Cenozoic megatooth sharks occupied extremely high trophic positions

Emma R. Kast1,2*, Michael L. Griffiths3†, Sora L. Kim4†, Zixuan C. Rao1, Kenshu Shimada5,6,7, Martin A. Becker8, Harry M. Maisch9, Robert A. Eagle9, Chelesia A. Clarke1, Allison N. Neumann3‡, Molly E. Karnes4, Tina Lüdecke10,11, Jennifer N. Leichliter10,12, Alfredo Martinez-García13, Aliiya A. Akhtar1, Xingchen T. Wang14, Gerald H. Haug13,15, Daniel M. Sigman1

Trophic position is a fundamental characteristic of animals, yet it is unknown in many extinct species. In this study, we ground-truth the $^{15}$N/$^{14}$N ratio of enameloid-bound organic matter ($\delta^{15}$N$_{EB}$) as a trophic level proxy by comparison to dentin collagen $\delta^{15}$N and apply this method to the fossil record to reconstruct the trophic level of the megatooth sharks (genus Otodus). These sharks evolved in the Cenozoic, culminating in Otodus megalodon, a shark with a maximum body size of more than 15 m, which went extinct 3.5 million years ago. Very high $\delta^{15}$N$_{EB}$ values ($22.9 \pm 4.4\%$) of O. megalodon from the Miocene and Pliocene show that it occupied a higher trophic level than is known for any marine species, extinct or extant. $\delta^{15}$N$_{EB}$ also indicates a dietary shift in sharks of the megatooth lineage as they evolved toward the gigantic O. megalodon, with the highest trophic level apparently reached earlier than peak size.

INTRODUCTION

The ecology of ancient marine vertebrates is often investigated with fossil evidence of predator-prey interactions, such as bite marks, preserved stomach contents, or coprolites (1). More frequently, feeding strategies and diet are inferred from the morphological characteristics of fossils, such as jaw size or tooth shape [e.g., (2–4)]. Fossil evidence of predator-prey interactions can be rare and typically captures only a snapshot in time, while morphological characteristics tend to group taxa into broad categories and are related not only to current diet but also to the accumulated history of millions of years of evolution [e.g., (5)]. In addition, the co-occurrence of taxa informs ecological reconstruction but does not confirm interactions among taxa. These approaches provide initial hypotheses for ancient ecosystems and animals, but methodological advances provide new opportunities for geochemical diet proxies. In this study, we use a novel geochemical method to fill some of these gaps in our knowledge, with a focus on the diets of Cenozoic megatooth sharks.

Our understanding of ancient and modern animal ecology has increased with stable isotope analysis. The stable carbon, oxygen, and strontium isotope composition of fossil bones and tooth enamel is used to investigate primary producers in the food web; to distinguish terrestrial, aquatic, and marine habitats; and to reconstruct physiology [(6) and references therein]. The calcium isotope composition ($^{44}$Ca/$^{42}$Ca) of tooth enamel(oid) and the zinc isotope composition ($^{64}$Zn) of bone are emerging proxies for trophic level in the marine setting (6–8). However, the determination of trophic level from the fossil record is still poorly developed, particularly on million-year time scales.

The nitrogen isotope composition ($\delta^{15}$N) of animal tissues is a powerful and well-studied tool in identifying trophic level in modern ecosystems [e.g., (6)]. Animals require nitrogen from their diet. The $\delta^{15}$N of animal tissues in turn reflects the $\delta^{15}$N of their dietary nitrogen, but with a roughly 3‰ elevation, often referred to as the “trophic discrimination factor” (TDF) (6). The TDF arises from isotopic discrimination associated with nitrogen metabolism and excretion, where preferential excretion of $^{14}$N as waste leaves the tissue nitrogen elevated in $\delta^{15}$N (6). Because of this discrimination, the $\delta^{15}$N of an organism’s tissue can be used as an indicator of its diet and trophic position.

Despite this solid basis for application, $\delta^{15}$N has only been measured in relatively recent (<14,000 years) marine vertebrates, specifically on fossil bone collagen [e.g., (9, 10)]. Collagen is not well preserved beyond a 10,000- to 100,000-year time scale because it is chemically labile and largely exposed, making it susceptible to alteration and loss during early diagenesis (11). Occurrences of million-year-old preserved collagen are rare [e.g., (12, 13)]. As a result, the application of $\delta^{15}$N-based trophic level proxies has been limited to the recent past.

In this study, we build on the well-established practice of using $\delta^{15}$N to understand ecology and diet by introducing a new substrate for $\delta^{15}$N-based paleoecological reconstructions on million-year time scales: the nitrogen-containing organic matter bound within the enameloid mineral matrix of shark teeth. Enameloid is a highly mineralized bioapatite structure with <5 weight % organic matter, analogous to enamel although with some differences in formation, structure, and composition (14, 15). Enameloid-bound organic
matter is composed of residual proteins from tooth formation (16). The method relies on technical advances in the isotopic measurement of nanomole quantities of nitrogen (N), specifically the coupled oxidation-denitifier method (17–19), which is necessary to measure the isotopic composition of the very low concentration of enameloid-bound organic matter. Recent analyses have shown that, similar to other tissues, the δ15N of modern terrestrial mammal tooth enamel organic matter records diet and trophic level enrichment (19). In contrast to other fossil types, the apatite mineral of enameloid is resistant to alteration (11, 20). Furthermore, other mineral-bound organic matter proxies such as foraminifera carbonate have preserved δ15N signals over million-year time scales (21), suggesting that enameloid-bound organic matter might have a similar preservation potential, far beyond the time scales possible with collagen in bone and tooth dentin.

To ground-truth the δ15N of enameloid-bound organic matter (enameloid-bound δ15N, δ15N_EEB), we compare δ15N_EEB and dentin collagen δ15N from modern sand tiger (Carcharias taurus) shark teeth. We then apply the δ15N_EEB method to the fossil record to reconstruct the trophic ecology of a group of large megatooth sharks (genus Otodus) that evolved during the Cenozoic (~66 to 3.5 million years ago).

The most notable megatooth shark is Otodus megalodon of the family Otodontidae, with a conservative estimated maximum body length of 15 to 20 m (22, 23), the largest known macrophagous shark. Its ancestors include other species of the genus Otodus that evolved from Cretalamna rooted in the Cretaceous (24). O. megalodon is well known for its large tooth, up to 16.5 cm in length of 15 to 20 m (22), family Otodontidae, with a conservative estimated maximum body length of 15 to 20 m (22, 23), the largest known macrophagous shark. Its ancestors include other species of the genus Otodus that evolved from Cretalamna rooted in the Cretaceous (24). O. megalodon is well known for its large tooth, up to 16.5 cm in length, but the conservative estimate life span is 22 million years (22, 23, 25). Climatic and ecological causes [e.g., (25–27)] have been proposed for its extinction approximately 3.5 million years ago (26), but so far, there is limited evidence as to the ecology of megatooth sharks.

O. megalodon is widely assumed to have been an apex predator of the Neogene ocean. Its large, triangular, serrated teeth [e.g., (2)] and bite marks in fossil cetacean and pinniped bones suggest that adult O. megalodon had a diet of marine mammals [28–30 and references therein]. While this evidence is compelling, the morphological trend observed in the megatooth shark lineage may not necessarily suggest any possible dietary preference or shift (31), and bite marks reflect brief events that may not represent the overall diet of O. megalodon. A high trophic level for O. megalodon has been inferred from low δ18O/δ13C values of two Pliocene teeth (7); however, this evidence is so far limited in scope with respect to sample size, temporal span, and spatial distribution. Identifying the trophic position of these megatooth sharks is crucial for characterizing their ecology and testing hypotheses about their evolution and extinction that involve their reliance on or competition with specific marine mammal taxa [e.g., (26, 28)].

**RESULTS AND DISCUSSION**

**Analytical precision of δ15N_EEB measurements**

We assessed the analytical precision of the δ15N_EEB measurement with a fossil enameloid standard that was run in triplicate alongside samples in every batch. For comparison, we also concurrently ran a coral carbonate standard that is regularly used as a general laboratory reference for mineral-bound δ15N measurements. The method for coral-carbonate–bound organic matter δ15N has been applied extensively, with analytical uncertainties that are well understood (32). For the same batches from 2017 to 2020, the long-term variability (1 SD) was 0.70‰ (average variability 0.37‰ within batches) for the fossil enameloid standard δ15N_EEB and 0.29‰ (average variability 0.21‰ within batches) for the coral carbonate standard δ15N (fig. S1, A and B). In terms of nitrogen (N) content, the long-term variability was 0.62 μmol N/g (7.4%, average variability 0.38 μmol N/g within batches) for the fossil enameloid standard and 0.11 μmol N/g (5.4%, average variability 0.08 μmol N/g within batches) for the coral carbonate standard (fig. S1, C and D).

The higher δ15N_EEB and N content variability of the fossil shark enameloid standard could be explained either by an intrinsic material property of enameloid that results in lower analytical precision or by the heterogeneous composition from preservation of the fossil enameloid standard. The long-term variability is higher than the variability within a batch of analyses, suggesting batch-to-batch effects of the enameloid cleaning: This is not captured by the nitrogen isotope standards used to correct our δ15N_EEB results. Despite these technical details, the analytical precision is sufficient, especially given the large magnitude of δ15N_EEB differences we observe among modern and fossil enameloid specimens.

**Modern ground-truthing of the δ15N_EEB proxy**

While dentin collagen is not a reliable source of organic nitrogen in ancient fossils, the δ15N of dentin collagen in modern sharks has been established as a trophic level proxy that robustly records variations in the dietary δ15N value (33, 34). As dentin collagen and enameloid are formed over similar time frames, we expect that the δ15N values of these tissues should covary in response to diet and physiology. The δ15N_EEB and dentin collagen δ15N values from 13 modern C. taurus teeth from different individuals are correlated (Pearson’s correlation 0.75, t = 3.76, df = 11, P = 0.0031; Fig. 1). The dentin collagen δ15N values range from 13.7 to 15.9‰, while δ15N_EEB values range from 15.4 to 18.0‰. A Deming regression (total least squares regression accounting for the ratio of errors between δ15N_EEB and dentin collagen δ15N) yields the relationship δ15N_EEB ~ 0.97 [95% confidence interval (CI): 0.44 to 1.5] × δ15N_dentin-collagen + 2.1‰.
The modern C. taurus δ15N values only span 2‰ but show promise in the close correspondence between enameloid-bound and dentin collagen δ15N (Fig. 1). The organic nitrogen within enameloid and dentin collagen is derived from diet and therefore might be expected to have closely overlapping δ15N values. The 1.7 ± 0.5‰ offset between the δ15N values of these tissues may be due to differences in amino acid composition. Shark enameloid mineralization occurs based on an organic protein matrix (14–16) and previous studies have identified noncollagenous protein within enameloid ([15, 16] and references therein). That protein is the main component of the enameloid-bound organic nitrogen and likely has a distinct amino acid composition from dentin collagen. This compositional difference may drive the δ15N of these tissues as amino acids can have distinct δ15N values [e.g., in sharks; (35)].

An additional consideration is the time over which enameloid-bound organic nitrogen and dentin collagen integrate the dietary δ15N signal. Enameloid mineralization precedes dentin formation and mineralization (15), with the exact timing suggestive to the rate of tooth replacement. The integration time will also be affected by the residence time of the tissue. A diet-switching experiment with captive leopard sharks (<1 m) had a residence time of 45 to 60 days for the carbon and nitrogen isotope composition of dentin collagen (33). Tissue incorporation rates scale metabolically (36, 37); therefore, the megatooth sharks in this study would likely have longer residence times. The difference in integration time of enameloid-bound organic nitrogen and dentin collagen would tend to reduce the correlation between δ15NEB and dentin collagen δ15N and may explain some of the variability of the observed offset of δ15NEB from dentin collagen δ15N (Fig. 1B). However, it is unlikely to explain the observed δ15N offset between the two tissues.

Shark dentin collagen has a TDF of 2.0 to 2.8‰ (33), implying a TDF of approximately 4% for enameloid-bound organic matter. This enameloid TDF is consistent with a recent controlled feeding experiment that estimated TDF values for rodent tooth enamel bound organic matter δ15N between 1.9 and 4.9‰ (19). The δ15NEB values of sharks can be related to δ15N measurements of other shark tissues (muscle, plasma, red blood cells, and fin) through their comparison to dentin collagen δ15N (33, 34). Notably, dentin collagen δ15N is on average 1.9 ± 0.7‰ lower than muscle δ15N (34). Combined with our observations of δ15NEB values that are on average 1.7 ± 0.5‰ higher than dentin collagen δ15N, this suggests that δ15NEB should closely match shark muscle δ15N. Future studies should sample a broader range of dentin collagen δ15N and δ15NEB to validate the offset we propose. In any case, the specimens in our study indicate a robust relationship between the δ15N values of these tissues and provide a framework to interpret trophic level from fossil shark teeth.

Enameloid-bound δ15N through time
We report δ15NEB measurements for the modern Carcharodon carcharias and Neogene C. carcharias, Carcharodon hastalis, Otodus chubutensis, and O. megalodon, from a variety of localities (Fig. 2). In addition, we report δ15NEB measurements for four O. megalodon ancestors: Late Cretaceous Cretalamna sp., Paleocene Otodus obliquus, Eocene Otodus auriculatus, and Oligocene Otodus angustidens (Fig. 2) (24). We compare these data to δ15NEB measurements of taxa with piscivorous diets (38): Late Cretaceous Scapanorhynchus spp.; Paleoecene Striatolamia spp., Scapanorhynchus elegans, and Palaeohypotodus rutovi; Eocene Striatolamia macrota and Carcharias sp.; Oligocene Carcharias sp.; Neogene Carcharias sp.; and modern C. taurus (Fig. 2). Distinct tooth morphologies and dentitions were used to infer prey preferences. In general, the teeth of piscivorous sharks are slender, elongated, and have smooth cutting edges, while those of macropredatory larger sharks, including megatooth sharks, are broad, robust, and serrated [(3) and references therein]. The average δ15NEB of piscivorous sharks varies between 13.4 and 16.5‰ across all epochs. High δ15NEB values are seen in Eocene O. auriculatus (24.4 ± 1.5‰), Oligocene O. angustidens (23.8 ± 3.2‰), Miocene O. chubutensis (24.9 ± 2.8‰), and O. megalodon from the Miocene (21.8 ± 5.8‰) and Pliocene (23.4 ± 3.6‰). Intermediate to these values are O. obliquus in the Paleocene (20.0 ± 1.9‰) and C. carcharias in the Pliocene (18.8 ± 2.1‰) and modern (19.2 ± 1.2‰), C. hastalis, which gave rise to extant C. carcharias (39), has similar δ15NEB values to C. carcharias in the Miocene (18.9 ± 2.5‰) but lower, more piscivore-like values in the Pliocene (16.1 ± 3.5‰). The ancestor of the megatooth (Otodus) lineage, Cretalamna sp., has δ15NEB values of 14.0 ± 0.7‰, similar to contemporaneous Late Cretaceous piscivorous sharks.

Interpreting the fossil δ15N signal
Before interpreting δ15NEB values of fossil teeth in terms of trophic level, we address the geochemical integrity of δ15NEB and the potential influence of baseline variations in δ15N. We explore the possibility for diagenetic alteration of the enameloid-bound organic matter by examining the N content of the enameloid. We found that the N content of fossil enameloid is on average lower than modern enameloid (4.8 ± 2.0 μmol N/g versus 7.4 ± 1.9 μmol N/g), although many fossil teeth have N content within the range of modern teeth (fig. S2, S3). O. megalodon, has a higher N content relative to the other fossil enameloid samples, not a surprising finding given previous observations of species-specific differences in biomineral-bound N content [e.g., in foraminifera (40), fish otoliths (41), and tooth enamel (19)]. The lower N content of some fossil samples may suggest loss of organic matter from the enameloid matrix. If this loss occurred with substantial isotopic fractionation, we would expect a negative correlation between δ15NEB and N content for these teeth as residual organic nitrogen would be left with a progressively higher δ15N value [e.g., in (42)]. However, this is not observed (fig. S2), and instead, we see a weak positive correlation in the fossil data overall with no significant correlation when considering the megatooth or piscivorous shark fossil data separately (fig. S2).

We hypothesize that the N content decline from modern to fossil enameloid occurred during early diagenetic maturation of the enameloid (e.g., small-scale recrystallization). During this process, a portion of the biomineral-bound organic matter could be exposed and fully degraded without preference for its chemical and/or isotopic composition and therefore have no substantial impact on δ15NEB, similar to what has been observed in modern foraminifera (40).

In addition to trophic position, the δ15N of animal tissues is influenced by the δ15N of nitrogen supplied to the base of the food web. This "baseline" δ15N signature is incorporated by autotrophs as they assimilate biologically available nitrogen from the environment, typically in the form of nitrate (NO3−) or ammonium (NH4+). Baseline δ15N can vary spatially (43) and through time [e.g., (21)] due to a variety of local and global nitrogen cycle processes in the ocean (43) and may complicate the interpretation of δ15NEB as a
trophic level proxy. Thus, considering baseline $\delta^{15}N$ is important when reconstructing trophic level.

To constrain the potential impact of baseline $\delta^{15}N$ variations on our $\delta^{15}N_{EB}$ results, we examine the $\delta^{15}N_{EB}$ values of the piscivorous sharks measured in this study. On the basis of their tooth morphology and comparisons to modern representatives, we assume that these piscivorous sharks maintained a similar diet and trophic level through time (38) and attribute $\delta^{15}N_{EB}$ variations in these taxa to baseline $\delta^{15}N$ variations propagating up the food chain. In the modern ocean, the $\delta^{15}N$ values of large marine animals have been shown to directly reflect geographic patterns of baseline $\delta^{15}N$ values (44), although an extensive temporal or geographic comparison has not been done for $\delta^{15}N$ values in modern sharks. The $\delta^{15}N$ measurements of individual amino acids can disentangle the trophic and baseline components of the $\delta^{15}N$ signature in modern stable isotope ecology ([35] and references therein). Unfortunately, this approach is not yet possible with enameloid-bound organic nitrogen due to analytical sample size requirements but could be an important path forward in the future upon methodological advancements. The use of piscivorous shark taxa, rather than taxa lower in the food web like foraminifera or bivalves, to constrain baseline $\delta^{15}N$ is motivated by the notion that these sharks are more likely to share an environmental range and therefore integrate similar spatial and temporal variations of baseline $\delta^{15}N$ as the *Otodus* sharks. Furthermore, there is added continuity in comparing $\delta^{15}N$ values from the same substrate, enameloid-bound organic nitrogen.

From piscivorous taxa, we observe minimal change over time in the average $\delta^{15}N_{EB}$ values (Fig. 2 and fig. S4). In addition, average $\delta^{15}N_{EB}$ differences are <3‰ for genera that were sampled at multiple locations, and these differences are small relative to differences between piscivores and the other taxa, which are conserved across localities (fig. S5). From these piscivorous shark $\delta^{15}N_{EB}$ data, we do not see evidence for large temporal or geographic baseline $\delta^{15}N$ effects, and so, we interpret the $\delta^{15}N_{EB}$ variations of the megatooth sharks in this study as dominantly a trophic level signal.

**Trophic level and diet of *Otodus megalodon***

*O. megalodon* $\delta^{15}N_{EB}$ values are very high, with a large range of values in both the Miocene and Pliocene (Fig. 2A). Average $\delta^{15}N_{EB}$ values for these two epochs are indistinguishable, and they are also consistent across locations; average *O. megalodon* $\delta^{15}N_{EB}$ in Japan, North Carolina, and California are within 3‰ of each other, and the differences are not significant (fig. S5). *O. chubutensis*, which gave rise to *O. megalodon* (2), has similar $\delta^{15}N_{EB}$ values (Fig. 2A).

There is a large range of $\delta^{15}N_{EB}$ values for *O. megalodon* (Fig. 2A). We can generally rule out spatial $\delta^{15}N$ variability as the cause for this large $\delta^{15}N_{EB}$ range, as the differences between regions are small (fig. S5). The large $\delta^{15}N_{EB}$ range may be driven by an ontogenetic
shift, where larger *O. megalodon* occupy a higher trophic level, as seen in modern *C. carcharias* (45). However, with this dataset, it is unlikely that we can detect ontogenetic changes or that ontogenetic changes are driving the large spread in these δ¹⁵N values of *O. megalodon*, as the samples studied here are mostly from mid-size individuals. We estimated the total animal length from the crown height of 12 fossil *O. megalodon* teeth that were also measured for δ¹⁵Nₑₑ. Estimated length ranged from 5.8 to 10.4 m, with an average length of 8 ± 1.5 m (fig. S6). We note that we often targeted fragmentary teeth in this study as we were performing destructive analyses, and so, our estimated sizes are less certain than those based on complete teeth. In any case, the estimated total lengths are all in the mid-size range of the overall size distribution of *O. megalodon* (46) and show no correlation to δ¹⁵Nₑₑ (fig. S6).

There are two outstanding possibilities to explain the large range in *O. megalodon* δ¹⁵Nₑₑ. First, we may be overlooking high-frequency baseline δ¹⁵N changes that are not evident when analyzing the specimens grouped by epoch. This possibility could be investigated with more precise specimen age information. Alternatively, and our favored explanation, the large range δ¹⁵Nₑₑ values for *O. megalodon* may reflect a fundamental aspect of their ecology, specifically, a generalist diet, with individuals feeding across many prey types and different trophic levels. Modern ecological studies have shown large δ¹⁵N variations between individuals in many apex predators, including *C. carcharias* and other sharks, attributed to generalist feeding behavior [e.g., (45, 47)]. As enameloid-bound organic matter integrates over relatively short time periods, we cannot distinguish between interindividual differences in diet preference and intra-individual generalist feeding behaviors.

The δ¹⁵Nₑₑ of *O. megalodon* is, on average, 7.3‰ higher than that of contemporaneous piscivorous sharks (Fig. 2B) and also significantly higher than the largest extant macrophagous shark, *C. carcharias*, indicating prey with a particularly high δ¹⁵N. Estimated TDFs for shark muscle, the predominant tissue type in modern specimens, range from 2.3 to 5.5‰, with most values on the lower end of this range (37, 48). Muscle TDFs are also relevant here because δ¹⁵Nₑₑ values are approximately equivalent to muscle δ¹⁵N (see modern ground-truthing discussion above). On the basis of the full range of estimated TDFs (2.3 to 5.5‰), *O. megalodon* is, on average, 1.3 to 3.2 trophic levels above the piscivorous sharks, with a high likelihood that it was more than 2 trophic levels higher (Fig. 2B). Considering that the modern piscivore *C. taurus* has an estimated trophic level of 4.4 (49), this implies an average trophic level of 5.7 to 7.6 for the *O. megalodon* (Fig. 2B) and a trophic level range from 3.3 in the lowest δ¹⁵Nₑₑ individual to 9.6 in the highest δ¹⁵Nₑₑ individual, using a mid-range TDF of 2.5‰. This conclusion provides quantitative, integrative geochemical evidence of a very high, and flexible, trophic level for *O. megalodon*, and generally supports previous inferences from tooth morphology, fossilized bite marks on marine mammal bones, and tooth enameloid δ⁴⁴/⁴²Ca data (2, 7, 28–30).

To contextualize these findings, we estimated dietary δ¹⁵N from the *O. megalodon* δ¹⁵Nₑₑ values by subtracting the 1.7‰ average offset between δ¹⁵Nₑₑ and dentin collagen δ¹⁵N (Fig. 1B) and an average TDF of 2.5‰ associated with dentin collagen (33, 45). This results in an estimated dietary δ¹⁵N of 18.8 ± 4.4‰ for *O. megalodon* (Fig. 3A). We compare this diet δ¹⁵N estimate to modern δ¹⁵N measurements of marine mammals and sharks from the literature (Fig. 3, B and C). The lower half of estimated dietary δ¹⁵N values corresponds well with the δ¹⁵N range of many marine mammal and shark species. On the other hand, the higher estimated dietary δ¹⁵N values are more difficult to match with the compilation of modern sharks and marine mammal δ¹⁵N values. Some marine mammal individuals do have δ¹⁵N values around the maximum *O. megalodon* estimated diet δ¹⁵N, predominantly eared seals (family Otariidae). The highest value, 26.2‰ for the bone collagen of a young South American sea lion (*Otaria flavescens*) (50), is higher than the highest estimated diet δ¹⁵N of *O. megalodon* by ~1‰ (Fig. 3). However, these high otariid δ¹⁵N values are driven in part by the locally highly elevated baseline δ¹⁵N off the east coast of South America (50). In addition, the reliance on a single regional species to explain the five highest *O. megalodon* δ¹⁵Nₑₑ values is likely insufficient. The highest δ¹⁵N values outside of this region are from a group of polar bears (*Ursus maritimus*, family Ursidae) with an average δ¹⁵N of 22.5‰ (51), an unlikely prey item for *O. megalodon* given its habitat and origins in the Pleistocene (52), and an individual orca (*Orca orca*, family Delphinidae) from California with a δ¹⁵N value of 22.5‰ (53). However, these fail to reach the highest estimated dietary δ¹⁵Nₑₑ values of 25‰.

There are possible explanations that could reconcile the very high δ¹⁵Nₑₑ of *O. megalodon* with modern marine mammal δ¹⁵N values. First, Neogene marine mammals may have fed at higher trophic levels than do modern representatives, perhaps due to changes in diversity (54) or functional strategies (55). One example may be the presence of raptorial sperm whales in the Miocene (4). Second, *O. megalodon* may have had a unique feeding strategy, such as targeting nursing marine mammal pups or intraspecific cannibalism, both of which could have elevated its δ¹⁵N [e.g., (56, 57)]. Cannibalism has been observed in modern sharks [e.g., (58)]. Third, we may have applied a TDF that is too small, artificially increasing estimated prey δ¹⁵N. While additional constraints will be necessary to establish exactly how individual *O. megalodon* achieved such elevated δ¹⁵Nₑₑ values, *O. megalodon* was at a high trophic level that is not represented in modern ocean food webs. If we look to the highest trophic level marine mammal predators, transient orcas and polar bears, their δ¹⁵N values do not cover the range of estimated *O. megalodon* diet δ¹⁵N (Fig. 3), let alone the δ¹⁵N of the *O. megalodon* individuals themselves.

These results have implications for possible biotic extinction mechanisms of *O. megalodon*. For example, hypotheses for the extinction of *O. megalodon* have invoked changes in the diversity and size of baleen whales (mysticetes) (26, 28). The high δ¹⁵Nₑₑ values of *O. megalodon* indicate that baleen whales were not the dominant prey of *O. megalodon*, as modern baleen whales have a low trophic level and a correspondingly low δ¹⁵N (Fig. 3, families Balaenopteridae and Balaenidae). Given the fossil evidence for baleen, Miocene and Pliocene mysticete species likely had similar feeding patterns to their modern counterparts (59–61). These observations do not rule out second-order interactions between *O. megalodon* and baleen whales, but they do argue that the extinction of *O. megalodon* was not due to the loss of baleen whales as its main prey.

Another proposed biotic extinction mechanism involves competition, rather than prey availability, namely, that competition with the white shark (*C. carcharias*) drove the extinction of *O. megalodon* (25, 26). The significant δ¹⁵Nₑₑ differences between *O. megalodon* and *C. carcharias* indicate that large individuals of these species were likely not competing for the same diet (Figs. 2 and 3). However, it has been suggested that *C. carcharias* may have competed with juvenile *O. megalodon* for resources (26), which could be plausible...
given that we do not report $\delta^{15}$N values for small (<5 m) *O. megalodon* in this study. We do note that the $\delta^{15}$N values of *Pliocene C. carcharias* and *Miocene C. hastalis* are similar, which suggests that the onset of this possible competition dynamic is earlier than the extinction timing of *O. megalodon* 3.5 million years ago (Fig. 2) (26). A larger $\delta^{15}$N dataset for *O. megalodon* and other shark taxa from the Miocene onward, especially with finer-scale age constraints and a focus on small individuals, may yield deeper insight into its demise in the Pliocene.

**Trophic evolution of the megatooth sharks**

From the Late Cretaceous to the Neogene, the $\delta^{15}$N of the megatooth (*Otodus*) lineage sharks diverges from that of contemporaneous piscivorous sharks (Fig. 2). Whereas *Cretalamna* sp. has similar $\delta^{15}$N values to the piscivorous *Scapanorhynchus* spp. of the time, Paleocene *O. obliquus* $\delta^{15}$N values are elevated by ~5‰ from contemporaneous piscivores, and in the Eocene, *O. auriculatus* values are elevated by 11‰ (Fig. 2B). This divergence in $\delta^{15}$N reflects the evolution of megatooth sharks toward the very high trophic level of *O. megalodon* and supports previous interpretations of dietary shifts based on tooth morphology (2).

The initial increase in $\delta^{15}$N values of the megatooth sharks occurs between the Cretaceous *Cretalamna* sp. and the Paleocene *O. obliquus*, before the emergence of marine mammals in the Eocene (62). This suggests that, at least for the megatooth sharks in the Paleocene, their evolution toward high trophic levels and larger size was disconnected from the evolutionary history of marine mammals. On the other hand, marine mammals were contemporaneous with the very high $\delta^{15}$N Eocene *O. auriculatus* (63). This coincidence may indicate a connection between the evolutionary history of marine mammals and the transition from the Paleocene *O. obliquus* to the very high $\delta^{15}$N values of Eocene through Pliocene *Otodus*.
species. Some Eocene cetacean taxa were likely feeding at a high trophic level, including eating smaller whales [e.g., (64, 65)], which should have resulted in high tissue $\delta^{15}N$ values. Predation on these taxa could have contributed to the very high $\delta^{15}N_{EB}$ values of Eocene $O. auriculatus$. Further studies of fossil shark tooth $\delta^{15}N_{EB}$ should help to disentangle the role of such macroevolutionary connections from oceanographic and climatic drivers of shark and marine ecosystem evolution over the Cenozoic.

Unexpectedly, the elevated $\delta^{15}N_{EB}$ values of $O. megalodon$ relative to contemporaneous piscivores had already been reached by $O. auriculatus$ in the Eocene and were maintained in the Oligocene by $O. angustidens$ (Fig. 2). These ancestors of $O. megalodon$ had an estimated maximum length of at least 8 m but were markedly smaller than $O. megalodon$ (Fig. 4) (22). This pattern suggests that the huge size of $O. megalodon$ was not a necessary condition for its very high trophic level. Instead, it is possible that the very high trophic level contributed to allowing $Otodus$ to evolve toward gigantism, which itself was encouraged by the benefits of regional endothermy and its embryos’ oophagy-based intrauterine cannibalism (22, 66).

**MATERIALS AND METHODS**

**Materials**

This study examined 162 tooth specimens of modern and extinct sharks housed in the following three repository institutions (data file S1): Calvert Marine Museum (CMM), Solomons, MD, USA; San Diego Natural History Museum (SDNHM), San Diego, CA, USA; and United States National Museum of Natural History (USNM; Smithsonian Institution), Washington, DC, USA. The following individuals helped secure or loaned the reposited specimens for destructive sampling: M.A.B., G. Cliff, T. Deméré (SDNHM), S. Godfrey (CMM), D. Fox, K. Fuji, Y. Kurihara, H. Maisch, A. Millhouse (USNM), J. Nance (CMM), A. Sekita, K. Shimada, S. Tanaka, H.-D. Sues (USNM), D. Ward, B. Welton, and T. Yamamoto. For fossil specimens, primary literature sources describing the geology and paleontology of field localities for teeth examined in this study include the following: NC, Purdy et al. (29), Maisch et al. (67), Maisch et al. (68); Japan, Shimada and Shimada (69, 70); and NJ, Maisch et al. (63).

The exact stratigraphic horizon for USNM 431694-1, 431694-2, and 431694-3 (fossil $C. carcharias$ teeth from Neuse River, NC, USA) is uncertain, but they are interpreted to have come from the Pliocene because $C. carcharias$ is regarded as a post-Miocene taxon (39) and Pliocene marine deposits are common in the area (71).

**Enameloid-bound organic matter $\delta^{15}N$ ($\delta^{15}N_{EB}$)**

The technique to measure $\delta^{15}N_{EB}$ employs methods developed to analyze the $\delta^{15}N$ of nanomole quantities of nitrogen (17, 72) that have been applied to other modern and fossil biominerals such as foraminifera shells (40, 73), diatom frustules (18), coral skeleton (32), fish otoliths (74), and mammalian tooth enamel (19) and now adapted for use with shark tooth enameloid.

Modern and fossil shark tooth enameloid powders were prepared by drilling from the enameloid layer, with care taken to avoid sampling the underlying dentin. Samples were drilled either at Princeton University (Princeton, NJ) or William Paterson University (Wayne, NJ).

Powdered enameloid samples were cleaned in three steps. Each sample was composed of approximately 20 mg of enameloid powders. Between each cleaning step, samples were rinsed three times with high-purity water (HPW). First, 10 ml of a 10% polyphosphate solution (adjusted to pH 8 with the stepwise addition of 4 N reagent grade HCl, ~2 ml) was added to samples in 15-ml centrifuge tubes, and samples were sonicated for 5 min to remove any clays or external detritus. Second, 10 ml of a sodium dithionite solution (31 g of sodium citrate + 10 g of sodium bicarbonate + 25 g of sodium dithionite in 500 ml of HPW, adjusted to pH 8 with the addition of 2 ml of 4 N reagent grade HCl) was added, and samples were placed in an 80°C water bath for 1 hour. The goal of this reductive cleaning step is to remove any oxidized coatings or contaminants. Third, samples were transferred to premuffled 12-ml borosilicate vials to which 5 ml of a basic potassium persulfate solution (2 g NaOH + 2 g potassium persulfate in 100 ml of HPW) was added, and samples were
autoclaved at 120°C for 1 hour. This last cleaning step serves to oxidize and remove any organic matter external to the mineral structure of the enameloid. Samples were rinsed four times with HPW and dried down overnight in an oven at 60°C.

Between 2 and 5 mg of cleaned enameloid powders was weighed into premuffled 4-ml borosilicate vials. To dissolve the enameloid and release the enameloid-bound organic matter, 80 µl of 4 N HPLC grade HCl was added, and samples were allowed to sit for up to an hour until they appeared completely dissolved. To oxidize the organic matter to nitrate, 1 ml of a basic potassium persulfate solution (2 g of high-purity NaOH + 1 g of four times recrystallized potassium persulfate in 100 ml of low-temperature distilled HPW) was added, and samples were autoclaved at 120°C for 1 hour to promote oxidation. Two amino acid isotope references, USGS40 (δ¹⁵N = −4.5‰) and USGS65 (δ¹⁵N = 20.68‰) (75, 76), and blanks were oxidized concurrently with the samples. Aliquots of these references with N and USGS65 (δ¹⁵N = 47.55 ± 0.08‰); Costech acetanilide (n = 4, δ¹⁵N = −0.38 ± 0.06‰)). Dentin collagen δ¹⁵N values are reported in data file S1.

**Dentin collagen δ¹⁵N measurements**

Collagen was isolated from tooth dentin to determine the stable isotopic composition of organic nitrogen. Modern shark tooth powders were prepared by drilling from the dentin layer. First, 3 to 4 mg of sample powder was transferred to microcentrifuge tubes. Next, 1.5 ml of chilled 0.1 M HCl was added, and samples were allowed to denaturize under refrigeration for 25 min. After de-mineralization, samples were rinsed five times with deionized water and freeze-dried overnight. Samples were weighed out to 0.4 to 0.5 mg in 3 × 5 mm tin capsules. Collagen samples were measured for δ¹⁵N values using a Costech 4010 Elemental Analyzer coupled to a Delta V Plus continuous flow isotope ratio mass spectrometer with a Conflo IV in the Stable Isotope Ecosystem Lab of (SIELO) University of California Merced. All data were corrected for linearity and drift using a suite of calibrated reference materials [USGS 40 (n = 9, δ¹⁵N = −4.52 ± 0.16‰); USGS 41a (n = 5, δ¹⁵N = 47.55 ± 0.08‰); Costech acetanilide (n = 4, δ¹⁵N = −0.38 ± 0.06‰)]. Dentin collagen δ¹⁵N values are reported in data file S1.

**O. megalodon size estimates**

The total lengths of *O. megalodon* individuals were estimated from the tooth crown heights (CH) of the fossil teeth. We used Perez et al.’s (23) reconstructed dentitions of *O. chubutensis* and *O megalodon* based on a disarticulated but associated tooth set to determine the approximate tooth position of each of our *O. megalodon* samples and to determine the missing portion in the case of fragmentary specimens. This process led to the determination of the approximate tooth position for a total of 12 tooth specimens.

For total length estimations, we used Shimada’s (81) linear functions that represent CH to total length relationships of extant *C. carcharias*. Perez et al.’s (23) total length estimation method, which also requires additional assumptions and knowledge of the crown width or its ratio with the crown height, was not readily applicable to our samples because many of them were fragmentary, making their crown width too uncertain. Among the 12 tooth specimens, CMM-V-10506, 10971, and 10974 were identified as upper anterior teeth because of their tall symmetrical, broad (= non-constricted) crown, and Shimada’s (2019) linear function for upper anterior teeth (“U”), found to still give robust total length estimates (23), was used (81). CMM-V-10505 was identified as a lower anterior tooth because of its tall symmetrical but constricted crown, and Shimada’s (81) linear function for lower anterior teeth (“L”) was used. CMM-V-10507, 10508, 10509, 105968, 10970, 10973, and SDNHHM 143306-C were identified to be equivalent to one of mesially located upper lateral teeth (specifically the third through sixth teeth in the reconstructed dentition of Perez et al.) (23) because of their asymmetrical (i.e., inclined) but broad crown showing a height similar to its width. Because the exact tooth position of these seven specimens could not be determined decisively, we used Shimada’s (80) linear function for the upper second lateral tooth (“L2”) that yielded the most conservative total length estimations among the mesially located upper lateral teeth. CMM-V-10502 with a symmetrical but constricted crown was determined to be equivalent to the second or third lower lateral tooth in *C. carcharias*, and we used Shimada’s (80) function for the second lower lateral tooth (“L2”), which gave a more conservative total length estimate relative to that based on the third lower lateral tooth. The estimated total length for each of these 12 specimens is reported in data file S1.

**Shark and marine mammal literature δ¹⁵N values**

Modern shark tissue δ¹⁵N measurements were compiled from the literature. Web of Science topic searches for [nitrogen isotope shark]
Modern marine mammal tissue δ^{15}N measurements were similarly compiled from the literature. Web of Science topic searches for [marine mammal nitrogen isotope] (293 results as of 24 March 2021), [nitrogen isotope and (whale OR dolphin OR porpoise OR sea lion OR seal OR walrus OR otter) NOT (marine mammal)] (488 results as of 24 March 2021), and [nitrogen isotope AND (polar bear OR sirenian OR manatee OR dugong)] (84 results as of 1 October 2021) were retrieved. The second searches were done to access papers that focus on a specific family of marine mammals and do not include the term “marine mammal,” meaning that they were missed in the original search. Of these results, 330 had relevant δ^{15}N data; data from samples older than ~1900, methods-focused studies, compound specific analyses, river dolphins, captive animals, and data reported in previous studies were all excluded. The database was then compiled from 225 papers for which the δ^{15}N data were available either in supplement tables or in in-text tables, or summarized with a mean and SD in the text.

For both the shark and the marine mammal literature compilations, the most individualized information possible was recorded: If a paper reported the isotope values by individual shark or marine mammal, either in the main text or in the supplementary materials, these were added to the database as unique observations. In many cases, papers only included summarized statistics for a group of individuals, in which case this information, along with the number of individuals and the standard deviation, was included as an observation. In addition, a variety of collection, tissue type, and taxonomic information were recorded. These two databases, descriptions of variables, and lists of citations are provided in data files S2 and S3.

**Data analysis**

Data analysis and visualization were done with R statistical software (82) using the tidyverse (83) and BSDA (84) packages. We report averages ± 1 SD unless otherwise stated. The regression between δ^{15}N_{NB} and dentin collagen δ^{15}N was done with a bootstrapped Deming regression, with the ratio of errors being set at 3.5 (0.7‰ for δ^{15}N_{NB} measurements and 0.2‰ 1 SD for δ^{15}N_{NB} measurements and 0.2‰ 1 SD for δ^{15}N measurements). The δ^{15}N_{NB} difference in Fig. 2B was calculated by subtracting the average of all piscivore shark δ^{15}N_{NB} from every other shark species average δ^{15}N_{NB}, for each epoch. Errors were fully propagated. Welch’s t tests were used to compare species δ^{15}N_{NB} with the δ^{15}N_{NB} of contemporaneous piscivore sharks (Fig. 2B and table S1).

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://science.org/doi/10.1126/sciadv.abl6529

**REFERENCES AND NOTES**

1. A. A. Klompmaker, P. H. Kelley, D. Chattopadhyay, J. C. Clements, J. W. Huntley, M. Kowalewski, Predation in the marine fossil record: Studies, data, recognition, environmental factors, and behavior. *Earth Sci. Rev.* **194**, 472–520 (2019).

2. V. J. Perez, S. J. Godfrey, B. W. Kent, R. E. Weems, J. R. Nance, The transition between *Carcharochthus chubutensis* and *Carcharochthus megalodon* (Otodontidae, Chondrichthyes): Lateral cusplet loss through time. *J. Verteb. Paleontol.* **38**, e1546732 (2019).

3. P. J. Motta, C. D. Wilga, Advances in the study of feeding behaviors, mechanisms, and mechanics of sharks. *Environ. Biol. Fishes* **60**, 131–156 (2001).

4. O. Lambert, G. Bianucci, K. Post, C. De Muizon, R. Salas-Gismondi, M. Urbina, J. Reumer, The giant bite of a new raptorial sperm whale from the Miocene epoch of Peru. *Nature* **466**, 105–108 (2010).

5. K. M. Melstrom, K. D. Angelichzyk, K. A. Ritterbusch, R. B. Irims, The limits of convergence: The roles of phylogeny and dietary ecology in shaping non-avian anhioan crania. *R. Soc. Open Sci.* **8**, 202145 (2021).

6. P. L. Koch, Isotopic study of the biology of modern and fossil vertebrates, in *Stable Isotopes in Ecology and Environmental Science*, R. Michener, K. Latjha, Eds. (Blackwell Publishing, ed. 2, 2007), pp. 99–115.

7. J. E. Martin, T. Tacail, S. Adnet, C. Girard, V. Balter, Calcium isotopes reveal the trophic position of extant and fossil elasmobranchs. *Chem. Geol.* **415**, 118–125 (2015).

8. J. McCormack, P. Szpak, N. Bourgon, M. Richards, C. Hyland, P. Méejan, J. J. Hublin, K. Jaouen, Zinc isotopes from archaeological bones provide reliable trophic level information for marine mammals. *Commun. Biol.* **4**, 683 (2021).

9. D. G. Vales, L. Cardona, A. F. Zangrando, F. Borella, F. Saporiti, R. N. P. Goodall, L. R. de Oliveira, E. A. Crespo, Holocene changes in the trophic ecology of an apex marine predator in the South Atlantic Ocean. *Oecologia* **183**, 555–570 (2017).

10. R. S. Feranec, M. E. Cournoyer, A. L. Kozlovski, 1^{13}C dates and stable isotope ecology of marine vertebrates in the Late Pleistocene-Early Holocene Chalmpain Sea. *Radiocarbon* **63**, 1259–1272 (2021).

11. T. Tüten, T. W. Venneemann, H.-U. Pfettschierz, Early diagenesis of bone and tooth apatite in fluvial and marine settings: Constraints from combined oxygen isotope, nitrogen and REE analysis. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **266**, 254–268 (2008).

12. P. Palmqvist, J. A. Pérez-Claros, C. M. Janis, D. R. Gröcke, Tracking the ecophysiology of ungulates and predator-prey relationships in an early Pleistocene large mammal community. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **266**, 95–111 (2008).

13. P. H. Ostrom, S. A. Macko, M. H. Engel, D. A. Russell, Assessment of trophic structure of Cretaceous communities based on stable nitrogen isotope analyses. *Geology* **21**, 491–494 (1993).

14. J.-Y. Sire, T. Davit-Béal, S. Delgado, X. Gu, The origin and evolution of enamel mineralization genes. *Cells Tissues Organs* **186**, 25–48 (2007).

15. I. Sagagawa, Mineralization patterns in elasmobranch fish. *Microsc. Res. Tech.* **59**, 396–407 (2002).

16. T. G. H. Diedrichsw, B. J. Berman, X. Anderton, B. Gurinsky, A. J. Ortega, P. G. Satchell, M. Williams, C. Arumugham, X. Luan, J. E. McIntosh, A. Yamane, D. S. S. Carlson, J.-Y. Sire, C. F. Shuler, Membranes, minerals, and proteins of developing vertebrate enamel. *Microsc. Res. Tech.* **59**, 373–395 (2002).

17. D. M. Sigman, K. L. Cacciotti, M. Andreani, C. Barford, M. Galanter, J. K. Böhkle, A bacterial method for the nitrogen isotopic analysis of nitrate in seawater and freshwater. *Anal. Chem.* **73**, 4145–4153 (2001).

18. R. S. Robinson, B. G. Brunelle, D. M. Sigman, Revisiting nutrient utilization in the glacial Antarctic: Evidence from a new method for diatom-bound N isotopic analysis. *Paleoceanography* **19**, PA3001 (2004).

19. J. N. Leichliter, T. Ludecke, A. D. Foreman, N. D. Duprey, D. E. Winkler, E. R. Kast, H. Vonhof, D. M. Sigman, G. H. Haug, M. Claus, T. Tüten, A. Martínez-García, Nitrogen isotopes in tooth enamel record diet and trophic level enrichment: Results from a controlled feeding experiment. *Chem. Geol.* **563**, 120047 (2021).

20. J. Lee-Thorp, M. Spoonheimer, Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. *J. Anthropol. Archaeol.* **22**, 208–216 (2003).

21. E. R. Kast, D. A. Stolper, A. Auderset, J. A. Higgin, R. Hen, E. X. Wang, A. Martinez-Garcia, G. H. Haug, D. M. Sigman, Nitrogen isotope evidence for expanded ocean subsidia in the early Cenozoic. *Science* **364**, 386–389 (2019).

22. K. Shimada, M. A. Becker, M. L. Griffiths, Body, jaw, and dentition lengths of macrophagous lamniform sharks, and body size evolution in Lamniformes with special reference to “off-the-scale” gigantism of the megatoothed shark. *Otodus megalodon*. *Hist. Biol.* **33**, 2543–2559 (2020).

23. V. Perez, R. Leder, T. Badaut, Body length estimation of Neogene macrophagous lamniform sharks (*Carcharodon* and *Otodus*) derived from associated fossil dentitions. *Palaeoentol. Electron.* **24**, a09 (2021).

24. K. Shimada, R. E. Chandler, O. L. T. Lam, T. Tanaka, D. J. Ward, A new elusive otodontid shark (Lamniformes: Otodontidae) from the lower Miocene, and comments on the taxonomy of otodontid genera, including the ‘megatoothed’ clade. *Hist. Biol.* **29**, 704–714 (2017).

25. C. Pimiento, B. J. MacFadden, C. F. Clements, S. Varela, C. Jaramillo, J. Velez-Juarbe, B. R. Silliman, Geographical distribution patterns of *Carcharocles megalodon* over time reveal clues about extinction mechanisms. *J. Biogeogr.* **43**, 1645–1655 (2016).
36. B. C. Weidel, S. R. Carpenter, J. F. Kitchell, M. J. Vander Zanden, Rates and components of carbon turnover in fish muscle: Insights from bioenergetics models and a whole-lake trophic enrichment factors of four large carnivorous fishes. J. Mar. Biol. Ecol. 43, 76–83 (2014).

37. C. Weidel, S. R. Carpenter, J. F. Kitchell, M. J. Vander Zanden, Rates and components of carbon turnover in fish muscle: Insights from bioenergetics models and a whole-lake trophic enrichment factors of four large carnivorous fishes. J. Mar. Biol. Ecol. 43, 76–83 (2014).

38. J. Kriwet, A. Engelbrecht, T. Mörs, M. Reguero, C. Pfaff, Ultimate Eocene (Priabonian) Carcharodon carcharias as revealed by biomechanical insights into the dentition of megatooth sharks (Lamniformes: Otodontidae). Sci. Rep. 11, 12321 (2021).

39. S. T. Win, D. M. Sigman, D. N. L. Sinclair, R. M. Sherrell, M. A. Weigand, D. V. Erler, S. S. Zeichner, A. S. Colman, P. L. Koch, C. Polo-Silva, F. Galván-Magaña, S. L. Kim, X. T. Wang, D. M. Sigman, A. L. Cohen, D. J. Sinclair, R. M. Sherrell, M. A. Weigand, D. V. Erler, J. Kriwet, A. Engelbrecht, T. Mörs, M. Reguero, C. Pfaff, Ultimate Eocene (Priabonian) Carcharodon carcharias as revealed by biomechanical insights into the dentition of megatooth sharks (Lamniformes: Otodontidae). Sci. Rep. 11, 12321 (2021).

40. J. A. Cahill, Polar bear taxonomy and evolution, in Ethology and Behavioral Ecology of Sea Otters and Polar Bears, R. W. Davis, A. M. Pagano, Eds. (Springer, 2021), pp. 207–218.

41. S. D. Newsome, M. A. Etnier, D. H. Monson, M. L. Fogel, Retrospective characterization of ontogenetic shifts in killer whale diets via δ15N and δ13C analysis of teeth. Mar. Ecol. Prog. Ser. 24, 229–242 (2009).

42. J. A. Cahill, Polar bear taxonomy and evolution, in Ethology and Behavioral Ecology of Sea Otters and Polar Bears, R. W. Davis, A. M. Pagano, Eds. (Springer, 2021), pp. 207–218.

43. S. M. Smart, H. Ren, S. E. Fawcett, R. Schiebel, M. Conte, P. A. Rafter, K. K. Ellis, J. A. Lueders-Dumont, D. M. Sigman, B. J. Johnson, O. P. Jensen, S. Oleynik, B. B. Ward, J. Möbius, N. Lahajnar, K. C. Emeis, Diagenetic control of nitrogen isotope ratios in fish otoliths from the submerged shelf of Onslow Bay, North Carolina, USA: Implications for processes of lag deposit formation. Ichnos 27, 122–141 (2020).

44. H. Shimada, Elasmobranchs from the Early Pliocene Naarai Formation, Choshi City, Chiba Prefecture, Japan, in Twenty-ninth Japanese Students Science Prize Complete Works, Japan Home Teacher Center, Tokyo (1986), pp. 357–359.

45. K. Shimada, elasmobranchs from the early Pliocene naarai formation, Choshi city, Chiba prefecture, Japan, in Twenty-ninth Japanese Students Science Prize Complete Works, Japan Home Teacher Center, Tokyo (1986), pp. 357–359.

46. K. Shimada, Elasmobranchs from the Early Pliocene Naarai Formation, Choshi City, Chiba Prefecture, Japan, in Twenty-ninth Japanese Students Science Prize Complete Works, Japan Home Teacher Center, Tokyo (1986), pp. 357–359.

47. K. Shimada, Elasmobranchs from the Early Pliocene Naarai Formation, Choshi City, Chiba Prefecture, Japan, in Twenty-ninth Japanese Students Science Prize Complete Works, Japan Home Teacher Center, Tokyo (1986), pp. 357–359.

48. K. Shimada, Elasmobranchs from the Early Pliocene Naarai Formation, Choshi City, Chiba Prefecture, Japan, in Twenty-ninth Japanese Students Science Prize Complete Works, Japan Home Teacher Center, Tokyo (1986), pp. 357–359.

49. M. A. Becker, J. A. Chamberlain, Macroborings in Otodus megalodon and Otodus chubutensis shark teeth from the submerged shelf of Onslow Bay, North Carolina, USA: Implications for processes of lag deposit formation. Ichnos 27, 122–141 (2020).

50. M. A. Becker, J. A. Chamberlain, Macroborings in Otodus megalodon and Otodus chubutensis shark teeth from the submerged shelf of Onslow Bay, North Carolina, USA: Implications for processes of lag deposit formation. Ichnos 27, 122–141 (2020).

51. M. C. Rogers, E. Peacock, K. Simac, M. B. O’Dell, J. M. Welker, Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol. 38, 1035–1047 (2015).

52. M. C. Rogers, E. Peacock, K. Simac, M. B. O’Dell, J. M. Welker, Diet of female polar bears in the southern Beaufort Sea of Alaska: Evidence for an emerging alternative foraging strategy in response to environmental change. Polar Biol. 38, 1035–1047 (2015).

53. J. A. Cahill, Polar bear taxonomy and evolution, in Ethology and Behavioral Ecology of Sea Otters and Polar Bears, R. W. Davis, A. M. Pagano, Eds. (Springer, 2021), pp. 207–218.

54. J. A. Cahill, Polar bear taxonomy and evolution, in Ethology and Behavioral Ecology of Sea Otters and Polar Bears, R. W. Davis, A. M. Pagano, Eds. (Springer, 2021), pp. 207–218.
75. H. Qi, T. B. Coplen, H. Geilmann, W. A. Brand, J. K. Böhlke, Two new organic reference materials for $\delta^{13}$C and $\delta^{15}$N measurements and a new value for the $\delta^{13}$C of NBS 22 oil. Rapid Commun. Mass Spectrom. 17, 2483–2487 (2003).
76. A. Schimmelmann, H. Qi, T. B. Coplen, W. A. Brand, J. Fong, W. Meier-Augenstein, H. F. Kempe, B. Toman, A. Ackermann, S. Assionov, A. T. Aerts-Bijma, R. Brejcha, Y. Chikaraishi, T. Darwish, M. Eslner, M. Gehre, H. Geilmann, M. Grö, J. F. O. Heie, S. Herrero-Martín, H. A. J. Meijer, P. E. Sauer, A. L. Sessions, R. A. Werner, Organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: Caffeines, n-alkanes, fatty acid methyl esters, glycines, $\gamma$-valines, polyethylenes, and oils. Anal. Chem. 88, 4294–4302 (2016).
77. R. S. Braman, S. A. Hendrix, Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. Anal. Chem. 61, 2715–2718 (1989).
78. J. K. Böhlke, T. B. Coplen, Interlaboratory comparison of reference materials for nitrogen-isotope-ratio measurements, in Reference and Intercomparison Materials for Stable Isotopes of Light Elements (IAEA, 1995), pp. 51–66.
79. T. B. Coplen, Report of Stable Isotopic Composition (USGS, 2016).
80. K. Shimada, The relationship between the tooth size and total body length in the white shark, Carcharodon carcharias (Lamniformes: Lamnidae). J. Foss. Res. 35, 28–33 (2003).
81. K. Shimada, The size of the megatooth shark, Otodus megalodon (Lamniformes: Otodontidae), revisited. Hist. Biol. 33, 904–911 (2021).
82. R Core Team, R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, 2020).
83. H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Yutani, Welcome to the tidyverse. J. Open Source Softw. 4, 1686 (2019).
84. A. T. Arnholt, B. Evans, BSDA: Basic Statistics and Data Analysis (2017); https://CRAN.R-project.org/package=BSDA.

Acknowledgments: We thank individuals listed in the “Materials” section for securing or loaning us specimens listed in data file S1, J. A. Higgins for general input, and C. Spence Morgan for Fig. 4. Funding: This study was supported by the Scott Fund of the Department of Geosciences, Princeton University, by grants from the National Science Foundation Sedimentary Geology and Paleobiology (1830581 to M.L.G. and M.A.B.; 1830638 to R.A.E.; 1830480 to S.L.K.; and 1830858 to K.S.), the European Research Council Consolidator Grant Agreement 681450 (to J.N.L., awarded to T. Tütken), the Max Planck Society (to A.M.-G. and G.H.H.), and the American Chemical Society Award, Petroleum Research Fund Undergraduate New Investigator Grant, PRF #54852-UNI2 (to M.L.G.).

Author contributions: Conceptualization: E.R.K., M.L.G., and D.M.S. Data curation: E.R.K., M.L.G., S.L.K., Z.C.R., K.S., M.A.B., H.M.M., and A.A.A. Formal analysis: E.R.K., K.S., and M.E.K. Funding acquisition: M.L.G., S.L.K., M.A.B., R.A.E., A.M.-G., G.H.H., and D.M.S. Investigation: E.R.K., Z.C.R., K.S., C.A.C., A.N.N., and M.E.K. Methodology: E.R.K., K.S., T.L., J.N.L., and X.T.W. Project administration: E.R.K., M.L.G., S.L.K., and K.S. Resources: M.L.G., S.L.K., M.A.B., H.M.M., R.A.E., A.M.-G., G.H.H., and D.M.S. Supervision: M.L.G., S.L.K., M.A.B., H.M.M., A.M.-G., G.H.H., and D.M.S. Validation: T.L., J.N.L., and A.M.-G. Visualization: E.R.K. Writing—original draft: E.R.K. and D.M.S. Writing—review and editing: All co-authors.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 29 July 2021
Accepted 5 May 2022
Published 22 June 2022
10.1126/sciadv.abl6529