Biogeochemistry (2009) 94:197–215
DOI 10.1007/s10533-009-9319-7

Compositional changes in fish scale hydroxylapatite during early diagenesis; an example from an abandoned meander

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Received: 16 November 2008 / Accepted: 27 March 2009 / Published online: 16 April 2009

Abstract The chemistry of recent fish scales (tench, common carp, amur and European perch) and subrecent fish scales (European perch and mostly taxonomically not determined) was studied by means of laser ablation inductively coupled plasma mass spectrometry and electron microprobe from shallow boreholes in the abandoned meander Certak near Uherské Hradiste. A detailed study of subfossil fish confirms extremely rapid changes in the chemical composition of hydroxylapatite during very early diagenetic processes in the first 2 years, which does not change significantly in the bottom of the boreholes about 25 years old. While there is an enormous increase in Na, K, Ba and Sr, Fe and Mn, there is a major decrease in Mg. The fish scales from 70 to 78 cm (6–8 years old) are approaching already the level of francolite-type hydroxylapatite. The high Na content indicates carbonate substitutions for OH or PO_4 group. Consequently, environmental interpretations based on the chemistry of fossil hydroxylapatites require extreme caution. In recent fish scales, the element concentrations reflect not only the position in the trophic pyramid but also a complicated interplay of the diet, physiological processes and environmental contamination.

Keywords Hydroxylapatite · Fish scale · LA-ICP-MS · Diagenesis · Trophic web · Abandoned meander · Morava River

Introduction

Scales of bony fish have two principal parts. A thin, hard, external, well mineralized layer which is composed chiefly of Ca-deficient dahllite-type hydroxylapatite containing small amounts of Na and Mg, as well as carbonate anions in the phosphate sites of the apatite structure, which overlays a thick poorly mineralized layer composed largely of collagen known as the basal or fibrillar plate (e.g., Fouda

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1979; Zylberberg and Nicols 1982; Helfman et al. 1997; Ikoma et al. 2003; Zylberberg 2004). Collagen usually predominates and the mineral content of teleost scales varies from 16 to 59% (Seshaya et al. 1963). Due to the presence of a significant amount of foreign ions and non-stochiometric formula, the biological apatites of fish scales have a small degree of crystallinity and a high surface area, which contributes to their high reactivity (Trueman 1999). Consequently, they are more prone to display a variety of chemical substitutions.

It is assumed that organic parts represented by the mucuous envelope and collagen are lost by hydrolysis and/or consumption by bacteria throughout earliest diagenesis (Lee-Thorp 2000; Pfretzschner 2004). During the subsequent diagenesis and fossilization F and Si are gradually incorporated into the biogenic hydroxylapatite structure replacing OH and P, respectively, in the dahllite-type hydroxylapatite and increasing its crystallinity (Zipkin et al. 1962; Eanes et al. 1965; Posner et al. 1984; Johnsson 1997). Tighter bonding of F makes F-enriched hydroxylapatite of francolite-type thermodynamically more stable and less soluble than dahllite-type hydroxylapatite and it is assumed that it reflects the replacement of biogeochemical reactions in living organisms. The gradual increase in F has been used as an indirect method of dating bones chemically (Johnsson 1997).

Hydroxylapatite in fish scales has been widely used recently for remediation of heavy metal pollution in water, soil and sediment (Admassu and Breese 1999; Wright and Conca 2000; Knox et al. 2006; Mustafiz 2002). There is a great deal of data on the rapidity of heavy metal retention by fish scale hydroxylapatite and on the reaction of artificial hydroxylapatite with heavy and other metals in duration from minutes and hours to days (Ma et al. 1993, 1994; Basu et al. 2007; Xu et al. 2007; Sheha 2007; Yasukawa et al. 2007; Nadeem et al. 2008). What is lacking, however, are the observations as to how a single fish scale reacts with its environment over a time span of several years, i.e. how rapid is the pace of the earliest diagenetic changes and absorptions.

The study of fish scales from well dated sediment in an abandoned meander of the Morava River and their comparison with scales of recent fish of the abandoned meander gives a reliable instrument for observing the fish scale behavior during the earliest diagenesis in a natural system over a short time interval from 1 to 26 years. Subfossil fish scales were mostly fragmented and specific determination was possible only in scales of European perch, recent fish scales belonged to amur, tench, common carp and European perch. The purpose of this paper is to concentrate on elements such as Fe, Mn, F, Sr, Mg, and Ba, which exhibit the most significant changes in concentration during the diagenesis/fossilization processes.

Materials and geological setting

The study material contains fish scales sampled from the abandoned meander lake Čerták located in the lowlands of the Lower Morava Valley, in the eastern part of the Czech Republic. The Čerták abandoned meander, about 1.2 km long and 50 m wide, is located in the SE outskirts of the town of Uherské Hradiště on the right bank of the Morava River, a left hand tributary to the Danube River (Fig. 1). The lake was created in the late 1930s by a river realignment and is still connected to an active channel of the Morava River by an underground pipe with a 0.8 m diameter located at its SE end (WGS-84: N 49°04′05.47″; E 17°26′09.69″). The fish scale samples were obtained from three boreholes, up to 4 m deep, drilled in the sedimentary fill of the lake (Bábek et al. 2008; Holá et al. 2009).

Fish scales were only obtained from depth intervals of 7–15, 18–26, 45–54, 70–78, 78–88 and 250–260 cm. The specimens were fragmented in nearly all of the cases, which prevented their taxonomic identification. The only exception was the whole scales of European perch (*Perca fluviatilis*), which were obtained from 18 to 26, 45 to 54 and 250 to 260 cm (Tables 2, 3). The fish scales of recent fishes in the abandoned meander—tench (*Tinca tinca*), common carp (*Cyprinus carpio*), amur—grass carp (*Ctenopharyngodon idella*) and European perch (*Perca fluviatilis*)—were obtained from the local Moravian fisherman association in the town of Staré Město.

The river channel deposits of the basal sequence were deposited in the original river meander before the the lake was formed in the late 1930s. The lake then turned into the lacustrine sedimentation mode. In 1981, sediments of the lake were dredged in a zone covering all the three cores to a level of about 2.2 m below the lake water level (Benícek 1981), which

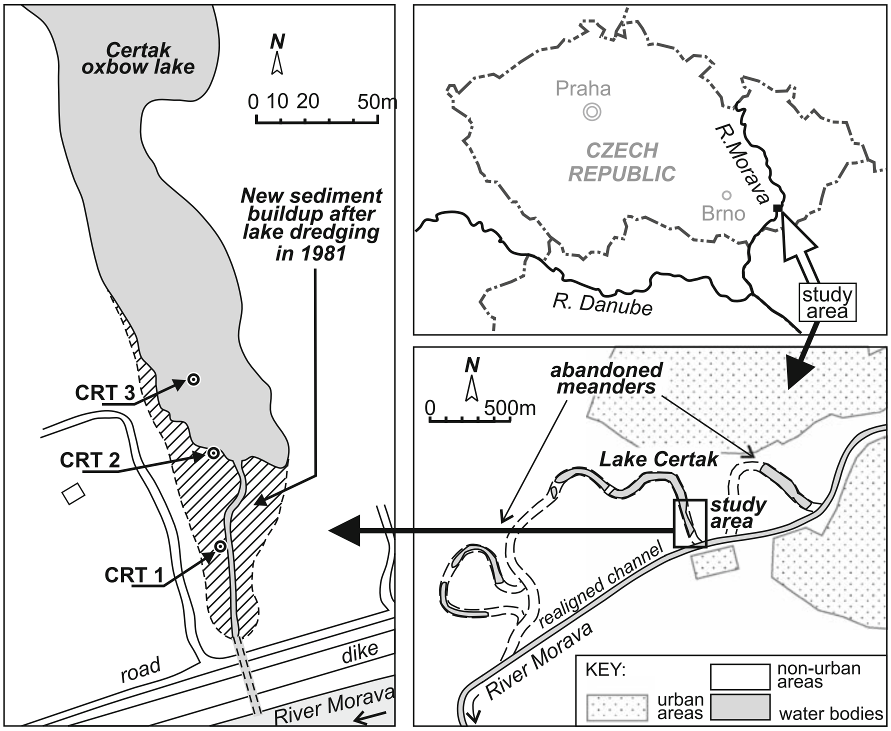

Fig. 1 Location of the Oxbow Lake Certak (CRT) near Uherske Hradiste

corresponds to the present-day boundary between the basal and the intermediate sequence. Since then, the lake has received a new sediment, which now stands more than 0.5 m above the water level in the most proximal part of the lake. The post-1981 sediments were dated based on the Chernobyl 1986 nuclear power plant accident using a gamma-ray spectrometric measurement of ^{137}Cs mass activity implying an average sedimentation rate of 7.0–7.7 cm per year for the intermediate and topmost sediment sequence. Sediments from the basal sequence are dated back to the pre 1930s based on core stratigraphy and local historical data.

Samples weighing from ~400 to ~900 g, which comprise some 8–15 cm of sediment thickness, were taken from the borehole in 15–70 cm intervals, and analyzed for concentrations of heavy metals and persistent organic pollutants (POPs) in the sediments. The wet sampled, lyophilized and sieved sediments were extracted and analyzed for heavy metals by inductively coupled plasma-mass spectrometry (ICP-MS) of Aqua Regia leachate, and for persistent organic pollutants by gas chromatography (GC-ECD and GC-MS).

Methods

Sample preparation

Subrecent fish scales were obtained from dried sediment, which was placed in 5-l buckets flushed with water and then wet-sieved on a 125 μm sieve. They were hand-picked from the air-dried oversize fraction. The scales were subsequently gently sonicated in a dilute solution (5%) of hydrogen peroxide and then triple rinsed in ultrapure water. The treatment was repeated until the scales were completely free of sediment. A similar approach was chosen for the cleaning of the recent fish scales, which were sonicated in a 5% solution of hydrogen peroxide several times and triple rinsed in ultrapure water. Finally, they were brushed gently with a soft bristle toothbrush until the mucous envelope was completely removed. Both the recent and subrecent scales were mounted onto petrographic slides using double-sided tape for subsequent LA-ICP-MS and EMP analysis. All instruments that came directly or indirectly into contact with the scales were non-metallic.

Laser ablation ICP MS

The trace elements were quantified using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A pulsed Nd:YAG laser system UP 213 (New Wave Research, Inc., Fremont, CA, USA) working with 213 nm wavelength and pulse duration of 4.2 ns was used. The ablation system is equipped with a 33-cm^3 SuperCell (New Wave Research) designed to enable rapid eluting of the ablation-generated aerosol in a large format cell. Helium was used as a carrier gas with the flow rate of 1 l min^{-1}. Aerosol is transported from the ablation cell with a 1-m long polyurethane transport tube (i.d. of 4 mm) to the ICP discharge of the quadrupole mass spectrometer (ICP-MS Agilent 7500ce, Agilent Technologies, Santa Clara, CA, USA).

Each ablation measurement was performed in the form of a line of spots creating a scan throughout the whole fish scale perpendicular to the growth rings. The spot diameter was 55 μm, scan speed 30 μm s^{-1}, repetition rate 10 Hz and fluence 5 J cm^{-2}. The following isotopes were selected and used for the LA-ICP-MS analyses: ^{26}Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{55}Mn, ^{51}V, ^{53}Cr, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{86}Sr, ^{111}Cd, ^{138}Ba and Pb as the sum of isotopes ^{206}Pb, ^{207}Pb and ^{208}Pb. The external calibration was performed using two standard reference materials (SRM)—SRM NIST 1486 (bone meal powder) and SRM NIST 612 (glass). Due to the matrix composition, SRM NIST 1486 was found to be suitable for the matrix-matched calibration. The bone meal powder was prepared for the ablation as a pressed pellet without any additional binder using a manual hydraulic press. Since the SRM NIST 1486 does not comprise all the elements of interest, certain trace elements (Table 2) were determined using the SRM NIST 612. Calcium was used as an internal standard; the Ca content in each sample was obtained from an EMP analysis.

Electron microprobe

Element abundances of Na, Ca, P, Fe, Mn, Mg, Sr, Zn, Pb, F and Cl in fish scales were determined using a CAMECA SX100 electron microprobe (Masaryk University and Czech Geological Survey Brno) equipped with five wavelength dispersive spectrometers, and with a CL detector. The analyses were performed on a carbon coated unpolished surface of scales under the following conditions: accelerating voltage 15 kV, beam current and 4 nA, and beam scanned area 50 × 35 μm. The homogeneous synthetic phases and well-defined minerals were used as standards. The raw data was converted into concentrations using appropriate PAP matrix corrections (Pouchou and Pichoir 1985). The peak counting times were 20 s for Ca and P and 40–60 s for other elements. At these conditions the average detection limits were as follows: ~1,000 ppm for F; ~700 ppm for Na, Fe, Mn, Zn; ~500–600 ppm for P, Mg, Ca, Cl, Sr.

Terminology for apatite

Apatite is a common mineral occurring in highly variable geological and biological environments but also commonly used in various chemical or physical experiments; hence, various terms are used in these scientific disciplines. In order to prevent misunderstandings, an explanation of the used terminology is given. An apatite group has the general formula $M_{10}(ZO_4)_6X_2$, where M = Ca, Sr, Pb, Na; Z = P and X = F, OH, Cl (only the most important elements are given). There are two distinct crystallographic sites for Ca—nine-coordinated Ca(1), and seven-coordinated Ca(2). Due to the fact that the apatite structure is remarkably tolerant to structural distortion, numerous substitutions enable highly variable compositions involving a wide spectrum of elements including e.g., REE, Ba, Mg, C and vacancy in the M site. Bioapatites, however, exhibit much simpler chemical compositions involving hydroxylapatite—$Ca_5(PO_4)_3(OH)$, carbonate-hydroxylapatite—$Ca_5(PO_4,CO_3)_3(OH)$ and carbonate-fluorapatite—$Ca_5(PO_4,CO_3)_3(F)$. Carbonate-hydroxylapatite (francolite-type with >1 wt% F) and carbonate-hydroxylapatite (dahllite-type with <1 wt% of F; Johnsson 1997; Hubert et al. 1996; Nemliher et al. 1997) are widely utilized chiefly in biological literature. Apatite minerals and their varieties are also abbreviated as FAP—(fluorapatite), HAP—(hydroxylapatite), Mg-CaHAP—(Mg-bearing hydroxylapatite) including complicated abbreviations such as CO_3/Na type-B defect in HAP (Na-bearing carbonate-hydroxylapatite) chiefly in chemical but also in biological literature (see e.g., El Feki et al. 2000; Yasukawa et al. 2002; Peroos et al. 2006).

Results

Sediment

The distribution of the majority of heavy metals in sediments shows a distinct stratigraphic pattern. The concentrations of most heavy metals increase from the sediment surface downwards to about 100 cm depth, which approximately corresponds to the mid 1990s (Bábek et al. 2008). The concentrations then continue to increase downwards, but less rapidly. Peak concentrations of Cr, Cu, Zn, As, Cd, Sb, Pb, and Hg were found in sediments at ~235 cm depth, deposited during the early to mid-1980s (Table 1; Fig. 2) as recalculated from the average sedimentation rate (Bábek et al. 2008). A sudden drop in the concentration of most heavy metals was detected at the boundary between the river and the abandoned meander sediments approximately at ~255 to ~265 cm depth, which corresponds to the 1981 datum level (Bábek et al. 2008). The lowest concentrations were found in the river sediments below ~265 cm depth, dating back to the pre-1930s. Concentrations of Pb, As, Zn, Cu, Cr, Sb, and Hg decreased twice to nine times and concentrations of Cd even 34-times with respect to the overlying, post-1981s sediments.

Concentrations of Mn, Mg, Na, K, Fe, and P in the sediment were recalculated from concentrations of major oxides (MnO, MgO, Na_2O, K_2O, Fe_2O_3, and P_2O_5) for the uppermost 90 cm of sediment thickness, which roughly span the interval from ~1995 to ~2005. The concentrations do not show any dramatic stratigraphic trends (Table 1; Fig. 2). Slightly increasing concentrations to the bottom were found for Fe (from 13,900 ppm in 15 cm depth to 19,300 ppm in 90 cm depth), P (from 600 to 1,300 ppm) and Mg (from 7,600 to 9,400 ppm), respectively. Slightly decreasing concentrations were found for Na (from 3,400 in 15 cm depth to 2,600 ppm in 90 cm depth) whereas K and Mn show no stratigraphic trends. No data is available for greater depths of sediment and for certain elements such as F, Sr, and Ba.

Fish scales

Although there are certain differences in the content of some elements in the fish scales of recent fish of tench, carp, amur (grass carp) and European perch (Tables 2, 3) which reflect variations in diet and the nutritional and environmental history of the individuals, a feature apparent at first glance is the substantial difference between the recent and subrecent scales on the whole (Tables 2, 3; Figs. 3, 4).

The most striking feature in the hydroxylapatite (HAP) chemistry is the enormous increase in F and Fe contents already in fish scales from the uppermost part of the borehole (7–15 cm sample) in comparison with the fish scales of recent fish (Fig. 3). These results show that after deposition in the sediment, the hydroxylapatite structure became very quickly (in 2 years) enriched in Fe, Mn and heavy metals (see Holá et al. 2009) attaining several orders-of-magnitude higher concentrations than the water of the Morava River (Fig. 3; Table 4). The concentrations of Fe increased approximately 100 times; Mn concentrations more than 10 times (Tables 2, 3; Fig. 3). The enormous increase in Fe content is evidently responsible for the color change of the fish scales from white in recent ones to brown in subrecent ones.

Disregarding calcium, Mg is the main bivalent cation found in biological apatites. However, its significant loss in fish scales during the earliest fossilization up to nearly 10-times (Fig. 3) is interesting because of high concentrations of Mg in the host sediments (Fig. 2). Another important feature is the variation of Mg content between the individual species (Table 2). The content in fish scales of European perch is less than a half than those of tench.

A considerable increase in Ba is already recorded in scales from 1 to 2-year-old sediment where its values are more than three times higher than in recent fish (Tables 2, 3; Fig. 3). Despite considerable differences in Ba content in the spectrum of studied recent fish scales, the data from the scales of recent and subrecent European perch confirms this trend.

The Sr content in subfossil fish scales of European perch is approximately four times higher than in recent ones (Tables 2, 3). This value is once again achieved very quickly in scales approximately 3–4 year old. The values remain nearly constant being approximately the same in the scale of common perch 25 years old. The reversed trend is in Mg where the content in scales of living fish is several times higher than in subfossil ones.

Albeit Na contents in fossil bone are usually lower relative to modern samples (Reiche et al. 2003), the

Table 1 Concentrations of elements in the sediments of the boreholes

Borehole interval	P (ppm)	Mn (ppm)	Fe (ppm)	Mg (ppm)	Ca (ppm)	Na (ppm)	K (ppm)	Cr (ppm)	Cu (ppm)	Zn (ppm)	As (ppm)	Cd (ppm)	Sb (ppm)	Pb (ppm)	Hg (ppm)
CRT 10/06 7 0–15 cm	570	700	13,920	7,580	16,740	3,430	7,690	46.9	31.4	118	6.0	0.82	0.61	26.0	0.14
CRT 10/06 7 15–30 cm	630	630	13,300	7,120	16,170	3,580	7,460	43.6	31.0	124	5.6	1.0	0.6	24.1	0.20
CRT 10/06 7 30–45 cm	860	690	15,370	8,040	15,370	3,000	6,850	57.3	34.3	151	7.6	1.0	0.7	28.4	0.22
CRT 10/06 7 45–60 cm	970	690	15,610	7,980	15,140	2,880	6,770	112	39.4	194	10.2	1.0	0.7	29.5	0.23
CRT 10/06 7 60–75 cm	760	620	17,340	8,940	12,150	2,600	8,010	98.9	37.5	175	11.3	1.4	0.9	32.2	0.24
CRT 10/06 7 75–90 cm	1,310	830	19,250	9,420	16,130	2,560	8,040	270	47.9	283	18.5	2.4	1.4	37.7	0.31
CRT 12/07 225–230 cm	980	580	15,730	9,830	16,290	3,710	9,340	348.4	60.1	302	14.9	3.7	1.5	47.7	0.54
CRT 12/07 260–265 cm	500	770	14,070	8,160	10,090	4,500	9,160	93.9	31.8	102	17.3	0.4	0.7	25.5	0.19
CRT 12/07 280–285 cm	350	430	16,670	10,480	6,800	3,510	8,970	66.2	23.0	85	6.5	0.1	0.2	17.4	0.06
CRT 12/07 330–335 cm	270	730	15,120	9,350	6,750	3,700	8,390	51.3	21.9	75	5.6	0.2	0.2	14.9	0.05
CRT 12/07 375–280 cm	250	880	14,930	8,410	6,160	4,020	9,320	49.4	22.8	69	7.1	0.2	0.2	14.4	0.05

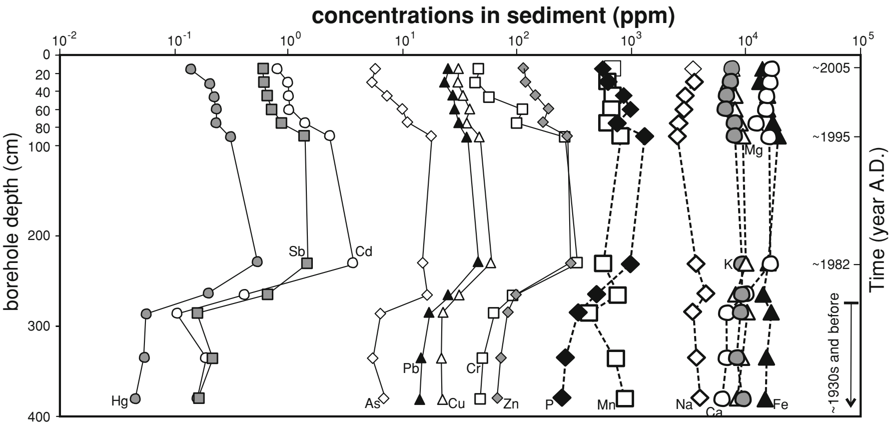

Fig. 2 Variation in the content of the studied elements (*gray lines*) in the boreholes. *Solid lines* heavy metals, *dashed lines* remaining elements

content of Na is increasing in an order of magnitude in Na-bearing subfossil fish scales. The elevated contents of K was also recorded, however, several times lower relative to the content of Na. It is in contradiction with the reverse situation in the sediment (Fig. 3).

The concentrations of trace heavy metal such as Cd, Zn, Cr, V, Co, Pb, Cu, Ni and As in the subrecent fish scales also substantially increased compared to the recent scales (Fig. 4) and are similar to the values found in the bulk sediment. Concentrations of V, Ni and Co do not vary considerably between the bottom and the top. The scales from the bottom layers are enriched in Cd, Zn and to a lesser extent Cr and As. This may reflect the fact that the sediment contamination for these elements gradually decreased from the late 1980s/early 1990s to 2005 (Bábek et al. 2008).

Discussion

Early diagenesis and its correlate—fossilization—brought about important changes i in hydroxylapatite chemistry. The most striking feature is an enormous increase in the F and Fe contents already in the fish scales of the uppermost part of the borehole (7–15 cm sample) (Fig. 3) in comparison with the scales of recent fish. A substantial increase was also found in Mn and Ba but less evident in Sr. Among the most apparent losses is the drop in Mg content and the anticipated depletion of OH, which is, however, difficult to prove (Elliott 2002) but which is widely assumed to be replaced by F (e.g. Nemliher et al. 1997). The behavior of the individual elements is discussed in detail as it follows.

Fluorine

The content of F in biological hydroxylapatite is time-dependent and during diagenesis the change from dahllite-type (<1 wt% F) to francolite-type (>1 wt% F) is accompanied by an increase in crystal size and stability (LeGeros and Suga 1980; Nemliher et al. 2004; Greene et al. 2004). It is assumed that F is completely incorporated into the hydroxylapatite structure (Nemliher et al. 2004). After this initial “recrystallization”, trace element signatures appear to be much more stable and resistant to later diagenesis (Grandjean and Albarede 1989; Grandjean-Lecuyer et al. 1993) relative to dahllite-type. Our results show a rapid increase in F downhole with the fish scale from the depth 70–78 cm already approaching the level defined for the francolite-type hydroxylapatite. Based on the Chernobyl anomaly (Bábek et al. 2008) and dredging, this level can be dated as approximately 6–8 years old. This result shows that early diagenesis in bones (Pfretzschner 1998, 2004) can be extremely rapid as francolite is much more stable in sediment than dahllite. This is quite promising for the interpretation of chemical signals given by the elements in a fossil hydroxylapatite.

Incorporation of F into the apatite structure via simple homovalent substitution F-OH-1 is generally assumed. It does not, in all likelihood, directly control entering of individual elements, although increasing of crystallinity of hydroxylapatite associated with this

Table 2 LA-ICP-MS data on concentrations (mg kg^{-1}) of elements in recent and subrecent fish scales

	Scan no.	Mg[a]	K[a]	Sr[a]	Ba[b]	Fe[a]	Mn[a]	Cu[a]	Zn[a]	As[a]	Pb[a]	V[b]	Cr[b]	Co[b]	Ni[b]	Cd[b]
Scales of recent fishes																
Fish																
Amur	1a	4,098	85	507	109	70	97	3.0	495	0.008	9.4	0.9	<0.1	0.4	2.8	<0.2
	1b	4,147	83	516	113	69	102	2.6	502	0.005	10	1.0	<0.1	0.4	2.9	<0.2
Common carp	2a	3,743	100	475	111	80	85	8.2	641	0.008	17	2.4	<0.1	0.4	5.1	<0.2
	2b	3,500	87	488	109	76	85	8.8	650	0.010	16	2.4	0.5	0.3	5.9	<0.2
Tench	3a	5,799	614	464	202	96	112	10.0	268	0.009	10	1.5	0.8	0.4	6.2	<0.2
	3b	4,912	669	443	220	97	99	11.7	257	0.004	10	1.6	1.0	0.4	7.9	<0.2
European perch	4a[c]	2,627	108	271	39	305	329	4.6	202	0.003	3.2	0.9	<0.1	0.2	2.8	<0.2
	4b[c]	2,761	159	279	42	344	449	5.8	290	0.002	3.4	1.0	0.4	0.3	3.0	<0.2
	5a[c]	2,457	229	286	44	322	422	3.6	225	0.005	2.2	0.9	<0.1	0.2	2.6	<0.2
	5b[c]	2,016	236	276	42	311	397	3.4	239	0.003	2.1	1.0	0.2	0.2	2.5	<0.2
Subrecent scales																
Interval of the borehole																
ČRT 10/06 7–15 cm	6a	1,003	1,968	858	729	32,739	2,755	44	323	0.7	180	13	7.4	3.7	11	0.6
	6b	1,042	2,243	809	644	36,082	2,779	59	490	1.1	265	16	10	3.6	13	1.5
ČRT 10/06 7–15 cm	7a	1,029	1,513	652	571	30,021	2,446	35	416	0.4	381	13	8.2	0.9	5.2	2.7
ČRT 10/06 18–26 cm	8a[c]	825	1,227	1,058	1,828	47,637	5,478	130	950	0.6	599	13	21	4.0	14	0.9
	8b[c]	845	1,162	1,048	1,928	46,150	5,615	84	1,026	0.6	520	14	18	3.2	11	1.2
ČRT 10/06 18–26 cm	9a	660	534	1,071	1,255	69,147	5,222	81	419	2.3	360	21	17	3.1	23	1.4
	9b	657	418	1,019	1,095	74,484	5,857	60	486	2.3	273	22	15	3.1	20	0.7
ČRT 10/06 45–54 cm	10a[c]	1,209	370	974	1,037	53,940	5,876	11	455	0.9	395	16	14	3.4	11	1.7
	10b[c]	1,011	214	837	1,030	41,045	4,923	12	446	0.9	549	14	9.3	17	8.8	1.1
ČRT 10/06 45–54 cm	11a	867	298	997	701	36,630	3,557	41	1,090	1.5	263	19	29	2.5	14	3.8
	11b	770	206	1,036	781	44,223	3,540	28	890	1.3	61	22	22	1.8	7.2	3.3
ČRT 10/06 70–78 cm	12a	580	522	779	672	37,757	4,249	30	241	2.9	83	21	100	<0.1	12	<0.2
	12b	884	1,240	717	720	44,794	3,554	37	530	4.2	138	55	216	3.4	23	0.9
ČRT 10/06 70–78 cm	13a	819	608	703	429	25,062	3,638	15	280	0.4	160	18	17	2.4	14	0.5
	13b	627	557	731	473	29,833	3,854	23	386	0.5	354	18	15	2.2	17	<0.2
ČRT 10/06 70–78 cm	14a	1,109	250	852	417	24,504	4,616	7.3	277	0.4	78	7.4	3.5	1.6	3.8	0.4
	14b	1,061	378	902	559	22,962	3,815	9.1	413	0.5	335	10	5.9	1.4	4.3	1.0

Table 2 continued

	Scan no.	Mg[a]	K[a]	Sr[a]	Ba[b]	Fe[a]	Mn[a]	Cu[a]	Zn[a]	As[a]	Pb[a]	V[b]	Cr[b]	Co[b]	Ni[b]	Cd[b]
ČRT 10/06 70–78 cm	15a	974	1,052	731	382	20,195	1,699	27	680	1.3	270	19	22	1.5	6.5	15
	15b	991	1,174	806	411	21,181	1,801	37	801	1.6	412	20	27	1.3	7.2	14
ČRT 10/06 70–78 cm	16a	615	192	824	384	17,709	2,341	28	313	0.7	58	11	12	0.5	3.9	2.2
ČRT 10/06 78–88 cm	17a	652	167	732	424	20,404	3,320	12	414	0.5	300	37	3.9	20	4.4	1.0
	17b	777	251	715	386	20,783	3,425	27	567	0.5	380	45	4.2	1.3	4.3	1.6
ČRT 10/06 78–88 cm	18a	904	234	768	344	15,345	2,271	35	275	0.3	301	9.3	8.0	0.7	2.9	2.5
	18b	1,167	261	829	435	22,404	2,669	38	436	0.6	277	11	8.9	0.8	2.7	2.7
ČRT 10/06 78–88 cm	19a	1,019	93	926	425	23,211	2,761	32	274	0.7	138	11	10	1.7	5.0	2.3
	19b	1,016	113	910	450	25,168	2,999	38	486	0.9	292	15	15	0.9	5.0	3.4
ČRT 10/06 78–88 cm	20a	759	277	747	212	8,673	2,556	22	596	0.3	128	31	6.7	1.0	4.6	1.4
	20b	722	243	728	242	10,004	2,416	26	656	0.5	218	24	6.8	1.5	4.6	1.2
ČRT 12/07 250 cm	21a	758	1,891	566	516	26,232	1,307	68	1,491	2.3	527	28	225	4.4	17	44
	21b	789	1,622	583	517	27,323	1,312	105	2,538	2.8	320	30	209	1.3	14	65
ČRT 12/07 250 cm	22a[c]	1,103	674	1,030	1,072	37,302	2,341	44	1,272	1.6	240	12	38	2.0	5.9	25
	22b[c]	977	814	945	982	32,478	2,021	43	1,886	1.9	214	13	38	1.5	5.8	32

[a] Quantification on SRM NIST 1486

[b] Quantification on SRM NIST 612

[c] Fish scale of European perch

Table 3 Electron microprobe data on concentrations (mg kg^{-1}) of elements in recent and subrecent fish scales

	Scan no.	Mg	Ca	Mn	Fe	P	Sr	Na	F	Cl
Scales of recent fishes										
Fish										
Amur	1	3,248	209,363	–	–	114,983	513	868	95	30
Common carp	2	3,180	204,975	–	–	105,053	388	698	25	313
Tench	3	5,340	218,808	–	–	120,065	540	2,743	690	325
European perch	4[a]	2,700	274,555	–	–	130,195	235	940	80	255
European perch	5[a]	2,428	265,263	–	–	123,865	280	715	190	190
Subrecent scales										
Interval of the borehole										
ČRT 10/06 7–15 cm	6	1,593	231,445	2,437	36,379	116,653	806	11,365	3,624	482
ČRT 10/06 7–15 cm	7	1,980	156,175	1,602	33,192	82,030	433	6,007	3,450	807
ČRT 10/06 18–26 cm	8[a]	830	214,200	5,480	46,120	107,840	880	9,760	6,150	490
ČRT 10/06 18–26 cm	9	798	177,903	5,891	78,722	98,358	694	7,988	3,540	456
ČRT 10/06 45–54 cm	10[a]	1,264	235,143	4,128	36,944	109,402	870	7,945	5,537	448
ČRT 10/06 45–54 cm	11	854	213,120	4,077	39,650	104,751	680	15,050	5,449	451
ČRT 10/06 70–78 cm	12	1,052	170,158	3,766	47,090	94,294	726	12,850	6,574	1,684
ČRT 10/06 70–78 cm	13	1,368	195,656	3,300	26,216	99,094	630	10,514	5,886	588
ČRT 10/06 70–78 cm	14	1,160	255,907	3,649	28,700	122,193	722	10,965	5,466	241
ČRT 10/06 70–78 cm	15	2,711	204,936	1,575	50,087	115,121	548	18,673	2,273	280
ČRT 10/06 70–78 cm	16	704	227,029	2,199	6,450	123,107	756	18,610	8,899	311
ČRT 10/06 78–88 cm	17	491	209,409	3,085	9,452	128,923	566	29,287	7,650	168
ČRT 10/06 78–88 cm	18	822	199,546	1,797	9,853	118,825	655	25,779	6,713	320
ČRT 10/06 78–88 cm	19	918	240,363	1,743	12,902	128,292	704	29,146	8,703	233
ČRT 10/06 78–88 cm	20	709	225,878	2,301	9,986	149,640	488	50,373	4,689	201
ČRT 12/07 250 cm	21	1,595	132,746	1,185	28,576	90,351	505	28,856	1,878	259
ČRT 12/07 250 cm	22[a]	905	240,688	1,490	28,519	122,776	752	12,449	5,015	371

[a] Fish scale of European perch

substitution generally influences entering of other elements into the crystal structure.

Iron and manganese

The contents of both elements in fish scales are higher than in the sediment (Figs. 2, 3) and are already achieved in the samples 1–2 years old and do not increase significantly downhole (Tables 2, 3; Fig. 3). The substantial increase in Fe already in the youngest subfossil scales is accompanied by the change of color from white in recent one to brown in subrecent ones. It has been given that Fe^{3+} is responsible for the brown tooth stains (Torell 1988) and Pfretzschner (2001) reports that a considerable amount of Fe seems to be incorporated into the crystal structure of Pleistocene bone hydroxylapatite staining the bone dark brown. Consequently, the change of color of our fish scales from white in recent ones to brown in subrecent ones seems to be connected with the Fe diffusion already in the course of the earliest fossilization.

In the synthetic hydroxylapatite, despite its smaller radius, Fe^{3+} is assumed to replace Ca^{2+} in the structure under low pH and Fe^{3+} is distributed in the whole of the particles (Wakamura et al. 2000). The character of Fe substitutions in biogenic hydroxylapatite is unclear as we do not know its valence. It has been reported that the microenvironment of bone and decaying collagen is characterized by high pH values and low redox conditions, which reduce Fe^{3+} to more mobile Fe^{2+}, and which can be later during the course of diagenesis once again reoxidized (Tanizawa et al. 1990; Pfretzschner 2001, 2004).

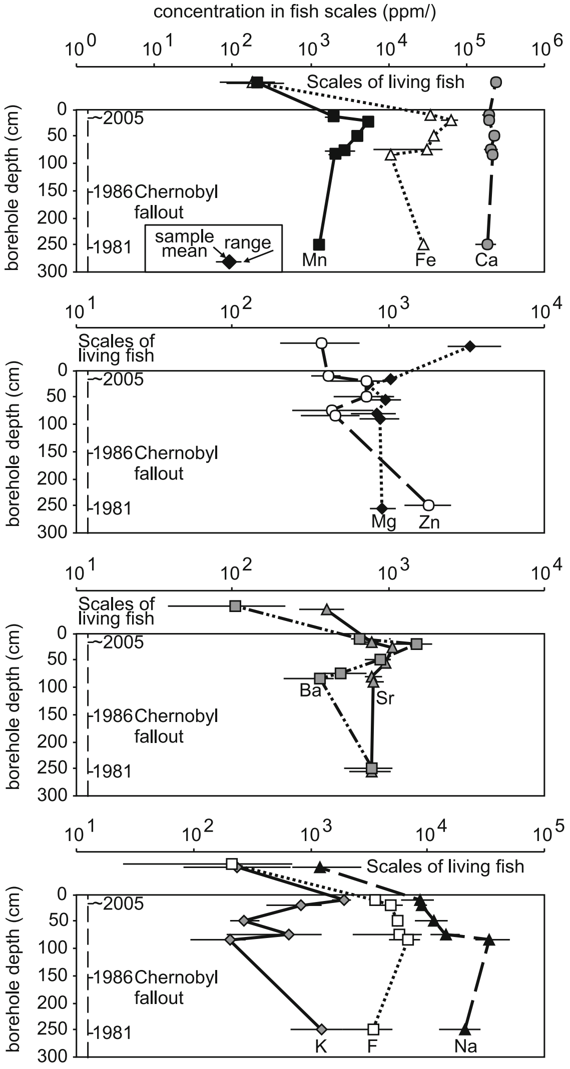

Fig. 3 Variation in the content of the studied non heavy metal elements in the scales of living fish and subfossil fish scales in the boreholes

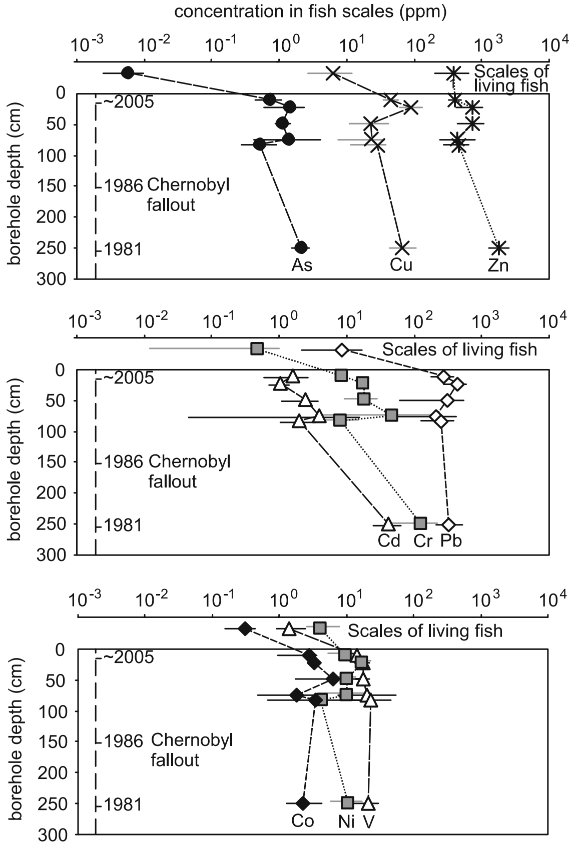

Fig. 4 Variation in the content of heavy metals in the fish scales of living fish and subfossil fish scales in the boreholes. Modified according to Holá et al. (2009)

A similar trend as with Fe can be observed in Mn, although the concentrations of Mn in fish scales are not as high as those for Fe. This is in contradiction with most natural apatites from a variety of rocks forming at a high temperature and pressure (Pan and

Table 4 Concentration of heavy metals and of the studied elements in the water of the Morava River in Spytihnev located close to the studied oxbow lake

	Heavy metals (μg l^{-1})								Cations (mg l^{-1})							
	As	Cd	Co	Cr	Cu	Ni	Pb	V	Zn	Ba	Fe	Mn	Mg	Ca	Na	K
Oct 2005	1.5	<0.1	0.4	3.7	2.0	3.0	0.8	1.5	5	0.053	0.513	0.108	10.6	65.9	25.2	6.4
May 2006	1.3	<0.1	0.7	1.3	2.3	2.5	1.0	1.6	18	0.053	0.513	0.108	10.6	65.9	25.2	6.4
Oct 2006	0.9	<0.1	0.3	0.7	2.4	2.1	0.6	1.1	16	0.057	0.351	0.093	12	73.6	30.1	6.6
Average	1.23	<0.1	0.47	1.9	2.23	2.53	0.8	1.4	13	0.054	0.459	0.103	11.1	68.5	26.8	6.47

Fleet 2002). The high affinity of both synthetic and biogenic apatite to sorb or precipitate Mn^{2+} was reported by Suzuki et al. (1982), Bostick et al. (2003) and Wells et al. (2000). There are different views on the substitution of Ca for Mn in the apatite structure. Manganese similarly to Fe is substituted into the Ca(2) site of synthetic hydroxylapatite according to Sutter et al. (2003) while previous authors advocate its preference for Ca(1) site (e.g., Pan and Fleet 2002).

Mn and Fe function rather as indicators of environmental contamination than paleodiet as no or low biomagnification from lower trophic levels to higher ones have been reported (Ezzo 1992; Borga et al. 2006). In spite of conflicting reports, our scales of carnivore European perch show much higher values of Fe and Mn than those of common carp, amur and tench which is consistent with the better absorption of Fe from animal than from plant food (Monson 1988; Tapiero et al. 2001; Miret et al. 2003). Considerably lower levels of Fe in herbivorous to omnivorous common carp, amur and tench correlate with higher levels of Ba which is well in agreement with experiments of Lambert and Weydert-Homeyer (1993). Higher contents of Mn are reported in plant food together with Mg (Stokes et al. 1988; Trancho and Robledo 2003; Howe et al. 2004). Consequently, the low Mn concentration in herbivores (Table 2) may reflect negative Mn interaction with Mg (Sanchez-Morito et al. 1999).

Barium and strontium

Quantitative relations between dietary intake and bone mineral composition have been demonstrated for Ba and Sr. Both have been widely used in biological hydroxylapatite for ecological and paleoecological reconstruction and for identification of herbivory, omnivory and carnivory as Ba/Ca and Sr/Ca ratios decrease at each successive trophic level (Elias et al. 1982; Sillen and Kavanagh 1982; Sealy and Sillen 1988; Sillen et al. 1989; Burton and Price 1990; Katzenberg and Harrison 1997; Burton et al. 1999; Sponheimer and Lee-Thorp 2006).

The strong affinity of Ba to vegetal foods (Lambert and Weydert-Homeyer 1993) is well in agreement with our fish scales as recent carnivore European perch show a several times lower content of Ba than herbivore to omnivore common and grass carp and especially tench (Tables 2, 3).

In fossil hydroxylapatite it is a rather naive approach to ignore fully the role of diagenetic processes and it has also been criticized (Kohn et al. 1999; Sponheimer and Lee-Thorp 2006). In fact hydroxylapatite is effective in reducing the water-soluble Ba in contaminated sediment and common substitutions of Ba for Ca (Goodwin et al. 2007). Our data reveals that extreme caution is necessary when interpreting the Ba content already in subfossil hydroxylapatite as the values of Ba in scales from 1 to 3-year-old sediment are more than three times higher than in recent fish (Tables 2, 3; Fig. 3). Despite certain differences in Ba content in the spectrum of studied recent fish scales, the data on subfossil scales of European perch confirms this trend. It is well in agreement with the observation of Hochstein and MacFadden (2006) who stress that fossil shark teeth are significantly enriched in Ba with one to two orders of magnitude greater content than modern shark. It is interesting that Ba contents are generally higher than Sr despite the fact that Sr ions can enter the crystal structure of hydroxylapatite and occupy a greater number of both Ca positions more easily than the larger Ba ions (Pate et al. 1989; Yasukawa et al. 2002). Another interesting feature is an association of high Ba content in subfossil fish scales with high content of Mn (Parker and Toots 1972). Also of interest is the fact that only small amounts of Ba are reported for natural fluorapatite and hydroxylapatite (Pan and Fleet 2002).

Strontium is one of the most common cation substituents in apatites with strong preference for Ca(2) sites and may also be incorporated into fossil bone via growth of authigenic apatite (Reynard et al. 1999; Trueman and Tuross 2002; Pan and Fleet 2002). Sr can vary significantly within fish scales of different taxons with different life strategies as shown by differences between our carnivore European perch and herbivore to omnivore common carp, grass carp (amur) and tench (see Tables 2, 3). It is well in agreement with the fact that strontium levels decrease as one moves up through the food chain (Elias et al. 1982; Sillen and Kavanagh 1982; Sillen et al. 1989; Burton and Price 1990; Katzenberg and Harrison 1997).

Of the greatest significance is the difference between recent and subrecent fish scales, best illustrated in the content in subfossil fish scales of European perch, which are approximately four times higher than in recent ones. This value is achieved very rapidly in scales approximately 3–4 years old with the values remaining nearly constant being approximately the same in the scale of European perch 25 years old. This trend is also supported by the much higher Sr content in other subfossil fish scales compared with recent ones. This data again documents a very rapid pace of diagenetic changes and questions the interpretation of Sr as an indicator of trophic behavior in fossil bones.

The addition Sr may also increase the solubility of hydroxylapatite (Christoffersen et al. 1997; Okayama et al. 1991), which is well apparent in the solid correlation of Ca content in recent species of European perch, tench, common carp and amur (Tables 2, 3). In subfossil fish scales this trend is not as evident as the Ca content may be also influenced by other substitutions proceeded during diagenetical processes.

Magnesium

Magnesium is the element revealing the most significant loss in fish scales during earliest fossilization (a nearly 10-times loss) despite its very high content in the host sediment (Tables 2, 3; Fig. 3), which is higher than the content in the water of the neighboring Morava River (Table 4). Fresh biologic hydroxylapatite contains a significant amount of Mg and this biogenic element can be mobilized from scales and bone, probably in order to guarantee muscle Mg homeostasis (Bijvelds et al. 1997). The substitution of Ca for Mg in the crystal structure is doubted because of the much smaller radius of Mg despite the fact that the limited incorporation of Mg appears to be possible (Bigi et al. 1996; Elliott 2002). More widespread is absorption on the hydroxylapatite crystal surface, where it is readily exchangeable (Neuman and Mulryan 1971; LeGeros 1991; Elliot 1994, 2002; Mayer et al. 1997). The rapid loss of Mg can be explained by the higher ion-exchangeability of Mg-CaHAP compared to CaHAP due to the high specific surface area of the former (Yasukawa et al. 2002). It results in a higher efficiency of metal removal (Shashkova et al. 1999), in our case Fe and Mn being the most probable favorites. A similar trend was observed in the archaeological bones, which are also depleted in Mg relative to the modern ones (Pate et al. 1989; Johnsson 1997). Another important feature is the variation of Mg content between recent species. The content in fish scales of carnivorous European perch is less than half those of tench and is also substantially lower than in amur and common carp. Even though the role of magnesium in dietary reconstructions is not unambiguously accepted (Francalacci and Borgognini Tarli 1988; Bijvelds et al. 1998) the main source of Mg is associated with predominately vegetal diet (Trancho and Robledo 2003). The negative correlation between Mg and Ca contents in recent fish scales (Table 2) is well in accord with observations that Ca may significantly inhibit the Mg uptake (Brink et al. 1992) and that a low dietary Mg intake induces high body Ca in fish (Bijvelds et al. 1998).

Calcium

The mineral phase in the fish scale is calcium-deficient hydroxylapatite containing a small and variable amount of magnesium and other ions, as well as carbonate anions in phosphate sites of the apatite lattice (Ikoma et al. 2003). The content of Ca is highly specific and can range considerably in recent species (Seshaya et al. 1963) especially with freshwater fish which appear to rely on internal stores of Ca in scales and/or bone (Rotllant et al. 2005) to increase availability of Ca. It is therefore difficult to follow any stratigraphical trends. The amount of Ca in our recent scales is within the range given by other authors both in fish scales and bones (Berntssen et al. 2003; Nishimoto et al. 2003; Kihara et al. 2007). Lower levels of Ca in fish scales of herbivorous to omnivorous common carp, amur and tench are well in accord with experiments of Lambert and Weydert-Homeyer (1993) according to which high levels of dietary fiber correlate with lower Ca and higher Sr and Ba in the bone.

During the early fossilization, a general decrease is connected to the substitution of Ca especially for Fe and Mn and also for Na, K, Ba and Sr. However, there can also be a certain increase in Ca connected with the loss of collagen, which can “dilute” the concentration of other elements.

Alkali metals

The substitutions of Ca for K and Na is connected with the replacement of phosphate group PO_4^{3-} by a carbonate one CO_3^{2-} to compensate the charge balance (LeGeros et al. 1968; Elliot 1994; Gibson et al. 1999; Gibson and Bonfield 2002). Generally, CO_3^{2-} can substitute both OH^- in the *c*-axis channel of apatite (type A) and for PO_4^{3-} (type B), respectively (Elliot 1994; Fleet and Liu 2007). Biological apatites are similar in chemical composition to synthetic Na-bearing type A–B apatites (with a high content of type A carbonate) with coupling between Na cations and channel (type A) and phosphate (type B) carbonate ions (Fleet and Liu 2007). The fossil bone content of Na is usually lower than that corresponding to modern samples (Reiche et al. 2003); however, in our samples, Na content increases being an order of magnitude higher in Na-bearing subfossil fish scales (Fig. 3). It is well in agreement with the reported increase of carbonate in the course of fossilization/diagenesis (Nemliher et al. 2004).

A similar role of K during carbonate substitutions is anticipated. However, due to the larger ionic radius of K, hydroxylapatite with CO_3/Na type-B defect is more stable than that with CO_3/K type-B defect (El Feki et al. 2000; Peroos et al. 2006). These conclusions are again well in agreement with our results as the content of Na is several times higher than K even though the reverse situation characterizes the sediment with much lower concentrations of Na and much higher concentrations of K than in fish scales (Figs. 2, 3).

Heavy metals

The concentrations of other trace heavy metal such as Cd, Zn, Cr, V, Co, Pb, Cu, Ni and As in the recent and subrecent fish scales has been discussed in detail elsewhere (see Holá et al. 2009 for a review). With the exception of biogenic element Zn, heavy metals are also substantially increased in subrecent scales (Fig. 4) compared to the recent ones (Holá et al. 2009). In brief, the fish scales seem to attain a certain degree of contamination in trace heavy metals in the uppermost layer and the contents of these metals do not change or change only slightly downwards (Fig. 4), even if the embedding sediment becomes increasingly contaminated.

Zinc, a typical biogenic element, has the highest representation among trace heavy metals. In our fish scales the carnivorous European perch has a considerably lower content of Zn than omnivorous and herbivorous tench, common carp, and amur (grass carp). This data is in contradiction with the higher concentration of Zn in a carnivore diet (Gilbert 1975; Ezzo 1992; Trancho and Robledo 2003) and can indicate a certain local source of pollution. However, the utility of Zn in paleodiet studies has been challenged because of the influence of other factors in an aquatic environment (salinity, thermal regime, pH, alkalinity and the amount of particulate matter in the water) and because the physiological model explaining its trophic performance is not yet fully understood (Ezzo 1994; Halden et al. 2000; Trancho and Robledo 2003). The considerably higher content of Zn in more herbivorous common carp and amur (grass carp) than in more omnivorous consuming tench (Petridis 1990; Brönmark 1994; Adámek et al. 2003) may indicate another explanation, i.e. that this reversed Eltonian pyramide of Zn can be connected with the high Zn content in plants. High Zn concentrations in common carp and amur accompanied by a lower Ca content in comparison with European perch (Table 2) may be also in accord with the assumed antagonism between the Ca and Zn uptake in fish (Sauer and Watabe 1988; Glover et al. 2004).

It is assumed that during diagenesis Zn content can influence the uptake of Ba distorting the surface of hydroxylapatite and thereby facilitating a replacement of Ca(2) by larger alkaline earth elements (Narasaraju and Phebe 1996; Bostick et al. 2003). In the subfossil scales the high concentrations of Ba often correlate with high concentration of Zn (Tables 2, 3), but this interdependence is not generally valid in all probability due to competition with alkali ions such as K or Na.

Conclusions

A detailed study of subfossil fish scales in the non-marine environment confirms very rapid changes in the chemical composition of hydroxylapatite during very early diagenetic processes and the composition does not change significantly downhole. The following results can be observed:

Alkaline earth elements in subfossil fish scales show an enormous increase in Ba and a significant increase in Sr while Mg exhibits a significant loss in subrecent fish scales. The contents of Ba are generally higher than Sr despite the Sr ions entering the hydroxylapatite crystal structure more easily relative to the larger Ba ions.

There is an enormous increase in alkali metals Na and K which is in accord with previous observations. The content of Na is several times higher than that of K albeit the reverse situation occurs in the sediment. The high content of Na may reflect either environmental factors or increases in the carbonate component in the hydroxylapatite structure during the course of fossilization/diagenesis.

Contrary to the reported modest affinity of biological apatite for Fe our fish scales show considerable and quite rapid incorporation of Fe accompanied by a change in color from white in recent to brown in subrecent scales. A similar trend as for Fe can be observed in Mn, although its concentration is much lower.

A rapid increase in heavy metal contents in subfossil fish scales does not change significantly downhole.

The fish scales from 70 to 78 cm (6–8 years old) enriched in F are approaching already to the level supporting growth of francolite-type hydroxylapatite but the increase is not linear. This raises some questions on the use of F content as a relative dating method of bones (Johnsson 1997; Gaschen et al. 2008).

A high increase in Na which indirectly indicates carbonate substitutions for OH or PO_4 group shows that oxygene isotopic studies of fossil hydroxyapatites require extreme caution.

The extent of diagenetic alteration in fish scales is so profound that paleoecological interpretations are not possible. Our observations thus throw serious doubts on the potential of chemical analyses of bones to provide information on paleoenvironmental conditions and paleodietary paterns, which is an approach long questioned but still often applied (Trueman and Tuross 2002; Martínez-García et al. 2005; Zapata et al. 2006). They also call in question the use of Sr and Ba as paleothermometers (Balter and Lécuyer 2004; Domingo et al. 2007).

Despite the fact that this was not the main purpose of the study, certain interesting results were obtained in recent fish scales concerning bioaccumulation and biomagnification of certain elements. The Sr, Ba and probably also Mn contents soundly reflect the position of the species in the Elton's trophic pyramid. Biomagnification of Fe from lower trophic levels (amur, common carp, tench) to higher ones (European perch) reflects a better absorption of Fe from animal than from plant foods. The lower Mn in herbivores, in contradiction with the higher Mn availability in plant food, may reflect a negative Mn interaction with Mg. In spite of higher concentration of Zn in the carnivore diet, European perch has considerably lower contents of Zn than omnivorous and herbivorous tench, common carp, and amur (grass carp). Further studies are needed to examine whether this reversed Eltonian pyramid in Zn is connected with a certain local source pollution or whether bioaccumulation of Zn in fish scales is related to physiological processes. To sum up, the contents of elements in recent fish scales reflect not only the position in the trophic pyramid but also complicated interplay of the diet, physiological processes and environmental contamination.

Acknowledgments This study was supported by the project MSM0021622412 (INCHEMBIOL). The authors would like to express their thanks to M. Kosik, and J. Horsak, the head of the Moravian fisherman's organization in Stare Mesto for their field and logistical assistance.

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