Use of nursery areas by the extinct megatooth shark *Otodus megalodon* (Chondrichthyes: Lamniformes)

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Abstract

Nursery areas are fundamental for the success of many marine species, particularly for large, slow-growing taxa with low fecundity and high age of maturity. Here we examine the population size-class structure of the extinct gigantic shark *Otodus megalodon* in a newly described middle Miocene locality from Northeastern Spain, as well as in eight previously known formations (Temblor, Calvert, Pisco, Gatun, Chucunaque, Bahía Inglesa, Yorktown and Bone Valley). In all cases, body lengths of all individuals were inferred from dental parameters and the size-class structure was estimated from Kernel probability density functions and Gaussian mixture models. Our analyses support the presence of five potential nurseries ranging from the Langhian (middle Miocene) to the Zanclean (Pliocene), with higher densities of individuals with estimated body lengths within the typical range of neonates and young juveniles. These results reveal, for the first time, that nursery areas were commonly used by *O. megalodon* over large temporal and spatial scales, reducing early mortality and playing a key role in maintaining viable adult populations. Ultimately, the presumed reliance of *O. megalodon* on the presence of suitable nursery grounds might have also been determinant in the demise of this iconic top predatory shark.

Keywords: *Otodus megalodon*, palaeoecology, sharks, nurseries, Miocene**.**

1. Introduction

Otodus megalodon is the largest macropredatory shark to have ever lived, with body length estimates of up to 15–18 meters for the largest adult individuals [1–4]. This species inhabited the warm and temperate waters of all major ocean basins, spanning a range of almost 20 million years, from the early Miocene to the Pliocene [5–7]. Most of the studies assessing the causes of the cosmopolitan distribution, the evolution of gigantism, and the extinction of otodontid sharks have focused on the impact of climatic factors and/or the abundance and migration patterns of their potential prey [1,8–11], competition with other macropredatory taxa [7,12], the availability of suitable habitats [13], and the presence of regional endothermy [6,14–16]. However, much less attention has been paid to important aspects of their reproductive biology [17].

Nursery areas are crucial for several marine groups, as they improve the probability of survival of juveniles, and allow a greater persistence and recruitment to the adult populations [18,19]. Shark nurseries are usually located in geographically discrete zones with high primary productivity, being defined by a comparatively high abundance of juveniles that tend to remain or return for extended periods of time [20,21]. The use of these areas has been documented in a number of living species [22], and may vary depending on the habitat (coastal, pelagic or deep-water nurseries) [22], the temporal patterns of occupation (permanent or seasonal) [23], or the number of taxa involved [24]. Some authors have also distinguished between primary nurseries, where gravid females give birth and neonates spend the first stages of their life, and secondary nurseries, with slightly older but still immature individuals [25].

Despite the utility of more recently proposed criteria in defining shark nursery areas, these are usually difficult to apply to extinct species and the recognition of nurseries in the fossil record is therefore challenging (see Heupel *et al.* [20,22] for a detailed discussion on the evolution of the concept for modern taxa). The existence of palaeonurseries has been recently supported by the presence of either abundant fossilized egg cases [26], biased representations of juveniles [8,27–35], or both [36,37]. Accordingly, the late Miocene Gatun Formation (Panama) has been proposed as a possible nursery area for *O. megalodon* based on the high proportion of juvenile individuals [27]. A second possible nursery area for *O. megalodon* could have been present in Borneo Island [38], but this observation, based only on three isolated juvenile teeth, is questionable. Although relevant, these sporadic findings are insufficient to evaluate the prevalence and the impact of such a reproductive strategy on the evolutionary history of *O. megalodon* and, as consequence, a more comprehensive body of evidence on nursery use patterns is required to reliably assess these questions. This is especially evident when considering the wide temporal and palaeobiogeographic distribution of this species.

In the present study, a new potential nursery of *O. megalodon* is described from the Langhian (middle Miocene) of Spain. We analyse a comprehensive database with records of this species revealing the plausible existence of multiple additional nursery areas, widely distributed in time and space. The broader implications of these findings are then contextualized in the light of some previous hypothesis about the success and later demise of this iconic extinct top predator.

2. Material and methods

The *O. megalodon* teeth studied first-hand in the present work were collected from Langhian outcrops (late middle Miocene) in the Reverté and Vidal quarries, which are situated in the southwestern part of the Vallès-Penedès Basin, close to the lowest stratigraphic layers of the El Camp de Tarragona Basin (see electronic supplementary material, figure S1*a* and supplementary methods for a detailed description of the geological context of the area). These quarries are located in the municipality of Castellet i La Gornal, in the province of Tarragona (Northeastern Spain) (electronic supplementary material, figure S1*b*). A total of 32 teeth, deposited in the Museu del Cau del Tauró de l'Arboç (Tarragona, Spain), have been collected over the last 20 years. Of these, 25 were considered in the present study due to their excellent preservation (figure 1 and electronic supplementary material, table S1). Their assignment to *O. megalodon* is well supported based on several morphological characteristics [27,39] (see electronic supplementary material, supplementary methods).

Teeth were assigned into jaw positions based on comparison with images in Pimiento & Balk [2], as well as an artificial tooth set figured in Applegate & Espinosa-Arrubarrena [40]. The population size-class structure of *O. megalodon* from the Reverté and Vidal quarries was inferred from dental parameters. According to the methodology described in Pimiento *et al*. [27], crown height was used to calculate total body lengths from the position-specific regressions established in the great white shark (*Carcharodon carcharias*) by Shimada [41] (electronic supplementary material, table S2 and supplementary methods).

The population size-class structure of *O. megalodon* was analysed by estimating Kernel probability densities of the inferred body lengths, with violin plots generated using the package 'ggplot2' [42] implemented in R [43]. Unimodality was checked by computing

Hartigans' dip statistic and its statistical significance in the R package 'diptest' [44]. Additionally, different modal parameters (mode(s), bimodality ratio and bimodality coefficient) were calculated using the R package 'modes' [45]. The bimodality ratio captures the difference in magnitude between the two identified peaks as the ratio between the amplitudes of the right and left peaks, respectively. The bimodality coefficient can vary between 0 and 1, where a value greater than 0.56 suggests the existence of bimodality and the maximum value of 1 indicates a fully bimodal distribution. The population size-class structure of *O. megalodon* was also evaluated with Gaussian mixture models using the R package 'Mclust' [46]. The optimal model and number of clusters was determined by the maximum Bayesian Information Criteria (BIC).

The population size-class structure of *O. megalodon* was assessed in eight additional formations covering a wide geographic area, including sites from the Atlantic, Caribbean and Pacific basins, which range from the middle Miocene to the Pliocene (i.e. the Temblor, Calvert, Yorktown and Bone Valley formations of the USA, the Pisco Formation of Peru, the Gatun and Chucunaque formations of Panama, and the Bahía Inglesa Formation of Chile) (figure 2). We analysed the dataset compiled in Pimiento & Balk [2], selecting only the formations therein containing more than 20 tooth records (see electronic supplementary material, supplementary methods). Analyses were first performed at formation level because this provided higher sample sizes and more statistically robust results. To obtain higher temporal and spatial resolutions, all the analyses were then repeated at locality level when more than 15 teeth were available (i.e., Shark Tooth Hill, Parkers Creek, Las Lomas, Lago Bayano, Mina Fosforita and Lee Creek Mine from Temblor, Calvert, Pisco, Chucunaque, Bahía Inglesa, and Yorktown formations, respectively). All the R scripts are available in the electronic supplementary material.

3. Results

Body length estimates derived from our teeth sample ranged from 2.61 meters (MCTA04205, upper anterior tooth) to 13.40 meters (MCTA04212, lower lateral tooth) (electronic supplementary material, table S1). Kernel probability density analysis and Hartigans' dip test (HDT) indicate that the population size-class structure of *O. megalodon* from Reverté and Vidal quarries shows a bimodal distribution, as suggested by estimated density graphs (figure 2), the high bimodality coefficient (0.73), and the comparatively low unimodality test p-value (0.22; although no statistical significance at 0.05 level is found). Higher densities of individuals are estimated within small and large body sizes (modes equal to 5.45 and 10.52 meters, respectively) (table 1). Similarly, the Gaussian mixture model analysis (GMM) supports bimodality within the optimal model, with modes equal to 5.28 and 11.10 meters, respectively (table 1).

The analysis of additional formations reveals a variety of size-class structures that can be grouped into two major types (figure 2 and table 1). The Temblor, Pisco, Bahía Inglesa and Yorktown formations show maximum density peaks in occurrences of large body sizes (modes from the HDT equal to 11.07, 11.05, 11.95 and 9.82 meters, respectively; modes from the GMM equal to 11.68, 11.40, 12.47 and 11.04 meters, respectively). Unimodal distributions are well supported by comparatively low bimodality coefficients (below 0.45), high unimodality test p-values (above 0.87), and BIC model selection. In contrast, the Calvert, Gatun, Chucunaque, and Bone Valley formations show maximum density peaks within smaller body sizes (modes from the HDT equal to 4.62, 6.56, 6.25, 5.62 meters, respectively; modes from the GMM equal to 4.29, 6.66, 7.69 and 6.71 meters, respectively). Bimodality coefficients are comparatively higher (above 0.53), and BIC values provide similar support for both unimodal and bimodal models in some instances. A second smaller peak can be recognized in the Gatun Formation (in both the HDT and the GMM), corresponding to large body sizes (modes equal to 17.15 and 14.79 meters, respectively), but is negligible when compared to the main left peak (bimodality ratio equals to 0.16). A second smaller peak is also detected in the Calvert Formation (only in the GMM), corresponding to comparatively small body sizes (mode equal to 7.54 meters). Despite this, unimodality is well supported in all these formations based on the unimodality test p-values (above 0.6). Results from the analyses performed at locality level are largely equivalent to those of the formations to which they belong to (electronic supplementary material, figure S2 and table S3).

4. Discussion

Our results reveal, for the first time, that nursery areas were commonly used by *O. megalodon* over large temporal and spatial scales. Five of the nine formations examined in this study (i.e., Reverté and Vidal quarries, Calvert, Gatun, Chucunaque, and Bone Valley formations) show size class structures that are consistent with the presence of nurseries, with the main size modes placed among body lengths typical of neonates and young juveniles (neonates ~ 4 meters and juveniles < 10.5 meters [1]) (figure 2 and table 1). Among these, the Calvert, Gatun, Chucunaque, and Bone Valley formations show unimodal distributions that are comparable to the age distributions present in most modern shark nursery areas ([47–54] among others). The population size-class structures estimated from the Reverté and Vidal quarries have bimodal distributions, with a second density peak among typical adult body sizes (> 10.5 meters [1]) that might represent gravid females. This distributional pattern is present in some living shark populations occupying areas that serve as primary nurseries [55–57]; however, a change in the use of this locality over time could also explain this size distribution, with juvenile and adult populations occupying this region at different times. In any case, the high proportion of neonates and juveniles supports the hypothesis that this area acted as a nursery ground for *O. megalodon* at some point during the Langhian. This interpretation is further supported by the fact that, during this interval, the Vallès-Penedès Basin was part of a shallow protected bay [58,59] with several taxa that could act as potential prey (i.e., marine mammals and a number fish species) and high primary productivity (as highlighted by the numerous fossil remains found in association with the teeth) (electronic supplementary material, table S4). The remaining four formations (i.e., Temblor, Pisco, Bahía Inglesa and Yorktown) demonstrate size-class structures typical of populations dominated by adults (> 10.5 meters [1]) (figure 2 and table 1), suggesting that these regions might correspond to feeding or mating areas [23].

Overall, our analysis based on Kernel probability density and Gaussian mixture model also supports the interpretation of the Gatun Formation as a nursery area [27], and reveals the presence of at least four additional nurseries for *O. megalodon* (i.e., Reverté and Vidal quarries, Calvert, Chucunaque, and Bone Valley formations). We further validate the results from the size-class structure analysis with a more integrative approach including stratigraphic, sedimentological and palaeontological evidence (electronic supplementary material, supplementary information), which is a fundamental procedure for the reliable recognition of new nursery areas [34]. Moreover, these findings are reinforced by analyses performed at locality level, with higher temporal and spatial resolutions (electronic supplementary material, figure S2 and table S3). This high proportion of potential nursery areas among the analysed localities may not be surprising as nurseries are zones with a high abundance of sharks and, as consequence, huge numbers of teeth can be shed, increasing the chances of subsequent fossil discoveries.

The use of nursery areas is likely to play a key role in the evolutionary history of some shark species [20,60]. This seems to be particularly true for large, slow-growing taxa with low fecundity and high age of maturity, which can benefit from this strategy of maximizing juvenile survival [22]. Evidence from analysis of vertebral growth rings suggests that the slowing or cessation of somatic growth in *O. megalodon* occurred around 25 years of age, denoting an extremely delayed sexual maturity [14]. It therefore seems plausible that the use of nursery areas could have been essential for *O. megalodon*, in order to reduce neonate and juvenile mortality and to provide maximum recruitment, thus maintaining viable populations on a long-term temporal scale. The widespread presence of *O. megalodon* nurseries supports this idea, demonstrating that this species benefited from this strategy at least from the Langhian (middle Miocene) to the Zanclean (Pliocene) in geographically separate regions (figure 2 and electronic supplementary material, figure S2). The presumed reliance of *O. megalodon* on the presence of suitable nursery grounds should be considered in future work as a potential disadvantage in light of the increasing competition of juveniles with the great white shark (*Carcharodon carcharias*) [6,7] and the drastic reduction in coastal habitats in the Pliocene [13]. More broadly, this work advances our understanding of the impact of reproductive strategies on the evolution of gigantic marine top predators and sheds light on the underlying factors of their evolutionary dynamics.

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Figure captions

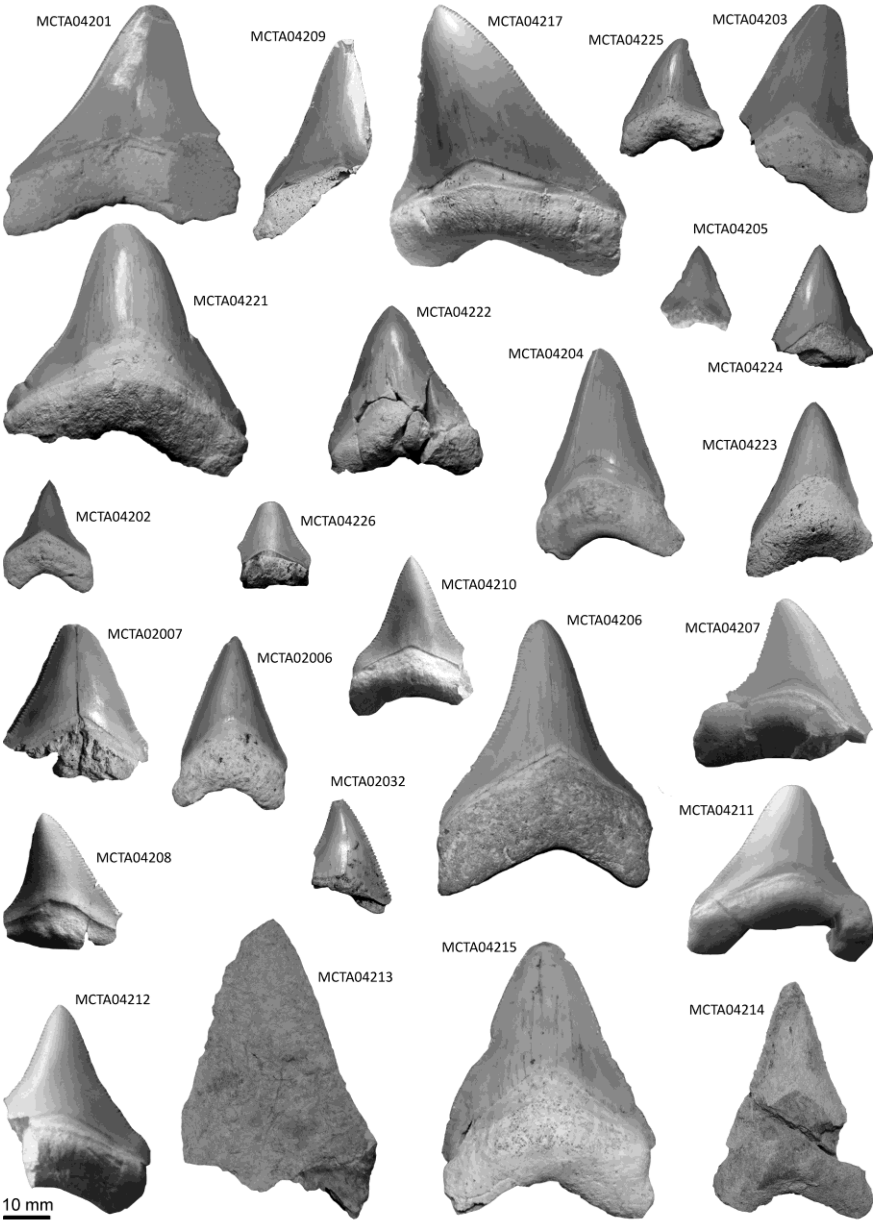

Figure 1. *Otodus megalodon* teeth collection from the Museu del Cau del Tauró de l’Arboç (MCTA, Tarragona, Spain), figured in lingual view.

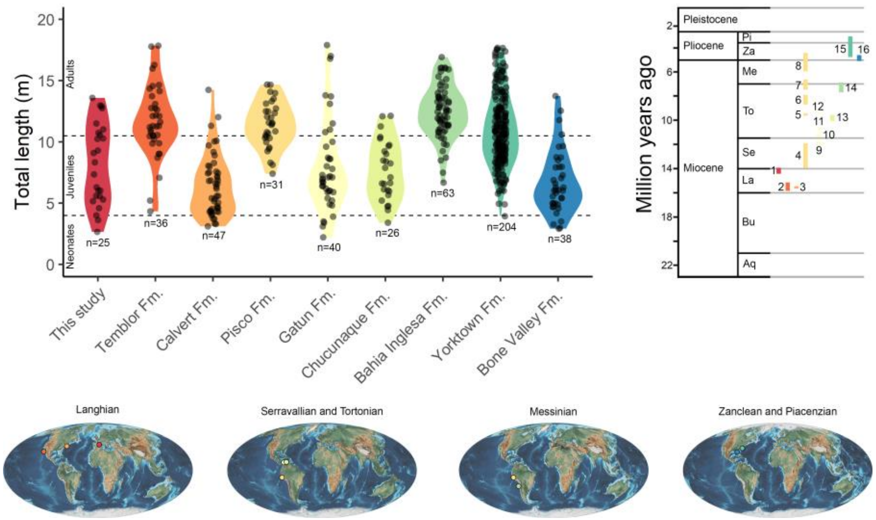

Figure 2. Size-class structure of *Otodus megalodon* populations from all the formations and localities analysed in this study (n > 20 tooth records), including information about their temporal and spatial distribution. Size-class categorization as neonates, juveniles and adults follows criteria from Gottfried *et al.* [1]. Palaeogeographic maps from Scotese [61]. Stages: Aq, Aquitanian; Bu, Burdigalian; La, Langhian; Me, Messinian; Pi, Piacenzian; Se, Serravallian; To, Tortonian; Za, Zanclean. Localities: 1, Reverté and Vidal quarries; 2, Shark tooth Hill; 3, Parkers Creek and Scientists Cliff; 4, Cerro La Bruja and Cerro Colorado; 5, Yesera de Amara; 6, Aguada de Lomas; 7, Cerro Los Quesos; 8, Montemar; 9, Texaco; 10, Alborada; 11, Las Lomas and San Judas; 12, Isla Payardi; 13, Lago Bayano; 14, Mina Fosforita; 15, Lee Creek Mine; 16, Achan Mine, Chicora Mine, Fort Green Mine, Four Corners Mine, Kingsford Mine, North Palmetto Mine, Palmetto Mine, and Payne Creek Mine (Tro Quarry).

Tables

Table 1. Modal parameters, Hartigans' dip test and Gaussian mixture models analysis results for estimated size-class density curves in all analysed formations. In Gaussian mixture models: E, equal variance; V, variable variance; 1, unimodal; 2, bimodal.

	Modal parameters and Hartigans' dip test				
	Mode 1 (m)	**Mode 2 (m)**	**Bimodality ratio**	**Bimodality coefficient**	**Unimodality test (p-value)**
This study	5.45	10.52	0.92	0.73	0.22
Temblor	11.07	4.86	7.66	0.29	0.91
Calvert	4.62	-	-	0.54	0.60
Pisco	11.05	-	-	0.45	0.87
Gatun	6.56	17.15	0.16	0.60	0.98
Chucunaque	6.25	-	-	0.56	0.87
Bahía Inglesa	11.95	-	-	0.35	0.92
Yorktown	9.82	-	-	0.43	0.98
Bonevalley	5.62	-	-	0.53	0.70

	Gaussian mixture models				
	Mode 1 (m)	**Mode 2 (m)**	**Top 3 models based on the BIC values**		
This study	5.28	11.10	E2 (-133.98)	E1 (-136.34)	V1 (-136.34)
Temblor	11.68	-	E1 (-187.01)	V1 (-187.01)	E2 (-194.20)
Calvert	4.29	7.54	V2 (-224.78)	E1 (-225.83)	V1 (-225.83)
Pisco	11.40	-	E1 (-133.44)	V1 (-133.44)	V2 (-139.79)
Gatun	6.66	14.79	E2 (-222.39)	V2 (-225.64)	E1 (-227.11)
Chucunaque	7.69	-	E1 (-125.57)	V1 (-125.57)	E2 (-129.23)
Bahía Inglesa	12.47	-	E1 (-283.76)	V1 (-283.76)	V2 (-292.02)
Yorktown	11.04	-	E1 (-1028.80)	V1 (-1028.80)	V2 (-1029.31)
Bonevalley	6.71	-	E1 (-186.33)	V1 (-186.33)	V2 (-186.99)