ELEMENTAL COMPOSITION OF INCISORS IN NOVA SCOTIA MOOSE: EVALUATION OF A POPULATION WITH ABNORMAL INCISOR BREAKAGE

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ABSTRACT: This study compared the concentrations of major and trace elements in the enamel of incisors from moose (*Alces alces andersoni*) in Cape Breton Highlands, where the incidence of incisor tooth breakage was believed to be unusually high, and moose in southwest Nova Scotia (*A. a. americana*) where there was no evidence of breakage. Our goal was to determine which elements, if any, might be related to the incisor breakage in moose from Cape Breton Highlands. There was a positive relationship between age and frequency of incisor breakage, and most moose had a broken I2 incisor by 4 years of age in the Cape Breton Highlands. We analyzed I2 incisors for 51 trace elements with Inductively Coupled Plasma-Mass Spectrometry. Concentrations of 8 elements, including barium, beryllium, cadmium, cobalt, lead, tin, strontium, and yttrium, were lower ($P < 0.05$) in incisors from Cape Breton Highlands; gallium had a higher concentration. Reduced intake of barium, beryllium, and strontium is linked to depressed growth and reduced calcification of bones and teeth.

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The role that major and trace elements play in the metabolism of organisms has been the focus of intense, well-documented research (Underwood 1977, Prasad 1993, Underwood and Suttle 1999, Bogden and Klevay 2000). Disease may result from either a toxic or a deficient dietary intake of elements, and is often a reflection of geo-environmental factors (Maisironi 2000). Teeth are good indicators of the abundance of many elements (Brown et al. 2004, Kang et al. 2004, Dolphin et al. 2005) due to the crystal structure of enamel (Sharaway and Yeager 1991, Simmelink 1994). Major and trace elements are incorporated within the hydroxyapatite crystal framework during the mineralization period (Sharaway and Yeager 1991, Simmelink 1994) and, due to the semi-permeable nature of hydroxyapatite, small ions and molecules are able to pass through the enamel framework preceding eruption (Cutress 1983). Therefore, to an extent, teeth remain in chemical equilibrium with the oral environment (Zimmerman 1976, Driessens 1982).

Strength and solubility of enamel is influenced by the concentration of major and trace elements (Zimmerman 1976), which is affected by various factors such as normal wear, food composition, and regional geochemistry (Cutress 1983). Therefore teeth are an excellent bio-indicator of local environmental conditions (Lee et al. 1999, Lochner et al. 1999, Gdula-Argasinska et al. 2004). This is especially true for non-migratory ruminant

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herbivores such as moose (*Alces alces*) that obtain their entire dietary mineral intake from distinct regional geological localities (Cederlund and Okarma 1988, Lepitch and Gilbert 1989, Hundertmark 1998).

Observations of physical and behavioral anomalies suggest that the moose population is stressed in the Cape Breton Highlands (*A. a. andersoni*) in Nova Scotia, Canada. Wildlife authorities have observed osteophagia (Roger and Nette 2002), an increased incidence of bark stripping, and tooth breakage (Fig. 1) in Cape Breton Highlands moose where densities are high and heavy browsing of preferred vegetation is evident. Distinct browse lines 2–3 meters off the ground extend several kilometers through the forest (Basquill and Thompson 1997).

The only previously reported case of incisor breakage was in a moose (*A. a. gigas*) population on the Seward Peninsula, Alaska. Smith (1992) documented 'incisiform breakage' over a 2-year period (1988-1990), which closely resembles the breakage observed in Cape Breton Highlands moose. The breakage observed in the Cape Breton Highlands is markedly different from the tooth wear frequently reported in other ungulate species (Hewison et al. 1999, Loe et al. 2003). It also differs from the incisiform wear reported in Kenai Peninsula moose by Peterson et al. (1982), and the unusual wear described by Young and Marty (1986) in a Manitoban moose population. The distinctive incisor breakage begins as a brown stained crack on the tooth surface (Fig. 1) that is a precursor to breakage. Where breakage has occurred, the tooth is subsequently rounded down and stained brown. This rounded, staining characteristic is important because it indicates that breakage occurred during the lifetime and not at, or after, death.

The purpose of this study was to determine whether concentrations of major and trace elements in incisors might explain breakage. To do this we compared trace element concentrations of teeth from Cape Breton Highlands moose to those of a control population on mainland Nova Scotia (*A. a. americana*) without evidence of abnormal incisor breakage.

STUDY AREAS

The Highlands region is located in northern Cape Breton Island, in northeastern Nova Scotia, and covers an area of approximately 3,900 km^2. The area is a characteristic boreal region (Pulsifer and Nette 1995) with a peak elevation of approximately 535 m above sea level (Canada: National and Historic Parks Branch 1970). Approximately 1,600 mm of precipitation is recorded annually, with > 400 cm falling as snow, resulting in snow pack that lasts 181-212 days (Phillips 1990).

The control area was the Tobeatic Wilderness Area, which is a characteristic Acadian Forest Region (Farrier et al. 1991). The area receives approximately 1,400 mm of precipitation annually, with 150-200 cm falling as snow, resulting in a snow pack that lasts 110-140 days (Phillips 1990).

METHODS

In the Cape Breton Highlands, incisor samples were obtained from hunters during the 2001 hunting season from management zones immediately north and south of Cape

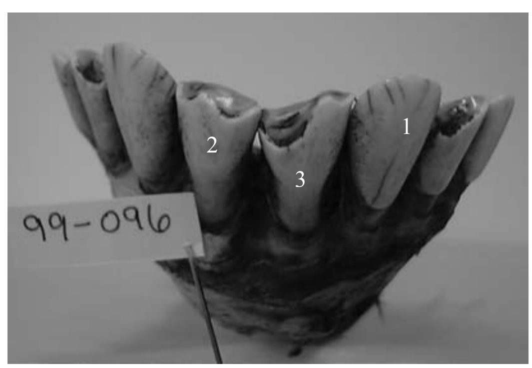

Fig 1. Lower mandible from a Cape Breton Highlands moose. Numbers on teeth represent breakage scores. Note the stained, polished, and rounded surfaces of the fractured teeth indicating breakage occurred during lifetime of the animal (Photo by Vince Power, 2001).

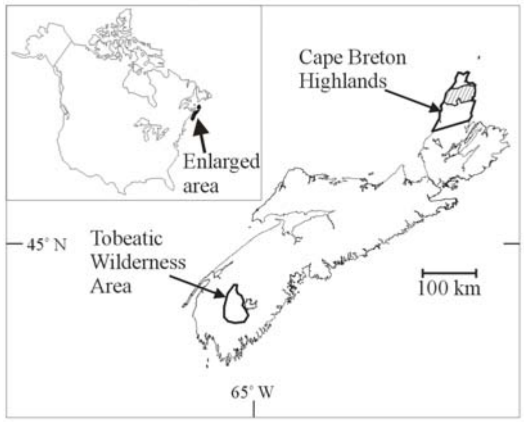

Fig. 2: Moose teeth sample collection areas in Nova Scotia, Canada. Note: No samples were taken from Cape Breton Highlands National Park indicated by the lined inset area.

Breton Highlands National Park (Fig. 2). In the Tobeatic Wilderness area in southwestern Nova Scotia, incisors were obtained by the Nova Scotia Department of Natural Resources (NSDNR) from accidental kills, illegal kills, and live extraction when collaring animals as part of ongoing studies (Fig. 2).

Based on the work of Smith (1992), Nette and Power (NSDNR 1999) devised a 3-level classification system for categorizing the degree of incisor breakage used in this study: slight – indicates < 30% of tooth material missing from the incisal surface; moderate – indicates 30-60% of tooth material missing from the incisal surface; and severe – indicates > 60% of tooth material missing from the incisal surface. Individual I1s were used to determine age from cementum annuli measurements (Matson 1981).

The concentrations of 37 elements and the 14 rare earth elements (REE) were determined from the I2s. The REE comprising the lanthanide series were summed and considered as one element (Brown et al. 2000). The root was separated from the crown with a Buhler Isomet low speed saw with a diamond tip blade. Crowns were crushed with a percussion cutter and enamel fragments were retrieved under a binocular microscope using a fine tipped paintbrush. Enamel was reduced to a fine powder using an agate mortar and pestle with ethanol as a lubricant. An ultrasound bath filled with distilled water was used to clean the agate mortar pestle between samples to prevent cross-contamination. Enamel was placed in drying dishes and dried at room temperature for 3-5 hours, recovered, and placed into labeled vials. Samples were sent to Geo Labs laboratory (Sudbury, Ontario, Canada) and analyzed for element content using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Two-sample *t*-tests were used to compare element concentrations of the two populations. Regression analysis was used to assess the relationship between age and breakage score (Sokal and Rohlf 1995) using 46 I2s collected during the 2001 hunting season. Age/breakage data compiled between 1996-2002 (NSDNR 2002) were analyzed using Chi-square analysis to test for significant differences in frequency of breakage between the I1s and I2s. Statistical significance was set at $\alpha = 0.05$.

RESULTS

There was a positive relationship between incisor breakage and age in Cape Breton Highlands moose (BREAKAGE SCORE = 1.28 + 0.158AGE, $P < 0.05$, $R^2 = 0.203$). The I1 had a higher frequency of breakage than the I2 ($\chi^2 = 189.7$, $P < 0.001$, df = 1). The frequency of breakage of I1 was 2-3x that of I2 at > 2.5 years old and 2 out of 3 moose had a broken I1 after 4.5 years. Breakage of I1 in moose > 3.5 years old was > 50% and more than doubled from 3.5 to 5.5 years old. Breakage of I2 was < 30% in moose < 6.5 years old (Table 1).

Relative to the control population in southwest Nova Scotia, Cape Breton Highlands moose had lower ($P < 0.05$) concentrations of barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), lead (Pb), strontium (Sr), tin (Sn), and yttrium (Y), and higher concentration of gallium (Ga) in the enamel of their teeth (Table 2). Largest absolute differences occurred in

Table 1. Sample size, age, and frequency of incisor breakage of harvested moose from the Cape Breton Highlands, 1999-2002 (NSDNR 2002).

Age	Incisor 1		Incisor 2	
	n	Breakage frequency (%)	*n*	Breakage frequency (%)
1.5	210	2 (1.0%)	214	1 (0.5%)
2.5	282	33 (11.7%)	287	8 (2.8%)
3.5	299	98 (32.8%)	308	24 (7.8%)
4.5	228	122 (53.5%)	232	49 (21.1%)
5.5	118	86 (72.9%)	120	32 (26.7%)
≥6.5	218	148 (67.9%)	220	71 (32.3%)

Cd, Co, Pb, Sn, Sr, and Y. Although not different, relatively large absolute differences were found in chromium (Cr), iron (Fe), and vanadium (V) (Table 2).

DISCUSSION

Breakage type and trends measured in Cape Breton Highland moose were similar to results from Alaskan moose that indicated that breakage severity increased with age, and breakage severity was greater in I1s than I2s (Smith 1992). There were no differences in elemental concentrations of 40 teeth collected from the Seward Peninsula and 20 teeth from a control population near Galena, Alaska (Smith 1992). The results for their microbeam analysis were not presented, therefore, comparisons between Nova Scotia and Alaska moose were not possible. Because of interspecific variation in mineral requirements, diagnosis of problems related to elemental deficiency/toxicity is not possible without species-specific information (Underwood and Suttle 1999).

However, the lower concentrations of Ba, Be, Co, Sr, and Y within the Cape Breton Highlands population are consistent with previous studies that concluded that a reduction of these elements may result in higher enamel solubility and compromised strength that lead to dental disease (Bibby and Losee 1970, Curzon 1983a, b, c, d). Barium, Be, Co, and Sr depletion result in depressed growth, reduced calcification of bones and teeth, and even evidence of hypoplasia within erythrogenic tissue and bone marrow (Underwood 1971, Curzon 1983a, d). Strontium may also share a synergistic relationship with fluoride that results in retardation of apatite dissolution (Curzon 1983d).

The effects that deficient/toxic concentrations of Cd, Ga, Pb, and Sn may have on enamel strength and solubility are unclear (Curzon 1983c, Stack 1983a, b), although Sn may inhibit the dissolution of enamel via acid neutralization within the oral environment (Curzon 1983c). Knowledge of essential vitamins and minerals within the diet of moose is limited (Schwartz and Renecker 1998). Frank et al. (2004) examined elemental concentrations from various tissues of Nova Scotia moose and found that Tobeatic moose displayed unusually high levels of Cd within kidney tissue as compared to Cape Breton Highlands moose; Cd concentration in enamel was also high in Tobeatic moose (Table 2). They also found that both Tobeatic and Cape Breton Highland moose had lower Co concentrations than Swedish and Alaskan moose. These data and the low concentration value of Co in the Cape Breton Highlands population (Table 2) suggest that Nova Scotia populations may suffer a Co deficiency.

Though not different, it is important to note that the relative concentrations of Cr, Fe, and V were low, and magnesium (Mg) high in the Cape Breton Highlands population (Table 2). With the exception of Mg, these results were not surprising because geochemical properties of elements are reflected by their position within the periodic table (Albarede 2003). Elements of the same group would be expected to have similar, relative availability within the environment. Cadmium, Fe, and V are all grouped in the transition metals of the periodic table with Cd, Co, and Y that

Table 2. Element concentrations in tooth enamel from 2 moose populations in Nova Scotia, Canada. Concentrations are expressed in ppm (except Ca, which is expressed as weight percent). Bold numbers illustrate significant differences between means.

Element	Cape Breton Highlands, *n* = 20			Mainland (control) population, *n* = 4		
	Mean	SE	Range	Mean	SE	Range
Ag	0.055	0.015	0.011 - 0.294	0.063	0.012	0.038 - 0.089
Al	65.3	18	12.9 - 329	72.5	8.4	59.9 - 95.8
As	4.842	0.22	3.870 - 4.212	5.685	0.58	4.71 - 7.37
Ba	**125.7**	**8.6**	**72.6 - 235**	**180.5**	**6.7**	**171 – 200**
Be	**0.015**	**0.002**	**0 - 0.034**	**0.029**	**0.01**	**0 - 0.045**
Bi	0.015	0.003	0.005 - 0.058	0.024	0.006	0.017 - 0.041
Ca (WT%)	34.8	0.4	30.8 - 38.5	35.2	0.7	33.9 - 37.2
Cd	**0.114**	**0.032**	**0.028 - 0.64**	**0.363**	**0.12**	**0.201 - 0.717**
Co	**0.017**	**0.015**	**0 - 0.297**	**0.824**	**0.31**	**0 - 1.15**
Cr	0.082	0.024	0 - 0.323	0.18	0.065	0.096 - 0.37
Cs	0.005	0.001	0.02 - 0.019	0.008	0.001	0.006 - 0.001
Cu	17.4	2.6	5.4 - 48.5	24.3	2.2	18 - 28.7
Fe	143.7	44	41.7 - 914	308	111	176 – 639
Ga	**0.207**	**0.007**	**0.144 - 0.267**	**0.169**	**0.009**	**0.144 - 0.182**
Hf	0.011	0.004	0 - 0.072	0.017	0.014	0.001 - 0.060
Li	1.031	0.17	0.270 - 3.16	1.216	0.21	0.902 - 1.81
Mg	3429	155	2640 - 4720	2767.5	86	2590 – 2960
Mn	56.8	8.8	14.4 - 168	34.5	11	14.7 – 65
Mo	0.055	0.012	0.015 - 0.242	0.061	0.006	0.047 - 0.076
Nb	0.02	0.005	0.009 - 0.104	0.02	0.002	0.015 - 0.023
Pb	**0.805**	**0.077**	**0.330 - 1.88**	**1.935**	**0.49**	**1.06 - 3.30**
Rb	0.607	0.054	0.287 - 1.25	0.847	0.053	0.689 - 0.926
Sb	0.027	0.007	0.004 - 0.141	0.026	0.009	0.007 - 0.048
Se	1.349	0.029	1.115 - 1.74	1.425	0.027	1.37 - 1.48
Si	173.2	26	62.2 - 552	152	9.3	137 – 179
Sn	**0.084**	**0.018**	**0.01 - 0.322**	**0.343**	**0.16**	**0.151 - 0.744**
Sr	**268.3**	**13**	**192 - 415**	**518.5**	**22**	**474 – 564**
Ta	0.001	0.000	0 - 0.008	0.001	0.000	0 - 0.001
Th	0.004	0.001	0 - 0.018	0.009	0.003	0.004 - 0.018
Ti	88.6	4.7	70 - 153	84.6	6.3	70.9 - 97.9
Tl	0.008	0.001	0.005 - 0.015	0.008	0.001	0.006 - 0.011
U	0.003	0.001	0.001 - 0.015	0.006	0.001	0.004 - 0.008
V	0.387	0.045	0.168 - 0.982	0.597	0.098	0.353 - 0.824
W	7.61	1.7	0.99 - 20.8	4.16	1.4	1.88 - 8.39
Y	**0.062**	**0.009**	**0.03 - 0.19**	**0.133**	**0.02**	**0.097 - 0.169**

Table 2 (continued). Element concentrations in tooth enamel from 2 moose populations in Nova Scotia, Canada. Concentrations are expressed in ppm (except Ca, which is expressed as weight percent). Bold numbers illustrate significant differences between means.

Element	Cape Breton Highlands, n = 20			Mainland (control) population, n = 4		
	Mean	SE	Range	Mean	SE	Range
Zn	54.7	3.3	37.9 - 85.9	64.6	5.6	53.6 - 80.3
Zr	0.456	0.19	0.027 - 3.35	0.865	0.8	0.027 - 0.084
REE	0.301	0.1	0.076 - 2.196	0.899	0.41	0.271 - 0.711

had lower concentration in the Cape Breton Highlands population. However, the alkaline – earth metal Mg has a higher concentration in the Cape Breton Highlands population than the Tobeatic population, unlike the other alkaline earth metals (Ba, Be, and Sr). The reason for this is unclear, however it may be a result of isobaric interference during the ICP-MS analysis, which is a common issue with biological applications (Taylor et al. 2005).

Mineral deficiency appears to be the underlying cause for the incisor breakage observed in the Cape Breton Highlands moose population. Osteophagia is generally considered to occur within ruminant species as a result of phosphorous deficiency (Bowyer 1983, Barrette 1985, Denton et al. 1986). Bark stripping by moose is generally considered to be a sign of starvation (Renecker and Schwartz 1998), and heavily browsed plants generally contain less nutritive value for moose (Schwartz and Renecker 1998). It is important for moose to consume a wide variety of plant species, or they may risk toxic/deficient mineral nutrition (Ohlson and Staaland 2001).

Whether tooth breakage is a result of osteophagia or bark stripping, or even a combination of the two, is unclear. However, these phenomena have been well documented in other ungulate species (Bowyer 1983, Barrette 1985, Denton et al. 1986, Duthie and Skinner 1986, Kierdorf 1994, Baker et al. 1997, Yokoyama et al. 2001, Ueda et al. 2002) with no reports of breakage similar to that observed in Cape Breton Highlands moose.

It was interesting that teeth from the control area were consistently richer in tin by one order of magnitude, which probably reflects differences in bedrock geology. Southwestern Nova Scotia contains significant (formerly economic) deposits of tin and the glacial deposits and soils are locally enriched with this and other elements common in granitoid intrusive rocks. This correlation of enamel geochemistry and bedrock geology may have valuable forensic usefulness.

Continued use of a broken incisor results in the incisal surface becoming rounded (Fig.1) and this probably reduces foraging efficiency. The gradual degradation of teeth likely has negative implications for the overall health and vitality of individual moose. To determine the influence of incisor breakage on population dynamics, it would be necessary to compare the mortality rate of older age classes and population age structure of several moose populations. We suggest that research on incisor breakage in the Cape Breton Highlands moose continue and be supplemented with more control samples from other populations.

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