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Pre-Lessepsian isotopic niche spaces: using paleoecological proxies to assess the impact of ongoing bioinvasions on fishes in the eastern Mediterranean Sea

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Abstract

Ongoing bioinvasions of Lessepsian species via the Suez Canal have profoundly altered marine coastal ecosystems in the eastern Mediterranean. In response to these Lessepsian migrations, some indigenous fishes have been observed to have widened their trophic niches and diversified their foraging strategies. Effects of invasive taxa are further compounded by modern anthropogenic drivers such as overfishing, habitat degradation, and pollution. The scale and characteristics of these recent changes in trophodynamics for broader ichthyofaunal communities are poorly understood due to a lack of data predating Lessepsian migrations; paleoecological data is, therefore, essential. Here, we present a substantial body of new carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic data from Middle to Late Holocene archaeological fish collagen (n=137), combined with previously published results (n=44) to establish paleoecological baselines for the region. An emphasis is placed on groupers (Epinephelidae) due to their high ubiquity in Mediterranean archaeological contexts and importance to rocky reef ecosystems today. We demonstrate that modern, indigenous Mediterranean fish have expanded their trophic niches *beyond* their pre-Lessepsian migrations baseline foraging ecology. Paleo data further show that fishes in middle and lower trophic levels have the highest degree of overlap in isotopic niche spaces, suggesting greater levels of competition for trophic resources. Our study highlights the importance of integrating data from historical ecological baselines into our assessments of bioinvasions and, more broadly, anthropogenically driven ecosystem alterations.

Keywords: Marine historical ecology; Lessepsian migrations; bioinvasions; stable isotopes; isotopic niche space; marine fish; trophic ecology.

Introduction

As a result of the opening of the Suez Canal in 1869, marine ecosystems in the eastern Mediterranean host the highest proportion of non-indigenous (often becoming invasive) species in the world (Edelist *et al.*, 2013; Corrales *et al.*, 2017). The effects of the presence of these nonindigenous species from the Red Sea and Indo-Pacific, commonly referred to as Lessepsian species (Por, 1971), has been exacerbated by overfishing and climate change (Boudouresque *et al.*, 2017; Rilov *et al.*, 2018). With rising sea temperatures, these thermophilic newcomers are increasingly suited to Mediterranean waters and are both replacing native taxa at an alarming rate and filling previously unoccupied ecological niches (Givan *et al.*, 2018). As a result, fish catches in the Levant region, which borders the eastern Mediterranean, are now largely dominated by Lessepsian species (Goren & Galil, 2005; Edelist *et al.*, 2013; Galil & Goren, 2014; Özyurt *et al.*, 2018; Rilov, 2016).

Archaeological contexts and historical collections provide useful tools to reconstruct past ecological conditions and set temporally defined ecological baselines for modern conservation and management strategies (Misarti *et al.*, 2009; Guiry *et al.*, 2016; Guiry *et al.*, 2020). A useful tool to study patterns and overlap is stable isotope analysis (SIA), which can be used to quantitatively define the isotopic niche of a predefined group (e.g., a local population, a species in a given geographic area). Isotopic niche space (INS) (Newsome *et al.*, 2007) and isotopic plasticity index (IPI) (Haubrock *et al.*, 2021) are used to understand trophic structures and relationships in ecological, paleoecological, and archaeological contexts (Yeakel *et al.*, 2016; Loponte & Corriale, 2020; Robinson, 2021). Calculating the INS for a group provides a means to quantify the spread of isotopic values observed and thus have a proxy for the size of a trophic or ecological niche. Whereas IPI, which is derived from INS calculations, can be used as an indicator of trophic plasticity and intra-group competition.

Modern isotopic evidence from the eastern Mediterranean shows that Lessepsian fishes have a smaller isotopic niche space than indigenous fishes. This finding has been used to argue that indigenous Mediterranean species have had to widen their trophic niches due to competition for trophic resources from Lessepsian migrants (Fanelli *et al.*, 2015). However, comparative isotopic data predating the opening of the Suez Canal is scarce (Vika & Theodoropoulou, 2012; Agiadi & Albano, 2020; Fuller *et al.*, 2020), yet it is vital to understanding the competition for trophic resources and niches presented by bioinvasions and whether or not a change in isotopic niche spaces has occurred.

We present bulk carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic data from the collagen of 181 ancient fish bones, representing at least thirteen families, sampled from three Middle to Late Holocene Levantine archaeological sites (geographic range Fig. 1). We aim to establish historical ecological baselines for foraging diversity among and resource overlap between marine ichthyofaunal trophic groups prior to Lessepsian migrations using novel statistical tools (Eckrich *et al.*, 2020). A particular focus is placed on groupers, an apex predator abundant in Mediterranean archaeological contexts, including our assemblages, to investigate ontogenetic changes in their foraging ecology in the context of a more 'pristine' ecosystem. More specifically, we ask the following questions:

- What are the ranges of δ^{13}C and δ^{15}N values observed in ancient marine fish in the Levant?
- Are ontogenetic changes in isotopic niche spaces observed in ancient groupers (Epinephelidae)?
- How do isotopic niche spaces, as a proxy for trophic resources, compare between different trophic groups in ancient Levantine marine ecosystems?
- How do isotopic niche spaces compare between ancient, indigenous Mediterranean marine fishes, modern indigenous Mediterranean marine fishes, and modern Lessepsian fishes?

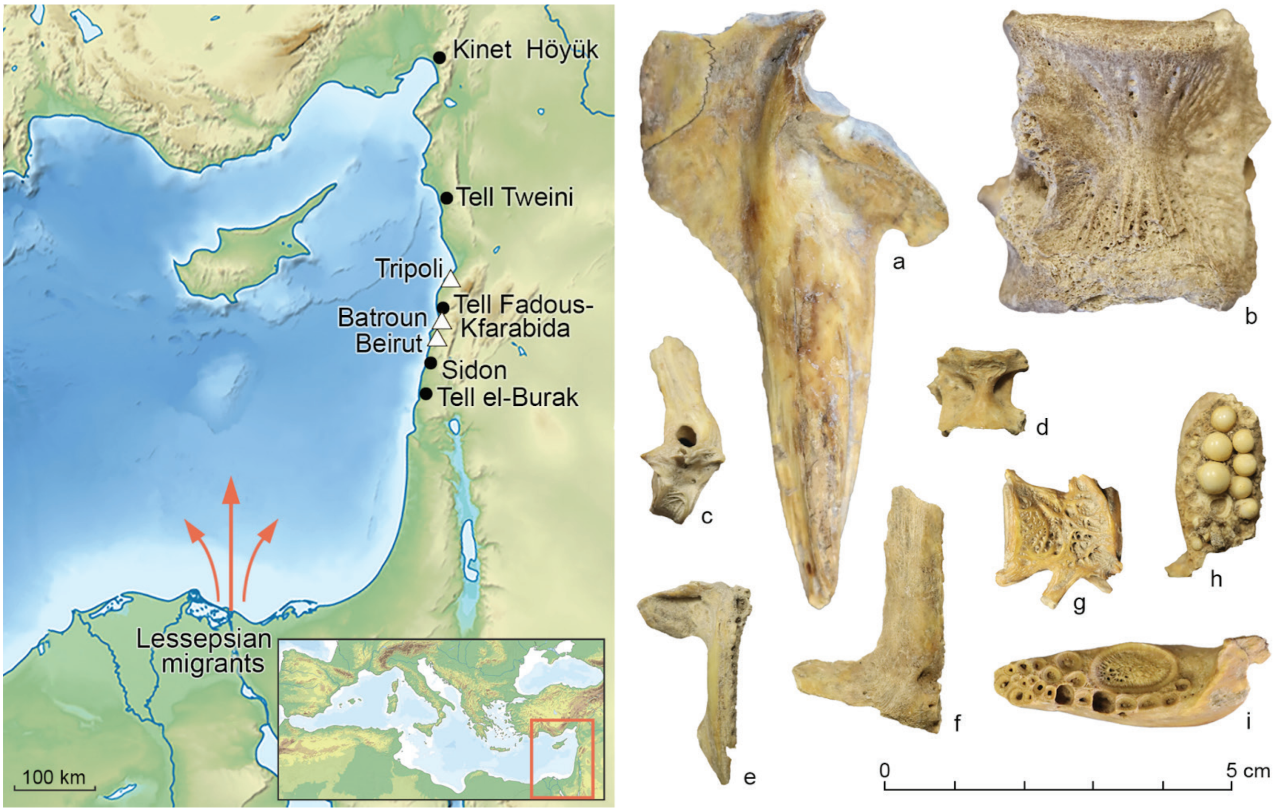


Fig. 1: Map of the eastern Mediterranean showing the archaeological sites that the ancient fish bones are from and the landing sites where commercially caught fish were purchased (Fanelli *et al.*, 2015). Archaeological sites (indicated by black circles) include Kinet Höyük in Turkey, Tell Tweini in Syria, and the remaining three sites, which are all in Lebanon: Tell Fadous, Sidon, and Tell el-Burak. Landing sites for the modern data (indicated by white triangles), Tripoli, Batroun, and Beirut, are all on the Lebanese coast. Ancient fish bones shown include: a. lateral view of a left articular of a grouper (Epinephelidae), b. Lateral view of a caudal vertebra of a jack (Carangidae), c. lateral view of a precaudal vertebra of a grey triggerfish (*Balistes capriscus*), d. lateral view of a caudal vertebra from a Scombridae, e. buccal view of a premaxilla of a grouper (Epinephelidae) or comber (Serranidae), f. buccal view of a premaxilla of a dentex (*Dentex* sp.), g. lateral view of a caudal vertebrae of a mullet (Mugilidae), h. occlusal surface of a premaxilla of a seabream or porgy (Sparidae), and i. occlusal surface of a dentary of a gilthead seabream (*Sparus aurata*). Figure created by Siebe Boersma from the Groningen Institute of Archaeology.

Materials and Methods

Sample selection

Two hundred and thirty-five fish bones were sampled from three archaeological sites in the eastern Mediterranean (ca. 3000 BC - 1300 AD) dating from the Middle to Late Holocene with 114 (49% of the full sample) of these samples being Epinephelidae or Serranidae. Robust bones which appeared visually well-preserved, spanning various occupational phases and marine ichthyofaunal taxa, were selected for isotopic analysis. Samples come from both hand collection and sieved material. The majority of the bones come from Kinet Höyük (n=166), with smaller assemblages from Tell Fadous-Kfarabida (hereafter, Tell Fadous) (n=44) and Tell el-Burak (n=25). Kinet Höyük, an ancient harbour site, sits in the most northeastern corner of the Mediterranean Sea, lying further north along the Levantine coast in Turkey. Fish bones sampled from Kinet date from the Early Bronze Age through Hellenistic (ca. 2800 - 50 BC) occupational phases as well as the Medieval period (ca. late 12th - early 14th centuries AD) (Gates *et al.*, 2015). Tell Fadous and Tell el-Burak are both small coastal sites about 1.5 hectares in size located in Lebanon. Fish bones sampled from Tell Fadous date to the Early Bronze Age II - IV (ca. 2800 - 2000 BC) (Genz, 2010) and from Tell el-Burak, they date to the Middle Bronze Age (ca. 1900 - 1700 BC) and Iron Age (ca. 725 - 350 BC) (Kamlah & Sader, 2019).

These assemblages are dominated by groupers (Epinephelidae) (n=92) due to their high ubiquity in Mediterranean archaeological contexts (Desse & Desse-Berset, 1999) and ecological importance today as large predatory fishes that are essential for maintaining healthy, well-balanced marine ecosystems (Prato *et al.*, 2013). Due to the foraging strategies of groupers, they have been proposed as a natural form of biocontrol for invasive species (Mumby *et al.*, 2011; Giakoumi *et al.*, 2019; Turan *et al.*, 2017). Recent work on groupers at one of our study sites indicates that Middle and Late Holocene marine ecosystems had well-balanced trophic structures, as evidenced by the recovery of fish bones from large, high-level predators (Winter *et al.*, 2022). Similarly, analysis of the taxonomic composition of surficial death assemblages dating to the Holocene in the Levant also provides evidence for past marine ecosystems having an abundance of carnivorous fishes (Agiadi & Albano, 2020). Thus, material from this time period is well suited to studying trophic niches and interactions in a context which has neither been impacted by Lessepsian migrations nor been overfished to the degree observed in the past centuries (Pauly *et al.*, 1998).

Samples were morphologically identified to the lowest (most precise) taxonomic level possible using the Groningen Institute of Archaeology (GIA)'s reference collection. Without the aid of biomolecular tools, when analysing small and often fragmented archaeological fish bones, groupers (Epinephelidae) are indistinguishable from combers (Serranidae) (Winter *et al.*, 2021). Combers exhibit similar behaviour to groupers but only reach maximum standard lengths of ca. 40 cm (ca. 47 cm TL) (Froese & Pauly, 2023). The typical size of sexual maturity for groupers in the Mediterranean is around 40 cm TL, thus this size is used as a cut-off for fishes which are either combers or sexually immature groupers (Froese & Pauly, 2023). Size estimates for groupers and combers were carried out as detailed in (Winter *et al.*, 2022). Where osteometrics were not possible for groupers and combers (i.e., bones and/or specific features were too damaged to take measurements), fish size was estimated to the broad categories of <40 cm TL, 40-80 cm TL, or >80 cm TL via comparison with the groupers in the reference collection at the GIA, corresponding with combers and juvenile groupers, adult groupers, and larger adult groupers, respectively.

To avoid analysing the bones of the same individual fish multiple times, samples identified as the same taxa were taken from different archaeological contexts. Where multiple samples were taken from the same archaeological context, these were either from different taxa or fish of drastically different sizes (i.e., a fish estimated to be 10-20 cm TL and one estimated to be 50-60 cm TL clearly represent two different fish). Being mindful of the precious nature of archaeological material, all samples were fully recorded and photographed prior to destructive sampling.

Collagen extraction methods

Whole bones were sampled for this study, each representing the average diet of a fish over its lifetime due to their continual growth (Wheeler & Jones, 1989), as opposed to the last few weeks or months (Hedges *et al.*, 2007). Due to limited laboratory access during the pandemic, specimens were isotopically analysed at two separate laboratory facilities. Initially, 44 fish bones from Kinet Höyük were analysed at the Centre for Isotope Research (CIO) at the University of Groningen, Netherlands, following the collagen extraction method described in Dee *et al.* (2020).

An additional 191 fish bones from Kinet Höyük (n=140), Tell Fadous (n=48), and Tell el-Burak (n=33) were analysed for $\delta^{13}C$ and $\delta^{15}N$ at the BioArCh facilities, Department of Archaeology, at the University of York, United Kingdom following a modified Longin (1971) method. Due to the fragility and porosity of fish bone that has been buried in a hot/dry environment, samples were demineralised using a range of acid concentrations between 0.1-0.4 M HCl. Published contemporaneous isotopic data from marine fishes in the eastern Mediterranean were also incorporated with our data (Schutkowski & Ogden, 2011; Sandias & Müldner, 2015; Fuller *et al.*, 2020).

Following collagen extraction, aliquots of 0.4 - 0.6 mg of collagen were weighed out and analysed in duplicate. Stable carbon and nitrogen isotopic compositions were determined for the bones extracted at the University of York using a Sercon 20-22 continuous flow isotope ratio mass spectrometer coupled to a Sercon GSL elemental analyzer at the University of York.

Accuracy and precision of IRMS analysis 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 ‰. Following convention, 'raw' values are those obtained from our analysis and 'true' values are the known values for the reference material. Our data was calibrated to these reference materials per recommended best practices in the field (Szpak *et al.*, 2017). In addition, an internal lab standard of homogenised bovine bone was extracted and analysed within the same batch as the samples and 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 bovine bone sample, which produced values of $\delta^{13}C$ = -23.09 ±0.26 and $\delta^{15}N$ = 6.27 ±0.38 and provides an indication of the accuracy and precision of the extraction method and mass spectrometer.

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 archaeological samples and reference materials across all runs was <0.21 ‰ for $\delta^{13}C$ and <0.30 ‰ for $\delta^{15}N$.

Comparing ancient and modern data

Data for the modern indigenous Mediterranean and non-indigenous Lessepsian samples have previously been reported (Fanelli *et al.*, 2015). Their sampling strategy was to collect the species most common in coastal rocky habitats in the Levant within the same region as the archaeological sites in our study (see Fig. 1). Due to the anthropogenic nature of zooarchaeological assemblages, the species in the ancient data set represent the species most commonly exploited in the past. Thus, while the ancient sample is anthropogenic in origin, the ancient data depicts trophodynamics in a comparatively pristine ecosystem, as evidenced by finds of large predatory fish from the archaeological sites included in this study (Fuller *et al.*, 2020; Winter *et al.*, 2022). Mitigating the discrepancies in species representation between these two samples is carried out by adopting and applying the trophic groups proposed by (Fanelli *et al.*, 2015) to the ancient data set. Using the trophic groups presented by Fanelli *et al.* 2015 (benthic carnivores (BC), generalised carnivores (GC), omnivores (O), piscivores (PI), planktivores (PL), and obligate herbivores (H)), enables direct comparison of ancient and modern data sets to then compare trophodynamics in past and present ecosystems. Only the BC, O, GC, and PI trophic groups could be used to categorise the ancient data based on the ichthyofauna present in the zooarchaeological assemblages. Table S1 details the taxa assigned to each trophic group and number of samples.

One of the challenges with categorising the Epinephelidae and Serranidae sample is that their bones cannot be morphologically distinguished from each other due to similar osteomorphology. However, groupers and combers have different foraging strategies. As such, the smaller fish in this category (<40 cm TL) are classified as generalised carnivores (consistent with the Fanelli *et al.* (2015) classification of *Serranus* sp.). The medium- and large-sized fish, presumably groupers, are then classified as piscivores, in accordance with (Fanelli *et al.*, 2015). In the case of one fish within the order Rajiformes, possessing a $\delta^{15}N$ of 12.1‰, higher than all of the groupers, the trophic grouping (PI) was informed by both the taxonomic identification and the $\delta^{15}N$ value. When the same species were present in both the modern and ancient data sets, trophic groups were determined based on the classifications used by Fanelli *et al.* (2015) (see Fanelli *et al.*, 2015) for a full list of species included in their study). For fish in the Sparidae family not represented in the modern sample, trophic groups were assigned upon surveying the literature for details of foraging ecologies with *Sparus aurata* being classified as omnivorous and *Pagrus* sp. and *Dentex* sp. as generalised carnivores. Within the Sparidae sample, there were twenty samples which could not be identified beyond family level. From herbivorous *Sarpa salpa* to the more carnivorous *S. aurata*, a wide range of foraging strategies exist within the Sparidae family, therefore the twenty samples which could not be more precisely identified have been excluded from the ancient trophic groups analysis due to uncertainty regarding how they should be classified.

rKIN package and Isotopic plasticity index (IPI)

Taking from the Hutchinsonian niche concept (Hutchinson, 1957), light stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) has been shown to be a useful means for determining the size of trophic niches (Bearhop *et al.*, 2004). In order to estimate INSs for this study, the rKIN package as developed by (Albeke, 2017) was used for data analysis in RStudio (version 2022.07.2+576). Code for the rKIN package was modified from (Robinson, 2021). The Kernel Utilisation Density (KUD) model is principally considered due to its demonstration of being best suited to archaeological data and greater sensitivity to outliers (Robinson, 2021) than the corrected standard ellipse areas used by Fanelli *et al.* (2015). Whilst samples greater than 15 samples are advised, sample sizes as small as 10 have been shown to reliably estimate isotopic niche size and overlap (Eckrich *et al.*, 2020). Isotopic spaces and overlap have been calculated at contour levels of 40%, 50%, 75%, and 95%, with a contour level of 40% considered to be the 'core' INS for a sample and 95% the 'total homing range'. When there is a high degree of overlap in INS between groups, it suggests sharing of trophic resources. Following (Haubrock *et al.* 2021), the Isotopic Plasticity Index (IPI), which is the INS at a contour of 95% divided by the INS at a contour of 40%, has been reported in this study to assess the efficiency of different taxonomic and trophic groups in utilising resources in their local environment and outside of their 'core' INS. A higher IPI value indicates lower intra-group competition and greater trophic plasticity; whereas a lower IPI value suggests dense INSs (high intra-group competition) and lower trophic plasticity.

When identification of species based on osteomorphology is difficult, other methods such as palaeoproteomics can improve identifications (e.g., Winter *et al.*, 2023), enabling direct comparisons between past and present ecological conditions for key taxa. For the purposes of this study, rather than needing to understand precisely the past isoscape (i.e., baselines for δ^{13}C and δ^{15}N), assessing the changes between past and present day involve comparing the *size* of the isotopic niche space and *spread* of values as opposed to comparing the isotopic values directly. Even when modern specimens may derive from the same location as archaeological fish remains, a direct comparison of isotope values from modern and archaeological specimens is not appropriate given uncertainties in how the isotopic baseline has changed over time (Garcia-Guixé *et al.*, 2010). Quantifying trophic ecology via INSs also enables a comparison between different tissue types as many modern studies are carried out on muscle or other soft tissues while archaeological studies are carried out on osseous tissue.

Results

Foraging ecology of Middle and Late Holocene Levantine ichthyofauna as inferred from δ^{13}C and δ^{15}N isotopes

Of the 235 fish bones sampled, 137 (59%) yielded good quality collagen (Fig. 2) (DeNiro, 1985; Guiry & Szpak, 2021; van Klinken, 1999) (see Supplementary Materials, Tables S1, S4, but in brief C:N of 2.9-3.6, 4.8% N by weight, and 13% C by weight). Values for δ^{13}C range from -13.4‰ to -6.2‰ and from 4.4‰ to 13.1‰ for δ^{15}N. These ranges are comparable with values obtained from other fish bones from archaeological contexts in the eastern Mediterranean (Vika & Theodoropoulou, 2012; Fuller *et al.*, 2020). To account for an atmospheric depletion in δ^{13}C since the industrial period (Suess Effect) we applied an estimated Suess correction of +2.61‰ (de Kock *et al.*, 2023), to the δ^{13}C values from modern eastern Mediterranean marine fishes (Fanelli *et al.*, 2015) for a range of -18.52‰ to -9.98‰, it is apparent that δ^{13}C values in the past were notably higher than they are today. Groupers and combers (considered as one group due to similarities in osteomorphology, *see prior discussion*) are the most abundant taxa in our data set, presenting the opportunity for a more nuanced look at this group's foraging ecology.

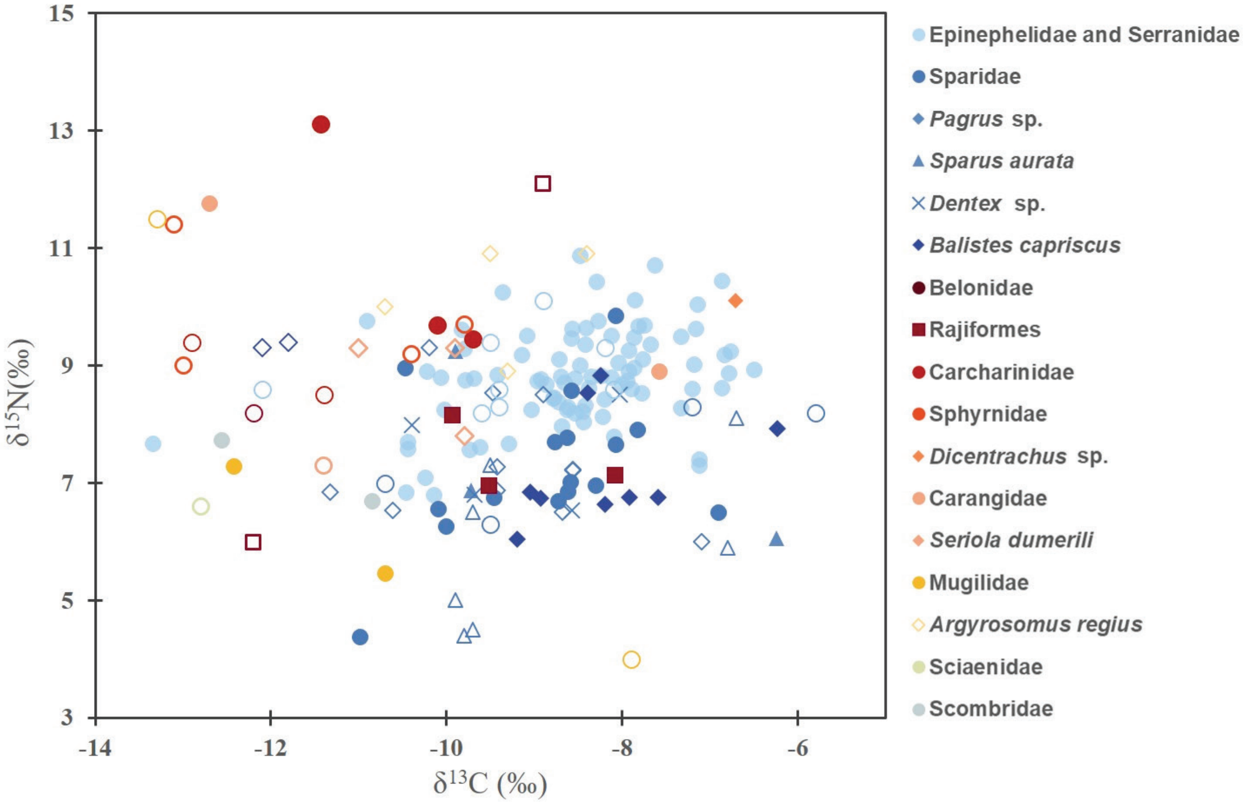


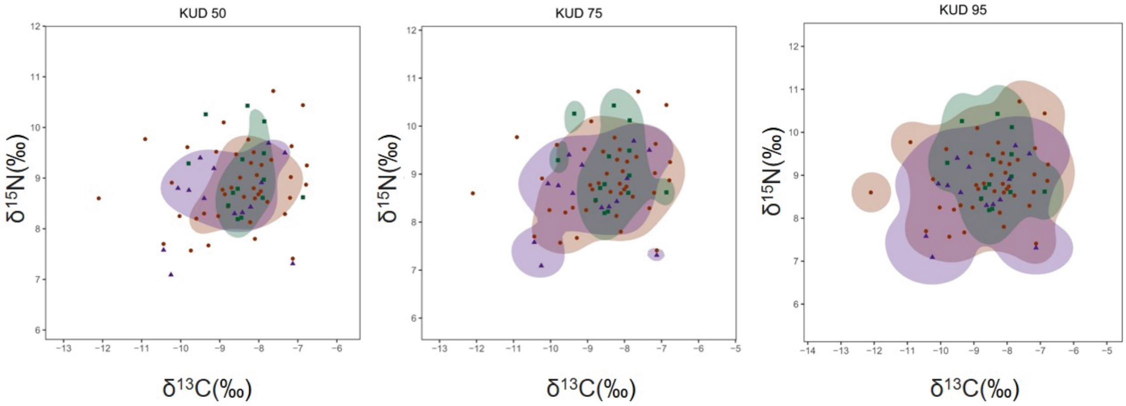
Fig. 2: Biplot of bulk δ^{13}C and δ^{15}N values from ancient marine fish bone collagen. Data combined from this study (n=137, solid markers) and (Fuller *et al.*, 2020; Schutkowski & Ogden, 2011) (n=44, outlined markers).

Ontogenetic changes in the trophic ecology of ancient groupers (Epinephelidae) in the Levant

By combining SIA of ancient groupers and combers with catch size reconstructions (Winter *et al.*, 2022), we were able to investigate whether or not foraging strategies changed over their life course. There is no strong correlation between total length and either δ^{13}C or δ^{15}N (Fig S1-S2). No trends are observed in the IPI, an indicator of trophic plasticity and intragroup competition, over the life course of these fishes, however, INS size decreases while δ^{13}C and δ^{15}N values rise with increasing fish size and age. Figure 3 shows that grouper foraging strategies become more specialised to relatively higher trophic level prey as they grow larger. Comparable IPI values of the three size categories show that these fishes exhibit similar degrees of trophic plasticity and intragroup competition for resources over their life courses.

Marine trophic groups in the Holocene

Trophic groups were assigned to different fish taxa and used to assess trophic niches and trophodynamics of Mediterranean marine ichthyofauna in the past. Trophic groups in order from smallest to largest INS are piscivorous fish (PS), generalised carnivores (GC), benthic carnivores (BC), and omnivores (O), corresponding respectively with the narrowest to widest trophic niches (Fig. 4). PS, GC, BC, and O are present in both the ancient and modern samples, with ancient samples lacking in herbivorous fishes and the modern sampling not containing adequate omnivorous fishes, which prevents the comparison of lower trophic levels between the past and present. Patterns for trophic groups in the Middle and Late Holocene are the same at all contour levels (Table S2). The piscivorous fishes, PI (8.22) have the highest IPI while the BC (6.32), GC (6.04), and O (5.81) groups are comparable with each other (Table S2), indicating the greatest niche breadth and lowest intragroup competition was among the piscivorous fishes in the past.



Size	Contour Level				IPI	Sample Size
	40%	50%	75%	95%		
▲ Combers and juvenile groupers	3.46	4.64	9.19	17.91	5.18	15
● Adult groupers	2.48	3.46	7.62	16.95	6.83	43
■ Older adult groupers	2.11	2.75	6.82	12.71	6.02	15

Fig. 3: Kernel Utilisation Density plot of groupers (Epinephelidae) and combers (Serranidae) divided by body sizes at contour levels of 50%, 75%, and 95%. The table provides the INS sizes at various contour levels, the IPI of each size category, and sample sizes.

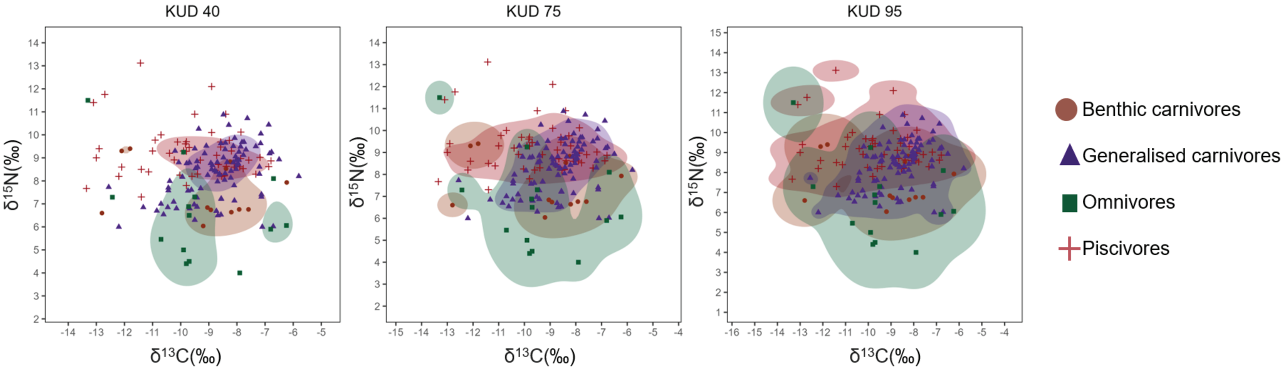


Fig. 4: Kernal Utilisation Density (KUD) model of INS at contour levels of 40%, 75%, and 95% of Middle and Late Holocene eastern Mediterranean ichthyofaunal trophic groups: Benthic carnivores (BC), Generalised carnivores (GC), Omnivores (O), and Piscivores (PS).

Overlap of INSs, indicating sharing trophic resources with similar isotopic values, was the highest within the O trophic group and the lowest within the PS trophic group, meaning that these groups experienced the most and least amount of trophic resource overlap, respectively (Table S3). High overlap between BC, GC, and O trophic groups further demonstrates that middle and lower trophic level groups were experiencing the highest amount of interspecific competition for the same resources, making them the most inextricably linked niches in the food web. INS size, IPI, and INS overlap all indicate that middle and lower trophic level taxa were the most interconnected ichthyofauna regarding trophic resources with greater competition for resources than was experienced by higher trophic level taxa.

Comparing isotopic niche spaces of modern and ancient marine fish

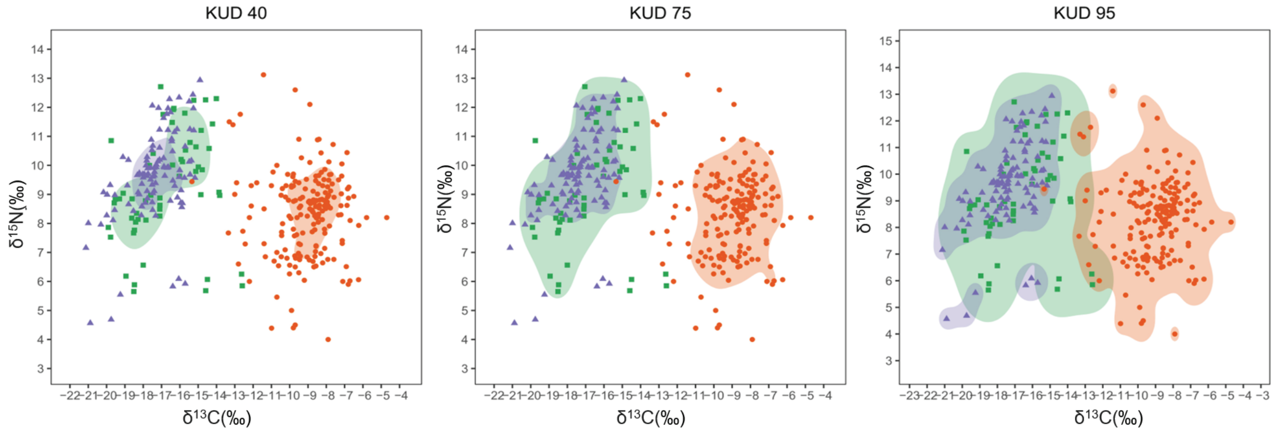
When comparing INS sizes of past and present ichthyofauna within eastern Mediterranean marine ecosystems (Fig. 5), two crucial findings are apparent:

1. The Lessepsian group has the smallest INS.
2. The modern indigenous Mediterranean group has the largest INS and it is notably larger than the Middle to Late Holocene INS.

The high IPI of 8.91 for the Middle to Late Holocene group compared to the modern ichthyofaunal groups, with 5.48 for the indigenous fishes and 7.01 for the Lessepsian fishes, may suggest the effects of long-term, heavy marine exploitation and Lessepsian migrations on eastern Mediterranean ichthyofaunal trophodynamics. Furthermore, the Middle to Late Holocene IPI is considerably larger than that of either modern ichthyofaunal group, demonstrating higher niche plasticity for marine ichthyofauna in the past. Modern, indigenous Mediterranean ichthyofauna have the lowest IPI indicating they have lower trophic plasticity than both the Lessepsian group and the Middle to Late Holocene group. Low IPI values additionally indicate a dense INS, implying high intragroup competition for resources. High levels of the Lessepsian group INS overlapping with the modern, indigenous ichthyofaunal group is further testament to indigenous fishes directly competing with Lessepsian species for resources. The high degree of overlap between the INS of the Lessepsian group and modern indigenous Mediterranean group confirms the utilisation of similar prey items of the two groups.

Discussion

Ichthyofaunal isotopic data from the Middle and Late Holocene establish trophic baselines for littoral Mediterranean marine fishes in ecosystems that have not yet been devastated by the effects of Lessepsian migrations and overfishing. The species composition of the ancient, archaeological assemblage is anthropogenic in nature, e.g., species composition is influenced by past fishing technologies (Morales-Muñíz, 2010), recovery methods in the field (Zohar & Belmaker, 2005), etc. However, the forag-



A

Group	Contour Level 40%	50%	75%	95%	IPI	Sample Size
🟩 Modern indigenous Mediterranean	12.39	16.58	32.76	67.90	5.48	56
▲ Modern Lessepsian	4.65	6.86	14.80	32.60	7.01	104
● Middle to Late Holocene Mediterranean	6.63	9.32	21.97	59.06	8.91	181

B

Contour Level	Modern indigenous Mediterranean (MED) 40%	50%	75%	95%	Modern Lessepsian (LES) 40%	50%	75%	95%
MED	-	-	-	-	0.26	0.34	0.45	0.44
LES	0.68	0.83	0.998	0.91	-	-	-	-

Fig. 5: Kernal Utilisation Density (KUD) model of INS at contour levels of 40%, 75%, and 95% comparing the full ranges of ichthyofauna for the Middle to Late Holocene, Modern Lessepsian, and Modern indigenous Mediterranean groups. Table A provides INS sizes, IPI, and sample sizes for each group and Table B shows the degree of INS overlap between the modern, indigenous Mediterranean fish (MED) and modern, Lessepsian fish (LES).

ing strategy of these ancient fish nonetheless reflects their behaviour in an ecosystem that has not been impacted by invasive species, industrial fishing, pollution, and habitat degradation.

Modern ichthyofauna in the eastern Mediterranean (average of -14.70±1.63‰ *with* a Suess correction) have lower $\delta^{13}C$ values compared to the Middle to Late Holocene ichthyofauna (average of -9.07±1.54‰), signifying a notable shift in the isotopic baseline for the region. In the absence of herbivorous fish in the archaeological assemblage or ancient seagrass, ancient green sea turtles (*Chelonia mydas*) have been used as a proxy for a Middle to Late Holocene $\delta^{13}C$ baseline in the Levant (de Kock *et al.*, 2023) with $\delta^{13}C$ values ranging from -15.52‰ to -4.95‰ (de Kock *et al.*, 2023). Green turtles predominantly feed on *Cymodocea nodos*a, a native seagrass in the region (Casale *et al.*, 2018). *C. nodosa* has more enriched $\delta^{13}C$ values, ranging from −8.2‰ and −6.2‰ (de Kock *et al.*, 2020) than the other native and endemic seagrass, *Posidonia oceanica*, which has a range of −15.8‰ and −12‰ (Apostolaki *et al.*, 2019). Similarly enriched $\delta^{13}C$ values are observed in other stable isotope studies including fish from archaeological contexts in the eastern Mediterranean (Vika & Theodoropoulou, 2012; Fuller *et al.*, 2020). The observed baseline shift could be a result of distinctive isotopic signatures being introduced to the food web, long-term changes in salinity (Grupe *et al.*, 2009; Fuller *et al.*, 2012;), a reduction in ^{13}C-rich phytoplankton (Magozzi *et al.*, 2017; Lorrain *et al.*, 2020), or (although highly unlikely to occur on such a large scale) an alien, less ^{13}C enriched seagrass, such as *H. stipulace*a, coming to dominate the base of the food web and replacing indigenous seagrasses (Apostolaki *et al.*, 2019; Vizzini *et al.*, 2002). Compound specific stable isotope analysis of several Lessepsian species found different isotopic signatures between migrated, Mediterranean populations and their Red Sea counterparts (Tsadok *et al.*, 2023). Thus, we can see that different foraging strategies are being adopted in the Mediterranean by Lessepsian species as they adapt to a new ecosystem. Future research applying compound specific analysis to ancient, lower trophic level fish may help to finesse the details and nuances contributing to this particular baseline shift.

Smaller INSs with increasing size and age of groupers indicates an ontogenetic shift to more specialised foraging strategies. The presence of groupers in an ecosystem has been shown to be effective at reducing the foraging behaviour of invasive siganids (Giakoumi *et al.*, 2019; Shapiro Goldberg *et al.*, 2021). In the eastern Mediterranean, herbivorous, invasive rabbitfishes (*Siganus rivulatus* and *S. luridus*) impose one of the greatest risks/disruptors to marine ecosystems through their intensive foraging which creates barren reefs (Bariche *et al.*, 2004; Sala *et al.*, 2011; Rilov, 2016). Well-enforced marine protected areas (MPAs) are effective tools for the recovery of abundant large-bodied, high trophic level predatory fishes, including groupers (Guidetti *et al.*, 2014), although limited evidence is currently available on their potential role in controlling the spreading of invasive fish species (Giakoumi *et al.*, 2019). The larger INS and lower $\delta^{15}N$ of combers and small groupers, indicative of eating prey lower on the trophic chain, indicates that all sizes of groupers may function as a natural form of biocontrol for these particular invasive species.

Large, apex and upper trophic level predators are often touted as the key target for conservation efforts (Pauly *et al.*, 1998; Estes *et al.*, 2011). As our results show, upper trophic level fish have the highest trophic plasticity and least overlap in isotopic niches with other trophic groups. Assessing INS of Middle to Late Holocene trophic groups shows that mid and lower trophic levels are more vulnerable to the effects of bioinvasions due to the high degree of overlap in INS. High overlap of INSs indicates these middle and lower trophic groups are competing with each other for the same trophic resources. Middle and lower trophic levels being the most vulnerable to competition for trophic niches is consistent with observations in the Levant (Goren *et al.*, 2016) and also in freshwater ecosystems (Schulze *et al.*, 2012). Isotopic niches of piscivorous and high trophic level fishes are likely less affected by Lessepsian species due to prior reductions in their abundance and biomass as a consequence of overfishing (Prato *et al.*, 2013; Boudouresque *et al.*, 2017).

The Lessepsian group has the smallest INS, indicating the use of less diverse trophic resources than indigenous Mediterranean fishes and their ancient counterparts. Additionally, Lessepsian species currently dominate the ichthyofaunal biomass of many eastern Mediterranean marine ecosystems (e.g., (Edelist *et al.*, 2013; Galil & Goren, 2014; Goren & Galil, 2005; Özyurt *et al.*, 2018)). Lessepsian species have previously been deemed and demonstrated (Edelist *et al.*, 2013; Fanelli *et al.*, 2015) to be better competitors for resources than indigenous species. The modern, indigenous Mediterranean fishes have a larger INS than the Lessepsian fishes *and* the Middle to Late Holocene fishes, which further demonstrates a wider array of trophic resources being consumed than would be expected based on a baseline which predates the opening of the Suez Canal. High overlap between the INSs of Lessepsian fishes and indigenous fishes further confirms the direct competition between these two groups for the same trophic resources. A higher IPI value for ancient Mediterranean fishes compared with modern Lessepsian and indigenous Mediterranean fishes indicates greater trophic plasticity and less intra-group competition if we are considering a more “pristine” ecosystem which has not yet experienced bioinvasions.

Conclusion

Returning to our original research questions, ancient ichthyofaunal isotopic data generates important baselines for trophic niches and interactions that predate Lessepsian migrations and overfishing. By turning to a more ‘pristine’ environment of the past, we see that middle and lower trophic levels face the highest levels of competition for trophic resources, making them the most vulnerable to ecosystem disruptors such as non-indigenous species. By comparing ancient and modern data sets, the degree of niche widening of indigenous fishes can be established. Better understanding of past trophodynamics in the eastern Mediterranean unlocks the potential for better informing management and conservation strategies in the face of the ongoing westward expansion of Lessepsian migrants.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Overview of the archaeological fish bones sampled in this study, (Fuller *et al.*, 2020), and (Schutkowski & Ogden, 2011) as well as the trophic groups assigned to each taxa.

Fig. S1: Comparison of Total Length (cm) and δ^{13}C values of Epinephelidae and Serranidae from Kinet Höyük, Tell Fadous, and Tell el-Burak.

Fig. S2: Comparison of Total Length (cm) and δ^{15}N values of Epinephelidae and Serranidae from Kinet Höyük, Tell Fadous, and Tell el-Burak.

Table S2. INS at various contour levels, IPI, and sample size of trophic groups of marine fishes from eastern Mediterranean archeological sites calculated using the KUD model in rKIN.

Table S3. Overlap of INS of archaeologically derived trophic groups of marine fishes utilising the KUD model in rKIN.

Table S4. Full quality control data pertaining to fish bones samples and analysed. Highlighted in light grey and *italicised* samples indicate unacceptable C% (minimum 13% by weight), N% (minimum 4.8% by weight), C:N ratio (outside of 2.9-3.6), or too much error between duplicates (indicated with * next to the problematic quality control criteria). Highlighted in grey and bold results indicate collagen yields which were too low or too low of quality for analysis. ** Due to low collagen yield, this sample could not be run in duplicate however due to meeting quality control criteria, it was included in our analysis.