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Can biochemical tracers reveal ontogenetic trophic shift and individual prey selection in white sharks from Guadalupe Island, Northeast Pacific?

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ABSTRACT

Refining the role of apex predators in marine food webs is a necessary step in predicting the consequences of their global decline under the footprint of fishing activities. White sharks (*Carcharodon carcharias*) are vulnerable predators, performing large migrations and able to forage on a variety of prey in different habitats. In the Northeast Pacific, juvenile and adult white sharks are found seasonally at the same aggregation sites, such as Guadalupe Island off Mexico. While adults are thought to target local pinniped colonies, very few predator-prey interactions have been documented and the diet of juveniles in this area remains poorly understood. Here we used carbon/nitrogen stable isotopes and fatty acids to characterize the trophic ecology of white sharks at Guadalupe Island. In contrast to the ontogenetic trophic shift paradigm, we detected no influence of size on muscle stable isotope and fatty acid composition, revealing no significant dietary variation between juvenile and adult sharks. Stable isotopes did not allow definitive conclusions to be drawn regarding the diet of white sharks at Guadalupe Island, due to significant variability in the contribution of different potential prey depending on the trophic discrimination factors used. However, most sharks were rich in polyunsaturated fatty acids (such as long-chain omega 3), suggesting a local diet of mainly pelagic prey (potentially large fish or cephalopods). A few individuals appeared to show recent consumption of pinnipeds, with higher proportions of saturated and monounsaturated fatty acids. These individual differences in fatty acid composition could reflect an ecological trade-off between consumption of prey rich in fat (marine mammals) *versus* prey rich in polyunsaturated fatty acids (pelagic prey), respectively meeting the energetic and physiological needs of white sharks. Although ontogenetic trophic changes were not able to be discerned, our results provide new insights into the physiological drivers of predator-prey interactions, which can benefit the definition of conservation strategies in a changing ocean.

1. Introduction

Over the last decades, the global risk of oceanic predatory fish extinction has steadily increased due to overfishing and bycatch (Dulvy et al., 2021; Juan-Jordá et al., 2022; Pacoureau et al., 2021). Population declines of some large sharks could lead to cascading trophic effects altering the structure and functioning of marine ecosystems (Ferretti et al., 2010; Heithaus et al., 2008; Pimiento et al., 2020). Improving knowledge on the trophic ecology of apex predators is therefore an essential step towards understanding their ecosystem role (e.g., top-down influence on potential prey populations) and implementing effective management and conservation programs (Bird et al., 2018; Hussey et al., 2015; Jorgensen et al., 2022). However, such studies pose a significant challenge in highly mobile, generalist predators such as white sharks (*Carcharodon carcharias*), which express their feeding behavior across a wide range of prey and habitats (Boustany et al., 2002; Carlisle et al., 2012; Huveneers et al., 2018; Le Croizier et al., 2022b).

Although they can forage on various prey items, white sharks are thought to show a marked ontogenetic shift in feeding behavior, from a predominantly piscivorous diet to a greater consumption of marine mammals after reaching a length of around 3 m (Estrada et al., 2006; Hussey et al., 2012). As a result, large white sharks are known to aggregate near coastal pinniped colonies across the world's oceans (Domeier, 2012; Huveneers et al., 2018). In the northeastern Pacific, adult individuals migrate seasonally from coastal and island aggregation sites (e.g., central California in the USA and Guadalupe Island in Mexico,

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respectively) - where they are believed to primarily target pinnipeds - to offshore oceanic areas in the North Pacific Subtropical Gyre where they may largely rely on prey from the mesopelagic zone (i.e., between 200 and 1000 m deep) (Carlisle et al., 2012; Jaime-Rivera et al., 2014; Jorgensen et al., 2012; Le Croizier et al., 2020b). In Mexico, young white sharks occupy coastal nursery areas near the mainland, before moving to aggregation sites such as Guadalupe Island after reaching a length of at least 1.8 m (Hoyos-Padilla et al., 2016). Unlike adults, juveniles do not perform seasonal offshore migrations and remain in the vicinity of Guadalupe Island throughout the year. While they are known to feed primarily on nearshore demersal prey in mainland nursery areas (García-Rodríguez et al., 2021; Tamburin et al., 2019), the diet of juvenile white sharks at Guadalupe Island is still poorly understood.

Carbon and nitrogen stable isotopes (expressed as δ^{13}C and δ^{15}N, respectively) have been widely used to characterize the trophic ecology of sharks (Besnard et al., 2022; Hussey et al., 2011; Le Croizier et al., 2020a; Shiffman et al., 2012). Since carbon stable isotope signatures vary substantially across primary producers at the base of food webs, δ^{13}C values allow to discriminate between marine ecosystems (e.g., coastal versus oceanic) and thus can trace the foraging habitat of predators (Bird et al., 2018; Cherel and Hobson, 2007). Additionally, δ^{15}N values increase significantly from prey to predator and are therefore considered a relevant proxy for assessing the trophic position of consumers in a given food web (Estrada et al., 2003; Hussey et al., 2015). The combination of δ^{13}C and δ^{15}N values can also be used to estimate the relative contribution of different prey items to the diet of a predator (e.g., using isotopic mixing models) (Carlisle et al., 2012; Raoult et al., 2019). These approaches require prior knowledge of the difference between the isotopic values of the consumer and its prey, called “trophic enrichment” or “trophic discrimination factor” (hereafter “TDF”), expressed as Δ^{13}C and Δ^{15}N for carbon and nitrogen, respectively. However, TDFs are known to depend on several sources of variation, such as environment, taxon, tissue and diet (Caut et al., 2009; Le Croizier et al., 2022a; Stephens et al., 2023). The use of different TDFs (e.g., Δ^{15}N ranging from 2.3 to 5.5 ‰) to study the diet of large sharks significantly affects the estimates, thus preventing any definitive conclusion as to the composition of shark prey (Hussey et al., 2010; Kim et al., 2012a; Olin et al., 2013).

Lipids form a large group of structurally heterogeneous molecules that support two major biological functions: structural lipids are involved in the constitution of cell membranes, while reserve lipids represent an important form of energy storage (Parrish, 2013; Sargent et al., 2003). Certain fatty acids (FAs), the major constituents of lipids, are essential for the proper functioning of organisms (e.g., some omega 3 and 6) (Vagner et al., 2014, 2015). Indeed, essential FAs play a major role in cellular synthesis, neuronal development, endocrine function and control, ionic regulation, immune function and reproduction (Sargent et al., 2003; Tocher, 2003). For instance, 22:6n-3 (docosahexaenoic acid, DHA) is necessary for the formation of neural membranes in the brain and eyes (Koven et al., 2018; Morais et al., 2011), while 20:5n-3 (eicosapentaenoic acid, EPA) and 20:4n-6 (arachidonic acid, ARA) are important modulators of inflammatory/immune responses and reproduction processes through the production of eicosanoids and prostanoids (Chapkin et al., 2009; Xu et al., 2022a,b). As essential FAs are poorly synthesized by marine fish, they must be obtained through food. In addition, when sufficient lipid intake is provided by diet, *de novo* synthesis of FAs is reduced or inhibited, and the majority of FAs (both essential and non-essential FAs) come from dietary sources (Sargent et al., 2003; Tocher, 2003). As the structural integrity of FAs is generally conserved during trophic transfers, they can be used to trace predator-prey relationships (Beckmann et al., 2013, 2014). Finally, since FA composition varies significantly between potential prey (e.g., marine mammals *versus* fish *versus* cephalopods), an increasing number of studies rely on FAs to assess the dietary composition of marine predators, including white sharks (Every et al., 2017; Meyer et al., 2019; Pethybridge et al., 2014).

Muscle is the most commonly used tissue to study shark trophic ecology based on biochemical tracers (e.g., Bird et al., 2018; Maurice et al., 2021; Meyer et al., 2019). As protein turnover is slower than lipid turnover in muscle tissue, stable isotope composition reflects shark diet over a longer period than fatty acid composition (months/years and weeks, respectively) (Munroe et al., 2018). In the present study, we investigated the trophic ecology of white sharks from Guadalupe Island, through the analysis of isotopic and FA compositions of the muscle from individuals of various sizes. Considering previous knowledge of white shark movements in the Northeast Pacific and the integration time of tissue compounds, we assume that isotope values of adult sharks reflect feeding over their entire migratory cycle, while isotope values of juveniles result mainly from foraging around Guadalupe Island. On the other hand, the fatty acid composition of white sharks is thought to be influenced primarily by recent prey consumption near Guadalupe Island, for both juveniles and adults. Given the ontogenetic dietary shift observed in other white shark populations, as well as the permanent presence of juveniles and the seasonal presence of adults in the waters of Guadalupe Island, we sought to answer three main questions: 1) Do isotope values vary with shark size, as a result of differences in habitat use between juveniles and adults and/or potential ontogenetic change in prey composition? 2) Can we identify the main prey of juvenile sharks around Guadalupe Island from isotope values? 3) Do fatty acid compositions make it possible to detect differences between juveniles and adults in the prey targeted during their simultaneous presence around Guadalupe Island? Our objective was to better understand the role of this aggregation site as foraging habitat during the life cycle of white sharks in the Northeast Pacific.

2. Materials and methods

2.1. Sample collection

White sharks (n = 25) were sampled at Guadalupe Island in the Mexican Pacific (Fig. 1), between the months of September and November in 2016, 2017 and 2018. Free-swimming white sharks were attracted with baits (yellowfin tuna, *Thunnus albacares*) near the scientific boat. Muscle samples were taken using a biopsy probe (1 cm diameter) targeting the tissue directly below the dorsal fin. The biopsy probe was cleaned and rinsed with alcohol before and between samples. After collection, tissue samples were immediately transferred to a −20 °C freezer on board the vessel. Once in the laboratory, muscle samples were freeze-dried and homogenized. Individual sharks were sexed (based on the presence or absence of claspers) and sized to the nearest 10 cm using visual size estimates. A previous study compared visually estimated total length measurements of white sharks during cage diving operations with measurements obtained from stereo-video cameras and assessed the accuracy of these estimates against suspected biases (e.g., shark size and observer experience) (May et al., 2019). The authors found that visual estimates were not influenced by shark size, and that scientists were the most accurate in their estimates, with a mean accuracy of around 20 cm. They conclude that visual estimates of length can be used in research that is not sensitive to 20 cm biases when the estimates are made by scientists. In our study, size differences between individuals were mostly larger (i.e., 50 cm) than the uncertainty associated with visual estimates, which were made by a researcher (MHP) with extensive experience in measuring white sharks at this specific site. We therefore assume that visual estimates are a reliable method with an acceptable degree of accuracy in the context of our study. The white sharks we sampled ranged from 2 m to 5 m total length (TL) and were composed of juveniles (<3 m TL), subadults (3–3.6 m TL for males and 3–4.8 m TL for females) and adults (>3.6 m TL for males and >4.8 m TL for females) (Domeier, 2012). Samples were collected from different individuals including 16 females (6 juveniles, 9 subadults, 1 adult), 6 males (2 juveniles, 2 subadults, 2 adults) and 3 unsexed sharks (1 adult, 2 unmeasured individuals).

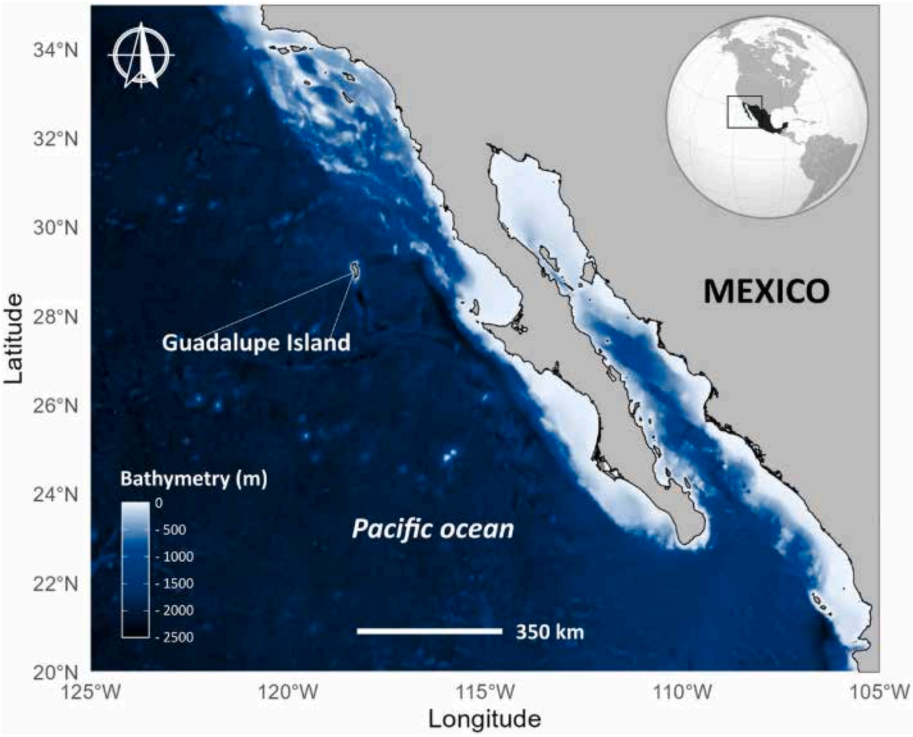

Fig. 1. Map of the sampling site (Guadalupe Island) off Mexico in the northeastern Pacific.

2.2. *Carbon and nitrogen stable isotope analysis*

Before stable isotope analyses, urea and lipid extractions were applied on powdered samples according to Li et al. (2016). Briefly, for urea removal, samples were vortexed in deionized water for 1 min, soaked for 24 h at room temperature, centrifuged for 5 min, and water removed with a medical needle. This process was repeated three times before the samples were dried again using an EZ-2 centrifugal evaporator (Genevac). For lipid extraction, urea-free samples were soaked in a 2:1 chloroform/methanol mixture, vortexed for 1 min, left overnight at room temperature, centrifuged for 10 min and decanted. This process was also repeated three times and the samples were dried again to remove excess solvent. Approximately 350 μg of sample powder was then weighed in tin capsules for isotopic analysis. The samples were analysed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyser coupled to a Delta V Plus mass spectrometer at the Pôle Spectrométrie Océan (Plouzané, France), according to a previously published protocol (Besnard et al., 2021; Le Croizier et al., 2020a, 2023). Results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$) following the equation $\delta^{13}C$ or $\delta^{15}N = [(Rsample/Rstandard) - 1] \times 10^3$ (in ‰), where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. International isotopic standards of known $\delta^{15}N$ and $\delta^{13}C$ were analysed: IAEA-600 Caffeine, IAEA–CH–6 Sucrose, IAEA-N-1 and IAEA-N-2 Ammonium Sulphate. A home standard (Thermo Acetanilide) was used for experimental precision (based on the standard deviation of the internal standard replicates) and indicated an analytical precision of ±0.31‰ for $\delta^{13}C$ and ±0.21‰ for $\delta^{15}N$.

2.3. *Fatty acid analysis*

Lipid extracts were flushed with nitrogen gas, vortexed, and stored at −20 °C. After the addition of tricosanoic acid (23:0) as an internal standard and evaporation to dryness under nitrogen, fatty acid methyl esters (FAMEs) were obtained using a method modified from Le Grand et al. (2014). A total of 0.8 ml of a sulphuric acid solution (3.4% in methanol) was added, vortexed and heated for 10 min at 100 °C. Before gas chromatography analysis, 0.8 ml of hexane was added and the organic phase containing FAMEs was washed three times with 1.5 ml of hexane-saturated distilled water. The organic phase was finally transferred to tapering vials and stored at −20 °C. FAMEs were analysed with a Varian CP 8400 gas chromatograph equipped with a Zebron ZB-WAX and a ZB-5HT column (both 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness, Phenomenex) and flame ionization detectors at the Lipidocean facility (Plouzané, France). FAMEs were identified by comparing sample retention times to those of commercial standard mixtures (Supelco 37-component FAME mix, BAME, and PUFA no. 1 and 3 mixes, Sigma-Aldrich) using Galaxie 1.9.3.2 software (Varian). FAME contents were converted to fatty acid (FA) contents based on 23:0 recovery. Individual FA proportions were expressed as the percentage (%) of the total FA content.

2.4. *Data analysis*

All statistical analyses were performed using the open-source software R (R Core Team, 2023). For group comparisons, data was first checked for normality (Shapiro-Wilk tests) and homogeneity of variances (Bartlett tests). When these conditions were met, one-way ANOVAs were performed to test for differences between groups, otherwise, non-parametric analogues were used (Wilcoxon tests with Holm p-value adjustments). After checking for data normality (Shapiro-Wilk tests), Pearson's correlation tests were used to assess relationships between $\delta^{13}C$ or $\delta^{15}N$ values and shark length. Among the 58 FAs identified in white shark muscle, 11 FAs with relative proportions above 1% (representing 87 ± 4% of the total FA content) were included in subsequent statistical analyses. Permutational multivariate analyses of variance (PERMANOVAs) were used to test the influence of sex and size on the FA composition of white sharks. Individual white sharks were classified based on their FA composition using a K-means cluster analysis. A similarity percentage analysis (SIMPER) was conducted to determine the FAs most involved in the dissimilarity between groups.

Bayesian stable isotope mixing models were constructed with $\delta^{13}C$

and δ^{15}N values to estimate the relative contribution of different prey groups to the diet of juvenile white sharks using the "simmr" package. Stable isotope values of potential prey were sourced from previous studies conducted near Guadalupe Island, and separated into four groups: three trophic groups of pelagic species previously determined in the region (Madigan et al., 2012), plus one group consisting of pinniped species present at Guadalupe Island (Table S1). A previous study in a controlled environment on hooded seals observed differences between isotopic values of fur and muscle (Pinzone et al., 2017). As the isotopic values reported in the literature on pinnipeds present at Guadalupe Island relate only to fur, we adjusted these values to resemble muscle values by using the differences between tissues observed experimentally (i.e., by subtracting 0.7 ‰ to δ^{13}C values and adding 0.7 ‰ to δ^{15}N values) (Pinzone et al., 2017). Different trophic discrimination factors were used according to a meta-analysis on carnivorous marine fishes (Stephens et al., 2023) and previous shark feeding studies under controlled conditions (Hussey et al., 2010; Kim et al., 2012a, c). The source data were incorporated in the mean ± SD form. Models were run with generalist prior distributions and Markov Chain Monte Carlo (MCMC) simulation methods (number of iterations = 100000, size of burn-in = 10000, amount of thinning = 100 and number of MCMC chains = 4). Convergence of the models was checked using Gelman-Rubin diagnostics. In all cases, the Gelman-Rubin diagnostic was 1, indicating good convergence.

A principal component analysis (PCA) was performed based on the FA composition of white sharks. Fatty acid profiles of potential prey species from the North and Central Pacific (species means as reported in the literature) were overlaid on the PCA as illustrative individuals. Potential prey were classified into four taxonomic groups (namely "Seals", "Tuna", "Sharks" and "Squid"), consisting of northern elephant seals, tuna species, pelagic sharks and vertically migrating large squid, respectively (Table S2). Based on the coordinates extracted from the first two dimensions (PC1 and PC2) of the PCA, the "nicheROVER" package was used to define niche regions and overlaps for white sharks and potential prey groups. Niche region was defined as the 95% probability region in multivariate space, while niche overlap was calculated as the probability that a randomly chosen individual from a prey group will be found in the white shark niche. Overlap uncertainty was accounted for by performing 1000 elliptical projections of niche region through Bayesian statistics.

3. Results and discussion

3.1. Stable isotopes

Due to slow protein turnover, isotopic composition of muscle can provide integrated dietary information over a long period (months to years) (Munroe et al., 2018). Although some juvenile sharks we analysed may have recently left mainland nurseries, both their δ^{13}C and δ^{15}N values were significantly lower than those of similarly sized conspecifics sampled along the Mexican coast (ANOVAs, $p < 0.01$; Table S3), suggesting that they arrived at Guadalupe Island long enough for their isotopic signature to stand out from that of juveniles still using the coastal habitat (García-Rodríguez et al., 2021). Thus, we assume that the isotopic values measured in our study primarily reflect feeding in the waters of Guadalupe Island for juvenile sharks, while they also reflect the offshore migration season for subadult and adult sharks (Hoyos-Padilla et al., 2016). No differences were detected in muscle isotopic composition (δ^{13}C and δ^{15}N values) between sexes (ANOVAs, $p > 0.05$), nor any variation in isotope values with shark length (Pearson's correlation test, $p > 0.05$; Fig. 2). These results contrast with the ontogenetic changes in isotopic composition previously observed in white sharks from the same northeast Pacific population sampled off the coast of California (Carlisle et al., 2012). A decrease in muscle δ^{13}C and δ^{15}N values with length was found in these white sharks, suggesting a size-based change in foraging habitat. Indeed, juvenile white sharks are known to feed primarily in coastal productive waters of the California Current, where baselines are enriched in ^{13}C and ^{15}N, while adults perform seasonal migrations to offshore regions where they exploit pelagic food webs with lower isotopic baselines (Jorgensen et al., 2012; Trueman and St John Glew, 2019; White et al., 2019). The authors therefore concluded that the variations in isotopic composition measured in California white sharks reflected changes in habitat use rather than an increase in the trophic level of adult sharks, which would have led conversely to increased δ^{13}C and δ^{15}N values. After leaving nurseries on the Mexican coast, juvenile white sharks that reach Guadalupe Island face an oceanic environment similar to offshore regions exploited by adults during their migrations (Fig. 1). Thus, the lack of marked contrast in isotopic baselines between habitats (i.e., Guadalupe Island and offshore habitats) could explain the similar isotopic compositions between juveniles and adults in our study, unlike California sharks which are exposed to stronger isotopic gradients during ontogenetic shift from coastal to offshore habitats. Still, C and N isotope baselines may be slightly lower in the offshore migration zone than in Guadalupe Island waters (Arnoldi et al., 2023; Trueman and St John Glew, 2019). In addition, it was recently observed that the increase in isotope values between trophic levels (i.e., trophic discrimination factor "TDF") could vary across regions in the North Pacific, suggesting spatial differences in the factors underlying trophic dynamics, such as variability in community composition or changes in diet based on resource availability (Arnoldi et al., 2023). For instance, it was estimated that top predators feeding offshore within the North Pacific Subtropical Gyre had significantly lower δ^{15}N values than predators of the same trophic level feeding in the Guadalupe Island region (Arnoldi et al., 2023). This difference of around 5 ‰ corresponds to the upper range of shifts in δ^{15}N values observed between two trophic levels (Hussey et al., 2010; Kim

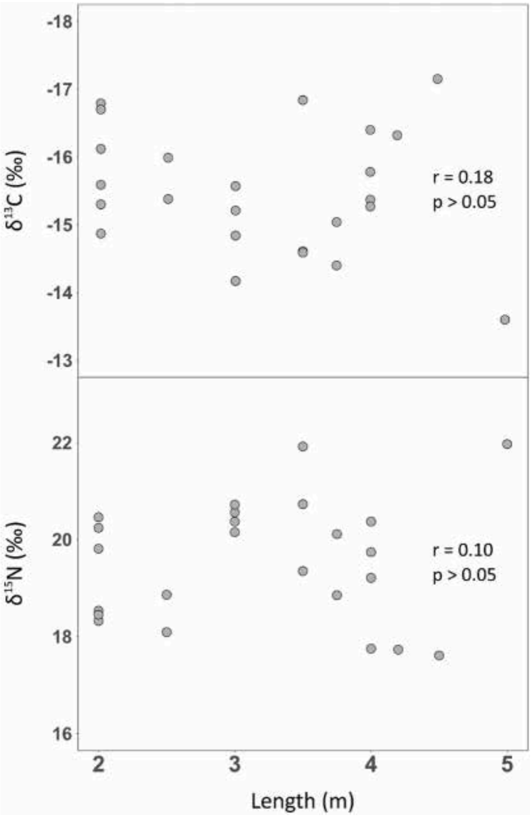

Fig. 2. Biplot of δ^{13}C or δ^{15}N values and total length of white sharks from Guadalupe Island. Pearson correlation tests detected no significant relationship between isotope values and shark length. Pearson correlation coefficients (r) and associated p-values are displayed in the panels.

et al., 2012c; Olin et al., 2013). Therefore, foraging offshore part of the year could lower the isotopic values of adult white sharks and, despite a potential higher trophic level, give them isotopic values similar to those of juvenile sharks that forage near Guadalupe Island throughout the year.

The lack of variation in isotopic compositions with size observed in our study could also result from a similar diet between juveniles and adults. Isotopic analysis of vertebrae from California white sharks has previously revealed that ontogenetic patterns in δ^{15}N values can vary considerably among individuals, with three trajectories observed: 1) δ^{15}N values increasing throughout life, 2) δ^{15}N values increasing until reaching a plateau and 3) constant δ^{15}N values throughout life (Kim et al., 2012b). The third pattern, which is similar to the one observed in our study (Fig. 2), resulted from the fact that juveniles were characterized by high δ^{15}N values, similar to those of adults. The authors therefore put forward the hypothesis of scavenging on pinniped carcasses or consumption of large squid by young sharks. The diet of juvenile sharks remains unknown in Guadalupe Island waters, as local predation on pinnipeds and squid has only been confirmed for larger sharks (Becerril-García et al., 2020; Hoyos, 2009). Accurate determination of diet composition using stable isotopes remains a challenge given the uncertainty associated with TDFs. In our study, estimates of the relative contribution of prey to the diet of juvenile white sharks varied significantly depending on the TDFs used. We estimated that 66% of juvenile sharks' diet consisted of pelagic meso-predators (Fig. 3A), using values of 3.5 ‰ and 5.5 ‰ for Δ^{13}C and Δ^{15}N respectively. Using lower TDFs, we found either a dominance of pelagic predators (i.e., 62% with $\Delta^{13}C = 0.9$ ‰ and $\Delta^{15}N = 2.3$ ‰, Fig. 3B) or an equivalent contribution of pelagic predators and pinnipeds to the diet of juvenile white sharks (Fig. 3C and D) (e.g., 43% and 42% respectively with $\Delta^{13}C = 1.7$ ‰ and $\Delta^{15}N = 2.5$ ‰). While three out of four models suggest that pelagic predators represent a significant, if not dominant, proportion of juvenile white shark prey, these results highlight the inherent limitations of traditional isotopic methods for determining the prey targeted by juvenile white sharks in the waters off Guadalupe Island. In future studies, the use of compound-specific isotope analyses could overcome TDFs by focusing on amino acids known to undergo negligible trophic fractionation (McMahon and Newsome, 2019; Skinner et al., 2021).

3.2. *Fatty acids*

3.2.1. *General trends*

Rapid lipid turnover implies that muscle fatty acid (FA) composition only reflects diet during the last weeks prior to sampling (Munroe et al., 2018), when sharks of all size classes occupied the waters off Guadalupe Island (Hoyos-Padilla et al., 2016). Of the 58 FAs identified in white shark muscle, 11 FAs had relative proportions above 1%, with 16:0, 22:6n-3 (docosahexaenoic acid, DHA), 18:0, 18:1n-9 and 20:4n-6 (arachidonic acid, ARA) as dominant FAs (in decreasing order; Table 1), and accounted for 87 ± 4% of the total FA content. Higher proportions of polyunsaturated FAs (PUFAs, 37 ± 9%) and saturated FAs (SFAs, 35 ± 5%) were found compared to monounsaturated FAs (MUFAs, 25 ± 5%). As with stable isotopes, no effect of sex or shark length was detected on total FA composition or individual FA proportions (PERMANOVAs, $p > 0.05$). Although no clear influence of size was also observed on the FA profiles of Australian white sharks (Meyer et al., 2019; Pethybridge et al., 2014), our results contrast with the paradigm of an ontogenetic trophic shift occurring in white sharks around 3–3.5 m in length, with an increased consumption of marine mammals beyond this size, as observed in other studies (Estrada et al., 2006; Hussey et al., 2012). In addition, dietary differences were expected as a result of the contrasting habitat use previously documented between juvenile and adult white sharks at Guadalupe Island (Hoyos-Padilla et al., 2016). It has been proposed that swimming close to shore in shallow water would allow juveniles to feed on demersal prey while avoiding larger sharks, whereas patrolling in deeper water would allow adults to ambush pinnipeds by taking advantage of reduced light (Aquino-Baleytó et al., 2021; Hoyos-Padilla et al., 2016; Papastamatiou

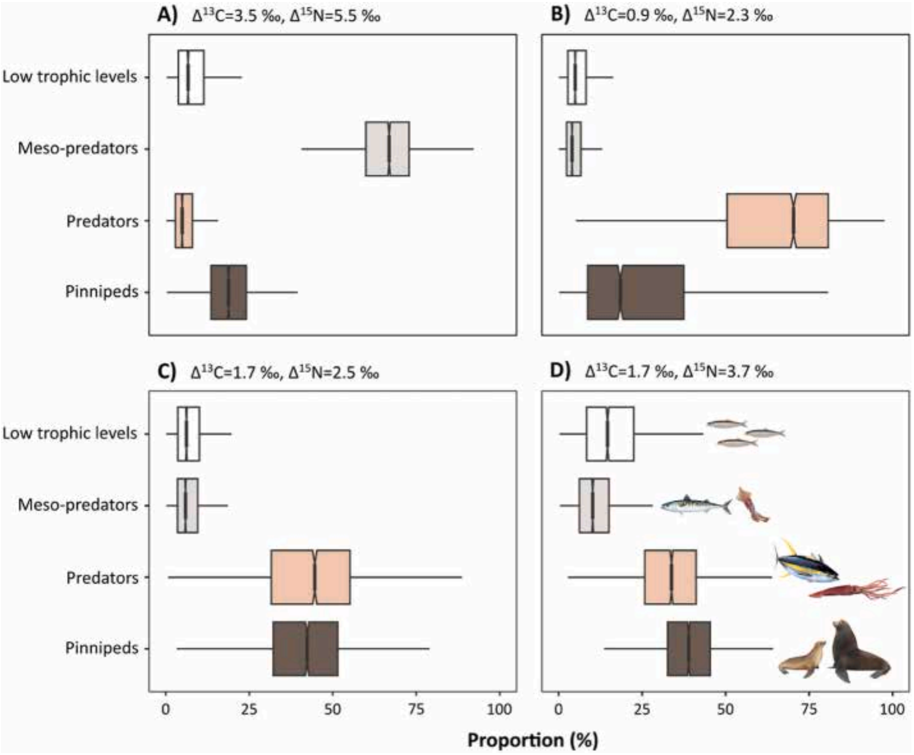

Fig. 3. Estimated proportions based on stable isotope values of pelagic species (low trophic level species, meso-predator species, predator species) and pinnipeds in the diet of juvenile white sharks ≤3 m in total length, known to stay year round near Guadalupe Island. Different trophic discrimination factors (TDFs) were used in the mixing models, according to previous studies: TDFs from A) Kim et al., 2012c, B) Hussey et al., (2010), C) Stephens et al., (2023) and D) Kim et al., 2012a, c.

Table 1
Fatty acid (%) and stable isotope (‰) compositions in white sharks from Guadalupe Island (mean ± SD). Individual white sharks were separated into two groups based on their FA composition (Groups 1 and 2, K-means clustering). ”*” indicates higher value compared to the other group (Wilcoxon tests with Holm p-value adjustments, $p < 0.05$).

	White sharks (n = 25)	Group 1 (n = 6)	Group 2 (n = 19)
Saturated fatty acids (SFAs)			
16:0	17.7 ± 2.6	20.2 ± 3.8 *	16.9 ± 1.5
18:0	15.3 ± 3.2	19.4 ± 3.8 *	14.1 ± 1.7
Σ SFAs	35.0 ± 5.3	42.0 ± 5.7 *	32.8 ± 2.5
Monounsaturated fatty acids (MUFAs)			
16:1n-7	1.6 ± 0.6	2.0 ± 0.8	1.5 ± 0.6
18:1n-9	11.5 ± 2.7	14.8 ± 2.5 *	10.4 ± 1.8
18:1n-7	6.2 ± 1.7	7.2 ± 2.8	5.9 ± 1.1
20:1n-9	1.6 ± 0.4	1.7 ± 0.5	1.6 ± 0.3
Σ MUFAs	24.7 ± 5.2	31.4 ± 4.7 *	22.6 ± 3.2
Polyunsaturated fatty acids (PUFAs)			
20:4n-6 (ARA)	9.2 ± 2.4	7.0 ± 2.9	9.9 ± 1.7 *
20:5n-3 (EPA)	1.1 ± 0.4	0.7 ± 0.2	1.2 ± 0.4 *
22:4n-6	3.2 ± 1.2	1.6 ± 0.8	3.6 ± 0.9 *
22:5n-3	3.8 ± 1.4	1.9 ± 0.5	4.4 ± 1.1 *
22:6n-3 (DHA)	16.1 ± 6.2	6.7 ± 2.8	19.0 ± 3.3 *
Σ PUFAs	37.2 ± 9.1	22.7 ± 4.9	41.8 ± 3.5 *
Stable isotopes			
$\delta^{13}C$	−15.4 ± 0.9	−15.3 ± 1.2	−15.5 ± 0.9
$\delta^{15}N$	19.7 ± 1.2	20.2 ± 1.4	19.6 ± 1.1

et al., 2022). Alternatively, both juvenile and adult sharks could feed on species occupying the mesopelagic zone (200–1000 m depth) during the day and reaching the surface at night, such as vertically migrating squid. While juveniles would only have access to these prey during the night, facilitated by the very steep topography of Guadalupe Island nearshore area, diel vertical migrations (i.e., use of deeper water during the day) observed in adult sharks may reflect foraging on mesopelagic prey both day and night off the island (Aquino-Baleytó et al., 2021; Hoyos-Padilla et al., 2016). This hypothesis was previously supported by mercury stable isotope compositions measured in Guadalupe white sharks, which suggested feeding on the mesopelagic compartment for both juveniles and adults (Le Croizier et al., 2020b, 2022b).

Here, we estimated the overlap between the trophic niche of white sharks based on FA composition and that of potential prey groups (i.e., elephant seals, tuna, pelagic sharks, squid; Fig. 5). The probability of finding these prey in the white shark niche was high for pelagic sharks and tuna (99% and 70% respectively), moderate for squid (30%) and low for elephant seals (10%) (Fig. 5). Large pelagic species could thus be targeted locally by white sharks, as evidenced by direct observations at Guadalupe Island. Notably, a large individual was spotted feeding on a 2-m blue shark (*Prionace glauca*) (Domeier and Nasby-Lucas, 2007), a juvenile was seen chasing a free-swimming yellowfin tuna (*Thunnus albacares*) (Hoyos-Padilla, *pers. comm.)* and white sharks were frequently observed preying on yellowfin tuna caught by recreational fishing boats (Domeier and Nasby-Lucas, 2007). These results corroborate stable isotope estimates, which placed pelagic predators such as tuna as the likely dominant prey of juvenile white sharks. Despite modest overlap between white shark and squid FA compositions, squid may also be important prey for white sharks in the Northeast Pacific, supported by cephalopod scars observed on Guadalupe white sharks that reveal predator-prey interactions (Becerril-García et al., 2020). In addition, the species of pelagic sharks used in our study (i.e., silky *Carcharhinus falciformis*, bigeye thresher *Alopias superciliosus*, pelagic thresher *Alopias pelagicus*, smooth hammerhead *Sphyrna zygaena* and blue sharks) and to which white sharks are very close in terms of FA composition (Fig. 5), are known to feed primarily on cephalopods in the Eastern Pacific (Galván-Magaña et al., 2013; Xu et al., 2022b). The gap between FA profiles of squid and pelagic/white sharks could therefore be due to taxa-specific FA concentrations, such as exceptionally high levels of DHA in cephalopods (Chen et al., 2020; Gong et al., 2018), which are not conserved in the same proportions in predators.

Finally, the presence of pinnipeds, such as northern elephant seals (*Mirounga angustirostris*), Guadalupe fur seals (*Arctocephalus townsendi*) and California sea lions (*Zalophus californianus*), is thought to drive the seasonal aggregation of white sharks around Guadalupe Island (Domeier et al., 2012; Domeier and Nasby-Lucas, 2008; Hoyos-Padilla et al., 2016). However, very few acts of predation by white sharks on pinnipeds have been documented at Guadalupe Island compared to other aggregation sites in South Africa or the Northeast Pacific, such as the Farallon Islands (Domeier and Nasby-Lucas, 2007; Hoyos, 2009). Supported by the weak overlap probability between FA compositions of white sharks and elephant seals (Fig. 5), we hypothesize that the capture of pinnipeds remains anecdotal in the area, potentially limited by the clarity of the oceanic waters surrounding Guadalupe Island which facilitates the detection of sharks by pinnipeds. Our results are also consistent with a previous study of FA profiles in eastern Australian white sharks, which suggested that the individuals analysed were not marine mammal specialists (Pethybridge et al., 2014). However, most attacks on pinnipeds are believed to take place in December, when pregnant elephant seals arrive at Guadalupe Island (Hoyos, 2009; Hoyos-Padilla et al., 2016). As white sharks were sampled from September to November, sampling later in the season would likely have revealed greater consumption of marine mammals, reflected in the FA composition of the sharks.

3.2.2. Interindividual variability

Although no ontogenetic changes were found, variability in FA profiles between individual sharks was detected. Two groups of individuals were separated based on FA composition (K-means clustering), with DHA, 18:0 and 18:1n-9 accounting mainly for the dissimilarity between groups (31%, 14% and 11% respectively, SIMPER). Group 1 (n = 6) had higher proportions of SFAs and MUFAs (16:0, 18:0, 18:1n-9), while group 2 (n = 19) was characterized by higher proportions of n-3 and n-6 PUFAs (ARA, DHA, EPA, 22:5n-3 and 22:4n-6) (Table 1, Fig. 4A). As in our study, significant intraspecific variability in FA composition was highlighted in eastern Australian white sharks, mainly driven by the opposition between 18:1n-9-rich and DHA-rich individuals (Pethybridge et al., 2014). The authors attributed these differences to varying contributions of mammalian blubber and fish/cephalopods to the diet of individual white sharks. Indeed, the blubber of marine mammals is particularly rich in 18:1n-9 and poor in DHA (e.g., 27% and 5% respectively in the northern elephant seal) (Goetsch et al., 2018), while the muscle of large pelagic fish and squid generally contains lower levels of 18:1n-9 and higher levels of DHA (e.g., 2% and 42% respectively in the jumbo squid *Dosidicus gigas*) (Chen et al., 2020). Here, the probability of finding elephant seals in the white shark niche was 36% for group 1 while it was zero for group 2 (Fig. 5). Conversely, the probability of niche overlap between white sharks and cephalopod-eating pelagic sharks was much higher for group 2 compared to group 1 (94% *versus* 5% respectively). These marked differences between groups could reflect individual dietary specialization, such as previously suggested in white sharks (French et al., 2018; Kim et al., 2012b; Pethybridge et al., 2014). However, we assume that consistent differences in predatory behavior would be revealed by long-term integrating stable isotopes, such as observed in other studies (French et al., 2018; Kim et al., 2012b), whereas no significant differences between groups were found here for either $\delta^{13}C$ or $\delta^{15}N$ (Table 1). Alternatively, rather than reflecting individual dietary specialization, this intergroup variability in FA composition could reflect the recent and opportunistic consumption of prey providing trophic inputs fulfilling different ecophysiological functions in white sharks. Since lipids are a major source of energy for metabolically active marine predators, white sharks are thought to rely primarily on marine mammal blubber to meet the energy requirements necessary to undertake long migrations (Del Raye et al., 2013; Moxley et al., 2019; Pethybridge et al., 2014). For instance, the consumption of northern elephant seals, in which fat can

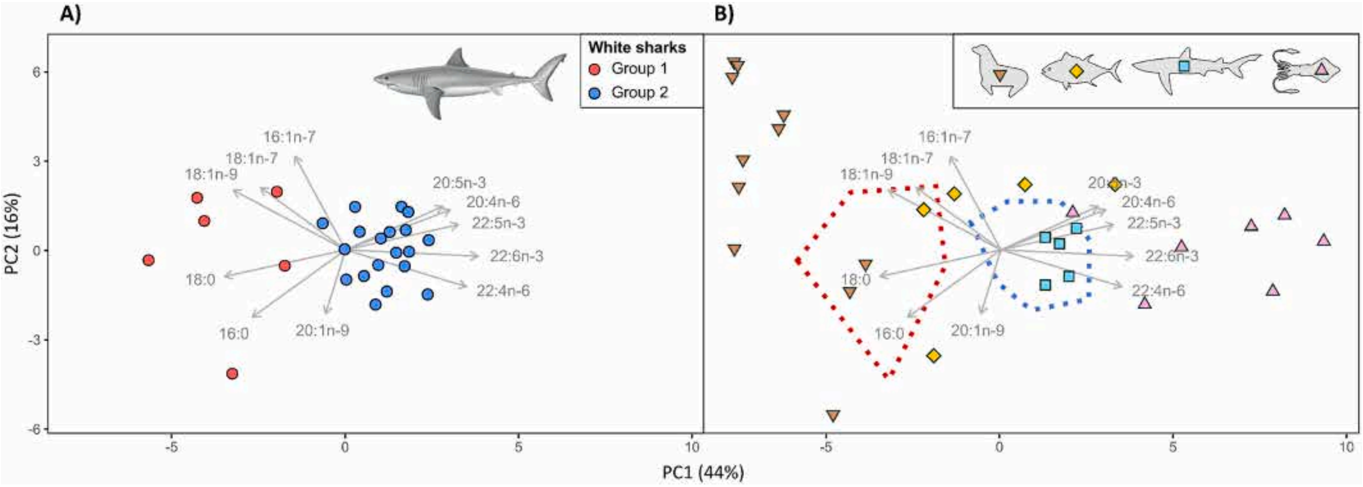

Fig. 4. **A)** Principal component analysis (PCA) of white sharks from Guadalupe Island, based on muscle fatty acid (FA) composition (FAs accounting for more than 1% of the total FA amount, shown as grey arrows). Individual white sharks were separated into two groups based on their FA composition (Group 1: red dots, Group 2: blue dots). **B)** FA profiles of potential prey items from the North and Central Pacific (sourced from the literature), shown as illustrative individuals on PCA based on FA composition of white sharks from Guadalupe Island (left panel). Brown inverted triangles: seals, yellow diamonds: tuna, light blue squares: pelagic sharks, pink triangles: squid. The convex hull areas of the two groups of white sharks are represented by red and blue dotted lines.

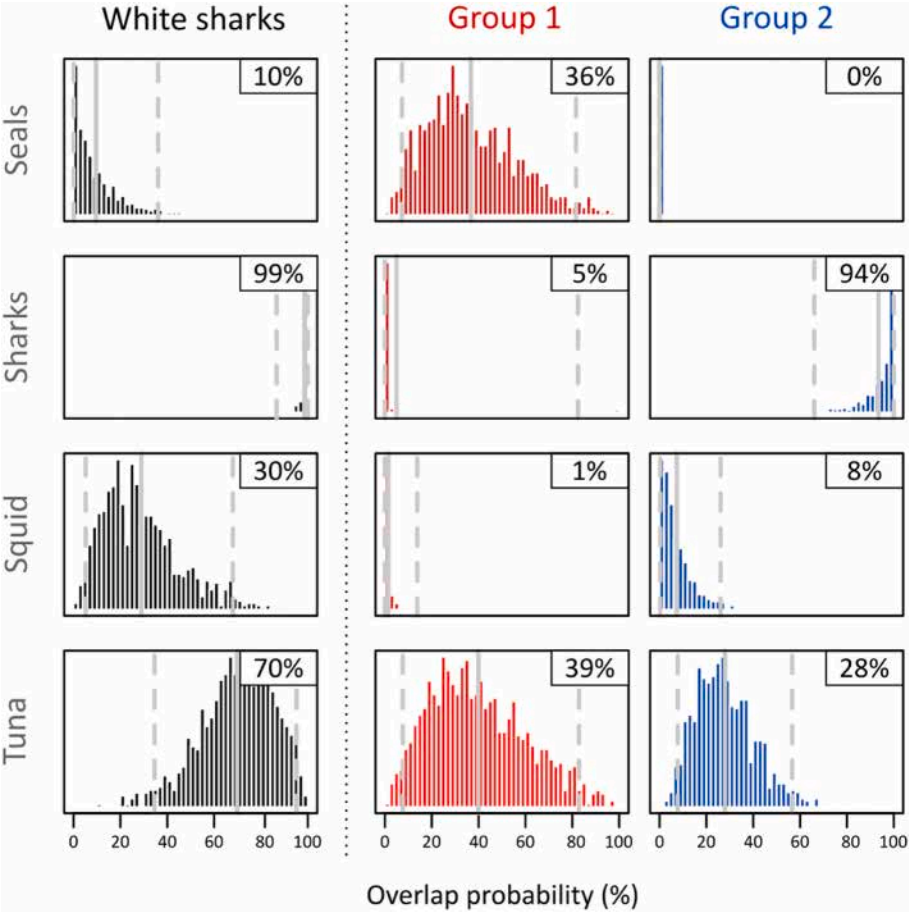

Fig. 5. Posterior distributions of probabilistic niche overlap metrics (%), for a niche region size of 95% and based on individual coordinates extracted from PCA of FA compositions (PC1 and PC2, Fig. 4). The posterior means and 95% credible intervals are displayed as solid and dashed grey vertical lines, respectively. The probability for an individual from a group of potential prey (tuna, squid, pelagic sharks, seals) to be found in the white shark niche (all individuals in black, group 1 in red, group 2 in blue) is indicated in a panel at the top right. For example, the probability of finding a seal in the white shark niche is 10% considering all individuals, 36% for group 1 sharks and 0% for group 2 sharks.

represent more than 30% of total body weight (Crocker et al., 2014), is likely to represent an important energy supply for white sharks before their offshore migration from Guadalupe Island (Hoyos-Padilla et al., 2016). On the other hand, some omega-3 PUFAs are necessary for the development and proper functioning of the brain, vision and nervous system (Glencross, 2009; Sargent et al., 2003; Tocher, 2003). Dietary limitation of these compounds is thus known to alter physiological performances in marine consumers (Vagner et al., 2014, 2015). While omega-3 PUFAs levels are low in marine mammal blubber in favor of SFAs and MUFAs (Goetsch et al., 2018), they can be much higher in lean prey such as cephalopods (e.g., 0.3% lipids composed of ~40% DHA in jumbo squid mantle) (Gong et al., 2018; Saito et al., 2014). Therefore, the observed opposition between SFAs/MUFAs and PUFAs among the two groups of white sharks (Table 1, Fig. 4A) may reflect an ecological trade-off between consumption of lipid-rich prey with higher levels of SFAs/MUFAs (i.e., marine mammals) *versus* consumption of lean prey with higher levels of PUFAs (i.e., pelagic fish and squid), respectively satisfying the energetic and physiological needs of white sharks.

4. Conclusion

Our study improves our limited knowledge of the trophic ecology of white sharks in a major aggregation site in the Northeast Pacific. Through a multi-tracer approach (stable isotopes and FAs), we observed similar feeding across three size classes (juveniles, subadults, adults). Our estimates based on FA profiles indicate that white sharks feed primarily on pelagic prey in Guadalupe Island waters. However, variability between individuals was found, with the FA composition of some sharks reflecting recent consumption of marine mammals. During their migratory cycle, white sharks may target different prey to meet their energetic and physiological needs. As fisheries and global change are likely to alter the distribution and abundance of marine species, potential impacts on the ecophysiology of apex predators such as white sharks should be carefully monitored in the future.

CRediT authorship contribution statement

Gaël Le Croizier: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mauricio Hoyos-Padilla:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **Felipe Amezcua-Martínez:** Writing – review & editing, Supervision, Investigation. **Marc Aquino-Baleytó:** Writing – review & editing, Resources, Investigation. **Lucien Besnard:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Fabienne Le Grand:** Writing – review & editing, Resources, Formal analysis. **François Le Loc'h:** Writing – review & editing, Resources, Funding acquisition. **Margaux Mathieu-Resuge:** Writing – review & editing, Resources, Methodology, Formal analysis. **Jean-Marie Munaron:** Writing – review & editing, Resources, Methodology, Formal analysis. **Arthur Ory:** Writing – review & editing, Formal analysis. **Fany Sardenne:** Writing – review & editing, Methodology, Investigation. **Gauthier Schaal:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization. **Anne Lorrain:** Writing – review & editing, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119507.

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