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Mercury concentrations and trophic relations in sharks of the Pacific Ocean of Colombia



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ABSTRACT

Sharks are fished for human consumption in Colombia, and fins are exported illegally to international markets. The goal was to identify differences in total mercury (THg) concentrations in fins and muscles of shark species seized in the Buenaventura port (Colombian Pacific), and to assess potential human health risks related to shark consumption. Seven species were considered in this study: Pelagic Thresher (*Alopias pelagicus*), Pacific Smalltail Shark (*Carcharhinus cerdale*), Brown Smoothhound (*Mustelus henlei*), Sicklefin Smoothhound (*Mustelus lunulatus*), Scalloped Bonnethead (*Sphyrna corona*), Scalloped Hammerhead (*Sphyrna lewini*), and Bonnethead Shark (*Sphyrna tiburo*), and THg was analyzed in shark tissues. 24% muscle samples concentrations were above international recommended limits for human consumption, especially for *A. pelagicus* and *S. lewini*. Stable isotopes (δ^{13} C and δ^{15} N) analysis showed a foraging overlapping in coastal habitats, where overfishing usually occurs. This study provides useful scientific information to develop management plans for sharks in Colombia and neighboring countries.

1. Introduction

Many shark species are currently threatened, due to the degradation of their habitat, overfishing, bycatch, and especially finning, considered as the main cause of shark population decline worldwide (Clarke et al., 2007; Dulvy et al., 2014). Finning is a fishing activity that consists in fishing sharks, removing their fins, and returning the rest of the body to the ocean (Clarke et al., 2007; Hernández Betancourt et al., 2011; Helfman and Burgess, 2014). Even if this is an illegal activity in many countries, finning continues on a large scale, and it is estimated that between 26 and 73 million of sharks are traded worldwide annually (Clarke et al., 2007). This is due to the high price and demand of this resource in Asian markets, particularly in Hong Kong (Clarke et al., 2004), where a shark fin costs ~US \$42.00 per kilo (Jaiteh et al., 2017), and a shark fin soup costs between US \$250 and US \$1800 (Nalluri et al., 2014; The 8 Restaurant, 2017).

In countries like Colombia, shark is part of food in some coastal locations, but fin soup is not consumed. However, finning is an activity that generates strong incomes due to their high commercial value in the Pacific region (Ross Salazar et al., 2019). Fin prices in Colombia are approximately US \$40 per kilo, in contrast to shark meat prices of US \$1.5 per kilo (Ross Salazar et al., 2019). Between 2005 and 2017, an average of 317,974 kg of sharks were caught in Colombia (Ross Salazar et al., 2019), even though in 2013 shark finning was prohibited in Colombia (Rodríguez Ortiz et al., 2016). Unfortunately, this activity has continued illegally, putting at high risk shark populations (Caldas Aristizábal et al., 2010; Jaiteh et al., 2017).

Recent studies suggest that prices of shark fins have fallen sharply

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since 2012 (Eriksson and Clarke, 2015) due, in part, to the conservation campaigns that have raised awareness of the effects of finning on shark populations (Whitcraft et al., 2014) and concerns about food safety (Fabinyi and Liu, 2014). Studies of THg concentrations in shark tissues have reported high concentrations of this metal (e.g., Kim et al., 2016; Le Bourg et al., 2019) that exceed the safety level for human consumption of 1000 ng g^{-1} ww determined by organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (FAO/WHO, 2011). These have generated a concern about human health risks to shark consumption, leading this distress as a conservation strategy to reduce shark fin demand (WHO, 2006; Escobar-Sánchez et al., 2010; Fabinyi and Liu, 2014; Man et al., 2014; Nalluri et al., 2014). The main concern is because THg is toxic for humans as it is a cumulative poison that bioaccumulates in the body (Matulik et al., 2017). Because THg is biomagnified by trophic transfers in food webs, top predators such as sharks, generally show very high THg concentrations in their tissues in comparison to other seafood products (Nalluri et al., 2014).

Because of both legal and illegal gold extraction (Tubb, 2015; Castellanos et al., 2016; Palacios-Torres et al., 2018), Colombia is the third country that emits more THg to the environment after China and Indonesia (Telmer and Veiga, 2009), exceeding the amounts issued by the three main gold producers together, minus China, which are Russia, Australia, and the United States (World Gold Council, 2020). The Chocó region, which is located in the Colombian North Pacific coast, is the second largest producer of gold in Colombia and it has been detected that 90% of the gold produced comes from alluvial operations (Massé and McDermott, 2017). The releases from gold mining may contribute to the THg contamination of the marine environment and consequently of the whole food webs up to sharks. Assessing THg concentrations in shark populations from the Colombian Pacific will allow to know the current contamination status in these species and the ecosystems they exploit and forage on, and also will provide relevant data about potential health risks related to shark consumption. International authorities such as FAO/WHO (Food and Agriculture Organization/World Health Organization) and Colombian agencies such as Invima (National Institute for Food and Drug Surveillance) are responsible for controlling these limits of THg in food and informing the population about the possible risks of THg in human health. Findings on this sense may also contribute to improve conservation and management strategies for shark populations that have been carried out in the Colombian Pacific, such as the National Action Plan for the Conservation and Management of Sharks, Rays, and Chimaeras of Colombia (PAN-Tiburones Colombia) (Caldas Aristizábal et al., 2010). Confirmation of THg concentrations in shark tissues from Colombia, could be used as a conservation strategy to reduce their international demand and national overfishing.

Therefore, for this study we assessed: (1) THg concentrations in fins and muscles of seven shark species seized in the Buenaventura port (Colombian Pacific), which include the Pelagic Thresher (*Alopias pelagicus*), Pacific Smalltail Shark (*Carcharhinus cerdale*), Brown Smoothhound (*Mustelus henlei*), Sicklefin Smoothhound (*Mustelus lunulatus*), Scalloped Bonnethead (*Sphyrna corona*), Scalloped Hammerhead (*Sphyrna lewini*), and Bonnethead Shark (*Sphyrna tiburo*); (2) potential human health risks related to fin and muscle consumption of any of these species; and (3) trophic ecology of the seven species of sharks by analyzing trophic interactions and possible overlaps of their ecological niche using chemical tracers of habitat and diet (δ^{13} C and δ^{15} N, respectively). Assessing trophic ecology and monitoring of THg in sharks captured in the Colombian Pacific and seized in the Buenaventura port, represents important precautionary measures to facilitate shark conservation in Colombia and neighboring countries.

2. Materials and methods

2.1. Study area

The Buenaventura port is located on the Colombian Pacific coast (Fig. 1). Fisheries on this region are characterized by sustenance fishing marketed in local markets (Pérez-Valbuena, 2007). Particularly for sharks, a large number of by-products such as jaw, vertebrae, meat, and liver are marketed in local markets (Caldas Aristizábal et al., 2010) as ornaments, food, or a nutritional source of omega-3 fatty acids (Maggie and Covington, 2004), respectively; however, fins are mostly marketed in international markets (Navia et al., 2016). These fins are exported illegally mainly from the Buenaventura port to Hong Kong and to China (Ross Salazar et al., 2019), so most of shark fin seizures in Colombia take place in this port (Estupiñán-Montaño et al., 2017: Ross Salazar et al., 2019). For this reason, samples used for this study came from shark fin seized on the coast of the Buenaventura port.

2.2. Shark tissue collection and sample preparation

A total of 175 samples of two different shark tissues were collected: 130 fin and 45 muscle samples. Shark fin and muscle samples seized at the Buenaventura port, were collected by researchers from the Malpelo Foundation and rangers from the Unit of National Natural Parks of Colombia between 2009 and 2013. For the purposes of this study, the seven species of sharks that had the highest collection frequency were selected.

Among the species selected to analyze fin samples, were included: (1) Pelagic Thresher (A. pelagicus) a highly migratory species from the epipelagic zone, which is in the Endangered category (EN) according to the IUCN (Rigby et al., 2019a, 2019b); (2) Pacific Smalltail Shark (C. cerdale), a coastal species that lives in estuarine ecosystems of the continental shelf, in shallow waters and with muddy substrates, cataloged by the IUCN as Critically Endangered (CR) (Pollom et al., 2020); (3) Brown Smoothhound (M. henlei), which inhabits mainly in coastal waters and in the open sea on the continental shelves, cataloged as Least Concern (LC) (Pérez-Jiménez et al., 2016a, 2016b); (4) Sicklefin Smoothhound (M. lunulatus), a species that is found near the coast and categorized as LC due mainly to incidental fishing (Pérez-Jiménez et al., 2016a); (5) Scalloped Bonnethead (S. corona), which resides on the continental shelf in shallow estuarine habitats, and is listed as Near Threatened (NT) mainly due to artisanal fishing (Mycock, 2004); (6) Scalloped Hammerhead (S. lewini), a coastal and semi-oceanic pelagic species found on continental and insular shelves, and in deeper waters up to 275 m depth, cataloged as Critically Endangered (CR) mainly due to finning (Rigby et al., 2019a); and (7) Bonnethead Shark (S. tiburo), an estuarine species found in shallow bays that is listed as LC mainly due to commercial, recreational, and incidental fisheries (Compagno, 1984; Cortés et al., 2016). Additionally, muscle samples were collected for A. pelagicus and S. lewini, two species whose threat status is the most critical (Supplementary Table S1).

Collected samples were preserved in 70% ethanol and stored at -20 °C for subsequent laboratory analysis. Following Barragán-Barrera et al. (2019), samples were left on a bench to let alcohol evaporated, then were homogenized, and freeze-dried to perform posterior THg and isotopic analyses (Fontaine et al., 2015; Barragán-Barrera et al., 2019).

2.3. Mercury analyses

THg measurements were conducted using an atomic absorption spectrometer AMA-254 (Altec © Advanced Mercury Analyzer-254). For each sample, at least a minimum of two aliquots of 1 to 5 mg of homogenized dry tissue subsamples were analyzed in order to estimate average THg concentrations (Aubail et al., 2013; Angel-Romero et al., 2018; Barragán-Barrera et al., 2019). Some measurements needed to be repeated until having analytical differences below 10%. The analytical



Fig. 1.. Map of study area showing Buenaventura, where is located the Buenaventura port in the Colombian Pacific coast.

quality of the THg measurements by the AMA-254 was evaluated by the analysis of blanks and certified reference material (CRM) TORT-2 (Reference Material of lobster hepatopancreas marine certified by the National Research Council of Canada) at the beginning and at the end of the analytical cycle, and after running the analysis of 15 samples. Blanks were negligible and agreement was considered satisfactory. The CRM measured concentration was $0.305\pm0.025~\mu g~g^{-1}~(n=20)$, with a percentage of average recovery of 104%. THg measurements are presented in ng g^{-1} on a wet weight basis (ww) and the detection limit was 0.5 ng.

2.4. Conversion of dry weight to wet weight in shark fins and muscles

Threshold values defined by regulatory guidelines of health agencies are established in wet weight (ww). However, concentration measurements of contaminants and stable isotope ratios C and N are usually made in dry material in order to avoid differences in results due to variations in water content of the individuals analyzed (Bryan, 1984; Fisk et al., 2002; Cresson et al., 2015; McKinney et al., 2016). For this reason, THg concentrations measured in dry weight (dw) in this study were transformed to wet weight (ww) based on the following formula:

$$C_{ww} = C_{dw} \times \left(\frac{100 - \%H}{100}\right)$$

where C_{ww} and C_{dw} are concentrations expressed in wet and dry weight respectively, and %H is the percentage of humidity that normally oscillates around 80% for fish species (Murray and Burt, 1969; Hislop et al., 1991; Payne et al., 1999; Environmental Protection Agency, 2011).

2.5. Risk assessment for consumption of shark fins and muscles

THg concentrations of each species in fins and muscles were

evaluated considering the maximum limit of THg in fish for human consumption by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), established as 1000 ng g^{-1} ww for top predators, such as sharks (FAO/WHO, 2011).

Human health risks derived from the consumption of fins and shark muscles were evaluated based on the non-carcinogenic risk ratio or Target Hazard Quotient (THQ). THQ is a specific dose ratio of a contaminant (in this case THg) at a reference dose level. THQ \leq 1 indicates that there are no adverse health effects, while THQ > 1 indicates possible adverse health effects (United States Environmental Protection Agency (USEPA), 1989, 2000). THQ is calculated using the following equation (United States Environmental Protection Agency (USEPA), 1989; Vieira et al., 2011):

$$THQ = \frac{EFr \times ED \times IR \times MC}{RfD \times BW \times AT}$$

where THQ is dimensionless; EFr is the frequency of exposure (365 days per year; Storelli et al., 2008); ED is the duration of the exposure (70 years, as the average human life time; Storelli et al., 2008); IR is the rate of food ingestion (33.4 10^{-3} kg per day per person for the world; Food and Agriculture Organization (FAO), 2009); MC is the THg shark tissue concentration (mg/kg); RfD is the oral reference dose (3.0 10^{-4} mg/kg/day for THg; Nadal et al., 2008; United States Environmental Protection Agency (USEPA), 2010); BW is the human body weight (kg): for this research, it was assumed an average body weight of 60 kg for an adult between 20 and 65 years and an average weight of 30 kg for a child between one and 19 years (Vieira et al., 2011); AT is the number of days during which the exposure is averaged (365 days × ED, according to REF). THQ was calculated with the average THg concentration (average THQ) and with the maximum and minimum values of THg concentrations for each species (maximum and minimum THQ, respectively).

Furthermore, the average, minimum, and maximum estimated weekly intake (EWI) of THg was calculated for each species, and these results were compared to the provisional tolerable weekly intake (PTWI) recommended by the Joint FAO/WHO Expert Committee on Food Additives for methylmercury (MeHg), since this organic form represents usually 90% of THg in fish muscles (Bloom, 1992; Adams and McMichael, 1999) and 70% in fins (Garcia Barcia et al., 2020). The Joint FAO/ WHO Expert Committee stated that the PTWI is 1.6 ng g⁻¹ body weight (bw) for the most susceptible human population such as children and women of childbearing age to protect an embryo (JECFA, 2004). Regarding the adult population, the committee considers that the intake of THg can be up to two times more (3.2 ng g⁻¹ of body weight) (WHO, 2006). The EWI was determined with the following equation (Mohammed and Mohammed, 2017):

$$EWI = \frac{Q_{fish} \times MO}{BW}$$

where Q_{fish} is the weekly amount of shark consumed (kg), MC is the concentration of the contaminant in the shark piece consumed (ng g⁻¹ ww), and BW is the human body weight (kg; 60 kg for adults between 20 and 65 years, and 30 kg for children between one and 19 years; Vieira et al., 2011). For adults, it was considered a consumption of 37.51 g of fins in a portion of fish soup (Man et al., 2014) and of 234 g of muscles in a plate (Food and Agriculture Organization (FAO), 2009). For children, knowing that food consumption at this early stage of life is lower than an adult (Leung et al., 2014). A rate consumption of one portion per week was considered for both adults and children (Man et al., 2014).

2.6. Stable isotope analysis

Because lipids are present in shark tissues and can alter values of δ^{13} C (De Niro and Epstein, 1977), all samples were delipidated before analysis of stable isotopes. For this, three repetitions of lipid removal in 4 ml of cyclohexane were carried out in each sample, stirring for 10 min, 5 min of centrifugation, and finally drying in an oven at a maximum temperature of 45 °C (Chouvelon et al., 2011). Then, between 0.2 and 0.4 mg of each delipidated sample were weighted in tin cups. Stable isotope measurements were performed on a Delta V Advantage IRMS isotopic mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to an EA 1112 Flash elemental analyzer (Thermo Scientific, Germany). The reference gas was calibrated with international reference materials including acetanilide and peptone. These two reference materials are passed at the beginning and at the end of the analytical cycle, and after running the analysis of 20 samples.

All results were expressed under the notation δ relative to Vienna PeeDee Belemnite and atmospheric nitrogen for δ^{13} C and δ^{15} N, respectively, according to the following equation (Peterson and Fry, 1987):

$$\delta \mathrm{X} = \left[\left(rac{R_{sample}}{R_{standard}}
ight) - 1
ight] imes 10^3$$

where X is δ^{13} C or δ^{15} N, and R is the isotopic ratio 13 C/ 12 C or 15 N/ 14 N, respectively. Replicate measurements of reference materials (caffeine: USGS61 and USGS62) indicated that analytical errors were < 0.15‰ for both δ^{13} C and δ^{15} N. Percentages of elemental composition C and N were also obtained by the elemental analyzer, and calculated C:N ratios were always below 3.5, which indicates a good efficiency of lipid removal (Table 1).

2.7. Data analysis

Shapiro-Wilk and Levene tests were performed to evaluate the assumptions of parametric tests (normality and homogeneity of variance, respectively). Transformations were performed so that the data fulfilled these assumptions (logarithmic transformation for THg), and nonparametric tests were performed when this was not possible (δ^{15} N and δ^{13} C). A one-way ANOVA test followed by a Tukey post-hoc test was used to evaluate significant differences in THg concentrations between species.

Table 1

Total mercury (THg in ng g⁻¹ ww) concentration, stable isotope ratios (δ^{13} C and δ^{15} N in ‰), C and N ratio (C:N) in sharks of the Colombian Pacific. Means and standard deviations were calculated (mean \pm SD). NA: data not available.

Species	Tissue	n	THg	δ^{15} N	$\delta^{13}\mathrm{C}$	C:N
			Mean \pm	Mean	Mean \pm	Mean
			SD	\pm SD	SD	\pm SD
Pelagic Thresher	Fins	30	$\textbf{88.07} \pm$	13.05	-15.14	3.04
(Alopias			97.66	± 0.56	\pm 0.74	$\pm \ 0.14$
pelagicus)	Muscles	25	566.78	14.19	-16.99	3.34
			±	± 0.52	\pm 0.60	$\pm \ 0.15$
			337.07			
Pacific Smalltail	Fins	9	45.56 \pm	13.64	-14.65	2.81
Shark			25.40	\pm 0.47	\pm 0.80	± 0.72
(Carcharhinus cerdale)	Muscles	NA	NA	NA	NA	NA
Brown	Fins	25	23.45 \pm	14.16	-15.50	3.08
Smoothhound			15.27	± 0.56	\pm 0.45	± 0.38
(Mustelus	Muscles	NA	NA	NA	NA	NA
henlei)						
Sicklefin	Fins	20	$18.38~\pm$	13.14	-14.81	2.52
Smoothhound			11.76	± 0.39	± 0.35	± 0.72
(Mustelus	Muscles	NA	NA	NA	NA	NA
lunulatus)						
Scalloped	Fins	10	$24.10~\pm$	12.74	-14.62	3.09
Bonnethead			16.16	± 0.42	± 0.30	$\pm \ 0.07$
(Sphyrna corona)	Muscles	NA	NA	NA	NA	NA
Scalloped	Fins	19	88.56 \pm	13.61	-14.83	2.66
Hammerhead			82.30	± 0.63	\pm 0.88	± 0.71
(Sphyrna lewini)	Muscles	20	809.29	15.46	-16.09	3.34
			±	± 0.67	\pm 0.85	± 0.14
			728.88			
Bonnethead	Fins	17	$34.69~\pm$	12.31	-15.64	3.04
Shark (Sphyrna			32.10	± 0.56	± 0.96	± 0.64
tiburo)	Muscles	NA	NA	NA	NA	NA

A two-way ANOVA followed by a Tukey post-hoc test was used to evaluate significant differences in THg concentrations between tissues. A Kruskal-Wallis test followed by a nonparametric multiple comparison post-hoc test was used to evaluate significant differences in δ^{13} C and δ^{15} N values between shark species.

A Bayesian approach based on multivariate ellipse metrics (SIBER — Stable Isotope Bayesian Ellipses within the R-siar package; Jackson et al., 2011) was used to assess the overlapping of isotopic niche between species. This analysis generates areas of standard ellipses corrected for small sample sizes (SEAc) that correspond to 40% of the data regardless of the sample size (Batschelet, 1981), which can be compared between species and thus determine differences in the central isotope niche space and the niche superposition (Jackson et al., 2011). The calculation of standard ellipses (SEAc) was performed for the data δ^{13} C - δ^{15} N, δ^{13} C - THg, and δ^{15} N - THg, supported by its associated covariance matrix:

$$\sum = \begin{bmatrix} \sigma_x^2 & cov(x,y) \\ cov(y,x) & \sigma_y^2 \end{bmatrix},$$

which defines the shape and size of the ellipses, and the averages of x and y define the location of the ellipse on the graph (Jackson et al., 2011). To know the degree of niche overlapping, that is, the overlap in the areas of the standard ellipses (SEAc) of the species for each combination of the above parameters, was used the OVERLAP command. This overlap value normally ranges between 0 and 1, and values closer to 1 represent greater overlap. The degree of overlapping was later transformed into percentages for a better visualization of the data (Jackson et al., 2011).

Finally, a nonparametric Spearman correlation test was performed to evaluate relationships between δ^{13} C - δ^{15} N, δ^{13} C - THg, and δ^{15} N - THg values. All data analyses were performed using the program R 1.1.423 (R Development Core Team, 2014).

3. Results

3.1. THg concentrations in shark fins and muscles

A total of 175 shark samples (130 fins and 45 muscles) were analysed. THg concentrations were measured in all samples (Table 1). Fin THg concentrations were measured in all species, showing significant differences between species (F = 5.55, df = 6, p < 0.05; Table 1, Fig. 2), of which *S. lewini* showed the highest values (88.56 ± 82.30 ng g⁻¹ ww). Muscle THg concentrations were only measured in *A. pelagicus* and *S. lewini* (Fig. 3). *S. lewini* also showed the highest concentrations (809.29 ± 728.88 ng g⁻¹ ww; Table 1, Fig. 3). These concentrations were significantly higher than fin THg concentrations for both species (F = 114.83, df = 1, p < 0.05, Fig. 3).

3.2. Risk assessment for consumption

Average THg concentrations were compared with the maximum permitted level (ML) of THg in predatory fish recommended by the FAO/WHO of 1000 ng g⁻¹ ww (FAO/WHO, 2011). THg fin concentrations were all below to ML (Figs. 2 and 3), and only for THg muscle concentrations some individuals of *A. pelagicus* and *S. lewini* exceeded the ML by two to almost three orders of magnitude (maximum values of 1893.46 and 2664.66 ng g⁻¹ ww, respectively; Fig. 3).

The minimum, maximum, and average values of the estimated weekly intake (EWI) and the average Target Hazard Quotient (THQ) values were calculated for two age-specific human categories (adults and children). EWI results in fins were below the PTWI for all species (Table 2). However, EWI results in muscles for *A. pelagicus* and *S. lewini* exceeded this limit for both age-groups, except for *A. pelagicus* in adults,

which presented a relatively high average but below to the recommended limit (2.21 ng g⁻¹ bw; Table 2). THQ results in fins for both agegroups analyzed were <1 while THQ results in muscles of *A. pelagicus* and *S. lewini* were >1 for both age-groups (Table 2).

3.3. Trophic interactions

Only fins were analyzed for all seven shark species for ecological niche overlapping. An overlapping of δ^{13} C - δ^{15} N ellipses among all species was observed, some of them with a greater percentage of overlapping than others (Table 3, Figs. 4A and 5A). Particularly, *S. tiburo* showed the lowest percentage of shared niche with other species (Table 3, Fig. 5A), while *A. pelagicus* and *S. lewini* showed the highest one (Table 3, Fig. 5A).

Results in standard ellipses of δ^{13} C-THg and δ^{15} N-THg (Tables 4 and 5, SEAc: Figs. 5B-C), showed for the genus *Mustelus* an important niche segregation in relation to other genera; particularly *M. henlei* showed lower percentages for δ^{13} C-THg and *S. tiburo* and *M. henlei* for δ^{15} N-THg (Tables 4 and 5, Fig. 5B in purple, Fig. 5C in dark blue and purple). Conversely, most of the species showed a considerable niche overlap for these results (Figs. 5B–C), in which *M. lunulatus* and *S. corona* presented the highest percentage values for δ^{13} C-THg and *M. lunulatus* the highest values for δ^{15} N-THg (Tables 4 and 5, Fig. 5B–C), in which *M. lunulatus* and *S. corona* presented the highest percentage values for δ^{13} C-THg and *M. lunulatus* the highest values for δ^{15} N-THg (Tables 4 and 5, Fig. 5B in neon green and neon blue, Fig. 5C in neon green). Likewise, for both δ^{13} C-THg and δ^{15} N-THg, *S. lewini* showed the largest ellipse area (229.45 and 168.14, in ‰², respectively), while *M. lunulatus* showed the smallest ellipse area (13.52 and 15.26, in ‰², respectively, Supplementary Table S2).



Fig. 2. THg concentrations (ng g^{-1} ww) in fins of seven shark species of the Colombian Pacific: *Sphyrna lewini* (purple), *Alopias pelagicus* (red), *Carcharhinus cerdale* (yellow), *Sphyrna tiburo* (pink), *Sphyrna corona* (blue) *Mustelus henlei* (neon green), and *Mustelus lunulatus* (mint green). The upper and lower ends of the boxes are the quartiles, the vertical lines are the maximum and minimum, the bold line represents the average and the points are the outliers. The horizontal lines are the paired combinations with significant differences (*: p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



Fig. 3. Fin (blue) and muscle (grey) THg concentrations (ng g⁻¹ ww) in *Alopias pelagicus* and *Sphyrna lewini* individuals of the Colombian Pacific. The upper and lower ends of the boxes are the quartiles, the vertical lines are the maximum and minimum, the bold line represents the average and the points are the outliers. The dotted horizontal line represents the limit of maximum consumption of Hg in predatory fish recommended by the FAO/WHO (2011). The horizontal lines are the paired combinations with significant differences (*: p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Estimated weekly intake (EWI) and Target Hazard Quotient (THQ) for seven shark species of the Colombian Pacific. The provisional tolerable weekly intake (PTWI) recommended by the Joint FAO/WHO Expert Committee (2004) is 1.6 ng g⁻¹ bw for children and 3.2 ng g⁻¹ bw for adults. THQ values >1 indicate possible adverse effects for human health.

Age group	Species	Tissue	Mean EWI (ng g^{-1})	Min EWI–Max EWI (ng g^{-1})	Mean THQ	Min THQ–Max THQ
Children (1-19 years) 30 kg	A. pelagicus	Fins Muscles	0.11 3.78	0.01–0.68 0.13–12.62	0.33 2.10	0.03–2.03 0.07–7.03
	C. cerdale	Fins	0.06	0.02-0.13	0.17	0.05-0.37
	M. henlei	Fins	0.03	0.01-0.09	0.09	0.03-0.28
	M. lunulatus	Fins	0.02	0.01-0.07	0.07	0.03-0.21
	S. corona	Fins	0.03	0.01-0.08	0.09	0.04-0.24
	S. lewini	Fins	0.11	0.02-0.40	0.33	0.07-1.20
		Muscles	5.40	0.30-17.76	3.00	0.17-9.89
	S. tiburo	Fins	0.04	0.01-0.16	0.13	0.02-0.47
Adult (25–65 years) 60 kg	A. pelagicus	Fins	0.06	0.00-0.34	0.16	0.01-1.01
		Muscles	2.21	0.07-7.38	1.05	0.04-3.51
	C. cerdale	Fins	0.03	0.01-0.06	0.08	0.03-0.19
	M. henlei	Fins	0.01	0.00-0.05	0.04	0.01-0.14
	M. lunulatus	Fins	0.01	0.01-0.04	0.03	0.02-0.10
	S. corona	Fins	0.02	0.01-0.04	0.04	0.02-0.12
	S. lewini	Fins	0.06	0.01-0.20	0.16	0.03-0.60
		Muscles	3.16	0.18-10.39	1.50	0.08-4.94
	S. tiburo	Fins	0.02	0.00–0.08	0.06	0.01-0.24

3.4. Relationship between $\delta^{13}C$, $\delta^{15}N$, and THg concentrations

Fin values of δ^{15} N and δ^{13} C varied significantly among species (Kruskal-Wallis, $X^2 = 77.60$, df = 6, p < 0.05 and $X^2 = 37.46$, df = 6, p < 0.05, respectively), but not varied significantly for THg concentrations. Spearman correlation tests were performed separately for fins and muscles since significant differences were encountered in THg

concentrations between fins and muscles. In fins, no significant (p > 0.05) correlations were found between δ^{13} C, δ^{15} N, and THg, when all species were considered. At species level with fin samples, only a significant positive correlation was found between δ^{15} N - THg for *S. tiburo* (Spearman: r = 0.60, p = 0.01). In muscles, no significant (p > 0.05) correlations were found between δ^{13} C, δ^{15} N, and THg, whether considering both species together or species individually.

Table 3

Overlapping percentage of corrected standard ellipses (SEAc) of δ^{13} C and δ^{15} N in fins of seven shark species of the Colombian Pacific, including: *Alopias pelagicus*, *Mustelus lunulatus, Sphyrna lewini, Sphyrna tiburo, Mustelus henlei, Carcharhinus cerdale*, and *Sphyrna corona*.

Species	A. pelagicus	M. lunulatus	S. lewini	S. tiburo	M. henlei	C. cerdale	S. corona
A. pelagicus	-	88.6	29.4	9.7	0.7	23.2	65.9
M. lunulatus	31.4	_	18.6	0.0	0.0	16.5	33.1
S. lewini	42.3	75.2	-	0.0	36.7	92.8	17.8
S. tiburo	13.2	0.0	0.0	-	0.0	0.0	9.7
M. henlei	0.4	0.0	16.5	0.0	-	6.6	0.0
C. cerdale	23.9	48.1	66.6	0.0	10.6	-	1.1
S. corona	21.7	30.8	4.1	2.3	0.0	0.3	-



Fig. 4. Relationship (mean \pm SD) between carbon and nitrogen isotopic signatures (‰) and THg concentrations (ng g⁻¹ ww) in fins for seven shark species of the Colombian Pacific, including: *Alopias pelagicus* (red), *Carcharhinus cerdale* (yellow), *Mustelus henlei* (neon green), *Mustelus lunulatus* (mint green), *Sphyrna corona* (blue), *Sphyrna lewini* (purple) and *Sphyrna tiburo* (pink). (A) represents relationship between δ^{13} C and δ^{15} N, (B) δ^{13} C and THg and (C) δ^{15} N and THg. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This is the first study that reports THg contamination levels for some of the most representative shark species on the Colombian Pacific, and that describes ecological aspects associated with their trophic ecology, and the partition of ecological and foraging niches. This information can be of great help for the implementation of future conservation plans and strategies to reduce the trade and export of shark fin in Colombia and other neighboring countries.

4.1. Levels of THg concentrations

In the Colombian Pacific, sharks showed moderate THg concentrations in comparison to other studies worldwide (Table 6) likely related to tissue samples being from juvenile sharks (see discussion Section 4.3). In muscles, these concentrations were similar to THg measurements reported for several shark species in the Pacific coast of Costa Rica, in the Mexican Pacific coast, in the Southeastern United States Caribbean coast, and in the Southwest Indian Ocean (Maz-Courrau et al., 2012; Bergés-Tiznado et al., 2015; Kiszka et al., 2015; Sandoval-Herrera et al.,



Fig. 5. Standard ellipses for corrected sample sizes (SEAc) of carbon and nitrogen isotopic signatures (‰) and THg concentrations (ng g⁻¹ ww) in fins for seven shark species of the Colombian Pacific, including: *Alopias pelagicus* (red), *Mustelus lunulatus* (neon green), *Sphyrna lewini* (dark green), *Sphyrna tiburo* (dark blue), *Mustelus henlei* (purple), *Carcharhinus cerdale* (orange) and *Sphyrna corona* (neon blue). (A) represents relationship between δ^{13} C and δ^{15} N, (B) δ^{13} C and THg and (C) δ^{15} N and THg. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4

Percentage of overlap (in %) of corrected standard ellipses (SEAc) of δ^{13} C vs THg for fins of seven shark species of the Colombian Pacific, including: Alopias pelagicus, Mustelus lunulatus, Sphyrna lewini, Sphyrna tiburo, Mustelus henlei, Carcharhinus cerdale, and Sphyrna corona.

Species	A. pelagicus	M. lunulatus	S. lewini	S. tiburo	M. henlei	C. cerdale	S. corona
A. pelagicus	-	100.0	68.8	40.6	37.4	67.4	90.9
M. lunulatus	6.5	-	4.9	7.9	4.6	7.1	58.8
S. lewini	76.0	83.8	-	27.8	9.1	87.8	99.7
S. tiburo	19.9	59.8	12.3	-	100.0	41.3	30.2
M. henlei	4.0	7.5	0.9	21.9	-	12.0	0.0
C. cerdale	18.1	29.1	21.3	22.6	29.9	-	42.1
S. corona	6.6	65.9	6.6	4.5	0.0	11.5	-

2016; O'Bryhim et al., 2017) (Table 6).

Differences in THg concentrations between fins and muscles could be explained by the high affinity that has THg with the thiol sulfuric groups present in the amino acids of muscles, showing high values for this tissue (Escobar-Sánchez et al., 2010; Pethybridge et al., 2010; O'Bryhim et al., 2017). However, fins also showed significant high THg concentrations for *A. pelagicus* and *S. lewini*, both species threatened according to the IUCN because of finning (Endangered and Critically Endangered, respectively; Rigby et al., 2019a, 2019b), which concerns about health

status of these species.

4.2. Effects on human health due to THg overload and risk assessment

According to FAO/WHO, the THg permitted concentration limit for humans is 1000 ng g⁻¹ ww (FAO/WHO, 2011). Our data showed THg concentrations in fins and muscles below this limit (46.12 and 688.04 ng g⁻¹ ww on average, respectively). However, our results showed that muscles of some individuals of *A. pelagicus* and *S. lewini* presented THg

Table 5

Percentage of overlap (in %) of corrected standard ellipses (SEAc) of δ^{15} N vs THg for fins of seven shark species of the Colombian Pacific, including: Alopias pelagicus, Mustelus lumulatus, Sphyrna lewini, Sphyrna tiburo, Mustelus henlei, Carcharhinus cerdale, and Sphyrna corona.

Species	A. pelagicus	M. lunulatus	S. lewini	S. tiburo	M. henlei	C. cerdale	S. corona
A. pelagicus	_	66.2	39.2	30.7	0.0	22.3	81.7
M. lunulatus	6.3	_	4.5	0.0	0.0	4.1	26.3
S. lewini	40.9	49.3	-	0.0	24.2	95.7	9.0
S. tiburo	10.5	0.0	0.0	-	0.0	0.0	45.0
M. henlei	0.0	0.0	3.9	0.0	-	16.7	0.0
C. cerdale	5.9	11.5	24.3	0.0	25.9	-	0.0
S. corona	11.8	40.2	1.3	19.2	0.0	0.0	-

Table 6

Total mercury	v (THg) concentrations (ng	q^{-1}	ww) in fins and	muscles	of shark	species rep	ported in	1 the	literature	and ir	this st	udv
roun mercur	, (1116) concentrations (ing	6	www.j m muo una	mascics	or bindin	opecies re	porteu n	i uic	menune	und m	i uno oc	aug.

Species	Ν	THg (mean \pm SD)	Tissue	Region	Reference
S. zygaena	37	7	Fins	Baja California Sur, Mexico	Escobar-Sánchez et al. (2010)
C. melanopterus	26	1100 ± 770	Fins	Korean Pacific Ocean	Kim et al. (2016)
P. glauca	15	2300 ± 1100	Fins	Korean Pacific Ocean	Kim et al. (2016)
A. pelagicus	13	1500 ± 510	Fins	Korean Pacific Ocean	Kim et al. (2016)
C. longimanus	4	530 ± 790	Fins	Korean Pacific Ocean	Kim et al. (2016)
S. zygaena	3	1600 ± 900	Fins	Korean Pacific Ocean	Kim et al. (2016)
M. manazo	2	320 ± 140	Fins	Korean Pacific Ocean	Kim et al. (2016)
S. tiburo	18	8 ± 6	Fins	Southeastern United States	O'Bryhim et al. (2017)
C. porosus	12	67.3-2368.4	Fins	Trinidad and Tobago	Mohammed and Mohammed (2017)
S. lewini	10	301.6-1465.8	Fins	Trinidad and Tobago	Mohammed and Mohammed (2017)
C. albimarginatus	26	56–32	Fins	Revillagigedo Archipelago	Le Croizier et al. (2020)
C. albimarginatus	28	63–48	Fins	Clipperton Atoll	Le Croizier et al. (2020)
A. pelagicus	30	88.07 ± 97.66	Fins	Colombian Pacific Coast	This study
C. porosus	9	45.56 ± 25.40	Fins	Colombian Pacific Coast	This study
M. henlei	25	23.45 ± 15.27	Fins	Colombian Pacific Coast	This study
M. lunulatus	20	18.38 ± 11.76	Fins	Colombian Pacific Coast	This study
S. corona	10	$\textbf{24.10} \pm \textbf{16.16}$	Fins	Colombian Pacific Coast	This study
S. lewini	19	88.56 ± 82.30	Fins	Colombian Pacific Coast	This study
S. tiburo	17	34.69 ± 32.10	Fins	Colombian Pacific Coast	This study
C. falciformis	15	680 ± 284	Muscles	Baja California Sur, Mexico	Maz-Courrau et al. (2012)
S. zygaena	31	196 ± 184	Muscles	Baja California Sur, Mexico	Maz-Courrau et al. (2012)
S. zygaena	37	730	Muscles	Baja California Sur, Mexico	Escobar-Sánchez et al. (2010)
P. glauca	38	1390 ± 1580	Muscles	Baja California Sur, Mexico	Escobar-Sánchez et al. (2011)
S. tiburo	42	474 ± 304	Muscles	Southeastern United States	O'Bryhim et al. (2017)
A. pelagicus	5	994 ± 720	Muscles	South-western Indian Ocean	Kiszka et al. (2015)
S. lewini	6	1172 ± 546	Muscles	South-western Indian Ocean	Kiszka et al. (2015)
S. lewini	40	630 ± 40	Muscles	Pacific Coast of Mexico	Bergés-Tiznado et al. (2015)
C. porosus	12	120-3328	Muscles	Trinidad and Tobago	Mohammed and Mohammed (2017)
S. lewini	10	208-1899	Muscles	Trinidad and Tobago	Mohammed and Mohammed (2017)
M. henlei	17	160 ± 90	Muscles	Pacific Coast of Costa Rica	Sandoval-Herrera et al. (2016)
S. lewini	44	2718 ± 1994	Muscles	Southwest Indian Ocean	Le Bourg et al. (2019)
A. pelagicus	5	4930 ± 3541	Muscles	Southwest Indian Ocean	Le Bourg et al. (2019)
A. pelagicus	25	566.78 ± 337.07	Muscles	Colombian Pacific Coast	This study
S. lewini	20	809.29 ± 728.88	Muscles	Colombian Pacific Coast	This study

concentrations that exceeded this safety limit for consumption.

EWI and THQ risk indexes for muscles of *A. pelagicus* and *S. lewini* also showed a risk for human health due to THg consumption in both adults and children. Particularly, the risks for children are greater because they consume three to four times more food in proportion to their body size than adults and, therefore, ingest larger amounts of chemicals per unit of body mass (United States Environmental Protection Agency (USEPA), 2008). However, since the fish ingestion rate in Colombia is unknown, it is possible the fish ingestion rate used is underestimated for the Colombian fishing community, and therefore, more specific studies including local fish and especially shark consumption should be carried out in the future.

Although fins are exported to external markets, muscles can be consumed by adults and children of local communities of the Colombian Pacific (Fuentes-Gandara et al., 2016). THg released into the environment by both natural and anthropogenic origins, this last one increased rapidly in Colombia due to gold mining during the last decade (Güiza and Aristizábal, 2013), accentuates a public health problem. Therefore, and following guidelines of European Commission and FAO/WHO, we suggest that daily consumption of *S. lewini* and *A. pelagicus* is not safe for

human health and should be regulated especially for children (Falcó et al., 2006). These findings have also conservation implications, because *A. pelagicus* and *S. lewini* have been reported as the most exploited species in the Colombian Pacific coast (Caballero et al., 2012).

4.3. Amplitude and overlapping patterns of ecological niche

Regarding the amplitude and overlapping patterns, only fin samples were considered since this tissue has been already employed in stable isotope and fatty acid analyzes in sharks (Every et al., 2016; Rangel et al., 2019), revealing feeding habits and habitat use over periods of time of more than a year due to its slow turn-over rate (Malpica-Cruz et al., 2012). Niche overlap of all species is not 100% in terms of trophic level (δ^{15} N). It was possible to identify three groups in which the first was *M. henlei* with the highest average of δ^{15} N, the second was *S. tiburo* with the lowest average of δ^{15} N, and the third group was the rest of the species that had a high overlap in terms of δ^{15} N (*A. pelagicus, C. cerdale, M. lunulatus, S. corona*, and *S. lewini*). The first group seems to have prey of relatively higher trophic level, while the second group appears to have prey of relatively lower trophic level, even though generally the

 δ^{15} N results are low for all the individuals. Previous studies on the diet of *M. henlei* and *S. tiburo* indicate that these species feed both on small fishes and crustaceans, which could explain these results (Cortés et al., 1996; Pérez-Jiménez et al., 2016a, 2016b).

 δ^{13} C values in fins ranged between -15.64 and -14.83 ‰. Although values for all the species were similar even for standard ellipses of δ^{13} C and THg, we suggest the existence of two shark groups in terms of habitat use. One group was only represented by S. tiburo (mean = -15.64 ‰) while remaining species formed the other group (range = -15.50 and -14.62 ‰). δ^{13} C values of shark species in this study were relatively high, indicating coastal habits (Hussey et al., 2011), with the exception of S. tiburo that showed a wide range of δ^{13} C values, suggesting a wider foraging area. This result differs from other studies for most shark species analyzed here, such as S. lewini, which usually inhabits pelagic zones when they are adults, for their development, growth, and foraging (Compagno, 1984; Estupiñán-Montaño et al., 2009, 2017, 2021). Because the Colombian Pacific mangroves are known as suitable areas for shark species during their first years (Quintanilla et al., 2015) and considering the relatively low δ^{15} N results, these results reinforce also the hypothesis that the S. lewini specimens analyzed in this study were juveniles feeding in coastal habitats.

Overall, δ^{13} C results suggest also a niche overlap in coastal areas for all the studied individuals, which could lead to a competition for food between these species. However, the significant differences found in isotopic relationships between δ^{15} N and THg, suggests diet differences among individuals analyzed (Maya Meneses et al., 2016). For instance, *S. lewini* prefers cephalopods, while *M. lunulatus* consumes more stomatopods (Estupiñán-Montaño et al., 2009; Galván-Magaña et al., 2013). These diet differences could thus allow coexistence of species in coastal areas, as has been reported for several shark species (Maya Meneses et al., 2016), even in other Colombian areas such as Malpelo Island (Estupiñán-Montaño et al., 2017). These results are also supported by SIBER-based analyses of the standard ellipses between δ^{13} C and δ^{15} N (with the exception of *S. tiburo*, see Fig. 5A).

4.4. Shark ecological patterns: implications for conservation

This study was limited by the origin of the samples, since they correspond to seizures, in which most of them were only fins already cut off from the sharks, which makes it impossible to know other important biological variables such as size. However, isotopic data showed insights about age of individuals analyzed in this study. The generally low δ^{15} N results suggest that individuals analyzed in this study could be juveniles (Estupiñán-Montaño et al., 2009; Torres-Rojas et al., 2006). Considering both, relatively low δ^{15} N and relatively high δ^{13} C values, these results reinforce also the hypothesis that most specimens analyzed in this study were juveniles feeding in coastal habitats.

Our findings suggest therefore two worrisome hypotheses: 1) most individuals collected, particularly A. pelagicus and S. lewini, were juveniles, and 2) biggest Colombian sharks fishing activity is concentrated in coastal areas. However, Payán et al. (2008) identified the pelagic zone as the most visited by the fishing fleet in the Colombian Pacific, where there is a greater incidental capture of elasmobranchs. Nevertheless, our findings suggest the potential shark fishing in coastal areas, likely in a small scale. The possibility of fishing coastal young Chondrichthyes or adults during their reproductive period should be considered as an important issue for fisheries management in Colombia and probably other neighboring countries with similar practices (Quintanilla et al., 2015), in order to maintain the population growth of these species that are in some state of threat. Particularly in the Colombian Pacific, a marine connectivity has been detected for adults and neonates of S. lewini between oceanic and coastal waters in Malpelo Island and the Sanquianga National Natural Park, respectively (Quintanilla et al., 2015; Estupiñán-Montaño et al., 2021). Therefore, potential extension of Marine Protected Areas mainly in coastal areas, or the inclusion of marine corridors between coastal and oceanic areas should be

considered as an important measure to protect sharks in Colombia (Estupiñán-Montaño et al., 2021).

Survival of juveniles is particularly important for shark conservation (Cortés, 2002). Sharks are highly vulnerable to increased mortality rates due to their slow growth, late age of sexual maturation, low reproduction rate, and an important parental investment in young pups (Ferretti et al., 2008). Juveniles are generally segregated from adults in coastal waters as breeding areas, a strategy that is supposed to improve survival by providing shelter from predation and prey abundance (Heupel et al., 2007). However, these areas, which are generally found in estuarine and mangroves ecosystems (Nakano and Stevens, 2008), are the most affected in Colombia by THg contamination due to anthropogenic activities, as has been reported, in South Baja California (Mexico) (Maz-Courrau et al., 2012). This coastal contamination could also affect adult sharks during pregnancy period because it has been reported that females are capable of carrying out excretion processes by transferring part of their bioaccumulated THg to their embryos (Cadena-Cárdenas, 2004; Pethybridge et al., 2010; Man et al., 2014). These situations may be happening in the Colombian Pacific coast. Species such as S. lewini are mobile and migrate in periods of rain constantly through the East Tropical Pacific Corridor between the islands of Cocos, Galapagos, and Malpelo (Bessudo et al., 2011). Since this corridor is a marine-protected area (MPA), it is possible that this regional conservation efforts carried out would be threatened by this THg contamination, in addition to coastal overfishing of juveniles and/or adults during their reproductive period. Further studies are needed to continue monitoring the THg exposure in sharks and local communities along the Colombian Pacific coast, as well as effective management of shark fishing in coastal areas, in order to guarantee their conservation in the long term.

5. Conclusions and recommendations

This study contributed to assess the status of THg contamination of shark populations, and knowledge of the trophic ecology of A. pelagicus, C. cerdale, M. henlei, M. lunulatus, S. corona, S. lewini, and S. tiburo in the Colombian Pacific. Overall, it was found that THg concentrations vary according to the species and were moderate, in comparison to other studies. However, ML, EWI, and THQ results suggested human health risks to shark consumption, which is a warning signal for consumers, especially for children and pregnant women. In addition, isotope analyses results suggested that coastal areas have the highest capture of sharks, which would be affecting the growth of shark populations. This information helps to understand the role of these shark species that are the most affected by overfishing and finning in this area of the country, with the aim of providing useful information to develop management and conservation plans for these species. Furthermore, the identification of key habitats like foraging areas can be also important evidence for ideal conservation and management policies such as potential extension of MPAs or creation of marine corridors.

It is recommended for future studies to include molecular analyses to complement the information obtained in this study such as sex identification, to assess if females accumulate higher THg concentrations than males, which could increase risks of THg transfer to the neonates (Cadena-Cárdenas, 2004; Pethybridge et al., 2010; Man et al., 2014). Additionally, shark size information would help explain possible bioaccumulation processes (Delshad et al., 2012; Maz-Courrau et al., 2012; Man et al., 2014; Kim et al., 2016), since adult sharks tend to accumulate more THg in their tissues than juveniles (Escobar-Sánchez et al., 2011). THg levels found here are an important issue for shark conservation, so pollution management should be considered as one of the most important issues for future management plans in Colombia or at regional level. Finally, for future investigations, the exact geographical fishing points would help to identify the areas of greatest threat of overfishing and finning for sharks in the Colombian Pacific and therefore prioritize fishing management plans for these areas.

CRediT authorship contribution statement

Natalia Vélez: Conceptualization, Methodology, Software, Investigation, Writing – original draft, Formal analysis, Visualization. Sandra Bessudo: Conceptualization, Resources, Supervision, Project administration, Funding acquisition. Dalia C. Barragán-Barrera: Conceptualization, Methodology, Funding acquisition, Writing – review & editing, Supervision. Felipe Ladino: Writing – review & editing. Paco Bustamante: Resources, Writing – review & editing. Andrea Luna-Acosta: Conceptualization, Supervision, Validation, Resources, Writing – review & editing.

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.

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Appendix A. Supplementary data

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