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THE SPATIAL DISTRIBUTION OF MOOSE (*ALCES ALCES*) INCISOR BREAKAGE IN ATLANTIC CANADA AND AN EVALUATION OF TOOTH CHEMICAL COMPOSITION FOR INFERENTIAL PURPOSES

By: Cynthia S. Kendall MacKenzie

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Approved:	Dr. Hugh Broders Supervisor Department of Biology
Approved:	Dr. Tamara Franz-Odendaal External Examiner Department of Biology Mount Saint Vincent University
Approved:	Dr. Ron Russell Supervisory Committee Member Department of Biology
Approved:	Dr. Marcos Zentilli Supervisory Committee Member Earth Sciences, Dalhousie University
Approved:	Dr. Jason Clyburne Supervisory Committee Member Department of Chemistry and Environmental Studies Program
Approved:	Dr. Genlou Sun Program Representative
Approved:	Dr. Pawan Lingras Graduate Studies Representative
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ABSTRACT

Mammalian teeth are used to obtain and consume food resources and, in some cases, for self-defence. As such, tooth quality is important for individuals to maintain body condition and meet daily nutritive requirements. This study investigates unexplained tooth breakage in the moose (*Alces alces*) populations of Atlantic Canada. By way of comparison of >5500 incisors from multiple North American moose jurisdictions, we found breakage frequency ranged from 1-6% except in Atlantic Canada (Cape Breton 6-34% and Newfoundland 24-47%). Population age structure effects were not detected. To investigate whether moose incisor elemental composition is related to high breakage in Atlantic Canada, elemental analyses and microhardness tests were performed on a subset of samples. Though tooth chemistry did not explain all of the existing variation, a negative relationship was found between Cu, Pb, Zn and breakage. Tooth microhardness did not significantly differ among regions. Other environment factors, such as density-related food resource declines, likely contribute to tooth breakage in Atlantic Canadian moose.

August 12, 2010

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This study was an ideal project in bringing together my many interests and enabling me to develop my skills as a researcher and scientist in ecology and environmental chemistry. For providing me with the chance to do so, I thank Dr. Hugh Broders for this wonderful opportunity and also for giving me the freedom to explore my interests, and, of course, for the support he provided throughout the entire process. Michael Clough was also instrumental in developing many of the methods that I used and providing assistance throughout the project, which was greatly appreciated. As well, the members of my supervisory committee, Dr. Russell, Dr. Zentilli, and Dr. Clyburne, were extremely helpful and provided assistance at several stages of this project, without hesitation.

I am also very grateful for the contributions of teeth and associated data from the Departments of Naturals Resources and Wildlife Divisions from each of Nova Scotia, Newfoundland and Labrador, and New Brunswick, and in particular to Barry Adams of Newfoundland whose determination to help me with data was greatly appreciated.

Funding support from the following agencies enabled this project, for which I am grateful; the Nova Scotia Department of Natural Resources, Cape Breton Highlands National Park, Newfoundland and Labrador Department of Environment and Conservation-Wildlife Division, the New Brunswick Wildlife Trust Fund, the Canadian Wildlife Federation, Saint Mary's University, and the Natural Science and Engineering Research Council of Canada (NSERC).

Finally, I am greatly indebted to my fellow lab mates, family, and my husband, Luke, for the unwavering support that they each provided throughout the duration of this project. Not only did they proofread reports, help with spreadsheets, or sit with me during some long hours in the laser lab; they encouraged me, provided me with helpful feedback, and ultimately listened to me talk about moose teeth for almost two years. To everyone who has assisted and supported me throughout this project, thank you.

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CHAPTER 1

THE SPATIAL DISTRIBUTION OF MOOSE INCISOR BREAKAGE IN ATLANTIC CANADA AND AN EVALUATION OF TOOTH CHEMICAL COMPOSITION FOR INFERENTIAL PURPOSES: AN INTRODUCTION

Mammalian dentition has evolved to aid in self-defence, prey capture, and for the handling and processing of food (Orr, 1961). Differences in diets and lifestyles of vertebrates have resulted in varied tooth forms (Romer, 1962; Peyer, 1968; Popowics & Fortelius, 1997). Carnivores have prominent canines used for defence and piercing the flesh of prey while herbivores have incisors designed for clipping and cropping vegetation and molars designed for the grinding of tough cellulose-rich plant material.

Mammalian teeth are designed to last an individual throughout the natural lifetime (Janis & Fortelius, 1988; Young & Marty, 1986; Hindelang & Peterson, 1994) and are influenced by such factors as age and diet (Solounias *et al.*, 1994; Fenton *et al.*, 1998). Herbivore cheek teeth often become worn by mastication; carnivoran teeth often become broken or chipped through conflict and/or contact with bone material of prey animals. Any individual may also incur unexpected stresses, such as falls, that reduce the quality of teeth (Van Valkenburgh, 1988). Therefore, tooth quality may change throughout an individual's lifetime, through regular use or from unexpected trauma. The ability to maintain chewing and masticatory efficiency depends highly upon tooth condition as impaired condition can lead to senescence and is thus highly related to selection pressures (Van Valkenburgh, 1988; Fenton *et al.*, 1998; Patterson *et al.*, 2003).

The microstructure and elemental composition of teeth, however, is not typically dynamic through an individual's life. Teeth are composed of two closely related, yet very different, matrices; enamel and dentine (Figure 1.1). Enamel, the outer shell of the tooth structure, once formed, is not subject to metabolic processes and remains relatively unchanged throughout life with the exception of the outermost surface (Cutress, 1983).

The surface area of the tooth remains in constant equilibrium with the oral environment throughout an individual's lifetime and may exchange and accumulate ions at the interface (Brudevold & Soremark, 1967; Curzon & Cutress, 1983). The inner portion of the tooth, the dentine, is less organized and thus more stochastic as a result of poor mineralization which increases susceptibility to impurities (Trautz, 1967; Zimmerman, 1976). The main constituent of both enamel and dentine is hydroxyapatite. Enamel hydroxyapatite is the hardest calcified tissue in the body with a higher mineral to collagen ratio than dentine or bone (Lazzari, 1976; Sharaway & Yaeger, 1991). The hardness of enamel may, however, render it brittle and breakage can occur. Tooth breakage is often seen in carnivores where frequent tooth to bone contact occurs and less frequently in herbivores because tooth are not expected to incur unexpected stress (Van Valkenburgh, 1988).

In Cape Breton, Nova Scotia, however, moose (*Alces alces*) incisor teeth were frequently broken upon submission of the lower jawbone of successful moose kills to wildlife officials, as required by provincial hunting regulations. The unusual occurrence of incisor tooth breakage in this large herbivorous species had previously only been reported in the Alaskan moose population of the Seward Peninsula and had been attributed to high moose densities (Smith, 1992; Stimmelmayr *et al.*, 2006).

Moose (*Alces alces*), as generalist browsers, consume leafy and woody plants and shrubs (Renecker & Schwartz, 1997; Schwartz & Renecker, 1997). Food is cropped between the bottom incisors and a tough, keratinous pad on the upper lip (Bubenik, 1997). Moose, at birth, have functional deciduous incisors which are replaced at approximately 6 months of age when the first permanent incisors erupt; full permanent dentition is attained by month 16 to 19 (Franzmann, 1981). The permanent incisors are used for cropping forage throughout the duration of the individual's lifetime, typically 10-15 years, sometimes up to 20 years (Van Ballenberghe & Ballard, 1997). As the lower incisors are an integral part of cropping food, extensive tooth breakage may be detrimental to an individual's ability to obtain browse and foliage, especially in the winter when browse is often frozen. The molar dentition of moose is known to exhibit excessive wearing with age, decreasing browsing and chewing efficiency and possibly limiting life span (Bubenik, 1997; Ericsson & Wallin, 2001), however, the possible effects of decreased incisor condition in moose are not well documented.

A study was undertaken by Michael Clough, in the form of an MSc research thesis, to examine the Cape Breton population more closely. As historical data were unavailable, teeth were compared to moose teeth from other regions. Clough found that the typical frequency of moose incisor breakage ranged from 1 to 6% (Clough, 2007). The frequency of breakage in Cape Breton was, in fact, elevated, increasing along a geographic gradient from 6% in the south to 34% in the north. Elevated frequencies of breakage were also found to occur in the moose population of Newfoundland with up to 47% of moose incisors examined from northern regions exhibiting breakage.

To further investigate the possible effects of an herbivore with defective incisors, Clough explored the hypothesis that age structure effects should be seen in moose populations with elevated frequencies of breakage. Also, Clough hypothesized that a relationship between tooth quality and elemental composition was present. Using laser ablation microprobe inductively-coupled-plasma mass spectrometry (LAM-ICPMS), Clough collected data regarding the elemental composition of moose incisor enamel for multiple specimens. Clough concluded that a relationship between lead, Pb, and enamel composition existed and recommended further investigation (Clough, 2007).

In view of the preliminary findings by Clough, further study was initiated of which this thesis is a result. The Natural Resource Departments of New Brunswick and Nova Scotia and the Newfoundland Department of Environment and Conservation-Wildlife Division provided additional moose incisor samples (a combined total of 4500) to be used for further investigation of the occurrence of moose incisor breakage in Atlantic Canada. In this thesis, data has been pooled with Clough to create a more robust study regarding the frequency of incisor breakage, the potential age structure effects which may be associated with breakage in a population, and the relationship between tooth elemental composition and tooth integrity. In addition, this thesis explores the hypothesis that tooth microstructure, in terms of hardness, may be an indicator of tooth integrity and provide further insight into the condition of moose teeth in Atlantic Canada.

The goal of this thesis is to further characterize the occurrence of deteriorating moose incisor tooth condition in Atlantic Canada and to determine whether the tooth microstructure is related to increased breakage. Specifically, in Chapter 2, I characterize the frequency of cracking, wear, and breakage in moose incisors from Atlantic Canadian regions to enable comparison with other regions in North America. As well, I examine whether population age structures differ in regions of frequent breakage. Secondly, in Chapter 3, I explore the possibility that the composition of teeth, in terms of chemical composition and microhardness, may differ among regions possibly enabling inference regarding possible factors related to tooth breakage. It is expected that the findings of this study and recommendations for future research will be used by provincial wildlife officials in jurisdictions of increased incisal breakage to create an awareness of issues which may impact future species management decisions. Annual moose harvests are important to local human populations, tourism operators and tourists, and provide a means of sustenance for many families across Atlantic Canada with longstanding traditions of participating in the yearly hunt. Abnormal conditions affecting such animals, whether deleterious to overall health condition, should be recognized by wildlife officials and information available to general public. This thesis characterizes the condition of increased frequencies of incisor breakage, makes inferences regarding possible related factors, and through the publication of findings will enable the dissemination of information to interested parties.

AUTHOR STATEMENT

The format of this thesis will be based upon the anticipated submission of two manuscripts for peer-reviewed publication. The first manuscript, Chapter 2, explores the spatial distribution of moose incisor breakage in North America and the potential for age structure effects. This manuscript, entitled "The spatial variation of extreme tooth breakage in an herbivore and potential age structure effects" was submitted (March 2010) to the journal *Annales Zoologici Fennici,* and subsequently revised and accepted (May 2010) for publication. It is included in this thesis with the permission of the Finnish

Zoological and Botanical Publishing Board. A second manuscript, which examines whether the chemical composition of moose incisors can explain unusual breakage patterns in Atlantic Canada, is presented in Chapter 3. It is anticipated that this manuscript will also be submitted for publication.

I, Cynthia S. Kendall MacKenzie, have completely written both manuscripts included herein as Chapter 2 and Chapter 3 and have completely prepared all figures and tables, with the exception of the photographs used to create Figure 2.1 as they were taken by Michael Clough. Prior to the submission of Chapter 2 for publication and the future submission of Chapter 3, each co-author of the paper did or will have the opportunity to edit, revise, or make suggestions for improvement of the final document. As such, Michael Clough and Hugh Broders have each contributed to revising and editing the second chapter of this thesis as well as two anonymous reviewers chosen by the publisher. The published manuscript was based on data collected and analyzed by both Michael Clough (3300 samples) and myself (an additional 4200 samples). Of these 7500 samples that were collected, approximately 2000 exhibited post-mortem damage and were thus excluded from further analyses. The remaining 5500 teeth were used in the analyses described in Chapter 2. As the groundwork, i.e. developing the methods of tooth characterization to be used in the study, was performed by Michael Clough, it was suitable that he assume lead authorship of the subsequent publication.

The manuscript presented in Chapter 3 of this thesis is intended to be published under the lead authorship of myself, Cynthia S. Kendall MacKenzie. Michael Clough contributed to the development of the methods for chemical analysis and analyzed approximately 500 samples. His data was pooled with the 800 samples that were analyzed by me with a refined methodology; slide preparation techniques were slightly altered and a fewer total number of elemental isotopes were determined. The addition of microhardness tests and the methods of statistical data treatment were developed solely by me and, as such, it is more appropriate that this chapter be submitted for publishing with myself assuming lead authorship.

The appendices provide summary results for chemical analyses performed by myself, rather than pooled with analyses by Michael Clough. Results from analyses performed by Michael Clough are presented elsewhere (Clough, 2007), and furthermore, each set of analyses were performed in different years thus it is not appropriate to combine data regarding quality control and reference materials or limits of detection. As such, the summary of elemental composition presented in Appendix F refers only to analyses by me to maintain consistency with the remainder of the appendices but summary data presented in Table 3.2 is pooled with Clough as both datasets were used in the development of the manuscript presented in Chapter 3 and all analyses therein.

As well, each document is intended as a stand-alone document, therefore, repetition occurs. An overall conclusion, Chapter 4, provides a synthesis of the findings of this study and that of Clough with the aim of making recommendations for future, complementary, research to investigate elevated frequencies of moose incisor breakage in Atlantic Canadian moose populations.

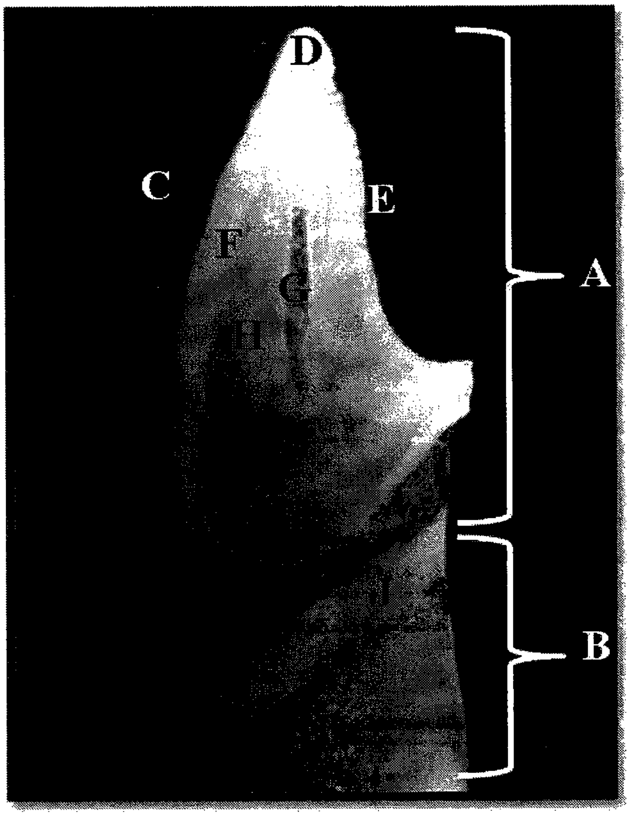

Figure 1.1. Cross-sectional view of a moose incisor tooth. Parts of the tooth are as labeled: A) crown, B) root, C) labial surface, D) incisal (occlusal) surface/edge, E) lingual surface, F) enamel, G) pulpal cavity, H) dentine, I) enamel-dentine junction, J) enamel-root junction.

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CHAPTER 2

THE SPATIAL VARIATION OF EXTREME TOOTH BREAKAGE IN AN HERBIVORE AND POTENTIAL AGE STRUCTURE EFFECTS

ABSTRACT

Teeth are essential in mammals for the capture, handling and processing of food, and self-defence. The rate of deterioration may affect longevity and indicate certain environmental conditions. The goal of this study was to characterize tooth conditions of moose (*Alces alces*) from multiple regions and to make inferences of possible causes of variation. An assessment of >5500 moose incisors found that the frequency of breakage and rate of decline in incisor integrity with age was much higher in Cape Breton and Newfoundland (breakage from 6 to 47%) than in New Brunswick, Ontario, New Hampshire, Vermont, and Yukon (breakage from 1 to 6%). Incisal degradation differed significantly, among jurisdictions, though population age structures did not appear different. The two jurisdictions most affected by incisal deterioration, Cape Breton and Newfoundland, are inhabited by genetically distinct subspecies at higher densities than other regions; therefore, breakage may be linked to local environmental conditions.

KEYWORDS: age structure, Atlantic Canada, density, moose, tooth breakage

INTRODUCTION

Teeth have evolved for the purposes of self-defence, capturing prey, and for the handling and processing of food (Orr, 1961). Tooth form varies among vertebrates because of differences in feeding adaptations (Romer, 1962; Peyer, 1968; Popowics & Fortelius, 1997). In mammals, teeth are classified as incisors, canines, premolars, or molars. Incisors, being in the front of the mouth, are used by herbivores for clipping and gnawing vegetation. Carnivores possess highly developed canine teeth used for securing prey and tearing flesh (Orr, 1961). Premolars and molars, collectively termed cheek teeth, display extensive variation in number and shape because of differing food habits (Hildebrand & Goslow, 2001).

The ability to acquire food resources and, in some cases, defend against predators is highly dependent upon the presence and condition of teeth; therefore, tooth condition is associated with animal senescence and there is strong selection pressure on teeth to withstand normal wear and tear (Van Valkenburgh, 1988, 2009; Fenton *et al.*, 1998; Patterson *et al.*, 2003). Teeth must maintain foraging and chewing efficiency over the expected lifespan of the species (Janis & Fortelius, 1988; Young & Marty, 1986; Hindelang & Peterson, 1994). The condition of teeth is influenced by factors such as diet and age (Solounias *et al.*, 1994; Fenton *et al.*, 1998) and tooth wear is, therefore, useful for aging purposes in many species (Fancy, 1980; Hindelang & Peterson, 1994). Rate of deterioration of teeth varies by species, tooth type, gender, and by geographical location due to dietary influence and major and trace element availability (Young & Marty, 1986; Van Valkenburgh, 1988, 2009; Bibby & Losee, 1970; Curzon & Cutress, 1983).

Tooth breakage is distinct from wear because the tooth may or may not remain effective and may also occur as a result of multiple environmental factors such as diet and usage patterns. However, it is generally accepted that tooth breakage occurs more frequently in carnivores, especially predators that consume bone material (Van Valkenburgh, 1988). Plant material consumption does not place as much stress on teeth, thus herbivores have a much lower potential for tooth breakage (Van Valkenburgh, 1988).

In Cape Breton, Nova Scotia, a high frequency of incisor tooth breakage has been observed in moose (*A.a.andersoni*) (Clough *et al.*, 2006). Though incisor breakage has been documented in Alaskan moose (*A. a. gigas*) for nearly two decades (Smith, 1992; Stimmelmayr *et al.*, 2006), this was the first report of breakage outside of Alaska and the first report in the *A.a.andersoni* subspecies of moose.

Moose (*Alces alces*) are generalist browsers that consume leafy plants and aquatic vegetation in the summer and woody plants and shrubs in the winter (Renecker & Schwartz, 1997; Schwartz & Renecker, 1997). To aid in food selection, moose have a narrow muzzle, a prehensile tongue, and lips (Renecker & Schwartz, 1997). Moose consume browse by placing it into their mouth with their prehensile tongue and cropping it between the bottom incisors and a tough, keratinous pad on the upper lip (Bubenik, 1997). Moose have the dental formula (top /bottom jaw) of 0/3 (Incisors) + 0/1 (Canines) + 3/3 (Premolars) + 3/3 (Molars) x 2 sides for a total number of 32 teeth (Bubenik, 1997). They have brachydont dentition, meaning that the crowns of the cheek teeth are low to accommodate continuous mastication of browse and foliage, including in the winter when browse is often frozen which leads to progressive wear as the individual ages (Bubenik, 1997). Browsing and chewing efficiency decreases with excessive wearing and breakage; possibly limiting life span (Bubenik, 1997; Ericsson & Wallin, 2001).

Previous research on cervid dentition has focused on the progressive wear of molar teeth and the associated life history consequences (Skogland, 1988; Hindelang & Peterson, 1994; Bubenik, 1997; Kojola *et al.*, 1998; Ericsson & Wallin, 2001; Mysterud *et al.,* 2001; Loe *et al.*, 2003; Carranza *et al.*, 2004; Loe *et al.*, 2006; Veiberg *et al.*, 2007a; Veiberg *et al.*, 2007b; Carranza *et al.*, 2008). A consensus among many of these studies is that tooth condition is suggested to be an important determinant of longevity and senescence (Skogland, 1988; Hindelang & Peterson, 1994; Ericsson & Wallin, 2001; Loe *et al.*, 2003; Carranza *et al.*, 2004). Individuals with severely degraded incisal conditions likely incur a reduced ability to obtain sufficient forage (Skogland, 1988; Kojola *et al.*, 1998; Ericsson & Wallin, 2001), potentially impairing reproductive success (Ericsson & Wallin, 2001) and, in the most severe cases, leading to gradual malnutrition and increased mortality relative to age (Fortelius, 1985). Offspring survival may also be impacted by the inability of a reproductive female with degraded dentition to obtain sufficient food resources (King *et al.,* 2005) or to maintain sufficient fat reserves (Kojola *et al.,* 1998).

The goal of this study was to determine whether effects on survivorship are seen in Cape Breton moose as a result of an increased frequency of incisor breakage. To do so, as historical data are unavailable, we will quantitatively characterize incisor tooth breakage from several North American moose populations. We will examine whether the spatial variation in rate of deterioration of incisors with age and sex can result in significant variation in population age structures. We hypothesize that any inter-regional variation in population age structure can be explained, at least in part, by rate of deterioration of tooth condition. We assume minimal hunting pressure bias on age structure among these populations. With the exception of a male-only moose harvest regime in the Yukon, hunting strategies within the studied regions (New Brunswick, Cape Breton, Newfoundland, Ontario, New Hampshire, Vermont, and the Yukon) are similar; hunting licences may be issued for either sex or any age. The given populations are also likely subjected to minimal predatory pressure, with wolves (*Canis lupus*) being absent in all studied populations except Ontario and Yukon, and brown bears (*Ursus arctos*) present only in Yukon (T. Nette; D. Sabine; K. Rines; C. Alexander; N. Dawson, (personal communication, 2007)). Although we recognize that hunter derived data are not ideal for the compilation of population age structures, they seem appropriate for our study given the following assumptions: 1) hunting biases that may exist are similar among regions and; 2) age-specific mortality threats do not significantly differ among regions (Begon *et al.*, 1996).

Additionally, we discuss our results in relation to possible effects of deteriorated tooth condition on reproductive success as well as differences in environmental conditions among regions such as population density and food resource availability.

METHODS

Incisors from moose of known age and gender were provided by wildlife officials from Newfoundland, New Brunswick, New Hampshire, Nova Scotia, Ontario, Vermont and the Yukon Territory. The age of each animal was determined by cementum annuli analysis (Sergeant & Pimlott, 1959), performed at an external laboratory. The average moose density (moose/km^2), as observed for the 2005 hunting season, was reported for each region.

The primary incisor, the I1, was examined for breakage from all regions. Incisors, after cataloguing, were visually inspected for post-mortem damage which was distinguished by the presence of breakage not accompanied by smoothing and staining along the broken edge(s) of the enamel surface. Damaged teeth were excluded from further characterization. The condition of remaining incisors was characterized according to cracking, wear, or breakage along the occlusal surface (Figure 2.1). Teeth with cracks which began or terminated along the occlusal edge of the tooth were recorded as exhibiting cracking. The presence of wear was recorded if tooth dentine was visible along the occlusal surface of the incisor with a characteristic band of discoloration where enamel had worn down. Breakage, distinguishable from post-mortem tooth damage, exhibited rounding of edges formed from breakage as well as staining along the newly formed occlusal surface of the tooth.

To quantitatively characterize cracking among regions, the length of all cracks on each tooth was summed and the average total length taken, by region, for those teeth which were well formed (mature) and did not exhibit excessive wear or breakage (incisal depth between 6 and 8 mm). Though broken teeth were excluded from the measure, this restriction on incisal depth was employed to avoid introducing bias by measuring cracks across an incomplete occlusal surface and comparing to the sum of measures taken on a tooth with a wider, complete, occlusal surface. The length of each labial surface crack terminating along the occlusal (cropping) surface was measured with a digital caliper. Additionally, the total number of cracks present on each tooth was averaged for all teeth within each region. An incisal depth measure was developed to quantify the functional impacts on incisor integrity of the cumulative effects of wear and breakage. Incisal depth was calculated as the sum of the depth of the tooth (front to back), 2 mm below the occlusal surface, in three locations across the surface; at ⅓, ½, and ⅔ the distance across the tooth's occlusal surface (Fig 2.2). Larger values for incisal depths were associated with excessive wear and/or breakage; generally, a less effective tooth with compromised integrity and an uneven surface for cropping. Characterization of incisors was performed consistently for all teeth.

To test whether the rate of deterioration of incisal depth with age differed between genders, a test of single covariate homogeneity of slopes (Borich, 1972) was conducted for each region using incisal depth as a dependent variable, age as an independent variable, gender as a categorical classification, and an interaction term, sex*gender. If a significant interaction existed, further consideration would treat males and females independently.

To assess the spatial variation in the relationship between incisal depth and age, linear regressions were constructed for each region. The slope coefficient (β_{Age}) and associated standard error measures from each region were used in conjunction with estimates of breakage frequency to characterize the tooth condition. To formally test spatial variation, we conducted test of homogeneity of regression slopes among regions. All statistics were performed using SYSTAT (Version 11.0, Systat Software Inc., Richmond, California, USA).

The population structure among regions was qualitatively characterized with static survivorship curves constructed with age data from hunter-killed moose to assess whether there was any meaningful level of variation. Hunting behaviours in each region were assumed to be similar.

RESULTS

There was significant spatial variation in wearing and breakage in moose incisors among regions (Figure 2.3). For example, frequency of breakage within the entire sample ranged from 1% in Ontario to 47% in northern Newfoundland (Figure 2.4). Wear, occurring less often than breakage or cracking, ranged from 3-41% across populations surveyed. The frequency of tooth crack presence was high in all regions, thus the total crack length measures were more valuable in assessing regional differences. For incisors with a depth of between 6 and 8 mm, the average total length of incisor cracks ranged from 5.8 mm to 12.1 mm (Table 2.1). The average number of cracks per tooth within each region ranged from 2.2 to 3.8 (Table 2.1). While there was a weak positive association ($R^2 = 0.107$) between average total crack length and frequency of breakage among regions, the association was stronger for total number of cracks and frequency of breakage within regions ($R^2 = 0.457$).

The homogeneity of slopes test including the gender*age interaction term indicated that incisal depth with age did not significantly depend upon the gender interaction ($p>0.05$, in all regions); therefore, further analysis considered males and females together in each region.

In New Brunswick, New Hampshire, Ontario, and Vermont, breakage ranged from 1 to 6% and the slope of regression lines of incisal depth with age ranged from 0.21 to 0.29, averaging 0.25 (0.05 SE). The regions with increased frequencies of breakage, northern Cape Breton and Newfoundland, are grouped together in a plot of all regression lines (Figure 5). The slopes of regression lines for these regions ranged from 0.40 to 0.56 (0.03 SE).

The overall frequency of breakage in New Brunswick was 2%; however, in the northwest area of the province (management zones 1 and 2), a breakage frequency of 11% was present in the teeth examined. In Cape Breton, a south to north gradient of increasing tooth deterioration was present when each moose management unit (MMU) in the region was considered separately. The slope of the regression lines ranged from 0.29 (0.05 SE) in the south to 0.51 (0.04 SE) in the north, corresponding to an increase in breakage from 6% to 34% in the north. The slope of the relationship between incisal depth and age in Newfoundland ranged from 0.40 (0.03 SE) in the Avalon region (where breakage occurred in 24% of the samples) to 0.56 (0.03 SE) in the northern region of the province (breakage in 47% of moose). The test of homogeneity of slopes indicated that the regression slopes, representing the extent of deteriorated tooth condition with age, differed significantly among regions ($p < 0.001$).

Visual assessment of survivorship curves (Figure 2.6) suggest that longevity and age structure of moose among regions is similar, despite variation in inter-regional tooth condition.

DISCUSSION

There was spatial variation in the frequency of tooth breakage among the North American moose regions surveyed (Figure 2.4) that was not significantly related to cracking, wear, or gender. High levels of breakage had previously been reported in Alaskan and Cape Breton populations (Smith, 1992; Stimmelmayr, 2006; Clough, 2006). In this study, we report that high levels of incisor breakage also occur in Newfoundland moose populations. The frequencies of moose incisor breakage in Newfoundland and northern Cape Breton are much higher than all other regions examined in this study, and thus appear to be anomalous for an herbivorous species. Cracking and wear of the occlusal surface, increasing in severity from use over the individual's lifetime, appear to be naturally occurring processes; however, it is possible that cracks extending deep into the enamel layer of teeth may reduce the integrity of the tooth thus leading to breakage and an overall decrease in incisal condition. Also, the total number of cracks is more closely related to breakage than the total cracking distance on a tooth; multiple cracks may lead to an overall decrease in ability to withstand stress. Among regions, differences in food and nutrient sources may explain the earlier onset of declining incisal condition, extent of cracking, and the likelihood that cracked teeth become broken teeth. A comparison among populations of molar condition may provide insight into the differences in diet of the given populations. It may be expected that individuals with markedly worn molars may consume diets composed of foods that are unsuitable or otherwise require disproportionate mastication, while individuals obtaining nutrients from sources such as mineral licks may be expected to exhibit less wear on cheek teeth.

The age structures among regions, however, were similar despite variation in incisor condition. The proportion of hunter-killed moose in older age classes was also similar. As we assumed that hunter biases were similar among regions, we would have expected fewer older individuals in regions of extreme breakage if survivorship was affected by deteriorated incisal condition. Since this was not true, the hunter-derived data do not support the contention that deterioration of incisor condition reduces survivorship in Atlantic Canadian moose. In this study, we assumed that hunting and non-hunting mortality biases were similar among regions; it is possible that these age structures may, in fact, be influenced by such factors as hunter biases, differences in predation, fecundity, or disease prevalence among regions.

It was hypothesized that incisor breakage would exert an effect on reproductive success only where the frequency is extremely high, such as that seen on the northern peninsula of Newfoundland. Tooth anomalies have been found to exert a larger effect on female body condition than male condition (Loe *et al.*, 2006). In a healthy population, female moose fecundity has been found to decrease as the individual ages beyond 12 years (Ericsson *et al.*, 2001). Therefore, the incisal depth of a 12 year old moose in a population unaffected by elevated incisor breakage may represent the upper limits of tooth quality necessary for obtaining the nutrients required to ensure reproductive success. According to linear regressions in this study, the average female moose in northern Newfoundland has an incisal depth greater than 10 mm by the age of 7, whereas females in regions without abnormally high frequencies of breakage do not exceed this depth during their reproductive years (i.e., <12 years old). It was not found, however, that the proportion of younger individuals in regions of frequent breakage was different than the proportion found in unaffected populations. There may be multiple explanations for this; offspring survival may not be impacted by degraded incisal conditions since nutrients required for development may not be limited, effects were not detected at this time with these methods, or, possibly, younger individuals are overrepresented in the data set for affected regions as a result of their higher susceptibility to hunting; a potential result of higher movement rates (Courtois *et al.,* 1998).

Each region affected by elevated frequencies of breakage (Alaska, Cape Breton, and Newfoundland), is populated by genetically distinct moose subspecies (*A.a. gigas, A.a. andersoni,* and *A.a. Americana*, respectively). Excessive breakage was not found, in this study or reported elsewhere, to be present across the entire range of each subspecies; therefore, it appears that breakage is unlikely to be attributed to genetic factors. This suggests that environmental factors may be more important in causing the breakage patterns found in this study. Moose incisor breakage previously documented in Alaskan moose was suggested to be a result of environmental factors; more specifically, lower food quality as a result of high moose densities (Smith, 1992).

We propose two hypotheses to explain the deterioration of tooth condition in Cape Breton and Newfoundland moose that are not mutually exclusive: 1) high mechanical stress is placed upon the teeth, possibly through the consumption of larger diameter browse, or 2) the quality of diet is insufficient to enable the formation of strong teeth able to withstand normal use. It is possible that both hypotheses are related to high densities of individuals and the subsequent lack of suitable (easily obtained and nutritious) food resources.

Ungulate densities that exceed carrying capacity may lead to an increase in the consumption of food with lower nutritional quality or that is otherwise unsuitable (Vivas & Saether, 1987; Freeland & Choquenot, 1990; Renecker & Schwartz, 1997). Tooth wear effects have been documented in other ungulates as a result of diet alterations during times of density-related food limitations (Skogland, 1988; Kojola *et al.,* 1998). In the regions of elevated tooth breakage (Cape Breton and Newfoundland), higher moose densities were reported from regional wildlife officials; 2.00 and 7.00 moose per km^2, respectively. Other regions, for which density information was available, are populated by moose at lower densities: New Brunswick, 0.32; New Hampshire, 0.46; Ontario, 0.15-0.40; and Vermont, 0.61 moose per km^2. The high moose densities found in regions of declining incisal condition likely limit the availability of preferred food resources, thus forcing individuals to consume less suitable supplies, perhaps those which place higher stress upon the incisors, for sustenance.

When faced with limited food supplies in the winter, moose use lower quality browse by consuming bites of larger diameter, and often lower digestibility (Vivas &

Saether, 1987; Renecker & Hudson, 1992). This may be exacerbated when competition for food increases, especially when food sources remain frozen for long periods of time. The availability of food resources is particularly important in the winter months, as woody browse often represents the only form of forage available in areas with persistent snow cover (LeResche & Davis, 1973). In addition, individuals may be unable to extract food resources with the appropriate nutrients when tooth condition has deteriorated (Ericsson & Wallin, 2001). Moose require a range of browse species to ensure mineral requirements are met; a diet consisting of few plant species may lead to elemental deficiencies or excesses (Ohlson & Staaland, 2001). Therefore, the availability of quality browse is important for the growth and maintenance of healthy teeth.

Nutritional excesses or deficiencies, either during development or throughout the lifetime, may render the composition of moose incisors in Atlantic Canada to be less conducive to typical external stresses experienced during cropping, thus elevating susceptibility to breakage. In certain regions of Newfoundland and Cape Breton, impaired browse regeneration and limited resources have been documented to result from elevated moose densities (Mercer & McLaren, 2002; Bridgland *et al.*, 2007). Previous study of both incisor and molar wearing in moose suggests that current ecological conditions, such as food quality, are important factors in incisor wearing (Veiberg *et al.*, 2007a). Food resource quality and availability among regions likely, in part, explains some proportion of the tooth integrity variability that we have seen in this study. The regions most affected by incisor breakage are high density areas with decreasing supplies of suitable food resources.

Further study of Atlantic Canadian moose should examine incisal condition in relation to density effects, body condition, and fecundity. Additionally, further studies should study the molar condition of individuals with compromised incisor integrity. Molar teeth are used for the continuous mastication of food resources and, thus, wear is generally perceived to decrease chewing efficiency, which impacts fitness and survival (Fortelius, 1985; Kojola, 1988; Skogland, 1988; Gaillard *et al.,* 1993; Hindelang & Peterson, 1994; Ericsson & Wallin, 2001; Loe *et al.,* 2003), though this was not supported in a recent study of red deer by Carranza *et al.* (2008). The relationship between the extent of molar wear and incisor integrity may provide insight regarding diet and feeding strategy when incisors are compromised.

CONCLUSION

In summary, this study characterized the spatial distribution of degraded moose incisor condition in eastern North America in terms of cracking, wear, and breakage. Potential effects on age structure were examined, as was the relationship between incisal condition and gender. We found abnormally high frequencies of incisor breakage, most notably in moose of northern Cape Breton and throughout Newfoundland that were positively correlated with age. Gender was not significantly related to degraded incisal condition. This study is the first to report the occurrence of moose incisor breakage in Newfoundland, Canada, which is present, in northern regions, at extremely high frequencies. Though population age structures appear unaffected in the regions examined when using hunter derived data sets, the high frequencies of breakage found in this study are anomalous for an herbivore species.

Over time, greater selection pressure on the dentition in large ruminant herbivores should be experienced by populations affected by elevated rates of incisor breakage where the animals are negatively impacted by such breakage. It may be suggested, as in the case of tooth wear in another wild ruminant (Veiberg *et al.,* 2007b), that foraging conditions are more extreme in certain current habitats than where this species previously evolved. It is important to be mindful of the temporal scale of the relatively recent observation of extreme breakage in this species when making inferences to possible effects on populations in the future. A change in diet does not lead to an immediate change in enamel structure; rather, selection pressure for optimal dentition is exerted over a much longer period of evolution (von Koenigswald, 1992). As such, understanding what environmental factors, which may include density, have changed in the recent past, contributing to the observed excessive breakage, will be important for future studies of these impacted populations.

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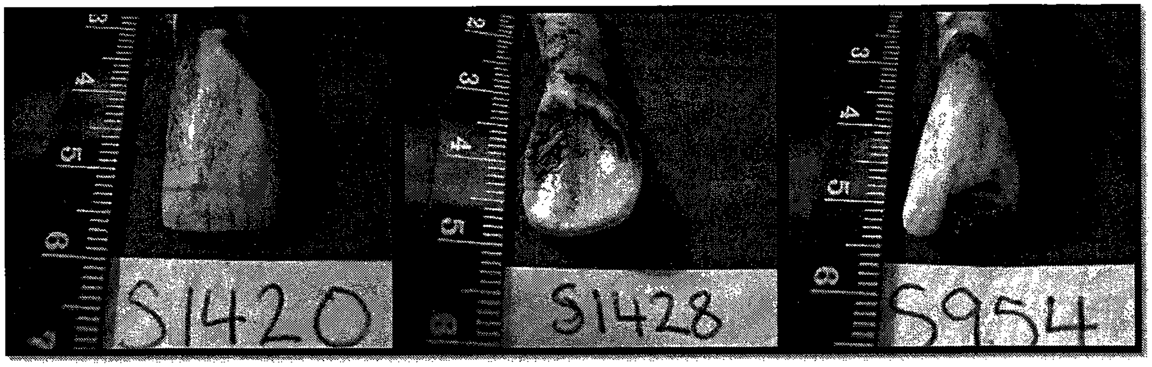

Figure 2.1 Moose incisor characterization conditions categorized by presence or absence of condition; cracking, wear, and breakage. Tooth S1420 demonstrates the presence of cracking, S1428 is worn along the occlusal surface as visible by a darker line along the worn edge, and S954 is broken.

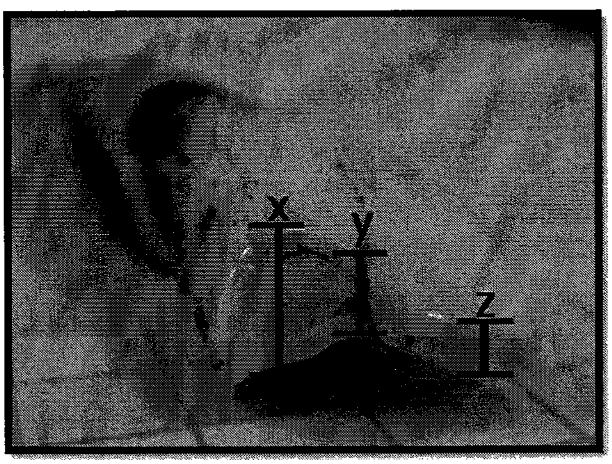

Figure 2.2 Method of incisal depth quantification for use as a proxy for moose incisor tooth condition as a function of broken and worn surfaces; x + y + z = Incisal depth (mm); measurements will be of a higher magnitude when the surface is broken, as shown in x.

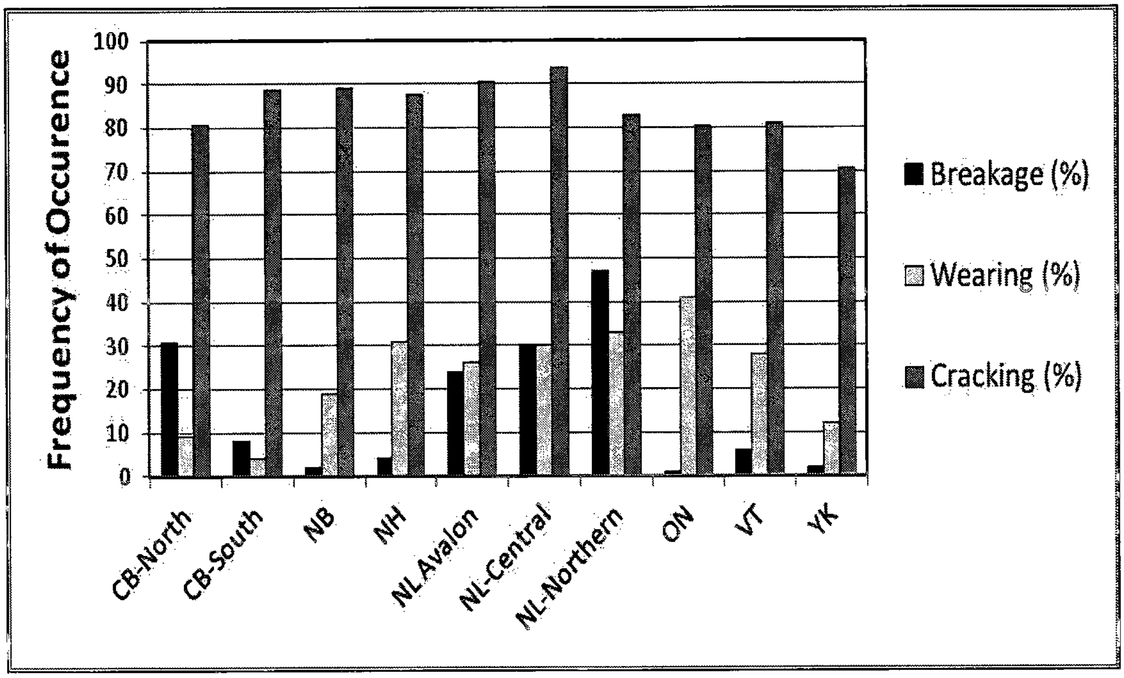

Figure 2.3 The frequency of moose incisor breakage, wearing, and cracking by region for animals harvested from 2004-2007, with all age classes and gender combined. (CB= Cape Breton, NB = New Brunswick, NH = New Hampshire, NL = Newfoundland, ON = Ontario, VT = Vermont, and YK = Yukon)

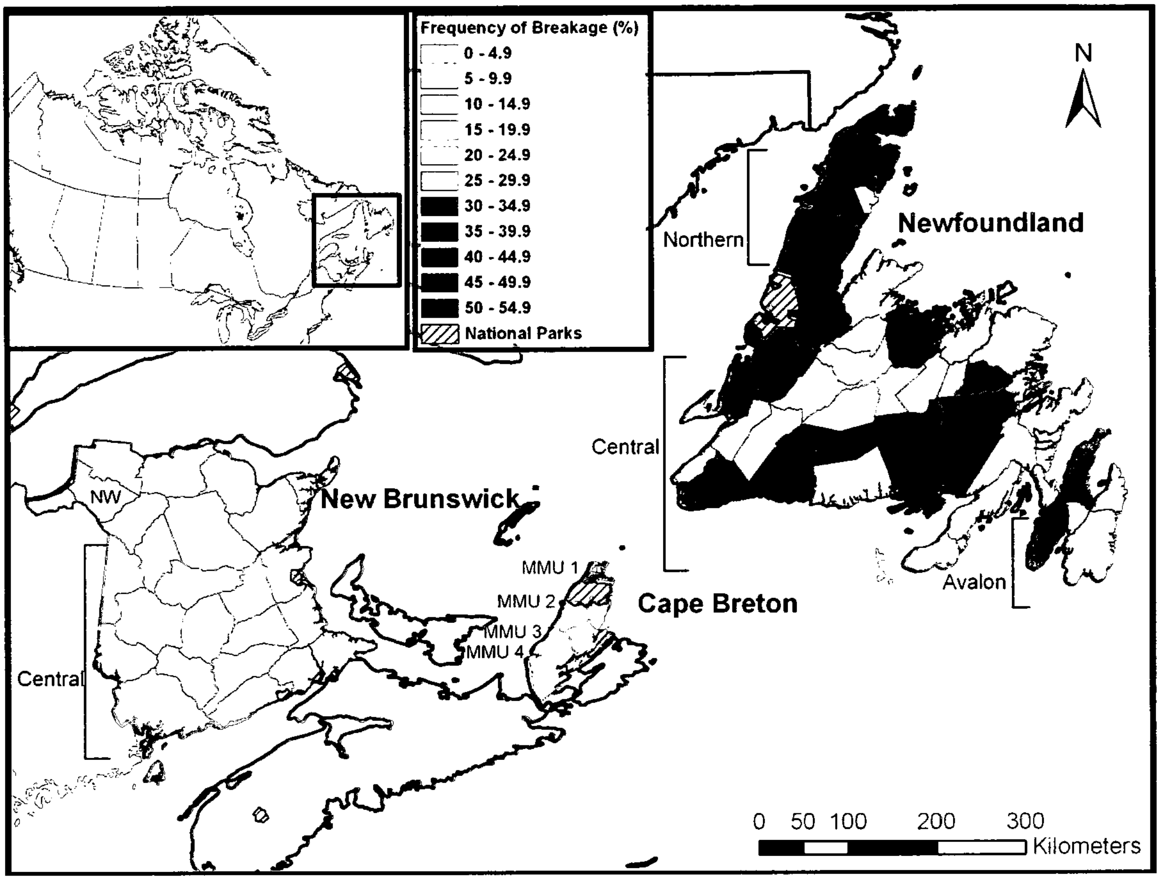

Figure 2.4 The spatial variation in the frequency of moose (*Alces alces*) incisor breakage in Atlantic Canadian regions for animals harvested from 2004-2007, with all age classes and genders combined. Each region has been subdivided by provincially maintained moose management units (MMUs) to indicate the variation of breakage within the region. Data sources: New Brunswick Department of Natural Resources, Nova Scotia Department of Natural Resources, Newfoundland Department of Environment and Conservation: Wildlife Division, and the Maritime Provinces Spatial Analysis Research Center.

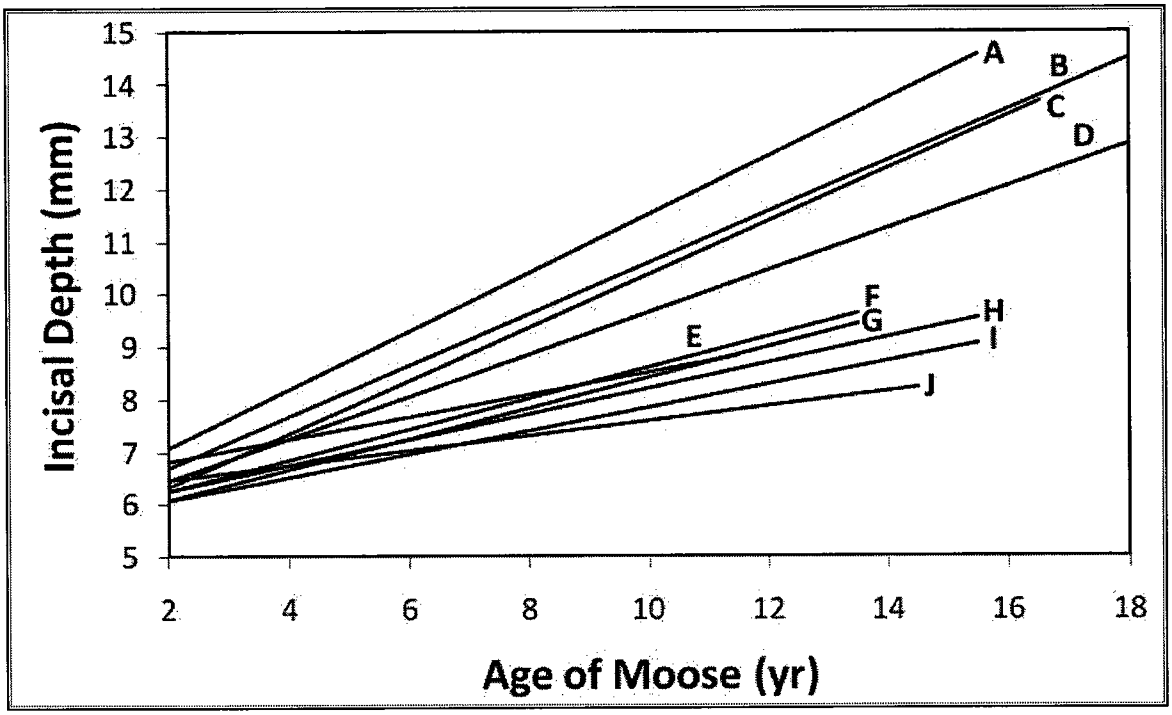

Figure 2.5 Regression lines of moose incisal depth and age, by region, where A-D are areas of increased frequencies of breakage and E-J represent regions where the frequency of moose incisor breakage is <6%. A) Newfoundland Northern, B) Newfoundland Central, C) Cape Breton North, D) Newfoundland Avalon, E) New Hampshire, F) Cape Breton South, G) Ontario, H) New Brunswick, I) Vermont J) Yukon.

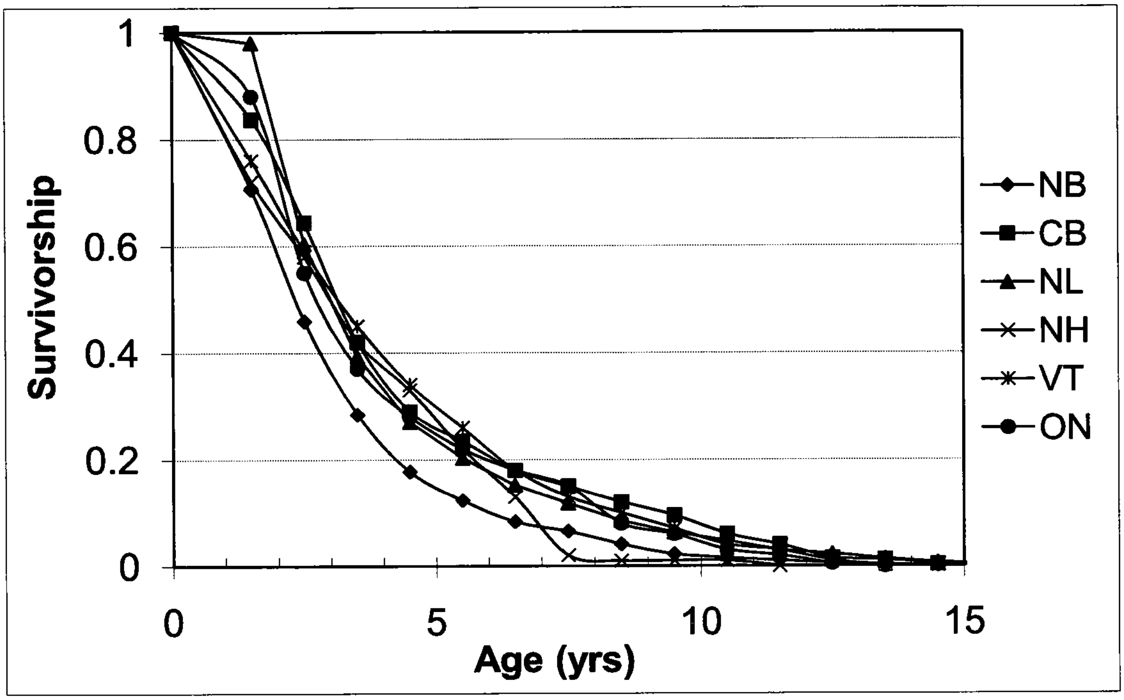

Figure 2.6 Survivorship curves of moose *(Alces alces)* populations from eastern North American regions using data acquired by age determination of hunter harvested individuals from 2004-2007.

Table 2.1 Moose (*Alces* alces) locations, sampling season(s), measures of tooth condition, and simple linear regressions results (β_{Age} and associated s.e.) of incisal depth (ID) as a function of age in multiple North American moose regions.

Region	n	Frequency of Breakage (%)	Frequency of Wearing (%)	Average Cracking Distance (mm)[1]	Average Number of Cracks	Regression Results β_{Age}	s.e.	r^2
New Brunswick								
Northwest	110	11	16	5.8	2.2	0.34	0.05	0.32
Central	2350	2	20	4.4	2.3	0.25	0.01	0.36
Cape Breton								
MMU1	288	34	12	12.1	2.7	0.51	0.04	0.42
MMU2	177	25	6	12.1	3.0	0.48	0.04	0.41
MMU3	85	9	6	9.2	2.8	0.29	0.05	0.28
MMU4	72	6	3	7.8	2.4	0.30	0.06	0.26
Newfoundland								
Avalon	350	24	26	10.4	3.8	0.40	0.03	0.39
Central	927	30	30	9.2	3.4	0.49	0.02	0.38
Northern	423	47	33	9.0	3.1	0.56	0.03	0.42
Ontario	97	1	41	7.3	2.2	0.29	0.02	0.57
New Hampshire	81	4	31	6.4	2.7	0.21	0.03	0.36
Vermont	501	6	28	6.6	2.2	0.22	0.01	0.37
Yukon	41	2	12	15.5	2.4	0.14	0.04	0.24

[1]To control for breakage-induced variation, only cracks on teeth with incisal depth between 6-8mm were summed and averaged by region.

CHAPTER 3

CHEMICAL COMPOSITION ANALYSES OF MOOSE (*ALCES ALCES*) INCISORS TO ENABLE INFERENCE REGARDING ANOMALOUS BREAKAGE IN ATLANTIC CANADA

ABSTRACT

Mammalian teeth can provide a great deal of information regarding local environmental conditions. For example, a high incidence of breakage and wear within a population may indicate that food quality is poor. Individuals consuming a diet causing high mechanical stress on the teeth or lacking the appropriate minerals for proper development could experience declines in tooth condition. Previously, we documented a high rate of tooth deterioration with age in two genetically distinct moose populations in Atlantic Canada. In this study, we test whether hardness, which is related to mineralization, varies among teeth from different regions and whether the concentration of a series of selected elements is related to deterioration of tooth condition. Hardness tests performed on incisors of the same age from New Brunswick, Cape Breton, and Newfoundland revealed no difference in the structural integrity of teeth. Laser ablation ICP-MS was used to determine the concentrations of multiple elements (^{11}B, ^{63}Cu, ^{64}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{111}Cd, ^{118}Sn, ^{137}Ba, ^{208}Pb, ^{232}Th, and ^{238}U) in the incisal teeth of moose from Cape Breton, Newfoundland, New Brunswick, New Hampshire, Ontario, Vermont, and Yukon Territory. A principal components analysis revealed that nearly 50% of the variation in the inner enamel matrix of moose teeth was explained by three groupings of elements. Regression models indicate that the elemental group that included Cu, Pb, and Zn was related to decreases in incisal integrity. Other environmental factors likely contribute to the occurrence of increased incisor breakage in affected populations. The relationship between changes in the quantity and quality of as a function of density should be further investigated.

KEYWORDS: *Alces alces*, enamel, principal component analyses, teeth

INTRODUCTION

The chemical characterization of mammalian teeth can provide a great deal of information about local environmental conditions (Bibby & Losee, 1970; Curzon, 1983). The chemical composition is determined, at least in part, by local geological conditions and the presence and concentration of certain elements and specific isotopes may be used to understand human and animal population migration patterns (Ericson, 1985; Price *et al.*, 2000; Evans, 2006; Montgomery *et al.*, 2000; Ezzo *et al.*, 1997; Grupe *et al.*, 1997). Tooth elemental composition may also reveal information such as trophic relationships (Sealy *et al.*, 1991; Skulan *et al.*, 1997; Hobson & Sease, 1998 and others) and enable the monitoring of historical heavy metal contamination (Cutress, 1983, Outridge *et al.*, 1997). Therefore, the relationship between tooth composition and lifetime exposure to elements can be a useful indicator of an individual's body burden of toxic elements and, consequently, its health status (Kinghorn *et al.*, 2008; Outridge *et al.*, 2000).

Teeth consist of two distinct mineral matrices; dentine surrounding the nerves and innermost pulpal cavity of the tooth, and enamel, which forms the outer protective layer. The enamel is formed of a highly organized crystalline lattice structure, characterized as hydroxyapatite ($Ca_3(PO_4)_5OH$) (Brudevold & Soremark, 1967; Curzon & Cutress, 1983), while the dentine layer displays much less organization (increased porosity) and is, in comparison, poorly crystallized hydroxyapatite and thus more susceptible to impurities (Zimmerman, 1976; Trautz, 1967). In enamel tissue, the elemental composition remains largely fixed at development. The isotopic composition and accumulation of heavy metals in tooth enamel reflects conditions present during development, remaining relatively static throughout life with the exception of the outer surface of the tooth (Balasse, 2002; Brudevold & Soremark, 1967; Cutress, 1983) where diffusion processes occur and the composition of the surface enamel becomes influenced by the oral environment (Cutress, 1983). Elements that tend to accumulate within the surface enamel include F, Zn, and Pb (Brudevold & Soremark, 1967). The enamel microstructure has a much higher mineral:collagen ratio than dentine, the latter more closely resembling the composition of bone and, consequently, enamel is a tougher, stronger material (Pasteris, 2008). Enamel is the hardest calcified tissue in the mammalian body (Lazzari, 1976; Sharaway & Yeager, 1991).

Despite the inherent strength of dental enamel, cracking and breakage of tooth structures may occur (Bajaj *et al.,* 2008). Tooth enamel fracture commonly occurs in carnivores where frequent tooth to bone contact occurs during the capture and consumption of prey (Van Valkenburgh, 1988). Animals may also incur tooth damage during displays of aggression, consumption of frozen or tough foods, or during unexpected stresses such as falls (Fenton *et al.*, 1998; Van Valkenburgh, 1988).

In an herbivore population a high rate of incisor tooth breakage may be considered anomalous. However, incisor tooth breakage has been previously documented in multiple moose populations of North America. Incisor breakage has been documented in Alaskan moose for nearly two decades (Smith, 1992; Stimmelmayr *et al.*, 2006) and more recently in Cape Breton, Nova Scotia (Clough *et al.*, 2006) and Newfoundland (Clough *et al*, 2010). As the three North American moose populations affected by incisor breakage represent three genetically distinct subspecies, and breakage has not been documented across the entire range of each subspecies, it is unlikely that breakage is related to genetic factors and is thus possibly related to local environmental conditions.

The goal of this study was to gain insight into the possible causes of broken teeth in moose. There are at least two potential hypotheses to explain an elevated frequency of moose incisor breakage in Atlantic Canada. The first hypothesis is that if moose have a poor diet (i.e., large diameter browse or highly abrasive grasses), which may or may not be related to density issues or nutritional distress, tooth quality may be compromised as a result of external stresses upon the tooth. Alaskan moose incisor breakage was attributed to high moose densities and a corresponding reduction in food quality (Smith, 1992). As well, Cape Breton moose incisor breakage has been documented to co-occur with behaviours such as bark stripping and osteophagia (Clough *et al.*, 2006). Barkstripping, the stripping of bark from a live tree, is considered indicative of nutritional distress (Miquelle & Van Ballenberghe, 1989; Renecker & Schwartz, 1997) while the consumption of bone or antler material, osteophagia, is considered to be a nutrient seeking behaviour in phosphorus-deficient animals. Osteophagia has been documented in both livestock (Green, 1925; Blairwest *et al.*, 1992) and wild ruminants of the deer family including elk (*Cervus elaphus*; Bowyer, 1983), wild axis deer (*Axis axis;* Barrette, 1985), and reindeer (*Rangifer tarandus;* Sutcliffe, 1973).

The second hypothesis, not mutually exclusive from the first, is that tooth tissues are defective as a result of nutritional excesses or deficiencies in the affected regions. Moose require a diverse, yet balanced, supply of food resources to meet nutritional needs

(Ohlson & Staaland, 2001). Local geochemical conditions influence the availability of elements in food and water resources (Underwood & Suttle, 1999; Maisironi, 2000; Chandrajith *et al.*, 2005; Howe *et al.*, 2005; Ljung *et al.*, 2006) which can impact overall health, including tooth condition (Bibby & Losee, 1970; Brown *et al.*, 2004; Garrott *et al.*, 2002; Curzon & Cutress, 1983). It is possible that if the tooth tissues of the permanent dentition are not formed adequately, as a result of sub-optimal diet, teeth may be physically and structurally inferior and thus more susceptible to breakage during typical wear and tear. Nutritional distress and other systemic stresses at the time of tooth development has been linked to the tooth condition, linear enamel hypoplasia (Goodman and Rose, 1990) and has been documented in the molar teeth of other ungulates (Franz-Odendaal *et al.,* 2003; Franz-Odendaal, 2004; Niven *et* al., 2004). In this study, moose incisor teeth are examined that do not visually exhibit linear enamel hypoplasia, therefore, we seek to examine whether non-visible structural properties (i.e., chemical composition) are related to increased frequencies of incisor breakage in Atlantic Canada. In addition to chemical composition, the mechanical and structural properties of a tooth are important to the ability of a tooth to function, distribute masticatory strain and are, thus, representative measures of tooth quality (Vieira, 2005). The masticatory strain distribution through the tooth affects the ability of the tooth to maintain composure; therefore, the mechanical properties of the tooth (i.e., microhardness) may allow a better understanding of strain (Kinney *et al.,* 1996). Reduced tooth hardness may result from demineralization and an increase in porosity and disorganization (Zioupos & Rogers, 2006), and is correlated with decreased tooth calcium content (Lazzari, 1976). Therefore, a comparison of the microhardness of teeth from regions of varying breakage frequencies may provide further insight regarding differences in tooth condition that could contribute to declining incisor conditions.

The intention of this study was to test the second hypothesis (defective tooth tissues) by examining whether the variation in the chemical and structural composition of moose tooth enamel can explain patterns of incisor deterioration which occur in Atlantic Canada. More specifically, I had two objectives; to examine the variability in tooth chemical composition in moose populations with different local environmental influences, and, second, to use tooth composition data to permit inference regarding increased frequencies of incisor breakage in Atlantic Canadian moose populations. Elemental concentrations are expected to be higher in the outermost portion of incisor enamel, particularly in older animals, because the outermost layer of the enamel is exposed to and involved in constant chemical exchange with the oral environment; ions are deposited and incorporated into enamel tissue at the outer surface (Brudevold & Steadman, 1956; Whittaker & Stack, 1984). Variations in tooth elemental concentrations among regions are expected as a result of different geochemical backgrounds and subsequently, different concentrations of biologically available elements in local water, soil and vegetation (Curzon, 1983; Berggren *et al.*, 1990). In the second objective, tooth chemical composition and microhardness data will permit inference regarding the relationship between tooth composition and dental declines. It is expected that elemental composition may be related to tooth integrity because tooth development processes and the subsequent chemical composition of teeth can be affected by nutritional deficiencies

(Kiely *et al.*, 1977; Nakamoto *et al.*, 1979; Fang *et al.*, 1980; Alvarez *et al.*, 1990; Alvarez, 1995). Microhardness measures will be used to determine whether the physical properties of tooth tissues are different among regions.

METHODS

Sample collection and preparation

Provincial, territorial, and state wildlife officials provided a combined total of approximately 7500 permanent incisors from harvested moose along with age data and location of origin. The teeth were catalogued by jurisdiction and those which exhibited post-mortem breakage or damage, distinguishable from ante mortem breakage by the absence of edge wearing and discoloration, were excluded from further consideration (approximately 2000). For the remaining 5500 incisors, incisal depth, a measure of dental integrity designed to combine the effects of both wear and breakage, was measured across the occlusal surface according to methods previously described Clough *et al.* (2010). A larger value for incisal depth is generally associated with a tooth that is worn and/or exhibits breakage along the occlusal surface.

A random subset of all incisor samples was chosen for chemical analyses with a minimum of 300 selected from each Atlantic Canadian region (New Brunswick, Cape Breton, and Newfoundland) to enable a more detailed examination of the region where tooth breakage has been documented to occur at high rates (Table 3.1). At least 20 samples were chosen from jurisdictions of low frequencies of breakage (New Hampshire, Ontario, Vermont, and Yukon Territory). All age groups were represented in the sample subset though few samples exceeded 8.5 years of age because the bulk of the samples were obtained from animals of younger ages.

Incisor teeth (n = 900) were sectioned longitudinally with a Dremel® tool (300 series) equipped with a 23.8 mm reinforced cut-off wheel at approximately halfway across the occlusal (incisal) surface of the tooth and set in epoxy on thin section glass slides (27x46 mm) (Appendix A). The labial edge of each incisor section was placed in the same orientation for each sample on a marked slide to facilitate consistency of analyses across the labial only, rather than lingual, surface of the tooth. The slides were lightly sanded, first using a 600 grit wet sanding cloth, followed by 1000 grit wet sanding cloth to remove epoxy from tooth surfaces. Fine polishing was performed using Mecaprex™ self-adhesive polishing discs on a Struers Planopol-V™ rotary automatic polisher in combination with Struers DP-Suspension™ diamond pastes ranging from 9 to 3 micrometers. Between stages of polishing, each slide was immersed in a clean ultrasonic water bath of deionized water for at least 15 minutes.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS)

Elemental analyses by LA-ICP-MS were performed at the Inco Innovation Centre at Memorial University of Newfoundland using a Finnigan ELEMENT XR; a high resolution double focusing magnetic sector inductively coupled plasma mass spectrometer with a GEOLAS 193 nm excimer LASER system. Elements were chosen for analysis based upon biological essentiality and toxicity in the mammalian body, and known capability to become incorporated into the hydroxyapatite structure. Specific isotopes of such elements were chosen based upon instrument capability. The following isotopes were chosen: ^{11}B, ^{63}Cu, ^{64}Zn, ^{75}As, ^{85}Rb, ^{88}Sr, ^{111}Cd, ^{118}Sn, ^{137}Ba, ^{208}Pb, ^{232}Th, and ^{238}U. The laser beam (energy 5 J/cm^2) was rastered across the labial enamel surface of each longitudinal tooth section from the outer edge to the dentine-enamel junction with a laser repetition rate of 10 Hz, producing ≈40 μm diameter sample spots across a total distance of 800 μm. The ablated material was sent to the ICP via a helium flow rate of 1.25 L/min. Argon was used in the ablation cell as the make-up gas. Peak jumping as well as pulse-count, analog, and Faraday modes were used to provide time resolved intensity data with one point per peak measured. The described method was employed for samples and quality control materials, NIST 612 glass and Durango apatite, with the exception of the lack of dentine-enamel junction in control materials; each surface was rastered without specific reference points as reference materials are assumed homogeneous. Each ablation required approximately 160 seconds, prior to which approximately 30 seconds of gas background data were collected.

Analysis quality control included instrument calibration with NIST 612 glass (Appendix B). An internal standard, CaO, was used to control for matrix effects and ablation yields in unknowns and calibration glasses; CaO concentration was assumed homogeneous at 49.1%. Durango apatite, a geologic material widely used as reference material for geochemical applications (McDowell *et al.*, 2005), was used as an unknown to monitor the precision and accuracy of collected data as its matrix is similar to tooth hydroxyapatite (Appendix C and D). The sequence for each run of 20 ablations was as follows; two rasters of the NIST 612 glass standard, one raster of the Durango apatite unknown, up to 14 tooth samples, and closing with a Durango raster and two NIST 612 glass ablations. The error of homogeneous materials, for this method, based upon the daily reproducibility of results in this laboratory, is estimated at less than 4%.

Data were converted from count rates to concentrations (ppm) using in-house programs, CONVERT and LAMTRACE, as described by Longerich *et al.,* (1996). CONVERT is a software program to create LAMTRACE-useable spreadsheet files from the ICP-MS data. LAMTRACE references standards, ablation yield differences, and instrument sensitivity drift during each session to convert count rates into concentrations based on user selected signal intervals, background subtraction intervals, internal standard correction, and calculated run detection limits (Appendix E). The selected signal intervals were chosen to represent both the surface (outer) enamel and the inner enamel. For the purposes of differentiating the elevated concentrations of elements that results from ion migration into the enamel surface from the homogeneous portion of the enamel, which remains relatively unchanged throughout the individual's lifetime, the outer matrix was classified as the distance from the labial surface of the enamel to a depth of 100 μm. The inner matrix extended from this depth to the dentine-enamel junction. Each ablation raster was initiated at approximately 20 to 40 mm below the occlusal (incisal) edge of the tooth.

In the case of elemental concentration values below the detection limit, those elements in which greater than 40% of the data were below limit of detection were eliminated from further analysis (Cd). The remaining left-censored elemental composition data set was flipped to become right-censored, enabling application of the nonparametric Kaplan-Meier method of calculating estimates for reported values below the detection limit (Helsel, 2005).

Microhardness Testing

Vickers' microhardness testing of tooth hardness (Potocnik *et al.*, 2000; Wongkhantee *et al.*, 2006) was conducted on thin section slides as previously prepared for LA-ICP-MS. Thirteen samples of the same age (6.5 years old) were chosen for microhardness analysis from Atlantic Canadian moose: 5 from New Brunswick, and 4 each from Cape Breton and Newfoundland. The selected samples were chosen at random to satisfy the condition that both incisors which had broken during the individual's lifetime and those that had not were chosen from each jurisdiction and that all samples were from individuals of the same age to control for a potential age-induced variation in hardness. Microhardness testing was performed by contract at Exova Laboratory (Pointe-Claire, Quebec, Canada) according to the following specifications: 100 gf load using a diamond tipped pyramid indenter, with three readings each in the dentine and the enamel portions of the tooth. For each indent, the same force, *f*, was applied (0.100 kg), producing square indents within the tooth surface from which the lengths (in mm) of each diagonal was used to find the mean diagonal length, *d*. The hardness value (HV), typically a unit-free measure, was then calculated according to the formula,

$$HV = \frac{\left(2F\,\frac{\sin 136^{\circ}}{2}\right)}{d^2}$$

Dentine readings, approximately 50 mm below the occlusal surface, were performed midway between the pulpal cavity and the dentine-enamel junction, while enamel readings were taken midway between the labial edge of the tooth and the dentine-enamel junction. Midpoint measurements were required as dentine adjacent to the pulpal cavity of the tooth has decreased hardness (Meredith *et al.*, 1996) compared to dentine in close proximity of the dentine-enamel junction of the tooth where the size, abundance, and distribution of dentinal tubules varies (Kinney *et al.*, 1996). The averages of three readings in the dentine and three readings in the enamel of each tooth were used for further analysis.

Statistical Analysis

Summary statistics were calculated for each elemental isotope for both inner and outer enamel. Paired sample t-tests were performed between inner and outer enamel concentrations for each element to see whether elements differed from the inner to the outer matrix.

To explore spatial and age-related trends in the chemical composition of moose incisors, MANCOVA's were performed on both inner and outer datasets with elemental concentrations as dependent variables and each of age, origin, and the interaction term, age*origin, as independent variables to determine the effect, if any, of age and origin on tooth composition.

Principal components analyses, a frequently used exploratory method for multivariate chemometric data (Varmuza & Filzmoser, 2009) were used to reduce dimensionality of the dataset and to determine which elements are most associated with variation among incisor composition. Log transformation of element data was performed to approximate normality for principal component analyses. Data scaling was not performed to avoid misrepresentation of elements which are present as noise (Varmuza & Filzmoser, 2009). A principal components analysis was performed for both inner and outer enamel data from all regions with an eigenvalue cutoff of 1.5, and varimax rotation to maximize the variance between the correlations (Thurston & Spengler, 1985). Plotting of principal component scores reveals the sample clusters and groups (Massart *et al.*, 1988), thus the primary principal component axes were determined and plotted with points coded by Atlantic Canadian jurisdiction and, in a separate plot, according to tooth condition to visually represent the differences in elemental composition among these regions where tooth condition is deteriorated.

For further analyses of Atlantic Canada (New Brunswick, Cape Breton, and Newfoundland) moose incisor breakage, principal component analyses were performed for inner enamel only because this matrix is homogenous, fixed at development, and represents the largest proportion of enamel. Analyses were performed separately for each region to generate principal component scores unique to the elemental environment and influences that exist within each region. Principal components scores are uncorrelated linear variables which eliminate multicollinearity issues in regression and also enable a reduction in the number of independent variables (Varmuza & Filzmoser, 2009), thus scores were retained for further analysis. Multivariate regression models for each Atlantic Canadian region were created with incisal depth, a quantitative measure of incisor wear and breakage, as the dependent variable and the first three principal component axes and age in each model as explanatory variables. Age was included in each model to control for age-induced deterioration of tooth tissues. Univariate regression models for elements from significant principal component axes were constructed for incisal depth and performed using only samples of the same age (6.5 years) to control for age effects on tooth deterioration. This specific age was chosen to represent a sample from an individual that was neither considered young nor old.

Microhardness tests provided mechanical property data used to make inference regarding the link between dental condition and dental declines. A one-way ANOVA was used to analyse microhardness data among regions for enamel and dentine. Significance testing for all tests used $\alpha = 0.05$. All statistics were performed using SYSTAT (Version 11.0, Systat Software Inc., Richmond, California, USA).

RESULTS

The incisor concentration of each element included in LA-ICPMS analyses is presented in Table 3.2 according to sampling region and location in the enamel. For all regions combined, multiple elements differed between the inner and outer enamel ($p < 0.005$): B, Cu, Zn, Sr, and, Pb (Table 3.3). Results from the MANCOVA analysis show that three elements significantly varied according to age; B in the inner enamel and Rb and Zn at the enamel surface. The elemental concentrations in the inner enamel which significantly differed by region of sample origin were B, Rb, Sr, Sn, Ba, and Th. Within the outer enamel, all elements significantly differed by sample origin. There were no significant effects for the interaction term, age*origin, for any elements in either enamel matrix.

The first three axes from principal component analyses, conducted on data from all regions, explained approximately 50% of variance in tooth elemental composition (Table 3.4). For both the inner and outer enamel of all samples, the three main element groupings consisted of the strontium group; Sr, Ba, and Rb, the trace metals group; Zn, Cu, and Pb, and the Th-U group, which consisted of Th and U. In the outer enamel Sn was also related to the variation explained by the Th-U group. Each of the three elemental groups of variation explained between 12.5 and 17.7% of the total variation in tooth composition (Table 3.4)

An overlapping cluster pattern by sample origin was revealed when the principal components of the inner enamel were graphed with Atlantic Canada samples placed upon the first three axes explaining the variation in tooth elemental composition (Figure 3.1). The clustering of New Brunswick samples varied the greatest, from the other Atlantic Canadian regions, along the Th-U elemental group axis. Newfoundland samples varied from the others along the Sr group axis. Despite clustering of regions along the principal axes of incisor composition variation and the differences among regions in the frequency of tooth breakage, plotting the Atlantic Canadian incisor samples according to tooth condition, in terms of incisal depth, did not reveal a specific clustering pattern (Figure 3.2).

When a principal components analysis was performed for Atlantic Canadian samples only and finally for each Atlantic Canadian region separately, the elemental groupings within the first three principal components differed for Cape Breton and Newfoundland samples (Table 3.5). In each of these aforementioned regions, the groupings of elements are similar but the relative importance, in terms of amount of variance explained by the given group's principal component, differs. The trace metals group has the highest loadings along the primary component for Newfoundland while Th, U, and As compose the element group explaining the most variation for Cape Breton incisor samples.

Multivariate regression models suggest that there is a significant relationship between incisal breakage with age and one principal component factors (Table 3.6). Age was significantly related to incisal depth in each model, as was the component for the trace element group (i.e., Cu, Zn, and Pb) for New Brunswick and Newfoundland. This group is also noteworthy in Cape Breton, though not statistically significant. The relationship between age and incisal depth is positive while the coefficients for the trace metal component in each region, as well as the Sr-group component in each region, were negative. Single element regressions (Table 3.7) varied in the magnitude and direction of effect and were not statistically significant. Microhardness values (Table 3.8) did not reveal significant differences, using ANOVA, among regions in tooth enamel or dentine hardness of broken and unbroken teeth.

DISCUSSION

The results suggest that there is variation in moose incisor elemental composition among regions. This variation likely results from the presence of different environmental influences throughout the individual's lifetime, including during tooth formation. Inter-regional variation in elemental concentrations in the inner enamel matrix may indicate differences in the availability of essential nutrients and elements during amelogenesis, the formation of tooth enamel. A geographical variation in tooth elemental composition has been previously demonstrated (Curzon *et al.*, 1975; Brown *et al.*, 2004) and was expected. However, in this study these variations provide information regarding the environmental influences among populations where tooth breakage occurs at different frequencies. This is important because differences in the regional availability of elements are essential considerations when investigating elemental influences on a tooth condition that varies geographically (Bibby & Losee, 1970). For example, we see that Pb, B, Cu, Zn, and Sr significantly differ from inner to outer enamel matrices in all teeth. This was expected as incremental deposition occurs at the tooth surface throughout the individual's lifetime; therefore, elemental concentrations at the tooth surface are expected to be higher in older individuals. This may represent a constant, or decrease in, exposure to such elements throughout the duration of the individual's lifetime, after the tooth formation. However, it must also be recognized that differences in the concentrations of elements at the surface of the enamel, the outer enamel, may be explained by multiple factors-differences in mineralization at development, differences in tooth porosity, rate of diffusion, and absorption as well as differences in the diet of the individuals (Lazzari, 1976).

As all elements were expected to increase in the outer enamel with cumulative deposition from the oral environment; it is unusual to see that, in this respect, Sr differed from previous tooth composition studies. The concentration of Sr was significantly less in the outer enamel matrix than in the inner enamel. Lazzari (1976) reports a relatively constant concentration of Sr in tooth enamel surface and subsurface in human teeth, with no expected increase in concentration with age. A constant concentration across the tooth enamel or an increase of Sr in the inner enamel may have indicated an increased uptake during amelogenesis (tooth formation) and thus, possibly, additional benefits for tooth integrity. Uptake of Sr during amelogenesis has been shown to modify tooth structure in a manner associated with a decrease in susceptibility to caries, such as dentin thickening (Bibby & Losee, 1970). Furthermore, fewer caries have been found to be correlated with high levels of Sr in local water supplies, while SrF is a more effective agent in reducing enamel solubility than NaF (Bibby & Losee, 1970).

The variation in the inner enamel, among regions, demonstrates that localized nutrient and elemental availability does, in fact, vary among regions and it is possible to expect that elements present in atypical concentrations may cause the tooth structure to be more susceptible to erosion (Labrada-Martagon *et al.*, 2007). Erosion of dentition is characterized by demineralization of the enamel surface followed by dissolution and the eventual loss of tooth structure (Barron *et al.*, 2003).

Differences among regions in environmental element availability result from differences in local geochemistry, such as bedrock composition and the acidity of precipitation which, in turn, influences the availability of elements from the soil and the extent of deposition from rocks (Berggren *et al.*, 1990). Water supplies, soil, and plants vary from region to region in the content of environmental background levels of elements. In the studied regions, the highest frequencies of tooth breakage occurred in moose populations which inhabited the more remote locations of each region, also of high elevations. Therefore, although environmental contaminants may also be deposited from anthropogenic activities and structures, it is more likely that in the regions of concern in this study, the origin of trace elements is from soil and bedrock and availability is strongly influenced by local climatic conditions and weathering processes.

The geographic variation of elements in water and dietary resources is further demonstrated by the relevant importance, or variation attributed by, three recurring groupings of elements which best characterize the variation among moose incisor elemental composition for all North America and, more specifically, each region within Atlantic Canada. Though the element groupings are consistent among regions, as well as the influence (variance explained ranged from 12.5 to 19.1%), in New Brunswick, where the breakage frequency is lower, the element group which explained the greatest amount of variation was the Sr group while in Cape Breton and Newfoundland, the Th-U group and the trace metals group, respectively, explained the greatest amount of variation.

The Sr-group was the first principal component for all North American samples. Sr, unless present in very high concentrations, is not generally associated with decreases in tooth condition. The other elements which are most related to Sr in characterizing these teeth, Ba and Rb, do not have a known biological role in teeth and/or are potentially toxic (Brown *et al.*, 2004). However, it can be seen that each of these elements follows a parallel profile to Ca in the tooth (Figure 3.3). Thus, based on the similar behaviour of these elements it is not unforeseen that they group together.

The second most prominent grouping of elements is the trace metals group; Cu, Zn, and Pb. The elements of this grouping also follow similar profiles, to each other, within the tooth (Figure 3.3); an increase, or peak of deposition at the surface of the tooth which subsides into a relatively homogeneous profile throughout the remainder of the enamel cross-section. These elements vary according to region in terms of abundance in the environment and are present in multiple environmental media. Though generally attributed to atmospheric deposition (Barnes, 1976), particularly in regions closest to industrial activities (Blanusa *et al.*, 1990), these trace metal elements have natural background levels which vary in vegetation across geographical regions (Driscoll *et al.*, 1988). Pb, a toxic element without known biological necessity in the body, is often stored in calcified tissues such as bones and teeth following ingestion (Stack, 1983). Cu and Zn are elements that are required for biological enzymatic processes (Curzon, 1983) such as the formation and activation of multiple metallo-enzymes (Fisher, 1975); however, excessive amounts may deposit in tooth and bone tissues as a result of increased availability in the external environment of the tooth (Bibby & Losee, 1970; Dreizen, 1976). A significant negative relationship was found between incisal depth and the trace metals group, for each region, which implies that, in moose, when incisal depth (wearing and breakage) is high, concentrations of these elements in the tooth are low. This may be explained by the deposition of Zn and Pb in the surface of defective enamel which may impede further development of carious lesions (Dreizen, 1976). As well, Fang *et al* (1980) suggest that, in rats, Zn may decrease susceptibility to caries and deficiency impairs post-eruptive tooth mineralization processes. The presence of Cu in the oral environment has been associated with reducing acidity, which may inhibit dissolution processes (Curzon, 1983). However, the bulk of the scientific literature implicates the trace elements, in particular Cu and Pb, with decreases in tooth quality or increases in decay or caries (Barmes *et al.*, 1970; Lawson *et al.*, 1971; Curzon & Losee, 1977; Gil *et al.*, 1996; Gerlach *et al.,* 2002). A study by Barmes *et al.* (1970) found higher levels of Ba and Sr in the soil of Papua-New Guinea regions with low caries prevalence while Pb and Cu presence was concluded to be likely harmful. Further research of the relationship between Sr and Ba presence in the tooth and caries prevalence has also supported these elements as positive components in the tooth microstructure (Bibby & Losee, 1970; Curzon *et al.,* 1978; Curzon, 1985; Zdanowicz *et al.,* 1987 and others). This suggests that the Sr group and incisal depth should have an inverse relationship from that existing between the trace metal group and incisal depth. This is not the case which indicates that the findings of this study are not consistent with the bulk of the scientific literature for trace elements in the dentition of human subjects. It is also possible, however, that despite individual relationships with tooth quality, interactions between elements can influence the toxicity, availability, and nutritive value of minerals (Underwood & Suttle, 1999). The presence of specific elements or groups of elements at specific concentrations during tooth development can act in concert to disrupt amelogenesis processes rendering the resultant product (tooth) more susceptible to breakage.

It is interesting to find that Th and U, two naturally occurring crustal components, explain some proportion of the variation among moose incisor elemental composition in each set of regions examined. These elements reflect local geochemical conditions (Berg *et al.*, 1995) and are not known to be essential elements in tooth composition or the mammalian body (Curzon, 1983). The influence of this element grouping in the Cape Breton region, as seen by the greater importance of this axis to principal component groupings, may explain why regressions with incisal depth were less supportive of the relationship between the trace metal group and breakage. The greater influence of Th and U in incisors from this region indicates a different geochemical background from which elements are available during development, and throughout the lifetime. As this region does not exhibit the extent of breakage frequency seen in Newfoundland moose (Clough *et al.*, 2010), and this component is not as important in explaining tooth composition variation in Newfoundland, it is possible that these elements are simply present in teeth without any influence on tooth condition. However, it cannot be overlooked that many factors likely influence incisor breakage in Atlantic Canadian moose and the relative importance of multiple factors may also differ among regions.

When plotting the principal axes of variation, clustering of Atlantic Canadian regions was revealed for samples coded by region but not for those coded by tooth condition. Furthermore, multiple linear regressions show that both age and the trace metal group contribute to increased incisal depth, though the strong correlation between age and incisal depth may explain most of the variation. Nonetheless, it is interesting to see that while increases in incisal depth are associated with increasing age of the individual, a decrease in concentration of the trace metal group is related to an increase in incisal depth. This relationship may be strongly driven by the presence of the essential trace metals Cu and Zn, as the third member of this grouping, Pb, is non-essential and toxic. However, none of the elements appeared to show consistent relationships with incisal depth in univariate regression models for each region of Atlantic Canada.

Microhardness tests did not reveal differences in tooth structure hardness among regions. This supports the argument that other factors are involved in the etiology of herbivore tooth breakage among Atlantic Canadian moose. We expected to see differences in microhardness if tooth mineralization processes had developed defectively. As incisor tooth hardness did not differ among regions and elemental composition explained only a proportion of variation among incisors, it is possible that tooth tissues are not defective and the variation in chemical composition does not exceed that which may be expected to exist among teeth from regions with different geochemical backgrounds/conditions. Other factors, such the quantity or quality of food resources, in the local environment must be considered when characterizing potential contributing factors in moose incisor tooth breakage in Atlantic Canada.

The anomalous breakage patterns seen in this large herbivore may be related to diet, tooth usage, and abnormal behaviour patterns. This exploratory analysis has shown that elemental intake varies among regions, possibly indicating differences in diet. The regional variation which exists in the quality of moose habitat, in terms of browse quality and species abundance (Risenhoover, 1989), may, in Atlantic Canada, result in the consumption of inadequate materials or lead to abnormal behaviours (i.e., barkstripping) where nutritional distress occurs after tooth tissues have developed. The regression results, which indicate that Cu, Pb, and Zn are negatively associated with tooth breakage, may indicate that moose with broken incisor teeth are not consuming preferred browse species as these elements are often present in typical browse (Kubota *et al.*, 1970; Tlustos *et al.*, 2007, Lagerwerff & Specht, 1970). Further consideration of this anomalous condition in the moose of Atlantic Canada should strongly consider the analysis of food consumption habits and the link between population density, resource availability and resource quality.

CONCLUSION

In the present study, LA-ICP-MS was used to determine the chemical composition of moose incisors from several North American populations. Of the 11 elements included in the analysis, the incisal concentrations of B, Rb, Sr, Sn, Ba, and Th significantly differed among regions. This demonstrates that different geological backgrounds exist among regions, thus influencing the environmental availabilities of the given elements in local water, soil, vegetation. Principal component analyses enabled reduction of elemental dataset into 3 components that explained nearly 50% of the variation in incisor composition among regions attributable to the given variables. The geographic variation in tooth composition was further supported by the clustering of regions across the principal component axes of variation. However, when samples were placed upon the axes of variation according to tooth condition, clustering did not occur. Regression analyses implicated age as a factor strongly associated with decreased incisal condition, while a significant negative association was found between the trace metal elements, Cu, Zn, and Pb, and deteriorated tooth condition in two regions, one with frequent tooth breakage (Newfoundland) and the other without (New Brunswick). The microhardness values of dentine and enamel tissues did not significantly differ among regions of varying tooth breakage frequency, therefore, it is likely that other factors contribute to elevated moose incisor breakage frequencies in Atlantic Canada. Abnormal diet, possibly related to a decrease in preferred food resources from high population densities, may be a contributing factor to tooth breakage. Further study should examine the food consumption habits of moose in affected regions. Analysis of food resource availability and/or evidence of abnormal consumption of unsuitable materials, through faecal pellet or stomach content analysis or direct observation may indicate consumption patterns or unusual habits which could contribute to declining dental condition. Additionally, trace element analyses of moose browse species throughout Atlantic Canada, with regard to the variation of preferred vegetation among local environments, would enable further examination of the observation that the trace metal intake (in particular, Cu, Zn, and Pb) is lower in regions with increased incisor breakage of moose.

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Table 3.1 Breakage frequency and average incisal depth measures for regions from which moose incisor chemical composition was determined using laser ablation ICP-MS.

Region	**n**	**Reported frequency of breakage**[1]	**Average incisal depth, ID (± s.d.)**
Cape Breton	323	6-34%	6.7(1.3)-7.9(2.4)
New Brunswick	359	2-11%	6.7(1.4)-7.1(0.9)
New Hampshire	30	4	7.5 (0.8)
Newfoundland	414	24-47%	7.4(1.8)-8.7(2.6)
Ontario	28	1	6.7 (1.1)
Vermont	124	6	6.9 (1.1)
Yukon	22	2	6.7 (1.1)

[1]Breakage frequency reported by Clough et al, 2010. In the case of significant variation within a region, the range is provided.

Table 3.2 Elemental concentrations (ppm) in inner and outer incisor tooth enamel matrices for multiple North American moose jurisdictions.

	New Brunswick		New Hampshire		Newfoundland		Cape Breton		Ontario		Vermont		Yukon	
n	359		30		414		323		28		124		22	
Enamel matrix	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration	Inner Concentration	Outer Concentration
Element	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)	mean (s.d)
B^{11}	6.13(2.90)	10.03(6.47)	7.97(2.78)	9.07(3.79)	5.55(2.56)	14.92(8.88)	5.41(2.13)	12.19(7.56)	6.60(6.09)	7.01(3.16)	6.21(3.00)	6.64(2.25)	2.82(1.05)	4.21(2.94)
Cu^{63}	2.49(2.45)	2.31(2.72)	1.62(1.51)	1.47(1.33)	2.50(3.31)	3.90(5.74)	2.88(2.84)	2.98(3.94)	1.33(0.80)	1.13(0.82)	2.08(1.54)	1.66(1.64)	1.03(0.59)	1.09(1.63)
Zn^{67}	20.01(6.68)	91.56(40.97)	24.84(8.20)	128.81(32.18)	18.83(18.81)	118.75(44.48)	20.34(6.69)	122.94(50.13)	18.58(6.93)	96.51(37.42)	20.09(6.95)	96.55(39.88)	17.76(8.21)	120.87(23.26)
As^{75}	0.45(0.46)	0.54(0.66)	0.71(0.20)	0.52(0.27)	0.48(0.89)	0.49(0.39)	0.47(0.72)	0.46(0.43)	0.41(0.30)	0.44(0.42)	0.51(0.51)	0.52(0.84)	0.63(0.42)	0.85(0.85)
Rb^{85}	0.53(0.28)	0.52(0.26)	0.89(0.40)	0.93(0.35)	0.36(0.22)	0.39(0.23)	0.53(0.24)	0.56(0.25)	0.60(0.28)	0.64(0.27)	0.97(0.36)	1.19(1.41)	0.49(0.20)	0.56(0.33)
Sr^{88}	219.91(95.44)	198.21(84.66)	164.43(49.48)	148.01(44.2)	150.34(77.63)	131.61(69.88)	223.72(54.67)	201.67(44.10)	163.33(51.42)	162.08(50.00)	186.34(47.64)	175.98(44.39)	196.46(43.24)	167.87(36.32)
Sn^{118}	0.34(0.27)	0.34(0.31)	2.92(1.58)	3.99(1.12)	0.21(0.23)	0.23(0.47)	0.44(0.42)	0.49(0.63)	0.76(0.36)	0.84(0.49)	1.30(0.97)	1.85(1.57)	0.54(0.86)	0.70(0.78)
Ba^{137}	143.00(83.15)	141.93(85.92)	95.70(33.50)	99.53(36.79)	56.50(29.45)	56.93(31.28)	82.1(34.02)	82.12(32.74)	113.16(67.91)	127.75(72.50)	88.14(35.55)	94.22(37.00)	120.98(79.67)	105.98(63.24)
Pb^{208}	0.55(0.56)	0.63(0.69)	0.50(0.30)	1.13(0.78)	0.52(0.59)	0.83(2.93)	0.84(1.17)	1.21(1.71)	1.54(1.56)	2.20(2.15)	0.80(0.86)	1.22(1.39)	0.47(1.06)	0.41(0.66)
Th^{232}	<0.00(0.01)	<0.00(0.01)	<0.00(0.00)	<0.00(0.01)	<0.00(0.00)	<0.00(0.01)	<0.00(0.01)	<0.00(0.02)	<0.00(0.00)	<0.00(0.00)	<0.00(0.01)	<0.00(0.00)	<0.00(0.00)	<0.00(0.00)
U^{238}	0.01(0.05)	0.01(0.04)	<0.00(0.01)	1.06(4.25)	0.02(0.10)	0.01(0.08)	0.01(0.04)	0.01(0.03)	<0.00(0.01)	0.01(0.06)	<0.00(0.01)	0.02(0.10)	0.01(0.02)	0.02(0.06)

Table 3.3 Mean concentrations (ppm) of elements in the inner and outer enamel matrices of moose incisors (n = 1300) from across North America, as determined by laser ablation ICP-MS, and paired sample comparison results by element.

Element	**Inner Enamel Concentration**	**Outer Enamel Concentration**	**Paired sample significance**
	Mean (s.d.)	Mean (s.d.)	p value
B^{11}	5.77(2.78)	11.25(7.65)	**<0.001**
Cu^{63}	2.48(2.75)	2.75(4.04)	**.005**
Zn^{67}	19.77(12.04)	108.88(46.06)	**<0.001**
As^{75}	0.48(0.68)	0.51(0.55)	.014
Rb^{85}	0.53(0.32)	0.56(0.55)	.011
Sr^{88}	192.60(81.11)	174.76(71.53)	**<0.001**
Sn^{118}	0.49(0.69)	0.60(0.97)	.606
Ba^{137}	92.98(63.09)	93.57(63.88)	.798
Pb^{208}	0.65(0.84)	0.95(1.94)	**<0.001**
Th^{232}	0.00(0.01)	0.00(0.01)	.022
U^{238}	0.01(0.07)	0.03(0.65)	.558

Table 3.4 Principal component analyses of North American moose incisor elemental composition data, log transformed. All rotated component loadings greater than 0.500 are indicated by bolded numerals.

Sample location	**Inner enamel**			**Outer enamel**		
Component	1	2	3	1	2	3
Eigenvalue	1.9	1.8	1.4	1.9	1.7	1.6
Explained Variance	17.7	16.7	12.5	17.6	15.3	14.8
Cumulative Explained Variance	17.7	34.4	46.9	17.6	33.0	47.8
Component	**Loadings**			**Loadings**		
B^{11}	0.246	0.223	0.147	-0.015	0.169	-0.011
Cu^{63}	-0.046	**0.783**	-0.054	-0.057	-0.080	**0.735**
Zn^{67}	0.135	**0.785**	-0.032	-0.141	-0.011	**0.593**
As^{75}	-0.043	-0.024	0.102	0.097	-0.406	0.518
Rb^{85}	**0.551**	0.113	0.013	**0.563**	0.343	0.002
Sr^{88}	**0.849**	-0.019	0.031	**0.846**	-0.037	-0.062
Sn^{118}	0.270	0.173	0.198	0.314	**0.689**	-0.019
Ba^{137}	**0.877**	0.041	-0.015	**0.870**	-0.019	-0.037
Pb^{208}	0.011	**0.714**	0.093	0.138	0.324	**0.679**
U^{238}	0.013	-0.007	**0.854**	-0.023	**0.674**	0.031
Th^{232}	0.015	0.014	**0.747**	-0.011	**0.578**	-0.032

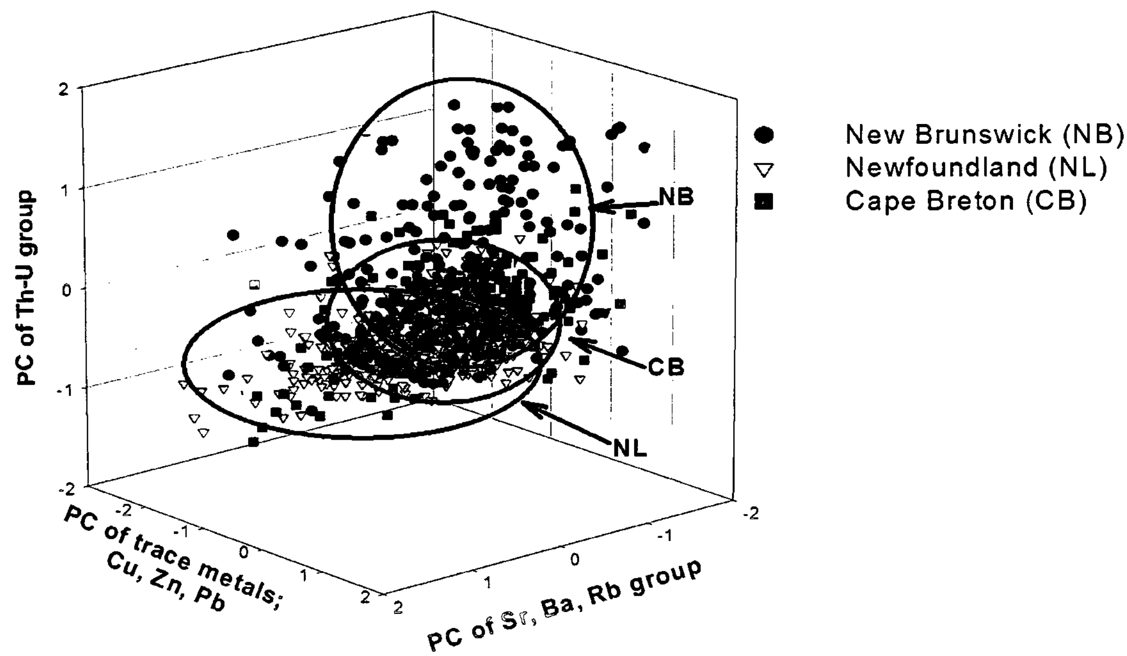

Figure 3.1 Plots of principal component scores for element groupings in the inner enamel of Atlantic Canadian moose incisors, different symbols are used to represent each province of sample origin.

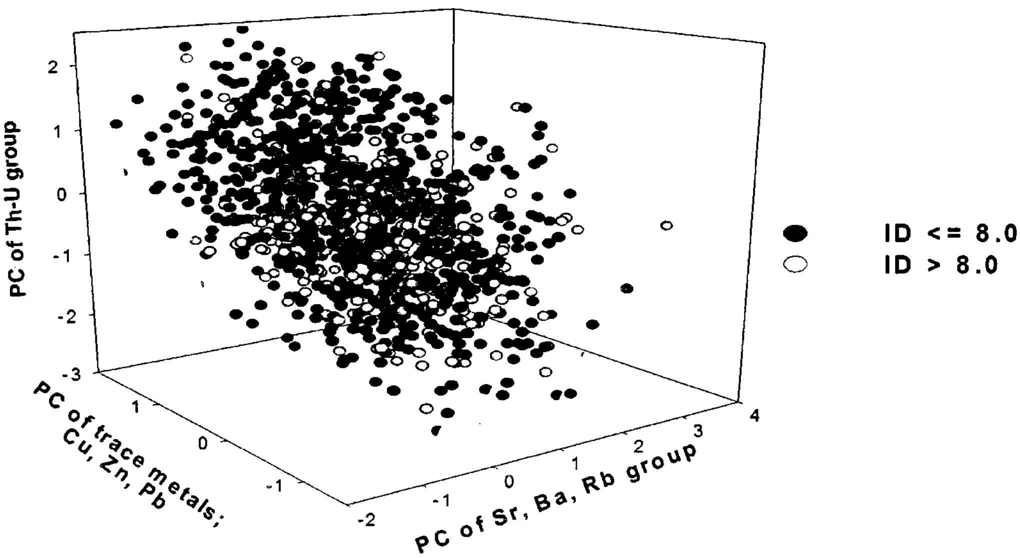

Figure 3.2 Principal component element groupings for moose incisor samples, plotted according to incisal depth (ID). Tooth samples are coded according to ID score: unshaded symbols represent teeth with excessive wear or breakage, those which typically have an incisal depth measure > 8, those teeth without physical deterioration of enamel, generally with an ID <= 8, are represented by shaded symbols.

Table 3.5 Principal component element groupings for the inner enamel of moose incisors from each Atlantic Canadian province. All component loadings greater than 0.500 are indicated by bolded numerals.

Sample location	**New Brunswick**			**Cape Breton**			**Newfoundland**		
Component	1	2	3	1	2	3	1	2	3
Eigenvalue	1.9	1.8	1.6	1.9	1.9	1.8	2.1	1.8	1.5
Explained Variance	17.0	16.6	14.9	17.7	17.4	16.3	19.1	16.8	13.5
Cumulative Explained Variance	17.0	33.6	48.5	17.7	35.1	51.4	19.1	35.9	49.4
Component	**Loadings**			**Loadings**			**Loadings**		
B^{11}	0.136	0.142	0.046	0.152	-0.035	0.340	0.260	0.188	-0.447
Cu^{63}	-0.048	**0.782**	0.086	-0.334	**0.773**	-0.082	**0.776**	-0.074	-0.092
Zn^{67}	0.205	**0.775**	0.086	0.154	**0.845**	0.130	**0.741**	0.090	-0.018
As^{75}	0.279	-0.183	**0.602**	0.496	-0.008	-0.057	0.272	-0.016	0.051
Rb^{85}	**0.514**	-0.017	0.319	0.102	0.078	0.410	0.192	**0.544**	-0.161
Sr^{88}	**0.819**	0.045	-0.060	-0.152	0.006	**0.836**	-0.149	**0.833**	0.157
Sn^{118}	0.293	0.084	0.392	**0.681**	-0.006	-0.015	0.394	0.140	0.497
Ba^{137}	**0.836**	0.158	-0.012	-0.183	0.007	**0.872**	-0.066	**0.889**	-0.013
Pb^{208}	0.056	**0.716**	-0.090	0.189	**0.765**	0.016	**0.761**	-0.004	0.176
U^{238}	-0.068	0.103	**0.726**	**0.640**	0.046	0.158	0.024	0.000	**0.586**
Th^{232}	-0.020	-0.054	**0.681**	**0.753**	0.093	0.029	0.106	0.011	**0.754**

Table 3.6 Multiple linear regression results using principal component factors and age as possible effects for declining incisal condition, incisal depth (ID). ID = Age + PC 1 + PC 2 + PC 3 + error.

Region	**n**	**Model**			**Coefficient**	**Element Grouping**	**β**	**s.e.**	**p**
		R^2	**F**	**p**					
New Brunswick	359	0.38	54.84	<0.001	Intercept		**5.839**	**0.101**	**<0.001**
					Age		**0.242**	**0.017**	**<0.001**
					PC1	Ba, Sr, Rb	-0.006	0.048	0.901
					PC2	**Zn, Cu, Pb**	**-0.135**	**0.050**	**0.008**
					PC3	U, Th, As	0.036	0.050	0.470
Cape Breton	371	0.40	52.41	<0.001	Intercept		**5.330**	**0.180**	**<0.001**
					Age		**0.480**	**0.034**	**<0.001**
					PC1	U, Th, Sn	-0.028	0.103	0.787
					PC2	Zn, Cu, Pb	-0.150	0.103	0.148
					PC3	Ba, Sr	-0.001	0.108	0.936
Newfoundland	323	0.46	77.76	<0.001	Intercept		**5.612**	**0.195**	**<0.001**
					Age		**0.491**	**0.029**	**<0.001**
					PC1	**Zn, Cu, Pb**	**-0.264**	**0.113**	**0.020**
					PC2	Ba, Sr	-0.179	0.108	0.100
					PC3	U, Th	0.180	0.118	0.127

Table 3.7 Regression results for trace metal elements in inner enamel of 6.5 year old moose incisors as a function of incisal depth (ID), a quantitative measure of the cumulative effects of wear and breakage. ID = element + error

Region	n	Element	β	s.e.	p
New Brunswick	60	Cu	0.075	0.368	0.840
		Zn	-0.546	1.064	0.610
		Pb	-0.809	0.437	0.069
Cape Breton	19	Cu	-0.519	4.500	0.910
		Zn	-7.492	6.656	0.276
		Pb	-1.873	2.090	0.383
Newfoundland	54	Cu	0.765	0.874	0.391
		Zn	1.287	2.647	0.629
		Pb	-0.269	0.979	0.784

Table 3.8 Vickers microhardness results for Atlantic Canadian moose incisor dentine and enamel hardness. An incisal depth < 8.0 is generally associated with an unbroken tooth; though extensive wearing of the tooth surface may result in an elevated incisal depth score.

Region of Sample origin	Broken	Incisal depth	Tissue	
			dentine	enamel
New Brunswick	no	6.8	79	299
New Brunswick	no	8.6	78	289
New Brunswick	no	7.3	63	319
New Brunswick	no	7.3	71	246
Newfoundland	no	7.7	64	320
Newfoundland	no	7.1	61	343
Cape Breton	no	7.0	75	324
Cape Breton	no	6.8	75	301
Average of unbroken (s.d.)		**7.3 (0.6)**	**71 (7)**	**305 (29)**
New Brunswick	yes	10.1	65	273
Newfoundland	yes	12.2	73	337
Newfoundland	yes	11.4	68	259
Cape Breton	yes	15.3	81	301
Cape Breton	yes	8.1	72	168
Average of broken (s.d.)		**11.4 (2.7)**	**72 (6)**	**268 (63)**

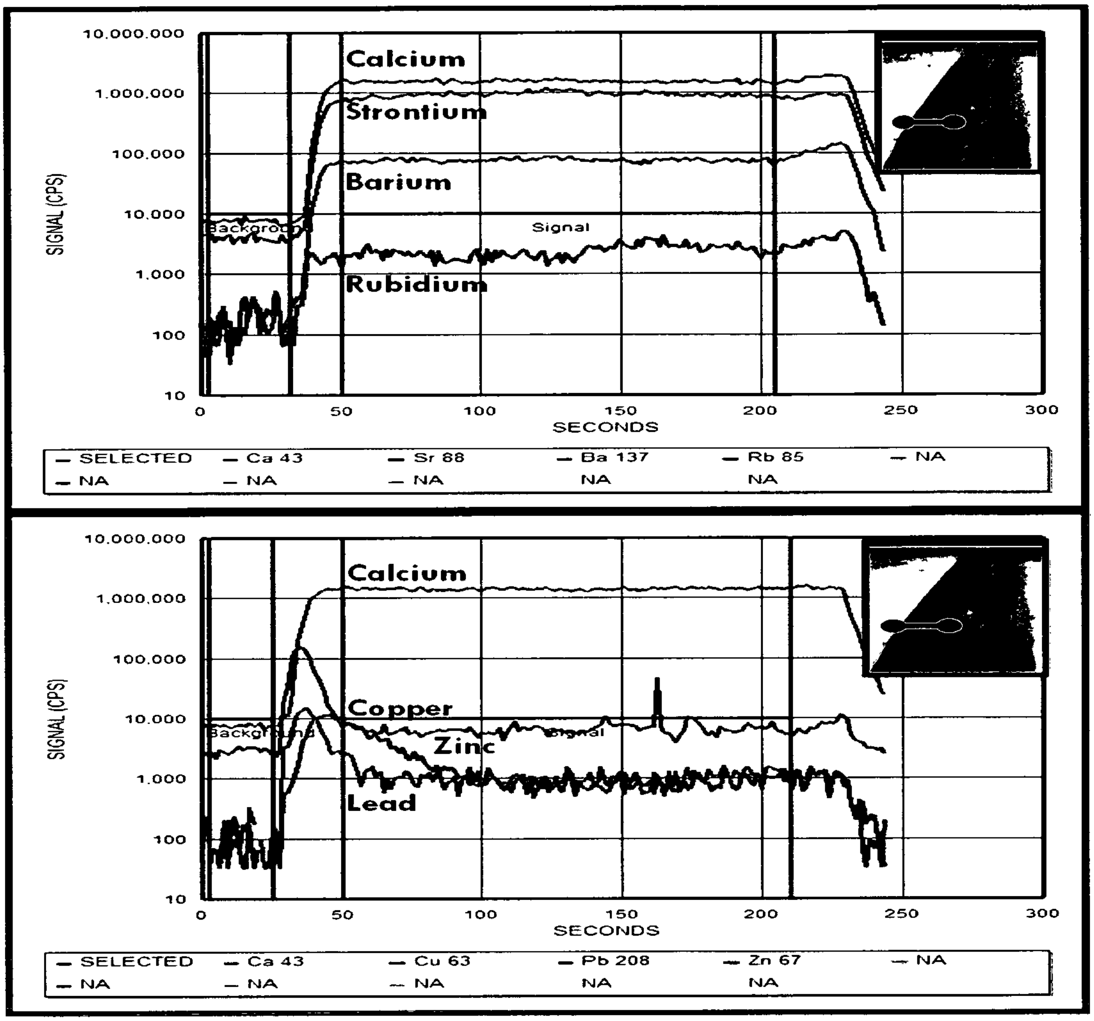

Figure 3.3 Laser ablation element profiles for chemical analysis of moose incisor from a 6.5 year old individual from Newfoundland with inset to demonstrate ablation path on tooth. Elements included in principal component analyses are displayed according to counts per second (CPS) for the duration of the tooth ablation which begins at the labial surface of the tooth after approximately 25 seconds of background data is collected. The signal length of approximately 150 seconds corresponds to a depth from tooth surface of approximately 700μm (5 μm/second).

CHAPTER 4

THE SPATIAL DISTRIBUTION OF MOOSE INCISOR BREAKAGE IN ATLANTIC CANADA AND AN EVALUATION OF TOOTH CHEMICAL COMPOSITION FOR INFERENTIAL PURPOSES: A SYNTHESIS

In this thesis, an analysis of moose incisor breakage in Atlantic Canada was performed to characterize patterns of occurrence and to enable inference regarding possible causes. The spatial distribution of moose incisor breakage in Atlantic Canada was characterized in the primary stage of the study (Chapter 2) to determine regions of high frequencies of moose incisor breakage. The moose populations of these regions were also examined in terms of population age structure. In the second stage of this study (Chapter 3), the hypothesis that defective tooth tissues were related to elevated frequencies of tooth breakage among Atlantic Canadian moose was explored.

In the primary stage of this study, it was found that the frequency of moose incisor breakage across North America typically varies from 1-6%. However, in northern Cape Breton (CB), Newfoundland (NL), and the northwest corner of New Brunswick (NB), higher frequencies of breakage were found; ranging from 9% (CB) to 47% (northern NL). The northern peninsula of Newfoundland, exhibiting the highest frequency of breakage in this study, was considered the region of greatest concern for potential effects on moose survival and reproduction which could result from a decreased ability, with broken incisor teeth, to maintain cropping efficiency. However, when survivorship tables were constructed for this area, and all other regions, there were no detectable differences in survivorship. Though it is possible that the data have inherent bias, in that the data were hunter derived, the dataset was deemed suitable for the purposes of this study. It appears that, at this time, using this data, an elevated frequency of moose incisor breakage in Atlantic Canada does not appear to impact survivorship.

In the second stage of this study, laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) was used as a tool to perform multi-element characterizations of incisor composition to examine the variation in local environmental element availabilities among regions and to provide data for inference on which environmental elements may be implicated in tooth breakage. Microhardness testing of tooth structures was used to further explore the hypothesis that the improper development of teeth, resulting from nutritional deficiency and/or elemental status, may have rendered tooth tissue mineralization defective and a tooth more susceptible to breakage or erosion (Labrada-Martagon *et al.*, 2007). Tooth microhardness can be used as an indicator of mineralization, demineralization, and remineralization (Lazzari, 1976).

Exploratory chemometric analyses, using principal component analysis, of the chemical composition data, identified three groupings of elements that cumulatively explained approximately 50% of the variation in tooth elemental composition. The elemental grouping most closely associated with breakage, when principal component axes were used in multiple linear regressions for each region, was the trace metals group, composed of Cu, Zn and Pb. This relationship was negative indicating that lower concentrations of the elements of this group are associated with higher frequencies of breakage. Further regression analyses, using each element singularly as a function of incisal depth, did not reveal whether one of these elements was disproportionately influential in determining the relationship of the group with incisal depth. Since Pb and Cu had previously been implicated with increasing the prevalence of caries in human teeth (Gil *et al.*, 1996; Curzon & Losee, 1977), this result was unexpected but may be explained by multiple factors. First, the interactions between elements may magnify or decrease the effects beyond that which is seen through additive effect models of the elements (Underwood & Suttle, 1999). Certain elements behave differently, biologically, when other elements are present and the overall effect can differ greatly from the sum of the effects that would be seen from the presence of each element separately. Second, the presence of these elements may inhibit or activate a biological process related to increasing or decreasing tooth susceptibility to breakage. The strong correlation, and significantly positive relationship between age and incisal depth appears to be the powerful factor in all multiple linear regression models for each Atlantic Canadian region. As incisal depth was explored in Chapter 2 it was not unexpected that incisal depth was significantly related to age in multivariate regressions, older individuals using their teeth over longer periods of time would be expected to have greater wear or chance of incurring breakage of tooth structures.

Hardness tests revealed that the structural integrity of teeth, unbroken and previously broken, from different regions was not different though the sample size was limited (13 samples).

Plotting of the Atlantic Canadian data points along axes of chemical groupings revealed clustering by region but not by tooth condition (Chapter 3). Together, the results obtained in chemometric and microhardness analyses indicate that it is probable that factors, other than tooth chemical and physical microstructure, contribute to the occurrence of increased incisor breakage in affected moose populations. Therefore, from this analysis it appears that the tooth tissues are not defective, and teeth likely display the amount of variation in chemical composition that may be expected when tooth development and usage occurs in regions with different geochemical conditions.

The alternative hypothesis regarding the aetiology of tooth breakage is that the manner in which the teeth are being used (consuming browse of exceptionally large diameter, unusual diet, etc) may be contributing to the deterioration of moose incisor teeth. Tooth wear effects have been documented to occur in other ungulate species as a result of changes in diet related to density-related food limitations (Skogland, 1988; Kojola *et al.*, 1998 and others). Incisor breakage in Alaskan moose was attributed to increased physical stress upon the teeth caused by density-related decreases in suitable food resources (Smith, 1992). The moose of Cape Breton and Newfoundland, particularly the northernmost regions of each province where breakage frequencies are the elevated, live in much higher densities than in the remaining regions studied (Chapter 2). Food resources may be inadequate for sustaining such large moose populations in these regions and subsequently, animals must rely on the consumption of unsuitable materials for sustenance. The incisor teeth may not be designed to accommodate the physical stress required for unusual food sources and consequently, breakage occurs. This alternative hypothesis remains unexplored but appears to warrant further investigation.

Future studies to explore possible causal factors in elevated frequencies of moose incisor breakage in Atlantic Canada should examine the diet and use of teeth by moose throughout the region, with focus on northern Newfoundland. Variation in the consumption of browse species, browse diameter, and unusual behaviours related to nutritional distress (ie. barkstripping, osteophagia) may be associated with deteriorating incisal condition. Such studies could be conducted through observation of animal consumption, or through faecal pellet or stomach content analysis. Additionally, it may be interesting to perform chemical analyses of browse material to determine whether the Cu, Zn, and Pb availability is, in fact, lower in regions of increased moose incisor breakage.

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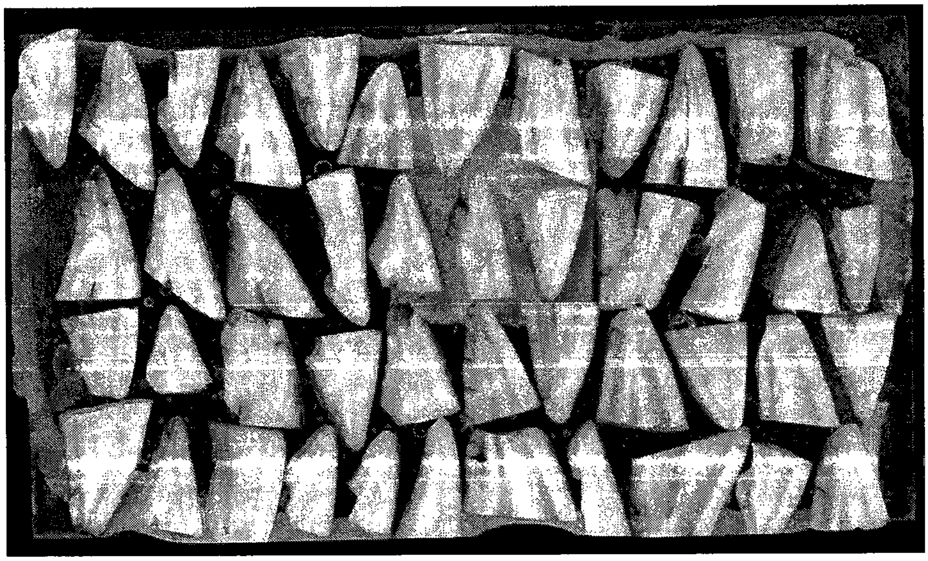
APPENDIX A

PREPARED SAMPLE SLIDE FOR LA-ICPMS

Prepared sample slide of 45 moose incisor tooth longitudinal sections for elemental analysis using LA-ICPMS. A total of 20 similar slides were prepared.



APPENDIX B

LA-ICPMS ANALYSIS RESULTS FOR QUALITY CONTROL MATERIAL, NIST 612 GLASS

Summary statistics for LA-ICPMS analysis quality control material, NIST 612 glass, statistics are provided for both inner and outer enamel analyses.

Element	n	Inner Concentration (ppm)					n	Outer Concentration (ppm)				
Isotope		Min	Max	Average	S.D.	RSD (%)		Min	Max	Average	S.D.	RSD (%)
Be^{9}	244	19.48	1527.83	43.76	95.41	218.01	244	1.94	75.30	37.74	3.65	9.66
B^{11}	244	21.84	47.84	34.73	1.62	4.67	244	21.84	47.84	34.73	1.59	4.57
Cu^{63}	244	32.63	40.78	36.71	0.72	1.97	244	32.37	41.08	36.71	0.92	2.51
Zn^{67}	244	25.36	50.72	37.92	1.93	5.09	244	24.48	55.88	38.01	2.29	6.01
As^{75}	244	33.57	41.10	37.33	0.95	2.53	244	32.58	42.05	37.33	1.04	2.77
Rb^{85}	244	30.79	32.47	31.63	0.30	0.93	244	29.53	33.70	31.63	0.42	1.32
Sr^{88}	244	74.39	77.91	76.15	0.62	0.81	244	69.94	82.39	76.15	0.83	1.09
Cd^{111}	244	27.19	29.45	28.32	0.39	1.38	244	25.58	31.08	28.32	0.47	1.65
Sn^{118}	244	32.21	43.76	37.96	0.64	1.70	244	32.21	43.76	37.96	0.73	1.93
Ba^{137}	244	34.00	41.50	37.74	0.54	1.43	244	31.75	43.79	37.74	0.91	2.42
Pb^{208}	244	37.59	40.33	38.96	0.42	1.08	244	31.03	46.99	38.96	0.84	2.15
Th^{232}	244	36.33	38.12	37.23	0.33	0.89	244	26.86	47.76	37.23	1.01	2.71
U^{238}	244	35.63	38.67	37.15	0.36	0.98	244	26.55	47.93	37.15	1.04	2.79

APPENDIX C

LA-ICPMS REFERENCE MATERIAL, DURANGO APATITE-SECTION 1, ANALYSIS RESULTS

Summary statistics for LA-ICPMS analysis reference material, Durango apatite-Section 1, for both inner and outer enamel matrices.

Element	n	Inner Concentration (ppm)					n	Outer Concentration (ppm)				
Isotope		Min	Max	Average	S.D.	RSD (%)		Min	Max	Average	S.D.	RSD (%)
Be^{9}	55	7.85	11.41	8.91	0.73	8.20	55	7.35	13.06	8.99	0.97	10.83
B^{11}	56	12.38	17.34	15.07	1.04	6.93	56	10.68	17.42	14.99	1.20	8.03
Cu^{63}	26	0.24	1.79	0.65	0.43	65.92	28	0.24	1.79	0.65	0.42	64.27
Zn^{67}	39	1.41	17.60	3.88	3.49	90.03	40	1.36	17.60	4.07	3.57	87.74
As^{75}	56	1068.61	1322.91	1207.88	66.74	5.53	56	1068.61	1377.71	1208.40	69.80	5.78
Rb^{85}	45	0.07	0.19	0.10	0.02	21.02	44	0.07	0.19	0.10	0.02	21.80
Sr^{88}	56	441.05	519.91	509.84	11.02	2.16	56	471.38	519.58	510.48	7.82	1.53
Cd^{111}	7	0.05	0.15	0.09	0.03	31.89	6	0.05	0.15	0.09	0.04	40.18
Sn^{118}	11	0.07	0.19	0.14	0.04	27.34	11	0.09	0.20	0.15	0.04	27.52
Ba^{137}	56	1.42	2.33	1.86	0.21	11.08	56	1.42	2.40	1.88	0.22	11.45
Pb^{208}	56	0.87	1.21	1.05	0.09	8.47	56	0.87	1.36	1.06	0.10	9.73
Th^{232}	56	176.46	272.06	263.47	13.21	5.01	56	222.72	273.90	264.52	8.01	3.03
U^{238}	56	10.61	12.23	11.81	0.31	2.65	56	10.89	13.44	11.88	0.35	2.96

APPENDIX D

ANALYSIS RESULTS OF LA-ICPMS REFERENCE MATERIAL:

DURANGO APATITE-SECTION 2

Summary statistics for LA-ICPMS analysis reference material, Durango apatite-Section 2, for both inner and outer enamel matrices.

Element	n	Inner Concentration (ppm)					n	Outer Concentration (ppm)				
Isotope		Min	Max	Average	S.D.	RSD (%)		Min	Max	Average	S.D.	RSD (%)
Be^{9}	61	6.71	10.91	9.53	0.76	7.93	56	6.85	10.91	9.52	0.73	7.70
B^{11}	61	10.93	17.06	13.68	0.99	7.24	56	10.93	15.55	13.66	0.91	6.64
Cu^{63}	36	0.28	4.01	0.88	1.00	112.95	34	0.28	4.01	0.88	1.00	114.26
Zn^{67}	31	1.65	5.53	3.62	0.98	27.03	29	1.65	5.54	3.50	1.03	29.61
As^{75}	61	969.90	1260.05	1135.90	55.97	4.93	56	970.17	1260.05	1135.66	56.72	4.99
Rb^{85}	46	0.05	0.28	0.10	0.04	35.21	42	0.07	0.28	0.10	0.03	33.97
Sr^{88}	61	497.85	531.76	509.99	6.25	1.23	56	497.85	531.76	510.19	6.02	1.18
Cd^{111}	2	0.06	0.08	0.07	0.01	20.50	2	0.06	0.08	0.07	0.01	14.45
Sn^{118}	12	0.08	0.38	0.13	0.08	60.98	10	0.10	0.38	0.14	0.08	58.54
Ba^{137}	61	1.57	2.87	1.91	0.23	11.94	56	1.54	2.35	1.91	0.23	11.86
Pb^{208}	61	0.74	2.40	1.10	0.41	37.29	56	0.74	2.40	1.11	0.42	37.86
Th^{232}	61	230.98	286.66	254.18	14.88	5.86	56	231.03	286.66	254.29	14.83	5.83
U^{238}	61	10.93	12.77	11.72	0.38	3.21	56	10.93	12.77	11.72	0.37	3.17

APPENDIX E

LA-ICPMS RUN DETECTION LIMITS FOR INNER AND OUTER ENAMEL ANALYSES

LA-ICPMS Run detection limits for inner and outer enamel analyses

Element	n	Inner Concentration (ppm)					n	Outer Concentration (ppm)				
Isotope		Min	Max	Average	S.D.	RSD (%)		Min	Max	Average	S.D.	RSD (%)
Be^{9}	61	0.14	0.37	0.22	0.04	16.73	61	0.10	0.20	0.16	0.02	12.37
B^{11}	61	0.09	0.22	0.15	0.03	19.04	61	0.06	0.20	0.11	0.02	21.99
Cu^{63}	61	0.08	0.19	0.12	0.03	21.89	61	0.05	0.14	0.09	0.02	20.71
Zn^{67}	61	0.26	1.58	0.79	0.25	31.42	61	0.18	1.10	0.57	0.16	28.71
As^{75}	61	0.06	0.14	0.10	0.02	17.87	61	0.04	0.64	0.08	0.07	89.54
Rb^{85}	61	0.01	0.04	0.02	0.01	36.68	61	0.01	0.03	0.01	0.00	34.03
Sr^{88}	61	0.02	0.07	0.05	0.01	18.42	61	0.02	0.14	0.04	0.02	44.43
Cd^{111}	61	0.02	0.07	0.04	0.01	24.88	61	0.01	0.05	0.03	0.01	23.15
Sn^{118}	61	0.02	0.08	0.05	0.01	25.53	61	0.02	0.06	0.03	0.01	22.78
Ba^{137}	61	0.04	0.12	0.08	0.02	20.36	61	0.03	0.18	0.06	0.02	37.95
Pb^{208}	61	0.00	0.04	0.01	0.00	53.56	61	0.00	0.02	0.01	0.00	39.59
Th^{232}	61	0.00	0.01	0.00	0.00	55.01	61	0.00	0.09	0.00	0.01	357.69
U^{238}	61	0.00	0.01	0.00	0.00	77.80	61	0.00	0.02	0.00	0.00	149.74

APPENDIX F

SUMMARY OF ATLANTIC CANADIAN MOOSE INCISOR ELEMENTAL COMPOSITION, BY AGE AND REGION

Summary statistics of New Brunswick moose incisor elemental composition, by age, for inner incisal tooth enamel.

New Brunswick Inner Enamel												
Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	359	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
1.5	29	5.32 (1.51)	2.06 (1.44)	19.18 (6.65)	0.52 (0.47)	0.46 (0.27)	226.68 (72.02)	0.42 (0.28)	126.21 (87.34)	0.59 (0.82)	0.005 (0.014)	0.020 (0.086)
2.5	91	6.83 (2.57)	2.66 (2.18)	21.65 (7.67)	0.42 (0.33)	0.45 (0.28)	221.35 (96.94)	0.26 (0.20)	147.57 (86.10)	0.53 (0.48)	0.001 (0.003)	0.005 (0.011)
3.5	21	4.95 (1.84)	1.82 (0.96)	18.44 (4.59)	0.59 (0.46)	0.59 (0.30)	258.37 (89.19)	0.51 (0.26)	163.45 (80.44)	0.67 (0.79)	0.001 (0.003)	0.010 (0.025)
4.5	21	5.13 (1.74)	2.28 (1.52)	17.08 (3.56)	0.49 (0.23)	0.51 (0.24)	214.69 (56.18)	0.58 (0.28)	166.46 (86.82)	0.56 (0.62)	0.001 (0.003)	0.003 (0.004)
5.5	53	6.23 (3.76)	2.72 (3.99)	19.08 (6.05)	0.50 (0.65)	0.62 (0.28)	230.42 (87.70)	0.32 (0.26)	142.37 (79.21)	0.53 (0.51)	0.002 (0.003)	0.024 (0.11)
6.5	60	5.81 (2.30)	2.86 (2.68)	19.48 (5.56)	0.43 (0.48)	0.56 (0.27)	203.42 (78.23)	0.32 (0.30)	133.88 (79.23)	0.50 (0.38)	0.004 (0.011)	0.016 (0.047)
7.5	21	5.79 (2.14)	2.75 (2.85)	21.46 (7.88)	0.48 (0.84)	0.59 (0.25)	202.55 (83.49)	0.34 (0.28)	137.44 (79.47)	0.48 (0.40)	0.002 (0.004)	0.015 (0.054)
8.5	18	6.09 (1.83)	1.96 (1.60)	20.27 (7.47)	0.49 (0.26)	0.46 (0.25)	203.34 (83.94)	0.36 (0.29)	149.15 (94.99)	0.52 (0.51)	0.002 (0.002)	0.003 (0.005)
9.5	24	7.13 (5.76)	2.33 (1.68)	20.80 (8.47)	0.41 (0.26)	0.59 (0.31)	247.05 (183.81)	0.36 (0.25)	136.73 (76.24)	0.67 (0.87)	0.001 (0.001)	0.009 (0.023)
10.5	9	6.64 (2.78)	2.11 (1.12)	19.83 (6.55)	0.32 (0.15)	0.51 (0.31)	239.52 (109.97)	0.32 (0.26)	164.13 (81.30)	0.62 (0.62)	0.001 (0.001)	0.004 (0.008)
11.5	4	6.01 (1.32)	2.53 (2.16)	20.70 (3.99)	0.34 (0.06)	0.69 (0.09)	142.48 (33.43)	0.18 (0.13)	90.35 (19.74)	0.61 (0.43)	0.0004 (0.0005)	0.010 (0.011)
12.5	4	5.56 (2.80)	1.39 (0.31)	19.88 (6.59)	0.26 (0.16)	0.43 (0.35)	169.07 (19.75)	0.26 (0.04)	152.72 (168.92)	0.46 (0.16)	0.001 (0.0013)	0.001 (0.001)
13.5	4	6.52 (2.52)	2.00 (1.09)	19.08 (4.57)	0.31 (0.16)	0.56 (0.31)	158.52 (63.67)	0.15 (0.07)	109.35 (48.87)	0.46 (0.24)	0.003 (0.003)	0.002 (0.003)
Mean (s.d.)		6.00 (2.53)	2.27 (1.82)	19.76 (6.12)	0.43 (0.35)	0.54 (0.27)	209.04 (81.41)	0.34 (0.22)	139.99 (82.21)	0.55 (0.53)	0.002 (0.004)	0.009 (0.030)

Summary statistics of New Brunswick moose incisor elemental composition, by age, for outer incisal tooth enamel.

New Brunswick Outer Enamel												
Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	359	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
1.5	29	7.48 (1.84)	1.82 (1.69)	107.13 (43.72)	0.45 (0.29)	0.46 (0.27)	208.28 (70.79)	0.44 (0.34)	124.46 (90.13)	0.51 (0.58)	0.006 (0.021)	0.005 (0.015)
2.5	91	12.76 (9.12)	2.86 (3.33)	110.26 (35.63)	0.43 (0.25)	0.44 (0.24)	197.48 (81.16)	0.26 (0.25)	149.53 (92.04)	0.66 (0.65)	0.001 (0.002)	0.005 (0.023)
3.5	21	6.97 (2.86)	1.64 (1.17)	79.12 (33.48)	0.58 (0.54)	0.56 (0.25)	237.97 (83.63)	0.58 (0.26)	165.97 (84.22)	0.76 (0.98)	0.001 (0.003)	0.004 (0.010)
4.5	21	7.49 (3.33)	2.40 (1.69)	78.35 (45.47)	1.07 (1.55)	0.49 (0.22)	194.91 (55.29)	0.59 (0.34)	160.38 (87.70)	0.66 (0.75)	0.001 (0.004)	0.002 (0.004)
5.5	53	9.77 (4.69)	2.00 (3.07)	81.94 (37.81)	0.52 (0.78)	0.62 (0.28)	205.94 (73.58)	0.30 (0.29)	143.31 (86.34)	0.59 (0.69)	0.001 (0.002)	0.008 (0.030)
6.5	60	10.48 (6.89)	2.53 (3.12)	87.91 (37.13)	0.46 (0.38)	0.55 (0.24)	182.32 (70.50)	0.29 (0.33)	128.74 (70.12)	0.57 (0.53)	0.006 (0.015)	0.016 (0.050)
7.5	21	8.13 (2.86)	2.35 (2.81)	86.47 (33.20)	0.73 (1.08)	0.58 (0.25)	176.24 (65.07)	0.31 (0.30)	130.23 (70.61)	0.51 (0.37)	0.003 (0.005)	0.024 (0.103)
8.5	18	8.88 (3.58)	1.81 (2.29)	86.87 (53.28)	0.59 (0.49)	0.52 (0.24)	186.74 (76.90)	0.37 (0.30)	144.02 (99.28)	0.71 (0.83)	0.002 (0.004)	0.001 (0.001)
9.5	24	9.62 (5.24)	2.31 (1.88)	75.50 (46.39)	0.68 (0.76)	0.58 (0.29)	228.61 (166.51)	0.36 (0.29)	137.65 (83.35)	0.77 (1.01)	0.001 (0.001)	0.003 (0.007)
10.5	9	7.52 (3.10)	1.62 (0.56)	65.73 (34.66)	0.38 (0.23)	0.50 (0.25)	223.64 (104.81)	0.34 (0.37)	164.93 (76.95)	0.83 (1.05)	0.001 (0.001)	0.003 (0.005)
11.5	4	8.61 (2.64)	2.00 (2.08)	76.65 (61.63)	0.34 (0.14)	0.56 (0.14)	127.94 (27.91)	0.25 (0.31)	92.60 (17.46)	0.78 (0.60)	0.002 (0.003)	0.003 (0.004)
12.5	4	15.61 (11.94)	2.16 (2.20)	94.65 (39.75)	0.28 (0.23)	0.44 (0.38)	155.25 (23.11)	0.24 (0.16)	179.04 (203.60)	0.61 (0.17)	0.001 (0.0005)	0.002 (0.002)
13.5	4	12.19 (2.87)	1.11 (0.72)	86.62 (21.28)	0.42 (0.03)	0.50 (0.24)	139.97 (55.51)	0.09 (0.03)	93.96 (37.97)	0.28 (0.14)	0.001 (0.001)	0.003 (0.003)
Mean (s.d.)		9.65 (4.69)	2.05 (2.05)	85.94 (40.26)	0.53 (0.52)	0.52 (0.25)	189.64 (73.44)	0.34 (0.27)	139.60 (84.60)	0.63 (0.64)	0.002 (0.005)	0.010 (0.020)

Summary statistics of Cape Breton moose incisor elemental composition, by age, for inner incisal tooth enamel. In place of the standard deviation for age classes with only one individual, (-) is indicated.

Cape Breton Inner Enamel												
Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	323	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
1.5	58	5.21 (1.25)	3.04 (2.53)	19.63 (5.45)	0.40 (0.66)	0.49 (0.17)	223.77 (54.91)	0.51 (0.58)	81.41 (34.84)	0.68 (0.91)	0.002 (0.005)	0.013 (0.063)
2.5	55	5.35 (2.59)	2.40 (2.5)	19.59 (7.19)	0.39 (0.28)	0.51 (0.17)	214.26 (50.77)	0.47 (0.47)	79.63 (30.60)	0.69 (1.00)	0.002 (0.005)	0.011 (0.027)
3.5	66	5.55 (2.36)	3.57 (3.07)	21.27 (7.53)	0.42 (0.41)	0.47 (0.20)	219.29 (49.80)	0.38 (0.31)	80.08 (31.42)	0.86 (1.17)	0.002 (0.007)	0.012 (0.035)
4.5	49	5.22 (1.73)	2.22 (1.71)	20.88 (6.88)	0.51 (0.65)	0.53 (0.19)	227.54 (62.39)	0.44 (0.36)	80.38 (36.20)	1.13 (1.54)	0.002 (0.005)	0.01 (0.027)
5.5	19	5.21 (1.42)	2.66 (1.73)	22.45 (9.00)	0.43 (0.31)	0.50 (0.21)	223.99 (46.73)	0.46 (0.35)	80.71 (26.56)	0.59 (0.49)	0.001 (0.003)	0.002 (0.003)
6.5	19	4.75 (1.18)	2.14 (0.67)	20.20 (4.52)	1.05 (2.20)	0.61 (0.31)	214.77 (48.87)	0.39 (0.23)	72.00 (22.20)	0.98 (1.23)	0.001 (0.004)	0.001 (0.002)
7.5	12	7.18 (4.61)	3.07 (2.43)	20.04 (6.53)	0.45 (0.21)	0.64 (0.54)	243.27 (51.18)	0.47 (0.51)	90.71 (36.22)	1.25 (2.01)	0.005 (0.009)	0.014 (0.035)
8.5	14	6.53 (2.36)	2.88 (2.49)	19.30 (6.19)	0.47 (0.31)	0.64 (0.28)	229.43 (53.67)	0.40 (0.27)	84.77 (23.19)	0.99 (1.44)	0.002 (0.004)	0.001 (0.001)
9.5	8	5.24 (2.08)	2.04 (1.56)	18.52 (3.18)	0.66 (0.75)	0.72 (0.32)	217.22 (54.01)	0.49 (0.51)	88.01 (51.24)	0.77 (0.51)	0.004 (0.007)	0.007 (0.009)
10.5	15	5.14 (1.16)	2.15 (1.63)	18.23 (3.67)	0.32 (0.26)	0.52 (0.17)	258.19 (74.16)	0.43 (0.36)	104.28 (50.42)	0.82 (1.14)	0.004 (0.01)	0.0004 (0.001)
11.5	1	4.72 (-)	1.10 (-)	15.86 (-)	0.32 (-)	0.64 (-)	277.33 (-)	0.04 (-)	151.37 (-)	0.20 (-)	0.001 (-)	0.002 (-)
12.5	3	5.64 (1.66)	12.86 (13.63)	28.7 (11.16)	0.74 (0.81)	0.85 (0.61)	251.21 (78.58)	0.30 (0.28)	105.78 (65.35)	0.78 (0.52)	0.000 (0.001)	0.001 (0.001)
13.5	1	4.45 (-)	13.27 (-)	29.03 (-)	0.42 (-)	0.53 (-)	171.80 (-)	0.16 (-)	36.81 (-)	0.62 (-)	0.001 (-)	0.001 (-)
14.5	3	5.60 (1.13)	3.00 (1.88)	17.82 (1.62)	0.39 (0.19)	0.73 (0.29)	198.39 (41.71)	0.17 (0.11)	80.99 (13.65)	0.34 (0.03)	0.015 (0.024)	0.027 (0.045)
Mean (s.d.)		5.41 (1.96)	4.03 (2.99)	20.82 (6.08)	0.50 (0.59)	0.60 (0.29)	226.46 (55.57)	0.37 (0.36)	86.92 (35.16)	0.76 (1.00)	0.003 (0.007)	0.007 (0.021)

Summary statistics of Cape Breton moose incisor elemental composition, by age, for outer incisal tooth enamel. In place of the standard deviation for age classes with only one individual, (-) is indicated.

Cape Breton Outer Enamel												
Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	323	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
1.5	58	14.03 (7.67)	3.71 (5.63)	139.07 (53.99)	0.35 (0.28)	0.52 (0.18)	202.21 (45.73)	0.64 (0.86)	80.85 (29.12)	1.03 (1.11)	0.004 (0.014)	0.012 (0.04)
2.5	55	10.99 (5.71)	2.48 (3.63)	118.15 (36.96)	0.51 (0.46)	0.54 (0.19)	196.38 (41.92)	0.52 (0.65)	79.86 (27.66)	0.95 (1.37)	0.004 (0.014)	0.003 (0.01)
3.5	66	12.96 (9.83)	3.38 (3.02)	127.21 (70.44)	0.43 (0.29)	0.50 (0.24)	196.11 (39.94)	0.43 (0.56)	80.05 (34.44)	1.37 (1.84)	0.007 (0.030)	0.011 (0.04)
4.5	49	11.48 (6.21)	2.78 (4.05)	116.35 (31.98)	0.52 (0.58)	0.55 (0.19)	209.15 (50.79)	0.46 (0.45)	81.75 (37.93)	1.57 (2.09)	0.003 (0.010)	0.010 (0.03)
5.5	19	10.87 (5.05)	2.35 (1.62)	125.45 (58.79)	0.69 (0.79)	0.58 (0.41)	200.23 (42.09)	0.68 (1.02)	81.54 (30.74)	0.85 (0.75)	0.001 (0.004)	0.010 (0.02)
6.5	19	8.93 (4.36)	2.05 (1.88)	99.63 (32.02)	0.57 (0.47)	0.64 (0.35)	199.97 (40.89)	0.36 (0.23)	76.36 (23.85)	1.19 (2.14)	0.0003 (0.001)	0.001 (0.001)
7.5	12	12.77 (4.79)	2.09 (2.12)	125.87 (36.27)	0.35 (0.23)	0.47 (0.13)	213.45 (51.65)	0.49 (0.54)	86.51 (32.14)	1.63 (2.50)	0.002 (0.004)	0.002 (0.003)
8.5	14	18.77 (11.42)	3.25 (3.31)	107.65 (40.31)	0.44 (0.23)	0.72 (0.33)	204.31 (38.27)	0.37 (0.35)	86.45 (18.24)	1.36 (2.84)	0.002 (0.004)	0.004 (0.006)
9.5	8	12.02 (7.50)	1.58 (0.90)	126.81 (52.26)	0.47 (0.28)	0.77 (0.42)	200.03 (43.27)	0.50 (0.76)	98.99 (54.45)	0.99 (0.39)	0.020 (0.051)	0.003 (0.003)
10.5	15	10.41 (6.09)	2.18 (1.54)	122.79 (37.81)	0.41 (0.24)	0.58 (0.21)	214.85 (47.03)	0.42 (0.36)	95.01 (43.62)	1.42 (1.86)	0.001 (0.002)	0.001 (0.002)
11.5	1	6.653 (-)	0.547 (-)	93.48 (-)	0.293 (-)	0.712 (-)	252.638 (-)	0.0575 (-)	153.813 (-)	0.169 (-)	0.000 (-)	0.0015 (-)
12.5	3	8.08 (2.47)	12.37 (13.22)	140.13 (16.43)	0.17 (0.01)	0.79 (0.36)	217.68 (56.85)	0.23 (0.15)	92.54 (45.61)	1.15 (0.78)	0.000 (0.001)	0.002 (0.001)
13.5	1	5.173 (-)	7.26 (-)	103.4 (-)	0.613 (-)	0.45 (-)	156.644 (-)	0.0815 (-)	33.799 (-)	0.521 (-)	0.001 (-)	0.002 (-)
14.5	3	8.02 (3.44)	1.97 (1.26)	101.08 (30.37)	0.47 (0.22)	0.88 (0.49)	175.85 (58.71)	0.18 (0.13)	78.28 (20.3)	0.43 (0.23)	0.018 (0.028)	0.032 (0.05)
Mean (s.d.)		10.80 (6.21)	3.43 (3.51)	117.65 (41.47)	0.45 (0.34)	0.62 (0.29)	202.82 (46.43)	0.39 (0.5)	86.13 (33.17)	1.04 (1.49)	0.004 (0.013)	0.006 (0.016)

Summary statistics of Newfoundland moose incisor elemental composition, by age, for inner incisal tooth enamel.

Newfoundland Inner Enamel

Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	369	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
2.5	150	6.25 (3.38)	2.36 (1.90)	18.05 (6.54)	0.46 (0.28)	0.29 (0.14)	153.17 (85.71)	0.15 (0.12)	55.20 (28.47)	0.48 (0.53)	0.003 (0.005)	0.020 (0.080)
3.5	11	5.50 (1.63)	1.51 (1.10)	16.43 (3.28)	0.45 (0.35)	0.31 (0.21)	145.40 (63.97)	0.13 (0.08)	69.07 (41.45)	0.48 (0.67)	0.003 (0.003)	0.010 (0.030)
4.5	11	5.04 (1.75)	1.73 (0.84)	16.08 (2.74)	0.23 (0.14)	0.28 (0.17)	198.23 (83.78)	0.12 (0.07)	58.86 (24.58)	0.34 (0.14)	0.002 (0.003)	0.001 (0.003)
5.5	31	5.71 (2.95)	2.63 (3.11)	18.08 (7.46)	0.27 (0.17)	0.29 (0.17)	117.01 (77.76)	0.14 (0.09)	47.12 (23.41)	0.40 (0.25)	0.002 (0.002)	0.010 (0.030)
6.5	54	5.44 (1.14)	3.01 (5.17)	17.77 (6.62)	0.39 (0.71)	0.48 (0.28)	153.70 (77.21)	0.20 (0.37)	68.34 (34.95)	0.43 (0.39)	0.002 (0.006)	0.030 (0.120)
7.5	40	5.57 (1.38)	3.28 (4.58)	18.35 (7.03)	0.93 (2.02)	0.44 (0.23)	139.42 (60.44)	0.17 (0.14)	62.19 (32.35)	0.46 (0.38)	0.004 (0.007)	0.040 (0.150)
8.5	8	5.59 (1.13)	2.32 (0.59)	63.64 (127.19)	0.33 (0.26)	0.44 (0.17)	161.62 (57.28)	0.22 (0.11)	68.59 (37.91)	0.58 (0.23)	0.002 (0.002)	0.030 (0.040)
9.5	16	5.89 (2.45)	4.03 (5.09)	18.46 (6.36)	0.31 (0.32)	0.41 (0.24)	154.83 (82.98)	0.16 (0.19)	53.40 (28.87)	0.40 (0.22)	0.002 (0.003)	0.010 (0.010)
10.5	12	4.78 (1.06)	1.77 (1.12)	16.33 (2.52)	0.32 (0.27)	0.31 (0.18)	146.30 (89.41)	0.11 (0.11)	48.59 (28.96)	0.45 (0.19)	0.003 (0.004)	0.020 (0.040)
11.5	11	5.22 (1.86)	4.29 (8.42)	18.67 (9.23)	0.36 (0.37)	0.36 (0.18)	116.72 (49.04)	0.19 (0.11)	47.77 (21.18)	0.54 (0.41)	0.003 (0.002)	0.110 (0.350)
12.5	9	4.92 (0.97)	3.21 (1.83)	18.82 (3.09)	1.39 (3.47)	0.34 (0.22)	119.80 (75.41)	0.21 (0.21)	48.30 (23.57)	0.58 (0.44)	0.004 (0.006)	0.030 (0.090)
13.5	6	5.07 (0.91)	1.99 (2.06)	25.42 (14.24)	0.60 (0.58)	0.61 (0.35)	129.09 (58.76)	0.38 (0.49)	47.72 (16.09)	0.68 (0.41)	0.010 (0.013)	0.010 (0.010)
14.5	5	5.15 (2.00)	2.76 (1.61)	19.50 (4.58)	0.36 (0.26)	0.37 (0.13)	106.14 (67.57)	0.17 (0.14)	39.88 (18.54)	0.53 (0.30)	0.001 (0.0004)	0.003 (0.002)
15.5	3	5.50 (3.07)	1.85 (2.03)	16.27 (3.45)	0.23 (0.10)	0.66 (0.67)	102.75 (77.97)	0.15 (0.14)	47.76 (49.76)	1.00 (1.14)	0.000 (0.000)	0.001 (0.001)
16.5	2	5.11 (1.43)	4.02 (0.57)	18.83 (4.61)	0.24 (0.01)	0.96 (0.51)	133.22 (5.00)	0.19 (0.14)	89.27 (36.04)	0.45 (0.09)	0.002 (0.000)	0.001 (0.001)
Mean (s.d.)		5.68 (1.81)	2.62 (2.67)	20.70 (13.93)	0.44 (0.62)	0.44 (0.26)	141.57 (67.49)	0.18 (0.17)	57.86 (29.74)	0.49 (0.39)	0.003 (0.004)	0.019 (0.064)

Summary statistics of Newfoundland moose incisor elemental composition, by age, for outer incisal tooth enamel.

Newfoundland Outer Enamel												
Age	n	B	Cu	Zn	As	Rb	Sr	Sn	Ba	Pb	Th	U
Total	369	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)	mean (s.d.)
2.5	150	16.73 (9.94)	3.88 (5.41)	131.1 (38.03)	0.60 (0.42)	0.34 (0.17)	137.91 (75.57)	0.24 (0.57)	55.11 (29.17)	1.03 (4.50)	0.003 (0.004)	0.01 (0.059)
3.5	11	11.56 (4.36)	1.79 (1.4)	112.32 (42.89)	0.56 (0.33)	0.34 (0.19)	133.94 (56.65)	0.13 (0.12)	71.70 (42.16)	0.66 (0.32)	0.004 (0.005)	0.013 (0.037)
4.5	11	14.12 (5.63)	2.13 (1.52)	97.28 (51.76)	0.23 (0.12)	0.33 (0.14)	176.28 (71.22)	0.24 (0.24)	58.55 (23.65)	1.03 (2.02)	0.002 (0.003)	0.001 (0.001)
5.5	31	16.24 (10.23)	3.64 (6.28)	126.42 (42.32)	0.39 (0.29)	0.31 (0.10)	101.94 (62.51)	0.15 (0.14)	45.51 (22.30)	0.65 (0.51)	0.002 (0.004)	0.006 (0.024)
6.5	54	14.34 (8.70)	3.66 (4.81)	112.96 (37.87)	0.39 (0.45)	0.46 (0.30)	138.17 (68.71)	0.26 (0.62)	67.63 (36.20)	0.54 (0.38)	0.004 (0.011)	0.004 (0.008)
7.5	40	14.83 (7.14)	3.81 (3.82)	115.61 (58.01)	0.42 (0.24)	0.47 (0.24)	125.24 (58.23)	0.18 (0.13)	64.24 (37.11)	0.69 (0.48)	0.002 (0.003)	0.002 (0.003)
8.5	8	11.65 (3.30)	2.95 (1.49)	122.23 (52.32)	0.34 (0.18)	0.46 (0.18)	150.36 (52.86)	0.26 (0.17)	70.09 (35.51)	0.61 (0.43)	0.002 (0.002)	0.002 (0.002)
9.5	16	13.04 (6.55)	3.64 (4.94)	94.20 (43.88)	0.34 (0.26)	0.45 (0.27)	141.38 (75.61)	0.34 (0.75)	52.27 (29.23)	0.55 (0.31)	0.002 (0.004)	0.002 (0.001)
10.5	12	11.34 (3.91)	2.16 (1.40)	110.77 (38.04)	0.40 (0.31)	0.35 (0.17)	130.35 (79.24)	0.11 (0.06)	49.37 (27.97)	0.57 (0.35)	0.004 (0.005)	0.017 (0.051)
11.5	11	9.32 (4.31)	3.69 (8.67)	81.07 (43.57)	0.65 (0.57)	0.30 (0.14)	108.34 (51.81)	0.12 (0.05)	45.05 (18.5)	0.56 (0.64)	0.004 (0.005)	0.143 (0.403)
12.5	9	10.88 (6.87)	6.58 (5.70)	111.65 (29.41)	0.50 (0.63)	0.35 (0.18)	103.50 (69.19)	0.31 (0.35)	43.94 (22.00)	0.96 (0.65)	0.003 (0.006)	0.013 (0.031)
13.5	6	15.63 (14.26)	7.69 (9.97)	115.58 (60.25)	0.51 (0.20)	0.70 (0.35)	116.05 (56.66)	0.28 (0.30)	43.38 (16.25)	1.15 (1.23)	0.006 (0.005)	0.003 (0.004)
14.5	5	10.15 (6.98)	4.76 (4.65)	65.54 (49.73)	0.34 (0.15)	0.44 (0.17)	100.79 (68.05)	0.18 (0.07)	46.59 (36.25)	0.73 (0.41)	0.001 (0.001)	0.003 (0.001)
15.5	3	7.02 (3.61)	8.94 (13.62)	126.48 (22.10)	0.41 (0.26)	0.67 (0.68)	88.47 (61.88)	0.30 (0.35)	44.29 (39.82)	3.44 (5.39)	0.002 (0.001)	0.004 (0.004)
16.5	2	11.25 (0.09)	25.04 (33.87)	110.48 (101.29)	0.31 (0.01)	0.73 (0.05)	106.04 (19.05)	0.13 (0.08)	86.00 (40.12)	0.75 (0.61)	0.001 (0.001)	0.001 (0.000)
Mean (s.d.)		12.54 (6.39)	5.62 (7.17)	108.91 (47.43)	0.43 (0.29)	0.45 (0.22)	123.92 (61.82)	0.22 (0.27)	56.25 (30.42)	0.93 (1.22)	0.003 (0.004)	0.015 (0.042)