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Research Article

Heavy metal concentrations of two highly migratory sharks (*Prionace glauca* and *Isurus oxyrinchus*) in the southeastern Pacific waters: comments on public health and conservation.

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Abstract

Despite the importance of sharks in structuring the marine food web, their biomass is declining dramatically throughout the world's oceans due to fishing pressures. Sharks caught as *by-catch* in long-line fisheries are sold for shark fins in the Asian fish market and secondarily as trunk sales for local consumption and fish meal. In order to determine the levels of heavy metals (mercury and lead) in oceanic shark populations in South Pacific waters, analyses of 39 *Prionace glauca* and 69 *Isurus oxyrinchus* were conducted. Mercury (Hg) and lead (Pb) were measured by cold vapor and via acetylene flame techniques, respectively. Mercury concentrations were similar in the studied sharks ($p=0.1516$), with $0.048 \pm 0.03 \mu\text{g}\cdot\text{g}^{-1}$ w/w for *P. glauca* and $0.034 \pm 0.023 \mu\text{g}\cdot\text{g}^{-1}$ w/w for *I. oxyrinchus*. *P. glauca* showed greater values of lead than *I. oxyrinchus* ($p<0.001$). Large specimens of both species showed high heavy metal concentration, while sexes showed no statistical differences ($p>0.05$). The metal concentrations reported in this work constitute a risk for human health, mainly from the high contributions of lead in tissues of *P. glauca* and *I. oxyrinchus*.

Key Words: Mercury, Lead, Sharks, Public health, Pacific Ocean.

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Introduction

Blue shark (*Prionace glauca*) and mako short fin shark (*Isurus oxyrinchus*) are pelagic, oceanic and highly migratory species [1]. These fishes have a wide geographic range and play an important role in marine food webs as apex predators, feeding on squid, tuna and other fishes [2–7]. The importance of these sharks in structuring marine food webs has been demonstrated, thus their biomass decrease throughout the world's oceans is a concern [8,9]. Acuña [10] reported that sharks comprise over 70% of the total catch in the swordfish long-line fisheries. Sharks caught as *by-catch* in long-line fisheries are sold for shark fins in the Asian market and as trunk sales for local meat consumption and fish meal [11].

Sharks accumulate trace elements in their tissues such as mercury (Hg), arsenic (As) and lead (Pb)[12,13], largely through the diet [12]. Although blue and mako short fin sharks are the most abundant and are highly exploited throughout the oceans, knowledge of their heavy metal concentrations is very poor. The mercury and lead concentration of *P. glauca* and *I. oxyrinchus* tissues since 1974 in different oceans is summarized in Appendix 1. Mercury is the most studied element, principally in the Northern Hemisphere; in contrast lead is the least studied. Mercury and lead are volatile and highly toxic environmental contaminants present in marine ecosystems [13] where sharks are more susceptible to uptake and biomagnification of these heavy metals, because they incorporate the metals very efficiently and eliminate them slowly.

In the southeastern Pacific Ocean concentrations of mercury and Lead in fresh fish tissues for human consumption are not regulated, but international agencies (European Union and Food and Agriculture Organization/World Health Organization) have established limits for these heavy metals, which are 1.0 $\mu\text{g}\cdot\text{g}^{-1}$ and 0.3 $\mu\text{g}\cdot\text{g}^{-1}$ wet weight for mercury and lead, respectively [15–18]. In addition, since well-documented incidents of heavy metal exposure of human communities in Japan and Iraq have resulted in severe toxic effects [19], there has been widespread public concern over the bioaccumulation of heavy metals through consumption of shark meat by humans as well as by animals. The main goal of this study was to determine the mercury and lead concentrations in different tissues of two highly migratory sharks that are consumed by humans.

Methods

During March 2011 and December of 2011 one hundred and eight sharks ($n=69$ *I. oxyrinchus* and $n=39$ *P. glauca*) were collected as *by-catch* from industrial long-line swordfish (*Xiphias gladius*) fisheries off Chile, in a geographic range between 21°- 35°S and 78°- 118°W. The total length (TL) was measured and sex determined on board. Muscles from the dorsal part and liver samples (1.0 g) were taken from both species of sharks; however, stomach tissue was obtained only from mako shark specimens. All tissue samples were stored at -80 °C until processing in the laboratory.

Laboratory analysis

All laboratory material was previously decontaminated for two days with HNO₃ (20%) [20] and washed with mili-Q water. Tissue samples were digested with 65% HNO₃ using a microwave system [21,22] and analyzed with a Shimadzu AA-6200 atomic absorption spectrophotometer (AAS). Mercury was analyzed by a hydride vapor system HV-1 (cold vapor technique), and lead was measured by acetylene flame [21,22]. The AAS was calibrated using Custom Grade standards, with detection limits of 0.007 $\mu\text{g}\cdot\text{g}^{-1}$ for mercury (Certipur Merck 1000 mg/L) and 0.0088 $\mu\text{g}\cdot\text{g}^{-1}$ for lead (PbCl₂). Further quality control included periodic blind analysis of an aliquot from a large sample of known concentration, and blind runs of duplicate samples (\pm 15%) during the analysis for each metal.

Data analyses

Based on size, studied specimens of blue shark were assigned into groups according to Lopez [2] as small (Ss): TL \leq 195 cm, and large (Ls): TL $>$ 195 cm. Similarly, individuals of Mako short fin sharks with TL \leq 285 cm were considered small, and specimens above 285 cm of TL were large. The Shapiro-Wilks test was used

to test the normality of data of concentrations of mercury and lead in blue and mako shark tissues, and a one-way ANOVA followed by a Tukey's *post hoc* test was used to compare heavy metal concentrations in different species, groups, sexes and type of tissues. All statistical analyses were performed using R software [23].

Results

Tissue/organ concentrations

Considering all tissues analyzed (muscle, liver and stomach), *P. glauca* showed 0.048 ± 0.03 (mean \pm standard deviation) $\mu\text{g}\cdot\text{g}^{-1}$ of Hg and 1.996 ± 0.67 $\mu\text{g}\cdot\text{g}^{-1}$ of lead, while *I. oxyrinchus* presented 0.034 ± 0.023 and 0.922 ± 0.44 $\mu\text{g}\cdot\text{g}^{-1}$ of mercury and lead, respectively. The statistical test did not find differences in the mercury concentration of specimens of blue and mako sharks ($F=2.08$; $p=0.1516$) (Fig 1a). However, blue sharks exhibited a significantly higher accumulation of lead than mako sharks ($F=24.7$; $p<0.001$) (Fig. 1b).

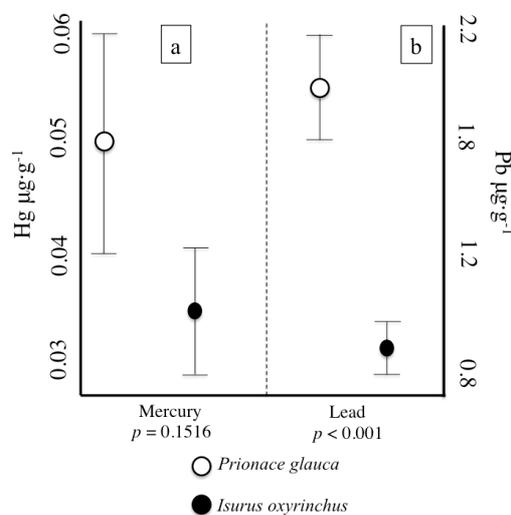


Fig. 1. Mercury and lead concentration comparison of blue sharks and mako shortfin sharks off Chile in 2011. a) mercury and b) lead concentration. *p*-value corresponding to one way ANOVA.

Analyzing metal concentrations separately, blue shark mercury concentrations in the liver (0.104 ± 0.03 $\mu\text{g}\cdot\text{g}^{-1}$) were greater than in muscle tissues (0.014 ± 0.09 $\mu\text{g}\cdot\text{g}^{-1}$), and significantly different ($F=40.6$; $p < 0.001$). In contrast, lead concentration was greater in muscle tissue (2.244 ± 0.81 $\mu\text{g}\cdot\text{g}^{-1}$) than in liver (1.602 ± 0.298 $\mu\text{g}\cdot\text{g}^{-1}$) but statistically similar ($F=2.17$; $p = 0.1491$).

In mako shark individuals, mercury concentrations were highest in the liver, followed by stomach tissue and muscle tissue with 0.108 ± 0.02 , 0.06 ± 0.01 and 0.006 ± 0.001 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. Lead concentration was greater in liver (1.67 ± 0.28 $\mu\text{g}\cdot\text{g}^{-1}$) than in muscle (0.848 ± 0.47 $\mu\text{g}\cdot\text{g}^{-1}$) and stomach tissues (0.448 ± 0.16 $\mu\text{g}\cdot\text{g}^{-1}$); the statistical analysis also revealed that differences in levels of mercury and lead were highly significant ($p < 0.001$).

Concentration by sexes and size

Male blue sharks had the highest concentration of mercury with 0.07 ± 0.03 $\mu\text{g}\cdot\text{g}^{-1}$, followed by female mako sharks with 0.04 ± 0.02 $\mu\text{g}\cdot\text{g}^{-1}$ (Appendix 2). Male blue sharks had statistically significant ($p=0.012$) greater mercury values than females. No differences were found in mercury concentration in males and females of mako shark ($p=0.2969$) (Appendix 3). In contrast to blue sharks, female mako sharks had higher mercury concentrations than males (Appendix 2). In comparison with individuals of mako shark, females and males of blue sharks had higher concentrations of lead with 2.07 ± 0.84 and 1.9 ± 0.4 $\mu\text{g}\cdot\text{g}^{-1}$, respectively (Appendix 3). Nevertheless, it should be noted that female blue and mako sharks had higher concentrations of lead than males.

In large-sized sharks (Fig. 2 and 3), the highest concentrations of mercury were found in blue sharks with $0.05 \pm 0.03 \mu\text{g}\cdot\text{g}^{-1}$, and $0.04 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$ for mako sharks. In contrast, the highest lead concentrations were encountered in small sized blue sharks and mako sharks (Appendix 2). Statistical differences were not found for mercury ($p=0.986$) or lead concentrations ($p=0.835$) between small and large blue shark specimens. A similar situation was found in mako sharks (Appendix 4).

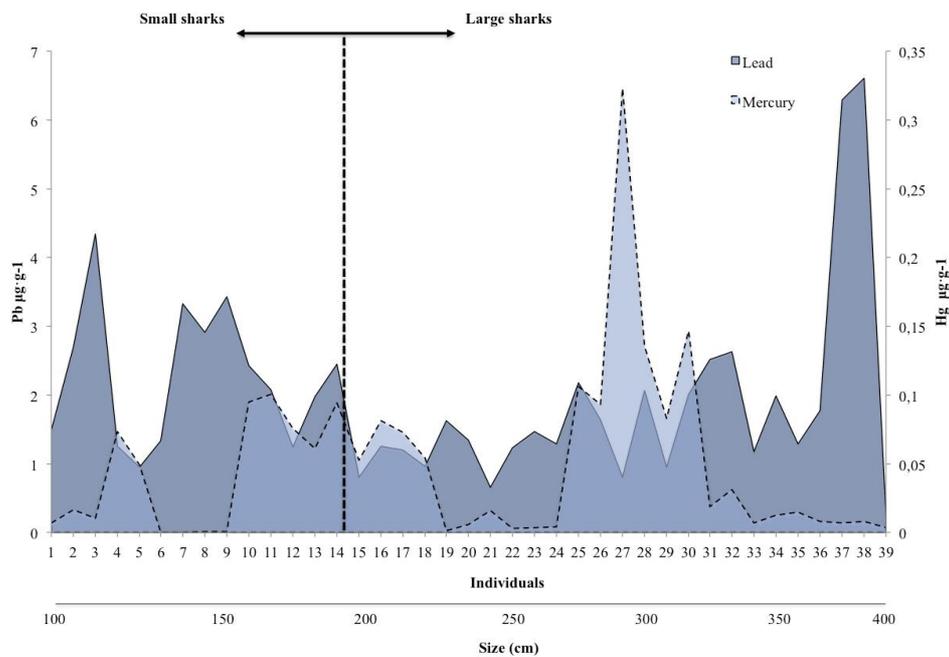


Fig. 2. Mercury and lead concentration found in different length individuals of blue shark off Chile, in 2011.

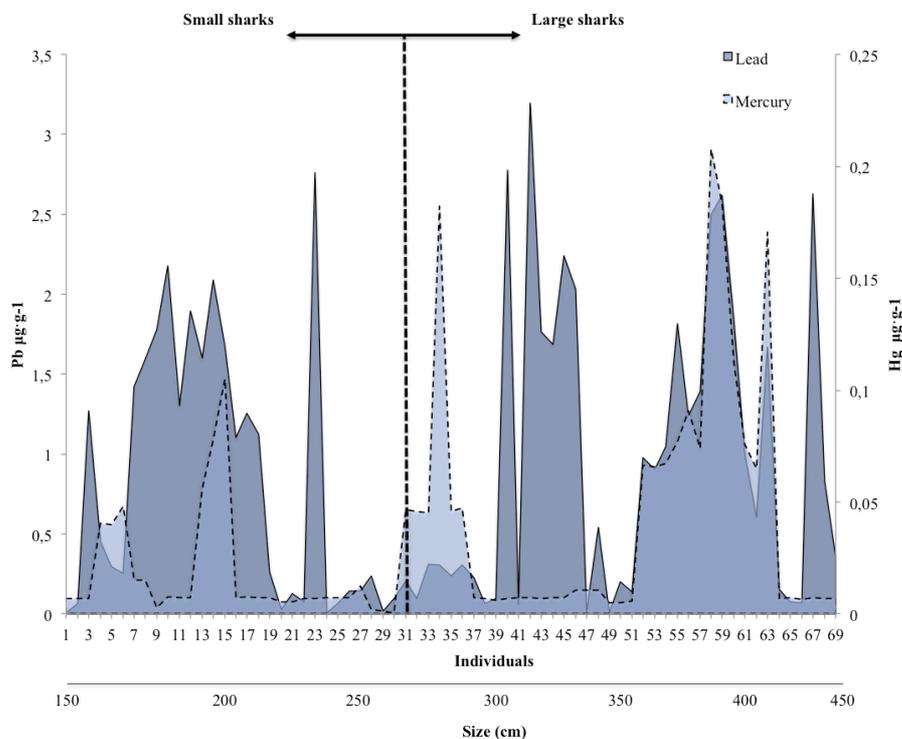


Fig. 3. Mercury and lead concentration found in different length individuals of Mako shortfin shark off Chile in 2011.

Discussion

The significant differences of mercury and lead found in tissues/organs between blue and mako sharks are due to an organotropism phenomenon in which mercury or lead are distributed differentially in shark organs [13]. In fact, organotropism in some marine species is related to the uptake (gill, stomach, intestine) or excretion rate (liver, kidney) [24], but in sharks it is well known that heavy metals are incorporated by dietary habits in a dose-dependent manner and consequently they accumulate in the internal organs (through the blood) [13], producing the organotropism phenomenon in these predators.

All tissues of blue and mako sharks showed lower concentration of mercury and were below the limit value proposed by international organizations ($1 \mu\text{g}\cdot\text{g}^{-1}$) and also with other reports [15-17]. In contrast, the mean lead concentration in both sharks was greater than the limit proposed by WHO/FAO and European commission ($0.3 \mu\text{g}\cdot\text{g}^{-1}$). A similar situation occurred in females and males of the studied sharks, with a lower mercury concentration than lead. The differences in mercury concentration found between female and male blue shark may be due to the type of food they consume, as suggested by Barrera-Garcia [12], who attributed these parameters to the differences between sexes and maturity stages, where females consume more invertebrates and males feed on fish such as mackerel.

Large size individuals of *P. glauca* had greater mercury and lead concentrations than small individuals. This is a well-documented process [12,13,20,25–34] known as bioaccumulation [25,28,35]. The bioaccumulation process can take different pathways, one of which is through the diet (biomagnification). Thus, the prey of small or large *P. glauca* and *I. oxyrinchus* individuals play an important role in size concentrations. In fact, the diet of these predators consists mainly of bony fishes in small individuals and squid in large specimens [3,4]; moreover, Maz-Courrau [28] found that different prey produced variable concentrations of heavy metals in blue sharks.

Risk for human consumption

Recently, the concentration of heavy metals in marine predators has been investigated because they form part of the diet of humans. In the last ten years sharks have been exploited in this region of the Pacific as target-species or by-catch [11]. Lamilla [11] identified the final destination of sharks caught in the fisheries as: sale of fins, local consumption and fishmeal. Thus the concentration of heavy metals reported in this study will constitute a risk for human health. Apparently, following the proposal by WHO/FAO, the mercury concentration will be not a problem for human consumption because it does not exceed the limit level, while lead concentration exceeded the limit value proposed by international organisms, which is $0.3 \mu\text{g}\cdot\text{g}^{-1}$ wet weight, thus indicating a risk for human health. However, recent studies [44-47] showed that even though the mercury concentration is low and in an acceptable range, there are synergistic effects of mercury and lead combined in tissues. Thus the mercury may be masked by another metal, which is probably cadmium because it presents a simultaneous synergism in tissues [44,45].

Another problem detected is the indirect consumption of sharks as fish meal [11]; its principal component is the internal organs such liver or stomach. This fish meal is used to make pellet food for farm fish, which are an important item in human diet. For example, Vizzini [48] compared the levels of heavy metals between wild and farmed tuna, finding no differences in both mercury and lead concentrations. It is well known that wild tuna accumulate high concentrations of these trace metals, thus for tuna from farms we would have expected lower concentrations. The unexpectedly high metal concentrations of farm fish may be due to pellet food, which is probably made with shark internal organs. Finally, possible risks to humans from lead and its synergistic effects with mercury due to fish consumption are poorly known; they probably involve cancer (mainly gastrointestinal), neurotoxicity, immunotoxicity, cardiotoxicity, reproductive toxicity, teratogenesis and genotoxicity [36].

Implications for conservation

Sharks are apex predators in all oceans; they play an important role structuring the ecosystems which they inhabit [2-4]. The ecological impacts of eliminating these top predators are already indicated in the literature [49], such as predator control and induction of subsequent cascades of indirect trophic interactions [50]. Fishing pressure has disproportionately reduced abundances of these predators mainly near the coast [51], which in turn could produce the above-mentioned effects. Despite a rich ecological and fishery literature on trophic cascades, consequences of removing oceanic apex predators remain uncertain. Moreover, exploitation of large sharks (principally *P. glauca* and *I. oxyrinchus*) has been intensified worldwide in recent decades, driven by an upsurge in demand for shark fins and meat [11] and in by-catch in many fisheries. Also, data to assess direct impacts of exploitation on these large sharks are limited, but consistently indicate that they have been driven to low levels of abundance [49,50]. In fact, when fisheries affect indirectly the mean trophic levels of the large sharks, an increase begins because they feed on high trophic level prey, which produce a major accumulation of the trace metals in their tissues, which is finally consumed by human as meat and fins or indirectly as fish meal.

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Appendix 1. Summary data of heavy metal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ w.w.) in different tissues of blue and mako shortfin shark in different oceans. Mean \pm standard deviation.

Specie	Hg	Pb	Region	References*
<i>Prionace glauca</i>	1.03 \pm 0.08	< 0.07 \pm 0.01	North East Pacific	Barrera-García et al. 2012
<i>Prionace glauca</i>	1.39 \pm 1.58	-	North East Pacific	Escobar-Sanchez et al. 2011
<i>Prionace glauca</i>	0.82 \pm 0.34	-	North Pacific	Maz-Courrau and López-Vera 2006
<i>Prionace glauca</i>	-	< 0.02	North Atlantic	Vas 1991
<i>Prionace glauca</i>	0.38	-	South Adriatic Sea	Storelli et al. 2001
<i>Prionace glauca</i>	0.22-2.5	-	Central Atlantic	Branco et al. 2007
<i>Prionace glauca</i>	0.27-1.2	-	South West Pacific	Davenport 1995
<i>Prionace glauca</i>	0.16-1.84	-	North East Atlantic	Branco et al. 2004
<i>Prionace glauca</i>	0.76	-	South East Atlantic	Dias et al. 2008
<i>Prionace glauca</i>	1.96 \pm 1.48	-	North Pacific	Maz-Courrau et al. 2012
<i>Prionace glauca</i>	-	< 0.02	North East Atlantic	Stevens and Brown 1974
<i>Isurus oxyrinchus</i>	0.4	0.29	North Pacific	Velez 2009
<i>Isurus oxyrinchus</i>	1.58	-	South West Pacific	Vlieg et al. 1993
<i>Isurus oxyrinchus</i>	> 0.45	-	South West Pacific	Menasveta and Siriyong 1977
<i>Isurus oxyrinchus</i>	1.05 \pm 0.82	-	North Pacific	Maz-Courrau et al. 2012

*Barrera-García[12], Escobar-Sanchez[25], Maz-Courrau and López-Vera[35], Vas[37], Storelli[38], Branco[20], Davenport[39], Branco[31], Dias[40], Maz-Courrau[28], Stevens and Brown[30], Velez[41], Vielg[42], Manasyeta and Siriyong[43].

Appendix 2. Mean concentration of Hg and Pb by sexes and size of *Prionace glauca* and *Isurus oxyrinchus* off Chile during 2011. BS: Blue shark (*Prionace glauca*) and MSf: Mako shortfin shark (*Isurus oxyrinchus*). Ss: Small size and Ls: Large size.

		Hg	Pb
		$\mu\text{g}\cdot\text{g}^{-1}$ w.w.	
Sexes	BS female	0.03 ± 0.01	2.08 ± 0.85
	BS male	0.08 ± 0.04	1.90 ± 0.40
	MSf female	0.04 ± 0.03	0.94 ± 0.41
	MSf male	0.02 ± 0.02	0.89 ± 0.53
Size	BS Ss	0.05 ± 0.02	2.81 ± 0.51
	BS Ls	0.05 ± 0.04	1.88 ± 0.80
	MSf Ss	0.02 ± 0.01	1.03 ± 0.42
	MSf Ls	0.04 ± 0.03	0.87 ± 0.45

Appendix 3. Differences in mercury and lead between males-females of *P. glauca* and *I. oxyrinchus* off Chile in 2011. Above the diagonal are *p-values* for Hg and below the diagonal is Pb from one-way ANOVA analysis.

	BS female	BS male	MSf female	MSf male
BS female	-	0.012	0.635	0.970
BS male	0.959	-	0.070	0.002
MSf female	< 0.001	0.013	-	0.296
MSf male	0.002	0.017	0.996	-

Appendix 4. Differences between small and large size of *P. glauca* and *I. oxyrinchus* individuals off Chile in 2011. Above the diagonal are *p-values* for Hg and below the diagonal is Pb from one-way ANOVA analysis.

	BS Ss	BS Ls	MSf Ss	MSf Ls
BS Ss	-	0.986	0.473	0.991
BS Ls	0.835	-	0.181	0.860
MSf Ss	0.008	0.035	-	0.416
MSf Ls	< 0.001	0.001	0.934	-