Archeologický ústav Akademie věd České republiky v Brně

Přehled výzkumů

50

Brno 2009

Přehled výzkumů

Recenzovaný časopis
Peer-reviewed journal

Ročník 50
Volume 50

Číslo 1–2
Issue 1–2

Software
Software Spencer Kimball, Peter Mattis, GIMP Development Team 2008: GNU Image Manipulation Program, 2.6.1
GRASS Development Team 2008: Geographic Resources Analysis Support System, 6.3.0
Kolektiv autorů 2008: Inkscape, 0.46
Kolektiv autorů 2005: LaTeX 2_ε

Fotografie na obálce
Cover Photography Fotografie levalloiského hrotu nalezeného při výzkumu paleolitické lokality Tvarožná-Za školou. Srov. studii P. Škrdly a kol. obr. 5:1. Foto J. Špaček.
A foto of the Levallois point found in the Paleolithic site Tvarožná-Za školou. See the study of P. Škrdla et. al. Fig. 5:1. Photo by J. Špaček.

Adresa redakce
Adress Archeologický ústav AV ČR, Brno, v. v. i.
Královopolská 147
612 00 Brno
E-mail: pv@iabrno.cz
Webové stránky s pokyny pro autory: http://www.iabrno.cz/pv

ISSN 1211-7250
MK ČR E 18648
Vydáno v Brně roku 2009

BEAR DIET, SEASONALITY AND MIGRATION BASED ON CHEMICAL MULTIELEMENTAL TEETH ANALYSIS

STRAVA, SEZONALITA A MIGRACE FOSILNÍHO MEDVĚDA HNĚDÉHO NA ZÁKLADĚ MULTIPRVKOVÉ ANALÝZY.

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Abstract
Laser Induced Breakdown Spectroscopy (LIBS) and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) were utilized for microspatial analyses of a fossil bear (Ursus arctos) tooth dentine. The distribution of selected trace elements (Sr, Ba, Fe) was measured on a 26 mm × 15 mm large and 3 mm thick transversal cross section of canine tooth. The Na and Mg content together with the distribution of matrix elements (Ca, P) was also monitored within this area. It is shown that LIBS, similarly to LA-ICP-MS can be successfully utilized for fast, spatially-resolved analysis of fossil teeth samples. Rate of Sr and Ca, Sr and Ba tracers were recognized and it is possible to say, that these elements are changing its concentration in according to the seasonal increments of dentine. The concentration is lower in winter, when the bear is in his hibernation, contrary to summer, which is affluent for its substances. Secondly, concentration of Sr shows us a seasonal migration between the place of hibernating and territory where bear searched for feed. From measurement of concentrations it is possible to claim, that the bear was hunted in his season of searching for feed, when he was ensuring his fat reserve for winter. From archaeological point of view, on the base of these measurements it was possible to reconstruct the ethology of the fossil brown bear, i.e. the nutrition, health and migration.

Keywords
Mammalia, Upper Palaeolithic, Migration, Diet, Seasonality, Multielemental analysis, LIBS, LA-ICP-MS

Introduction

Teeth and bones consist of an inorganic calcium phosphate mineral approximated by hydroxylapatite $Ca_{10}(PO_4)_6(OH)_2$ and matrix proteins. The physical and chemical properties of these "bioapatite" crystals are different from those of geologic hydroxylapatite because of the way they are formed. These unique properties are required for fulfilling the biological functions of bones and teeth (Boskey 2007). The chemical constituents of the tooth tissue layers – enamel, which is the hardest and most highly mineralised substance of the body (Ross *et al.* 2003) and dentine that comprises the bulk of a tooth, are tolerant to substitution by a range of trace elements. Close to 40 trace elements have been reported to be present in tooth tissues, some of them (*i.e.* Zn, Sr, Fe, Al, B, Ba, Pb...) in the $\geq$ 1000 ppm range (Runia 1985).

Permanent tooth enamel and dentine begin its calcification around animal birth and continue to calcify into adolescence. The composition of sub-surface enamel is already fixed before tooth emergence, and is therefore able to provide a retrospective and relatively permanent record of the trace elements absorbed during the period of enamel formation (Humphrey *et al.* 2008). Spatially resolved analysis of trace element concentration has a broad relevance in disciplines ranging from dentistry and health care (Lochner *et al.* 1999) to forensics (Bush *et al.* 2008), anthropology (Dolphin *et al.* 2005), zoology (Humphrey *et al.* 2008) and archaeology (Cucina *et al.* 2007).

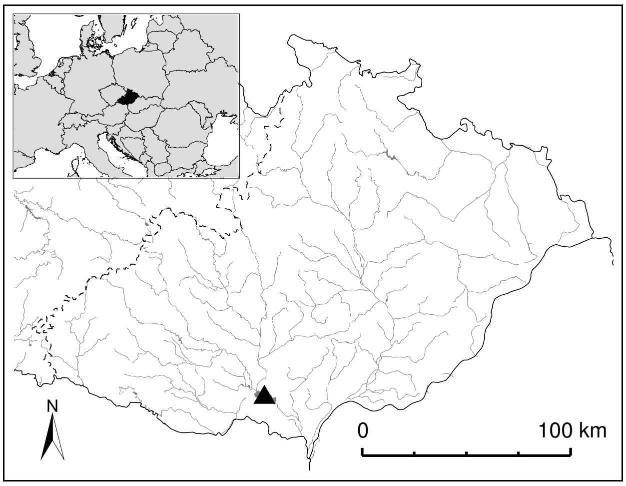

Fig. 1: Localisation of the Dolní Věstonice II site on the map of Moravia. *Obr. 1: Lokalizace sídliště Dolní Věstonice II na mapě Moravy.*

The trace element concentration of dental tissues is traditionally studied by a bulk sampling approach (Perez *et al.* 2004; Copeland *et al.* 2008). Further techniques include the mechanical (grinding, drilling) or chemical (acid dissolution) separation of successive enamel layers and their analysis (Adachi *et al.* 1998). Such layers have been studied with X-ray fluorescence (XRF) (Carvalho *et al.* 2000; Martin *et al.* 2007), electron microprobe X-ray microanalysis (EPXMA) (Johnson 1972; Grman, Andrik 1978), particle (proton) induced X-ray

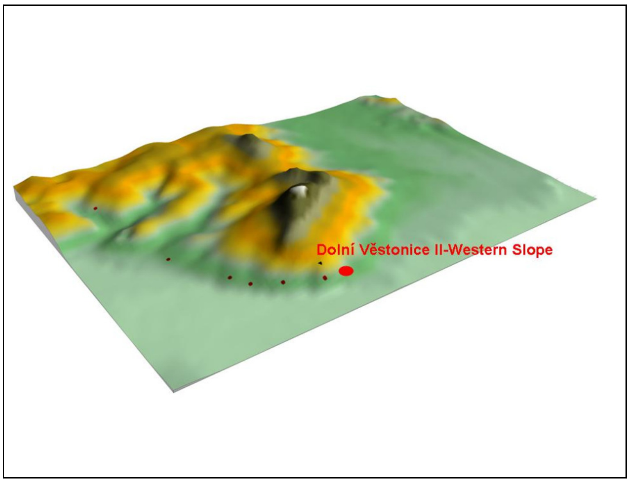

Fig. 2: Localisation of Dolní Věstonice II – Western Slope. *Obr. 2: Lokalizace sídliště Dolní Věstonice II – západní svah.*

Fig. 3: Studied canin of fossil brown bear (*Ursus arctos*) from Dolní Věstonice II – Western Slope, field II. *Obr. 3: Studovaný špičák fosilního medvěda hnědého (Ursus arctos) z Dolních Věstonic II – západního svahu, pole II.*

emission spectroscopy (PIXE) (Brenn *et al.* 1999) or secondary ions mass spectrometry (SIMS) (Stermer *et al.* 1996; Jälevik *et al.* 2001) for many years. The feasibility of laser-ablation based analytical methods for quantitative and qualitative microspatial analysis of teeth samples has been also demonstrated (Samek *et al.* 1999; Prohaska *et al.* 2002; Daniel *et al.* 2004). LIBS and LA-ICP-MS allow depth profiling and surface area scanning/mapping that make them suitable for analysis of the spatial distribution of elements (Lee *et al.* 1999; Lochner *et al.* 1999; Novotný *et al.* 2008; Kaiser *et al.* 2009). Additionally, LA-ICP-MS provides high sensitivity and low detection limits.

Here we present the results on microspatial analyses of a 26 mm × 15 mm large and 3 mm thick transversal cross section of fossil brown bears (*Ursus arctos*) permanent canine tooth (Fig. 2). The investigated sample consisted of dentine covered by a thin cementum layer. The distribution of selected trace (Sr, Ba, Fe) and (Ca, P) matrix elements was monitored. The spreading of further elements (Na, P, Mg) was also measured. The intertooth distribution of these elements derived mainly from LA-ICP-MS measurements is reported in several former works (Lee *et al.* 1999; Lochner *et al.* 1999; Kang *et al.* 2004). The choice of investigated chemical elements was further motivated by their importance in palaeozoology.

It has been observed that the strontium content decreases in the dentine towards the pulp (Molleson 1988; Frank *et al.* 1989; Reitznerová *et al.* 2000). Some deviations in the Sr/Ca and Sr/Ba ratios indicate population mobility (Burton *et al.* 2003) or a social status in the childhood (Grupe 1998). The metabolism of strontium and barium is analogical to the calcium one. The Sr/Ca and Ba/Sr ratios generally show on the character of the nutrition (Burton, Price 1990; Sponheimer *et al.* 2005). In fossil findings, the barium and strontium contents may be increased by post depositional processes (Kohn *et al.* 1999). Iron contamination is important for archaeological materials (Kohn *et al.* 1999; Rodríguez-Fernández *et al.* 1999; Carvalho *et al.* 2000). The sodium content decreases in the dentine (Besic *et al.* 1969; Shaw, Yen 1972; Grman, Andrik 1978). Potassium shows similar behaviour (Shaw, Yen 1972; Grman, Andrik 1978).

Magnesium plays an important role at the mineralization, mainly in its early phase. It is progressively replaced with calcium and behaves similarly to strontium (*e.g.* rat). Its content slightly increases from the surface through the dentine. The dentine content is substantially higher than the enamel one (Johnson 1972; Steinfort *et al.* 1991). It was verified for macaque, mastodont and human (Besic et al. 1969; Shaw, Yen 1972; Grman, Andrik 1978) respectively. An increase from the apical towards the cervical end of the dentine was also reported (Arany *et al.* 2004). The differences among the outer, inner and pulpal parts of the dentine were negligible.

Material and sample preparation

Dolní Věstonice II is one of a series of large settlements of mammoth hunters on the loess elevations at an altitude of about 180–240 m a.s.l., rising above the Dyje river and sloping further to the top of Pavlovské Hills (550 m a.s.l.) – Fig. 2. Archaeological excavations at this site were organized by B. Klíma and J. Svoboda between 1985 and 1989 (Svoboda 1991), and additional excavations took place in 1991, 1999 and 2005 (Svoboda 2001; Svoboda *et al.* 2006). The site became world-famous for human paleontological finds including the triple burial (DV 13–15) discovered at the top of the site, the DV 16 burial on the western slope, and individual human remains scattered at various places in the cultural layer. In terms of chronology, the site probably results from repeated occupations, extended over the time span of 28–24 ka ^{14}C BP (all ^{14}C data are uncalibrated). The first series of radiocarbon dates, around 29–27 ka ^{14}C BP, were obtained from charcoals in the underlying paleosols, with little or no evidence of human occupation, where only two dates in the lower part of the site are associated with artifacts. The second series of radiocarbon dates, all from clearly anthropogenic cultural layers and from artificial structures (settlement units) cluster around 27 ka ^{14}C BP (Early Pavlovian). A third series of dates fall into the time span of 27–25 ka ^{14}C BP (Evolved Pavlovian). This time-span is framed by a series of earlier and later luminescence dates from paleosols and loess below and above, which make Dolní Věstonice II the best dated Gravettian site

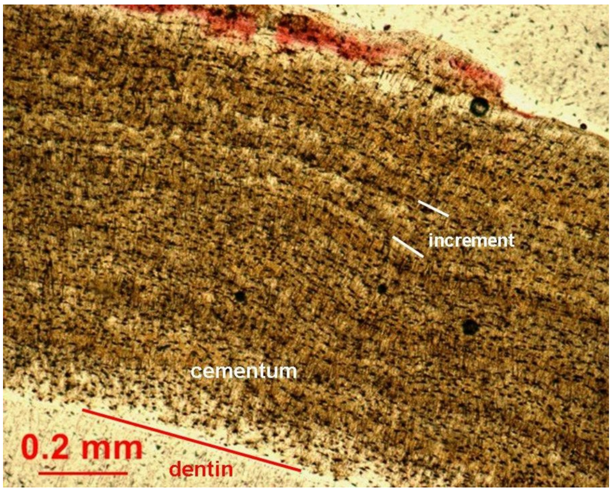

Fig. 4: Cross section of bear's canin. This bear died at the age of 14 years and it is possible to appoint the term of death from unfinished summer increment and absence of winter increment in between summer and autumn season (August to October). *Obr. 4: Výbrus kořene špičáku medvěda hnědého. Tento medvěd zahynul ve věku 14 let a podle nedokončeného letního přírůstku a absence zimního přírůstku lze dobu smrti určit do období mezi létem a podzimem (od srpna do října). Increment=přírustek; cementum=cement; dentic=zubovina.*

in the region (Svoboda, 1996a; 1996b; 2001; Svoboda *et al.* 2000).

The bear tooth analysed in this paper (Fig. 3) originates from field II, which is a longitudinal zone on top of the site, adjacent to the triple burial, and excavated in 1986. It may be related to the third series of radiocarbon dates obtained from the same area. The analysed tooth (canin – C1) belongs to brown bear (*Ursus arctos*). Abrasion of tooth's oclusal area and tooth's root cement increments (Nývltová Fišáková 2007) were studied to determine the age and seasonality of bear death. This bear died at the age of 14 years and it is possible to appoint the term of death from unfinished summer increment and absence of winter increment in between summer and autumn season (August to October) – Fig. 4.

Instrumentation

Comparing the two laser-ablation based techniques applied, it should be noted that LIBS gives practically an instantaneous signal directly related to the location at which the single ablation event occurred. However LA-ICP-MS has in general a lower detection limits, this technique involves sample transport and diffusion so that the signal produced in the mass spectrometer is not directly attributable to a specific location on the sample, without considerable care in the analysis (Kaiser *et al.* 2009).

LIBS device

The second harmonic (532 nm) of a pulsed Q-Switched Nd:YAG laser with homogeneous energy along the beam cross section (Spectron, model SL 284, pulse width 5 ns, beam diameter 4 mm) was used to generate microplasmas on the sample surface in air at atmospheric pressure. The beam was 3× expanded by an optical system consisting of two lenses (a diverging lens BK7 with 25 mm of focal length and a converging lens BK7 with 75 mm of focal length) and then focused onto the sample surface with 100 mm focal length BK7 lens. Plasma emission was collected by a fiber optic (length = 5 m, diameter = 600 μ m, NA = 0.22) and guided onto the entrance slit of a 0.5 m focal length Czerny-Turner imaging spectrograph (Chromex, model 500 IS, f-number 8, fitted with interchangeable gratings of 300, 1200 and 2400 grooves.mm^{-1}). Spectral emission was detected by an intensified charge-coupled device (ICCD, Stanford Computer Optics, model 4Quik 05) with 768 × 512 pixels, each 7.8 × 7.8 μ m^2. This configuration provides a spectral window of 15 nm and a spectral resolution of 0.02 nm.pixel^{-1} using an entrance slit width of 50 μ m and the grating of 2400 grooves.mm^{-1}. Operation of the detector was controlled with 4Spec software. The sample was positioned on two crossed motorized stages (PI Physik Instrumente) for both X and Y displacement. Moreover, a viewing system for assisting in examination and sample positioning was also used.

The LIBS spectra were acquired in appropriate spectral windows from region 266–598 nm. The following spectral lines were used in analysis: P (I) (253.56 nm), Mg (II) (279.55 nm, 280.27 nm), Mg (I) (285.21 nm), Fe (I) (302.40 nm), Ca (II) (315.89 nm, 317.93 nm, 370.60 nm, 373.69 nm, 393.36 nm, 396.85 nm,), Ca (I) (422.67 nm, 518.89 nm, 616.22 nm), Ba (II) (455.40 nm), Sr (I) (460.73 nm) and Na (I) (589.00 nm, 589.59 nm).

LA-ICP-MS setup

Instrumentation for LA-ICP-MS consists of a laser ablation system UP 213 (New Wave, USA) and an ICP-MS spectrometer Agilent 7500 CE (Agilent, Japan). A commercial Q-switched Nd:YAG laser ablation device works at the 5th harmonic frequency (213 nm). The ablation device is equipped with programmable XY-stages to move the sample along a programmed trajectory during ablation. Target visual inspection is accomplished by means of built-in microscope/CCD-camera system. A sample was enclosed in the SuperCell (New Wave, USA) and was ablated by the laser beam, which was focused onto the sample surface through a quartz window. The ablation cell was flushed with helium (carrier gas), which transported the laser-induced aerosol to the inductively coupled plasma. A sample gas flow of argon was admixed to the helium carrier gas flow after the laser ablation cell. Therefore, the total gas flow was 1.6 l.min^{-1}. Optimisation of LA-ICP-MS conditions (gas flow rates, sampling depth, electrostatic lenses voltages of the MS) was performed with the glass reference material NIST SRM 612 in respect to maximum S/N ratio and minimum oxide formation (ThO^+/Th^+ counts ratio 0.2%, U^+/Th^+ counts ratio 1.1%).

In order to analyse specific locations in the sample, for LA-ICP-MS measurements the ablation laser was used in hole drilling mode (fixed sample position during laser ablation), for the duration of 6 seconds for each spot. Distance between them was 200 μ m. Time delay between end of laser ablation of one spot and initiation of laser ablation of following spot was 10 seconds. Laser ablation

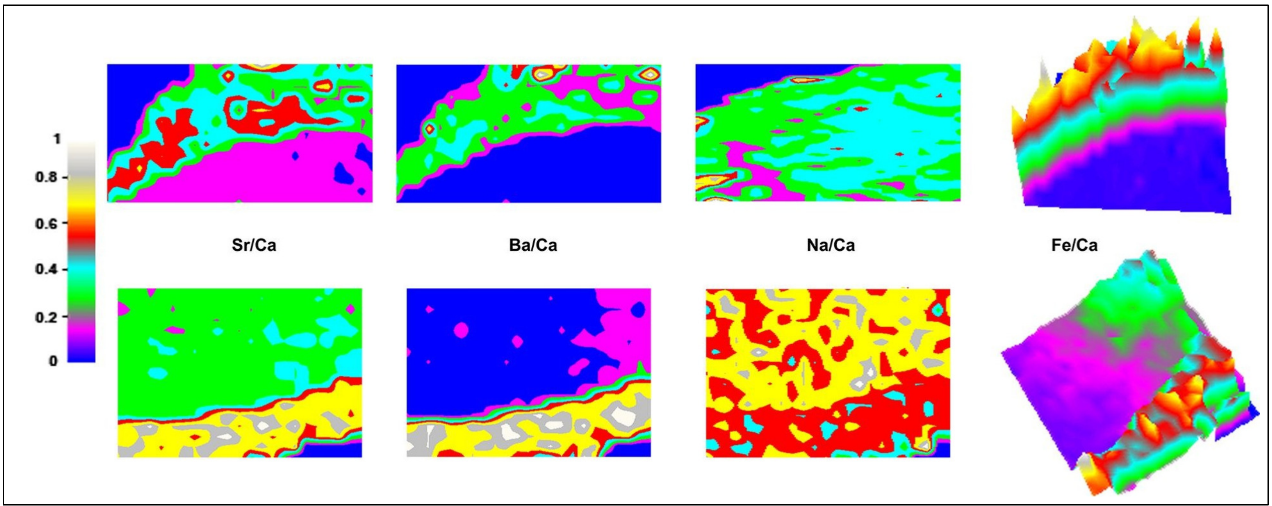

Fig. 5: The results of LA-ICP-MS elemental mapping in two different areas of root of tooth (a), (b). The bar has a length of 400 μ m. *Obr. 5: Výsledky LA-ICP-MS distribuce prvků ve dvou oblastech kořene zubu. Škála má délku 400 μ m.*

was performed with laser spot diameter 100 μ m, laser fluence 12 J.cm^{-2} and repetition rate 10 Hz. The isotopes 23Na, 24Mg, 31P, 43Ca, 57Fe, 86Sr and 135Ba were measured with integration time 0.1 s/isotope.

Results and Discussion

LIBS and LA-ICP-MS line scans and mapping

Typical single-shot LIBS spectra are shown in Fig. 3. For LIBS lines scanning, spectral lines routinely used in LIBS analysis (online on: *http://www.appliedphotonics.co.uk/Libs/capabilities_libs.htm*) were chosen. In order to properly relate the detected emission lines intensities with the species amount, *i.e.* to avoid self-absorption, the microplasma emission and the detection temporal interval were optimised by preliminary measurements. After 3 cleaning shots the LIBS signal was averaged from 7 shots fired to the same sample position. During the data analysis, the continuum background determined for each shot from five data points on both sides of the monitored spectral line by a linear background fit method was subtracted from the intensity value of every data point forming the spectral line.

The line scans derived from LIBS and LA-ICP-MS analysis of macro element Na and trace element Fe are compared. Both laser-ablation based techniques revealed similar accumulation for all of the tracked elements across the scanned lines. Similar behaviour of elemental distribution inside the dentine to that reported in literature was observed, *i.e.* the differences of among the magnesium signal in outer, inner and pulpal parts of the dentine were negligible. The inhomogeneity in Na signal can indicate contamination from outside, which was further confirmed with the line and area scans of Fe.

The 100 μ m ablation crater diameter used for LA-ICP-MS technique allowed mapping the sample with higher-spatial resolution. The results of this elemental mapping in two different regions of the tooth cross section are shown in Fig. 6. The (normalized) variation of the Sr/Ca, Ba/Ca, Na/Ca and Fe/Ca is plotted. The different chemical composition of the cementum (1 mm thick layer) can be clearly distinguished. On the frame of ongoing work we would like to exploit also the LIBS capabilities for mapping, mainly by optimization of LIBS ablation crater diameter and applying double-pulse LIBS or LIBS+LIFS techniques in order to decrease detection limits.

Using of Sr/Ca and Sr/Ba ratios to determine the bear migration

The line and area scans of the sample were used to reconstruct the ethology of this fossil brown bear. The seasonal fluctuations of the Sr/Ca and Sr/Ba detected by both laser-ablation based techniques, showed the migration of this bear between his hibernaculum location and the place, where the fossils were found. From measurement of concentrations it is possible to claim, that the bear was hunted in his season of searching for feed, when he was ensuring his fat reserve for winter. Moreover, he could approach closer to the settlement of hunters, because he was attracted by a lot of remains from their hunting. As an example, Fig. 6 shows these ratios for both, LIBS line scan and LA-ICP-MS mapping. The incremental strips in the LA-ICP-MS maps are marked with the dashed lines. The dark areas are well correlated with the lower Sr/Ba ratio in the map. They are rather related to the narrow winter strips, which are well identified in the photographs superimposed on the Sr/Ba and Sr/Ca line scans above acquired by LIBS. The correlation of the Sr/Ca ratio with that strips is not so apparent because they are both higher and lower Sr-doped wide strips in the mapped area. If the tooth is not very affected by diagenesis it can be concluded that i) the bear consumed a plant food mainly in the hot seasons by the Sr/Ca increased ratio visible mainly in the LIBS scans, ii) the bears migration is characteristic not only for the years seasons but also for different years which is visible in comparison of the Sr/Ba ratios in the particular wide strips from the outer to the inner part of the dentine in the LA-ICP-MS maps and by the Sr/Ba decrease ob-

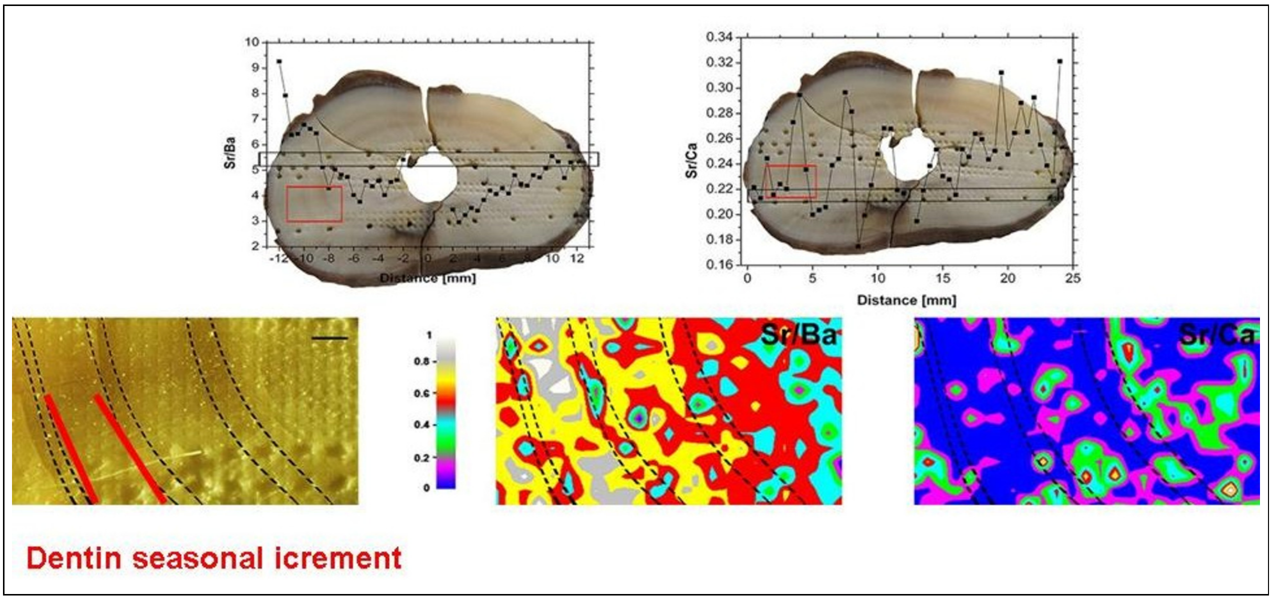

Fig. 6: The Sr/Ca and Sr/Ba ratios derived from LIBS line scan and LA-ICP-MS mapping. With dotted lines the different regions on the teeth cross section are shown. The bar has a length of 500 μ m. *Obr. 6: Poměr Sr/Ca a Sr/Ba odvozený z line scanu měřeného pomocí metod LIBS a LA-ICP-MS mapování. Tečkovanou čárou jsou vyznačeny oblasti zimních a letních přírůstku dentínu. Škála má délku 500 μ m. Dental seasonal increment= sezónní přírůstky dentinu.*

servable in the LIBS scan. The bear probably changed its living territory in one direction.

Conclusions

Laser-ablation based analytical techniques were used for mapping and line-scanning of fossil animal tooth section. LIBS, similarly to LA-ICP-MS, was proved suitable for fast, spatially-resolved analyses of such calcified tissues.

From archaeological point of view, on the base of these measurements it was possible to reconstruct the ethology of the fossil brown bear, *i.e.* the nutrition, health and migration. The measured Sr/Ca and Sr/Ba profiles across the sample showed seasonal fluctuations and showed the migration of this bear between his hibernaculum location and the place, where the fossils were found. Together with the results from other techniques (*i.e.* study of cementum increments), we can conclude that this bear specimen was most probably hunted in the time when it was foraging before winter dormancy and was migrating near the human settlement (where the fossils were found). In terms of seasonality, the settlement of Dolní Věstonice II provided a large amount of skeletal materials of fur-bearing animals, namely of foxes, as well as lithic and bone tools suitable for hide working. This has been interpreted as an evidence of human activities during a cold season (*cf.* discussion in Svoboda *et al.* 2000; Opravil 1994), basing on evidence from tree rings of the preserved charcoal, suggested a winter occupation as well. However the location of human activity areas both inside and outside the reconstructed dwellings would suggest, rather, an all-year-round occupation. Teeth's roots cement increments of various mammals were analysed in order to support this statement (Nývltová Fišáková 2007, 2008). Occupation during the growing season was confirmed also by element analysis of bear's tooth which show us, that the bear was hunted in between summer and autumn season, when he was in his feed searching for winter period. This finding is consistent with cement increments analysis on the bear's canine root.

It was shown that LIBS and LA-ICP-MS could be successfully applied as direct or complementary techniques in spatially-resolved microchemical analysis of fossil samples.

Acknowledgements

M.N.F. and J.S. acknowledge the grant of GA AV ČR KJB800010701-„Hunting Strategies of Upper Palaeolithic People“ and the grant of Institute of Archaeology Academy of Science of the Czech Republic No. AVOZ80010507.

M.G., J.K. and R.M. acknowledge the Ministry of Education, Youth and Sports of the Czech Republic for research project MSM 0021630508. K.N. and A.H. acknowledge the Ministry of Education, Youth and Sports of the Czech Republic for research project MSM 0021622411 and ME08002.

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Resumé

LIBS a LA-ICP-MS byly využity pro mikroskopickou distribuci prvků v dentinu a cementu špičáku fosilního medvěda hnědého (*Ursus arctos*). Distribuce vybraných stopových prvků (Sr, Ba, Fe) byly naměřeny na výbrusu kořene medvědího špičáku o velikosti 26 $\times$ 15 mm a 3 mm šířky. Byly sledována distribuce sodíku (Na), hořčíku (Mg) spolu s rozložením matričních prvků vápníku a fosforu (Ca a P). Ukázalo se, že pomocí LIBS a stejně tak i s LA-ICP-MS lze rychle analyzovat distribuci prvků ve fosilním zubu. Poměry stroncia a vápníků (Sr/Ca) a stroncia a baria (Sr/Ba) byly změřeny a zjištěny změny obsahuv souvislosti se sezónními přírůstky dentinu. Obsah je nízký v zimě, kdy medvěd je v zimním spánku, oproti letním přírůstkům, kde koncentrace stoupá díky bohaté potravní nabídce během vegetačního období. Obsah stroncia (Sr) nám vypovídá také o sezónních migracích studovaného zvířete mezi místem jeho zimního spánku a oblastí, kde si hledal potravu. Z analýz přírůstku cementu na kořeni špičáku a z koncentrací zkoumaných prvků lze říct, že zvíře uhynulo na rozhraní léta a podzimu, kdy medvěd hledal

potravu pro vytvoření zásob na zimu. Zmíněné metody ukazují využití pro archeologii, paleontologii a paleoekologii, které umožňují zrekonstruovat etologii fosilního medvěda hnědého včetně výživy, zdraví a migrace.