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In situ high spatial resolution ⁸⁷Sr/⁸⁶Sr ratio determination of two Middle Pleistocene (c.a. 580 ka) Stephanorhinus hundsheimensis teeth by LA–MC–ICP–MS

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Graphical abstract



Highlights

- ⁸⁷Sr/⁸⁶Sr determination by LA–MC–ICP–MS of biogenic apatites strengthened.
- Using of three matrix-matched in-house reference materials.
- Two Middle-Pleistocene rhino fossil teeth analysed in high-spatial resolution.

Abstract

Bone and tooth tissues are important biological archives to study eating habits and provenance of ancient humans and animals. By taking advantage of the high spatial resolution offered by the Laser Ablation Multi Collector Inductively Coupled Plasma Mass Spectrometry (LA-MC-ICPMS) technique, we investigated the ⁸⁷Sr/⁸⁶Sr intratooth variability of two Stephanorhinus hundsheimensis tooth fragments from the Middle Pleistocene site of Isernia La Pineta. We detected significant Sr isotopes variability within the rhinos tooth enamel (enamel average ±20: sample RH-IS30 0.70951 ± 0.00014; sample RH-IS26 0.70976 ± 0.00015) with values higher than the "local" 87 Sr/ 86 Sr ratio (dentine average $\pm 2\sigma$: sample RH–IS30 0.70918 \pm 0.00013; sample RH-IS26 0.70934 ± 0.00009). This is likely linked to a different water and food intake with a Sr isotopic signature similar to volcanic soils nearby (Roccamonfina: from 0.7093 to 0.7100; Colli Albani: > 0.7100) and supports the idea that the Stephanorhinus hundsheimensis species moved around seasonally. The improvement of non-destructive, accurate and precise analytical methods to decrypt the information hidden within bone and tooth hard tissues of archeological material is crucial to- unravel critical questions about evolution, migration and ecology of human and animals. We have successfully took upon this challenge using three matrixmatched standards, with variable Sr concentration (c.a. 100 to 1000 ppm), to correct unresolved interferences arising from LA analyses.

Keywords: LA-MC-ICP-MS; strontium isotopes; bioapatite; enamel; rhino

1. Introduction

Strontium isotopes have become an important tool in palaeontological research to track ancient human and animal migratory patterns and provenance (e.g. Price et al., 2002; Bentley, 2006; Evans et al., 2006; Montgomery et al., 2007; Copeland et al., 2011; Bentley et al., 2012). Tooth enamel, if available, is the best material for geochemical investigations because it is made of a ~ 96% mineralized hydroxyapatite more resistant to diagenesis (Bentley, 2006) and is an elemental and Sr isotope archive of the individual life.

Strontium substitutes calcium in the hydroxyapatite of bones and teeth (Burton et al., 1999) and its ⁸⁷Sr/⁸⁶Sr ratio is directly related to the ratio of plants and water ingested by humans and animals (e.g. Pate, 1994; Burton and Wright, 1995; Bentley, 2006).

Strontium ions enter the ecosystem through the weathering of the local bedrock, which usually retains a peculiar ⁸⁷Sr/⁸⁶Sr ratio related to the petrogenetic process, the age and the initial rubidium content of the rock itself. Thus, analysing the ⁸⁷Sr/⁸⁶Sr ratio of dental and bone tissues can help identify the place where the tissue has formed or remodelled. Given that enamel forms during the individual growth and preserves incremental features of its formation, small scale isotopic investigations allow to detect temporal variations in the Sr isotopic composition of the animal life even within one tooth (e.g. Balasse, 2003; Tafforeau et al., 2007; Martin et al., 2008; Britton et al., 2011). In such sense, the growth lines or bands visible by microscopic observations of teeth, the so-called Retzius lines (or striae of Retzius), are the best-known long-term period incremental features of the dental enamel and represent the successive apposition of two striae of Retzius is between 2 and 12 days in primate teeth and about 7 days in rhino teeth (e.g. Tafforeau et al., 2007; Smith and

Tafforeau, 2008). This time-span, namely periodicity, can be estimated counting the visible laminations (i.e. daily isochrones of the enamel deposition) per Retzius band (Tafforeau et al., 2007).

Traditionally, Sr isotopes have been analyzed by Thermal Ionization Mass Spectrometry (TIMS) and MC–ICPMS requiring total dissolution of a portion of the sample and chemical separation of strontium (e.g. Li et al., 2013). The recent development of the Laser Ablation system (LA) coupled with a Multi Collector Inductively Coupled Plasma Mass Spectrometer (MC–ICPMS) has allowed high spatial resolution analyses with a micro-destructive approach, with the opportunity to analyze even small tooth fragments and unravel possible isotopic heterogeneity at the small scale within the tooth itself (Horstwood and Nowell, 2005; Copeland et al., 2008; Horstwood et al., 2008; Richards et al., 2008; Simonetti et al., 2008; Copeland et al., 2010; Copeland et al., 2011; Balter et al., 2012). This technique can be particularly helpful when dealing with small ancient enamel fragments of migratory animals or precious remains that cannot be destroyed by canonical dissolution methods.

Despite the clear advantage of this technique, LA–MC–ICPMS analyses have some issues mainly related to analytical artefacts. In particular, during analysis several isobaric interferences are known to form; whilst some are routinely and properly corrected by many laboratories (e.g. Rb and Kr), others may hinder the achievement of precise and accurate results (e.g. REE²⁺, ⁴⁰Ca⁴⁶Ca, ⁴⁶Ca⁴⁰Ar, ⁴⁰Ca³¹P¹⁶O and ⁴⁰Ar³¹P¹⁶O; Copeland et al., 2008; Horstwood et al., 2008; Vroon et al., 2008; Willmes et al., 2016).

In this work, we investigated the 87 Sr/ 86 Sr ratio intra-tooth variability of two Stephanorhinus hundsheimensis teeth (LA samples n = 82) from the Middle Pleistocene site of Isernia La Pineta (Molise, Italy), to unravel possible variations within the tooth enamel and to try to reconstruct a migratory pattern for the *Stephanorhinus hundsheimensis* species in the area. Beside the *Bison schoetensacki,* this is the second most represented taxa in the area (Ballatore and Breda, 2013; Peretto et al., 2015). We analyzed our two samples in order to detect if incremental fluctuations of the ⁸⁷Sr/⁸⁶Sr ratio, i.e. representing temporal variations in the Sr isotopic composition, were preserved in the enamel and if they correlated with the structural growth features of the tooth.

2. Materials and Methods

Two fragments (a longitudinal and a transversal section) of *Stephanorhinus hundsheimensis* molars (RH–IS30 and RH–IS26) were collected among the nonmusealized samples found at the Isernia La Pineta site. Sample RH–IS30 was collected from the archaeological layer called 3s10 and sample RH–IS26 from the 3c layer (for more details about the stratigraphy see Peretto et al., 2015). Argon–argon dating (⁴⁰Ar/³⁹Ar) suggests an age of about 580 ka for both layers (Peretto et al., 2015). The surface of both samples was analyzed in detail, with 80 LA short linear scans composing four profiles (Fig. 1 and Fig. 2; Profiles A, B, C and D). We noticed that short linear scans gave better results than spots in terms of signal stability. Of these short scans, 60 were performed on the enamel surface and 20 on the dentine surface.

Dentine is usually not a good indicator of provenance because of its high susceptibility to diagenetic contamination (e.g. Bentley et al., 2006) whereas enamel is generally less affected by diagenesis and represents the best target. In the case of samples that due to their uniqueness cannot be cut to expose their inner parts, scientists have to rely on analyses of the outer enamel (e.g. Copeland et al., 2011). Sample RH–IS26 was also tested with a slightly different method using continuous long scan profiles for the entire length of the enamel portion. We obtained two profiles: one (profile E) on the inner and one (profile F) on the outer surface of the enamel as shown in Fig. 3.

Following Horstwood et al. (2008), we have also selected and analyzed four bioapatites with different Sr concentration to establish our matrix-matched standard materials given the unavailability of solid bioapatite standards on the market. We selected a shark tooth with a Sr concentration of ~1100 ppm (average 1101 ± 47 ppm), a bovine tooth with a Sr concentration of \sim 350 ppm (average 347 ± 18 ppm), a swine tooth with a Sr concentration of ~250 ppm (average 253 ± 31 ppm) and a human tooth with a Sr concentration of ~100 ppm (average 104 ± 25 ppm). The shark tooth (Carcharhinus leucas, Müller and Henle, 1839) was purchased from an online specialized shop. Shark tooth enameloid is composed largely of fluorapatite $(Ca_5(PO_4)F)$ and minor hydroxyapatite $(Ca_5(PO_4)OH)$ (Vennemann et al., 2001), however, their Ca/P molar ratio and the relative content of Ca²⁺ and PO₄³⁻ are similar to mammal enamel (Enax et al., 2012). The swine tooth (SUS-Sr1) and the bovine tooth (BOS-Sr1) were collected from a roman archaeological site in Ravenna (Italy), while the human tooth (ROCht421) was selected among the best-preserved mummies teeth buried in the archaeological site of Roccapelago (16th and the 18th century AD, Modena, Italy; Lugli et al., accepted). Prior to LA-analyses, portions of our in-house bio-standards were analysed in solution by MC-ICP-MS to obtain the reference ⁸⁷Sr/⁸⁶Sr value.

2.1. Thin section preparation

Incremental growth features of the transversal section of tooth RH–IS26 were studied under a transmitted-light petrographic microscope (Fig. 5). A petrographic thin section of the sample was prepared by embedding the tooth in polyester resin, gluing onto a microscope glass section, cutting in a ~1 mm section with a diamond saw and manually polishing to a thickness of 100 µm with decreasing-size polishing paper and aluminium oxide (Tafforeau et al., 2007). Observation and counting of Retzius lines was performed in polarized transmitted light with an Olympus BH-2 petrographic microscope.

2.2. Dissolution method MC–ICPMS

Samples were prepared at the Department of Chemical and Geological Sciences of the University of Modena and Reggio Emilia. The tooth enamel was manually separated from the dentine using a dentist drill. After a macroscopic cleaning with small brushes, each enamel chunk was boiled in MilliQ[®] for 1h to remove any possible superficial residue of organic matter, rinsed repeatedly with MilliQ[®] and then sonicated in an ultrasonic bath for 30 minutes. Before dry down, samples were rinsed 3 times with MilliQ[®] and once with high purity acetone. Samples were digested in 1ml of suprapure 14N HNO₃, evaporated to dryness and dissolved in 3ml of 3N HNO₃. After centrifuging, samples were loaded into 300 µl volume columns filled with Eichrom Sr spec–resin and washed with 3N HNO3. Sr was finally eluted with several reservoirs of MilliQ[®] water (Deniel and Pin, 2001).

The ⁸⁷Sr/⁸⁶Sr isotopic composition of the samples was determined using a double focusing MC–ICPMS with a forward Nier–Johnson geometry (Thermo Fisher

Scientific, Neptune[™]) housed at the Centro Interdipartimentale Grandi Strumenti (CIGS) of the University of Modena and Reggio Emilia.

Seven of the nine Faraday detectors, fitted with 10¹¹Ω resistors, were used to collect signals of the following masses: ⁸²Kr, ⁸³Kr, ⁸⁴Sr, ⁸⁵Rb, ⁸⁶Sr, ⁸⁷Sr, ⁸⁸Sr. Solutions were diluted to 200 ppb of Sr and introduced into the Neptune via quartz spray chamber and a 100 microliter/min nebulizer. The ⁸⁸Sr signal of the shark tooth (~1100 ppm), after proper dilution to 200 ppb, was ~15 V and the blank level (0.5 HNO₃) 0.02 V. Samples and standards were analyzed in a static multi–collection mode in a single block of 100 cycles, with an integration time of 8 s per cycle. A classic bracketing sequence [blank/standard/blank/sample/blank] has been used to monitor any eventual drift of the instrument.

Masses 82 and 83 were collected to monitor the presence of Kr in the argon. Data were corrected using a 86 Kr/ 83 Kr ratio of 1.505657. Mass 85 was used to correct the signal on mass 87 for the presence of isobaric Rb, using a 87 Rb/ 85 Rb ratio of 0.3856656. Mass bias normalization, through exponential law, was carried out using a 88 Sr/ 86 Sr ratio of 8.375209. Mass fractionation for both Kr and Rb has been assumed equal to that one of Sr. The 87 Sr/ 86 Sr ratios were corrected for instrumental bias to an NBS–987 value of 0.710260 ± 0.00002 (Ehrlich et al., 2004; Durante et al., 2015). Repeated analyses of the NBS–987 yielded a 87 Sr/ 86 Sr ratio of 0.710272 ± 0.000013 (2 σ ; *n* = 8, corresponding to an external reproducibility of 18 ppm). The long–term reproducibility (*c.a.* three years of analyses; *n* > 2000) of the instrument yielded a 87 Sr/ 86 Sr ratio of 0.71027 ± 0.00002 (2 σ ; Durante et al. 2015).

2.3. Laser ablation method MC–ICPMS

The teeth portions chosen for laser analysis were cleaned with brushes, washed with

MilliQ[®] water and carefully dried down before introduction into the LA sample holder. Sr data were acquired with the Neptune MC–ICPMS, coupled to a 213 nm Nd:YAG laser ablation system (New Wave Research[™]) housed at CIGS–UNIMORE (Table 1). Before LA analyses, the instrument was tuned using the NBS–987 standard solution, monitoring both the signals and the significant isotopic ratios.

All nine Faraday detectors were used to acquire peaks of the following masses ⁸²Kr, ⁸³Kr, ⁸⁴Sr, ⁸⁵Rb, ⁸⁶Sr, ⁸⁷Sr, ⁸⁸Sr and half masses 85.5 and 86.5. Half masses 86.5 and 85.5 were monitored to check for the contribution of doubly charged Rare Earth Elements (¹⁷¹Yb²⁺ and ¹⁷³Yb²⁺). Usually these signals are below the detection limit of the instrument (LOD), therefore the correspondent detectors were equipped with 10¹² Ω resistors.

We used the "on peak zero" method to correct for Kr interferences (Davidson et al., 2001; Vroon et al., 2008) by measuring a 60 s gas background for each mass before each analysis and subtracting the signal of each detector to the measured value of the sample. After background subtraction, the remaining signal of mass 82, was used to check the formation of Ca dimers and argides, isobars to masses ⁸⁴Sr, ⁸⁶Sr, ⁸⁷Sr and ⁸⁸Sr (Woodhead et al., 2005). Typically, Ca dimer and argide signals on mass 82 are around 0.1 mV. Likewise, the remaining signal of mass 83 (after Ca dimers argides subtraction) was used to correct for the presence of ¹⁶⁶Er²⁺ (Copeland et al., 2010). Rb interferences were corrected using the same procedure followed for the dissolution method. We note here that the ⁸⁵Rb signal during our analytical sessions has always been lower than 1 mV.

We employed a linear ablation pattern (100 x 500 μ m) to sample the tooth enamel surfaces in order to limit laser-induced elemental fractionations and to improve the beam size stability (Vroon et al., 2008; Guillong and Günther, 2002).

Our daily LA–MC–ICPMS tuning is based on a 3-step protocol using our ST1 in house-reference material. During the first step, ST1 is analysed to optimize the machine parameters tuning for the maximum signal intensity on ⁸⁸Sr. Second, background signal intensities on ⁸³Kr and ⁸²Kr are monitored. Ideally they have to be as low as possible and, as also observed by Jackson and Hart (2006), the ⁸³Kr/⁸²Kr ratio has to be maintained near ~1 to obtain the best results in term of precision and accuracy. Finally, the ⁸⁷Sr/⁸⁶Sr ratio of the ST1 standard is measured and machine parameters are tuned until the desired ratio (~0.7092) is obtained. Laser data were acquired with a block of 200 cycles, an integration time of 0.5 s and a laser spot size of 100 µm. The surface of the tooth was pre–ablated before each analysis. Summary of the typical LA parameters used during our sessions are presented in Table 1.

The two continuous long LA scan profiles obtained for tooth RH–IS26 were acquired with a block of 2000 cycles and an integration time of 0.5 s. We used a laser spot size of 100 μ m and a line length of about 6 mm. We calculated a moving average of the cycles with a period of 100.

As reported by several authors, during LA analyses is difficult to address the specific interferences that contribute to the fractionation of the ⁸⁷Sr/⁸⁶Sr ratio (e.g. Whoodhead et al., 2005; Balter et al., 2008; Horstwood et al., 2008; Simonetti et al., 2008; Vroon et al., 2008; Nowell and Horstwood, 2009; Lewis et al., 2014). The proposed ⁴⁰Ca³¹P¹⁶O interference on mass 87 is still a matter of debate. In fact, while several authors claim its existence (e.g. Horstwood et al., 2008; Simonetti et al., 2008; Lewis et al., 2014), others never experienced it (e.g. Copeland et al., 2008; Müller and Anczkiewicz, 2016). More recently, Willmes et al. (2016) have attributed the interference on mass 87 to the ⁴⁰Ar³¹P¹⁶O molecule, because of the omnipresence of Ar in the plasma atmosphere. They correct this polyatomic

interference adding a flux of nitrogen gas to reduce the formation of oxides within the plasma and monitoring mass 71 (40 Ar³¹P) as a proxy of the 40 Ar³¹P¹⁶O oxide production. This method seems to solve the interference problem related to mass 87 but largely reduces (factor of 2–3) the intensity of Sr signals, a serious problem when dealing with low-Sr samples. They partially resolve this issue by employing a larger spot size (~250 µm), resulting in a more intense signal because of the increased volume of ablated material (Vroon et al., 2008), but with the obvious consequence of a more destructive analysis. They also suggest that a valid approach to correct for the bias arising from mass 87-interferences is a calibration correction with a series of known standards (Willmes et al., 2016; Horstwood et al., 2008).

In addition to mass-87 interference, LA isotope analyses often endure the so-called laser-induced elemental/isotope fractionation, as observed for other isotopic systematics (i.e. U–Pb), which may bias the final isotope ratio (see Horn 2008; Vroon et al., 2008). Moreover, many unknown interferences may form in the MC–ICP–MS atmosphere, mainly because of the bonds of oxygen and argon with elements of the sample matrix ionised within the plasma (Vroon et al., 2008). No matter how or why interferences arise during Sr LA analysis of a particular material, the concurrent analysis of a set of matrix-matched standards is a proper approach to monitor and correct the Sr fractionation issues, as demonstrated by Horstwood et al. (2008) and reiterated by Willmes et al. (2016). Standards with matrix equal to the samples are ideal because their behaviour during analysis is supposed to be the same of the sample (Sylvester, 2008). Thus, each of our bio-apatite in-house standard was analyzed several times (at least 5) during the whole session to observe the external reproducibility on different Sr-concentration materials. Following the method proposed by Horstwood et al. (2008), we built with our bio-standards a calibration

curve based on the accuracy of the ⁸⁷Sr/⁸⁶Sr ratio (measured value/true value) vs. the inverse of the ⁸⁸Sr signal (1/⁸⁸Sr). Three of the apatites (the shark tooth, the bovine tooth and the swine tooth) were used to build the daily calibration curve, while the human tooth was used only to check the precision and accuracy of the analysis on low-Sr samples. This daily LA calibration line ($r^2 = 0.82$; p < 0.00001; n = 18) results in a slope of 0.0005954 and an intercept of 1.0000077 (Fig. 4). We also built a calibration curve ($r^2 = 0.99$) based on the 2 σ of the standard materials vs. the inverse of the Sr concentration (1/Sr) to obtain the external reproducibility of an unknown sample with a given Sr concentration. Sr concentrations of our standards have been previously measured by LA–ICP–MS for comparison.

The daily ⁸⁴Sr/⁸⁶Sr ratio is 0.05609 \pm 0.00088 (2 σ), which is, within error, equal to the natural 0.0565 ratio (for a detailed review of the significance of this ratio see Horstwood et al., 2008; Vroon et al., 2008).

All LA results of our standard materials are reported in Table 2. The LA analysis of the human tooth (Sr c.a. 100 ppm) yielded an average value of 0.70917 ± 0.00050, which is identical to the true value by dissolution MC–ICP–MS (0.70917 ± 0.00001; 2SE). The swine tooth (Sr c.a. 250 ppm), after the calibration correction, yielded a ⁸⁷Sr/⁸⁶Sr ratio of 0.70897 ± 0.00012 (2 σ), equal to the dissolution result (0.70900 ± 0.00001; 2SE). The bovine tooth standard (Sr c.a. 350 ppm), after correction, yielded a mean ⁸⁷Sr/⁸⁶Sr ratio of 0.70881 ± 0.00008 (2 σ), equal within error to the dissolution value (0.70877 ± 0.00001; 2SE). The shark tooth (Sr c.a. 1100 ppm), yielded a mean corrected ⁸⁷Sr/⁸⁶Sr ratio of 0.70916 ± 0.00004 (2 σ), identical within error to the modern Indian Ocean seawater (~0.70918; Mokadem et al., 2015) and to the ratio obtained by dissolution method (0.70919 ± 0.00001; 2SE).

3. Results

3.1. Retzius line counting

Retzius lines were counted only in sample RH–IS26 because it was the best available tooth fragment with the proper orientation for this type of observations (Fig. 5).

Relying on visible striae, we have estimated the presence of c.a. 30-35 Retzius lines along the entire length of the enamel and of about 10-15 lines along the OES. The number of laminations per Retzius band (i.e. periodicity) is 7, in agreement with the observations of Tafforeau et al. (2007). Thus, sample RH–IS26 should cover a period of about 200–250 days along the EDJ and of about 80–100 days along the OES. In the upper part of the sample (nearest to the apex), we approximately calculated an outward extension rate of the enamel of about 0.5 mm/month (~ 15 µm/d) in agreement with literature data (e.g. Tafforeau et al., 2007; Bendrey et al., 2015).

3.2. Dissolution Sr isotopes

The enamel portions of the two rhinos yielded bulk 87 Sr/ 86 Sr ratios of 0.70952 ± 0.00001 (2SE, 100 cycles per analysis) and 0.70979 ± 0.00001 (2SE, 100 cycles per analysis), for RH–IS30 and RH–IS26 respectively.

All dissolution results, including those of the standard materials, are reported in Table 2.

3.3. In situ LA Sr isotopes

The whole dataset of RH–IS30 and RH–IS26 87 Sr/ 86 Sr ratios is reported in Table 3. The mean 87 Sr/ 86 Sr ratio of all LA analyses of RH–IS30 enamel is 0.70951 ± 0.00014 $(2\sigma, n = 42)$, i.e., identical to the ratio obtained by dissolution (0.70952 ± 0.00001). The ⁸⁷Sr/⁸⁶Sr ratios, however, vary significantly within the RH–IS30 enamel ranging from 0.70966 to 0.70938. The mean ⁸⁷Sr/⁸⁶Sr value of LA dentine analyses is 0.70918 ± 0.00013 (2σ , n = 10), a ratio quite similar to modern seawater (~0.70917; Dia et al., 1992). Using our standard materials with known Sr concentrations, we have also calculated a Sr concentration of 361 ± 49 ppm (2σ) in the enamel and 447 ± 76 ppm (2σ) in the dentine for sample RH–IS30.

The mean LA ⁸⁷Sr/⁸⁶Sr value of RH–IS26 enamel is 0.70976 ± 0.00015 (2σ , n = 20), identical to the true value by dissolution (0.70979 ± 0.00001). Similarly to sample RH–IS30, we observe a high variability of the ⁸⁷Sr/⁸⁶Sr ratios within the enamel, with values ranging between 0.70997 and 0.70950. The mean ⁸⁷Sr/⁸⁶Sr ratio of LA dentine analyses is 0.70934 ± 0.00009 (2σ , n = 10), a value more radiogenic than modern seawater (~0.70917). The interpolated Sr concentration for RH–IS26 enamel is 509 ± 131 ppm (2σ) while the dentine is 544 ± 131 ppm (2σ).

3.4. Diagenesis

Both teeth show significant isotopic variability along the enamel surface (Fig. 1 and 2). Enamel, unlike dentine (e.g. Lee-Thorp and Sponheimer, 2003; Bentley, 2006), is rarely affected by diagenetic contamination even in prehistoric samples (Budd et al., 2000), we can, therefore, consider these variations as real. The ⁸⁷Sr/⁸⁶Sr ratio of dentine, however, is still an important information because is used as the reference value for the "local" substrate isotopic signature (e.g. Bentley, 2006; Britton et al, 2011). This is due to the large pores (1µm tubules) of dentine that make this tissue highly susceptibility to diagenetic alteration (Kohn et al., 1999) especially in prehistoric samples. Any significant Sr enrichment in dentine can be due to the effect

of local diagenetic contamination and/or animal physiology (see Evans et al., 2006). Sample RH–IS30 shows a dentine-enamel difference of 86 ppm, while sample RH–IS26 of 35 ppm. The smaller Sr concentration difference of sample RH–IS26 might be interpreted as a minor diagenetic alteration for this sample that in fact retains a ⁸⁷Sr/⁸⁶Sr ratio (0.70934) higher than sample RH–IS30 (0.70918) and perhaps less affected by the modern seawater signal of 0.7092, that locally dominates the Sr rainwater isotopic composition (e.g. Montgomery, 2010). Therefore, we can infer that the local Sr isotope signature should be lower than ~0.7093, probably close to modern seawater.

3.5. External reproducibility

The external reproducibility is the error associated with repeated measurements of reference standard materials, generally expressed as a multiple of the standard deviation.

We calculated an external reproducibility of 0.00008 (2 σ) for sample RH–IS30, whereas the interpolated external reproducibility is 0.00005 (2 σ) for sample RH–IS26 (see section 2.3 of this paper for more details). Therefore, we consider all values outside this range as significantly different Sr isotopic compositions that can be related to morphological growth features of the teeth as discussed in the following section.

4. Discussion

4.1. Rhino tooth growth and Sr isotopic composition

The estimated crown formation time of rhino species ranges between about 1.5 and 3

years (Tafforeau et al., 2007; Hillman-Smith et al., 1986), a long enough period during which the animal may have migrated through the landscape. For all the profiles we studied at the microscope and analysed by LA, we approximately followed the growth direction of the enamel (e.g. Tafforeau et al., 2007; Bentley, 2006; Khon et al., 2002), trying to observe possible seasonal variations both in the growth features and in the Sr isotopic composition. Because dentine growth direction is inward from the EDJ, those portions of the profiles that include dentine samples are inverted in terms of tissue growth. However, because of the observed diagenetic contamination of dentine, we considered this tissue as a homogeneous matrix, ignoring any possible incremental feature.

4.1.1. Tooth sample RH–IS30

The RH–IS30 sample is a horizontal section of a rhino molar fragment, but given that enamel nucleates at EDJ and grows downward from the tooth crown and outward from the EDJ itself, it is difficult to determine the precise time-span that this tooth section covers. In large herbivores, namely horses from Kohn et al. (2002), the outward growth rate is about 1 mm/month (Kohn et al., 2002). Given that the enamel thickness of RH–IS30 sample is about 2 mm, the time recorded by this sample should be at least 2 months, or even longer considering that rhino enamel growth seems slightly slower than the one of a horse (Bendrey et al., 2015; Tafforeau et al., 2007; Kohn et al., 2002). RH–IS26 sample, based on the observation of laminations, revealed an outward growth rate of about 0.5 mm/month in agreement with the growth rate of rhino teeth (Tafforeau et al. 2007). This suggests a recorded time span of about 3-4 months for sample RH–IS30. Both Sr isotope profiles obtained for this sample follow the outward direction (from the EDJ) of the enamel growth. Profile A,

composed by 32 small linear analyses, covers the entire length of the sample with the first line near the EDJ and the last line close to the external surface (Fig. 1). This profile includes relatively regular cyclic variations of the ⁸⁷Sr/⁸⁶Sr ratio, from highly radiogenic ratios (>0.70960) to less radiogenic ratios (<0.70945). A simple t-test (>0.70960, single sample one-tailed t-test p = 0.02; <0.70945, p = 0.04) shows that these ratios are statistically different from the mean of the profile (0.70953). Profile B, measured half within the enamel and half within the dentine, also shows cyclic variations of the Sr isotopic ratios but with higher periodicity and clearly displays the chemical boundary between the enamel and the dentine. The enamel part of the profile includes only one value (0.70957) statistically different from the mean value of the profile itself.

4.1.2. Tooth sample RH–IS26

Sample RH–IS26 is a vertical section of a rhino molar fragment. For this sample, we analyzed both the exposed inner surface (Fig. 2) and the external surface (OES, Fig. 3) of the enamel. We observe cyclic variations of the Sr isotopic composition in both profiles with different periodicity. Theoretically, the OES and the exposed internal surface should encrypt similar incremental information. However, Tafforeau et al. (2007) showed how the external and the internal surface (along the EDJ) of the rhino enamel have different growth rate. When Retzius lines reach the OES of the enamel, they form ridges called perikymata (e.g. Smith and Tafforeau, 2008). Our sample represents the last ~ 8 mm (distance from the cervix) of the crown. Hence, the internal exposed surface of the enamel based on rates by Tafforeau et al. (2007) should represents a time-span of about 300–350 days, while the external surface a time-span of about 100 days. Our counting of Retzius lines agrees well with these

estimates, revealing at least a time-interval of about 200–250 days along the EDJ and of 80 days along the OES.

According to the different growth rate between OES and inner surface, we observe that the second half of the Sr isotopic composition profile E seems to reflect the same fluctuations of the entire length of the F profile. In Figure 3c we compared the two profiles and shifted them to obtain the strongest possible correlation ($r^2 = 0.56$; p < 0.00001), confirming the fact that the two profiles show equivalent temporal variations in Sr isotopic composition. In addition, the other two profiles (C and D), which should encrypt fewer (about 2) months of the rhino life, display similar cyclical variations.

4.2. Rhino seasonal movements

Given that the Sr isotopic composition of the growing rhinos teeth derives from the land where the animal lived, these cyclic variations of the ⁸⁷Sr/⁸⁶Sr ratio likely indicate different water and food intake and, therefore, seasonal movements of the two animals to different lands, e.g. with different Sr isotopic composition. Even the shorter time range (about 100 days) observed seems to be characterized by cyclic variations of the ⁸⁷Sr/⁸⁶Sr ratio and consequently of the migration pattern. Moreover, both tooth samples show an enamel ⁸⁷Sr/⁸⁶Sr ratio considerably higher than its relative dentine ratio that represents the local water/food signal (0.70951 vs. 0.70918 for RHIS–30, one-tailed Mann-Whitney U-test p = 0.04; 0.70976 vs. 0.70934 for RH–IS26, one-tailed Mann-Whitney U-test p = 0.06).

We try now to identify the Sr reservoir responsible for the chemical variations observed in the rhinos. The site of Isernia and the surrounding area are characterized by Holocene and Pleistocene fluvial deposits and limestone and dolostone ranging between the Cretaceous and the Holocene (Peretto et al., 2015). Therefore, the ⁸⁷Sr/⁸⁶Sr isotopic composition of these rocks should range approximately between 0.7073 and 0.7092 according to the global Sr isotopes seawater curve of McArthur et al. (2001). This is a big interval, however, our samples have even higher ⁸⁷Sr/⁸⁶Sr ratios. One of the possible sources of the more radiogenic ratios of our samples can be identified in the Roccamonfina volcano, located 50 km to the southeast of Isernia. Previous geological work has shown that the lavas from the 630 ka eruption of the Roccamonfina volcano have a ⁸⁷Sr/⁸⁶Sr isotopic signature ranging between about 0.7093 and 0.7100 (Conticelli et al., 2009). In addition, located about 150 km to the northeast of Isernia, the Middle-Pleistocene Colli Albani volcano lavas show ⁸⁷Sr/⁸⁶Sr ratios higher than 0.7100 (Giaccio et al., 2013; Boari et al., 2009). Likely, our Isernia Rhinos have travelled to these volcanic grounds and fed themselves with water/food "contaminated" with highly radiogenic ⁸⁷Sr/⁸⁶Sr ratios.

According to palaeoenvironmental reconstructions, the Isernia La Pineta environment consisted of an association of closed forests, grasslands and semi-open woodlands (Orain et al., 2013; Thun Hohenstein et al., 2004), seemingly a good ground for rhinos grazing. However, based on our data, these rhinos were very mobile and seasonally reached the fertile substratum offered by the volcanic soils (Ugolini and Zasoski, 1979). Understanding the specific direction of the rhinos movements (either to the Roccamonfina volcano or to the Colli Albani area) remains no more than a speculation. Yet, we can certainly state that the Isernia rhinos have seasonally migrated as shown by the Sr isotopic composition temporal variations measured in their teeth, covering a distance of at least 50 km and returned cyclically to Isernia La Pineta or to a different site, but farther away from the isotopic influence of the volcanos.

Conclusions

We exploited the LA–MC–ICP–MS technique to study ancient animal migration patterns of two *Stephanorhinus hundsheimensis* teeth discovered in the Middle Pleistocene site of Isernia La Pineta, where the most ancient human Italian tooth has been unearthed. The two teeth, here analysed in high-spatial resolution, revealed time-related fluctuations of the Sr isotopic composition, likely linked to seasonal movements of the rhinos, probably towards the more fertile volcanic soils of Roccamonfina and/or Colli Albani. Moreover, while dentine has been possibly altered by diagenetic processes, enamel seems to be pristine and therefore suitable for reliable Sr isotopic analysis. As expected, both the inner and the outer enamel surface of the vertically sectioned sample, RH–IS26, showed highly correlated variations of the ⁸⁷Sr/⁸⁶Sr ratio, but with different time spans, related to the differential growth rate of the tooth portions. Additionally, we have successfully investigated the ⁸⁷Sr/⁸⁶Sr ratio of low-Sr (to c.a. 100 ppm) bioapatites, using three matrix-matched standards to correct unresolved interferences arising from LA analyses.

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Fig. 1. a. RH–IS30 sample photo, with visible Hunter-Schreger bands. **b.** Profile A is composed by 32 small (100 x 500 μ m) LA analyses; blue line is the average of the analyses; grey area is the 2 σ -error of 0.00008 calculated from the standard materials; x-error bars are the 2-standard errors. **c.** Profile B starts from the dentine and proceeds outward to the OES; while for enamel the profile follows the tooth growth, for dentine the direction is inverted (see text for details); symbols are the same as in profile A. **d.** Example of a tooth horizontal section (redrawn from Ballatore and Breda, 2013).



Fig. 2. a. RH–IS26 sample photo of the exposed inner surface. **b.** Profile C cover a short portion of the enamel near the OES and is composed by 11 LA analyses; blue line is the average of the analyses; grey area is the 2σ -error of 0.00005 calculated from the standard materials; x-error bars are the 2-standard errors. **c.** Profile D starts from the dentine and proceeds outward to the OES; while for enamel the profile follows the tooth growth, for dentine the direction is inverted (see text for details); symbols are the same as in profile C. **d.** Profile E is a single LA analysis which cover all the length of the enamel, reported as a moving average with a period of 100; symbols are the same as in profile D. **e.** Example of a tooth vertical section (redrawn from Ballatore and Breda, 2013).



Fig. 3. a. RH–IS26 sample photo of the outer enamel surface (OES). **b.** Profile F is a single LA analysis which cover all the length of the outer enamel, reported as a moving average with a period of 100; blue line is the average of the analyses; grey area is the 2σ -error of 0.00005 calculated from the standard materials. **c.** Comparison between profile F and the second half of profile E (Fig. 2d); the two profiles show a high correlation ($r^2 = 0.56$). For a better visual comparison, profile E, which has relatively less data, is smoothed with a Savitzky–Golay filter.



Fig. 4. Correlation between the accuracy of the ⁸⁷Sr/⁸⁶Sr analysis (observed/true) and the inverse of the ⁸⁸Sr signal. This high correlation is attributed by some authors to the Ca–P–O interference which occurs during LA analysis of bioapatites (see text for details). The line equation is used to correct our LA data.



Fig. 5. Retzius line observation under polarized transmitted light with a petrographic microscope on RH-IS26



Parameters	Values
Laser ablation New Wave Up 213	
Wavelength	213 nm
He flow rate	0.6 L/min
Ablation	
Spot size	100 µm
Frequency	10 Hz
Fluence	~20 J/cm ²
Sampling scheme	Line (dynamic)
Line translation rate	5 µms⁻¹
Pre-ablation	
Spot size	100 µm
Frequency	10 Hz
Line translation rate	40 µms ⁻¹
MC–ICP–MS Neptune	
Cool gas flow rate	15 L/min
Auxiliary gas flow	0.8 L/min
Sample gas flow	0.9 – 1 L/min
Plasma power	1200
Resolution	Low (450)
Collected masses	⁸⁸ Sr (H4), ⁸⁷ Sr (H3), 86.5 (H2), ⁸⁶ Sr (H1), 85.5 (C), ⁸⁵ Rb (L1), ⁸⁴ Sr, (L2) ⁸³ Kr (L3), ⁸² Kr (L4)
Data collection	1 block, 200 cycles, 0.5 s integration

Table 1. Parameters and operating conditions for Sr isotope determination by laser ablation MC-ICP-MS

Table 2	
MC-ICP-MS ⁸⁷ Sr/ ⁸⁶ Sr data and Sr concentrations of the bioapatites us	ed as standard materials.

Sample	Solution ⁸⁷ Sr/ ⁸⁶ Sr (±2SE)	LA corrected ⁸⁷ Sr/ ⁸⁶ Sr (±2σ)	Sr concentration (ppm)	Characteristics	Provenance
ST1 – A	0.70918 ± 0.00001	0.70916 ± 0.00004	1101 ± 47	Modern shark tooth	Indian Ocean
ST1 – B	0.70919 ± 0.00001	-	-	-	-
ST1 – C	0.70918 ± 0.00001	-	-	-	-
BOS– Sr1	0.70877 ± 0.00001	0.70881 ± 0.00008	347 ± 18	Archaeological cattle tooth	P.zza Anita Garibaldi Roman site, Ravenna
SUS– Sr1	0.70899 ± 0.00001	0.70897 ± 0.00012	253 ± 31	Archaeological swine tooth	P.zza Anita Garibaldi Roman site, Ravenna
ROCht4 21*	0.70917 ± 0.00001	0.70917 ± 0.00050	104 ± 25	Archaeological human tooth	Roccapelago post-medieval site, Modena

Letters after the sample name indicate duplicate analyses of a different portion of the same tooth. *This sample was used only to check the accuracy of the correction. It was not used to build the daily calibration line.

Tooth sample	Solution ⁸⁷ Sr/ ⁸⁶ Sr (± 2SE)	LA sample ID	Sr (ppm)	LA corrected ⁸⁷ Sr/ ⁸⁶ Sr	2SE
RH–IS30	0.70951 ± 0.00001				
		1	412	0.70953	0.00004
		2	398	0.70966	0.00005
		3	386	0.70956	0.00005
		4	385	0.70956	0.00005
		5	393	0.70958	0.00005
		6	391	0.70953	0.00005
		7	384	0.70965	0.00004
		8	394	0.70955	0.00005
		9	406	0.70953	0.00006
		10	404	0.70961	0.00005
		11	402	0.70949	0.00005
		12	373	0.70957	0.00005
		13	370	0.70955	0.00005
		14	376	0.70952	0.00005
		15	377	0.70949	0.00005
		16	362	0.70952	0.00005
		17	365	0.70939	0.00005
		18	360	0.70941	0.00006
		19	356	0.70949	0.00005
		20	361	0.70951	0.00005
		21	337	0.70945	0.00006
		22	345	0.70960	0.00006
		23	341	0.70953	0.00006
		24	335	0.70945	0.00006
		25	338	0.70958	0.00006
		26	353	0.70954	0.00005
		27	339	0.70955	0.00006
		28	346	0.70941	0.00006
		29	344	0.70951	0.00006
		30	338	0.70950	0.00005
		31	342	0.70946	0.00006
		32	340	0.70961	0.00007
		33*	494	0.70924	0.00004
		34*	484	0.70916	0.00004
		35*	460	0.70918	0.00005
		36*	454	0.70914	0.00004
		37*	498	0.70923	0.00004
		38*	428	0.70928	0.00005
		39*	443	0.70919	0.00005
		40*	414	0.70914	0.00004

 Table 3

 ⁸⁷Sr/⁸⁶Sr results for samples RH–IS30 and RH–IS26. Each analysis represents a short linear scan except for profiles E and F that are long continuous scans

41* 388 0.70905 0.00005 42* 408 0.70918 0.00005 43 375 0.70930 0.00006 45 335 0.70939 0.00006 45 335 0.70938 0.00006 46 338 0.70944 0.00006 47 346 0.70947 0.00006 48 338 0.70948 0.00006 50 338 0.70947 0.00006 51 340 0.70975 0.00004 52 341 0.70975 0.00004 54 486 0.70975 0.00004 55 488 0.70968 0.00004 56 482 0.70976 0.00004 57 519 0.70976 0.00004 58 505 0.70978 0.00004 59 515 0.70989 0.00004 58 505 0.70976 0.00004 66* 559 <t< th=""><th></th><th></th><th></th><th></th><th></th></t<>					
42* 408 0.70918 0.00005 43 375 0.70940 0.00006 44 337 0.70938 0.00006 45 335 0.70934 0.00006 46 338 0.70944 0.00006 47 346 0.70957 0.00006 48 338 0.70946 0.00006 50 338 0.70947 0.00006 51 340 0.70973 0.00007 52 341 0.70947 0.00004 54 486 0.70973 0.0004 55 488 0.70968 0.0004 56 482 0.70968 0.0004 57 519 0.70972 0.0004 58 505 0.70978 0.0004 59 515 0.70976 0.0004 59 515 0.70978 0.00004 60 559 0.70976 0.00004 61 467 679 <td></td> <td>41*</td> <td>388</td> <td>0.70905</td> <td>0.00005</td>		41*	388	0.70905	0.00005
43 375 0.70940 0.00005 44 337 0.70938 0.0006 45 335 0.70939 0.00006 46 338 0.70944 0.00006 47 346 0.70944 0.00006 48 338 0.70947 0.00006 50 338 0.70947 0.00006 51 340 0.70947 0.00006 52 341 0.70943 0.00007 0.00001 52 341 0.70943 0.00004 52 341 0.70943 0.00004 55 53 504 0.70975 0.0004 55 488 0.70958 0.0004 56 482 0.70976 0.0004 56 482 0.70978 0.00004 58 505 0.70978 0.00004 60 559 0.70976 0.00004 61 467 0.70973 0.00003 6		42*	408	0.70918	0.00005
44 337 0.70938 0.0006 45 335 0.70939 0.0006 46 338 0.70944 0.0006 47 346 0.70957 0.0006 48 338 0.70945 0.0006 50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70948 0.0007 52 341 0.70975 0.0004 54 486 0.70975 0.0004 55 488 0.70968 0.0004 56 482 0.70985 0.0004 56 482 0.7098 0.0004 56 482 0.70985 0.0004 57 519 0.70976 0.0004 58 505 0.70985 0.0004 61 467 0.70978 0.0006 64* 555 0.70933 0.0004 65* 579 0.70934		43	375	0.70940	0.00005
45 335 0.70939 0.0006 46 338 0.70944 0.0006 47 346 0.70946 0.0005 48 338 0.70946 0.0006 50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70943 0.0004 54 486 0.70975 0.0004 54 486 0.70973 0.0004 55 488 0.70968 0.0004 56 482 0.70985 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70985 0.0004 59 515 0.70985 0.0004 59 515 0.70985 0.0004 60 559 0.70976 0.0004 61 467 0.70973 0.0006 62 464 0.70978		44	337	0.70938	0.00006
46 338 0.70944 0.0006 47 346 0.70944 0.0005 48 338 0.70946 0.0006 50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70977 0.00004 52 341 0.70975 0.0004 54 486 0.70975 0.00004 55 488 0.70968 0.00004 56 482 0.70968 0.00004 57 519 0.70972 0.00004 58 505 0.70985 0.0004 59 515 0.70985 0.0004 60 559 0.70976 0.0006 61 467 0.70973 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.0006 64* 555 0.70938 0.00004 65* 579 0.70943		45	335	0.70939	0.00006
47 346 0.70944 0.00005 48 338 0.70945 0.00005 50 338 0.70948 0.00006 51 340 0.70947 0.00006 52 341 0.70973 0.00007 0.70979 ± 53 504 0.70975 0.00004 54 486 0.70973 0.00004 55 488 0.70968 0.0004 56 482 0.70985 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70985 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70973 0.00004 63 455 0.70938 0.00004 64* 555 0.70936 0.00004 65* 635 0.70936 0.00004 66* <t< td=""><td></td><td>46</td><td>338</td><td>0.70944</td><td>0.00006</td></t<>		46	338	0.70944	0.00006
48 338 0.70946 0.0005 49 340 0.70957 0.0006 50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70947 0.0007 0.70979 ± 53 504 0.70975 0.0004 54 486 0.70973 0.0004 55 488 0.70968 0.0004 56 482 0.70985 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70985 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70973 0.0004 63 454 0.70935 0.0004 64* 555 0.70935 0.0004 65* 635 0.70936 0.0003 66* 635		47	346	0.70944	0.00006
49 340 0.70957 0.0006 50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70943 0.0007 0.70979 ± 0.00001 0.70975 0.00004 54 486 0.70973 0.00004 55 488 0.70986 0.0004 56 482 0.70985 0.0004 55 488 0.70986 0.0004 56 482 0.70986 0.0004 57 519 0.70972 0.0004 59 515 0.70989 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70978 0.00004 63 454 0.70938 0.0003 64* 555 0.70938 0.0003 65* 579 0.70943 0.0004 69* 530 0.7		48	338	0.70946	0.00005
50 338 0.70948 0.0006 51 340 0.70947 0.0006 52 341 0.70943 0.0007 0.70979 ± 0.00001 53 504 0.70975 0.0004 54 486 0.70973 0.0004 55 488 0.70969 0.0004 56 482 0.70985 0.0004 57 519 0.70972 0.0004 58 505 0.70989 0.0004 59 515 0.70976 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70978 0.0004 63 454 0.70978 0.0004 64* 555 0.70938 0.0004 65* 579 0.70943 0.0004 66* 635 0.70936 0.0004 67* 620 0.70938 0.0004 70* 5		49	340	0.70957	0.00006
51 340 0.70947 0.00001 52 341 0.70943 0.0007 0.70979 ± 0.00001 53 504 0.70975 0.00004 54 486 0.70973 0.00004 55 488 0.70968 0.00004 56 482 0.70968 0.00004 57 519 0.70972 0.00004 58 505 0.70985 0.00004 59 515 0.70980 0.00004 60 559 0.70976 0.00004 61 467 0.70970 0.00006 62 464 0.70973 0.00004 63 454 0.70978 0.00004 64 555 0.70933 0.00004 65* 579 0.70943 0.00004 66* 635 0.70936 0.00004 67* 620 0.70936 0.00004 67* 620 0.70936 0.00004 70* </td <td></td> <td>50</td> <td>338</td> <td>0.70948</td> <td>0.00006</td>		50	338	0.70948	0.00006
52 341 0.70943 0.00007 0.70979 ± 0.00001 53 504 0.70975 0.00004 54 486 0.70973 0.00004 55 488 0.70968 0.00004 56 482 0.70968 0.00004 57 519 0.70972 0.00004 58 505 0.70985 0.00004 59 515 0.70986 0.00004 60 559 0.70976 0.00004 61 467 0.70970 0.00004 61 467 0.70970 0.00004 62 464 0.70973 0.00004 63 454 0.70978 0.00004 64* 555 0.70933 0.00004 65* 579 0.70943 0.00003 66* 635 0.70936 0.00004 67* 620 0.70938 0.00004 70* 536 0.70936 0.00004 71*		51	340	0.70947	0.00006
0.70979 ± 0.00001 53 504 0.70975 0.00004 54 486 0.70973 0.00004 55 488 0.70969 0.00004 56 482 0.70972 0.00004 57 519 0.70972 0.00004 58 505 0.70985 0.00004 59 515 0.70970 0.00004 60 559 0.70970 0.00004 61 467 0.70970 0.00006 62 464 0.70973 0.00004 63 454 0.70978 0.00004 64* 555 0.70933 0.00004 65* 579 0.70943 0.00003 66* 635 0.70936 0.00004 67* 620 0.70938 0.00004 67* 620 0.70936 0.00004 67* 530 0.70936 0.00004 70* 506 0.70936 0.00004 <td< td=""><td></td><td>52</td><td>341</td><td>0.70943</td><td>0.00007</td></td<>		52	341	0.70943	0.00007
53 504 0.70975 0.0004 54 486 0.70973 0.0004 55 488 0.70969 0.0004 56 482 0.70968 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70976 0.0004 60 559 0.70976 0.0006 61 467 0.70970 0.0006 62 464 0.70973 0.00006 63 454 0.70978 0.00006 64* 555 0.70933 0.00004 65* 579 0.70943 0.00003 66* 635 0.70936 0.00003 66* 580 0.70936 0.00004 70* 506 0.70936 0.00004 70* 536 0.70931 0.00004 71* 483 0.70977 0.0004 72* 536 0.70	0.70979 ± 0.00001				
54 486 0.70973 0.0004 55 488 0.70969 0.0004 56 482 0.70968 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.00004 65* 579 0.70933 0.00004 65* 579 0.70933 0.00003 66* 635 0.70933 0.00003 66* 635 0.70938 0.00003 66* 635 0.70938 0.00004 67* 620 0.70938 0.00004 70* 506 0.70936 0.0004 70* 506 0.70937 0.0004 71* 483 0.70977 0.0004 72* 536 0.70977 0.0004 75 460 0.70977 0.0004 <td></td> <td>53</td> <td>504</td> <td>0.70975</td> <td>0.00004</td>		53	504	0.70975	0.00004
55 488 0.70969 0.0004 56 482 0.70968 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 60 559 0.70976 0.0004 61 467 0.70970 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.0004 65* 579 0.70933 0.0004 65* 579 0.70933 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0004 67* 620 0.70938 0.0004 70* 506 0.70936 0.0004 70* 506 0.70937 0.0004 71* 483 0.70977 0.0004 73* 415 0.70977 0.0004 74 415 0.70977 0.0004		54	486	0.70973	0.00004
56 482 0.70968 0.0004 57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70976 0.0004 60 559 0.70976 0.0006 61 467 0.70970 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.0004 65* 579 0.70933 0.0003 66* 635 0.70935 0.0004 65* 579 0.70938 0.0003 66* 635 0.70936 0.0003 66* 635 0.70936 0.0004 67* 620 0.70938 0.0004 67* 620 0.70938 0.0004 70* 506 0.70936 0.0004 70* 506 0.70931 0.0004 71* 483 0.70927 0.0004 72* 536 0.70937 0.0004 75 460 0.70977 0.0004		55	488	0.70969	0.00004
57 519 0.70972 0.0004 58 505 0.70985 0.0004 59 515 0.70976 0.0004 60 559 0.70976 0.0006 61 467 0.70970 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.0006 64* 555 0.70933 0.0003 65* 579 0.70943 0.0003 66* 635 0.70935 0.0004 67* 620 0.70936 0.0003 68* 580 0.70936 0.0004 69* 530 0.70936 0.0004 70* 506 0.70936 0.0004 71* 483 0.70927 0.0004 72* 536 0.70931 0.0003 73* 415 0.70977 0.0004 74 415 0.70977 0.0004 76 545 0.70974 0.0003 78 573 0.70974 0.0003		56	482	0.70968	0.00004
58 505 0.70985 0.0004 59 515 0.70976 0.0004 60 559 0.70976 0.0006 61 467 0.70970 0.0006 62 464 0.70978 0.0006 63 454 0.70978 0.0006 64* 555 0.70933 0.0004 65* 579 0.70943 0.0003 66* 635 0.70936 0.0003 66* 635 0.70936 0.0004 67* 620 0.70936 0.0003 68* 580 0.70936 0.0004 70* 506 0.70936 0.0004 70* 506 0.70936 0.0004 71* 483 0.70928 0.0004 72* 536 0.70931 0.0004 74 415 0.70977 0.0004 75 460 0.70977 0.0004 76 545 0.70978 <td></td> <td>57</td> <td>519</td> <td>0.70972</td> <td>0.00004</td>		57	519	0.70972	0.00004
59 515 0.70989 0.0004 60 559 0.70976 0.0006 61 467 0.70970 0.0006 62 464 0.70978 0.0006 63 454 0.70978 0.0006 64* 555 0.70933 0.0004 65* 579 0.70935 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0004 67* 620 0.70938 0.0004 69* 530 0.70936 0.0004 70* 506 0.70936 0.0004 70* 536 0.70931 0.0003 73* 415 0.70927 0.0004 74 415 0.70977 0.0004 76 545 0.70978 0.0004 76 545 0.70974 0.0003 78 573 0.70974 <td></td> <td>58</td> <td>505</td> <td>0.70985</td> <td>0.00004</td>		58	505	0.70985	0.00004
60 559 0.70976 0.00004 61 467 0.70970 0.00005 62 464 0.70973 0.00005 63 454 0.70978 0.00004 64* 555 0.70933 0.00004 65* 579 0.70943 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0003 66* 635 0.70938 0.0003 66* 580 0.70938 0.0003 68* 580 0.70936 0.0004 70* 506 0.70936 0.0004 70* 506 0.70936 0.0004 71* 483 0.70927 0.0004 72* 536 0.70937 0.00004 74 415 0.70977 0.00004 75 460 0.70977 0.00004 76 545 0.70974 0.00003 78 573		59	515	0.70989	0.00004
61 467 0.70970 0.0006 62 464 0.70973 0.0006 63 454 0.70978 0.0006 64* 555 0.70933 0.0003 65* 579 0.70943 0.0003 66* 635 0.70935 0.0004 67* 620 0.70938 0.0003 68* 580 0.70936 0.0004 69* 530 0.70936 0.0004 70* 506 0.70936 0.0004 70* 536 0.70931 0.0003 73* 415 0.70927 0.0004 74 415 0.70977 0.0004 76 545 0.70977 0.0004 76 545 0.70978 0.0004 76 545 0.70974 0.0003 78 573 0.70974 0.0003 80 570 0.70974 0.0004 79 645 0.70972 0.0003 80 570 0.70974 0.0003 <		60	559	0.70976	0.00004
62 464 0.70973 0.00005 63 454 0.70978 0.00006 64* 555 0.70933 0.00003 65* 579 0.70943 0.0003 66* 635 0.70935 0.0004 67* 620 0.70938 0.0003 68* 580 0.70936 0.0004 69* 530 0.70936 0.0004 70* 506 0.70936 0.0004 70* 506 0.70936 0.0004 71* 483 0.70928 0.0004 72* 536 0.70937 0.0004 73* 415 0.70927 0.0004 74 415 0.70977 0.0004 75 460 0.70977 0.0004 76 545 0.70974 0.0003 78 573 0.70974 0.0003 80 570 0.70974 0.0004 79 645 0.70972 0.0003 80 570 0.70974 0.0004		61	467	0.70970	0.00006
63 454 0.70978 0.00004 64* 555 0.70933 0.00003 65* 579 0.70943 0.00003 66* 635 0.70938 0.00003 67* 620 0.70938 0.00003 68* 580 0.70936 0.00004 69* 530 0.70936 0.00004 70* 506 0.70936 0.00004 71* 483 0.70928 0.00004 71* 536 0.70931 0.00003 73* 415 0.70927 0.00004 74 415 0.70977 0.00004 75 460 0.70977 0.00004 76 545 0.70978 0.00004 77 655 0.70974 0.00003 78 573 0.70972 0.00003 80 570 0.70974 0.00004 79 645 0.70974 0.00004 80 570 0.70974 0.00004 80 570 0.70971 0.00004		62	464	0.70973	0.00005
64*5550.709330.000465*5790.709430.000366*6350.709350.000467*6200.709380.000368*5800.709360.000469*5300.709340.000470*5060.709360.000471*4830.709280.000472*5360.709310.000373*4150.709270.0004744150.709770.0004754600.709770.0004765450.709780.0004776550.709740.0003785730.709690.0004796450.709740.0004Profile E4370.709710.0001Profile F4380.709710.0002		63	454	0.70978	0.00006
65*5790.709430.000366*6350.709350.000467*6200.709380.000368*5800.709360.000469*5300.709340.000470*5060.709360.000471*4830.709280.000472*5360.709310.000373*4150.709270.0004744150.709770.0005754600.709770.0004765450.709780.0004776550.709740.0003785730.709690.0004796450.709720.0003805700.709740.0004Profile E4370.709710.0001Profile F4380.709710.0002		64*	555	0.70933	0.00004
66* 635 0.70935 0.0004 67* 620 0.70938 0.0003 68* 580 0.70936 0.0004 69* 530 0.70936 0.0004 70* 506 0.70936 0.0004 71* 483 0.70928 0.0004 72* 536 0.70931 0.0003 73* 415 0.70927 0.0004 74 415 0.70977 0.0004 75 460 0.70977 0.0004 76 545 0.70978 0.0004 77 655 0.70974 0.0003 78 573 0.70972 0.0004 79 645 0.70972 0.0004 79 645 0.70972 0.0003 80 570 0.70974 0.0004 Profile E 437 0.70971 0.0004 Profile F 438 0.70971 0.0002		65*	579	0.70943	0.00003
67*6200.709380.0000368*5800.709360.0000369*5300.709340.0000470*5060.709360.0000471*4830.709280.0000472*5360.709310.0000373*4150.709270.00004744150.709770.00004754600.709770.00004765450.709780.00004776550.709740.00003785730.709720.00004796450.709740.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.0002		66*	635	0.70935	0.00004
68*5800.709360.0000369*5300.709340.0000470*5060.709360.0000471*4830.709280.0000472*5360.709310.0000373*4150.709270.00004744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.0002		67*	620	0.70938	0.00003
69*5300.709340.0000470*5060.709360.0000471*4830.709280.0000372*5360.709310.0000373*4150.709270.00004744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		68*	580	0.70936	0.00003
70*5060.709360.000471*4830.709280.000472*5360.709310.000373*4150.709270.0004744150.709970.0005754600.709770.0004765450.709780.0003776550.709740.0003785730.709690.0004796450.709720.0003805700.709740.0004Profile E4370.709710.0002		69*	530	0.70934	0.00004
71*4830.709280.0000472*5360.709310.000373*4150.709270.00004744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		70*	506	0.70936	0.00004
72*5360.709310.0000373*4150.709270.00004744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		71*	483	0.70928	0.00004
73*4150.709270.00004744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.0001Profile F4380.709710.0002		72*	536	0.70931	0.00003
744150.709970.00005754600.709770.00004765450.709780.00004776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		73*	415	0.70927	0.00004
754600.709770.0004765450.709780.0004776550.709740.0003785730.709690.0004796450.709720.0003805700.709740.0004Profile E4370.709710.0001Profile F4380.709710.0002		74	415	0.70997	0.00005
76 545 0.70978 0.00004 77 655 0.70974 0.00003 78 573 0.70969 0.00004 79 645 0.70972 0.00003 80 570 0.70974 0.00004 Profile E 437 0.70971 0.00001 Profile F 438 0.70971 0.00002		75	460	0.70977	0.00004
776550.709740.00003785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		76	545	0.70978	0.00004
785730.709690.00004796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		77	655	0.70974	0.00003
796450.709720.00003805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		78	573	0.70969	0.00004
805700.709740.00004Profile E4370.709710.00001Profile F4380.709710.00002		79	645	0.70972	0.00003
Profile E 437 0.70971 0.00001 Profile F 438 0.70971 0.00002		80	570	0.70974	0.00004
Profile F 438 0.70971 0.00002		Profile E	437	0.70971	0.00001
		Profile F	438	0.70971	0.00002

RH–IS26

* Dentine samples otherwise enamel.