



Do marine protected areas influence mercury exposure? Insights from a shark community in the tropical Northeast Pacific[☆]

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ABSTRACT

Biomass depletion caused by overfishing is likely to alter the structure of food webs and impact mercury transfer to marine predators. Although marine protected areas (MPAs) are spared from fishing pressure, their influence on biota mercury levels is poorly understood. Here, we used carbon and nitrogen stable isotope compositions as well as mercury concentrations in fin clips to characterize foraging habitat and mercury exposure of a shark community composed of migratory and resident species of the Revillagigedo archipelago, an offshore MPA in the Northeast Pacific off Mexico. We found that the probability of finding migratory sharks in the isotopic niche of Revillagigedo-resident sharks was low, likely reflecting the use of habitats outside the archipelago by highly mobile species. Community-wide variations in mercury were primarily explained by shark length, revealing that bioaccumulation was the main driver of Hg concentrations. We failed to detect a clear effect of foraging habitat on shark mercury exposure, which may be related to migratory species using both exploited and protected areas when moving outside the Revillagigedo MPA. More similar studies on the potential mitigation of Hg contamination by MPAs are needed in the future if fishing pressure increases to satisfy the growing global human population.

1. Introduction

Mercury (Hg) is a global pollutant emitted into the atmosphere by natural and anthropogenic sources (e.g., volcanic and industrial emissions, respectively), and deposited on the surface of the world's oceans (Outridge et al., 2018). Once in the marine environment, a fraction of Hg is converted by microbial transformation to the methylmercury (MeHg) form (Gilmour et al., 2013; Podar et al., 2015), which is primarily assimilated by phytoplankton and transferred along food webs. The bioaccumulation (i.e., increase over time) and biomagnification (i.e., increase with trophic position) properties of MeHg lead to high levels of this potent neurotoxicant in long-lived top predators such as sharks

(Besnard et al., 2021; Le Bourg et al., 2019; Le Croizier et al., 2022b). The potential alterations caused by MeHg on shark neurophysiology are still poorly understood (Ehnert-Russo and Gelsleichter, 2020; Rodrigues et al., 2021) and some species are thought to possess metabolic mechanisms allowing them to reduce toxicity, such as *in vivo* demethylation of MeHg (Le Croizier et al., 2020c, 2020b; Maurice et al., 2021). However, the high MeHg concentrations generally found in sharks probably induce deleterious effects and represent an additional pressure for some large species whose populations are already globally depleted by overfishing (Dulvy et al., 2021; Juan-Jordá et al., 2022; Pacoureaux et al., 2021).

Overfishing results in several negative impacts on marine

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ecosystems, including the direct removal of significant biomass and the indirect modification of ecological linkages (Daskalov et al., 2007; Myers et al., 2007; Myers and Worm, 2003). Marine protected areas (MPAs), where fishing activities are restricted or prohibited, have proven to be effective in achieving conservation and management objectives to mitigate the degradation associated with overfishing. The restoration of natural communities in marine reserves generally leads to an increase in biomass and species abundance, as well as a shift in food web structure compared to exploited areas (Aburto-Oropeza et al., 2011; Micheli et al., 2004; Soler et al., 2015). Regarding metal contamination, biodilution of MeHg in large biomasses of lower trophic levels is supposed to reduce MeHg transfer to top predators (Chouvelon et al., 2018; Le Croizier et al., 2022a). In addition, modifications of ecosystem structure (e.g., change in trophic chain length) are likely to influence the bioamplification of MeHg along the food web (Lavoie et al., 2013). Despite the potential differences in terms of biomass and food web structure between marine reserves and exploited areas, Hg exposure remains to be assessed for predators using habitats with varying levels of protection.

The Revillagigedo Archipelago consists of three volcanic islands and one islet, and is located more than 400 km south of the Baja California peninsula (Mexico) in the Pacific Ocean. This offshore archipelago is home to a great biodiversity and for this reason has been classified as a protected natural area since 1994, and listed as a UNESCO World Heritage site since 2016. In 2017, the surface of the Revillagigedo MPA was expanded, becoming the Revillagigedo National Park, the largest marine reserve in North America with nearly 150,000 square kilometers of no-take zone (Kerlin, 2019; Mpatlas, 2017). Among the 28 different species of chondrichthyans frequenting the archipelago (Becerril-García et al., 2020), some are full-time residents while others are thought to use the MPA intermittently. For instance, silvertip sharks (*Carcharhinus albimarginatus*) display a reef-associated behavior and strong site fidelity to the MPA, moving between the different islands of the archipelago according to their life stages (Ketchum et al., 2020; Le Croizier et al., 2020a). In contrast, it was observed that tiger sharks (*Galeocerdo cuvier*) spent less than 50% of the time at the Revillagigedo Archipelago and were able to perform long-distance movements within the archipelago, as well as round trips between the MPA and the coasts of the Mexican mainland (Ketchum et al., 2020). Finally, highly migratory species are less resident to the area, such as Galapagos (*Carcharhinus galapagensis*) and silky (*Carcharhinus falciformis*) sharks, capable of traveling several thousand kilometers south to reach other oceanic islands in the tropical eastern Pacific (i.e., Clipperton Atoll and Galapagos Islands) (Ketchum et al., 2020; Lara-Lizardi et al., 2020).

Stable isotope analyses have been widely used to characterize the trophic ecology of sharks species (Bird et al., 2018; Carlisle et al., 2012; Young et al., 2015). The carbon isotope composition ($\delta^{13}\text{C}$) is subject to spatial gradients related to the use of different carbon sources and/or metabolic pathways by primary producers during photosynthesis (Laws et al., 1995; Rau et al., 1996). Regarding nitrogen stable isotopes, the use of nitrogen in different forms (e.g., nitrate, nitrite, ammonia or gaseous nitrogen) by marine primary production is responsible for geographical variations in $\delta^{15}\text{N}$ values at the base of food webs (Lorrain et al., 2015). These spatial variations of isotopic baselines make it possible to trace the foraging habitat of marine predators (Graham et al., 2010; Trueman and St John Glew, 2019). Additionally, since $\delta^{15}\text{N}$ values (and to a lesser extent $\delta^{13}\text{C}$ values) increase significantly between prey and predators, this tracer is commonly employed as a proxy for trophic position (Cherel et al., 2008; Hussey et al., 2015). Finally, the combination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values constitutes a relevant proxy for the trophic niche (Layman et al., 2007; Newsome et al., 2007), allowing the assessment of dietary overlap between different predator populations or species (Besnard et al., 2022, 2021; Madigan et al., 2021).

Various types of shark tissues are analyzed in ecological tracer-based studies, providing insight into feeding habits at different temporal scales based on tissue turnover rate (Kim et al., 2012; Malpica-Cruz et al.,

2012). Frequently sampled for genetic analyses, fin clips are increasingly used for biochemical analyses in sharks (Chan et al., 2022; Every et al., 2016; Rangel et al., 2019), allowing the characterization of feeding habits and habitat use over a long period (i.e., probably more than a year) (Malpica-Cruz et al., 2012). Moreover, since Hg concentration in fins is strongly correlated with Hg levels in other tissues such as liver and muscle, the analysis of fin clips represents a relevant, minimally invasive method for estimating Hg contamination in sharks (O'Bryhim et al., 2017).

Here, we analyzed carbon and nitrogen stable isotopes, as well as total Hg concentrations in fin clips of four predatory species (Galapagos, silky, silvertip and tiger sharks) sampled in the Revillagigedo National Park. The objectives of our study were to i) assess the use of the MPA as a foraging habitat for these species and ii) test for possible differences in Hg exposure between sharks using the MPA and exploited areas. Specifically, we considered the isotopic niche of silvertip sharks, known to be residents of the Revillagigedo Archipelago (Ketchum et al., 2020; Le Croizier et al., 2020a), as a proxy for MPA habitats. We estimated niche overlap between silvertip sharks and migratory sharks (Galapagos, silky, and tiger), to infer MPA reliance of highly mobile species and their resulting Hg levels. We expected to find differences in Hg concentrations between species using the Revillagigedo Archipelago extensively and species depending more on habitats beyond the MPA.

2. Materials and methods

2.1. Sampling

Seventy seven individuals from four shark species (Galapagos sharks *Carcharhinus galapagensis*, silky sharks *Carcharhinus falciformis*, silvertip sharks *Carcharhinus albimarginatus* and tiger sharks *Galeocerdo cuvier*) were captured using drumlines at the Revillagigedo Archipelago in the Mexican Pacific (Fig. 1), during annual campaigns from 2006 to 2010

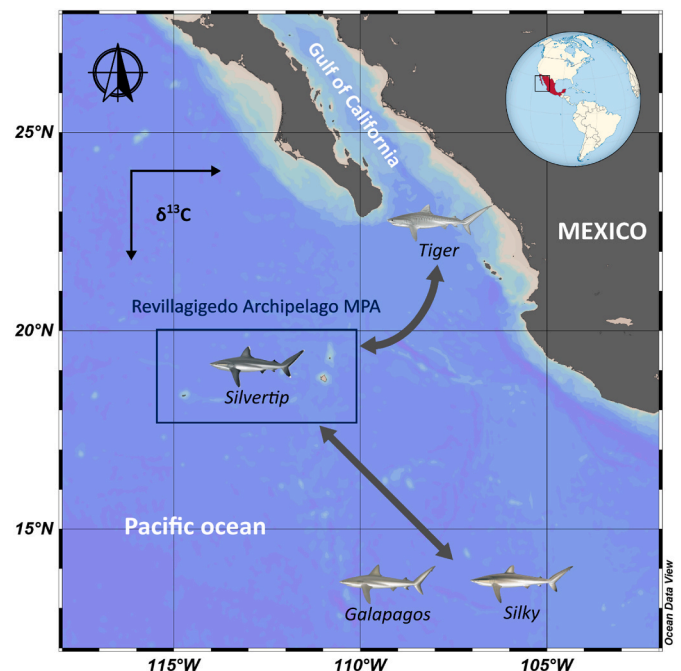


Fig. 1. Map of the sampling site, the Revillagigedo Archipelago Marine Protected Area (MPA) in the Northeast Pacific off the coast of Mexico. This region is subject to spatial isotopic gradients, with $\delta^{13}\text{C}$ values increasing eastward and southward (Le Croizier et al., 2020a; Magozzi et al., 2017; Tamburin et al., 2019). The proposed movements of the four shark species studied here are shown, based on previous tracking data (Ketchum et al., 2020; Lara-Lizardi et al., 2020) and isotopic results of the present study.

(2006: n = 2 *C. galapagensis*, n = 11 *C. falciformis*, n = 6 *C. albimarginatus*, n = 6 *G. cuvier*; 2007: n = 3 *C. galapagensis*; 2008: n = 2 *C. galapagensis*, n = 5 *C. albimarginatus*; 2009: n = 2 *C. galapagensis*, n = 10 *C. albimarginatus*; 2010: n = 25 *C. falciformis*, n = 5 *C. albimarginatus*). Following the recording of biometric data (total length and sex), a fin clip sample from the rear tip of the dorsal fin was collected for each individual. Sampled sharks ranged from young-of-the-year to adults and the majority of individuals were females (Table 1). Tissue samples were immediately transferred to a -20°C freezer onboard. Once at laboratory, fin clip samples were freeze-dried and homogenized.

2.2. Total mercury concentration

As total mercury (THg) is strongly correlated to methylmercury (MeHg) in the tissues of most predatory fish species (Bosch et al., 2016), including shark fins (Nalluri et al., 2014), THg was used as a proxy for MeHg concentrations in shark tissues. THg determination was carried out on an aliquot (around 10 mg) of sample powder by combustion, gold trapping and atomic absorption spectrophotometry detection using a DMA80 analyzer (Milestone, USA). A 10-point calibration curve (correlation coefficient of 0.998), encompassing the variability of Hg concentrations in shark samples, was produced from a freeze-dried reference biological material (lobster hepatopancreas; TORT 3, NRCC) and used for the analyses. Mercury concentrations in samples are expressed on a dry weight basis ($\text{ng}\cdot\text{g}^{-1}\text{ dw}$). Each sample was analyzed twice, with relative standard deviation (RSD) below 10%. The accuracy and reproducibility of the method were established using the TORT certified reference material, which was analyzed in addition to a blank every 10–15 samples. The certified values for TORT 3 ($0.292 \pm 0.022 \mu\text{g}\text{ g}^{-1}\text{ dw}$) were reproduced (measured value: $0.286 \pm 0.024 \mu\text{g}\text{ g}^{-1}\text{ dw}$) within the confidence limits. Average TORT recovery reached 98% with a RSD of 8%. The limit of detection was $0.005 \mu\text{g}\text{ g}^{-1}\text{ dw}$.

2.3. Carbon and nitrogen stable isotope analysis

Before 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 a Genevac centrifugal evaporator. 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 $350 \mu\text{g}$ of sample powder was then weighed in tin capsules for isotopic analysis. The samples were analyzed by continuous flow on a Thermo Scientific Flash EA 2000 elemental analyzer coupled to a Delta V Plus mass spectrometer. Results are expressed in standard δ notation based on international standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$) following the equation $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(\text{Rsample}/\text{Rstandard}) - 1] \times 10^3$ (in ‰), where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. International isotopic standards of known $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were analyzed: 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 $\pm 0.11\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.07\text{‰}$ for $\delta^{15}\text{N}$.

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) (Shapiro and Wilk, 1965) and homogeneity of variances (Bartlett tests) (Bartlett, 1937). When these conditions were met, one-way ANOVAs followed by Tukey's HSD tests were performed to test for differences in isotopic values and log (Hg) concentrations between species, otherwise, non-parametric analogues were employed: Kruskal-Wallis (KW) tests followed by Conover-Iman multiple comparison tests with Bonferroni's adjustment (Conover and Iman, 1979).

Standard ellipse areas encompassing 95% and 40% of the data were performed using the package "SIBER" based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, to quantify niche overlap between species (Jackson et al., 2011). Isotope ellipses provide a proxy for trophic niche, and overlapping ellipses allow estimation of trophic overlap between species. Trophic overlap between two species is expressed as a proportion of the non-overlapping area of species ellipses.

Based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, the "nicheROVER" package has been used to define niche regions and overlaps for silvertip sharks and migratory species (Galapagos, silky and tiger sharks) (Swanson et al., 2015). Niche region was defined as the 95% probability region in bivariate space, while niche overlap was calculated as the probability that an individual migratory shark will be found in the silvertip niche (proxy for the Revillagigedo Archipelago MPA). Overlap uncertainty was accounted for by performing 1000 elliptical projections of niche region through Bayesian statistics.

Depending on data normality (Shapiro-Wilk tests), Pearson or Spearman correlation tests were applied to assess relationships between variables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\log(\text{Hg})$, shark length). Linear models were used to evaluate the influence of species, shark length, sex, and isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) on community-wide $\log(\text{Hg})$ concentrations. The models were built using backward stepwise selection, ranked based on Akaike's Information Criteria adjusted for small sample sizes (AIC_c) and compared using ΔAIC_c and Akaike weights (w_i) (Burnham and Anderson, 2002).

3. Results

3.1. Interspecific differences

Stable isotope compositions varied significantly among shark species. Silvertip sharks had lower $\delta^{13}\text{C}$ values than the three other species (Galapagos, silky and tiger sharks) (ANOVA, $p < 0.001$) (Table 1, Fig. 2A). Silky and Galapagos sharks had similar $\delta^{13}\text{C}$ values (ANOVA, $p > 0.05$), while tiger sharks had higher $\delta^{13}\text{C}$ values than the three other species (silvertip, Galapagos and silky sharks) (ANOVA, $p < 0.05$). Regarding nitrogen stable isotopes, we found lower $\delta^{15}\text{N}$ values in silky sharks compared to the other species (KW, $p < 0.001$), whereas

Table 1

Data summary (mean \pm SD) for shark species sampled at the Revillagigedo Archipelago. "TL" refers to total length, "F" indicates the proportion of female individuals. Life stages are derived from Compagno et al. (2005) (YOY: young-of-the-year), movement patterns are from Ketchum et al. (2020) and habitat use is from Bizzarro et al. (2017) (D: demersal, N: nearshore, P: pelagic, O: oceanic).

Species	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	THg ($\text{ng}\cdot\text{g}^{-1}$)	TL (m)	F (%)	Stage	Movement	Habitat
Silvertip	26	-14.7 ± 0.5	15.4 ± 0.6	222 ± 127	1.10 ± 0.23	67	YOY-juvenile	resident	DPN
Silky	36	-13.9 ± 0.4	14.8 ± 0.5	307 ± 191	1.98 ± 0.14	97	juvenile-adult	migratory	PNO
Galapagos	9	-13.8 ± 0.3	16.0 ± 0.6	226 ± 215	2.08 ± 0.70	75	juvenile-adult	migratory	DPNO
Tiger	6	-13.2 ± 0.4	17.3 ± 1.2	573 ± 384	3.50 ± 0.47	100	adult	migratory	DPN

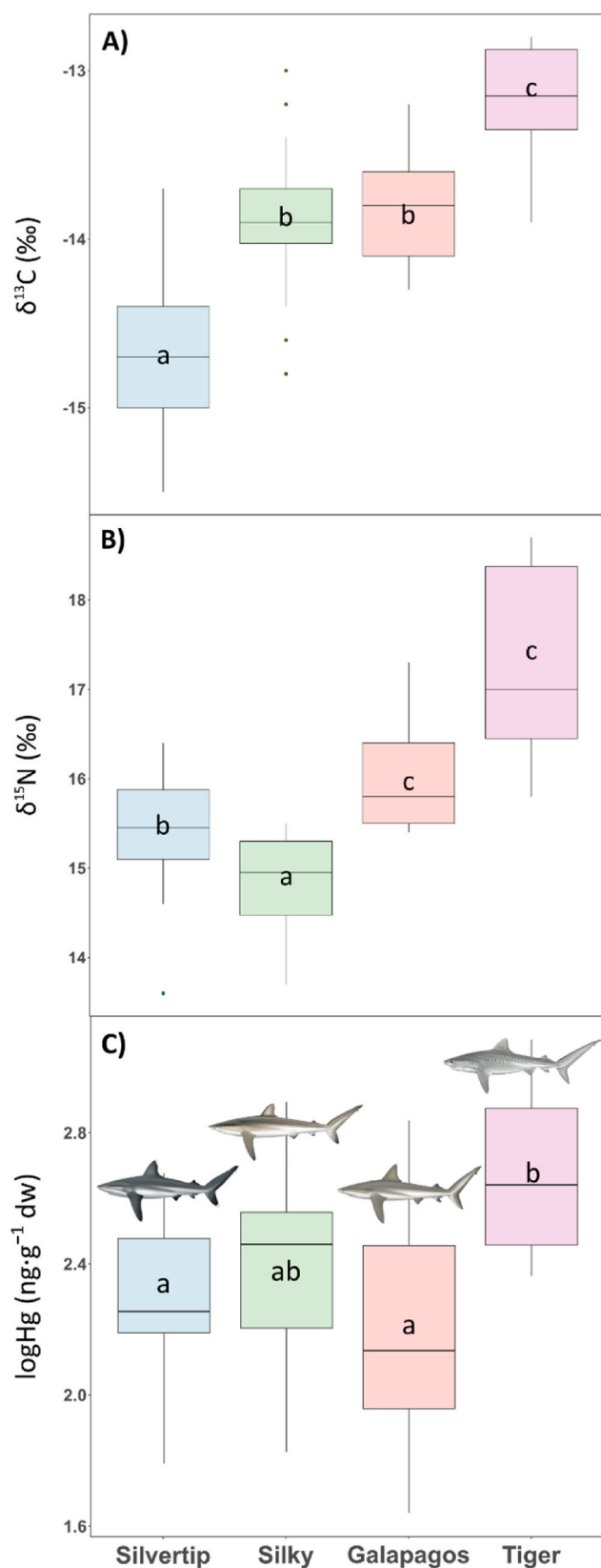


Fig. 2. Boxplots of **A)** carbon and **B)** nitrogen isotope compositions, as well as **C)** mercury concentrations in fin clips of shark species from the Revillagigedo Archipelago. Different letters indicate significant differences between species ($p < 0.05$; **A**) and **C)** one-way ANOVAs followed by Tukey's HSD tests; **B)** Kruskal-Wallis test followed by Conover-Iman multiple comparison test with Bonferroni's adjustment).

Galapagos and tiger sharks exhibited higher $\delta^{15}\text{N}$ values than silvertip and silky sharks (KW, $p < 0.05$) (Table 1, Fig. 2B). Mercury concentrations were similar between silvertip, silky and Galapagos sharks (ANOVA, $p > 0.05$), while tiger sharks displayed higher Hg levels than silvertip and Galapagos sharks (ANOVA, $p < 0.05$) (Table 1, Fig. 2C).

3.2. Community trends

Considering 40% niche regions, we found no overlap in the isotopic niches of the four species (Fig. 3, Table S1). Isotope overlap remained limited using 95% niche regions, reaching a maximum of 31% between silky and silvertip sharks (Table S1). We assessed the probability of finding migratory sharks (Galapagos, silky and tiger sharks) in the isotopic niche of silvertip sharks, known to be resident in the Revillagigedo Archipelago (Ketchum et al., 2020; Le Croizier et al., 2020a). Using 95% niche regions, we estimated that the probability of finding a migratory shark in the silvertip shark niche was 59% for silky sharks, compared to only 6% and 5% for Galapagos and tiger sharks, respectively (Fig. 3, Table S2). With niche region sizes reduced to 40%, the probability of finding a silky shark in the silvertip shark niche dropped to 6% (1% for both Galapagos and tiger sharks, Fig. 3 and Table S2).

At the community scale, we observed a positive correlation between $\delta^{13}\text{C}$ values and shark length (Pearson, $r = 0.69$, $p < 0.001$; Fig. 4A), while no significant correlation was detected between $\delta^{15}\text{N}$ values and shark length, nor between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Spearman, $p > 0.05$). We found a significant correlation between $\log(\text{Hg})$ concentrations and shark length (Pearson, $r = 0.46$, $p < 0.001$; Fig. 4B), and between $\log(\text{Hg})$ concentrations and $\delta^{13}\text{C}$ values (Pearson, $r = 0.25$, $p < 0.05$; Fig. 4C), whereas no relationship was detected between $\log(\text{Hg})$ and $\delta^{15}\text{N}$ (Spearman, $p > 0.05$). Using linear models, we found that the best model for predicting variations in $\log(\text{Hg})$ concentrations only included length as explanatory variable, and explained 21% of the observed variability (Table S3).

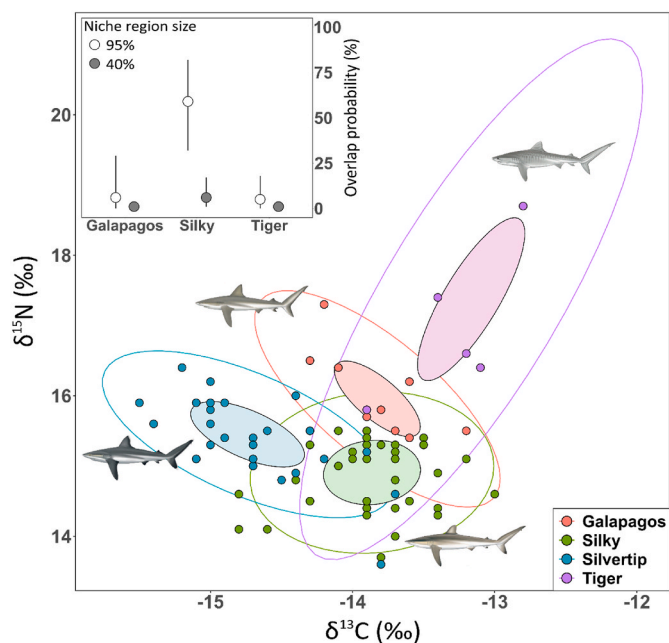


Fig. 3. Biplot of carbon and nitrogen isotope values, standard ellipse areas at 95% and 40% are figured for each species. The upper left panel displays the probability (%) of finding a migratory individual (galapagos, silky or tiger shark) in the silvertip shark niche, using niche region sizes of 95% and 40%.

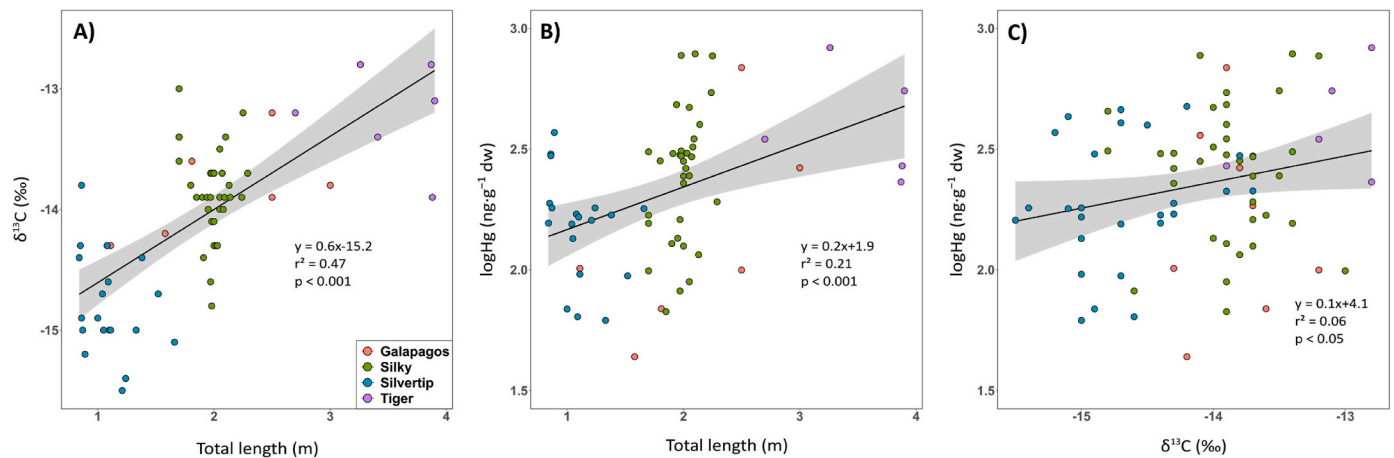


Fig. 4. Biplots of A) carbon isotope values and total length, B) $\log(\text{Hg})$ concentrations and total length, and C) $\log(\text{Hg})$ concentrations and carbon isotope values. Data fits linear regressions.

4. Discussion

4.1. Shark trophic ecology

In our study area, isotopic baselines follow latitudinal and longitudinal gradients, with $\delta^{13}\text{C}$ values known to increase from west to east and from north to south (Magozzi et al., 2017; Trueman and St John Glew, 2019) (Fig. 1). Marine predators foraging in the different habitats of this region have been shown to integrate these spatial isotopic variations (Le Croizier et al., 2020a; Madigan et al., 2021; Olson et al., 2010). While $\delta^{13}\text{C}$ values primarily reflect foraging habitat (Bird et al., 2018), $\delta^{15}\text{N}$ values are known to increase significantly with trophic level, masking potential spatial variations in predator $\delta^{15}\text{N}$ values (Pethybridge et al., 2018). Silvertip sharks are characterized by a high degree of site fidelity and typically reside around oceanic islands (Ketchum et al., 2020), where they forage on both reef and pelagic prey (Curnick et al., 2019; Le Croizier et al., 2020a). They display minimal dispersal and perform limited excursion outside their residence site, as observed in different locations such as the Chagos and Fijian Archipelagos (Indian Ocean and South West Pacific, respectively) (Bond et al., 2015; Carlisle et al., 2019). In the Revillagigedo Archipelago, silvertip sharks perform inter-island movements within the MPA, with a high residency index at different islands according to life stage (Ketchum et al., 2020; Muntaner López, 2016). Here, the isotopic niche of silvertip sharks, considered as a proxy for the Revillagigedo MPA, was distinguished from that of other species by lower $\delta^{13}\text{C}$ values (Fig. 2A). This result suggests that the archipelago is fueled by ^{13}C -depleted offshore inputs, in accordance with known $\delta^{13}\text{C}$ baselines in the region (Magozzi et al., 2017). Although sampled within the MPA, the other three species have been shown to perform large-scale movements across different habitats. For example, Galapagos and silky sharks tagged in the Revillagigedo Archipelago have been detected at Clipperton Atoll (Lara-Lizardi et al., 2020), which lies almost 1000 km further south and is characterized by higher $\delta^{13}\text{C}$ baselines (Le Croizier et al., 2020a; Magozzi et al., 2017) (Fig. 1). Similarly, tiger sharks have been observed performing round trips between the Revillagigedo Archipelago and the coasts of the Gulf of California in the Mexican mainland (Fig. 1) (Ketchum et al., 2020), which also display higher $\delta^{13}\text{C}$ baselines than offshore habitats (Tamburini et al., 2019). Therefore, the isotopic values we found in the migratory species are consistent with their known movements in the region. Higher $\delta^{13}\text{C}$ values compared to silvertip sharks probably reflect the use of pelagic habitats lying further east and/or south of the Revillagigedo Archipelago for Galapagos and silky sharks, and the use of mainland coastal habitats for tiger sharks (Figs. 1 and 2A). Alternatively, the observed interspecific variability in isotope

values could also result from different feeding habits within the Revillagigedo National Park. For instance, as silvertip and tiger sharks are known to use similar habitats (Table 1), the higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in tiger sharks (Fig. 2A–B) could reflect their belonging to the same food web as silvertip sharks within the MPA, but at a higher trophic position. This hypothesis is all the more likely given the size class differences between these two species (i.e., young-of-the-year to juveniles for silvertip sharks *versus* adults for tiger sharks, Table 1). Similarly, although using comparable habitats (Table 1), silky sharks are known to feed mainly on cephalopods (Galván-Magaña et al., 2013) while Galapagos sharks show a diet that also includes demersal prey (Wetherbee et al., 1996), which could lead to differences in trophic level between these two species, reflected by different $\delta^{15}\text{N}$ values (Fig. 2B).

As a result of significant variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, we observed weak isotopic niche overlap between species (Table S1, Fig. 3), likely reflecting a high degree of resource partitioning within this shark community. We also observed low probabilities of finding Galapagos, silky and tiger sharks in the silvertip shark niche (Table S2), supporting the hypothesis that these migratory species do not use the Revillagigedo MPA permanently and may forage outside the archipelago. Given the different movement patterns displayed by the species studied here (Fig. 1), our results suggest the use of discrete foraging habitats limiting interspecific competition for dietary resources. Our findings are consistent with a previous study of a shark community (including Galapagos and tiger sharks) in the Hawaiian archipelago, which found that interspecies trophic competition was mitigated via spatial segregation (Papastamatiou et al., 2006). Finally, we found an increase in $\delta^{13}\text{C}$ values with shark length at the community scale (Fig. 4A), which is unlikely to be related to trophic level since no size-based variation in $\delta^{15}\text{N}$ values, nor any significant relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were detected. Instead, these results could reflect a higher frequency of movements out of the archipelago as individuals grow, consistent with the previously found ontogenetic increase in i) pelagic habitat use for Galapagos and silky sharks (Bonfil, 2009; Meyer et al., 2010), and ii) spatial dispersal of tiger sharks (Afonso and Hazin, 2015; Ajemian et al., 2020).

4.2. Factors explaining mercury variability

Mercury (Hg) concentrations in marine predators vary according to many parameters, including trophic level, age/size, prey composition, foraging habitat, and Hg metabolism (Kiszka et al., 2015; Le Bourg et al., 2019; Le Croizier et al., 2020c). Notably, prey biomass and food chain length influence Hg transfer to higher trophic levels, related to Hg biodilution and biomagnification processes, respectively (Chouvelon

et al., 2018; Le Croizier et al., 2022a). Because fisheries exploitation of unprotected areas is likely to alter biomass and food web structure (Aburto-Oropeza et al., 2011; Micheli et al., 2004; Soler et al., 2015), we hypothesized that these potential impacts may lead to differences in Hg exposure for predators using MPAs versus exploited areas. In our study, only tiger sharks displayed higher Hg concentrations compared to other sharks (Fig. 2C). As this species also exhibited the highest $\delta^{15}\text{N}$ values and body length, higher trophic position and age (all individuals were adults) could account for increased Hg levels. Satellite tracking of tiger sharks at the Revillagigedo Archipelago has also shown a high degree of residence near beaches where sea turtles come to lay eggs (Ketchum et al., 2020). Moreover, tiger sharks spend a large amount of time near the surface where they might hunt birds, as evidenced by the adult red-footed boobies that were regurgitated by two tiger sharks during a tagging campaign (Ketchum et al., 2020). Thus, the consumption of large prey such as turtles and seabirds could also expose tiger sharks to higher Hg levels. Apart from this case, no difference was found in Hg concentrations between silvertip sharks restricted to the MPA and Galapagos and silky sharks (Fig. 2C), despite a larger size for these migratory sharks (Table 1). Therefore, the lack of a marked difference in Hg concentrations between resident and highly mobile species would not support the hypothesis of higher Hg exposure for sharks foraging outside the Revillagigedo MPA.

Previous studies using Hg isotopes have revealed that sharks exhibit Hg detoxification capabilities (Besnard et al., 2021; Le Croizier et al., 2022b, 2020b; 2020c), probably through *in vivo* demethylation of methylmercury, which would set them apart from teleost fish and bring them closer to marine mammals in terms of Hg metabolism (Bolea-Fernández et al., 2019; Li et al., 2020). It has also been suggested that methylmercury demethylation abilities may vary between co-occurring species (Besnard et al., 2021; Le Croizier et al., 2020c), which could partly explain the interspecific variability in Hg levels that we observed at Revillagigedo (Fig. 2C). Of note, although metallothioneins appear to be poorly implicated in Hg detoxification in sharks (Hauser-Davis, 2020; Hauser-Davis et al., 2021), metal binding to these proteins has been shown to influence contaminant accumulation and excretion in marine fish (Le Croizier et al., 2019, 2018). Thus, differences in Hg storage may also have contributed to the interspecific variability in Hg concentrations observed here.

Very few studies have analyzed Hg concentrations in shark fin clips, which limits the comparison of our results with those from previous work. It is also interesting to note that Hg concentrations in fin clips are generally much lower than those observed in other tissues such as muscle (O'Bryhim et al., 2017). Hg concentrations of $50 \text{ ng g}^{-1} \text{ dw}$ were found in the fin clips of silky sharks from two different regions (Southeast Coast of the USA and the Red Sea) (O'Bryhim et al., 2017), which is 6 times less than the Hg levels observed in this species for our study site (i.e., $\sim 300 \text{ ng g}^{-1} \text{ dw}$, Table 1). Although the tropical Northeast Pacific is not subject to strong local anthropogenic Hg inputs (Amezcuca et al., 2022; Médiéu et al., 2022), the Revillagigedo Archipelago lies within the largest and shallowest oxygen minimum zone in the global ocean. Local hypoxic conditions are known to favor natural microbial production of methylmercury, leading to higher Hg exposure for marine predators from this region compared to other areas of the Pacific Ocean (Médiéu et al., 2022).

Shark length was found to be the best predictor of Hg variations at the community level, although explaining a modest part (i.e., 21%) of the observed variability (Table S3). As shown in our study (Fig. 4B), positive correlations between Hg concentrations and length (a proxy for age) are commonly found in shark species and other long-lived marine predators, reflecting Hg bioaccumulation (Kiszka et al., 2015; Le Bourg et al., 2019). Indeed, Hg is highly assimilated by marine predators, primarily in the methylmercury form, and slowly excreted due to strong affinity and binding to thiol-containing amino acids in proteins (Harris et al., 2003; Lemes and Wang, 2009; Thera et al., 2019). As assimilation exceeds elimination, Hg concentrations generally increase over time and

can reach particularly high levels in long-lived shark species (Besnard et al., 2021; Le Croizier et al., 2022b; Maurice et al., 2021). Overall, our results thus suggest that bioaccumulation is the main parameter dictating Hg concentrations in the shark community we analyzed, as commonly found in shark assemblages from other regions such as the Southwest Indian Ocean (Kiszka et al., 2015; Le Bourg et al., 2019; McKinney et al., 2016). However, a more complete sampling, involving a larger number of individuals per species and similar size classes, may reveal the influence of parameters other than size on the variability of Hg concentrations in sharks in this region.

We detected a slight, yet statistically significant, positive correlation between Hg levels and $\delta^{13}\text{C}$ values (Fig. 4C). As $\delta^{15}\text{N}$ values did not covary with Hg concentrations nor $\delta^{13}\text{C}$ values, this correlation is unlikely to be influenced by trophic level, which could have increased both $\delta^{13}\text{C}$ values and Hg levels. Rather, this result may suggest an increase in Hg exposure at the community scale as individuals forage in exploited habitats outside of MPA protection, characterized by higher $\delta^{13}\text{C}$ baselines (Fig. 1). While supporting our initial hypothesis, the influence of foraging habitat on Hg exposure appeared marginal, as $\delta^{13}\text{C}$ values were not kept in the best model to explain Hg concentrations in our dataset (Table S3). Since $\delta^{13}\text{C}$ values and Hg levels were both correlated with shark length (Fig. 4A–B), the link between $\delta^{13}\text{C}$ and Hg may have resulted from the confounding effect of size. However, a potential influence of foraging habitat (expressed as $\delta^{13}\text{C}$ values) on Hg exposure may have been partially masked by the fact that the highly mobile species analyzed in our study can use other regional MPAs when moving away from the Revillagigedo Archipelago, such as the MPAs of Cabo Pulmo (tiger sharks), Clipperton Atoll (Galapagos and silky sharks) and the Galapagos Islands (Galapagos sharks) (Ketchum et al., 2020; Lara-Lizardi et al., 2020). This connectivity between different MPAs could therefore have prevented a clear test of the influence of the protection status of the area on the Hg exposure of marine predators in this region. Contrary to our study involving migratory species sampled within an MPA, the comparison of less mobile species (whether predators or prey), residents of areas exploited by fisheries versus protected ones, would allow a better understanding of the role of MPAs in mitigating the Hg contamination of marine ecosystems. Finally, future studies should carry out an in-depth comparison of exploited areas versus MPAs in terms of abundance, biodiversity and trophic structure, which are all parameters likely to influence Hg concentrations in marine predators.

5. Conclusion

Marine protected areas can serve as trophic refuges for predators performing large-scale movements across regions subject to different degrees of fishing exploitation. Using isotopic niches as proxies for foraging habitat, dietary segregation among four shark species sampled in the Revillagigedo National Park was highlighted. Highly mobile species (Galapagos, silky and tiger sharks) displayed a low probability of isotopic overlap with silvertip sharks residing in the archipelago, suggesting the use of foraging habitats outside the MPA. Community-wide Hg concentrations were primarily influenced by shark length, with no clear influence of foraging habitat on Hg exposure, possibly due to the complex migratory behavior of the studied species moving between protected and exploited areas. Given that the combination of climate change and overfishing may increase Hg levels in marine predators (Booth and Zeller, 2005; Schartup et al., 2019), and fishing pressure is unlikely to abate under increasing human populations, our work paves the way for further studies on how conservation and management tools such as MPAs could help restore biomasses and reduce Hg contamination of marine food webs.

Credit author statement

Gaël Le Croizier: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. Anne Lorrain:

Resources, Writing – review & editing, Supervision, Funding acquisition. **Mauricio Hoyos-Padilla:** Resources, Writing – review & editing, Funding acquisition. **James T. Ketchum:** Resources, Writing – review & editing, Funding acquisition. **Felipe Amezcua-Martinez:** Writing – review & editing, Supervision. **François Le Loc'h:** Resources, Writing – review & editing. **Jean-Marie Munaron:** Formal analysis. **Gauthier Schaal:** Resources, Writing – review & editing, Funding acquisition. **David Point:** Resources, Writing – review & editing, Supervision, Funding acquisition.

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 related to this article can be found at <https://doi.org/10.1016/j.envpol.2023.122352>.

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