Ann. Zool. Fennici 50: 89–99 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 12 April 2013

Trace elements in claw keratin as temporally explicit indicators of geographic origin in terrestrial mammals

Danielle M. Ethier[1], Christopher J. Kyle[2], T. Kurt Kyser[3] & Joseph J. Nocera[4]

[1)] *University of Guelph, Science Complex, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada (corresponding author's e-mail: ethier.danielle@gmail.com)*
[2)] *Trent University, DNA Building, 2140 East Bank Dr., Peterborough, Ontario, K9J 7B8, Canada*
[3)] *Queen's University, Department of Geological Sciences and Geological Engineering, 36 Union Street, Kingston, Ontario, K7L 3N6, Canada*
[4)] *Ontario Ministry of Natural Resources, Trent University, DNA Building, 2140 East Bank Dr., Peterborough, Ontario, K9J 7B8, Canada*

Received 30 Oct. 2012, final version received 30 Oct. 2012, accepted 14 Nov. 2012

Ethier, D. M., Kyle, C. J., Kyser, T. K. & Nocera, J. J. 2013: Trace elements in claw keratin as temporally explicit indicators of geographic origin in terrestrial mammals. — *Ann. Zool. Fennici* 50: 89–99.

Biogeochemical markers in ecology are useful for indicating geographic origin and movement patterns of species on various temporal and spatial scales. By assessing these markers in a tissue that is chemically inert once formed (e.g., claws) and is grown incrementally (i.e., deposited in layers), changes in an individual's foraging environment would be captured in the chemical signatures in these tissues. To determine whether trace elements can be used as a marker to track movement in terrestrial carnivores, we resolved multi-elemental data at a fine spatial scale in the claw keratin of a model species (*Taxidea taxus*) using high resolution laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS). The unique chemical signatures we detected in claws suggested that these chemical variations were, in part, a reflection of the chemical environment and were not be attributable to sex or random deposition. Neighbouring claw samples were not more chemically related than distant ones, which suggests that local differences in chemical composition (e.g., habitat type) may have a stronger influence on claw chemistry than large-scale patterns in trace element variation associated with underlying geology. Our findings illustrated that temporally-explicit chemical profiles in the blade horn keratin of mammalian claws may be used to examine endogenous uptake of trace element signatures from the local environment and serve as tool to assist in reconstructing animal movement pathways.

Introduction

Central to our understanding of ecological and evolutionary processes is a comprehension of how individuals acquire resources and interact with their surrounding environment. Traditional tracking methods (e.g., mark-recapture and radiotelemetry) have provided valuable information about such ecological relationships, but are often burdened by low sample sizes, high financial costs, and considerable logistical barriers (Webster *et al*. 2002). The search for a biochemi-

cal indicator that could be used to assign individuals to geographic origin, and subsequently resolve other aspects of species ecology, began in the 1960s with stable isotopes (e.g., Devine & Peterle 1968, Hanson & Jones 1968) and was soon followed by trace elements (e.g., Kelsall & Calaprice 1972, Hanson & Jones 1976, Parrish *et al*. 1983, Burger *et al*. 2001, Szép *et al*. 2003, Donovan *et al*. 2006, Gómez-Diaz & González-Solis 2007, Norris *et al*. 2007, Ramos *et al*. 2009, Torres-Dowdall *et al*. 2010). Trace element techniques rely on the premise that chemical signatures are incorporated into an animal's tissues through dietary uptake in proportion to local environmental concentrations (Gartner 1989, Driessens & Verbeeck 1990, Szép *et al*. 2003), which are in turn reflective of the chemical composition of the surface geology, water, soil, and vegetation of a particular area (Bortolotti *et al*. 1989, Szép *et al*. 2003). In general, element signatures in tissues integrate information on local habitat over the period the tissue was synthesized (Hobson & Clark 1992, Chamberlain *et al*. 1997, Hobson & Wassenaar 1997, Bearhop *et al*. 2002). By selecting a biological tissue that is chemically inert once formed (e.g., shells, otoliths, hair, claws) and is grown incrementally (i.e., deposited in layers on a daily, seasonal, or annual basis), it is hypothesized that temporally-explicit shifts in an individual's foraging environment would be captured in the chemical variation in these tissues.

Trace element profiles in incrementally grown tissues have been used as coarse indicators of foraging location (fish otoliths; Campana *et al*. 1994), migratory routes (fish otoliths; Kalish 1989), shifts in local climatic conditions (reef coral; McCulloch *et al*. 1999), and point-source contamination (reef coral; Guzman & Jarvis 1996, tortoise sutures; Seltzer & Berry 2005). A remaining challenge is to determine whether continuously grown tissues, such as claws, can provide spatial and temporal resolution for geographic assignment of individuals. Evidence suggests that the lateral portions of some mammal claws may be reliable temporal indicators of previous foraging environments (Ethier *et al*. 2010). The blade horn keratin covering the lateral aspects of the claw are deposited continuously from the germinal matrix at the base of the claw (like human fingernails) and are uncomplicated by additional keratin layers (Homberger *et al*. 2009). And, like other hard biological tissues, claws are metabolically isolated and chemically inert once formed (Takagi *et al*. 1988). These tissues, therefore, should encapsulate an unbroken time-series of ecological data, which may allow for finer-scale studies of movement history of individuals throughout their annual cycles.

The advent of laser ablation inductively-coupled plasma mass spectrometry (LA ICP-MS; Gray 1985) has made high resolution multi-elemental detection at fine spatial scales possible (Gunther *et al*. 2000, Russo *et al*. 2002). In addition, the ability to operate the laser in a continuous scanning mode permits the assessment of micro-spatial resolution of elemental variation along a temporal axis. Here, we examine the capability of LA ICP-MS for assessing the spatial distribution of twelve trace elements (Ba, Pb, Mg, P, Cr, Mn, Fe, Cu, Zn, Sr, Zr, and K) in the claw keratin of a model terrestrial mammal species; the American badger (*Taxideus taxus*). Our objectives were to (1) describe a new technique for evaluating spatially-explicit trace element profiles in claw keratin, (2) evaluate whether element composition of claws can be attributed large-scale geographic trace element variation associated with underlying geology or fine-scale spatial differences related to foraging habitat, (3) determine whether element profiles along the length of an individual's claw were autocorrelated (i.e., occurring in a predictable or random pattern), and (4) provide spatially resolved multi-elemental information that could be used to assess movement history and habitat association in terrestrial mammals. American badgers are an appropriate case study because the species is highly mobile in its environment, has long sturdy claws, and consumes a varied diet (ensuring variability in biochemical exposure and integration).

Material and methods

Sample preparation and laser ablation

Claw samples were obtained from archival and road-killed badger specimens with known col-

lection coordinates in southwestern Ontario, Canada (n = 28; Table 1 and Fig. 1). Cleaning procedures were considered carefully, as environmental chemical exposure may result in substantial exogenous contamination of biological tissues, obscuring patterns of endogenous elemental distribution associated with metabolic uptake of trace elements. Washing procedures alone are insufficient for removing exogenous contamination on human fingernails (Rodushkin & Axelsson 2003) and tortoise shells (Seltzer & Berry 2005). We therefore combined surface scraping (with a scalpel) to remove the superficial keratin layers, washing with an organic solvent (1:1, methanol: chloroform) in an ultrasonic bath, and pre-ablation (laser ablation performed at a reduced power) to ensure surfaces were clean prior to ablation for data collection. Claws were mounted in epoxy to ensure the ablation surface was level, so that the laser remained in focus during ablation along the length of the blade horn.

We used a Thermo Scientific ELEMENT XR high-resolution inductively coupled plasma mass spectrometer (ICP-MS) equipped with a Newwave 213 nm NdYAG laser system to make all elemental measurements. The ICP-MS was optimized for nebulizer gas flows, X-Y torch position, and lens voltage prior to each measurement session by monitoring the multi-elemental signals arising from ablation of a glass standard reference material, SRM 612 from the National Institute of Standards and Technology (NIST).

Transects were ablated by the laser along the lateral surface (blade horn) of individual claws to examine the intrinsic elemental composition.

Table 1. American badger (*Taxidea taxus*) claw samples collected from archival and road-killed individuals in southwestern Ontario for trace element analysis by laser ablation inductively coupled plasma mass spectrometry, including information on sex, age, location and date of collection (when available). Notes: F = female; M = male; Unk = unknown, Universal Trans Mercator datum = WGS 84, Zone 17T.

Badger ID	Easting	Northing	Sex	Age	Collection date
AB01	516377	4764522	F	Adult	11 Oct. 2007
AB03	568095	4776878	F	Adult	27 Mar. 2006
AB04	551757	4778766	M	Adult	23 May 2005
AB05	540397	4792401	M	Juvenile	7 June 2004
AB06	553934	4773416	F	Adult	15 Sep. 2005
AB07	536732	4745038	M	Adult	28 Sep. 2005
AB08	556285	4759143	M	Adult	7 May 2005
AB09	586260	4801232	M	Adult	1 July 2007
AB11	546419	4754191	M	Adult	21 June 2006
AB12	524110	4744320	F	Adult	20 Apr. 2009
AB13	519360	4735082	F	Adult	4 June 2008
AB14	454306	4759154	F	Adult	1970
AB15	479831	4727507	M	Adult	Autumn 1963
AB16	479831	4727507	M	Adult	Spring 1964
AB17	454503	4740667	M	Adult	1992
AB18	525660	4744460	M	Adult	23 Aug. 2009
AB19	424994	4733468	F	Adult	20 Nov. 1979
AB20	536549	4728993	F	Adult	21 Oct. 1997
CS	528807	4734311	Unk	Adult	11 June 2004
StJohn	558111	4738620	Unk	Unk	1 Jan. 1996
LeeBrown	541802	4714350	Unk	Adult	1 Jan. 1995
Langton	534504	4731913	F	Juvenile	1 Jan. 1988
Villa Nova	562597	4755632	F	Adult	1 Aug. 2008
UWO #M440	450706	4716495	M	Adult	1 Sep. 1955
UWO #M441	484757	4727689	F	Adult	1 July 1964
ROM 19630	441369	4726373	Unk	Adult	1 Aug. 1948
ROM 93103	410179	4693075	M	Adult	12 Oct. 1985
ROM 97069	468760	4769504	Unk	Adult	8 Apr. 1990

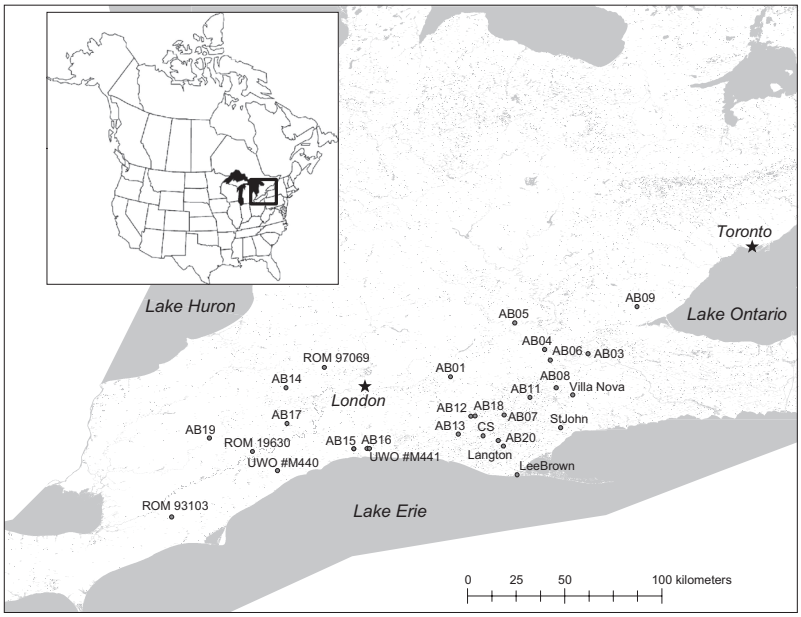

Fig. 1. Collection locations of American badger (*Taxidea taxus*) claw samples from southwestern Ontario, Canada (n = 28).

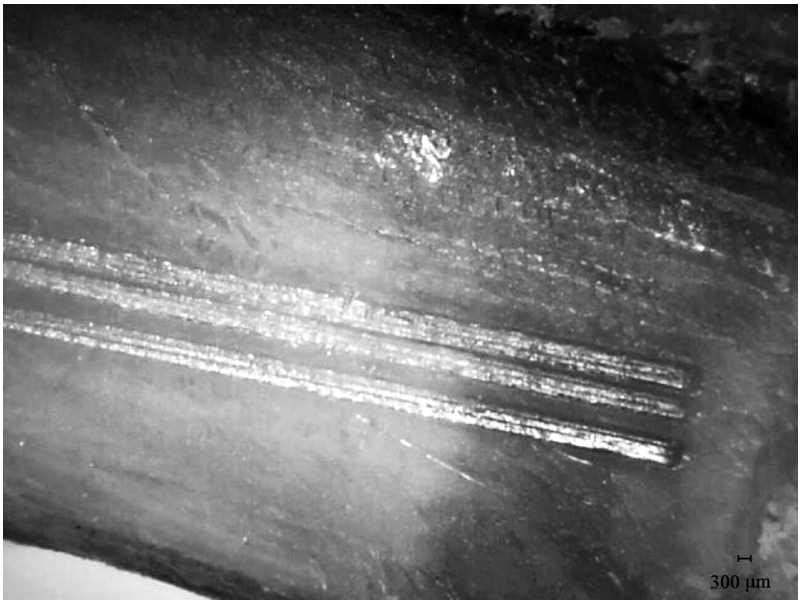

Fig. 2. Digital photo of three parallel laser ablation transects on the lateral wall of an American badger (*Taxidea taxus*) claw.

Transects were initiated at the base of the claw, or otherwise youngest tissue, and proceeded toward the distal end. Thus, the ablation transect was orthogonal to the direction of growth, and would represent an unbroken temporal profile of elemental concentrations. Ablation patterns consisted of three parallel 300 µm diameter lines (Fig. 2), with a laser repetition of 20 Hz at a pulse rate of 30 μm s^{-1}.

A major challenge in using laser ablation-based spectroanalytical techniques is the lack of matrix-matched calibration standards (e.g., a keratin-based standard) on which to base absolute concentration data. Ideally, the standard and the sample should have the same matrix, since the intensity of peaks from a constant signal (measured in counts per second; cps) can vary dramatically between the SRM (e.g., NIST

glass) and the ablation surface of biological tissues (Seltzer & Berry 2005). To correct for this difference in peak intensity caused by variations in ablation yield, it is necessary to normalize the analyte intensity to that of an internal standard, an element of known concentration in the sample and reference material. This semi-quantitative technique is a practical alternative for samples that contain a reasonably homogeneous matrix (Van de Weijer *et al*. 1992, Craig *et al*. 2000). Claws are appropriate for this approach, since cystine (a sulphur-containing amino acid) forms a major component of claw keratin (7.1 wt% in hoof-horn; Zoccola *et al*. 2009). For this application, it is assumed that measured ion intensities of sulphur and other trace element isotopes, used in conjunction with the percent isotope abundance and the ICP-MS instrument response curve, could be used to estimate trace element concentrations in the claw samples (Seltzer & Berry 2005). The average sulphur concentration of badger claws was determined independently using solution nebulisation ICP-MS ($n = 5$, mean ± SD = 26 597 ± 9550 ppm). The use of an internal standard accounted for any variation in the amount of ablated material reaching the plasma between samples and the reference material.

Following laser optimization, ablation scans were performed on the NIST glass SRM using conditions identical to those used on the claws. The same ion intensities are measured on the SRM as the badger claws (Ba, Pb, Mg, P, Cr, Mn, Fe, Cu, Zn, Sr, Zr, and K), where isotope intensities (cps) are measured sequentially during ablation along the transects. Gas blanks were subtracted in each instance to obtain net ion intensities. Since sulphur is a poor ionizer relative to other transition metals, it was necessary to estimate interelemental sensitivity factors to enable semi-quantitative analysis (Seltzer & Berry 2005). Interelemental sensitivity factors were calculated based on the average ion intensity from the NIST glass ablation transects, which varied daily based on instrument response. These interelemental sensitivity factors reflect differences in the sample induction and instrument response, and serve as a surrogate for badger claws. We used the following formula, derived by Seltzer and Berry (2005), to determine trace element concentrations based on the relative ion intensities:

$$\left(C_{\mathrm{E}}\right)_{\mathrm{claw}} = \left(C_{\mathrm{S}}\right)_{\mathrm{claw}} \times \frac{I_{\mathrm{E}}}{\left(I_{\mathrm{S}}\right)_{\mathrm{claw}}} \times \frac{F_{\mathrm{S}}}{F_{\mathrm{E}}} \times \frac{A\left(^{*}\mathrm{E}\right)}{A\left(^{32}\mathrm{S}\right)}$$

where C = concentration (ppm), I = intensity (cps), A = abundance of isotope (%), F = relative sensitivity factor, *E = element analyte, S = internal standard = ^{32}S, claw = claw sample.

Pre-analyses data treatment

Extreme outliers were identified as those exceeding the 1.5 interquartile range of the individual(s) displaying the greatest variation for that particular element, which are likely a result of analytical error rather than real variation. In total, 66 of 2200 (0.3%) extreme outliers were identified and removed before multivariate analysis. We ensured the data met the necessary analytical assumptions prior to all multivariate analyses. We applied a Box–Cox power transformation (Box & Cox 1964) to better approximate a normal distribution and diminish the extremity of outliers, while reducing the relative distribution of the data so that elements with higher abundances are brought into the same range as elements with lower abundances.

Statistical analyses

To determine if there are bulk chemical differences between claws of male and female badgers (n = 15 animals of known sex) we used multivariate ANOVA on median element concentrations. We then assessed whether trace element concentrations in claws can be used to assess whether neighbouring samples were more chemically related than distant ones. This would be expected if trace element variability in claws were influenced by large-scale geographic trace element variation associated with underlying geology rather than fine-scale spatial differences related to foraging habitat. For this, we used univariate ANOVA on each chemical element separately (Bonferroni-adjusted $\alpha = 0.0001$) and a Tukey HSD post-hoc test (with adjusted 95% family-wise confidence level) to identify the relative number of pairwise elemental differences contributing to individual differentiation.

Using a linear regression, we investigated the relationship between geographic distances and the number of pair-wise differences in mean element concentrations.

We applied a stationary time-series analysis on raw elemental data, making the assumption that growth rates of the claw are constant, to determine if patterns in chemical profiles were predictable or random. Using the median element concentration from the three ablation transects, we applied an autoregressive integrated moving average (ARIMA; Box & Jenkins 1976) model to describe temporal patterns in the data by quantifying the number of autoregressive (AR) components (p) in the model and the number of moving average (MA) components (q) in the model. Because we sought only to differentiate between a random deposition and measurable patterns, we set d in all models equal to zero. The best-fit model was selected using Akaike's Information Criterion (AIC; Akaike 1974). If an AR or MA process was identified in the model (i.e., p or q were > 0), then the pattern was not deemed a function of random deposition. Elements with the greatest number of AR terms across individuals are considered to show the strongest trends. We then plotted time-series element concentrations from laser ablation transects to illustrate their spatial variation along the length of the claw. We selected claws with the greatest number of pair-wise chemical differences and elements that showed the strongest ARIMA trends for comparative display.

Table 2. Twelve trace elements quantified in American badger claws (*Taxidea taxus*), including: mean, median, and median absolute deviation (MAD). All element concentrations in parts per million.

	Mean	Median	MAD
Ba	524	203	235
Pb	505	303	338
Mg	11695	5190	5424
P	37716	21193	17683
Cr	3429	54	73
Mn	222	56	66
Fe	4284	600	761
Cu	352	183	151
Zn	2008	1455	906
Sr	241	93	100
Zr	51	20	23
K	1102	595	435

Results

Summary statistics for claw element concentrations are given in Table 2. We report the median and the median absolute deviation (MAD) in addition to the mean, since these measures are inherently more stable against outliers and deviations, and in most cases, give a more realistic value for location and spread (Reimann & Filzmoser 2000). Magnesium (median ± MAD = 5190 ± 5424 ppm) and P (21193 ± 17683 ppm) were the most abundant elements, and Cr (54 ± 73 ppm) and Zr (20 ± 23 ppm) were the least abundant.

We did not detect a difference in the mean chemical concentrations between male and female badger claws (MANOVA: $F_{1,12} = 0.53$, $p = 0.64$). We therefore pooled genders for subsequent analysis. There was no relationship between geographic distance and the number of elemental differences between individuals (linear regression; $y = 4.796 + 0.002x$, $t_{376} = 0.581$, $p = 0.56$), which would have been expected if landscape-level chemical variation was driving chemical differentiation of claws. The badgers that displayed the greatest number of pair-wise chemical differences were AB01, AB08, AB11, ROM19630. The ARIMA models indicated that all elements displayed some degree of spatial autocorrelation in one or more claws (Table 3). Given that an autoregressive coefficient of zero implies no autocorrelation, the elements which displayed the strongest trends were Mn, Fe, P, Sr, K, with autoregressive coefficients ranging from –1.15 to 1.33 (Fig. 3).

Discussion

We have illustrated that temporally explicit chemical profiles in the blade horn keratin of mammalian claws may be used to examine endogenous uptake of trace element signatures from the local environment. Differences in the element composition of claws could not be attributed to sex. Work with other species has shown that element concentrations in biological tissues can vary between the sexes (e.g., Kelsall & Pannekoek 1976, Kelsall & Burton 1979, Bortolotti & Barlow 1988, however c.f. Szép

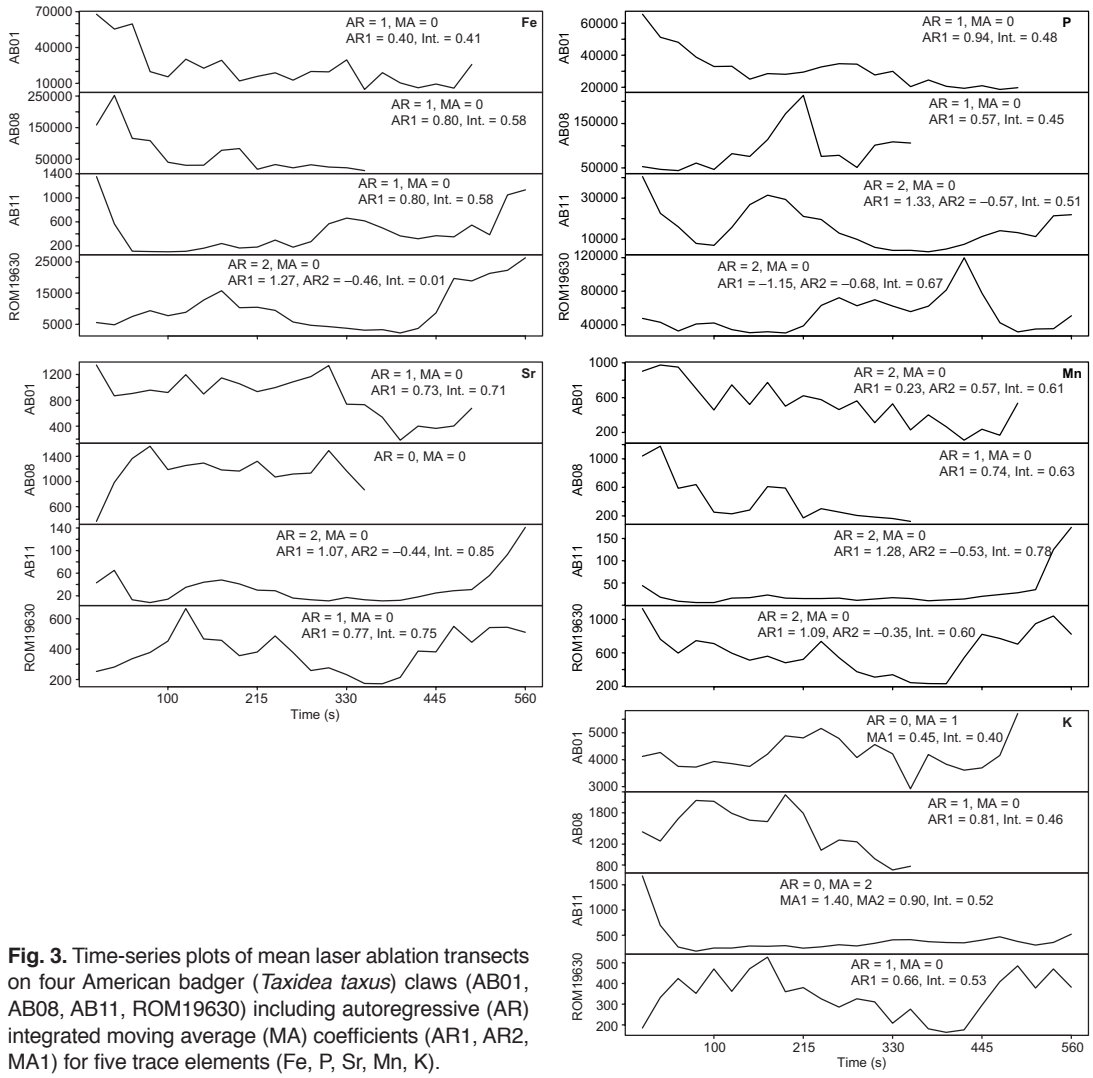

Fig. 3. Time-series plots of mean laser ablation transects on four American badger (*Taxidea taxus*) claws (AB01, AB08, AB11, ROM19630) including autoregressive (AR) integrated moving average (MA) coefficients (AR1, AR2, MA1) for five trace elements (Fe, P, Sr, Mn, K).

et al. 2003), which is attributed to inter-sexual behavioural divergences in food choice and habitat preference (Hanson & Jones 1974, Kelsall *et al*. 1975, Kelsall & Burton 1979). Previous work found that food preferences do not seem to differ substantially between male and female American badgers (Azevedo *et al*. 2006), which substantiates our result.

Individual claws were distinct from one another chemically, although this difference was not explained by geographic distance. For example, chemical signatures in the claw of AB08 were quite distinct from AB11, although these claws were collected close geographically. Given that both individuals were adult males (not dispersing juveniles) this result suggests that local differences in chemical foraging habitat may have a stronger influence on claw chemistry than large scale patterns in trace element variation associated with underlying geology. A similar finding was noted by Bortolotti *et al*. (1989) in their classification of spruce grouse (*Falcipennis canadensis*) to origin, where they demonstrated that chemical profiles in feathers were highly specific to certain forest stands.

For claws to be reliable recorders of environmental conditions, elements within the claws must be deposited in proportion to concentrations found in the local foraging environment. Our ARIMA models suggest this is indeed the case,

as they revealed high levels of autocorrelation (Table 3) wherein 89% of the AR coefficients estimated were ≥ 1. Transect profiles of trace element concentrations exposed patterns that could not be attributed to a random deposition [indicated by AR coefficients equal to zero, which occurred in only 11% of our models (Table 3)], suggesting these profiles reflect chronological elemental uptake. Thus, laser ablation transects orthogonal to the axis of claw growth can reveal distinct patterns in chemical variability, comparable to those observed in tortoise scutes (Seltzer & Berry 2005), fish otoliths (Kalish 1989), and tooth enamel (Evans *et al*. 1994, 1995, Cox *et al*. 1996, Lee *et al*. 1999).

We recognize two important sources of analytical error in our study. First, the sensitivity of LA ICP-MS analysis means some variation among repeated scans can be expected. Consider that, even though parallel transects were ablated along the length of the blade horn, the ICP-MS resolves each chemical element sequentially, and thus all elements are quantified in a transect before the next transect begins and some shifts in sensitivity may occur. Second, the blade horn keratin is very thin and non-distinct from the underlying parietal horn. Mechanical removal of the superficial keratin layers (*see* Material and methods) may thin the blade horn keratin in certain areas, resulting in the inadvertent ablation of the underlying parietal horn. The parietal horn is not deposited chronologically like that of the blade horn and would represent a different time period of chemical acquisition (Ethier *et al*. 2010). Nonetheless, despite these potential causes of spurious results, trends in chemical profiles along the length of the claw are rather distinct for most elements.

Table 3. Autoregressive integrated moving average (ARIMA) model results for mean laser ablation transects on individual American badger (*Taxidea taxus*) claws. Temporal patterns in the data are quantified by the number of autoregressive (AR, *p*) and moving average (MA, *q*) components in the model (displayed as *p*, *q*).

Claw ID	Ba	Pb	Mg	P	Cr	Mn	Fe	Cu	Zn	Sr	Zr	K
AB01	1, 0	1, 0	1, 0	1, 0	0, 0	2, 0	1, 0	1, 0	1, 0	1, 0	0, 0	0, 1
AB03	1, 1	0, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	2, 0	1, 0	1, 0	1, 0
AB04	1, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0
AB05	1, 0	1, 0	1, 0	1, 1	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0
AB06	0, 0	1, 0	1, 0	2, 0	1, 0	2, 1	1, 2	1, 0	1, 0	2, 0	1, 0	1, 0
AB07	1, 0	2, 0	1, 0	1, 0	1, 0	2, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0
AB08	0, 0	0, 0	0, 0	1, 0	1, 1	1, 0	1, 0	1, 0	0, 0	0, 0	0, 0	1, 0
AB09	1, 0	0, 1	1, 0	1, 0	1, 0	1, 0	2, 0	0, 1	2, 0	1, 0	1, 0	1, 2
AB11	2, 0	2, 0	1, 1	2, 0	1, 0	2, 0	1, 0	2, 1	2, 0	2, 0	1, 0	0, 2
AB12	1, 0	2, 0	1, 0	1, 0	1, 0	2, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0
AB13	1, 0	1, 0	1, 0	1, 1	1, 0	1, 0	0, 1	1, 0	0, 0	2, 0	1, 0	1, 0
AB14	1, 0	1, 0	1, 0	1, 0	2, 0	1, 0	0, 1	0, 1	2, 0	0, 2	1, 0	1, 0
AB15	1, 0	0, 0	0, 1	1, 0	1, 0	1, 0	1, 0	1, 1	1, 0	1, 0	1, 0	2, 0
AB16	1, 0	0, 0	2, 0	2, 0	1, 0	2, 0	1, 0	1, 0	1, 0	2, 0	2, 0	1, 0
AB17	1, 0	1, 0	1, 0	2, 0	1, 0	1, 1	1, 0	1, 0	2, 0	1, 0	1, 0	1, 0
AB18	1, 0	1, 0	1, 0	1, 0	1, 0	2, 0	2, 0	1, 0	0, 1	2, 0	1, 0	1, 0
AB19	1, 0	0, 0	1, 0	1, 0	0, 1	2, 0	0, 2	1, 0	1, 0	1, 0	0, 1	1, 0
AB20	1, 0	2, 0	1, 0	1, 0	1, 0	2, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0
StJohn	1, 0	1, 0	1, 0	1, 0	1, 0	1, 1	1, 0	1, 0	0, 1	1, 0	1, 0	2, 0
CS	1, 0	1, 0	1, 0	1, 0	0, 1	1, 0	1, 0	0, 0	1, 0	1, 0	1, 0	1, 0
Langton	1, 0	1, 0	2, 0	2, 0	1, 0	1, 0	1, 0	1, 0	2, 0	3, 0	2, 0	1, 0
LeeBrown	1, 0	2, 0	1, 0	2, 0	2, 0	1, 0	2, 0	1, 0	1, 0	1, 1	1, 0	2, 0
UWOM440	1, 0	1, 0	0, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	2, 0
UWOM441	2, 0	0, 1	1, 0	0, 1	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0
ROM97069	1, 0	0, 0	1, 1	1, 1	1, 0	0, 1	0, 0	1, 0	1, 0	2, 0	0, 0	0, 0
ROM19630	0, 1	1, 0	2, 0	2, 0	1, 0	2, 0	2, 0	1, 0	0, 0	1, 0	1, 0	1, 0
ROM93103	1, 0	1, 0	1, 0	2, 0	0, 0	1, 0	1, 0	1, 0	1, 0	1, 0	1, 0	2, 0
Villa	1, 0	1, 0	1, 0	1, 0	1, 0	1, 1	1, 0	1, 0	0, 1	1, 0	1, 0	2, 0

Transect profiles of trace element concentrations reveal patterns that should reflect chronological elemental uptake, although we cannot completely rule out a physiological basis for differences in claw chemistry for structurally important elements. High concentrations of P and Mg can be attributed to their structural role in claw keratin (Goldblum *et al*. 1953, Finlay *et al*. 1980). Other minerals, such as Zn, Cu, Fe, and Mn are also functionally important due to their associations with enzymes (Goldblum *et al*. 1953, Finlay *et al*. 1980). For example, Zn is a component of over 200 enzymes, several of which are involved in horn production (Müelling 2009). Due to physiological function, these elements may be less reliable for geographic assignment of individuals relative to heavy metals (e.g., Cr, Pb, Hg) and non-essential elements (e.g., Ba, Rb, Mo, Sr). Seasonal trends in trace element sequestration may be influenced by an individuals' metabolic condition. For example, reproductive status can affect trace Mg and Ca concentrations in cow blood due to milk production (Lemus & Rivera 2009). Other factors can also affect element sequestration into biological tissues, such as young animals that differ from adults with respect to their physiological use of various elements (Kelsall *et al*. 1975, Bortolotti & Barlow 1988, Szép *et al*. 2003). In young birds, Zn is required for feather and skeletal development (Underwood 1971) and has been reported in lower concentrations in feathers of young geese relative to adults (Kelsall *et al*. 1975). We did not attempt to determine if age influenced claw chemistry due to sample size restrictions. The impact of extended physiological stress caused by long distance dispersal, migration, and malnutrition on element concentrations in biological tissues also warrants further investigation.

Although we now have a better understanding of how the chemical variability in claws can provide temporal information on geographic location, it is important to bear in mind that the exact function of many trace elements in claw keratin is not known (Harvey & Markwell 1996). Understanding the physiological role of elements in a biological tissue is of particular importance, especially when working with metabolically active elements. Decoupling of trace elements during physiological processing may obscure our ability to directly link the chemical environment to element profiles in tissues. Further, certain tissues will disproportionately sequester elements, particularly those that can be toxic (Honda *et al*. 1985, 1986, Braune & Gaskin 1987, Burger 1993). Future research should consider feeding trials on captive animals, as these data may provide valuable "real-time" information on element sequestration into the claw keratin, as it has with stable isotopes in various bird tissues (DeNiro & Epstein 1978, 1981).

Acknowledgments

We would like to thank Justina Ray, Josh Sayers, and Jim Schaefer for helpful comments and edits on earlier drafts of this manuscript. The Ontario Badger Recovery Team, Royal Ontario Museum, University of Western Ontario, and numerous private collectors donated claws for this study. Funding was provided in part by the Ontario Ministry of Natural Resources, Ontario Graduate Scholarship, Species at Risk Research Fund for Ontario/World Wildlife Fund, Species at Risk Stewardship Fund, and the Natural Sciences and Engineering Research Council of Canada. Thanks to Bill MacFarlane and personnel at the Queen's University Facility for Isotope Research for their technical assistance.

References

Akaike, H. 1974: A new look at the statistical model identification. — *IEEE Transactions on Automatic Control* 19: 716–723.

Azevedo, F. C. C., Lester, V., Gorsuch, W., Lariviere, S., Wirsing, A. J. & Murray, D. L. 2006: Dietary breadth and overlap among five sympatric prairie carnivores. — *Journal of Zoology* 269: 127–135.

Bearhop, S., Waldron, S., Votier, S. C. & Furness, R. W. 2002: Factors influencing simulation and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. — *Physiological and Biochemical Zoology* 75: 451–458.

Bortolotti, G. R. & Barlow, J. C. 1988: Some sources of variation in the element composition of bald eagle feathers. — *Canadian Journal of Zoology* 66: 1948–1951.

Bortolotti, G. R., Szuba, K. J., Naylor, B. J. & Bendell, J. F. 1989: Mineral profiles of spruce grouse feathers show habitat affinities. — *Journal of Wildlife Management* 53: 811–817.

Box, G. E. P. & Cox, D. R. 1964: An analysis of transformations. — *Journal of the Royal Statistical Society Series B: Methodological* 26: 211–252.

Box, G. E. P. & Jenkins, G. M. 1976: *Time series analysis: Forecasting and control*. — John Wiley & Son, Inc.,

New Jersey.

Braune, B. M. & Gaskin, D. E. 1987: Mercury levels in Bonaparte's gulls (*Larus philadelphia*) during autumn molt in the Quoddy Region, New Brunswick, Canada. — *Archives of Environmental Contamination and Toxicology* 16: 539–549.

Burger, J. 1993: Metal in avian feathers: Bioindicators of environmental pollution. — *Reviews of Environmental Contamination and Toxicology* 5: 203–311.

Burger, J., Shukla, T., Dixon, C., Shukla, S., McMahon, M. J., Ramos, R. & Gochfeld, M. 2001: Metal in feathers of sooty tern, white tern, gray-backed tern, and brown noddy from islands in the North Pacific. — *Environmental Monitoring and Assessment* 71: 71–89.

Campana, S. E., Fowler, A. J. & Jones, C. M. 1994: Otolith elemental finger-printing for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICP-MS. — *Canadian Journal of Fisheries and Aquatic Science* 51: 194–1952.

Chamberlain, C. P., Blum, J. D., Holmes, R. T., Feng, X., Sherry, T. W. & Graves G. R. 1997: The use of isotope tracers for identifying populations of migrating birds. — *Oecologia* 109: 132–141.

Cox, A., Keenan, F., Cooke, M. & Appleton, J. 1996: Trace element profiling of dental tissue using laser ablation-inductively coupled plasma-mass spectrometry. — *Fresenius' Journal of Analytical Chemistry* 354: 254–258.

Craig, C.-A., Jarvis, K. E. & Clarke, L. J. 2000: An assessment of calibration strategies for the quantitative and semi-quantitative analysis of calcium carbonate matrices by laser ablation-inductively couple plasma-mass spectrometry (LA-ICP-MS). — *Journal of Analytical Atomic Spectrometry* 15: 1001–1008.

DeNiro, M. J. & Epstein, S. 1978: Influence of diet on the distribution of carbon isotopes in animals. — *Geochimica et Cosmochimica Acta* 42: 49–506.

DeNiro, M. J. & Epstein, S. 1981: Influence of diet on the distribution of nitrogen isotopes in animals. — *Geochimica et Cosmochimica Acta* 45: 341–351.

Devine, T. & Peterle, T. J. 1968: Possible differentiation of natal area of North American waterfowl by neutron activation analysis. — *Journal of Wildlife Management* 32: 274–279.

Donovan, T., Buzas, J., Jones, P. & Gibbs, L. 2006: Tracking dispersal in birds: assessing potential of elemental markers. — *Auk* 123: 500–511.

Driessens, F. C. M. & Verbeeck, R. M. H. 1990: *Biominerals*. — CRC Press, Florida.

Ethier, D. M., Kyle, C. J., Kyser, T. K. & Nocera, J. J. 2010: Variability in the growth pattern of the cornified claw sheath among vertebrates: implications for using biogeochemistry to study animal movement. — *Canadian Journal of Zoology* 88: 1043–1052.

Evans, R. D., Richner, P. & Outridge, P. M. 1994: Applications of laser ablation inductively coupled mass spectrometry to the determination of environmental contaminants on calcified biological structures. — *Journal of Analytical Atomic Spectrometry* 9: 985–989.

Evans, R. D., Richner, P. & Outridge, P. M. 1995: Microspatial variation of heavy metals in the teeth of walrus as determined by laser ablation ICP-MS: The potential for constructing a history of metal exposure. — *Archives of Environmental Contamination and Toxicology* 28: 55–60.

Finlay, A. Y., Frost, P., Keith, A. D. & Snipes, W. 1980: An assessment of factors influencing flexibility of human finger nails. — *Journal of Investigative Dermatology* 103: 357–365.

Gartner, L. P. 1989: *Oral histology and embryology*. — Jen House Publishing.

Goldblum, R. W., Derby, S. & Lerner, A. B. 1953: The metal content of skin, nail and hair. — *Journal of Investigative Dermatology* 20: 13–18.

Gómez-Diaz, E. & González-Solis, J. 2007: Geographic assignment of seabirds to their origin: combining morphological, genetics, and biogeochemical analyses. — *Ecological Applications* 17: 1484–1498.

Gray, A. L. 1985: Solid sample introduction by laser ablation for inductively coupled plasma source mass spectrometry. — *Analyst* 110: 551–556.

Gunther, D., Horn, I. & Hattendorf, B. 2000: Recent trends and developments in laser ablation ICP-mass spectrometry. — *Fresenius' Journal of Analytical Chemistry* 368: 4–14.

Guzman, H. Z. & Jarvis, K. E. 1996: Vanadium century record from Caribbean reef corals: a tracer of oil pollution in Panama. — *Ambio* 25: 523–526.

Hanson, H. C. & Jones, R. L. 1974: An inferred sex differential in copper metabolism in Ross' geese (*Anser rossi*): biogeochemical and physiological considerations. — *Arctic* 27: 111–120.

Hanson, H. C. & Jones, R. L. 1976: *The biochemistry of blue, snow, and Ross' geese. Illinois natural history survey*. — Southern University Press, Carbondale, IL.

Hanson, H. C. & Jones, R. L. 1968: Use of feather minerals as biological tracers to determine the breeding and molting grounds of wild geese. — *Illinois Natural History Survey Biological Notes* 60: 3–8.

Harvey, R. G. & Markwell, P. J. 1996: The mineral composition of nails in normal dogs and comparisons with shed nails in canine idiopathic onychomadesis. — *Veterinary Dermatology* 7: 29–34.

Hobson, K. A. & Clark, R. G. 1992: Assessing avian diets using stable isotopes I: turnover of $\delta^{13}C$ in tissues. — *Condor* 94: 181–188.

Hobson, K. A. & Wassenaar, L. I. 1997: Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. — *Oecologia* 109: 142–148.

Homberger, D. G., Ham, K., Ogunbakin, T., Bonin, J. A., Hopkins, B. A., Osborn, M. L., Hossain, I., Barnett, H. A., Matthews, K. L., Butler, L. G. & Bragulla, H. H. 2009: The structure of the cornified claw sheath in the domestic cat (*Felis catus*): implications for the claw shedding mechanism and the evolution of the cornified digital end organ. — *Journal of Anatony* 214: 620–643.

Honda, K., Min, B. Y. & Tatsukawa, R. 1985: Distribution of heavy metals and their age-related changes in the eastern great white egret, *Egretta alba modesta* in Korea. — *Archives of Environmental Contamination and Toxicol-*

ogy 16: 539–549.

Honda, K., Nasu, T. & Tatsukawa, R. 1986: Seasonal changes in mercury accumulation in the black-eared kite, *Milvus migrans lineatus*. — *Environmental Pollution* 42: 325–334.

Kalish, J. M. 1989: Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. — *Journal of Experimental Marine Biology and Ecology* 132: 151–178.

Kelsall, J.P. & Burton, R. 1979: Some problems in identification of origins of lesser snow geese by chemical profiles. — *Canadian Journal of Zoology* 57: 2292–2302.

Kelsall, J. P. & Calaprice, J. R. 1972: Chemical content of waterfowl plumage as a potential diagnostic tool. — *Journal of Wildlife Management* 36: 1088–1097.

Kelsall, J. P. & Pannekoek, W. J. 1976: The mineral profile of plumage in captive lesser snow geese. — *Canadian Journal of Zoology* 54: 301–305.

Kelsall, J. P., Pannekoek, W. J. & Burton, R. 1975: Chemical variability in plumage of wild lesser snow geese. — *Canadian Journal of Zoology* 53: 1369–1375.

Lee, K. M., Appleton, J., Cooke, M., Keenan, F. & Sawicka-Kapusta, K. 1999: Use of laser ablation inductively coupled plasma mass spectrometry to provide element versus time profiles in teeth. — *Analytica Chimica Acta* 395: 179–185.

Lemus, R. & Rivera, D. 2009: Spring grazing and grass tetany in beef cattle production. — *Forage News*, February 2009: 1–5.

McCulloch, M. T., Tudhope, A. W., Esat, T. M., Mortimer, G. E., Chappell, J., Pillans, B., Chivas, A. R. & Omura, A. 1999: Coral record of equatorial sea surface temperatures during the penultimate deglaciation at Huon Peninsula. — *Science* 283: 202–204.

Mülling, C., Bragulla, H., Reese, S., Budras, K.-D. & Steinberg, W. 1999: How structures in bovine hoof epidermis are influenced by nutritional factors. — *Anatomia Histologia Embryologia, Journal of Veterinary Medicine Series C* 28: 103–108.

Norris, D. R., Lank, D. B., Pither, J., Chipley, D., Ydenberg, R. C. & Kyser, T. K. 2007: Trace element profiles as unique identifiers of western sandpipers (*Calidris mauri*) populations. — *Canadian Journal of Zoology* 85: 579–583.

Parrish. J. R., Rogers, D. T. & Ward, F. P. 1983: Identification of natal locales of peregrine falcon (*Falco peregrines*) by trace-element analysis of feathers. — *Auk* 100: 560–567.

Ramos, R., González-Solís, J., Croxall, J. P., Oro, D. & Ruiz, X. 2009: Understanding oceanic migrations with intrinsic biogeochemical markers. — *PLoS ONE* 4(7): e6236. doi:10.1371/journal.pone.0006236.

Reimann, C. & Filzmoser, P. 2000: Normal and lognormal data distribution in geochemistry: death of a myth. Consequences for the statistical treatment of geochemical and environmental data. *Environmental Geology* 39: 1001–1014.

Rodushkin, I. & Axelsson, M. D. 2003: Application of double focusing sector field ICP-MS for the multielement characterization of human hair and nails: Part III Direct analysis by laser ablation. — *Science of the Total Environment* 305: 23–39.

Russo, R. E., Mao, X., Liu, H., Gonzalez, J. & Mao, S. S. 2002: Laser ablation in analytical chemistry — a review. — *Talanta* 57: 425–452.

Seltzer, M. D. & Berry, K. H. 2005: Laser ablation ICP-MS profiling and semiquantitative determination of trace element concentrations on desert tortoise shells: documenting the uptake of elemental toxicants. — *Science of the Total Environment* 339: 253–265.

Szép, T., Møller, A. P., Vallner, J., Kovacs, B. & Norman, D. 2003: Use of trace elements in feather of sand martin *Riparia riparia* for identifying moulting areas. — *Journal of Avian Biology* 34: 307–320.

Takagi, Y., Matsuda, S., Imai, S., Ohmori, Y., Masuda, T., Vinson, J. A., Mehra, M. C., Puri, B. K. & Kaniewski, A. 1988: Survey of trace elements in human nails: an international comparison. — *Bulletin of Environmental Contamination and Toxicology* 41: 690–695.

Torres-Dowdall, J., Farmer, D. H., Abril, M., Bucher, E. H. & Ridley, I. 2010: Trace elements have limited utility for studying migratory connectivity in shorebirds that winter in Argentina. — *Condor* 112: 490–498.

Underwood, E. J. 1971: *Trace elements in animal and human nutrition*, 3rd ed. — Academic Press, New York.

Van de Weijer, P., Baeten, W. L. M., Bekkers, M. H. J. & Vullings, P. J. M. G. 1992: Fast semiquantitative survey analysis by laser ablation inductively coupled plasma mass spectrometry. — *Journal of Analytical Atomic Spectrometry* 7: 599–603.

Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S. & Holmes, R. T. 2002: Links between worlds: Unravelling migration connectivity. — *Trends in Ecology and Evolution* 17: 76–83.

Zoccola, M., Aluigi, A. & Tonin, C. 2009: Characterisation of keratin biomass from butchery and wool industry waste. — *Journal of Molecular Structure* 938: 35–40.