14	1.815	0.754	1.082	P<0.001	0	0	0.002	0.007	0.4738	0.002	0.005	0.002	0.004	0.3717
3.5	11	2.027	2.022	1.416	1.051	0.0484	77.948	18.816	72.249	18.511	0.4863	1.286	1.621	0.869	1.159	0.0145	0	0	0.001	0.002	0.1913	0.001	0.002	0.001	0.001	0.5556
4.5	16	1.253	1.121	0.938	0.649	0.0432	80.944	39.494	77.487	43.021	0.2260	1.247	1.353	0.925	1.018	0.0084	0.002	0.006	0.004	0.016	0.3357	0.008	0.022	0.003	0.006	0.5248
5.5	10	1.254	1.194	1.056	1.342	0.2181	87.707	43.368	81.95	35.277	0.7809	0.644	0.61	0.481	0.71	0.0111	0	0	0.001	0.003	0.3434	0.004	0.012	0.001	0.002	0.5963
6.5	15	2.02	1.888	1.416	0.961	0.0009	97.359	38.067	95.349	54.043	0.6735	1.353	1.332	0.829	0.848	0.6434	0	0	0.002	0.004	0.2981	0.011	0.023	0.005	0.01	0.5643
7.5	15	2.111	1.344	1.35	0.779	0.0006	109.27	39.915	99.99	33.5	0.0046	1.544	1.263	0.935	0.809	0.2133	0	0	0.001	0.002	0.3343	0.079	0.245	0.004	0.012	0.4970
8.5	12	1.585	1.431	1.142	0.943	0.2619	87.414	26.918	80.803	23.854	0.1798	0.839	1.074	0.679	0.724	0.3165	0	0	0.004	0.01	0.1950	0	0	0.001	0.003	0.2889
9.5	9	1.908	1.679	1.246	1.11	0.4571	89.581	29.988	85.4	28.275	0.1793	1.453	1.811	0.751	0.905	0.3231	0	0	0	0	0.9938	0.054	0.152	0.002	0.003	0.9709
10.5	10	2.638	1.721	1.76	1.142	0.7788	100.77	33.681	98.072	40.012	0.8707	1.06	0.785	0.742	0.332	0.2562	0.001	0.004	0.002	0.004	0.1093	0.017	0.029	0.014	0.02	0.6569