Impacts of the end Pliocene marine megafaunal extinction on North Atlantic trophic structure

Amy E. Shipley[1], Andrew P. Beckerman[2], Catalina Pimiento[3,4], Jack O. Shaw[5,6], Jennifer A. Dunne[6], Tracy Aze[7] & Alexander M. Dunhill[1]

[1]University of Leeds, UK. [2]University of Sheffield, UK. [3]University of Zurich, Switzerland. [4]Swansea University, UK. [5]University of Colorado Boulder, USA. [6]Santa Fe Institute, USA. [7]University of Plymouth, UK.

Corresponding authors: Amy E. Shipley, email: amyshipley730@gmail.com
Alexander M. Dunhill, email: a.dunhill@leeds.ac.uk

Abstract

Marine megafauna are experiencing widespread population declines and these losses are expected to have cascading effects, impacting ecosystem structure and function. However, quantifying these ecological consequences at a global scale remains challenging, requiring an understanding of long-term trophic dynamics. Palaeoecology can provide useful insights into how communities respond to taxonomic losses through reconstructing food webs across extinction events. Here, we investigate the end Pliocene marine megafaunal extinction (~2.6 Ma) which saw the loss of one third of marine megafaunal genera globally, including the large apex predator *Otodus megalodon*. The trait-based Paleo Food web Inference Model (PFIM) is used to assess the impact of these extinctions on North Atlantic food web structure and function in the Pliocene and Pleistocene alongside the consequences of apex predator loss on other top predator trophic positions. Despite substantial taxonomic loss, no major structural changes were found, with the trophic positions of top predators being unaffected by the extinction of *O. megalodon*. Minor shifts in food web structure suggest more densely connected post-extinction communities characterised by a larger proportion of generalists. These findings suggest that, contrary to expectations, the Pliocene marine megafaunal extinction had minimal impact on long-term trophic structure and function in the North Atlantic.

Main

Marine megafauna are currently experiencing significant population declines and are under increasing threat of extinction on a global scale[1–3]. These declines have been driven by increasing direct human pressures (e.g. overfishing[4,5]) and anthropogenic climate change[6,7], and are concerning due to the importance of these species within marine ecosystems[8–10]. Marine megafauna typically occupy key ecological roles, modifying habitats, controlling prey populations and transporting nutrients. Consequently, localised extirpations of marine megafauna can cause cascading impacts resulting in mesopredator release[11,12], prey behavioural shifts[12,13], changes in habitat composition[9,14,15], and secondary extinctions[16]. With over one third of marine megafaunal species currently at risk of global extinction[3], it is expected that any future broadscale losses will have significant ecological implications. However, despite well-studied regional examples of marine megafaunal extirpations, modern and historical records offer limited insight into the ecological consequences of global extinction.

Documented global extinctions of marine megafauna are rare, have occurred in isolation and are temporally staggered[3,9]. Crucially, there has not been a global extinction of a marine apex predator throughout human history[17], yet current declines are disproportionately impacting predator populations[4,14]. These constraints of recent analogues mean it remains unclear what the ecological consequences would be if widespread losses across multiple taxonomic groups were to occur simultaneously. As such, the fossil record is vital for addressing such questions, providing records of metacommunity changes across long-term ecological timescales[18,19].

A marine megafaunal extinction event at the end of the Pliocene (~2.6 Ma) resulted in ~36% of all marine megafauna genera going extinct globally[20]. This extinction is hypothesised to have been driven by global cooling, leading to sea level drop and a consequent loss of productive neritic habitats[20]. Losses were seen across the taxonomic groups of mammals (predominantly cetaceans), sea birds, turtles, sharks and rays, and included the extinction of the giant shark *Otodus megalodon*. Estimated to have reached lengths of 16-24 metres[21–23], *O. megalodon* inhabited the global ocean for approximately 20 million years and was one of the largest apex predators to ever exist. Apex predators occupy the highest trophic level in a food web and experience very little (if any)

predation upon reaching full maturity[24]. Due to the large size, it is expected this shark had notable impacts on trophic interactions (including top-down predation pressure) and may have even impacted global nutrient transfer[25].

In addition to taxonomic losses, a reduction in functional trait diversity also occurred across this event, with a net change of 16% in functional space from the Pliocene to the Pleistocene[20]. However, questions remain regarding the long-term ecological consequences of these extinctions from the perspective of ecosystem and trophic structure. Understanding how this geologically recent extinction event altered marine food web structure, and function, may provide useful insight into how the global extinction of significant numbers of marine megafaunal species can impact marine ecosystems.

Extinction events are primarily considered from the perspective of taxonomic loss, and while incorporating functional diversity allows for a more informative approach, additionally understanding the impacts on ecological interactions can provide clearer insight into whole community dynamics[20,26–28] and allows for more applicable comparison to extant ecosystems. The trophic structural and functional changes that occurred across extinction events are rarely considered yet may often be decoupled from the patterns seen in diversity changes alone[27]. Reconstructing ecosystems in the form of food webs is a useful way to integrate this functional approach into a structural trophic framework[28–34]. Structural food webs represent the interactions among taxa within a community through feeding links and can be informative in detangling the relationship between diversity loss and changes to ecosystem structure.

Here, we assess the consequences of diversity loss on trophic structure and function following the end Pliocene marine megafaunal extinction event. To achieve this, trait-based reconstructions of food webs were generated for pre- and post-extinction time periods for North Atlantic pelagic communities. First, metacommunity food webs were constructed, which record all possible feeding links. These webs were then downsampled by reducing the number of links to produce ‘realised’ webs which align these networks to known levels of structural complexity in extant communities. This ensures important structural patterns are not masked by high link density of the metacommunity webs. Pre- and post-extinction food webs were then compared using

commonly applied measures of structure and function to assess (1) the change in community structure following extinctions, and (2) the impact of marine apex predator extinction (*Otodus megalodon*) on the trophic positions of other top predators.

Results

North Atlantic food webs

Using the trait-based Paleo Food web Inference Model (PFIM), metacommunity food webs (topological webs showing all feasible feeding links) were constructed for Pliocene and Pleistocene North Atlantic pelagic communities (Fig. 1). The magnitude of marine megafauna extinction in these communities is close to the taxonomic loss found in global assemblages albeit with slightly lower overall generic losses (~25% in the North Atlantic vs 36% globally; Fig. S1). The Pliocene and Pleistocene North Atlantic food webs consist of 267 and 242 individual vertebrate genera respectively, as well as 21 invertebrate nodes (see Methods) and a primary productivity node.

The distribution and position of taxa in the metacommunity food webs are similar before and after the extinction event (i.e., Pliocene and Pleistocene; Fig. 1). Further, the extant taxa present in these ancient food webs occupy trophic positions comparable to those seen in modern ecosystems. Trophic position is measured here by the average length of the multiple chains (feeding links) required to reach the primary node from the taxa. The genera that fall within the same trophic levels also mostly occupy the same broad ecological guilds. Herbivores and the macroinvertebrate and microinvertebrate nodes are the sole occupants of trophic level two. Mesopredators of various sizes are spread between trophic levels three and four, with these genera largely being piscivorous, durophagous, or filter-feeders. Taxa that would be considered top predators in extant ecosystems, mostly larger sharks and toothed cetaceans, are positioned in trophic level five (i.e. trophic position = 5.0-5.9) alongside the largest predatory fish and cephalopods and consist of both specialist and generalist feeders. In the Pliocene, trophic levels six (i.e. trophic position = 6.0-6.9) consists of *O. megalodon* and the extant sperm whale, *Physeter* (see *Top predator trophic changes* below; Fig. 4). In the Pleistocene, following the extinction of *O. megalodon*, *Physeter* solely occupies trophic level six. To account for

uncertainties in *O. megalodon* palaeoecology, we re-ran the Pliocene model with variable feeding and depth distribution traits for *O. megalodon* yet found these had negligible impacts on trophic positioning and structure. The Pliocene food webs displayed here are based upon the recent interpretations of *O. megalodon*[25,35] as a transoceanic generalist, and is thus assigned the broadest feeding habit and a depth distribution up to 1000m.

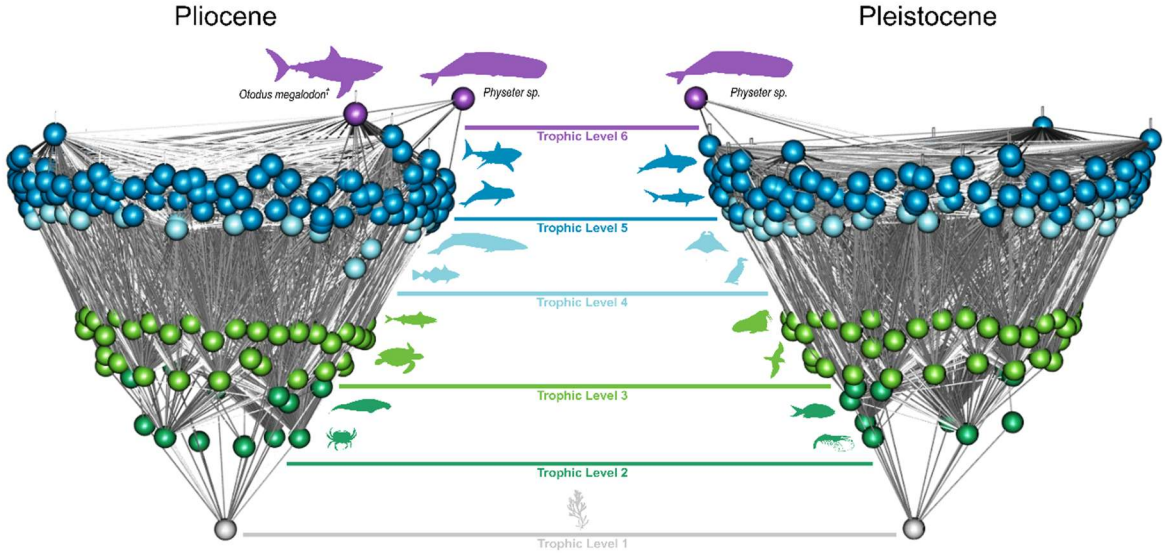


Fig. 1: Metacommunity food webs of North Atlantic marine communities for the Pliocene (left) and Pleistocene (right). Nodes represent individual vertebrate genera, the primary productivity node (in trophic level 1) and the invertebrate nodes. Different node colours denote different trophic levels. Example taxa are presented for each of the trophic levels using silhouettes from www.phylopic.org and consist of *Fucus serratus* for trophic level 1; *Metacarcinus magister*, *Metaxytherium*, *Caridea* and Acanthuridae for trophic level two; *Eretmochelys imbricate*, *Scomber scombrus*, *Pachyptila* and *Odobenus rosmarus* for trophic level 3; *Melanogrammus aeglefinus*, *Balaenoptera physalus*, *Pinguinis impennis* and *Mobula alfredi* for trophic level 4; *Grampus griseus*, *Carcharodon carcharias*, *Carcharhinus obscurus* and *Orcinus orca* for trophic level 5; *Otodus megalodon* and *Physeter microcephalus* for trophic level 6.

As the metacommunity food webs exhibit all feasible feeding links, the connectance of the webs (the proportion of expressed links out of all possible links in a network) at ~0.2 is higher than would be expected in most observed modern communities (which usually fall within the range of 0.01 and 0.1[36,37]), potentially masking structural signals. Consequently, these webs were downsampled to produce realised webs with a target mixed exponential-power law in-degree distribution[38,39] by randomly removing links to

reach this desired link distribution. Of the 1000 realised webs generated for each epoch, 858 and 834 iterations recovered realistic web structures (see Methods) for the Pliocene and Pleistocene respectively. These realised webs exhibit a wide range of structure with connectance values close to those observed in modern communities, ranging from 0.030 to 0.048 (Fig. 2).

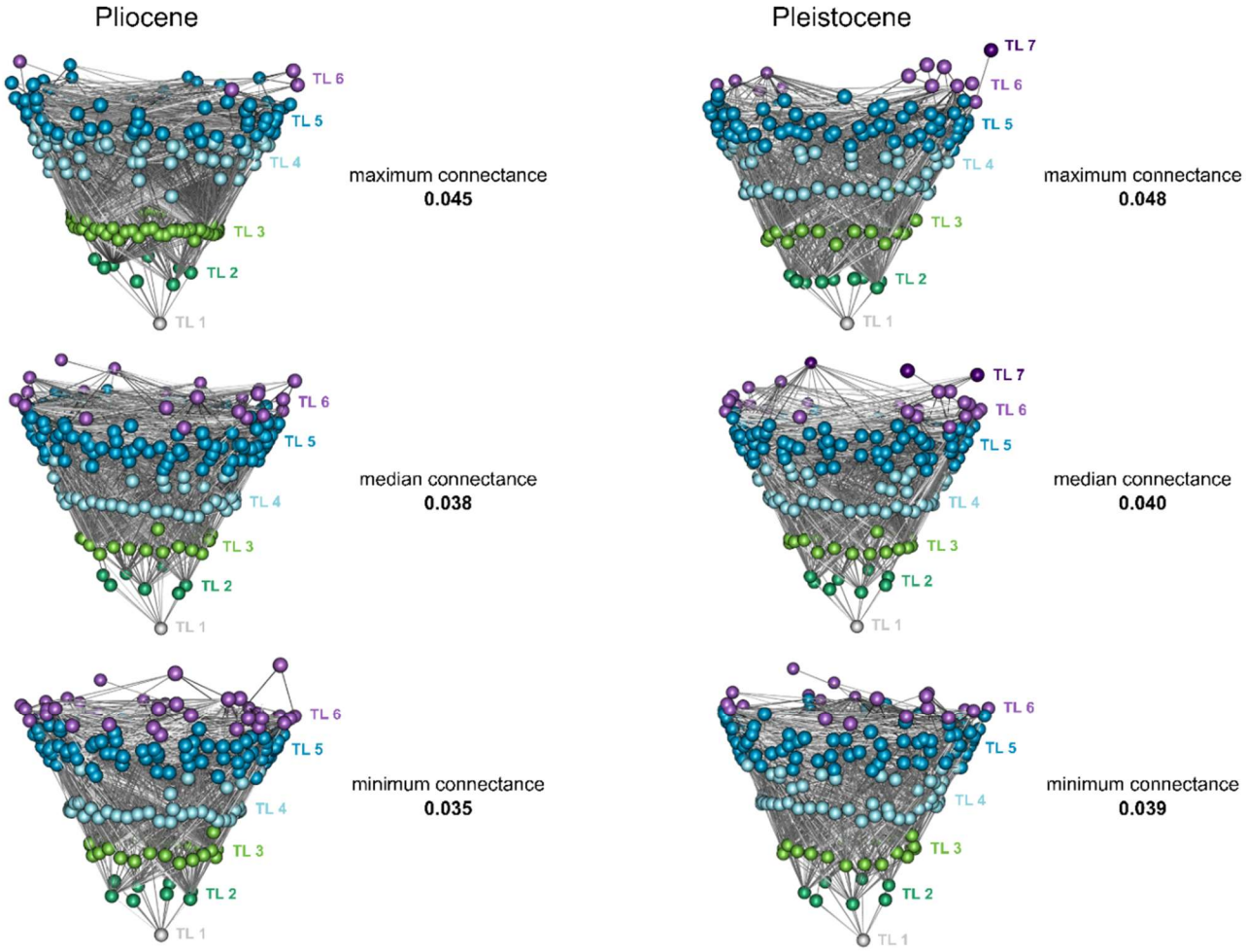


Fig. 2: Iterations of the realised webs representing the range of connectance for the Pliocene (left) and Pleistocene (right). The realised web iterations here exhibited the maximum, median and minimum values of connectance out of all iterations (858 Pliocene webs and 834 Pleistocene webs) for each epoch. Nodes represent individual vertebrate genera, the primary productivity node (in trophic level 1) and the invertebrate nodes. Different node colours denote different trophic levels.

The realised webs follow a similar pattern of taxa distribution to the metacommunity webs, with the ecological guilds shifting from herbivores to mesopredators to top predators with increasing trophic level. However, the removal of a significant number of feeding links in the realised webs results in longer pathways to the primary node, thus these webs have a greater number of trophic levels reaching a maximum trophic position of 8.7 (Fig. 2). This is reflected in a greater spread of the guilds across the trophic levels; herbivores and invertebrates are still restricted to trophic level two, however, mesopredators are now present from trophic levels three to five (and occasionally six) and top predators from levels five to eight.

Changes to food web structure and function across the megafaunal extinction

Our results suggest that, despite the loss of 25% of megafaunal genera, including apex predator *O. megalodon*, there was only minimal change in North Atlantic food web structure. Food web structure was assessed via comparing commonly used network metrics and motifs for both the metacommunity and realised food webs across the Plio-Pleistocene boundary (Table 1). These metrics measure overall structural properties of the food webs, from the density of links (i.e. connectance), the mean and maximum number of trophic levels, the availability of resources to predators (number of prey available per predator; generality) and the predation pressure on prey (number of consumers per taxon; vulnerability). The motifs assess functional changes through measuring the proportion of specific interactions between three connected nodes compared to the whole network. The interactions considered here are the number of vertical linear chains (linearity), the extent of feeding across multiple trophic levels (omnivory) as well as measures of competition, both indirect competition where two prey species, which share the same predator, indirectly compete for survival (apparent competition) and direct competition, where two predators compete for the same prey (direct competition). Further description of these metrics and motifs and how they are calculated are detailed in the Methods (Table 2).

Table 1: Calculated metrics and motifs for the metacommunity and realised food webs. Mean values are provided for the realised webs (see Supplementary Information for full table of results) alongside the calculated Cohen's d value and associated effect size. Definitions for the metrics and motifs are found in Table 2.

Metric/Motif	Metacommunity webs		Realised webs (means)			
	Pliocene	Pleistocene	Pliocene	Pleistocene	*Cohen's d*	*Effect size*
Connectance	0.22	0.23	0.038	0.040	0.79	medium
Mean trophic level	4.62	4.54	4.637	4.536	0.42	small
Maximum trophic level	6.31	6.22	6.885	6.721	0.37	small
Generality	0.17	0.18	0.048	0.050	0.54	medium
Vulnerability	0.24	0.24	0.045	0.046	0.62	medium
S1: Linear chains	1.24	1.05	0.212	0.207	0.20	small
S2: Omnivory	4.62	4.53	0.064	0.065	0.03	small
S4: Apparent competition	3.53	3.56	0.427	0.433	0.08	small
S5: Direct competition	6.78	6.49	0.384	0.383	0.03	small

These structural metrics and functional motifs are a useful way of measuring community changes across time in both the realised and metacommunity food webs. The wide range of food web structures generated for the realised webs is reflected in the calculated metrics and motifs (Fig. 3), however, the measure of effect size allows for the strength of the changes over the boundary to be quantified. The overall directional change seen across the extinction event in the realised webs match that of the metacommunity webs, with the exception of omnivory.

The most notable shift seen in the metrics is that of connectance, which increases across the extinction and exhibits a borderline large effect size (Cohen's d of 0.79). This signifies an increase in the density of links amongst the surviving taxa, corresponding to increases in both generality (more feeding links per predator taxa) and vulnerability (more feeding links per prey taxa). Despite the generality and vulnerability metrics showing little change in the metacommunity webs, they both exhibit medium effect sizes

(Cohen's d values of 0.54 and 0.62 respectively) for the realised webs. Although these effect sizes indicate differences over the extinction boundary, shifts in these three metrics are still no greater than 15%, highlighting that the changes, whilst significant directional signals, are only minor in terms of magnitude of change.

Both mean and maximum trophic level decrease over the extinction in the metacommunity and realised food webs, although this is only a marginal shift with a low effect size. Interestingly, the largest shift seen in the metacommunity webs is that of a decrease in the linearity motif which signifies a decrease in the number of linear feeding chains. However, with a small effect size of Cohen's d value 0.2, this reflects a weaker signal and thus a smaller sign of change in the realised food webs. Both apparent and direct competition show very small effect sizes, suggesting overall little change in either direct or indirect competition occurred from the Pliocene to the Pleistocene. Finally, the omnivory motif presents a contrary pattern, decreasing in the metacommunity webs and increasing in the realised webs. However, with a very small effect size, there was likely very little change, if any at all, in the levels of feeding from predators across different trophic levels following the extinction.

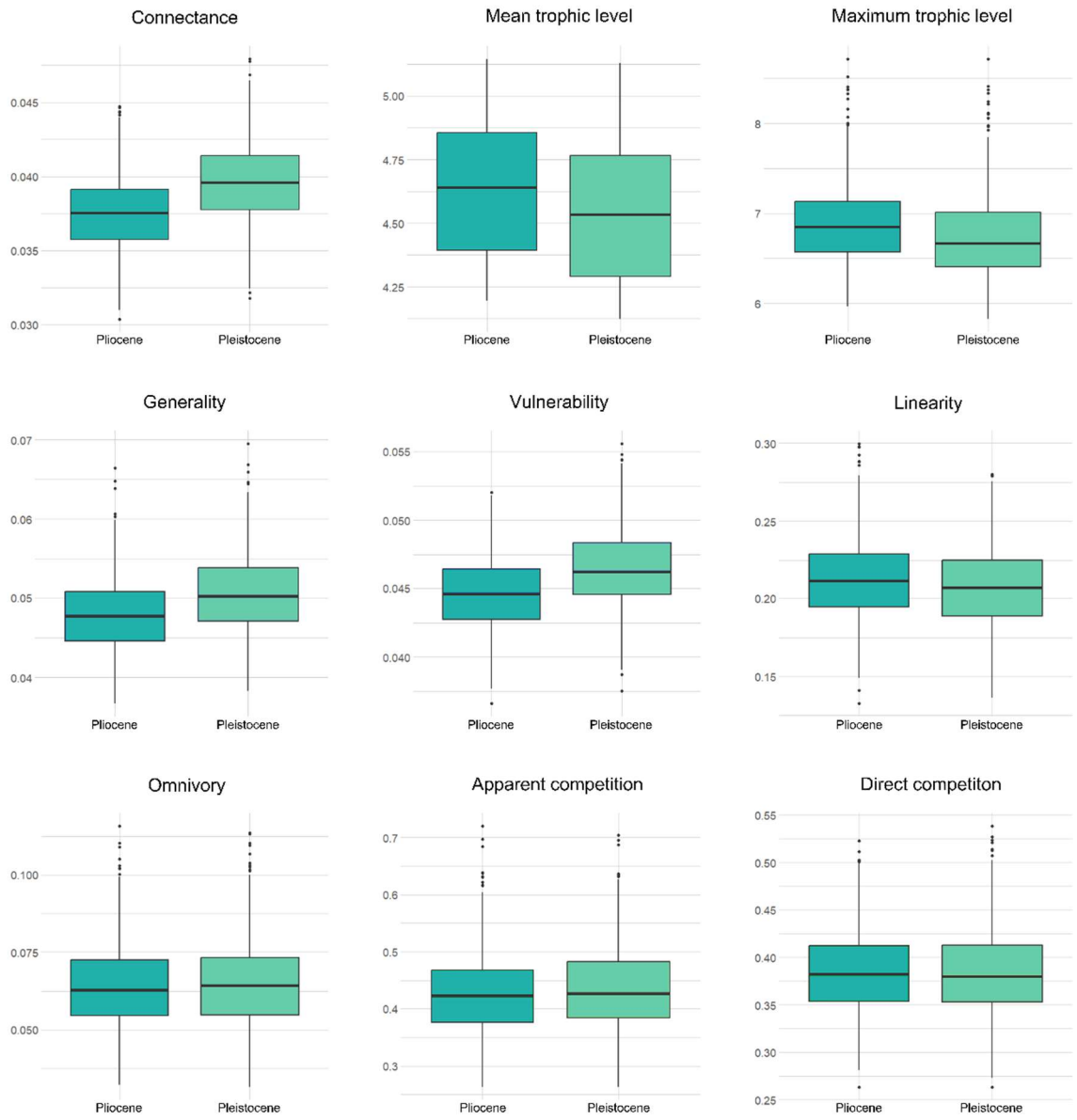


Fig. 3: Realised food web metrics and motifs variation across the extinction event. Boxplots show the range in values recovered across the 858 Pliocene realised webs and 834 Pleistocene realised webs.

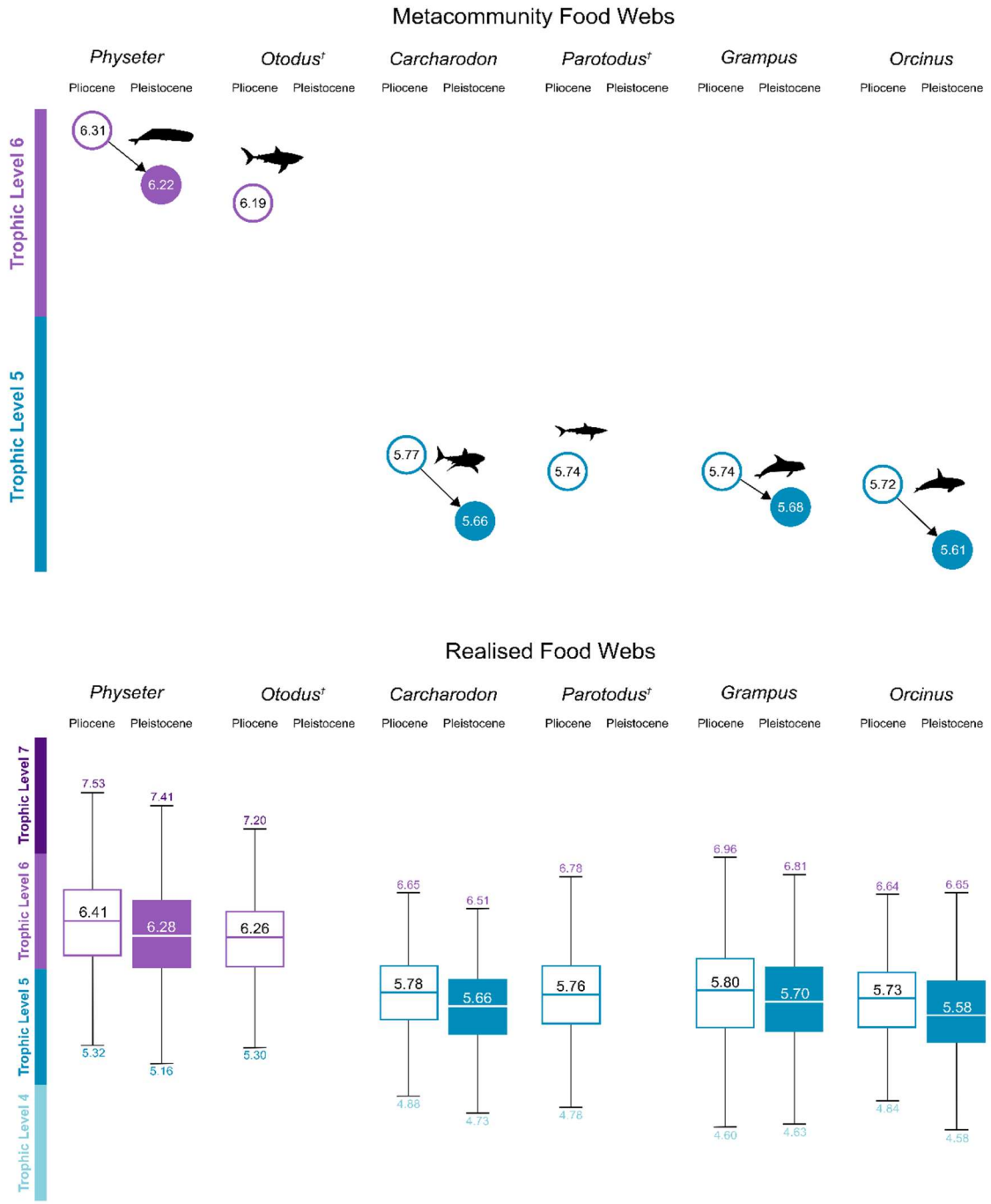
Top predator trophic positions

Both the metacommunity and realised food webs record no major shifts in the trophic positions of the top predators following the marine megafaunal extinction, despite the extinction of *O. megalodon* (Fig. 4). In the Pliocene metacommunity food web, *O. megalodon* holds the position of apex predator being in the highest trophic level (with a trophic position of 6.19) and is not predated upon (aside from cannibalism). Surprisingly,

the sperm whale *Physeter* is also positioned in the highest trophic level with a position even higher than that of *O. megalodon* (at 6.31). Despite this slightly higher position in the food web, *Physeter* is not considered an apex predator due to predation from *O. megalodon*, as seen in the feeding links. The other top predators (taken here as trophic position 5.7 and above) in the Pliocene metacommunity web consist of the extant predators *Carcharodon* (great white shark), *Orcinus* (orca) and *Grampus* (Risso's dolphin) alongside the lamniform shark *Parotodus* which went extinct in the marine megafauna extinction event.

In the Pleistocene metacommunity food web, the extinction of *O. megalodon* leaves *Physeter* as the sole occupant of trophic level six with a trophic position of 6.22. The lack of predation from *O. megalodon* or any other predator now results in *Physeter* positioned as the apex predator in the Pleistocene. The other surviving top predators hold similar trophic positions following the extinction event, though with the predatory cetacean *Grampus* now occupying the highest position in trophic level five, above *Carcharodon*. Overall, all predators undergo as slight decline in trophic position, corresponding to the drop in mean and maximum trophic level mentioned previously.

The realised food webs record a wide range of trophic positions for the top predators identified in the metacommunity webs (Fig. 4). The highest trophic positions recovered by these predators are due to the longer pathways to the primary node, a facet of downsampling the metacommunity webs. While *Physeter* still maintains an average trophic position higher than *O. megalodon* in the Pliocene, the range of *Physeter* trophic positions now fits more closely within the extant sperm whale range (see Discussion). Overall, the average trophic positions of the individual taxa and their relative positions to each other before and after the extinction event in the realised webs fit closely to that seen in the metacommunity webs. This suggests top predator trophic positions were not affected by the high connectance of the metacommunity food webs.



260 **Fig. 4. Trophic position changes in trop predators for the metacommunity (top) and realised**
261 **(bottom) food webs.** For the metacommunity food webs, arrows show directional shift of trophic
262 position of surviving top predators from the Pliocene to the Pleistocene. For the realised food
263 webs, boxplots show range of trophic positions recovered across the 858 Pliocene webs and 834
264 Pleistocene webs. Outliers have been removed with the maximum, minimum and median values
265 labelled. Colours denote different trophic levels and representative silhouettes from
266 www.phylopic.org consist of *Physeter microcephalus*, *Otodus megalodon*, *Carcharodon*
267 *carcharias*, *Carcharhinus obscurus* (used to represent *Parotodus*), *Grampus griseus* and *Orcinus*
268 *orca*.

To assess the impact of *O. megalodon* as an apex predator in these marine communities, additional food webs for the Pleistocene were constructed, simulating the presence of *O. megalodon* post-extinction. This was done for both the metacommunity and realised food webs, for which 867 iterations of 1000 recovered realistic food web structures. The trophic position of *O. megalodon* did decrease from the Pliocene, from 6.19 to 6.09 in the metacommunity webs and from a median of 6.26 to 6.14 in the realised webs (Fig. S2). These shifts are in line with the decreases in trophic positions seen amongst the other surviving top predators over this boundary. Further, the key metrics and motifs did not significantly change following the inclusion of *O. megalodon* in the Pleistocene (Table S1).

Discussion

Food webs were reconstructed for Pliocene and Pleistocene North Atlantic marine communities to assess structural and functional change following the end Pliocene marine megafaunal extinction event. Analysis and comparison of the food webs before and after the extinction finds only minor shifts in structure and function. Post extinction Pleistocene communities are more densely connected, characterised by more generalist taxa and exhibit a decrease in average trophic level. However, despite these minor changes, food web structure remains fairly consistent across the Pliocene and Pleistocene, lacking any of the major shifts that may be expected across an extinction boundary. The stability of trophic network metrics across this event are striking given that marine megafauna fulfil important ecological roles in marine ecosystems[8–10]. Such high taxonomic losses might be expected to trigger cascading effects through marine ecosystems, significantly impacting food web topology, especially given the notable impacts documented from extant localised extirpations[9,12–14].

The apparent robustness of these ecosystems may reflect high trophic redundancy (where multiple taxa occupy the same feeding niche) which can act as a buffer against extinction cascades, making an ecosystem less vulnerable to further secondary extinctions[40]. The Pliocene food web contained high levels of trophic redundancy, thus the feeding-trait combinations exhibited by the taxa that went extinct were mostly maintained among surviving taxa in the Pleistocene. Complete losses of feeding trait

combinations were rare, which is in contrast to the substantial losses seen in unique functional entities (trait combinations using a different framework) as measured in Pimiento *et al.* (2017). Thus, although generic and functional diversity may have declined, food web composition, specifically its structure, function and trophic organisation appears to have remained largely intact, with most feeding niches still occupied (albeit by fewer genera). This distinction highlights that vulnerability inferred from functional diversity metrics does not necessarily equate to diminished ecological functioning at the metacommunity level.

It should be noted the lack of major trophic changes may be partly due to the conservative nature of topological structural food webs[41,42]. Topological food webs will intrinsically underestimate certain elements of ecosystem structural change, as they only consider the presence/absence of taxa and interactions without the nuances of changing population sizes, biomass or interaction strength between nodes. Further, extant ecological studies have found, in general, marine food webs are particularly resilient to losses of both well-connected and random taxa[37] and this may be reflected in the results presented here.

The minor shifts exhibited in food web structure are predominantly seen in the metrics of connectance, generality and vulnerability which exhibited large and medium effect sizes. Increases in these three metrics in modern ecological theory are often attributed to more robust and stable ecosystems[43,44]. This is perhaps contrary to what would be expected of communities following a major faunal turnover[45,46]. However, these increases in connectance, generality, and vulnerability are comparable to patterns exhibited following other extinction events in the fossil record which, rather than corresponding to increasing ecosystem stability and robustness, reflect extinction selectivity against specialist species and increased survivorship of opportunist generalists[28]. This is likely the case in these Pleistocene communities where approximately two thirds of the genera lost from the Pliocene food web had a narrow diet breadth, feeding on only one or two feeding categories. Predators with a broader diet breadth are more likely to retain adequate prey compared to specialist feeders when extinction rates are high. Thus, increased survivorship of generalists supports the increase in generality and connectance, as surviving predators each feed upon a broader range of prey which results in more feeding links per node across the community. The increase in

vulnerability can similarly be explained due to the increase in predation pressure on the lower trophic level prey, where a larger proportion of generalist predators are feeding upon more of these prey nodes. These justifications do not preclude the inferences from modern ecology and it is possible these more densely connected Pleistocene communities may have been more stable following the extinction. As there is still some debate around what inferences can be made from these metrics (particularly connectance)[44,47,48], these minor shifts could reflect varying ecosystem dynamics.

Further structural shifts were seen in the metacommunity food webs, however, due to the small effect sizes of these metrics and motifs in the realised food webs, we cannot assign certainty to these results. The drop seen in the mean and maximum trophic levels likely reflects the loss of higher trophic level prey, resulting in top trophic level predators having shorter feeding chains to the primary node. This is further supported by the decreases in trophic level predominantly impacting trophic level three taxa and above. An increase in the apparent competition motif may support the apparent increase in generalism seen (supporting a greater diet breadth of taxa), however this change is more likely due to a reduction in predator numbers compared to prey, with extinction concentrated in the higher trophic levels. Further, direct competition decreases over the extinction boundary in the metacommunity webs, indicating a drop in competition between predators for the same prey, as would be expected with fewer predators now present in these post-extinction communities. Linearity presents the largest decrease in the metacommunity webs, dropping notably across the extinction boundary. This indicates the Pleistocene food webs were less vertically complex, with fewer linear feeding chains signifying a loss of nodes and connections in the higher trophic levels. This curtails food chain lengths and possible vertical pathways through the web. The loss of vertical complexity supports the idea that loss of neritic habitat was a primary driver of these extinctions[20], as the decline of productive habitats created more energetically constrained ecosystems with reduced energy flow through the food web. This reduction in vertical complexity, often inferred through linearity, serves as a proxy for these constraints[49], with fewer taxa able to persist at the highest trophic levels. However, this may also be as result of the loss of *O. megalodon* from these Pleistocene webs, reducing energy flow pathways to trophic levels six. Regardless, as the effect size

for this motif was small in the realised webs, only limited support may be taken from this metacommunity food web result.

The impact of a marine apex predator extinction, *Otodus megalodon*, on the trophic positions of other top predators was also assessed. As apex predators are known to exert top-down predation pressure[13,50,51], it may be expected the extinction of this shark would cause trophic shifts in the other top predators. However, the trophic positions of top predators appeared unaffected by the presence or absence of *O. megalodon* in both the metacommunity and realised webs. This was also the case in the simulated Pleistocene webs where *O. megalodon* is present. These negligible relative shifts may be due to any resultant cascading effects (such as mesopredator release) primarily impacting population dynamics, which cannot be interpreted from structural webs alone[52]. As such, perhaps trophic structure is maintained following marine apex predator loss despite the impacts this may have on biomass distribution across trophic levels[11,12,14], and is similarly buffered by the trophic redundancy present in these Pliocene ecosystems[40].

Additionally, some studies have hypothesised the extinction of *O. megalodon* was driven by interspecific competition with other predators, notably *Carcharodon carcharias* (the great white shark) following the extinction of its primary prey (mostly large whales)[17,53,54]. However, the stability of top predator trophic positions following the extinction do not suggest trophic release or restructuring typically associated with competitive displacement. Further, if direct competition were a main driver, it may be expected the trophic position of *O. megalodon* would be lower in the simulated Pleistocene webs to reflect the similar feeding interactions now occurring between the two sharks as they compete for the same, smaller prey[53]. The trophic position of *O. megalodon* does decrease in the simulations, however this shift is of a similar magnitude to the drop in trophic position seen in all top predators over this extinction boundary.

The reconstructed food webs also present further interpretation of the trophic positions of all top predators during the Pliocene and Pleistocene. Most interpretations of extinct predator trophic positions are derived from stable isotope analysis on fossil samples (usually teeth), where diet is inferred from isotope ratios and thus provides a measure of a taxon's position in a food web (e.g.[53,55,56]). The differences between this approach and

how trophic positions are determined from food webs, may give differing insights into the positions of key species. In the Pliocene food web, *O. megalodon* is positioned as the apex predator in trophic level six, a level higher than apex predators in modern marine communities where pelagic systems only consist of four to five trophic levels[37,57,58]. The trophic position of 6.19 for *O. megalodon* aligns with isotopic analyses which also place this species in trophic level six or above[55]. The trophic level six assignment of *Physeter,* the sperm whale, in our modelled food webs, is perhaps surprising as this genus is usually positioned in trophic level five in modern ecosystems[58,59]. However, research using stable isotope analysis[60] estimates an extant trophic position of 5.7±0.4, close to its position in the metacommunity webs, and falls well within the realised webs range. The positioning of *Physeter* above *O. megalodon* (despite being predated upon by the shark) is due to the sperm whale's prey being positioned solely in trophic level five, albeit the lower end of this level, whereas *O. megalodon* has feeding links for a broader range of prey which span multiple trophic levels, predominantly baleen whales[61].

In the Pleistocene food web, following the extinction of *O. megalodon*, *Physeter* is now positioned as the apex predator, facing no predation. In modern ecosystems, the sperm whale is not an apex predator as it is predated upon by orcas through pack hunting, a feeding method this food web model does not account for. However, there is currently no fossil evidence to support such predation in the fossil record and the feeding behaviour of *Orcinus* is known to have changed within the last million years[62]. The trophic positioning of the other top predators during both epochs are in line with their ecological lifestyles. The extant *Carcharodon*, *Grampus* and *Orcinus* are macropredators in modern ecosystems and also positioned in the higher trophic levels (in most cases trophic level five). Understanding of the ecology of the extinct shark *Parotodus* is limited[63], though it likely had similar macropredatory feeding preferences to the other top predators, supporting its position in this high trophic level.

The end Pliocene marine megafaunal extinction event resulted in surprisingly limited reorganisation of North Atlantic food web structure and function, with only minor shifts in network topology and negligible changes in the relative trophic positions of top predators. Although post-extinction communities became more highly connected and increasingly dominated by generalist taxa, marine ecosystems remained structurally robust despite the loss of multiple megafaunal species, including a marine apex

predator. These findings provide important long-term evidence that marine food webs can retain functional cohesion following concurrent megafaunal extinctions, largely because broad trophic redundancy buffers the loss of some key ecological roles. The broad comparability of Pliocene, Pleistocene and extant communities in both guild composition and trophic organisation further underscores the resilience of marine ecosystem structure through time. However, this resilience should not be interpreted as immunity. Contemporary biodiversity loss is occurring under intense and interacting anthropogenic pressures[4–6] at an unprecedented scale[64], with rates and drivers that differ fundamentally from past extinction events. Consequently, future marine megafaunal declines may generate more complex outcomes, where trophic frameworks may persist even as populations collapse, biomass is depleted, and ecosystem functioning is profoundly diminished.

Methods

Fossil occurrence data

Taxon occurrence data were compiled from the North Atlantic for the Pliocene (5.2-2.58 Ma) and Pleistocene (2.58-0.012 Ma) to represent pre- and post-extinction marine metacommunities. This region was chosen due to better sampling in the North Atlantic compared to other ocean basins across this time period. Vertebrate taxa were collected at genus level from fossil occurrence data sourced from literature[65–70] and the Paleobiology Database (PBDB). From the PBDB, genera were downloaded from North American and European marine sediments for the taxonomic groups of Actinopterygii, Chondrichthyes and Sarcopterygii (see Supplementary Information). The sarcopterygians were subsequently split into the groups of Aves, Reptilia (non-avian) and Mammalia. The dataset was cleaned to remove any terrestrial taxa and those that were not found in the North Atlantic.

Incompleteness of fossil datasets is an endemic problem faced by all palaeontological researchers, with the fossil record not only being incomplete but also exhibiting sampling bias[71,72], which can vary both temporally and spatially[72–74]. To account for this, the dataset was expanded to include ranged-through occurrences based on first and last occurrence

456 data as well as knowledge of extant species ranges. By ranging-through occurrences this
457 regional study is making the assumption that taxa did not become extirpated from the
458 North Atlantic for a period of time and were present in this area across both epochs as
459 extirpation is assumed to be less likely than the absence of taxa being the result of a
460 sampling failure. These 'inferred occurrences' are denoted in each dataset and make up
461 5% of the Pliocene and 21% of the Pleistocene datasets (Supplementary Information).

462 Invertebrate taxa are represented initially in the dataset by two nodes, one to represent
463 microinvertebrates (e.g. plankton and larvae) and one to represent macroinvertebrates
464 (e.g. crustaceans and molluscs). Individual invertebrate genera were not included as
465 many invertebrates represented by these nodes are not found in the fossil record[75], such
466 as plankton. Also, the focus of this study is on changes within pelagic communities with
467 most macroinvertebrates being benthic and not interacting with these vertebrate species
468 other than as a prey source. However, squids are an exception to this assumption as it is
469 known from extant pelagic communities that squid play important ecological roles as
470 both predators and prey within pelagic marine communities[76], with several vertebrate
471 genera in the dataset interpreted to be specialist cephalopod consumers. Due to their
472 predominantly soft-bodied nature, Cenozoic squids have a poor fossil record and no
473 fossil occurrences have been recorded in the North Atlantic for either the Pliocene or
474 Pleistocene. To address this data gap, 19 nodes were added to represent each of the
475 unique feeding guilds of modern North Atlantic squids (Table S2). These guilds were
476 identified based on the traits detailed below. As the food webs in this research are
477 structural, only showing presence/absence of taxa without consideration of abundance,
478 the amount of primary productivity entering the web does not need to be quantified.
479 Consequently, primary productivity is represented as just a single node to root the web,
480 an approach previously used in palaeo food web reconstructions[28].

481

482 ## Ecological trait data

483 Functional trait data was assigned to each node in each community and consisted of
484 body size, vertical position in the water column, depth distribution and feeding habits.
485 These traits were selected as they are informative of feeding interactions in modern
486 pelagic systems and represent predator ability to subdue and consume prey, predator-

prey encounter rates, and predator foraging choice. Trait data was primarily sourced from the published literature, however, if little or no information could be found for a particular fossil organism, traits for extant species of the same genus that presently reside in the North Atlantic were used, sourced from extant species databases[77,78]. Trait data for all categories could not be found for every genus in the dataset and these genera were thus removed from the analysis as the model framework requires a complete set of trait data for each taxon (see below).

Body size is arguably the most important trait in determining trophic interactions in marine communities, underpinning much modern ecology theory, such as optimal foraging theory[79]. Here body size refers to the maximum length of the largest known specimens. In the case of birds, this was taken as the distance from head to tail as opposed to wingspan and for turtles this was taken as the carapace length due to the poor preservation and lack of size estimates which include head and neck length[80–82]. These lengths were taken from direct measurements of fossil specimens or body size estimates from published literature. If no size estimate for fossil specimens was given, the length of the largest extant North Atlantic member of the genus was used. Taxa were then placed into discrete body categories: tiny (<1m), small (1-3m), medium (3-6m), large (6-9m), giant (9-12m) and mega (>12m). Suspension feeders within Mammalia and Chondrichthyes were separated into their own size categories: medium suspension (3-6m), large suspension (6-9m), giant suspension (9-12m) and mega suspension (>12m). It was necessary to differentiate this group due to these predators operating under different size feeding rules than other taxa (see model description below). Body size was also used to define the marine megafauna in the dataset, with all vertebrates with a body length >1m considered megafauna based upon Pimiento *et al.*[83].

The traits of vertical position and depth distribution were selected to cover all spatial aspects of the marine taxa in order to parameterise encounter likelihood within the modelling framework. The vertical position of where a taxon is found in the water column was classified as being either on the bottom of the ocean floor (benthic), out in the open ocean (pelagic) or a combination of the two (benthopelagic). Similarly, depth distribution corresponds to the maximum depth at which a genus would usually reside, split into three broad categories of neritic (<200m), twilight (200-1000m) and midnight (>1000m). A genus can be assigned a combination of any of these three.

The preferred diet of taxa (the usual feeding behaviour of adult individuals) is accounted for by feeding habit. Feeding habits were restricted to what classes of organism a taxon could feed on: Actinopterygii, Chondrichthyes, Mammalia, Aves, Reptilian, Cephalopoda, macroinvertebrates and microinvertebrates. Feeding interactions were determined using fossil evidence such as tooth morphology, gut contents or predation marks and inferences from modern taxa. Herbivores were assigned to feed directly on the primary productivity node.

Model framework

Food webs were reconstructed using the Paleo Food web Inference Model (PFIM)[28,38]. This trait-based model was designed specifically for the reconstruction of ancient food webs using only fossil data. In addition to a list of taxa and associated traits, the model requires defined rules specifying which traits are 'compatible' and allow one taxon to feed upon another. These rules are grounded in optimal foraging theory[84,85], where links are assigned based on whether taxa are likely to interact and if it is energetically beneficial for a consumer to feed on a resource. The original framework used traits and rules based on Bambach's ecospace cube[39,86] used to establish potential modes of life of marine fauna, predominantly invertebrates. As this study focusses on pelagic vertebrates, this prior framework was not appropriate as it does not allow for sufficient separation between the diverse modes of life of pelagic marine vertebrates. Consequently, the traits and rules used here are tailored to these pelagic marine communities.

The rules assigned in this study firstly consist of a size hierarchy. For body size, it is generally not beneficial nor often physically possible for taxa to hunt and feed on resources larger than themselves as they will often expend more energy than they recoup from eating that prey item. Similarly feeding upon a prey item too small will also result in an energetic imbalance. While some extant species engage in cooperative hunting to take down larger prey (although this is uncommon), here only individual behaviours are considered. As such, consumers are assigned to only feed upon taxa which are in the same size category or up to three sizes smaller. This size restriction means that while any taxon within the category 'large' can feed on any resource smaller

than themselves, the bigger predators are restricted to how small their prey can be. The three categories limit was chosen based upon the feeding range of modern top predators, in addition to the known feeding range of *O. megalodon*[54,87]. Suspension feeders have been incorporated separately in this rule. Regardless of size, all chondrichthyan and mammalian filter feeders are only permitted to feed on taxa in the 'tiny' size category, due to their ecological lifestyle.

For the traits of vertical position and depth distribution the rules dictate that a consumer must share spatial distribution with its prey; they must co-occur together in the same location, water depth and position in the water column. The strict feeding habits assigned to each genus finally dictate which classes of organism they can feed upon.

All modelling was performed in R[88] (R version 4.3.1). The PFIM generates pairwise combinations between all taxa in the community. This includes combinations between two of the same genera to allow for the possibility of cannibalism. For a feeding link to then be assigned for each combination, all the rules stated above must be satisfied. The PFIM then returns an edge-list of all feasible interactions and constructs a network, assigning each genus a trophic position. These networks are then plotted as metawebs, showing all feasible interactions, using the R package "igraph"[89]. Metacommunity food webs were constructed for the Pliocene and Pleistocene North Atlantic.

This research is interested in the trophic position of *O. megalodon*, however some of the functional traits of this shark have been subject to speculation. Whilst size is well estimated[21–23] and a pelagic lifestyle is well accepted, the other traits in this dataset are less well constrained. The fossil record places *O. megalodon* solely in coastal habitats and gives no indication of depth ranges. Research hypothesising a similar ecology to extant great white sharks (*Carcharodon carcharias*)[25] would imply migration to deeper oceanic regions with depths beyond the neritic zone and this is supported by the recent discovery of a fossil tooth from a deep-sea locality in the Pacific Ocean[90]. Isotopic studies also indicate a generalist feeding habit[35,53,55], suggesting a greater prey breadth than recorded by feeding traces in the fossil record. Due to these uncertainties, the metacommunity webs for the Pliocene were run several times to determine whether different traits for depth distribution and feeding habit returned different outcomes. Additionally, to further assess the role of *O. megalodon* in these marine communities,

another metacommunity web was also reconstructed for the Pleistocene including the presence of this shark post-extinction.

Food web downsampling

The metacommunity webs modelled for the Pliocene and Pleistocene show every feasible feeding interaction. It is unlikely that all of these interactions would have occurred, thus meaning the metacommunity webs are likely over connected with regard to what would be expected. It is possible that a high density of interactions could mask any changes detected when analysing the food webs, potentially underestimating the extent of community change following the extinction event. To account for this possibility, 'realised' webs were also modelled which exhibit a reduced link distribution.

The realised webs were constructed using a downsampling function which sets a target degree (link) distribution based on the mixed-exponential power law found to represent the structure of modern and some extinct communities[33,38,39,43]. During downsampling, links are removed randomly where feeding links attached to more highly connected nodes have a higher probability of removal. While this method relies on the assumption of consistency of food web degree distribution through time, this Pliocene extinction is relatively recent in geological terms and, therefore, modern marine communities are likely to have exhibited a similar pattern to these earlier Neogene communities.

Initially 1000 iterations of realised webs were generated for each time bin. Due to the random removal of links, downsampling resulted in multiple web topologies with two or more primary nodes. This occurred when taxa with few feeding links (notably squid) had all of their prey links removed and was thus moved to the base of the webs. These webs were removed from the dataset as they do not represent realistic food web structures.

As with the metacommunity food webs, an additional sample of realised webs were also generated simulating the presence of *O. megalodon* in the post-extinction Pleistocene communities. A further 1000 iterations of the realised webs were generated and those without realistic food web structures removed, as described above.

Food web analysis

The metacommunity and downsampled food webs were analysed to assess changes in structure across the extinction event. Commonly used network-level and node-level metrics and motifs (patterns of interaction between three connecting nodes[91]) were calculated in R (Table 2). Additionally, effect size was calculated for each of the metric and motif values from the realised webs using the Cohen's d measure[92] where values <0.49 signify a small effect size, 0.5-0.79 a medium effect size and >0.8 a large effect size. As the realised food webs were downsampled to achieve a target connectance, effect size for this metric is considered with caution.

Table 2: Metrics and motifs used in the food web analysis. Select metrics and motifs detailing what they measure and how they are calculated. Table modified from Shaw *et al.*, 2024[38].

Metric/Motif	Description	Explanation
Connectance	Realised feeding links expressed as a proportion of all links in a completed network	Measure of connectivity, often used as a proxy for ecosystem complexity
Normalised in-degree i.e. generality	Average number of resource nodes a consumer node is linked to	Measure of the range of prey items consumed by predators in the community
Normalised out-degree i.e. vulnerability	Average number of consumer nodes a resource node is linked to	Measure of the range of predators that feed upon prey taxa within the community
Number of linear chains (S1) (motif)	Normalised number of linear three-node chains in the network	Measure of linear energy pathways, often used as a proxy for vertical complexity
Omnivory (S2) (motif)	Normalised number of three-node chains with a consumer feeding on two resources at different trophic levels	Measure of predation across trophic levels, an indicator of specialism

Apparent competition (S4) (motif)	Normalised number of three-node chains where a consumer is linked to two resource nodes	Measure of predator choice
Direct competition (S5) (motif)	Normalised number of three-node chains where two consumers share a resource node	Measure of competition between predators

Data availability

The taxonomic and trait datasets alongside food web modelling rules used in the analyses are publicly available via Zenodo at https://doi.org/10.5281/zenodo.20473881.

Code availability

The R code used in the food web modelling and analysis are publicly available via Zenodo at https://doi.org/10.5281/zenodo.20473881.

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Impacts of the end Pliocene marine megafaunal extinction on North Atlantic trophic structure

Supplementary Figures and Tables

Table S1 Trophic level positions of *Otodus megalodon* from Pliocene metacommunity food webs for different trait combinations of depth distribution and feeding habit. Specialist refers to the diet of *O. megalodon* confirmed from fossil evidence (mammalia and reptilia), semi-generalist refers to the highly accepted diet based upon modern shark ecology (mammalia, reptilia, chondrichthyes and actinopterygii), and broad-generalist refers to the widest range of feeding habit based upon most-recent studies (mammalia, reptilia, chondrichthyes, actinopteryii, aves and cephalopoda). Trait combinations have no impact on calculated structural metrics of the food webs and combination six is presented in the Results.

Trait Combinations	Depth Distribution	Feeding Habit	*O. megalodon* Trophic Level
Combination 1	Neritic	Specialist	6.27
Combination 2	Neritic + Twilight	Specialist	6.27
Combination 3	Neritic	Semi-generalist	6.30
Combination 4	Neritic + Twilight	Semi-generalist	6.29
Combination 5	Neritic	Broad generalist	6.20
Combination 6	Neritic + Twilight	Broad generalist	6.19

Table S2 Unique feeding guilds of extant North Atlantic squid, inserted as individual nodes into the Pliocene and Pleistocene metacommunity and realised food webs.

Squid Nodes	Size Category	Feeding Habit	Vertical Position	Depth Distribution	Extant Genera Examples
Cephalopoda 1	tiny	Macroinvertebrates Actinopterygii	Benthopelagic	Neritic	*Lolliguncula*
Cephalopoda 2	tiny	Macroinvertebrates Actinopterygii Cephalopoda	Benthopelagic	Neritic	*Doryteuthis* *Sepioteuthis*
Cephalopoda 3	tiny	Macroinvertebrates Actinopterygii Cephalopoda	Pelagic	Neritic	*Stenoteuthis*
Cephalopoda 4	tiny	Macroinvertebrates Actinopterygii	Benthopelagic	Neritic Twilight	*Alloteuthis* *Ancistroteuthis* *Ancistroteuthis*

Cephalopoda 5	tiny	Macroinvertebrates Actinopterygii Cephalopoda	Benthopelagic	Neritic Twilight	*Illex* *Loligo* *Sepia* *Todaropsis*
Cephalopoda 6	tiny	Microinvertebrates	Pelagic	Neritic Twilight	*Discoteuthis*
Cephalopoda 7	tiny	Macroinvertebrates Actinopterygii Cephalopoda	Pelagic	Neritic Twilight	*Eucleoteuthis* *Hyaloteuthis* *Ornithoteuthis*
Cephalopoda 8	tiny	Actinopterygii Cephalopoda	Pelagic	Neritic Twilight	*Ommastrephes* *Onychoteuthis*
Cephalopoda 9	tiny	Macroinvertebrates Actinopterygii Cephalopoda	Benthopelagic	Neritic Twilight Midnight	*Gonatus* *Stigmatoteuthis*
Cephalopoda 10	tiny	Microinvertebrates	Pelagic	Neritic Twilight Midnight	*Sandalops*
Cephalopoda 11	tiny	Macroinvertebrates	Pelagic	Neritic Twilight Midnight	*Abralia* *Brachioteuthis*
Cephalopoda 12	tiny	Actinopterygii Cephalopoda	Pelagic	Twilight Midnight	*Grimalditeuthis*
Cephalopoda 13	small	Actinopterygii Cephalopoda	Pelagic	Neritic Twilight	*Thysanoteuthis*
Cephalopoda 14	small	Macroinvertebrates Actinopterygii Cephalopoda	Benthopelagic	Neritic Twilight Midnight	*Histioteuthis* *Todarodes*
Cephalopoda 15	small	Macroinvertebrates Actinopterygii Cephalopoda	Pelagic	Neritic Twilight Midnight	*Lepidoteuthis*
Cephalopoda 16	small	Actinopterygii Cephalopoda	Pelagic	Neritic Twilight Midnight	*Megalocranchia*
Cephalopoda 17	small	Macroinvertebrates Actinopterygii Cephalopoda	Pelagic	Twilight	*Tanigia*
Cephalopoda 18	medium	Macroinvertebrates Actinopterygii Cephalopoda	Pelagic	Neritic Twilight	*Onykia*
Cephalopoda 19	mega	Actinopterygii Cephalopoda	Pelagic	Twilight Midnight	*Architeuthis*

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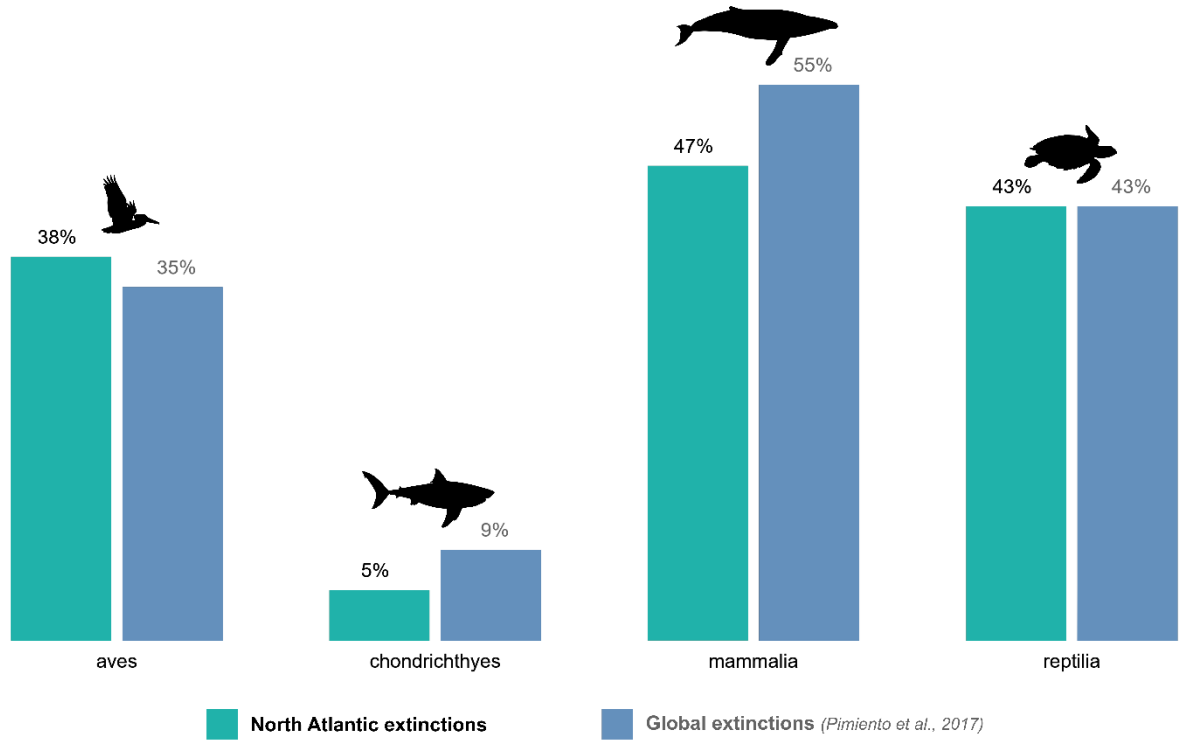

Figure S1 Proportional extinction of megafauna during the Pliocene marine megafaunal extinction. Extinction proportion of the different vertebrate groups is compared between the North Atlantic, as the focus of Chapter 2, and globally, using data from Pimiento *et al.* (2017). Silhouettes represent *Pelecanus occidentalis*, *Otodus megalodon*, *Balaenoptera novaeangliae* and *Eretmochelys imbricata*.

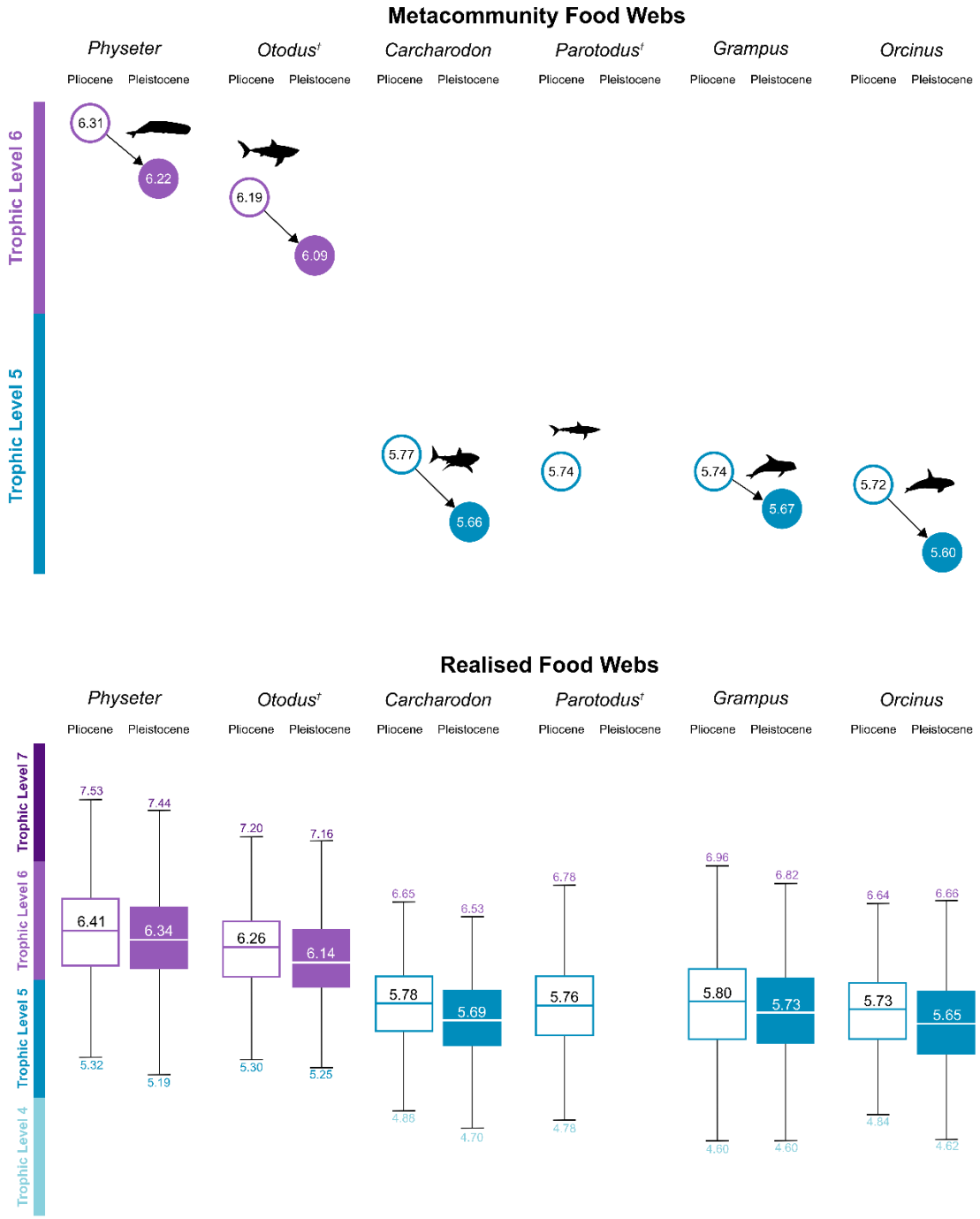

Figure S2 Trophic position changes in trop predators with simulated presence of *Otodus megalodon* in the Pleistocene for the metacommunity (top) and realised (bottom) food webs. For the metacommunity food webs, arrows show directional shift of trophic position of surviving top predators from the Pliocene to the Pleistocene. For the realised food webs, boxplots show range of trophic positions recovered across the 858 Pliocene webs and 834 Pleistocene webs. Outliers have been removed with the maximum, minimum and median values labelled. Colours denote different trophic levels and representative silhouettes from www.phylopic.org consist of *Physeter microcephalus*, *Otodus megalodon*, *Carcharodon carcharias*, *Carcharhinus obscurus* (used to represent *Parotodus*), *Grampus griseus* and *Orcinus orca*.