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A 4000-year record of parallel collapse in the ecological niche of Black Sea top predators

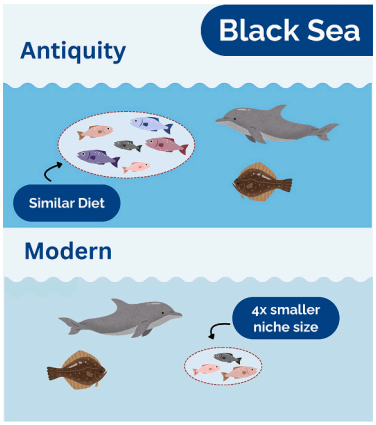
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HIGHLIGHTS

- Stable isotopes are compared between archaeological and modern Black Sea cetaceans and turbot.
- Archaeological populations had highly overlapping isotopic niches, whereas modern species are more distinct.
- Modern taxa show pronounced isotopic niche compression across species.
- Patterns are consistent with long-term changes in Black Sea food-web structure.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine ecosystems face multifaceted threats stemming from historical overexploitation and environmental degradation. Despite existing research, there are notable gaps in understanding the long-term dietary dynamics of marine organisms in the context of anthropogenic impacts. To address these gaps, we utilized stable isotope analysis as a proxy for dietary niche and examined changes in the Black Sea over a 4000-year period, focusing on ancient and contemporary samples from cetaceans (*Phocoena phocoena*, *Delphinus delphis*, *Tursiops truncatus*) and

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Overexploitation
Cetacean

fish (*Scophthalmus maximus*). Among the ancient samples, the isotopic data from all four species revealed a wide and generally overlapping average dietary niche. In contrast, contemporary cetacean and fish samples exhibited niche compression, indicative of a fundamental shift in the Black Sea marine ecosystem from Antiquity to the present day. Our analyses provide ecologically significant temporal perspectives, shedding light on critical trends and changes essential for conservation efforts aimed at addressing contemporary ecological challenges in degraded marine systems.

1. Introduction

The health of the world's oceans is critical for climate regulation, ecosystem services, and human livelihoods. The relatively isolated Black Sea has been a centre for human activities for millennia, although major impacts are thought to mainly have occurred in the mid-to late-20th century in the form of overexploitation, pollution, physical modification of the environment, food web changes, and multiple bioinvasions (Birkun, 2002; Birkun et al., 2014; Bologa, 2012; Keskin et al., 2015; Oguz and Gilbert, 2007; Tsikliras et al., 2015; Oguz and Velikova, 2010) Additionally, excessive nutrient input from Black Sea tributaries due to agriculture and urban waste has resulted in the eutrophication of shallow coastal waters leading to algal blooms, hypoxia, anoxia, mass mortality of benthic fishes and invertebrates, and an overall decline in marine biodiversity (Bologa, 2001; Bănăduc et al., 2016; Birkun, 2002; Keskin et al., 2015; O'Higgins et al., 2014).

There has been a significant decrease in Black Sea cetacean and turbot populations. A large number of cetaceans were killed in the Black Sea in the 20th century, perhaps in the hundreds of thousands, corresponding to a 10-fold decrease in the Black Sea harbour porpoise subspecies (*Phocoena phocoena relicta*) population abundance over five generations (Tsikliras et al., 2015; Bologa and Sava, 2012; Bologa, 2001; Birkun, 2002; Keskin et al., 2017; Sánchez-Cabanes et al., 2017; Daskalov, 2002; Fontaine et al., 2012; Paiu et al., 2024). Turbots (*Scophthalmus maximus maeoticus*) have been subjected to similar overexploitation resulting in multifold decrease in catches: e.g., an estimated 33-fold decrease (330 t per year to 12 t per year) from the 1960s to 1980s in Bulgarian waters (Keskin et al., 2017, United Nations, 1998). These decreases coincided with a collapse of many of the prey species populations due to overexploitation of the fish stocks and the effects of eutrophication and bioinvasions beginning in the 1950s (Tsikliras et al., 2015; Bologa and Sava, 2012; Bologa, 2001; Birkun, 2002; Keskin et al., 2017; Sánchez-Cabanes et al., 2017; Daskalov, 2002; Paiu et al., 2024). Key prey species that underwent severe commercial or ecological collapses in the late 20th century include anchovy (*Engraulis encrasicolus*) and horse mackerel (*Trachurus mediterraneus*) (Kideys, 1994; Demirel et al., 2020; Ulman et al., 2020). Additionally, larger predators have been severely affected, including the local extirpation of the Mediterranean monk seal (*Monachus monachus*) in the 1990s (Kıraç, 2011), the bluefin tuna (*Thunnus thynnus*) in the 1980s (Di Natale, 2015), and the swordfish (*Xiphias gladius*) in the 1990s (Öztürk, 1999).

Recent changes in the biotic structure of the Black Sea ecosystem, such as the decline of large-sized fish, have been identified (e.g., Gladilina and Gol'din, 2014), however, these studies often cover a period of a relatively short time span, impeding a clear understanding of the onset, duration, and long-term consequences of substantial anthropogenic changes. To bridge these temporal gaps, this study addresses a central research question: how have the dietary niches and trophic interactions of key Black Sea marine predators changed across a 4000-year timeline of accelerating human impact? Specifically, we aim to determine if modern ecological degradation has forced a contraction or shift in the trophic resources available to these species compared to a historical baseline.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis is increasingly being used in cetacean ecological studies to study foraging behaviours (Teixeira et al., 2022). Generally, $\delta^{13}C$ values are commonly used as representative markers of producers at the base of the food web with a gradient between benthic and pelagic marine systems (Szpak et al., 2019; Raven et al., 2002; MacKenzie et al., 2011; McConnaughey and McRoy, 1979; Alter et al., 2012; Louis et al., 2021; Pinela et al., 2010). $\delta^{15}N$ values vary according to the trophic level, demonstrating a stepwise enrichment of approximately 3–5 ‰ per level up the food chain (Minagawa and Wada, 1984; Bocherens and Drucker, 2003; Rau et al., 1989). In archaeological contexts, where preservation is often limited to osteological material, stable isotope analysis is a key method for the study of foraging ecology.

To evaluate these long-term ecosystem changes, we analysed the stable isotopic composition of carbon and nitrogen in bone collagen samples obtained from ancient cetacean and fish specimens from archaeological sites. These isotopic values were then compared with contemporary data from previously published isotope studies (Das et al., 2004; Bănaru and Harmelin-Vivien, 2009).

2. Methods

2.1. Samples

The stable isotope analyses were conducted on 79 ancient cetacean specimens from the Black Sea, including 18 bottlenose dolphins (*Tursiops truncatus ponticus*), 27 common dolphins (*Delphinus delphis ponticus*), and 34 harbour porpoises (Table 1; Supplementary Table 1). The skeletal elements come from kitchen middens of seven archaeological sites along the Black Sea coast, mostly from the north-western Black Sea coast (Fig. 1; Supplementary Table 1). They were dated from their archaeological contexts (Bronze Age, 2900–700 BCE; Archaic Greek, 700–480 BCE; Roman, 62–641 CE; or Medieval, 641–1475 CE) and stored dry at room temperature (Table 1; Aiken et al., 2023; Aiken et al., 2024). Bone samples were selected based on their preservation and skeletal maturity (evaluated by epiphyseal fusion), and species identification was determined by morphological assessment and/or genetic analysis (see below). In addition to the ancient cetacean samples, 14 ancient samples of turbot were also analysed (Table 1; Supplementary Table 1). Turbot and cetacean remains were analysed concurrently to offer an integrated view of high-trophic-level taxa exploited in the Black Sea that allow for comparison between benthic (turbot) and pelagic (cetacean) foodsystems. All of the samples come from morphologically distinct specimens representing different individuals. Although samples derive from different skeletal elements (primarily vertebrae; Supplementary Table 1), inter-element isotopic variation in bone collagen is generally low (~0.5 ‰; Hyland et al., 2021) and unlikely to meaningfully affect dietary interpretations.

2.2. Ancient DNA analysis

Ancient DNA analysis was used to evaluate morphological species identifications of 27 of the 79 ancient specimens that were difficult to identify to species by morphological examination as they consisted of incomplete vertebrae and other bone fragments (Biard et al., 2017). All pre-PCR procedures were performed in dedicated animal biomolecule clean laboratories at the Globe Institute (University of Copenhagen, Denmark) following strict contamination protocols (Cooper and Poinar, 2000; Gilbert, 2005). Samples were manually cleaned and drilled with a Dremel hand drill and DNA was extracted from the samples using a lysis buffer of EDTA, Tween 20, and Proteinase K following Dabney et al.

Table 1
Summary table of samples by species including site (archaeological site or sampling location for modern samples), period, date (where applicable), tissue type, and the number of samples.

Species	Site	Period	Date	Tissue	*n*
Bottlenose Dolphin	Chersonesos	Roman	400–530 CE	Bone	5
		Medieval	1000–1100 CE	Bone	2
	Gleiki 2	Bronze Age	2000–1800 BCE	Bone	1
	Kerch	Bronze Age	Bronze Age	Bone	1
	Olbia	Bronze Age	Bronze Age	Bone	1
		Roman	1–450 CE	Bone	6
	Ukrainian Coast	Modern	1997 CE	Muscle	4
Common Dolphin	Berezan	Archaic	550–500 BCE	Bone	1
	Chersonesos	Roman	400–530 CE	Bone	14
		Medieval	1000–1100 CE	Bone	1
	Choloki	Bronze Age	1500–1300 BCE	Bone	1
	Lapsi	Medieval	700–900 CE	Bone	1
	Olbia	Roman	1–450 CE	Bone	9
	Ukrainian Coast	Modern	1995 CE	Muscle	2
	Berezan	Archaic	550–500 BCE	Bone	1
Harbour Porpoise	Chersonesos	Roman	400–530 CE	Bone	20
	Gleiki 2	Bronze Age	2000–1800 BCE	Bone	4
		Roman	1–450 CE	Bone	1
	Olbia	Roman	1–450 CE	Bone	6
	Techirghiol	Neolithic	5500–5000 BCE	Bone	1
	Ukrainian Coast	Modern	1997–1998 CE	Muscle	46
Turbot	Chersonesos	Roman	400–530 CE	Bone	10
		Medieval	900–1300 CE	Bone	4
	Romanian Coast	Modern	2004–2006 CE	Muscle	40

(2013). Extracts were concentrated using Roche columns and eluted in Monarch® DNA Cleanup Columns. Libraries were built following the single-tube BEST protocol (Carøe et al., 2018). Following PCR amplification and bead purification, the DNA concentrations were measured on a Qubit high-sensitivity kit, and fragment lengths were tested on a Bioanalyzer using a high-sensitivity kit. Samples with low DNA concentrations were not sequenced. The samples were pooled and sequenced at the GeoGenetics Sequencing Core, University of Copenhagen, Denmark on an Illumina NovaSeq 6000.

The Paleomix v.1.3.6 (Schubert et al., 2014) pipeline was used to map all raw data against reference mitogenomes for the harbour porpoise (NCBI Accession ID: NC_005280.1), common dolphin (NCBI Accession ID: MF669498.1), and bottlenose dolphin (NCBI Accession ID: MF669485.1). Prior to mapping, a 50 base pair pad was manually added to the opposing end of the circular reference mitogenomes to increase coverage of the artificial break created when performing linear analysis of mitogenomes. AdapterRemoval v.2.3.3 (Schubert et al., 2016) was used to trim adapters, low-quality bases, reads shorter than 25 nucleotides, and collapse overlapping mate-pairs. Trimmed and collapsed reads were mapped to the reference mitogenomes using the BWA-MEM algorithm (Li and Durbin, 2009) with a filter keeping only reads of a minimum mapping quality score of 25. PCR duplicates were removed using Picard v. 2.27.4 MarkDuplicates and mapping statistics were visualized in R. A specimen was assigned the taxonomic identity of the species for which it had the greatest endogenous content (proportion of mapped reads to a reference mitogenome out of total reads retained after quality filtering). In cases of a specimen having similar endogenous content with multiple reference mitogenomes, consensus sequences were called in ANGSD v.940 (Korneliussen et al., 2014) with a majority base rule and a minimum depth of three reads per site for further assessment. Consensus sequences were aligned with the reference mitogenomes using the Mafft algorithm (Katoh and Standley, 2013) and manually inspected in Geneious Prime 2020.1.2 (https://www.geneious.com) to determine which species the sequence most closely resembled.

2.3. Stable isotope analysis

Collagen extraction and mass spectrometry were performed at the Trent Environmental Archaeology Laboratory (TEAL, Trent University, Canada). Bone samples were first demineralized in 0.5 M hydrochloric acid (HCl) at room temperature under constant motion from an orbital shaker until soft (21–72 h). Samples were assessed for humic contamination based on color. Following Wilson and Szpak (2022) samples that were observably stained (discolored) underwent humic extraction through the application of 0.1 M sodium hydroxide (NaOH) for 20 min. If the solution became discolored the procedure was repeated, to a maximum of three applications. Finally, the samples were refluxed in 0.01 M HCl at 75 °C for 36 h and lyophilized.

All samples were analysed using a EuroEA 3000 Elemental Analyzer coupled with a Nu Horizon continuous flow isotope ratio mass spectrometer at the Trent University Water Quality Center. As a quality control, 10% of the samples were analysed in duplicate, to assess the homogeneity of the analysis. The duplicated values were averaged for downstream analysis. Calibration to the international reference scales Vienna Pee Dee Belemnite (V-PDB) and AIR was completed using USGS40 and USGS66 for $\delta^{15}N$ and USGS40 and USGS63 for $\delta^{13}C$ (Supplementary Table 2). Accuracy and precision were assessed through internal standards SRM-1 (caribou bone collagen), SRM-2 (walrus bone collagen), SRM-14 (polar bear bone collagen), and SRM-16 (seal bone collagen). The total analytical uncertainty was ±0.12 ‰ for $\delta^{13}C$ and ± 0.13 ‰ for $\delta^{15}N$. Four standardized quality assessment measures were used to determine sample quality: percent collagen yield (>2% and between 1 and 2% if no other indicators were marginal), carbon and nitrogen content by weight (>15% for carbon and > 5% for nitrogen), and atomic C:N ratio (values between 3.1 and 3.5; van Klinken, 1999; Ambrose, 1990; DeNiro and Weiner, 1988; DeNiro, 1985).

All statistical analyses were completed using R v.3.6.3 (R Core Team, 2020). For each species, means and standard deviations for $\delta^{13}C$ and $\delta^{15}N$ were calculated (Supplementary Table 3). The normality of the data was tested using the Shapiro-Wilk normality test. Levene's test was used to test for homogeneity of variance. Differences between taxa from ancient samples were compared using a one-way analysis of variance (Welch's ANOVA), which checks for unequal group means. Follow-up Tukey's honestly significant difference (HSD) test was performed to identify specific differences between groups.

To evaluate whether the archaeological samples could be treated as a single 'ancient' group, we tested isotopic differences by archaeological site and time period using both multivariate and univariate modelling. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed to examine the combined $\delta^{13}C$ and $\delta^{15}N$ values by species over time (years before present; BP), by archaeological site and the interaction between these two factors. Univariate analysis was also performed to examine the relationship between $\delta^{13}C$ and $\delta^{15}N$ values individually.

To investigate the potential linearity of the temporal trends, the data were fit to a Generalized Additive Model (GAM), with the degrees of freedom estimated to examine the linearity of the relationship between time and isotopic composition. Permutational Analysis of Multivariate Dispersions (PERMDISP) was used to assess whether differences detected in PERMANOVA were due to differences in group centroids (mean values) or within-group variance. To evaluate the robustness of the temporal differences observed in the turbot isotopic data, a subsampling analysis was performed to address potential concerns about unequal sample sizes across time periods. In this analysis, a random subset of four samples from the late time period (900–1300 common era; CE) was

selected in each iteration, while all the samples from the early time period (400–530 CE) were retained. This procedure was repeated 100 times to ensure the consistency of the observed differences in isotopic composition. For each subsample, PERMANOVA was performed to assess multivariate differences in $\delta^{13}C$ and $\delta^{15}N$ between the early and late time periods. In addition, univariate ANOVAs (analysis of variance) were conducted on the individual isotopic variables ($\delta^{13}C$ and $\delta^{15}N$) to test for significant differences between the two time periods. The *p*-values from the PERMANOVA and ANOVAs were stored for each iteration to evaluate the robustness of the temporal differences.

The modern Black Sea cetacean and turbot isotopic data (also mostly originating from the north-western Black Sea) were extracted from muscle samples analysed in previous studies (Das et al., 2004; Bănaru and Harmelin-Vivien, 2009; Supplementary Table 4). To account for the depletion in oceanic ^{13}C caused by the release of carbon (CO_2) from the combustion of fossil fuels, the modern comparative data was corrected for ^{13}C-Suess effects; as there are currently no correction factors for the Black Sea, we followed Sisma-Ventura et al. (2019) and used the Mediterranean Sea correction factor of 1.2 ‰ set to the year 1850, prior to major industrial developments in Europe. Furthermore, due to differential fractionation between the tissues, a correction factor was applied to the modern cetaceans. Two published bone-muscle offsets were considered: (i) paired tissue measurements from fin whale (*Balaenoptera physalus*; +1.82 ‰ $\delta^{13}C$ and − 0.70 ‰ $\delta^{15}N$; Borrell et al., 2012) and (ii) population-level muscle and bone collagen averages from North Sea harbour porpoises (*Phocoena phocoena*; +3.9 ‰ $\delta^{13}C$ and − 0.95 ‰ $\delta^{15}N$; Jansen et al., 2012). Because harbour porpoises are more similar to the focal cetacean species of this study in body size, metabolism, and trophic ecology (Clark et al., 2019; Matthews, 2024; Horstmann-Dehn et al., 2012), their muscle-bone offset, though derived from unpaired samples, was used as the primary correction, with the fin whale offset reported only for comparison (Supplementary Fig. 1, Supplementary Table 3). No offset was applied to the turbot as there are no suitable correction factors available. We note that bone has lower turnover rate than muscle tissue and isotope values thus represent an averaged diet of a period of multiple years versus multiple months, respectively (Caut et al., 2011; Teixeira et al., 2022; Dalerum and Angerbjörn, 2005). Thus, we would expect a priori that the muscle stable isotope values would show greater short-term within-tissue variability than bone collagen. However, the North Sea harbour

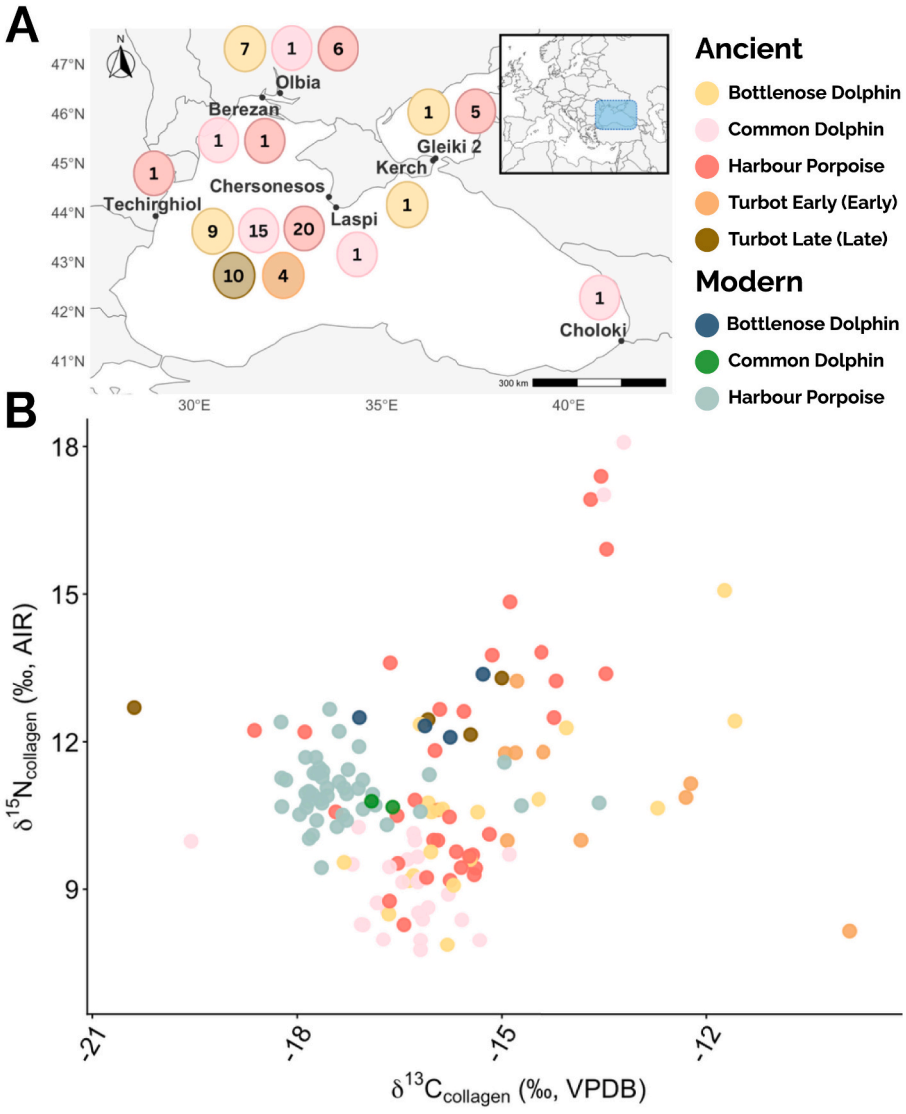


Fig. 1. A) Map of archaeological sites including the number of ancient samples included per species. B) Carbon (δ13C) and nitrogen (δ15N) isotopic values for ancient and modern Black Sea cetacean and turbot samples. Modern sample data has been corrected for the Suess effect and the porpoise-derived correctional factor has been applied.

porpoise dataset (Jansen et al., 2012) shows that the two tissues can exhibit very similar standard deviations for both $\delta^{15}N$ and $\delta^{13}C$, with the greatest variation occurring within tissues (e.g., between sexes) rather than between tissues indicating that muscle and bone may integrate largely similar dietary signals.

Isotopic niche region (N_R) and pairwise niche overlap between the species were calculated following Borrell et al. (2012) using the method developed by Swanson et al. (2015) with the R package "nicheROVER" (Lysy et al., 2021). NicheRover uses a probabilistic method to calculate a multidimensional niche region in a Bayesian framework. The N_{R95} value is the ellipsoidal volume representative of the species' isotopic niche size (Swanson et al., 2015; Lysy et al., 2021; Yurkowski et al., 2020). Species niche size is the probability (calculated at 95%) of finding a specific individual of that species in the ellipses. Niche overlap is the probability of an individual from a species falling into the N_{R95} of another species, which is a one-way calculation meaning the proportional niche overlap of species A to B may be different from the niche overlap of species B to A. We calculated N_{R95} using uninformative priors with 10,000 iterations. Following Brewster et al. (2016), we define high niche overlap as a median overlap greater than 50% between two populations.

3. Results and discussion

3.1. Genetic species identification

We performed genetic identification of 27 cetacean samples that were difficult to identify morphologically, comprising newly sequenced samples and re-analysis of raw data from Biard et al. (2017). These were successfully identified as 10 common dolphins, 8 bottlenose dolphins, and 9 harbour porpoises (Supplementary Table 6). Two samples identified morphologically to "Cetacea" were genetically identified to common dolphin and harbour porpoise and eight samples were genetically identified to species different than their preliminary morphological assessment, including four harbour porpoises reclassified as common dolphins and three bottlenose dolphins reclassified as common dolphins (Supplementary Table 6).

3.2. Temporal and spatial trends

We examined the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of ancient and modern Black Sea cetaceans, as well as ancient turbot. All samples met the quality control standards and were used for subsequent analyses. The isotopic values remained relatively stable, with average shifts in $\delta^{13}C$ and $\delta^{15}N$ values between the ancient and modern specimens of 1 ‰ and 2 ‰, respectively (Fig. 1; Table 2; Supplementary Table 3).

The multivariate analysis, PERMANOVA, showed that cetacean species exhibited no significant temporal or spatial trends in $\delta^{13}C$ or $\delta^{15}N$, except for the bottlenose dolphin, which exhibited site-specific variation. However, this site-specific variation was based on only two samples (one from Kerch and one from Gleiki 2, both located at the Kerch Strait in the east of Crimea, Ukraine; Fig. 1). This difference is not unexpected, as bottlenose dolphins are highly philopatric and exhibit strong site fidelity (Bolaños-Jiménez et al., 2021; Nykänen et al., 2018; Passadore et al., 2018), which can lead to fine-scale geographic isotopic differentiation (Nicholson et al., 2021; Browning et al., 2014). Due to the small sample size limiting the robustness of the conclusions, these results were not considered further and were removed from niche size calculations.

Table 2
Summary of ancient and modern isotopic data for bottlenose dolphin, common dolphin, harbour porpoise, and turbot including if ancient or modern, the number of samples, the average $\delta^{13}C$ and $\delta^{15}N$ values, and the standard deviations. The turbot data is reported separated by the early (E; Roman) and late (L; Medieval) samples.

Species	Period	*n*	$\delta^{13}C$ VPDB ($\pm 1\sigma$)	$\delta^{15}N$ AIR ($\pm 1\sigma$)
Bottlenose Dolphin	Ancient	18	−15.2 ± 1.7	10.5 ± 1.7
	Modern	4	−16.1 ± 0.8	12.6 ± 0.6
Common Dolphin	Ancient	27	−16.2 ± 1.2	9.6 ± 2.4
	Modern	2	−16.7 ± 0.22	10.7 ± 0.1
Harbour Porpoise	Ancient	33	−15.6 ± 1.2	11.6 ± 2.4
	Modern	46	−17.3 ± 0.9	11.0 ± 0.6
Turbot	Ancient - E	10	−13.8 ± 1.81	10.9 ± 1.4
	Ancient - L	4	−16.7 ± 2.5	12.6 ± 0.5
	Modern	40	−18.5 ± 0.8	16.3 ± 0.8

While no significant overall temporal trends in $\delta^{15}N$ values were found, $\delta^{13}C$ values exhibited a decreasing pattern over time, from Antiquity to the present day, with all three cetacean species showing lower modern $\delta^{13}C$ values (Table 2; Fig. 2; Supplementary Table 3). This suggests a possible shift towards a more oceanic or pelagic diet in recent times. Alternatively, it may reflect ancient exploitation practices that targeted near-shore rather than offshore individuals. While fishing was export oriented in the Black Sea as early as the 5th century BCE (Bekker-Nielsen, 2020), fishing vessels are thought to have been smaller until the 11th century CE during the Byzantine period (Marzano, 2013). Instead of open sea, deep-water fishing, much fishing likely occurred in lagoons, estuaries, and along the coast (Marzano, 2013; Mylona, 2021). Indeed, a study in the Aegean found that between the Bronze Age and Classical periods, 90% of the fish recovered were shallow water dwellers (Mylona, 2008). However, due to the limited sample size of the modern bottlenose and common dolphins, and the relatively small temporal change of a few parts per thousand, the strength of these conclusions is limited.

For the turbot samples from Chersonesos, PERMANOVA revealed significant temporal differences in $\delta^{13}C$ and $\delta^{15}N$ ($p = 0.009$; Table 2). PERMDISP confirmed that these temporal differences were driven by differences in group centroids (mean values) rather than differences in within-group variance ($p = 0.883$). To assess whether the temporal differences in turbot isotopes were influenced by unequal sample sizes, a 100-iteration balanced subsampling analysis was performed. The results indicated that the multivariate differences detected by PERMANOVA were highly robust, remaining statistically significant in 78% of the random balanced subsets, suggesting that the observed differences were robust and not due to sample size disparities. Additionally, the GAM demonstrated that these temporal shifts were driven predominately by carbon. Turbot showed a highly significant, strictly linear relationship between time (BP) and $\delta^{13}C$ (EDF = 1.00; $p = 0.006$) In contrast, there was no significant temporal trend for $\delta^{15}N$ (EDF = 1.00; $p = 0.279$) While the subsampled nitrogen ANOVAs picked up minor fluctuations across some iterations (retaining significance in 55% of subsets), the overarching trend confirms that the turbot's trophic position remained stable despite a shifting carbon base.

Given the broad temporal span of the ancient datasets, it is important to consider how this might influence isotopic variability. While the ancient samples represent long periods of ecological and foraging conditions, which could mask short-term dietary fluctuations, the PERMANOVA on the samples from the "ancient" period shows that the temporal aggregation does not significantly alter the overall isotopic trends (the exception being the turbot, see above). The modern samples, while more temporally restricted, provide a snapshot of modern ecological dynamics, which serve as a useful comparison for understanding long-term deep-time shifts in foraging habitats and dietary patterns. While the temporal resolution of the datasets varies, these differences do not appear to significantly affect the overall conclusions drawn from the data.

3.3. Reduction in cetacean and turbot isotopic niche size

The isotopic niche size was found to be large for all ancient cetaceans characterised by high pairwise overlap ranging from 68% to 88% (Fig. 3A; Table 3). The isotopic niche size was the largest for the ancient

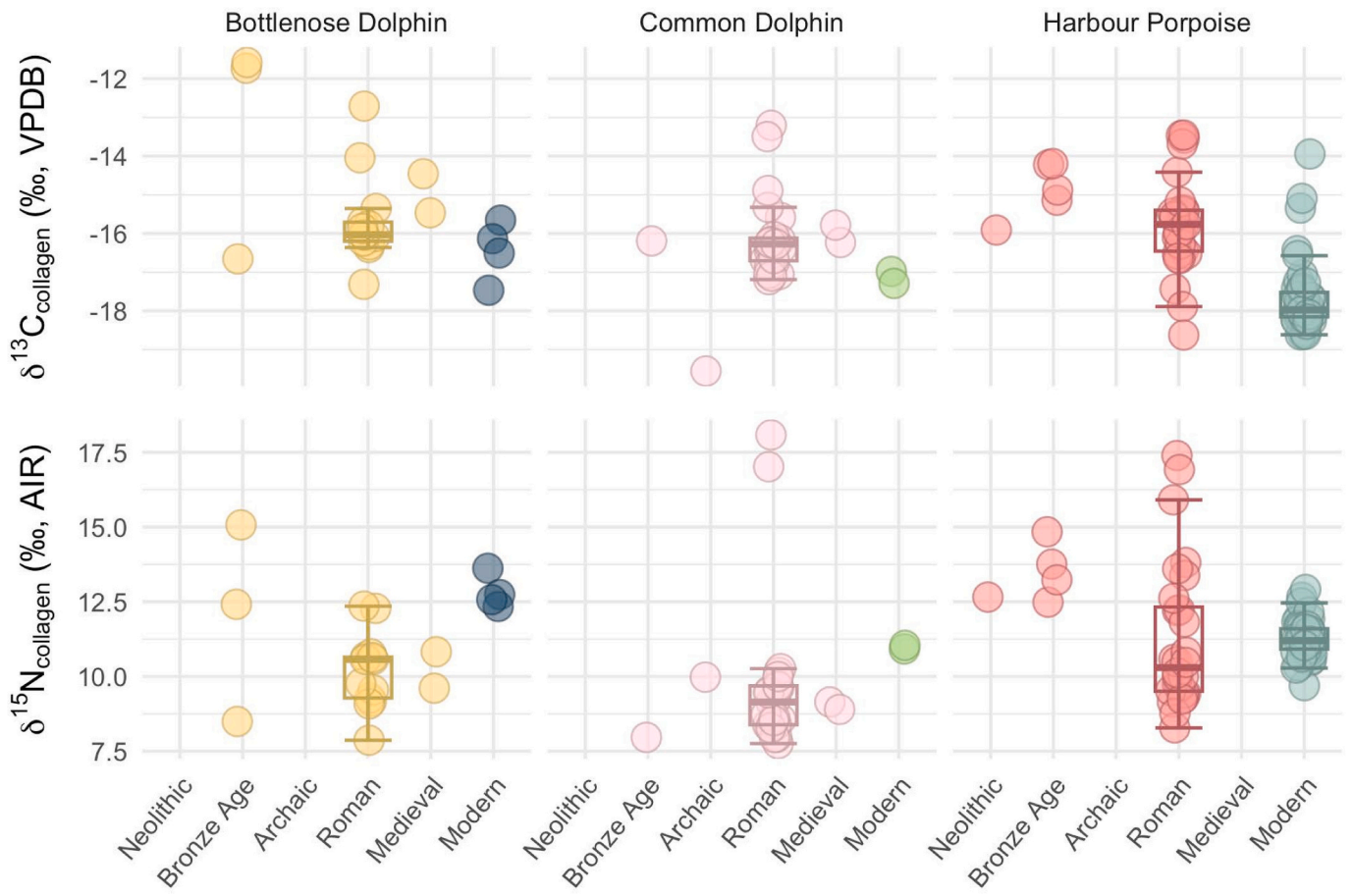


Fig. 2. Boxplots of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic values by species and temporal period. Boxplots are only shown for groups with more than 10 observations to ensure reliable summary statistics. Note that the temporal periods are chronologically ordered but non-contiguous.

harbour porpoise (45 ± 8 ‰^{2}) followed by the ancient common dolphin (40 ± 8 ‰^{2}), the early turbot (34 ± 11.5 ‰^{2}), the ancient bottlenose dolphin (22 ± 8 ‰^{2}) and finally the late turbot (17.0 ± 9.5 ‰^{2}; Fig. 3B, Table 3). There was a dramatic reduction in the isotopic niche size in modern specimens compared to ancient ones. This aligns with the results of the Levene's test, which showed a significant difference in variance between the modern and ancient periods, driven by the tight clustering of the modern data. The modern niche sizes ranged from 12 ± 2 ‰^{2} for the modern turbot to 10 ± 2 ‰^{2} for the modern harbour porpoise and finally 5 ± 3 ‰^{2} for the modern bottlenose dolphin (Table 3). Modern common dolphin niche size is not reported because the available published dataset included too few individuals to reliably estimate a modern niche. This corresponds to an average 4-fold decrease from ancient to modern cetaceans whereas the turbot exhibit a more gradual pattern of change, with a 2-fold decrease within the archaeological samples from early to late turbot and a further 1.7-fold decrease from late to modern. Taken together, this represents a 3.4-fold overall decrease from the earliest samples to the modern (Fig. 3A, B, Table 3). Due to the small size of the modern niches, the directional niche overlap metric becomes highly asymmetric, yielding low ancient to modern overlap (5.8–35.7%) but substantially higher modern to ancient overlap (23.1–97.3%; Table 3).

The large isotopic niche size and the high degree of overlap observed in ancient Black Sea cetacean and turbot specimens suggests overall similarities in their ecological niches. It suggests that each species had a wide dietary range, feeding on various isotopically diverse prey species. Although isotopic niche space can be influenced by spatial or temporal resource shifts rather than diet alone and high niche overlap can occasionally mask the consumption of different prey with identical isotopic signatures, the consistency of this pattern across multiple taxa strongly points to a complex, interconnected historical food web rather than isolated species-specific differences. In contrast, contemporary Black Sea cetaceans show less isotopic niche overlap, which is consistent with previous studies that suggest modern Black Sea cetaceans can have distinct habitat preferences, at least during some of the seasons: common dolphins are primarily found in deep water and where the surface temperatures are relatively low, bottlenose dolphins are found mainly in shallower and warmer waters than common dolphins, and harbour porpoises are distributed in shallower waters but are more tolerant to lower sea surface temperatures than bottlenose dolphins (Sánchez-Cabanes et al., 2017; Paiu et al., 2024). Indeed, most studies of contemporary cetacean species or populations have found limited overlap in isotopic niche which is usually interpreted as ecological niche partitioning due to adaptation to different habitats and/or prey species (Fernández et al., 2011; Buss et al., 2022; MacKenzie et al., 2022; Linnebjerg et al., 2016; Fraleigh et al., 2023; Neves et al., 2023, for an exception see Borrell et al., 2024).

The question arises as to why ancient cetacean and fish populations exhibited such wide and overlapping isotopic niches while contemporary populations do not. We hypothesize that the substantial reduction in niche sizes between the ancient and modern cetaceans and turbot reflects consequences of an ecological collapse of the Black Sea ecosystem. Small niche sizes, as seen in modern specimens, are often linked to specialization, either forced or chosen (Das et al., 2017), which may be explained by the observed structural impoverishment of food webs (Bănaru et al., 2010). Such reductions in dietary niche breadth may also have important implications for population vulnerability, as species with more specialized diets and constrained resource use can be

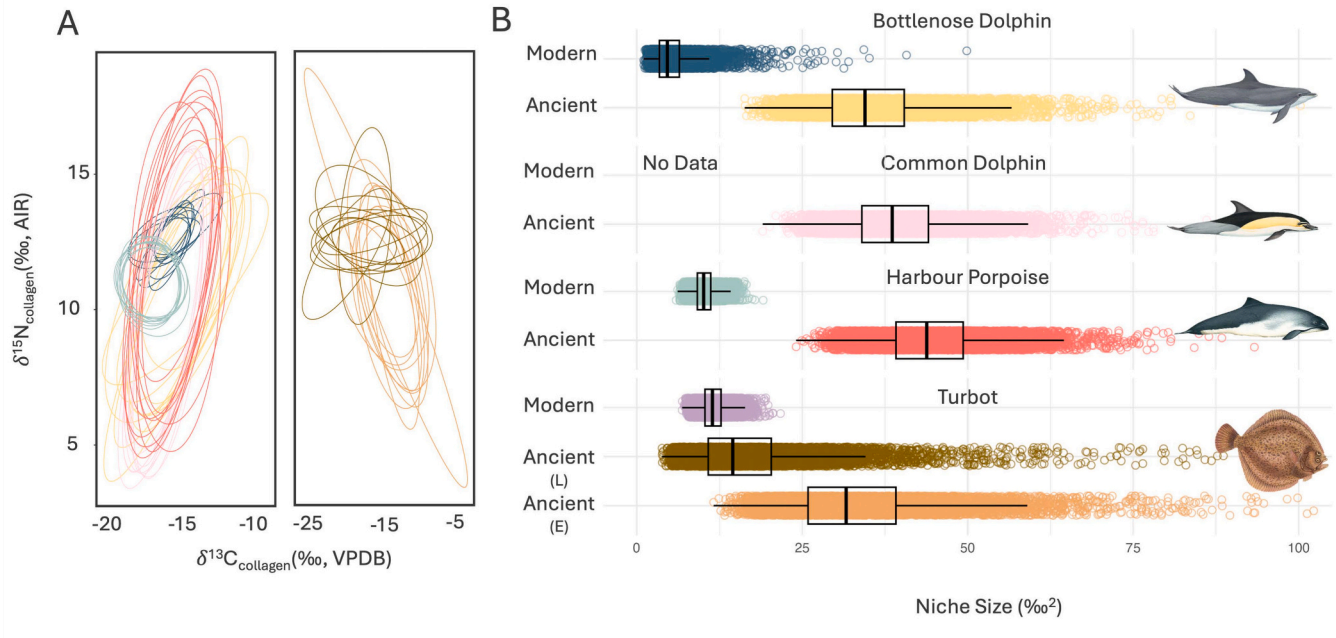


Fig. 3. Isotopic niche size for ancient and modern bottlenose dolphins, harbour porpoises, common dolphins, and turbot in the Black Sea. A: The ecological niche estimated for each of the ancient and the modern cetaceans (left) and the ancient turbot (right) by two-dimensional elliptical projections of the isotopic niche range (NR95; 10 iterations; turbot not included). B: The niche size estimated for modern and ancient cetaceans and turbot as the area of the two-dimensional elliptical projections over 10,000 iterations (no data from modern common dolphins). For turbot, Ancient (L) refers to samples dating to 900-1300 CE and Ancient (E) refers to samples dating to 400-530 CE.

Table 3
Estimation of dietary niche overlap. Pairwise percentages of directional overlap between dietary niches (ellipses) were calculated using nicheROVER and posterior mean (95% intervals). Pairwise percentages over 50 are underlined. Niche region size and associated standard error are along the diagonal in parts per mil.

		Ancient					Modern		
		Bottlenose dolphin	Common dolphin	Harbour porpoise	Turbot		Bottlenose dolphin	Harbour porpoise	Turbot *
					E	L			
Ancient	Bottlenose dolphin	**22 ± 8 ‰²**	78.4	83.6	57.9	14.6	5.28	20.6	–
	Common dolphin	76.2	**40 ± 8 ‰²**	87.8	38.2	13.0	10.3	24.9	–
	Harbour porpoise	68.7	82.5	**45 ± 8 ‰²**	53.1	24.9	19.2	25.7	–
	Turbot E	63.4	46.2	54.8	**34 ± 11 ‰²**	20.4	11.3	8.4	–
	Turbot L	33.5	46.6	58.6	56.3	**17 ± 9.5 ‰²**	35.7	14.8	–
Modern	Bottlenose dolphin	48.1	88.8	97.3	85.2	82.7	**5 ± 3 ‰²**	25.1	–
	Harbour porpoise	37.07	53.2	66.4	34.5	23.1	14.1	**10 ± 2 ‰²**	–
	Turbot *	– –	–	–	–	–	–	–	**12 ± 2 ‰²**

* Modern Turbot data were excluded from the pairwise comparison due to the absence of an appropriate tissue correction factor.

more sensitive to environmental disturbances (Cloyed et al., 2021). A study of Pacific beluga (*Delphinapterus leucas*) shows a similar, albeit smaller, reduction in niche size from the 1800s to the 1900s (Fraleigh et al., 2023).

Because our study looks at these long-term trends by focusing on top predators, it is important to consider how other environmental factors might also play a role. For instance, the narrowed modern niches could be amplified by severe historic population declines within the cetacean populations themselves which can alter competitive pressures and foraging opportunities (Aiken et al., 2023). At the same time, long-term climatic or environmental shifts can introduce baseline changes. Indeed, long-term isotopic shifts in marine apex predators are frequently a complex reflection of combined harvesting pressures, demographic changes, and shifting baselines (Zenteno et al., 2015).

While we are the first to use isotopic analyses of ancient and contemporary samples to demonstrate long-term changes in the Black Sea ecosystem, others have previously suggested a regime shift caused by historical overexploitation, habitat degradation, and collapse of fish stocks in the Black Sea (Oguz and Gilbert, 2007; Birkun, 2002; Tsikliras et al., 2015; Smith, 1978; Oguz and Velikova, 2010). This regime shift resulted in the dominance of a few fish species, such as planktivorous fishes like anchovy and sprat (*Sprattus sprattus*); or mesopredators: horse mackerel (*Trachurus mediterraneus*) and whiting (*Merlangius merlangus*; Daskalov, 2002, 2003; FAO, 2020; Ulman et al., 2020). Indeed, stomach content analyses of contemporary samples indicate a dietary shift in all three cetacean species towards pelagic feeding on a few abundant fish species, including sprat, anchovy, horse mackerel, whiting, and, possibly, sand smelts (*Atherina boyeri*; Bushuev., 2002; Tonay and Dede,

2007; Gladilina and Gol'din, 2014). This shift towards pelagic fish species raises concerns due to commercial fishing activities and the potential for increased mortality through by-catch (Popov et al., 2023). Taken together, the stepwise contraction in turbot isotopic niche from Late Antiquity to the Medieval period, and the more recent collapse in cetacean niches points towards a multi-causal, long-term degradation of the Black Sea ecosystem. Punctuated by seven localized species extirpations since the 15th century, intensive 20th-century eutrophication, and invasive species, the data are consistent with a progressive loss of ecological complexity, driven by historical exploitation, climatic shifts, habitat change, and modern industrial impacts.

3.4. Pre-industrial human and climatic impacts on the Black Sea

The earlier niche contraction, seen in ancient early-late turbot, likely reflects both environmental and anthropogenic pressures. Climatic changes during the transition to the Medieval Warm Period (Neumann, 1991; Lüning et al., 2019) may have altered benthic habitats by increasing stratification, reducing bottom-water oxygenation, and shifting nutrient delivery, thereby decreasing benthic prey diversity and availability. Concurrently, archaeological and historical records indicate that Roman and Byzantine communities harvested fish intensively (Bekker-Nielsen, 2020; Čechová, 2014; Højte, 2005), with strong pressure on coastal and demersal resources. The reduction of large-bodied prey may have led to a simplification of benthic food webs, reducing the isotopic breadth of the prey available to benthic predators such as turbot. These factors likely initiated an early, stepwise simplification of benthic food webs, identified in the reduced isotopic niche of Medieval turbot relative to the earlier specimens.

3.5. Post-industrial and contemporary impacts on the Black Sea

The later niche contraction of both turbot and cetacean isotopic niches reflects the cumulative impact of modern industrial and biological pressures that reshaped the Black Sea food web. Overfishing of both pelagic and demersal stocks removed the large-bodied and diverse prey species, disproportionately targeting historically abundant benthic and mid-water fish (Demirel et al., 2020; Ulman et al., 2020). This reduction in prey diversity forces predators to rely on the remaining abundant species, narrowing their dietary breadth and compressing isotopic niche space. Bottom trawling further damages benthic habitats by physically disrupting sediment structure, reducing habitat complexity, and removing benthic invertebrates, which are key components of turbot diets (Pusceddu et al., 2014; Hiddink et al., 2011). This habitat simplification removes important habitats for key prey species and homogenizes benthic trophic interactions, leaving top predators with fewer, more isotopically similar prey options.

Eutrophication-driven hypoxia starting in the 1980s has exacerbated these effects by creating oxygen-depleted zones that reduce benthic and demersal species richness and force prey into smaller, more constrained habitats (O'Higgins et al., 2014). Biological invasions have likely also played a major role in the restructuring of the Black Sea food web, particularly the introduction of the ctenophore *Mnemiopsis leidyi* (comb jelly) from the western Atlantic in the early 1980s, dramatically affecting the recruitment of both pelagic and demersal fish species by predation (Shiganova et al., 2001). As prey distributions become compressed, predators are forced to feed in overlapping areas on a limited suite of species, reducing both the spatial and trophic diversity that would normally contribute to larger isotopic niche widths. Interestingly, while overall prey diversity is reduced, the modern bottlenose dolphin and harbour porpoise exhibit far less isotopic overlap than their ancient populations. The ancient samples show isotopic niches that range in overlap from ~68% to ~88% (Table 3). Modern direction overlap values are much lower, ~14% to 25% (Table 3), indicating stronger partitioning of the reduced prey base. Alternatively, this pattern may reflect behavioural specialization, spatial segregation, or competitive expulsion in response to a simplified ecosystem, highlighting that even within a collapsed foodweb where resources are limited, predators can maintain distinct foraging strategies.

Finally, climatic changes, including the increased stratification and warming of the Black Sea have likely altered productivity patterns and seasonal prey availability amplifying these effects (Cannaby et al., 2015). Stratification of the water reduces vertical mixing, limiting nutrient delivery to surface waters and affecting the abundance and diversity of pelagic and benthic food sources (Ağırbaş et al., 2026). Similar mechanisms have been observed in other marginal seas, where increasing stratification intensifies existing gradients (Jung et al., 2025).

4. Conclusion and perspectives

The observed reduction in the dietary niche of Black Sea cetaceans and turbot is likely the result of multiple collapses of key species in the Black Sea food web and a transition from a healthy to an unhealthy marine ecosystem. While recent studies using physical and chemical metrics indicate that the Black Sea ecosystem may be slowly recovering (Zhang et al., 2020), its biota remains fundamentally altered both in its composition (Ulman et al., 2020) and, as seen here, ecological structure.

These changes can be understood through the thresholds framework (Atmore et al., 2021), in which marine systems are viewed not as static baselines, but as environments that cross cultural, ecological, and evolutionary thresholds over time. The stepwise contraction of the turbot niche suggests an early ecological threshold was crossed during the Roman-Byzantine period when intensive coastal exploitation and technological explosion simplified foodwebs. Later, modern pressures, including overfishing, eutrophication, and invasive species, likely pushed the system past another ecological threshold, causing a whole-system homogenization. By situating stable isotope-based reconstruction within this framework, long-term human environment interactions are shown to lead to persistent degradation of the Black Sea, not just a shifting baseline. This perspective emphasizes that the observed niche reduction represents not only a record of loss but also a marker of systemic transformation, with important implications for ecosystem-based management and restoration.

Isotopic analysis of bones from archaeological contexts provides the advantage of time depth not normally available to ecological studies allowing a comparison of pre-industrial and recent samples. As a next step, it may be beneficial to analyse additional Black Sea historical specimens to pinpoint the timing and speed of alterations more precisely and perhaps correlate these changes to known events (i.e., the collapse of fisheries). Compound-specific isotopic analyses may also prove to be beneficial as the approach offers fine-scale resolution capable of distinguishing isotopic niches (Yun et al., 2022; Larsen et al., 2022). Additional isotopic analysis of top predators in the Black Sea and elsewhere may also shed more light on the intriguing observation that ancient specimens often show largely overlapping dietary niches. In particular, it raises the question of whether widely reported niche partitioning among contemporary, ecologically similar cetacean species is a recent phenomenon driven by the overexploitation of marine ecosystems.

CRediT authorship contribution statement

Magie Aiken: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paul Szpak:** Writing – review & editing, Supervision, Resources. **Morgan L. McCarthy:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Jennifer Routledge:** Writing – review & editing, Methodology. **Daniela Bănaru:** Writing – review & editing, Resources. **Krishna Das:** Writing – review & editing, Resources. **Elena Gladilina:** Writing – review & editing. **Pavel Gol'din:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Morten Tange Olsen: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2026.182331.

Data availability

The data is available in the supplementary materials

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