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What about the delta ($\delta^{13}C$)? A case for brackish fish in Mediterranean palaeodietary isotopic baselines

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A B S T R A C T

While a lot of paleodietary studies in the Mediterranean region have reported $\delta^{13}C$ and $\delta^{15}N$ values for humans, similar data for aquatic resources is much more limited. The aim of our paper is to make relevant data available for isotopic faunal baselines and future paleodietary research in the eastern Mediterranean. We analysed the collagen extracted from the bones of freshwater and brackish fish from two coastal archaeological sites in the Levant, Kinet Höyük (n = 21) (1550 BCE – 14th c. AD, present day Turkey) and Tell el-Burak (n = 8) (975–332 BCE, present day Lebanon). From the original 29 bones sampled, 17 (59 %) produced collagen which met quality control criteria and we present the corresponding $\delta^{13}C$ and $\delta^{15}N$ values here. Whereas freshwater and marine ecosystems in an area typically have distinctive $\delta^{13}C$ values, paleodietary interpretations regarding aquatic resource consumption can be challenging when isotopic values reflecting the range of exploitable aquatic environments are not available. Bearing this in mind, our data set is used to demonstrate the potential range of $\delta^{13}C$ values (−27.97 ‰ to −10.87 ‰) of freshwater and brackish environments along the Levantine coast, encouraging future palaeodietary studies in the region to consider this more robust isoscape for aquatic resources.

1. Introduction

Stable isotope analysis has been used extensively to explore the role of marine and terrestrial resources in Mediterranean palaeodiets (e.g., Alexander et al., 2015; Budd et al., 2018; Craig et al., 2009; Vika and Theodoropoulou, 2012). For decades, the capacity for having a robust aquatic isotopic baseline for such studies has been limited by recovery methods, few specialists, and access to adequate reference collections for ichthyofaunal material (Van Neer et al., 2005; Zohar and Belmaker, 2005). Along the Levantine coast in particular, while there is ample zooarchaeological evidence for fish consumption on archaeological sites (e.g., Çakırlar et al., 2016; Lernau et al., 2021; Van Neer et al., 2004; Zohar and Artzy, 2019), the published stable isotope values for fish from the region is comparatively scarce.

The most robust publications of stable isotope values from fish remains from the Levant are composed predominantly of marine fishes (Fuller et al., 2020; Winter et al., 2025). Of these two papers, only Fuller et al. (2020) has stable isotope data from freshwater (n = 8) and brackish (n = 6) fish. In the Aegean, (Vika and Theodoropoulou, 2012) include fish that can be found in marine, brackish, and/or fresh waters, providing insight into a broader range of aquatic isoscapes encountered in a Mediterranean context. As has been demonstrated by other studies (e.g., Dierickx et al., 2024; Fuller et al., 2012; Robson et al., 2016), having fish which occupy a range of ecological niches and local environments helps to better inform the construction of a local isotopic baseline.

Many coastal sites in the Mediterranean are geographically positioned so that past inhabitants would have been able to exploit aquatic resources from the sea, estuaries, lagoons, river deltas, and/or freshwater ecosystems. With the addition of new stable isotope data from

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Table 1
Fish taxa represented in this study, their aquatic ecosystems, and generalised foraging ecology. References for foraging ecology: 1. Kadye and Booth (2012) 2. Spataru et al. (1987) 3. Mkumbo and Ligtvoet (1992) 4. Nkalubo et al. (2014) 5. Denis et al. (2022) 6. Deelder (1984) 7. Alp (2017) 8. Al-Shamma'a (2006) 9. Lammens and Hoogenboezem (1991) 10. Cardona (2006).

Scientific name	Common name	Aquatic habitat	Diet (sexually mature fish)
Clarias gariepinus	North African catfish	freshwater	Omnivorous, increasing piscivory with size[1,2]
Lates niloticus	Nile perch	freshwater	Piscivorous[3,4]
Anguilla anguilla	European eel	Freshwater, brackish, and marine	Generalised carnivores[5,6]
Siluridae	Sheatfishes	Freshwater, brackish	Carnivorous[7], some species omnivorous[8]
Cyprinidae	Minnows and carps	Freshwater mostly, some species can also be in brackish waters	Many species generalists, some being specialised herbivores or predators[9]
Mugilidae	Mullets	Freshwater, brackish, and marine	Mostly herbivores and detritivores[10]

brackish and freshwater fishes from Levantine archaeological contexts, we aim to add to the corpus of data already available and further expand the existing knowledge of $\delta^{13}C$ and $\delta^{15}N$ values for future faunal isotopic baselines. An overview of the aquatic environments utilised by the taxa presented here and their general foraging patterns are provided in Table 1. The novelty of this work lies in providing the most robust sample of isotopic values from archaeologically-derived freshwater and brackish fish in the eastern Mediterranean to date.

2. Materials and methods

Twenty eight fish bones from two eastern Mediterranean archaeological sites were analysed for stable carbon and nitrogen isotopic ratios (see Fig. 1 for examples). The fish originate from Kinet Höyük (n = 20) and Tell el-Burak (n = 8) and are taxonomically composed of *Clarias gariepinus* (n = 17), *Lates niloticus* (n = 3), Mugilidae (n = 3), and Siluridae (n = 3). Most of the material is from contexts spanning the Late Bronze Age to the Late Iron Age, corresponding to ca. 1550 BCE – 332 BCE, with two *C. gariepinus* from Kinet Höyük dating to the period of medieval occupation (ca. 8th/9th – 14th c. AD). Kinet Höyük is a coastal site located adjacent to Iskenderun Bay in present day Turkey. Kinet Höyük had an estuary to the south of the site fed by the river Deli Çay, however this was filled with alluvial sediment in the Hellenistic period (332——50 BC), eliminating the preexisting estuary (Beach and Luzzadder-Beach, 2008). Tell el-Burak, in present day Lebanon, is situated on an alluvial floodplain with mountains to the east and the Mediterranean Sea to the west. The local geomorphology of both sites would have readily provided access to aquatic resources from marine, brackish, and freshwater environments.

Precise details of which skeletal element was sampled and the context of each is provided in Table S1. Recovery methods for both sites include hand collection and sieving (Winter et al., 2022). Prior published work on the fish remains from both sites is largely focused on groupers (family: Epinephelidae) and includes taphonomic analysis (Çakırlar et al., 2016), palaeoproteomics (Winter et al., 2023), and catch size reconstruction (Winter et al., 2022). Fig. 1 *(right)* shows the location of the sites included in this study and discussed later in the text. Samples were morphologically identified using the reference collection at the Groningen Institute of Archaeology. All samples were documented and photographed prior to destructive analysis. Bones were sampled in a way to encapsulate all growth layers when possible.

Twenty four of these bones were analysed at the BioArCh facilities at the University of York, United Kingdom with the remaining four analysed at the Centre for Isotope Research (CIO) at the University of Groningen, Netherlands. Sample preparation and collagen extraction at the CIO followed the protocol published in Dee et al. (2020). The samples processed at BioArCh followed a modified Longin (1971) method for collagen extraction, modified to include Ezee filtering (Bronk Ramsey et al., 2004) and the use of a weaker acid for demineralisation (0.1–0.4 M HCl) to accommodate poor sample preservation. A key difference between the two methods is that the Longin (1971) method does not include a treatment step with a base, which the Dee et al. (2020) method does.

Duplicate aliquots of 0.4——0.6 mg of extracted collagen were weighed out and analysed. Isotopic compositions (carbon and nitrogen) were determined using a Sercon 20–22 continuous flow isotope ratio mass spectrometer coupled to a Sercon GSL elemental analyzer at the University of York. At the CIO, aliquots of 0.16–5.77 mg of collagen, contingent on sample yields, were weighed out for analysis via an Elemental Analyser (Elementar Vario Isotope Cube™) connected to an Isotope Ratio Mass Spectrometer (IRMS, IsoPrime 100™). None of the samples analysed at the CIO both produced adequate collagen and met quality control criteria and thus these samples are not further discussed.

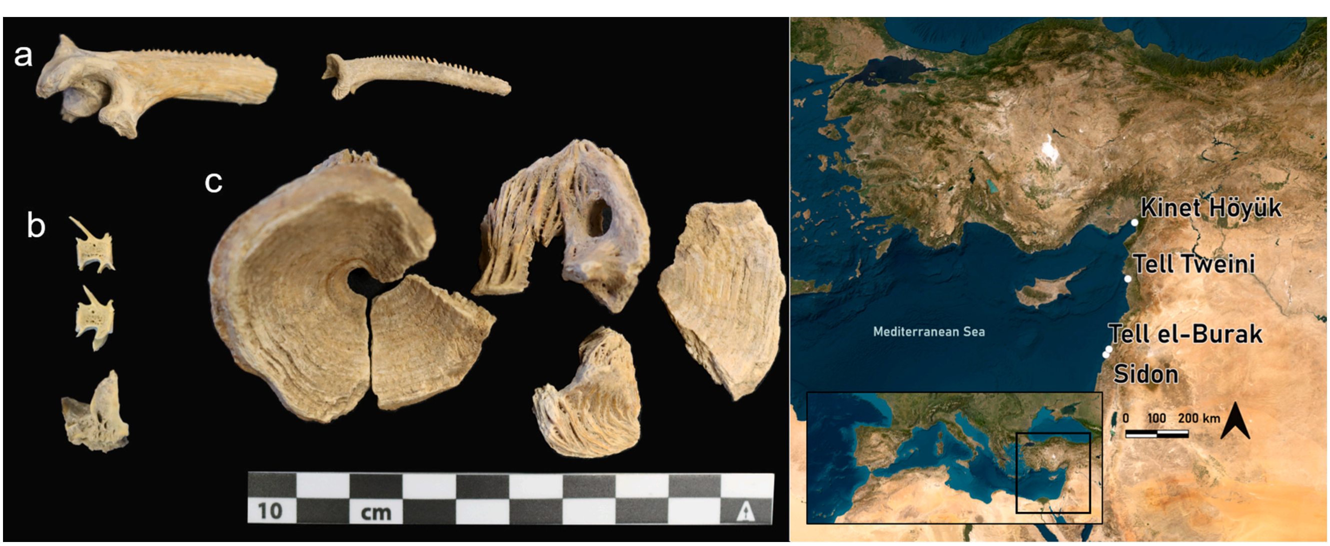

Fig. 1. Selection of the fish bones sampled a) pectoral spines (x2) of *Clarias gariepinus*, b) vertebrae (x3) of Mugilidae, and c) damaged vertebra of a *Lates niloticus (left panel)*. Map showing where the archaeological sites discussed, Kinet Höyük, Tell el-Burak, Sidon, and Tell Tweini, are in relation to each other and within the broader context of the Mediterranean basin *(right panel)*.

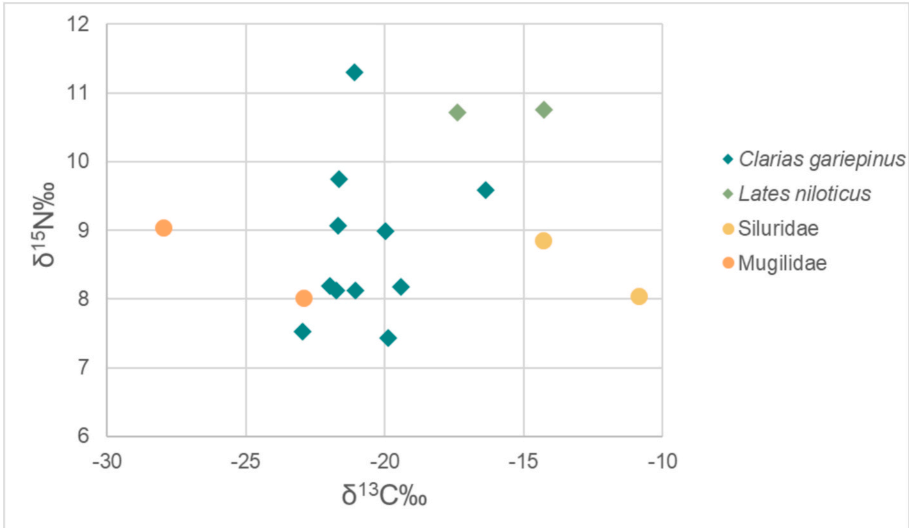

Fig. 2. $\delta^{13}C$ and $\delta^{15}N$ values of freshwater and brackish fish from Kinet Höyük and Tell el-Burak.

Table 2
Bulk $\delta^{13}C$ and $\delta^{15}N$ values from fish bone collagen of ichthyofauna recovered from the archaeological sites of Kinet Höyük and Tell el-Burak.

Site	Taxa	$\delta^{13}C$‰	$\delta^{15}N$‰	C:N ratio
Kinet Höyük	Mugilidae	−27.97	9.04	3.2
Kinet Höyük	Mugilidae	−22.91	8.02	3.13
Kinet Höyük	*Clarias gariepinus*	−19.87	7.43	3.23
Kinet Höyük	*Clarias gariepinus*	−21.68	9.07	3.23
Kinet Höyük	*Clarias gariepinus*	−21.64	9.74	3.15
Kinet Höyük	*Clarias gariepinus*	−21.09	11.3	3.28
Kinet Höyük	*Clarias gariepinus*	−21.75	8.12	3.29
Kinet Höyük	*Clarias gariepinus*	−19.43	8.18	3.17
Kinet Höyük	*Clarias gariepinus*	−21.96	8.19	3.35
Kinet Höyük	*Clarias gariepinus*	−21.05	8.13	3.19
Kinet Höyük	*Clarias gariepinus*	−19.96	8.99	3.23
Kinet Höyük	*Clarias gariepinus*	−22.95	7.53	3.16
Tell el-Burak	*Clarias gariepinus*	−16.36	9.58	3.41
Tell el-Burak	*Lates niloticus*	−14.26	10.75	3.3
Tell el-Burak	*Lates niloticus*	−17.39	10.72	3.25
Tell el-Burak	Siluridae	−14.31	8.85	3.3
Tell el-Burak	Siluridae	−10.87	8.05	3.19

For the BioArCh samples, accuracy was determined by measurements of international standard reference materials within each analytical run. These were IAEA 600 $\delta^{13}C_{raw}$ = -27.69 ± 0.08 ‰, $\delta^{13}C_{true}$ = -27.77 ± 0.043 ‰, $\delta^{15}N_{raw}$ = 0.78 ± 0.19 ‰, $\delta^{15}N_{true}$ = 1 ± 0.2 ‰; IAEA N2 $\delta^{15}N_{raw}$ = 20.58 ± 0.32 ‰, $\delta^{15}N_{true}$ = 20.3 ± 0.2 ‰; IA Cane, $\delta^{13}C_{raw}$ = -11.68 ± 0.09 ‰; $\delta^{13}C_{true}$ = -11.64 ± 0.03 ‰. We calibrated our data to these reference materials, as per recommended best practices (Szpak et al., 2017).

The overall uncertainties on the measurements of each sample were calculated based on the method of Kragten (1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials. These are expressed as one standard deviation. The maximum uncertainty for all successful samples across all runs was 0.21 ‰ for $\delta^{13}C$ and 0.30 ‰ for $\delta^{15}N$.

Additionally, at the BioArCh laboratory, a homogenised bovine bone extracted and analysed within the same batch as the samples produced the following average values; $\delta^{13}C$ = –23.01 ± 0.05; $\delta^{15}N$ = 5.75 ± 0.20. This was within the overall mean value from 50 separate extracts of this bone sample, which produced values of $\delta^{13}C$ = –23.09 ± 0.26 and $\delta^{15}N$ = 6.27 ± 0.38.

3. Results

Seventeen of the twenty nine (59 %) bones sampled passed quality control, with C:N ratios between 2.9–3.6 and being at least 13 % C and 4.8 % N by weight (Ambrose 1990; DeNiro 1985; Guiry and Szpak 2021; van Klinken 1999). Isotopic values range from −27.97 ‰ to −10.87 ‰ for $\delta^{13}C$ and 7.43 ‰ and 11.3 ‰ for $\delta^{15}N$ (Fig. 2). Full details can be found in Table 2 (see Supplementary Materials Table S1 for additional details, e.g., skeletal element, quality control data). The average collagen yield for the samples which passed quality control was 4.6 %. Whilst our sample sizes per taxa is small, the fish in our study reflect their expected ecological niches in freshwater and brackish environments.

4. Discussion

While there is a recognition for the need of a well-constructed faunal isotopic baseline for paleodietary studies, an aquatic component to that baseline which encapsulates the full range of environments locally available is often challenging to obtain. The $\delta^{15}N$ values obtained here suggest that these fishes were not feeding at a wide range of trophic levels. This can be attributed to the small range of taxa, which are mostly of comparable sizes (ca. 20–40 cm Standard Length), being present in this sample. The largest taxonomic group present in this data set is the North African catfish, *C. gariepinus*, (n = 11). Most of the *C. gariepinus* in this sample (n = 10) come from Kinet Höyük with the final individual being from Tell el-Burak, notably this particular fish has the lowest $\delta^{13}C$ value (−16.4 ‰). The range of $\delta^{15}N$ values within this group, which encompasses sizes ca. 20–50 cm Standard Length, is most likely attributed to feeding at higher trophic levels with ontogeny, which has been observed in modern studies of *C. gariepinus* (Kadye and Booth, 2012). With the exception of the Nile perch (*Lates niloticus*), which would have most likely have been imported from Egypt (Van Neer et al. 2004), the taxa present were locally available at the archaeological sites discussed here. However, it is also possible that the Siluridae may have also been imported from either Egypt or the Euphrates or Tigris rivers both to the east.

The largest fish datasets published from the Levant, Fuller et al. (2020) and Winter et al. (2025), are composed predominantly of marine fish. Among the freshwater and brackish fish from (Fuller et al. 2020) are *Anguilla anguilla* (n = 1), *Lates niloticus* (n = 5), Cyprinidae (n = 2), and Mugilidae (n = 6). The Nile perch and Mugilidae are the only taxa present in both of our data sets that have, for the most part, comparable

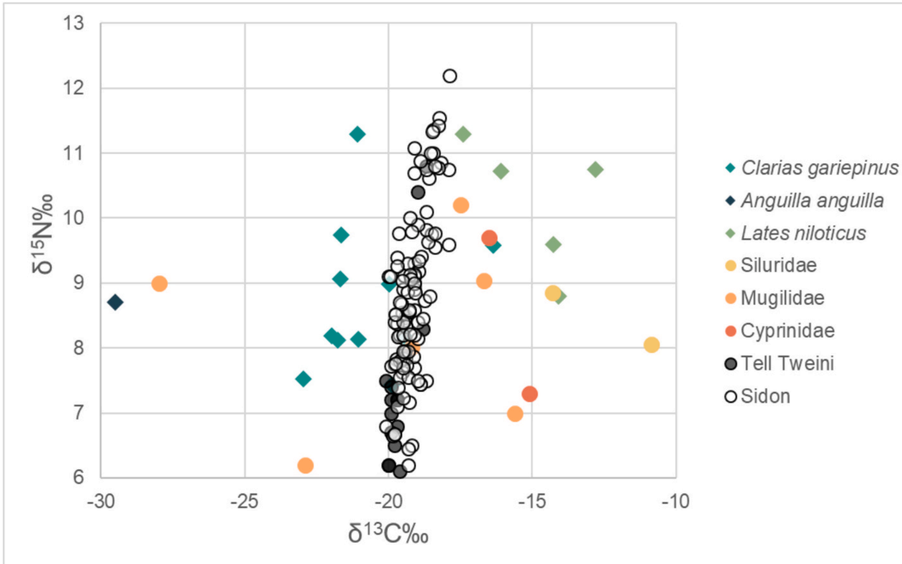

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ values for freshwater and brackish fish from the Levant including data from our study (n = 17) and previously published by Fuller et al. (2020) (n = 14) with the addition of human isotope values from Sidon (n = 108; Stantis et al., 2021) and Tell Tweini (n = 16; Fuller et al., 2024).

isotopic values. However, with the addition of our data, we have shown a wider range of $\delta^{13}C$ values for Nile perch and wider range of $\delta^{13}C$ and $\delta^{15}N$ values for mullets. Two of the Mugilidae have notably depleted $\delta^{13}C$ values of −27.98 ‰ and −22.91 ‰, consistent with these two fish having passed their full lives in freshwater indicating this sample belongs to a species which can tolerate freshwater such as *Chelon labrosus*, *Liza ramada*, or *Mugil cephalus*. A notable taxonomic addition that our data offers is providing isotopic values for Siluridae and *Clarias gariepinus*, which in turn will aid in greater robusticity of future faunal baselines in the Levant. The taxonomic composition of these data sets combined is fairly representative of common fresh and brackish water found in eastern Mediterranean archaeological contexts, however some taxa, such as cichlids (perch-like fish, including tilapia), are absent from our data sets despite being regular finds in eastern Mediterranean archaeological contexts (Van Neer et al., 2005). Furthermore, this data set represents two sites and spans multiple millennia, future aquatic faunal baselines would benefit from more biodiversity, larger sample sizes of key taxa, time periods, and geographic regions in the Levant.

Our data are combined with the previously published freshwater and brackish fish isotope data from Levantine archaeological sites (Fuller et al. 2020) and two paleodietary studies in the Levant (Fuller et al. 2024; Stantis et al. 2021) in Fig. 3. Isotopic analysis of inhabitants of Middle Bronze Age (ca. 2000–1600 BCE) Sidon suggests minimal marine resource consumption, though overlap with brackish/freshwater isotopic signatures still leaves an aquatic component possible (Stantis et al., 2021). Similarly, Fuller et al. (2024) found no isotopic evidence of marine diets at Bronze and Iron Age Tell Tweini. Our additional data points, whilst a small sample size, they add further support for the range of $\delta^{13}C$ values encountered in the Levant and provide further data for key taxa (e.g., *C. gariepinus*) in the ancient Levantine region. As demonstrated with ancient freshwater fish from the inland archaeological site of Sagalassos in southwest Anatolia (Van Neer et al., 2024), a local ecosystem may unexpectedly exhibit a wide range of $\delta^{13}C$ values, including those typically associated with marine signals (Schoeninger & DeNiro, 1984). This highlights the need for caution when considering aquatic ecosystems and thus, trophic resources that might have been locally exploited or accessed through trade routes. The use of compound-specific stable isotope analysis, rather than bulk collagen analysis, has proven essential for achieving a more refined understanding of palaeodiet in the western Mediterranean (Fontanals-Coll et al., 2023) and could lend further insight to the eastern Mediterranean.

5. Conclusion

This study adds to the meagre freshwater fish isotopic data from Levantine archaeological contexts that currently exists. We have increased the number of data points available to illustrate the range of carbon and nitrogen values encountered in Levantine estuarine and freshwater environments in the Late Holocene. With our data for freshwater and brackish ecosystems in the Levant, we hope to encourage future research to move beyond seeing aquatic resource consumption as a dichotomy of either marine or freshwater resources and also consider the in-between environments of lagoons, estuaries, and deltas.

Data Statement

All isotope and quality control data for bones analysed are provided in the Supplementary Materials and have additionally been submitted to the IsoArCh database.

CRediT authorship contribution statement

Rachel M. Winter: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft preparation, Writing – review & editing, Visualization, Project administration. **Matthew von Tersch:** Validation, Investigation, Data curation, Writing – review & editing. **Michael W. Dee:** Writing – review & editing, Validation, Resources, Investigation, Data curation. **Canan Çakırlar:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Michelle Alexander:** Writing – review & editing, Supervision, Resources, Investigation.

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Data availability

Data is available through the IsoArcH initiative and is provided in the Supplemenatry Materials.

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