

Life on a troubled border: Preliminary results from stable isotope investigations at a late medieval site in Dalmatia, Croatia

Authors: Brianna J. Muir, Emily Zavodny, Jure Šućur, & Željka Bedić

Supplementary materials: Study methodology

Study Materials

Long bones and teeth (M2's and M3's) from five individuals (Table 1) were sampled for light isotope analyses of collagen (carbon, nitrogen) and radiogenic isotope analysis of enamel bioapatite (strontium, lead). These skeletal elements were chosen in order to produce a sequence of isotopic values from across the life span of the sampled individuals. Bone constantly remodels during life, and thus holds isotopic signals from the last 5-10 years of life (Fahy, et al. 2017; Sims & Martin, 2020). In comparison, tooth tissues, particularly enamel, are metabolically inactive and will not remodel once formed – thus, teeth reflect the isotopic values of the individual at the time the tooth was forming (Lacruz et al., 2017; Sealy, Armstrong, & Schrire, 1995).

As such, the collagen analysis of a second molar (tooth roots = ~9 to 15.5 years), third molar (~15 to ~22 years), and bone sample (~5-10 years prior to death) allows for the assessment of isotopic change throughout an individual's life (Beaumont and Montgomery 2015; Eerkens et al. 2018). Similarly, by analysing bulk enamel bioapatite samples for strontium and lead, we can also assess mobility based on the values of the second (enamel formation = ~2.5 to 8.5 years) and third (~8.5 to 14.5 years) molars (AlQahtani et al. 2010). Bioarchaeological analysis (sex estimation, age at death, and paleopathological analysis) followed standard anthropological criteria (Buikstra and Ubelaker 1994; Ortner 2003).

Table 1: Demographic profile of the Maksanova gomila sample.

Individual	Estimated sex	Estimated age	Bone sample	Teeth sampled
MG-01	F	33	Radius	M ₂ , M ₃ (L)
MG-02	M	40	Radius	M ₂ , M ₃ (L)
MG-03	M	33	Ulna	M ² (L), M ₃ (R)
MG-04	M	28	Ulna	M ² , M ³ (L)
MG-05	F	45	Radius	M ₂ , M ₃ (R)

Stable Carbon and Nitrogen (collagen)

Bulk bone and tooth dentine samples were prepared using standard procedures for collagen extraction at the Zavodny Isotope Geochemistry and ZooArchaeology Group (ZIGZAG) lab at the University of Central Florida. Briefly, sampled dry bone or tooth root was crushed to increase the area of reactive surface before being washed in UltraPure water and demineralized in 0.5N HCl at 5°C for several days. Samples were then prepared for collagen extraction and purification using a modified Longin (1971) method before being frozen and lyophilized.

Collagen stable isotope concentrations and ratios were analyzed at the Light Stable Isotope Mass Spectrometry Laboratory at the University of Florida using a Thermo Electron Delta V Advantage isotope ratio mass spectrometer fitted with a ConFlo IV interface. Sample gas was measured relative to laboratory reference N₂ and CO₂ gasses. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are reported in standard ‰ notation relative to VPDB and atmospheric nitrogen, respectively. Bone collagen sample quality was evaluated with %C, %N, and C:N ratios. C:N ratios fall between 3.14 and 3.28, denoting good preservation for isotopic analyses (Table 2; DeNiro 1985; van Klinken 1999).

Table 2: %C, %N, and C:N ratios by individual.

Tissue	Individual	%C	%N	C:N
Bone	MG-01	41.82	15.15	3.19
	MG-02	42.66	15.53	3.17
	MG-03	42.3	14.87	3.28
	MG-04	42.94	15.79	3.14
	MG-05	37.56	13.5	3.21
M2	MG-01	40.11	14.55	3.18
	MG-02	38.53	13.56	3.28
	MG-03	36.44	13.15	3.20
	MG-04	41.63	15.01	3.20
	MG-05	37.67	13.4	3.24
M3	MG-01	40.6	14.76	3.17
	MG-02	13.82	4.93	3.23
	MG-03	38.43	13.85	3.20
	MG-04	39.57	14.3	3.19
	MG-05	40.03	14.61	3.16

Strontium and Lead Preparation (bioapatite)

Each tooth was sampled using a Dedeco carbide blade and cleaned of residue with a diamond-tipped Brasseler NSK Z500 drill. Samples were then processed in a class-500 clean lab in the Department of Geological Sciences at the University of Florida. Briefly, samples were dissolved in pre-cleaned Teflon vials using 2mL of 8N nitric acid (HNO₃, Optima). A small portion of each solution was removed to perform the elemental concentration analyses using an Element2 HR-ICP-MS (Kamenov et al. 2018). From the remaining solution Sr and Pb were separated from single aliquots using ion chromatography and Eichrom Sr and Pb-spec resins, respectively (Valentine et al. 2015). Samples were analyzed for ⁸⁷Sr/⁸⁶Sr and ²⁰Pb/²⁰⁴Pb using a NuPlasma multiple-collector inductively coupled plasma mass spectrometer (MC-ICP-MS), and compared to NBS standards 981 and 987.

Strontium and Lead Baselines

Baseline data is critical for contextualizing isotopic results and reconstructing past environments and climate. To date there have been no radiogenic strontium or lead isotope ratios reported for archaeological or modern material in Dalmatia, but co-authors plan to assay for local isotope values by collecting environmental samples (e.g., land snail shell, archaeological sediments, modern plants, non-weathered rocks) near the site.

Statistical methodology

All statistical analyses were performed using RStudio Version 2023.6.0.421 for Windows (R Core Team 2023; RStudio Team, 2023). The packages ‘tidyverse’ (Wickham et al. 2019) and ‘ggplot2’ (Wickham 2016) were also utilised for analysis and figure generation, respectively. Basic descriptive statistics (mean, standard deviation, minimum value, maximum value, interquartile range) were calculated. In the absence of an established isotopic baseline, a statistical “local range” was defined as the median, $\pm 1.5 * \text{IQR}$ (e.g., Lightfoot and O’Connell 2016; Stantis et al., 2021). Any samples that fall outside of this range are considered potentially non-local.

All comparative data used for the poster come from Lightfoot and colleagues (2012).

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