Dietary Variability in the Varna Chalcolithic Cemeteries

BISSERKA GAYDARSKA, JOE ROE AND VLADIMIR SLAVCHEV

SUPPLEMENTARY MATERIAL

S1: ISOTOPIC DIETARY STUDIES

Stable isotope data ($\delta^{13}C$, $\delta^{15}N$) for the burials were drawn from previously published sources: Higham et al. (2018: tab. 1) and Gaydarska et al. (2021: tab. 2). The carbon and nitrogen stable isotope analyses methods for both published sources were conducted by the Oxford Radiocarbon Accelerator Unit and are described by Brock et al. (2010: 110).

The determination of probable ancient diet is based on the isotopic profiles of $\delta^{13}C$ and $\delta^{15}N$ from skeletal samples of individuals interred in the Varna cemeteries. These isotopic profiles were input to the proportional diet mixing model FRUITS (Fernandes et al., 2014) to estimate individual diet proportions with reference to baseline isotopic data on likely regional food sources. The FRUITS model settings are the same as in our initial Varna study (Gaydarska et al., 2022). The modelling employs a simple whole fraction format, without macronutrient routing. As there are few animal and no plant remains that have been recovered from the Varna vicinity, proxies for foods that were likely to have been available at Varna were collated from previously published work on regional sources. "Cereal/Pulses" are from carbon and nitrogen values for archaeobotanical samples of wheat, barley, lentil, and bitter vetch from sites within 500 km of Varna (Azmak, Karanovo, Slatina, and Kapitan Dimitrievo; Bogaard et al., 2013: tab. 2). Mean values and errors are −24.0±0.2‰ $\delta^{13}C$ and 3.7±0.4‰ $\delta^{15}N$. "Terrestrial Animals" are herbivore and omnivore/carnivore animal bone from the Durankulak and Varna cemeteries (Honch et al., 2006: tab. 1). Here the mean isotopic values for animal bone were averaged, and then adjusted by −3.7‰ for $\delta^{13}C$ and −0.6 for $\delta^{15}N$ to reflect estimated flesh values (cf. Beavan-Athfield et al., 2008). The resulting adjusted values for regional terrestrial animal sources are −23.2±0.1‰ for $\delta^{13}C$, and 6.4±0.3‰ for $\delta^{15}N$. The baseline for "Black Sea Fish" consists of flesh values of modern free-range Black Sea sprats, mackerel, and anchovies (Bănaru & Harmelin-Vivien, 2009). These modern fish values are adjusted by +0.86‰ to reflect the temporal variations in $\delta^{13}C$ incorporated into marine organisms relative to modern waters (the 'Suess effect'; Suess, 1958; see also Böhm et al., 2002). The resulting Black Sea fish values are

−18.7±0.1‰ for $\delta^{13}C$, and 12.7±0.1‰ for $\delta^{15}N$. The isotopic offset between diet and consumers was 4.8± 0.5‰ for $\delta^{13}C$ (Fernandes et al., 2014, 2015), and 5.5±0.5‰ for $\delta^{15}N$ (O'Connell et al., 2001). The weight and concentration of each of the three diet sources were set at 100%.

S2: Clustering Methodology

We delineated clusters of burials with similar stable isotope ratios using the HDBSCAN algorithm (Campello et al., 2013) with $m_{pts}=3$. HDBSCAN (Hierarchical Density-Based Spatial Clustering of Applications with Noise) is a non-parametric clustering and outlier detection algorithm that seeks the 'most stable' clusters in a given dataset. In other words, it selects those natural clusters in the data that are least affected by the choice of a particular density or distance threshold. HDBSCAN is well suited to stable isotope data because it performs well with non-linear clusters, is robust to noise, and does not rely on a pre-specified number of desired clusters (Campello et al., 2015). We applied the modified algorithm suggested by Malzer and Baum (2020), where clustering below a certain threshold distance is ignored. In our case, we selected this threshold to collapse together clusters that were only visible at distances under the maximum measurement error of the isotope ratios (0.03). Clustering was conducted with the R package dbscan (Hahsler et al., 2019); the data and R code to reproduce this analysis is deposited with Zenodo at https://doi.org/10.5281/zenodo.11203467

S3: Isotopic Dietary Characteristics of Varna 1 Sub-groups

Two smaller clusters (A and B, Figure 1) consist of ten individuals, including one child (Grave 158, the 5–7-year old whose aDNA was initially claimed to have a steppe ancestry: Mathieson et al., 2018, a claim revised by a later study: Penske et al., 2023). These individuals plot above the core group due to having among the highest $\delta^{15}N$ values of the Varna 1 and Varna 3 populations, and $\delta^{13}C$ trending towards more enriched values. Combined, clusters A and B have a mean 11.4±0.3‰ $\delta^{15}N$ and mean $\delta^{13}C$ of −19.2±0.2‰. For clusters A and B, the average proportions of FRUITS estimated terrestrial animal meat (40.1%±22.6 %) and fish (7.5%±5.4%) combines for 47.6% animal and fish proportion, compared to the core group's 29.3%.

Group C has four burials which have mean $\delta^{15}N$ similar to that of the core population (10.5±0.3‰) but are slightly depleted in $\delta^{13}C$ (mean −19.3±0.2‰) as compared to the core group mean δ^{13}C of −18.9±0.2‰. FRUITS estimated proportions of terrestrial animal (28.3%±18.8%) and Black Sea fish (6.0%±4.6%) indicate a combined meat and fish diet proportion of 34.3%.

Cluster D consists of three burials from Varna 3 (Grave12, a 13-15-year old female, and Grave G1 and Grave G11VR3, both 20–30 years old, and male and female respectively). As a group they have the lowest δ^{15}N and are in the depleted range of ^{13}C. Group D mean δ^{13}C and δ^{15}N are −19.9±0.2‰ and 9.4±0.3‰ respectively. FRUITS estimates for cereal/pulses make up the greater proportion of the diet (82.6%±11.3%), with terrestrial animal (14.2%±11.5%), and fish (3.2%±2.7%) contributing only a combined 17.4%. Cluster D indicates people with more basic cereal and pulse diets, with far lower meat and fish sources than other groups.

Turning from the graves clustered by similar isotopic profiles, we examine the seventeen outlier points, which are individuals who exhibit singular variations in Varna diets. These points can be broadly divided into six individuals (Graves 51, 28, 111, 43, 32, and 25) with enriched δ^{13}C (minimum/maximum −18.7‰ to −17.8‰) and δ^{15}N (minimum/maximum 10.0‰ to 11.7‰). Among these is the richest burial in the necropolis, Grave 43 from Varna 1, the 40–60-year-old male with δ^{15}N of 11.1±0.3‰, who was buried with over a thousand items, including gold artefacts weighing nearly 1.6 kg (Rusev et al., 2010). Driving higher nitrogen and enriched δ^{13}C for these individuals are the mean FRUITS estimates of terrestrial animals (34.7%±22.2%) and Black Sea fish (10.0%±6.4%) for a combined 44.7% animal and fish component in these diets, suggesting access to higher-status foods.

The second trend, consisting of ten individuals (Graves 225, 179, 137, 129, 126, 87, 84 from Varna 1, Graves G13, G9, and G6 from Varna 3), is marked by depleted δ^{13}C (minimum/maximum −20.0‰ to −19.3‰) and a broader range of δ^{15}N (minimum/maximum 8.3‰ to 10.9‰). These individuals' range of isotopic profiles seem to show a varying story of access or dietary preference, with lower FRUITS estimated terrestrial animal (mean 17.5±13%) and minimal proportions Black Sea fish (mean 3.9±2.8%), implying diets which rely mainly on non-flesh diet sources. Delving further into these results, two children (Grave 84, a 7–8-year old, and Grave 179, a 6–8-year old child) have the lowest δ^{15}N values in the entire population of only 8.3‰ and 8.8‰ respectively and are matched only by a δ^{15}N of 8.4‰ for Grave 126, a 25–30-year old of indeterminate sex. For these, both depleted ^{13}C and lower ^{15}N are substantiated by FRUITS estimations that suggest less animal flesh in their diets (from 8.4±7.3% to 11.8±9.7%). and negligible Black Sea fish (2.4±2.1% to 3.1±2.7%). As for Graves 225, 137, and G9, they have higher δ ^{15}N of 10.1‰ to 10.9‰, and their FRUITS estimated diet proportions of terrestrial animals (from 21.9±15.8% to 33.4±20.7%) and, again, a negligible proportion of Black Sea fish (from 4.0±3.3% to 5.4±4.3%). We suggest that, for these outlier points with a range of δ ^{15}N, either access or dietary preference was for diets much lower in animal flesh, and with little or no fish.

Grave 44 was a 13-year old male with δ^{13}C −19.6‰ and the most enriched of all individuals in the Varna population, with δ^{15}N of 11.9‰. FRUITS estimations suggests that terrestrial animal contribute to 54.6%±24.3% of diet, and 7.2%±5.6% Black Sea fish. Grave 44's closest isotopic neighbours in Figure 1 is cluster B, which groups five of the burials with the highest δ^{15}N in the cemetery population (mean 11.4‰) and a mean δ^{13}C of −19.4‰. FRUITS estimations for terrestrial animals for cluster B is a mean of 44.9%, while Black Sea fish for the cluster is comparable to Grave 44 at 7.1%. The individual in Grave 44 may be of particular interest, with the most enriched δ^{15}N and the highest estimated proportion of terrestrial animal component in the overall population.

We also consider factors that support the assumption that marine fish (i.e. our baseline Black Sea fish) rather than freshwater fish are the aquatic food source in Varna diets. Freshwater fish diet sources have a terrestrial δ^{13}C range of −28.2‰ to −20.2‰, compared to marine and brackish water fish of −14·9 to −9·4‰ (see Fuller et al., 2012; Robson et al., 2016) due to contributions to freshwater environs from dissolved inorganic carbon and terrestrial runoff. With the trophic effect factors that we have used in the FRUITS modelling—4.8± 0.5‰ for δ ^{13}C (Fernandes et al., 2014, 2015), and 5.5±0.5‰ for δ^{15}N (O'Connell et al., 2001)—freshwater fish would contribute to δ^{13}C values of consumer isotopic profiles in the range of −23.4 to −15.4, as compared to similar trophic enrichment that would see marine/brackish water fish contribute in the −10.1‰ to −4.6‰ range, as well as drive higher δ^{15}N values.

Table S1. *Isotopic and FRUITS modelling results for human bone samples from sixty individuals from Varna 1 and Varna 3 cemeteries. Note 1: Human bone, initially sampled as animal bone. Subsequent zooarchaeology by mass spectrometry (ZooMs) confirmed that this is a human. Note 2: Initially sampled as animal bone and no material was left for ZooMs. Based on the isotopic values and the confirmed misidentification in Graves 28 and 286, we consider this as human* *bone. Note 3: Human bone, initially sampled as animal bone. Subsequent ZooMs confirmed that this is a human. 'Reference' cites the original published source of the stable isotope analyses.*

REFERENCES

Bănaru, D. & Harmelin-Vivien, M. 2009. Trophic Links and Riverine Effects on Food Webs of Pelagic Fish of the North-Western Black Sea. *Marine and Freshwater Research*, 60: 529–40. https://doi.org/10.1071/MF08005

Beavan-Athfield, N., Green, R.C., Graig, J., McFadien, B. & Bickler, S. 2008. Influence of Marine Sources on 14C Ages: Isotopic Data from Watom Island, Papua New Guinea Inhumations and Pig Teeth in Light of New Dietary Standards. *Journal of the Royal Society of New Zealand*, 38: 1–23. https://doi.org/10.1080/03014220809510543

Bogaard, A., Fraser, R., Heaton, T.H.E., Wallace, M., Vaiglova, P., Charles, M. et al. 2013: Crop Manuring and Intensive Land Management by Europe's First Farmers. *Proceedings of the National Academy of Sciences*, 110: 12589–94. https://doi.org/10.1073/pnas.1305918110"https://doi.org/10.1073/pnas.1305918110

Böhm, F., Haase-Schramm, A., Eisenhauer, A., Dullo, W.C., Joachimski, M.M., Lehnert, H. & Reitner, J. 2002: Evidence for Preindustrial Variations in the Marine Surface Water Carbonate System from Coralline Sponges. *Geochemistry Geophysics Geosystems*, 3: 1019. https://doi.org/10.1029/2001GC000264

Brock, F., Higham, T., Ditchfield, P. & Bronk Ramsey, C. 2010. Current Pretreatment Methods for AMS Radiocarbon Dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon*, 52: 10312. 10.1017/S0033822200045069"https://doi.org/10.1017/S0033822200045069

Campello, R.J.G.B., Moulavi, D. & Sander, J. 2013. Density-Based Clustering Based on Hierarchical Density Estimates. In: J. Pei, V.S. Tseng, L. Cao, H. Motoda & G. Xu, eds. *Advances in Knowledge Discovery and Data Mining* (Lecture Notes in Computer Science, 7819). Berlin & Heidelberg: Springer, pp. 160–72. https://doi.org/10.1007/978-3-642-37456-2_14

Campello, R.J.G.B., Moulavi, D., Zimek, A. & Sander, J. 2015. Hierarchical Density Estimates for Data Clustering, Visualization, and Outlier Detection. *ACM Transactions on Knowledge Discovery from Data*, 10: 1–51. https://doi.org/10.1145/2733381

Fernandes, R., Millard, A., Brabec, M., Nadeau, M.-J. & Grootes, P. 2014. Food Reconstruction Using Isotopic Transferred Signals (FRUITS): A Bayesian Model for Diet Reconstruction. *PloS One* 9: e87436. https://doi.org/10.1371/journal.pone.0087436

Fernandes, R., Grootes, P., Nadeau, M.-J. & Nehlich, O. 2015. Quantitative Diet Reconstruction of a Neolithic Population Using a Bayesian Mixing Model (FRUITS): The Case Study of Ostorf (Germany). *American Journal of Physical Anthropology*, 158: 325–40. https://doi.org/10.1002/ajpa.22788"https://doi.org/10.1002/ajpa.22788

Fuller, B.T., Müldner, G., Van Neer, W., Ervyncke, A., & Richards, M.P. 2012. Carbon and Nitrogen Stable Isotope Ratio Analysis of Freshwater, Brackish and Marine Fish from Belgian Archaeological Sites (1st and 2nd Millennium AD). *Journal of Analytical Atomic Spectrometry*, 27: 807–20. https://doi.org/10.1039/C2JA10366D

Gaydarska, B., Bayliss, A. & Slavchev, V. 2021. Contemporary Copper Age Burials from the Varna Mortuary Zone, Bulgaria. *The Antiquaries Journal*, 101: 1–15. https://doi.org/10.1017/S0003581521000032

Gaydarska, B., Beavan, N. & Slavchev, V. 2022. Lifeway Interpretations from Ancient Diet in the Varna Cemetery. *Oxford Journal of Archaeology*, 41: 22–41. https://doi.org/10.1111/ojoa.12236"https://doi.org/10.1111/ojoa.12236

Hahsler, M., Piekenbrock, M. & Doran, D. 2019. dbscan: Fast Density-Based Clustering with R. *Journal of Statistical Software*, 91: 1–30. https://doi.org/10.18637/jss.v091.i01

Higham, T., Slavchev, V., Gaydarska, B. & Chapman, .J 2018. AMS Dating of the Late Copper Age Varna Cemetery, Bulgaria. *Radiocarbon*, 60: 493–516. 10.1017/RDC.2018.9"https://doi.org/10.1017/RDC.2018.9

Honch, N., Higham, T., Chapman, J., Gaydarska, B. & Hedges R.E.M. 2006. A Palaeodietary Investigation of Carbon ($^{13}C/^{12}C$) and Nitrogen ($^{15}N/^{14}N$) in Human and Faunal Bones from the Copper Age Cemeteries of Varna I and Durankulak, Bulgaria. *Journal of Archaeological Science*, 33: 1493–504. https://doi.org/10.1016/j.jas.2006.02.002

Malzer, C. & Baum, M. 2020. A Hybrid Approach to Hierarchical Density-Based Cluster Selection. *2020 IEEE International Conference on Multisensor Fusion and Integration for Intelligent Systems (MFI):* 223–28. https://doi.org/10.1109/MFI49285.2020.9235263

Mathieson, I., Alpaslan Roodenberg, S., Posth, C., Szécsényi-Nagy, A., Rohland, N., Mallick, S et al. 2018. The Genomic History of Southeastern Europe. *Nature*, 555: 197–203. https://doi.org/10.1038/nature25778"https://doi.org/10.1038/nature25778

O'Connell, T.C., Hedges, R.E.M., Healey, M.A.. & Simpson, A.H.R.W. 2001: Isotopic Comparison of Hair, Bone AND Nail: Modern Analyses. *Journal of Archaeological Science*, 28: 1247–55. https://doi.org/10.1006/jasc.2001.0698

Penske, S., Rohrlach, A., Childebayeva, A., Gnecchi-Ruscone, G., Schmid, C., Spyrou, M.A. et al. 2023. Early Contact between Late Farming and Pastoralist Societies in Southeastern Europe. *Nature*, 620: 358–65. https://doi.org/10.1038/s41586-023-06334-8

Robson, H., Andersen, S., Clarke, L., Craig, O., Gron, K., Jones, A., et al. 2016. Carbon and Nitrogen Stable Isotope Values in Freshwater, Brackish and Marine Fish Bone Collagen from Mesolithic and Neolithic Sites in Central and Northern Europe. *Environmental Archaeology*. 21: 105–18. 10.1179/1749631415Y.0000000014"https://doi.org/10.1179/1749631415Y.0000000014

Rusev, R., Slavchev, V., Marinov, G. & Boyadzhiev, Y. 2010. *Varna – praistoricheski tsentar na metaloobraotkata*. Varna: Dangrafik.

Suess, H. 1958. The Radioactivity of the Atmosphere and Hydrosphere. *Annual Review of Nuclear Science*, 8: 243–56. https://doi.org/10.1146/annurev.ns.18.120168.002203