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Trace Metals Distribution in Tissues of 10 Different Shark Species from the Eastern Mediterranean Sea

Eleni Roubie [1], Sotirios Karavoltsos [2,*], Aikaterini Sakellari [2], Nikolaos Katsikatsos [2], Manos Dassenakis [2] and Persefoni Megalofonou [1,*]

[1] Department of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, 157 84 Athens, Greece
[2] Laboratory of Environmental Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, 157 84 Athens, Greece; esakel@chem.uoa.gr (A.S.); nikkatsi@chem.uoa.gr (N.K.); edasenak@chem.uoa.gr (M.D.)
* Correspondence: skarav@chem.uoa.gr (S.K.); pmegalo@biol.uoa.gr (P.M.)

Abstract: As long-living apex predators, sharks tend to bioaccumulate trace metals through their diet. The distribution of Al, As, Cd, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, V, Zn and Hg in different tissues (muscle, liver, heart, gills and gonads) of large-size (58–390 cm) sharks, some of which rare, of the eastern Mediterranean Sea was studied. Trace metals analyses in samples originating from ten different Chondrichthyes species were performed by inductively coupled plasma–mass spectrometry (ICP-MS) and Cold Vapor Atomic Absorption Spectrometry (CVAAS) for Hg. Data on trace metal levels are for the first time reported herewith for the species *O. ferox* and *H. nakamurai*. Higher median concentrations of trace metals were generally determined in the liver. The concentrations of Hg, Cs and As in the muscle increased proportionally with body length. Statistically significant differences between sexes were recorded for Hg, Cr, Ni and As (p = 0.015) in the muscle tissues of *P. glauca*. Muscle tissue Hg concentrations exceeded the EU maximum limit (1 μg g^{-1} wet weight) in 67% of the individuals sampled, with the highest concentrations detected in *O. ferox* and *S. zygaena*, whereas regarding Pb (limit 0.30 μg g^{-1} ww), the corresponding percentage was 15%. Arsenic concentrations were also of concern in almost all shark tissues examined.

Keywords: trace metals; mercury; sharks; tissues; eastern Mediterranean

Key Contribution: The present study is focused on trace metal analyses of five different tissues of ten Chondrichthyes species and presents a statistical study of the correlation between metal content and gender and body length.

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1. Introduction

The eastern Mediterranean Sea is an oligotrophic sea, where Aegean waters, which differ from Ionian and Levantine in nutrient and oxygen concentrations [1], constitute a great habitat for Chondrichthyes species, with only a few being endemic. The Mediterranean Sea has been subject to fishing pressures, particularly near the coastal zone, where shark populations have declined dramatically over the last two centuries [2], as a by-catch of commercial Mediterranean fisheries, representing 1–2% of total landings [3].

Overexploitation of fishing species, principally near costal zones, leads top predators such as sharks towards searching for food at higher levels, resulting in the bioconcentration of metals in their tissues [4]. As apex predators, Chondrichthyes are susceptible to bioaccumulation of contaminants such as metals. Metal toxicity may potentially affect growing embryos, having a further impact on shark populations, particularly those which are limited to coastal distributions [5]. Anatomy and life cycle features of Chondrichthyes can potentially affect the intake and retention of certain metals. Since non-essential metals

are not subject to regulatory mechanisms, their concentrations in organisms can reveal the degree of marine pollution [6].

The increase in demand for seafood, combined with a lack of cheap alternatives, has led developing countries, albeit considering shark meat as a low-quality product, to commercialize it under brands which consumers are familiar with and relate to animals. Hence, several buyers are often unaware of what they consume labelled under these brands. In addition, governments finance the use of shark meat in public school meals as seafood [7].

Due to the growing consumption of sharks and shark products, information on food safety is required, particularly in relation to toxic elements [8]. Most studies, however, focus on mercury concentrations [9,10], although studies with toxic and essential metals attract a similar interest [11,12]. Regarding Chondrichthyes, available relevant data still remain limited [6,13–16] and more species need to be studied.

The principal aim of the present study was the evaluation of Al, As, Cd, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, V, Zn and Hg concentrations in the tissues of 10 species of large mainly pelagic Chondrichthyes, of differing ecology and biology, harvested in the eastern Mediterranean Sea (Greece). Pertinent available data referring to the species *Prionace glauca, Isurus oxyrinchus*, *Sphyrna zygaena*, *Heptranchias perlo* and *Hexanchus griseus* are limited, whereas regarding the species which are relatively rare in the Mediterranean, *Mobula mobular*, *Alopias supercilliosus* and *Oxynotus centrina,* scarce data are available. For *Odontaspis ferox and Hexanchus nakamurai* no relevant data are available. The results obtained were compared to those of previous studies and to the limits imposed by European legislation.

2. Materials and Methods

2.1. Sample Collection and Pretreatment

A total of 33 individual large sharks, consisting of 10 different large sized species, were collected from the eastern Mediterranean Sea, specifically from the Aegean, Ionian and Libyan Seas, between July 2015 and August 2016 (Table 1, Figure S1 Supplemental Material). All specimens were incidentally caught by commercial trawlers or long-liners in Greece and samples were kindly offered by fishermen. Species identification was carried out by Compagno catalogue [17] and morphological parts were recorded individually.

The species identified were the oceanic *M. mobular* (n = 2), an epipelagic, endemic and endangered species of the Mediterranean Sea considered as rare [3], the pelagic and oceanic species regularly observed in Mediterranean fisheries *I. oxyrinchus* (n = 1) and *P. glauca* (n = 14) [18] and the semi-pelagic *A. superciliosus* (n = 2) distributed in temperate and tropical seas worldwide, with its presence in the Mediterranean considered as uncommon by its sparse records [19]. Also examined were *O. ferox* (n = 2), both a demersal and pelagic species occurring "infrequently but regularly" in Mediterranean fisheries and considered as globally vulnerable; *S. zygaena* (n = 1), a pelagic and semi oceanic species regularly seen in tropical and warm temperate waters, although not so commonly in Mediterranean waters due to its population's decline in biomass [2]; and *H. griseus* (n = 5), the presence of which in the Mediterranean Sea is well documented, although it is more common in the western basin [18]. The demersal or benthopelagic species identified were *O. centrina* (n = 1), an uncommon to relatively rare bottom shark for Mediterranean waters [20,21]; *H. perlo* (n = 4), a bathypelagic species of temperate and warm temperate seas [22], with its presence being well documented in both western and eastern waters and considered as rare in the Mediterranean Sea due to lack of data [19]; and *H. nakamurai* (n = 1), considered as rare in the Mediterranean Sea [19], although data for this species are insufficient due to misidentification with *H. griseus*.

For each specimen obtained, the location and date of capture, total length (LT) and gender were recorded (Table 1). In the case of *M. mobular,* length was recorded as the width of its disc (WD). Three unsexed specimens were characterized due to the lack of pelvic fins. Muscle samples were obtained from all 33 individual sharks and due to the lack of internal organs, 27 gills, 13 livers, 15 hearts and 5 gonad tissues were collected.

Table 1. Shark species collected, classified per family.

Family/Species	Sex [1]	Length (cm)	Sampling Area
Mobulidae			
Mobula mobular	F	203WD [2]	Ionian Sea
Mobula mobular	M	285WD	Ionian Sea
Oxynotidae			
Oxynotus centrina	F	58	Aegean Sea
Hexanchidae			
Heptranchias perlo	F	108	Aegean Sea
Heptranchias perlo	F	100	Aegean Sea
Heptranchias perlo	F	111	Aegean Sea
Heptranchias perlo	M	66.5	Aegean Sea
Hexanchus griseus	F	247	Aegean Sea
Hexanchus griseus	M	256	Aegean Sea
Hexanchus griseus	F	380	Aegean Sea
Hexanchus griseus	M	238	Aegean Sea
Hexanchus griseus	F	266	Aegean Sea
Hexanchus nakamurai	M	72	Aegean Sea
Alopias superciliosus	M	171	Libyan Sea
Alopias superciliosus	F	321	Ionian Sea
Odontaspidae			
Odontaspis ferox	F	377	Aegean Sea
Odontaspis ferox	F	285	Aegean Sea
Lamnidae			
Isurus oxyrinchus	M	97	Aegean Sea
Sphyrinidae			
Sphyrna zygaena	M	326	Libyan Sea
Carcharhinidae			
Prionace glauca	U	300	Aegean Sea
Prionace glauca	U	240	Aegean Sea
Prionace glauca	U	250	Aegean Sea
Prionace glauca	F	270	Aegean Sea
Prionace glauca	M	190	Ionian Sea
Prionace glauca	M	214	Ionian Sea
Prionace glauca	F	281	Ionian Sea
Prionace glauca	F	266	Ionian Sea
Prionace glauca	M	129	Ionian Sea
Prionace glauca	M	130	Aegean Sea
Prionace glauca	M	219	Aegean Sea
Prionace glauca	F	204	Aegean Sea
Prionace glauca	F	172	Aegean Sea
Prionace glauca	F	390	Gulf of Corinth

[1] F: female; M: male; U: unsexed, [2] Width disk for *Mobulidae* family.

Throughout the treatment of the samples, ceramic tools were utilized, thoroughly cleaned prior to their use and between each sample processing with ethanol 95% (Merck, Darmstadt, Germany), HNO_3 10% (Merck) and MilliQ ultra-pure water of 18.2 MΩ.cm (Millipore, Bedford, MA, USA).

Samples were weighed, placed into clean plastic vials, sealed and maintained frozen at −20 °C. Tissue samples were subsequently lyophilized in a Virtis Freeze Dryer System (The VIRTIS Company, New York, NY, USA) for 48 h, ground with a pestle and mortar and homogenized. Wet weights to dry weights were calculated by measuring the moisture loss in each tissue type. The average wet to dry weight ratio was 4.2:1 for muscle tissue, 4.6:1 for gill tissue, 4.8:1 for gonad tissue, 5.4:1 for heart tissue and 2.1:1 for liver tissue.

2.2. Trace Metals Determination

For the determination of Al, As, Cd, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, V and Zn, 0.1–0.2 g tissue samples were digested with the addition of 5 mL HNO_3 65% supra pure and 1 mL H_2O_2 30% supra pure in a microwave digestion system (Anton Paar Multiwave GO Plus, Graz, Austria) [23]. Trace metals determination of the digested samples was performed by inductively coupled plasma–mass spectrometry (ICP-MS), employing a Thermo Scientific ICAP Qc (Waltham, MA, USA) instrument. Measurements were carried out in a single collision cell mode, with kinetic energy determination (KED) using pure He. Matrix-induced signal suppressions and instrumental drift were corrected by internal standardization (^{45}Sc, ^{103}Rh).

For Hg determination, the tissue samples were digested with HNO_3 65% supra pure (Merck), according to the procedure of Meador et al. [24], slightly modified. Mercury measurements were performed by cold vapor atomic absorption spectrometry (CVAAS), employing a Varian SpectrAA 200 with VGA-77 instrument (Varian, Mulgrave, Australia).

Measurements were performed in triplicate. The limits of detection (LODs) were calculated equal to 21, 14, 11, 7.0, 2.8 ng g^{-1} w.w. for Fe, Al, Hg, Zn, As, respectively, 1.4 for Mn and Cu, 1.0 for V, 0.70 for Cr, Ni, Cd, Pb and 0.14 for Co, Cs [25].

Quality assurance of the determinations was provided by the analysis of a certified reference material (CRM) (IAEA-436; tuna fish flesh homogenized, IAEA Reference Materials), with the recoveries of the trace metals measured being in the range 100 ± 10%. Within each batch of samples analyzed, a procedural blank was included, in which the detected analytes concentrations were lower than the respective LODs, with the exception of Zn, where the concentrations were corrected.

All metal concentrations were converted to μg g^{-1} wet weight (ww) for comparison to those of other studies and to EU legislation limits. The equation used for the conversion to wet weight is Concentration μg·g^{-1} ww = Concentration μg·g^{-1} dw x ((100-Moisture loss %)/100).

2.3. Statistical Analysis

The Kolmogorov–Smirnov and Shapiro–Wilk tests were used in order to evaluate the normality of the data. Both tests provided p values lower than 0.05 and the null hypothesis (that data are normally distributed) was rejected. Therefore, the Mann–Whitney statistical comparison of values among two groups and Kruskal–Wallis test for more than two groups were used. In the Mann–Whitney and Kruskal–Wallis tests, the null hypothesis is that the medians of two or more groups, respectively, are equal. The tests were two-sided and p values lower than 0.05 were considered statistically significant. Principal component analysis (PCA) was used in order to obtain an overview of the potential relationships existing between the metals and the different tissues studied. For the statistical treatment of the data, values lower than the LOD were assigned the value of LOD divided by two (the percentage of metal concentrations under LOD was less than 10% per tissue).

3. Results and Discussion

3.1. Trace Metals Concentrations

Mean concentrations exceeding 10 μg g^{-1} ww were determined for As, Fe and Zn in muscle tissues (M), for Fe in gills (B), for Fe and Zn in hearts (H), for As and Fe in gonads (G) and for Fe, Hg, Zn and As in livers (L). Concentrations ranging between 1 and 10 μg g^{-1} ww were obtained for Hg and Al in muscle tissues, for Zn, As, Al and Hg in gills, for As, Hg, Cu and Al in hearts, for Zn in gonads and for Al and Cu in livers. For the rest of the trace metals examined in the five different tissues, concentrations lower than 1 μg g^{-1} ww were measured (Figure 1, Table S1 Supplemental Material).

Among the five different tissues examined, statistically significant differences were recorded regarding the median concentrations of Al (Kruskal–Wallis test, p = 0.003), V (0.003), Cu (0.005), As (0.010) and Mn, Fe, Co, Zn, Cd, Cs (0.001). The case is different for Hg, Cr, Ni and Pb (Kruskal–Wallis test, $p > 0.05$). Higher concentrations of most metals

were generally determined in the liver tissue, especially of *H. griseus* and *H. perlo,* for which more samples were available (Figure 1). Metals are absorbed in blood and then distributed to target organs with high metal-accumulating capacity, such as the liver. The liver is a lipid store accumulating lipophilic toxic metals, considered the main target organ of toxic and essential metals, also having a detoxification role [26,27]. The high levels of MT (metalothionein) contained in the liver indicate binding of metals, reducing their availability for participation in metabolic interactions [28,29].

Data on metal levels are for the first time reported herewith for the species *O. ferox* and *H. nakamurai,* whereas for *O. centrina* and *A. supercilliosus,* available data are scarce [30,31] (Table S1). The *M. mobular* (WD = 285 cm) sample, a species for which data are rare for the Mediterranean [32], which remains severely understudied (especially concerning metal evaluations) [33], exhibited the highest concentrations of Fe, Zn, As and Cs in the muscle tissue and of V in the gills. These values are significantly higher compared to those of other species and of the other examined specimen of *M. mobular* (WD = 203 cm). For *H. perlo* (TL = 100 cm) the highest values of Cr and Ni were exhibited in the liver. The case was similar also for Co and Cd in *H. perlo* (TL = 111 cm) and for Hg in *H. griseus* (TL = 238 cm). Regarding *P. glauca* (TL = 129 cm, 266 cm), the highest values of Cu and Pb were measured in the muscles and gills, respectively. In the gills of *O. ferox,* the highest value of Mn was found. Trace metal concentrations measured in the gills of *H. griseus* (TL = 247 cm) were lower than the limits of detection, excluding Hg, V, As, Cd and Cs. High Pb concentrations were observed only in individual samples of *P. glauca* gills and *H. griseus* muscle.

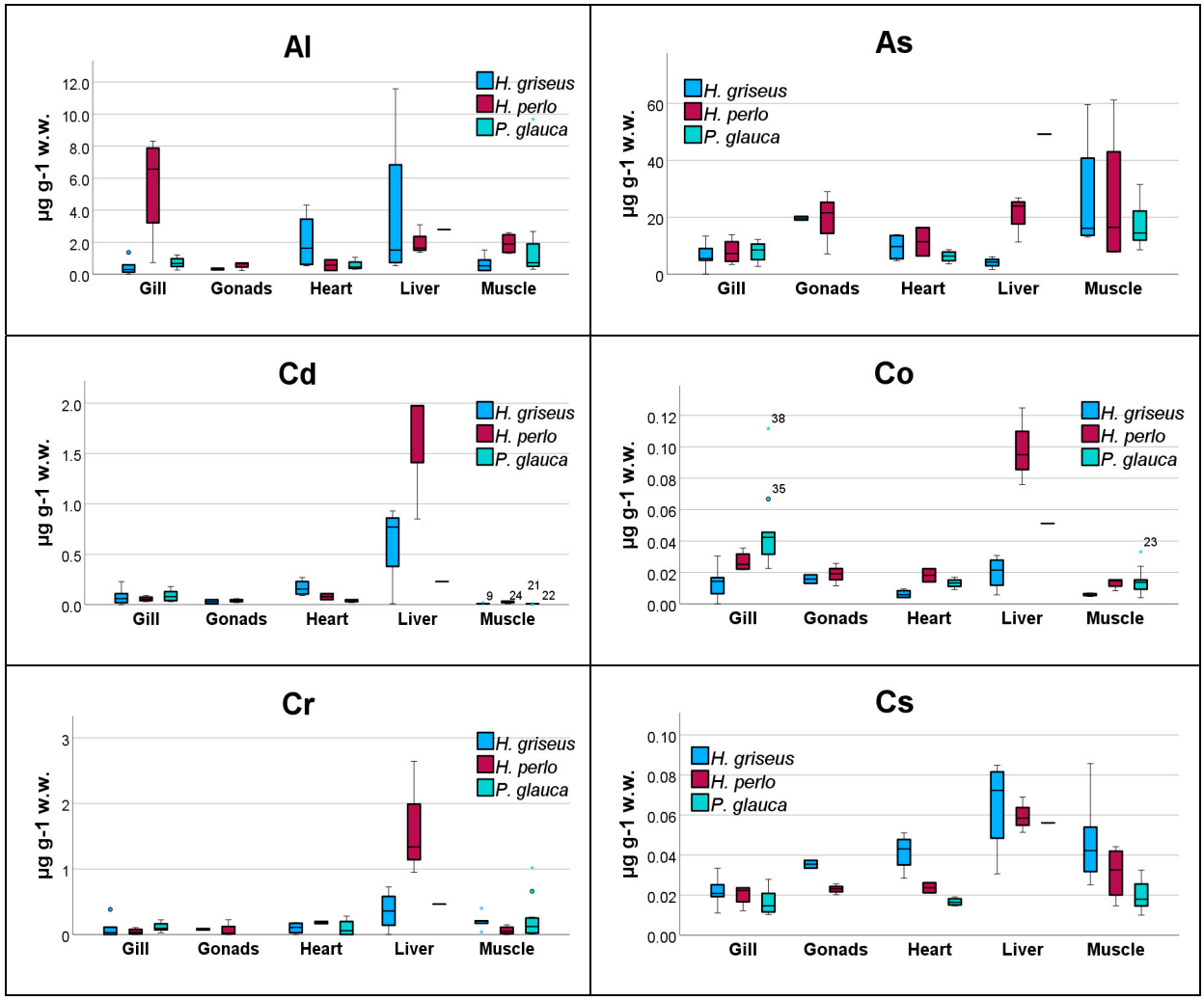

Figure 1. *Cont.*

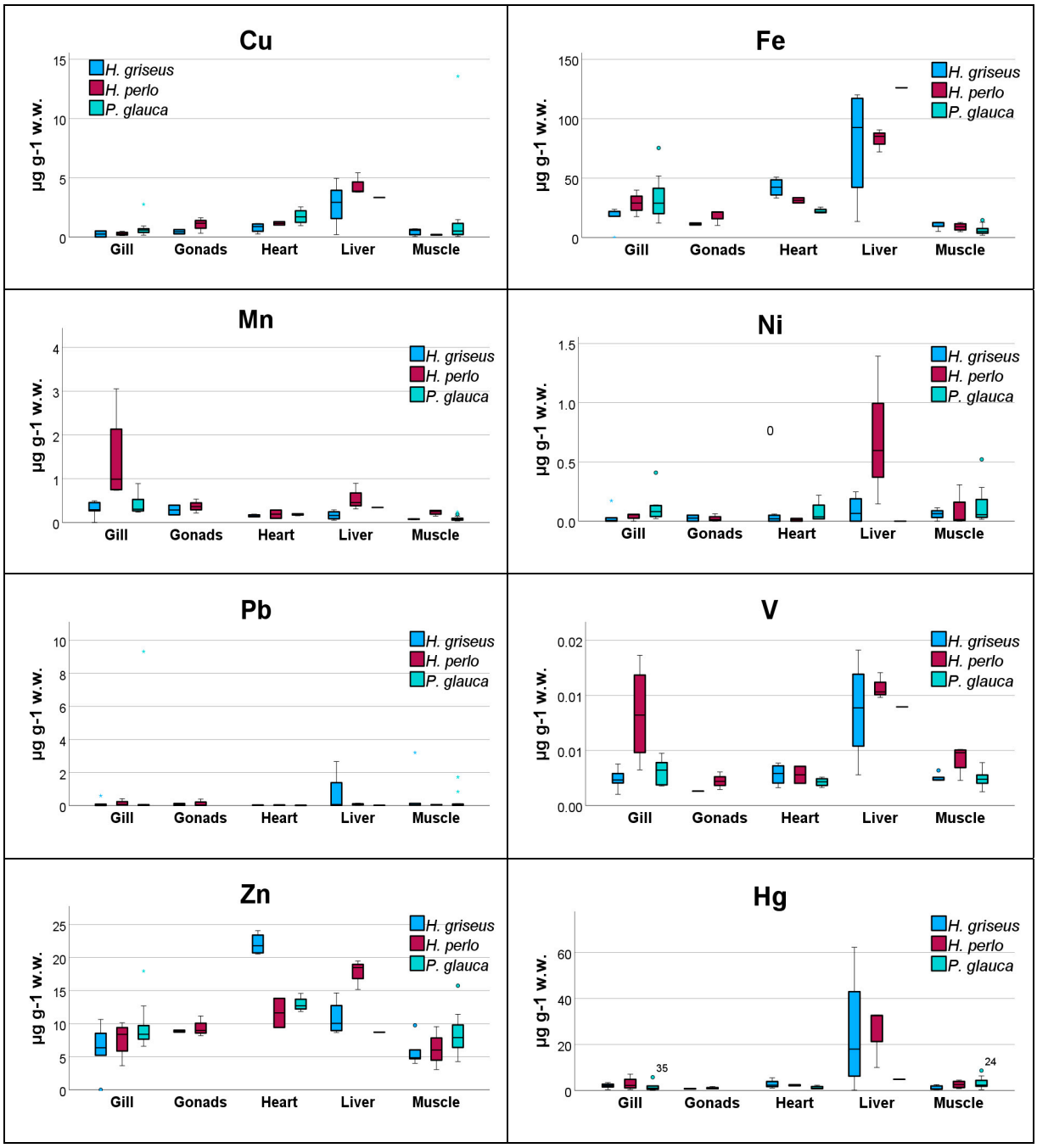

Figure 1. Box plot of metal concentrations (µg g^{-1} ww) in muscle, gill, heart, liver and gonads tissues of *P. glauca* (n = 14 for muscle, n= 10 for gills, n = 1 for liver, n = 4 for heart, n = 0 for gonads), *H. griseus* (n = 5, 5, 4, 4, 2) and *H. perlo* (n = 4, 4, 3, 2, 3).

In the principal component analysis (PCA) performed for trace metals, in each tissue individually studied, the value of the Keiser–Meyer–Olkin (KMO) statistical criterion was calculated equal to 0.835 in the muscle tissue and <0.5 for the other tissues. In the PCA for trace elements in the muscle tissue, two principal components (PCs) with eigenvalues > 1 were extracted, where PC1 explained up to 67.1% of the total variance and PC2 explained up to 9.1% (Table S2). According to the component plots obtained (Figure 2), a strong correlation of all metals in muscles was derived, excluding Hg, Pb, Cu and Cr. For the rest of the tissues investigated (liver, heart, gills), a scatter plot is presented, showcasing these appearing even higher in gonads. On the basis of the aforementioned strong correlation,

the muscle appears to be the most appropriate tissue for the evaluation of metal levels and generally of their bioconcentration in sharks. This stems from the fact that the muscle is not an active metabolic tissue, such as the gills or liver, capable of accumulating metals, excluding Hg [34].

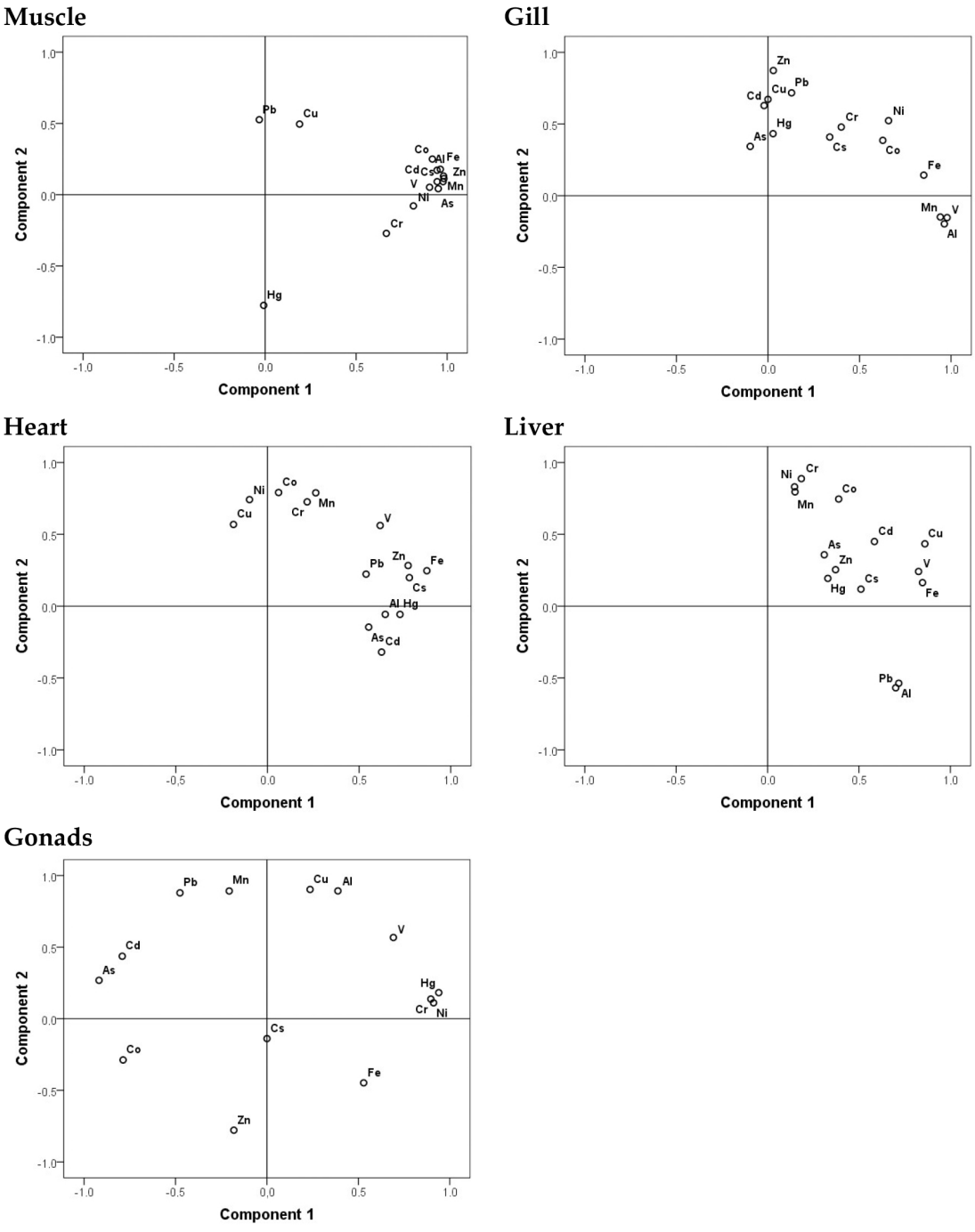

Figure 2. Principal component analysis (PCA) of trace metals in shark tissues examined.

Regarding Hg, quite high concentrations in muscles were detected in *O. ferox* (mean concentration 13 μg g^{-1} ww; n = 2) and *S. zygaena* (9.1 μg g^{-1} ww; n = 1), whereas in all other species examined, the mean concentrations were lower than 4 μg g^{-1} ww (Table S1). High Hg concentrations may reflect a diet of large carnivorous teleost fishes and other shark species [35], which are a great proportion of *O. ferox's* [36] and *S. zygaena*'s [37] diet, which may explain the differences characterizing the specific species compared to the others

studied. High Hg levels in the muscle of *S. zygaena* are linked with interspecific biological mechanisms enhancing metal retention in muscles [16]. Regarding metallothionein detoxification efficiency in liver, these metalloproteins seem to be poorly involved in Hg detoxification, since the total Hg bound to the heat-stable fraction was very low, as also previously reported for dolphins [38]. However, most of the species investigated exhibited higher Hg levels in the liver in comparison to the muscles (liver > muscle > gills > heart > gonads), with *H. griseus* having the highest value, presumably attributed either to recent exposure to Hg, accompanied by subsequent accumulation in the liver, or to strong binding of Hg to MT [39]. Mercury is highly species specific and the differences characterizing only *H. perlo* and not *H. griseus* or *P. glauca* may be attributed to prey preferences [40], local environmental conditions [41], habitat, the capability to induce metal-binding proteins such as MT [42] and physiological differences in metal digestion and metabolic capacity of methylmercury (MeHg) [43]. Hg concentrations may increase with the preference of the benthic diet to the pelagic one and at higher levels of the trophic web [44]. Low hepatic Hg may be linked to Se excess, in comparison with Hg, which is related to Hg detoxification in marine organisms [29,45]. Hauser-Davis et al. (2021) [29] calculated Se:Hg molar ratios of 12:1 in liver and 1.5:1 in the muscles of blue sharks, indicating excess Se in relation to Hg in both tissues, denoting another possible Hg detoxification pathway, in addition to MT metal binding. This is in contrast with other studies, e.g., of Alves et al. (2023), where all blue sharks sampled presented negative Se/Hg ratios in the muscles. This difference may be due to differences in sex, age and geographic distribution of the assessed blue shark specimens [46]. According to Kazama et al. (2020) [47], the detoxification of MeHg in the liver of blue shark is highly developed, explaining the lower Hg concentration.

Variations in metal concentrations can be observed among species, even of the same genus, being attributed mainly to interspecific regulation mechanisms [48]. Among the different shark species studied, statistically significant differences in metal concentrations in muscle tissues were recorded for Hg, Cd, Co and Cs (Kruskal–Wallis test, $p = 0.014$, 0.025, 0.028, 0.039, respectively). The differentiation characterizing Co concentrations among species may be attributed either to ineffective metabolic regulation of basic metals, the concentrations of which are affected by environmental factors, or to different physiological requirements of the species despite the effective regulation [49].

The trace metals V, Fe, Cd and Cs exhibited liver differentiation in *P. glauca*, *H. perlo* and *H. griseus* (Kruskal–Wallis test, $p < 0.05$). Food ingestion does not represent a V source and in accordance to relatively low V concentrations detected, it does not appear to have any prominent ecological or biological role [49].

Iron is essential to living organisms as a constituent of many enzymes, electron transfer complexes and oxygen carriers. Higher Fe values were detected in the liver and gills, partially attributed to blood irrigation. The blood of Chondrichthyes, compared to other marine organisms, has hemoglobin, an Fe-containing molecule. Gills receive a great amount of blood for oxygen transportation. The liver has an important role in blood purification processes. In addition, exposure to Fe can be enforced by nutrient absorption from dietary paths via bioaugmentation, engendering high Fe levels in local sharks [50].

Cephalopods are considered as the main factor of Cd transfer to apex predators. High bioavailability of Cd in their digestive gland cells suggests a high prospect of trophic transport of the metal to their predators [51]. Increases in Cd concentrations induce the synthesis of metallothioneins in the liver, with Cd successively binding to MT along with Zn and Cu [52].

Cs is ingested from fishes through the water phase and is transported to upper levels of the food web [53]. Indications of its biomagnification in the marine food web have been reported [54], albeit no biological function is known for Cs so far. However, it has been reported to act as proportional to plasma K^+ [54].

Manganese is an essential element participating in many metabolic activities. Absorption from the water column constitutes an insufficient explanation of its presence in internal tissues [55]; nevertheless, diet contribution, together with Mn absorption from

the environment, may explain the increased values detected [56]. Copper provokes a protective response by inducing MT synthesis in the liver [57]. It can be toxic under specific circumstances, causing swimming alterations and even mortality [50].

The concentrations measured for Ni, as well as Cr, despite their essential role, were relatively low. Chromium differentiations occurred only among *H. perlo's* tissues, whereas hepatic Ni variations were found also in *H. perlo*. Mehrim [58] suggests that Cr exerts a positive effect on fish health, since it inhibits pests growth. Elevated Ni concentrations in ion-regulating organs such as gills do not jeopardize their function but indicate Ni absorption by Chondrichthyes. Ni is responsible for distractions in osmoregulation, with increases in plasma K^+ and urea [57]. Concentrations of As may vary among species due to endogenous factors or target-specific diets, with some species accumulating As more than others [15]. Regarding Pb, its levels in Chondrichthyans tissues remain quite low. Gills are the organs usually affected by Pb [50].

3.2. Correlation with Gender

Statistical analyses between gender (F, females; M, males) and trace metal concentrations were conducted using only *P. glauca* data due to the insufficient sampling size of the rest of the species examined. Statistically significant differences of median values between genders (F: n = 6; M: n = 5) and metal concentrations were found for Hg, Cr, Ni and As (Mann–Whitney, $p = 0.015$) for muscle tissues. A comparison of median concentrations indicated higher trace metal values in female specimens. The differentiation of metal concentrations in Chondrichthyes due to gender can be attributed to diet preference, season change in diet or level of maturity, especially in cases of mature pregnant females [59]. Some species can transfer metals during spawning or deliver them directly to the fetus [60]. In accordance with the findings of the present work, significantly higher concentrations of Hg and As were detected in females than males. Females have a higher degree of growth, being larger than males (sometimes up to 40%), exhibiting racial dimorphism that may reflect a higher degree of pollutants bioaccumulation [12].

3.3. Correlation with Size

The regression analysis for all sizes of species combined exhibited a positive correlation in the muscles between total length (TL) and Hg concentrations (R = 0.464, $p = 0.007$, n = 33) and in the gills between TL and Fe (R = 0.436, $p = 0.023$, n = 27). For a further explanation of the fitted model, regression analysis was conducted individually in *P. glauca,* with the majority of samples in the muscles (n = 14) and gills (n = 9). Regression analysis exhibited a moderately strong relationship between total length and concentrations of Hg (R = 0.816, $p < 0.001$), As (R = 0.718, $p = 0.004$) and Cs (R = 0.551, $p = 0.041$) in the muscles and of As in gills (R = 0.837, $p = 0.003$) (Figure S2). Riesgo et al. (2023) [61] also reported a positive correlation of Hg with size in muscle samples of *P. glauca*. A positive correlation between muscle tissue and size reveals metal bioaccumulation [62], while a negative one presents a small representation of all length values [63]. Additionally, a negative correlation in muscle tissue is linked with different absorption and more efficient secretion rates [64]. A lack of essential metals and size correlation suggests that requirements of essential elements do not vary with size, consequently nor with age [49].

3.4. Comparison with Literature

The metal concentrations detected in the present work regarding *H. perlo*, *H. griseus*, *P. glauca*, *M. mobular*, *I. oxyrinchus* and *S. zygaena*, for which several samples were examined, are generally within the range of values reported for the same species worldwide (Table 2). However, the Hg levels in sharks from the eastern Mediterranean appear to be higher than those detected in the same species from other areas, such as the Atlantic Ocean [29,65], the Pacific Ocean [66,67] and western Italian coasts [68]. Correspondingly, the As levels were higher in sharks from the Mediterranean than in those from other regions [11,35,69] but differed even among sharks originating from different Mediterranean regions [14,15,70].

Table 2. Comparison of trace elements concentrations (μg g^{-1} ww) measured in different tissues of *H. griseus*, *H. perlo* and *P. glauca*, with available literature.

Area	Tissue	Species	Al	As	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn	Source
Eastern Mediterranean	muscle				0.04				0.58	4.10	0.41–4.55	0.18				4.28	[68]
	liver				1.06				2.58	79.3	0.42–13.3	1.21				15.0	
Pacific coast of Canada	muscle			2.2–37.8													[71]
Mediterranean (north Aegean)	muscle			10.85 ± 3.19	0.007 ± 0.004	0.006 ± 0.003			0.357 ± 0.077		1.98 ± 0.59	0.231 ± 0.052	0.040 ± 0.017	0.192 ± 0.082	0.029 ± 0.010	5.92 ± 0.98	[72]
Mediterranean (Aegean)	muscle	*H. griseus*	0.68 ± 0.54	29 ± 21	0.011 ± 0.003	0.006 ± 0.001	0.21 ± 0.13	0.048 ± 0.024	0.43 ± 0.29	11 ± 3	1.2 ± 1.0	0.08 ± 0.01	0.06 ± 0.04	0.69 ± 1.4	0.003 ± 0.001	5.9 ± 2.3	Present study
	gills		0.52 ± 0.61	7.0 ± 5.6	0.047 ± 0.048	0.009 ± 0.007	0.04 ± 0.05	0.021 ± 0.009	0.20 ± 0.25	15 ± 10	1.7 ± 1.0	0.31 ± 0.22	0.009 ± 0.013	0.17 ± 0.28	0.002 ± 0.001	5.0 ± 3.6	
	heart		2.0 ± 1.8	9.5 ± 4.7	0.17 ± 0.08	0.006 ± 0.003	0.10 ± 0.09	0.041 ± 0.010	0.79 ± 0.40	42 ± 8	2.8 ± 1.9	0.15 ± 0.03	0.03 ± 0.03	0.03 ± 0.01	0.003 ± 0.001	22 ± 2	
	liver		3.8 ± 5.2	4.1 ± 1.8	0.62 ± 0.41	0.020 ± 0.011	0.36 ± 0.30	0.065 ± 0.024	2.8 ± 2.0	80 ± 49	25 ± 27	0.16 ± 0.10	0.10 ± 0.12	0.70 ± 1.3	0.009 ± 0.005	11 ± 3	
	gonads		0.33 ± 0.07	20 ± 1	0.031 ± 0.026	0.016 ± 0.004	0.08 ± 0.02	0.035 ± 0.003	0.45 ± 0.25	11 ± 1	0.80 ± 0.10	0.28 ± 0.15	0.03 ± 0.04	0.07 ± 0.08	0.001 ± 0.001	8.9 ± 0.2	
Ionian Sea	muscle			10.88 ± 2.52													[15]
Ionian Sea	liver			6.22 ± 1.73													
Ionian Sea	muscle										1.27 ± 1.70						[13]
SE Australia	muscle										1.33 ± 0.38						[73]
East China Sea	muscle	*H. perlo*		44	5.52	0.06	1.2	0.03	6.16		0.30	2.85		0.152	0.057	32.6	[69]
Mediterranean (Aegean)	muscle		1.9 ± 0.6	26 ± 25	0.029 ± 0.009	0.013 ± 0.003	0.06 ± 0.06	0.031 ± 0.013	0.12 ± 0.03	9.0 ± 3.3	2.6 ± 1.6	0.23 ± 0.06	0.08 ± 0.15	0.05 ± 0.02	0.004 ± 0.001	6.2 ± 2.6	Present study
	gills		5.5 ± 3.4	8.0 ± 4.6	0.060 ± 0.023	0.03 ± 0.01	0.04 ± 0.05	0.020 ± 0.005	0.28 ± 0.15	29 ± 9	3.0 ± 2.9	1.4 ± 1.1	0.04 ± 0.03	0.14 ± 0.19	0.008 ± 0.004	7.6 ± 2.8	
	heart		0.57 ± 0.47	11 ± 7	0.078 ± 0.045	0.02 ± 0.01	0.18 ± 0.02	0.024 ± 0.004	1.2 ± 0.2	31 ± 3	2.3 ± 0.3	0.19 ± 0.13	0.01 ± 0.02	0.03 ± 0.01	0.003 ± 0.001	12 ± 3	
	liver		2.0 ± 0.9	21 ± 8	1.6 ± 0.6	0.10 ± 0.02	1.6 ± 0.9	0.060 ± 0.009	4.4 ± 0.9	83 ± 10	25 ± 13	0.55 ± 0.30	0.71 ± 0.63	0.08 ± 0.07	0.011 ± 0.001	18 ± 2	
	gonads		0.54 ± 0.28	19 ± 11	0.04 ± 0.02	0.02 ± 0.01	0.08 ± 0.12	0.023 ± 0.003	1.0 ± 0.7	18 ± 6	1.1 ± 0.5	0.37 ± 0.16	0.02 ± 0.03	0.14 ± 0.22	0.002 ± 0.001	9.4 ± 1.5	

Table 2. *Cont.*

Area	Tissue	Species	Al	As	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn	Source
Atlantic (Santa Catarina, Brasil)	muscle	*P. glauca*									0.40 ± 0.29						[74]
English channel (between Atlantic and North Sea)	muscle				0.45				0.24	6.34		1.55	2.58	<0.02			[75]
	liver				0.25				0.65	4.02		0.65	3.23	1.14			
	gills				0.99				0.55	21.71		0.55	1.91	0.36			
S. Atlantic	muscle		1.70				0.14		0.98							5.38	[7]
Ionian Sea	muscle			7.20 ± 3.05													[15]
	liver			5.95 ± 2.67													
Baja California	muscle			6.66 ± 0.55	0.2 ± 0.12				1.64 ± 0.13	27.4 ± 3.57	1.03 ± 0.08			n.d.		6.10 ± 0.37	[35]
Baja California	liver			10.62 ± 4.76	34.7 ± 29.6				9.28 ± 8.39	196 ± 96	0.22 ± 0.35			0.37 ± 0.37		49.9 ± 27.1	[26]
Pacific	muscle										0.55–7.0						[76]
South Adriatic	muscle										0.38						[6]
Atlantic, Azores	muscle										0.22–1.3						[44]
	liver										0.032–0.96						
Atlantic, Equator	muscle										0.68–2.5						
	liver										0.15–2.2						
Pacific, Mexico	muscle										0.27						[77]
Pacific, Mexico	muscle										1.39 ± 1.58						[78]
SE Pacific	muscle										0.014 ± 0.1			2.24 ± 0.81			[79]
	liver										0.10 ± 0.03			1.60 ± 0.3			
SW Indian	muscle																[9]
SE Australia	muscle										0.41						[75]
Mid Atlantic, Azores	muscle			10.02 ± 0.69			0.50 ± 0.03		0.70 ± 0.21		0.33 ± 0.02					3.95 ± 0.15	[12]
Pacific, California	muscle										1.96 ± 1.48						[80]
NE Atlantic	muscle										0.60–4.04						[81]

Table 2. *Cont.*

Area	Tissue	Species	Al	As	Cd	Co	Cr	Cs	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn	Source
NE Atlantic	muscle	*P. glauca*			<0.05				4.4					<0.2		35	[82]
	liver				<0.05				5.7					<0.2		35	
	gonads				<0.05				5.6					<0.2		88	
Basque, Spain	muscle								0.142			0.033				1.952	[70]
Basque, Spain	muscle			0.144	0.003						0.350			0.004			[83]
Brazil	muscle										0.46–2.40						[84]
Atlantic	muscle		23.8 ± 47.0	78.2 ± 22.0	0.006 ± 0.028		2.58 ± 3.27		1.15 ± 0.55	28.2 ± 26.2	1.36 ± 0.83	0.633 ± 0.579	0.341 ± 0.573	0.125 ± 0.109		24.6 ± 15.5	[11]
	liver		24.4 ± 40.1	40.0 ± 27.8	4.52 ± 3.60		1.61 ± 0.12		6.81 ± 3.89	99.8 ± 55.8	0.28 ± 0.35	2.46 ± 1.14	0.041 ± 0.152	1.30 ± 4.35		44.0 ± 39.6	
NE Atlantic, Azores	muscle										0.14–1.71						[85]
Pacific, Peru	muscle				0.009 ± 0.002									0.009 ± 0.007			[86]
Western North Atlantic Ocean	muscle		1.49 ± 0.60	60.40 ± 34.04	0.19 ± 0.15	0.005 ± 0.002	0.49 ± 0.27	0.077 ± 0.047	0.60 ± 0.74	3.86 ± 2.69	1.27 ± 0.53	0.057 ± 0.03	0.11 ± 0.13	0.052 ± 0.027	0.03 ± 0.017	7.84 ± 4.73	[29]
	liver		0.24 ± 0.15	23.46 ± 13.14	1.83 ± 1.34	0.025 ± 0.020	0.152 ± 0.125	0.007 ± 0.002	1.05 ± 1.08	38.6 ± 33.6	0.27 ± 0.22	0.46 ± 0.25	<0.0007	0.035 ± 0.005	0.020 ± 0.022	6.54 ± 3.85	
Mexican Pacific Coast	muscle				0.25 ± 0.20						0.44 ± 0.35						[67]
	liver				1.50 ± 0.72						0.02 ± 0.02						
Colima Coast, Mexico	muscle			114.9 ± 83.26	0.76 ± 0.3	0.26 ± 0.07	2.1 ± 4.78		22.7 ± 15.95	445.3 ± 673	0.36 ± 0.1	7.35 ± 11.31	2.14 ± 2.91	2.89 ± 2.8	1.08 ± 0.78	169.2 ± 82	[66]
Mediterranean (Ionian)	muscle		0.65 ± 0.47	14 ± 5	0.006 ± 0.001	0.013 ± 0.012	0.051 ± 0.040	0.014 ± 0.003	2.9 ± 6.0	3.4 ± 1.2	3.4 ± 2.5	0.07 ± 0.07	0.04 ± 0.03	0.046 ± 0.047	0.002 ± 0.001	8.2 ± 4.6	Present study
	gills		0.65 ± 0.24	8.6 ± 3.6	0.070 ± 0.062	0.041 ± 0.016	0.090 ± 0.077	0.015 ± 0.007	1.0 ± 1.0	26 ± 13	2.0 ± 2.2	0.46 ± 0.26	0.12 ± 0.16	1.90 ± 4.15	0.003 ± 0.001	9.7 ± 4.7	
Mediterranean (Aegean)	muscle		2.3 ± 3.1	17 ± 6	0.008 ± 0.003	0.015 ± 0.004	0.31 ± 0.35	0.022 ± 0.007	0.85 ± 0.52	8.3 ± 3.9	2.4 ± 1.2	0.11 ± 0.06	0.17 ± 0.17	0.35 ± 0.62	0.003 ± 0.001	7.7 ± 1.7	
	gills		0.78 ± 0.35	7.3 ± 2.7	0.107 ± 0.040	0.055 ± 0.032	0.13 ± 0.04	0.018 ± 0.005	0.51 ± 0.29	43 ± 19	1.0 ± 0.8	0.36 ± 0.19	0.098 ± 0.040	0.024 ± 0.019	0.004 ± 0.001	9.4 ± 2.0	
	heart		0.57 ± 0.33	6.3 ± 2.1	0.040 ± 0.013	0.013 ± 0.003	0.099 ± 0.132	0.017 ± 0.002	1.7 ± 0.7	22 ± 2	1.3 ± 0.7	0.18 ± 0.02	0.078 ± 0.095	0.015 ± 0.016	0.002 ± 0.001	13 ± 1	

Overexploitation has affected the life strategies of Chondrichthyes, which in order to adjust are forced to enter the reproduction age earlier, facing the consequence of a maximum size reduction. According to Ferretti et al. [2], a gradual decrease in size of Mediterranean Chondrichthyes over time is observed, with the average size of Mediterranean sharks being the smallest compared to that recorded in other regions worldwide [19]. Consequently, an individual species from the Mediterranean is expected to be older and characterized by a higher metal content compared to identical species of the same size from other geographical regions.

3.5. Legislation Limits

According to the European legislation's [87] maximum levels of contaminants in foodstuffs, 67% of the raw muscle shark tissue samples examined had concentrations exceeding the maximum limit for Hg (1 μg g^{-1} ww) (Figure 3) and 15% for Pb (limit 0.30 μg g^{-1} ww), whereas were 0% for Cd (limit 0.050 μg g^{-1} ww). Although As limits are not included in the European legislation, the As concentrations determined in all shark tissues were considerable in comparison to the rest of the trace metals examined. Nevertheless, even in cases where legislative limits are not exceeded, shark meat consumption should be still considered with caution.

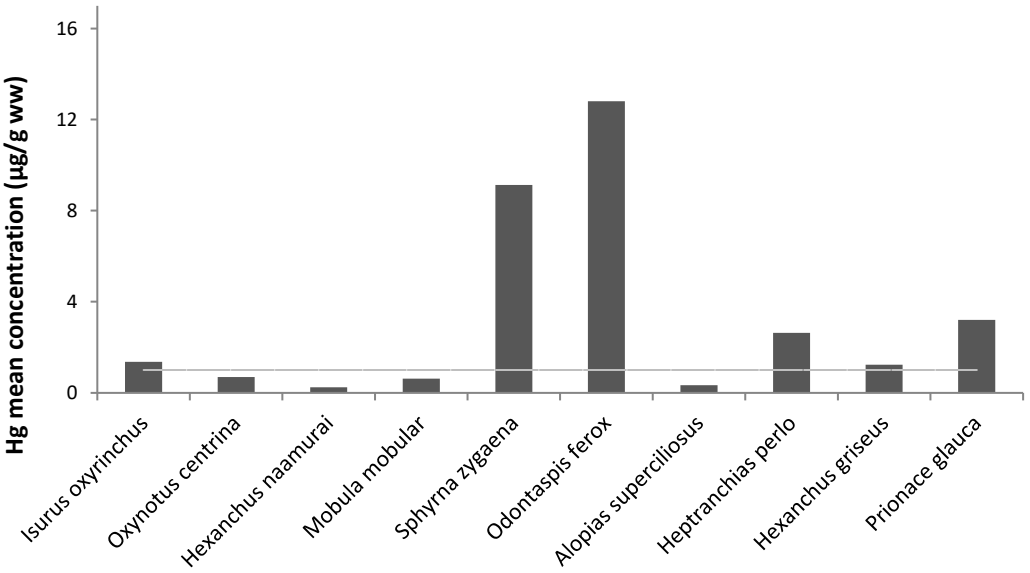

Figure 3. Mean Hg concentrations (μg g^{-1} ww) in muscle tissue of the species examined. The dotted line indicates the European legislation maximum limit set at 1.0 μg g^{-1} ww.

The combined exposure of Hg with metals such as Al, Cu, Pb, Cd and Mn may act synergistically with other factors [8], causing such dysfunctions in metabolic activities and biological processes that a single metal, even in high doses, could not engender [88]. In order to quantify the limits of consumption, the hypothesis was made that the dose of consumption is equal to contaminant absorption and that it is not affected by thermal treatment [89]. However, Matos et al. [90] demonstrated differences in metal concentrations following culinary treatments, with total Hg concentrations in the muscle tissue of *P. glauca* following the decreasing order of grilled (3.57 ± 0.96) > steamed (3.12 ± 1.37) > raw (2.25 ± 0.71). Kalogeropoulos et al. [91] found that the concentrations of metals in heat-treated fish and shellfish followed a similar sequence of fried > baked > raw.

4. Conclusions

The results of the present study contribute to the metal concentration database of several Chondrichthyes species, for which information is limited due to their either scarce or uncommon occurrence.

A wide variation in metal concentrations among tissues, species and genders was highlighted, together with positive correlations between Hg concentrations and total length in muscle tissues. Regarding *P. glauca*, correlations among Hg, As and Cs in the muscles suggest a possible bioaccumulation of metals with age. The food chain is the main route of uptake for most metals, which are either bioaccumulated or biomagnified, whereas their soluble phase may affect the metal content of their tissues. Mediterranean Chondrichthyes are exposed to higher Hg levels, compared to those originating from other marine areas, due to natural geochemical processes and the occurrence of anthropogenic sources. Indicatively, in >50% of the individual samples examined, the Hg concentrations measured in the present work exceeded the European legislation maximum limit.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9020077/s1, Figure S1: Sampling sites of sharks studied. Table S1: Mean concentrations (μg g^{-1} ww) of trace elements in different tissues of *I. oxyrinchus*, *O. centrina*, *M. mobular*, *S. zygaena*, *O. ferox*, *A. superciliosus* and *H. nakamurai*. The number of specimens is in parenthesis. Figure S2: Positive correlation between total length of *P. glauca* and metal concentrations. A: Hg in muscle tissue; B: Cs in muscle tissue; C: As in muscle and gill tissue. Table S2: Statistical parameters of principal component analysis (PCA) of trace metals in shark tissues examined.

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References

1. Souvermezoglou, E.; Krasakopoulou, E.; Pavlidou, A. Temporal variability in oxygen and nutrient concentrations in the southern Aegean Sea and the Straits of the Cretan Arc. *Prog. Oceanogr.* **1999**, *44*, 573–600. [CrossRef]
2. Ferretti, F.; Myers, R.A.; Serena, F.; Lotze, H.K. Loss of large predatory sharks from the Mediterranean Sea. *Conserv. Biol.* **2008**, *22*, 952–964. [CrossRef]
3. Bradai, M.N.; Said, B.; Enajjar, S. *Elasmobranchs of the Mediterranean and Black Sea: Status, Ecology and Biology: A Bibiliographic Analysis*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2012.
4. Bendall, V.A.; Barber, J.L.; Papachlimitzou, A.; Bolam, T.; Warford, L.; Hetherington, S.J.; Silva, J.F.; McCully, S.R.; Losada, S.; Maes, T.; et al. Organohalogen contaminants and trace metals in North-East Atlantic porbeagle shark (*Lamnanasus*). *Mar. Pollut. Bull.* **2014**, *85*, 280–286. [CrossRef]
5. Moore, A.B.M.; Bolam, T.; Lyons, B.P.; Ellis, J.R. Concentrations of trace elements in a rare and threatened coastal shark from the Arabian Gulf (smoothtooth blacktip *Carcharhinus leiodon*). *Mar. Pollut. Bull.* **2015**, *100*, 646–650. [CrossRef]
6. Storelli, M.M.; Giacominelli Stuffler, R.; Marcotrigiano, G.O. Total mercury and methylmercury in tuna fish and sharks from the south Adriatic Sea. *Ital. J. Food Sci.* **2001**, *13*, 101–106.
7. Vignatti, G.; Schneider, V.E.; Poletto, M. Biological assessment and metals concentration in blue shark (*Prionace glauca*) caught in the southeast-south coast of Brazil. *Sci. Cum Ind.* **2018**, *6*, 7–11. [CrossRef]
8. Kim, S.W.; Han, S.J.; Kim, Y.; Jun, J.W.; Giri, S.S.; Chi, C.; Yun, S.; Kim, H.J.; Kim, S.G.; Kang, J.W.; et al. Heavy metal accumulation in and food safety of shark meat from Jeju island, Republic of Korea. *PLoS ONE* **2019**, *14*, e0212410. [CrossRef]
9. Kiszka, J.J.; Aubail, A.; Hussey, N.E.; Heithaus, M.R.; Caurant, F.; Bustamante, P. Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean revealed by stable isotope and mercury analyses. *Deep Sea Res* **2015**, *96*, 49–58. [CrossRef]

10. McKinney, M.A.; Dean, K.; Hussey, N.E.; Cliff, G.; Wintner, S.P.; Dudley, S.F.; Zungu, M.P.; Fisk, A.T. Global versus local causes and health implications of high mercury concentrations in sharks from the east coast of South Africa. *Sci. Total Environ.* **2016**, *541*, 176–183. [CrossRef]
11. Alves, L.M.; Nunes, M.; Marchand, P.; Le Bizec, B.; Mendes, S.; Correia, J.P.; Lemos, M.F.L.; Novais, S.C. Blue sharks (*Prionace glauca*) as bioindicators of pollution and health in the Atlantic Ocean: Contamination levels and biochemical stress responses. *Sci. Total Environ.* **2016**, *563*, 282–292. [CrossRef]
12. Torres, P.; da Cunha, R.T.; dos Santos Rodrigues, A. Mid-Atlantic elasmobranchs: Suitable metal scouts? *Mar. Pollut. Bull.* **2017**, *117*, 203–213. [CrossRef]
13. Storelli, M.M.; Giacominelli-Stuffler, R.; Marcotrigiano, G.O. Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. *B Environ. Contam. Tox.* **2002**, *68*, 201–210. [CrossRef]
14. Storelli, M.M.; Ceci, E.; Storelli, A.; Marcotrigiano, G.O. Polychlorinated biphenyl, heavy metal and methylmercury residues in hammerhead sharks: Contaminant status and assessment. *Mar. Pollut. Bull.* **2003**, *46*, 1035–1048. [CrossRef]
15. Storelli, M.M.; Marcotrigiano, G.O. Interspecific variation in total arsenic body concentrations in elasmobranch fish from the Mediterranean Sea. *Mar. Pollut. Bull.* **2004**, *48*, 1145–1167. [CrossRef]
16. Storelli, M.M.; Cuttone, G.; Marcotrigiano, G.O. Distribution of trace elements in the tissues of smooth hound *Mustelus mustelus* (Linnaeus, 1758) from the southern–eastern waters of Mediterranean Sea (Italy). *Environ. Monit. Assess.* **2011**, *174*, 271–281. [CrossRef]
17. Compagno, L.J.V. *Sharks of the World: An Annotated and Illustrated Catalogue of the Shark Species Known to Date*; Bullhead, mackerel and carpet sharks (*Heterodontiformes, Lamniformes* and *Orectolobiformes*); FAO Species Catalogue for Fisheries Purposes No. 1; Food and Agriculture Organization of the United Nations: Rome, Italy, 2001; Volume 2.
18. Sperone, E.; Parise, G.; Leone, A.; Milazzo, C.; Circosta, V.; Santoro, G.; Paolillo, G.; Micarelli, P.; Tripepi, S. Spatiotemporal patterns of distribution of large predatory sharks in Calabria (central Mediterranean, southern Italy). *Acta Adriat.* **2012**, *53*, 13–23.
19. Megalofonou, P.; Damalas, D.; Yannopoulos, C. Composition and abundance of pelagic shark by-catch in the eastern Mediterranean Sea. *Cybium* **2005**, *29*, 135–140.
20. Megalofonou, P.; Damalas, D. Morphological and biological characteristics of a gravid angular rough shark (*Oxynotus centrina*) and its embryos from the eastern Mediterranean Sea. *Cybium* **2004**, *28*, 105–110.
21. Kousteni, V.; Megalofonou, P. Observations on the biological traits of the rare shark *Oxynotus centrina* (Chondrichthyes: *Oxynotidae*) in the Hellenic Seas. *J. Fish Biol.* **2017**, *89*, 1880–1888. [CrossRef]
22. Başusta, N. New records of neonate and juvenile sharks (*Heptranchias perlo, Squatina aculeata, Etmopterus spinax*) from the North-eastern Mediterranean Sea. *Mar. Biodivers.* **2016**, *46*, 525–527. [CrossRef]
23. de Macêdo, G.R.; Tarantino, T.B.; Barbosa, I.S.; Pires, T.T.; Rostan, G.; Goldberg, D.W.; Pinto, L.F.B.; Korn, M.G.A.; Franke, C.R. Trace elements distribution in hawksbill turtle (*Eretmochelys imbricata*) and green turtle (*Chelonia mydas*) tissues on the northern coast of Bahia, Brazil. *Mar. Pollut. Bull.* **2015**, *94*, 284–289. [CrossRef]
24. Meador, J.P.; Ernest, D.W.; Kagley, A.N. A comparison of the non-essential elements cadmium, mercury and lead found in fish and sediment from Alaska and California. *Sci. Total Environ.* **2005**, *339*, 189–205. [CrossRef]
25. USEPA, US Environmental Protection Agency. *Guidelines Establishing Test Procedures for the Analysis of Pollutants (App. B, Part 136, Definition and Procedures for the Determination of the Method Detection Limit): US Code of Federal Regulations*; Title 40; National Archives; USEPA: Washington, DC, USA, 1997; pp. 265–267.
26. Barrera-García, A.; O'Hara, T.; Galván-Magaña, F.; Méndez-Rodríguez, L.C.; Castellini, J.M.; Zenteno-Savín, T. Trace elements and oxidative stress indicators in the liver and kidney of the blue shark (*Prionace glauca*). *Comp. Biochem. Phys. A* **2013**, *165*, 483–490. [CrossRef]
27. Bezerra, M.F.; Lacerda, L.D.; Lai, C.T. Trace metals and persistent organic pollutants contamination in batoids (Chondrichthyes: Batoidea): A systematic review. *Environ. Pollut.* **2019**, *248*, 684–695. [CrossRef]
28. Schlenk, D.; Li-Schlenk, R. Characterization of liver flavin-containing monooxygenase of the dogfish shark (*Squalus acanthias*) and partial purification of liver flavin-containing monooxygenase of the silky shark (*Carcharhinus falciformis*). *Comp. Biochem. Phys. B* **1994**, *109*, 655–664. [CrossRef]
29. Hauser-Davis, R.A.; Rocha, R.C.C.; Saint'Pierre, T.D.; Adams, D.H. Metal concentrations and metallothionein metal detoxification in blue sharks, *Prionace glauca* L. from the Western North Atlantic Ocean. *J. Tr. Elem. Med. Biol.* **2021**, *68*, 126813. [CrossRef]
30. Carrasco-Puig, P.; Colmenero, A.I.; Ruiz-García, D.; Molera- Arribas, A.J.; Hernandez-Martínez, A.M.; Raga, J.A.; Barría, C. Heavy metal concentrations in sharks, rays and chimaeras from the western Mediterranean Sea. *Mar. Pollut. Bull.* **2024**, *199*, 115942. [CrossRef]
31. Çiftçi, N.; Cicik, B.; Ayas, D. First Report on the Elemental Composition of the Bigeye Thresher Shark Alopias superciliosus Lowe, 1841 from the Mediterranean Sea. *Nat. Eng. Sci.* **2023**, *8*, 106–118. [CrossRef]
32. Bordbar, L.; Strogyloudi, E.; Hatzianestis, I.; Paraskevopoulou, V.; Chatzispyrou, A. First evidence of trace metals and persistent organic contaminants from an endangered marine species, *Mobula mobular* (Bonattere, 1788) caught in Hellenic waters (*Saronikos Gulf*). *Mar. Biol. Res.* **2023**, *19*, 399–406. [CrossRef]
33. Hauser-Davis, R.A.; Amorim-Lopes, C.; Araujo, N.L.F.; Reboucas, M.; Gomes, R.I.; Rocha, R.C.C.; Saint'Pierre, T.D.; dos Santos, L.N. On mobulid rays and metals: Metal content for the first Mobula mobular record for the state of Rio de Janeiro, Brazil and a review on metal ecotoxicology assessments for the Manta and Mobula genera. *Mar. Pollut. Bull.* **2021**, *168*, 112472. [CrossRef] [PubMed]

34. Company, R.; Felícia, H.; Serafim, A.; Almeida, A.J.; Biscoito, M.; Bebianno, M.J. Metal concentrations and metallothionein-like protein levels in deep-sea fishes captured near hydrothermal vents in the Mid-Atlantic Ridge off Azores. *Deep Sea Res.* **2010**, *57*, 893–908. [CrossRef]
35. Barrera-García, A.; O'Hara, T.; Galván-Magaña, F.; Méndez-Rodríguez, L.C.; Castellini, J.M.; Zenteno-Savín, T. Oxidative stress indicators and trace elements in the blue shark (*Prionace glauca*) off the east coast of the Mexican Pacific Ocean. *Comp. Biochem. Phys. C* **2012**, *156*, 59–66. [CrossRef] [PubMed]
36. Fergusson, I.K.; Graham, K.J.; Compagno, L.J. Distribution, abundance and biology of the small tooths and tiger shark *Odontaspis ferox* (Risso, 1810) (Lamniformes: *Odontaspididae*). *Environ. Biol. Fish.* **2007**, *81*, 207–228. [CrossRef]
37. Compagno, L.J.V. FAO Species Catalogue. In *Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. Parts 1 and 2*; FAO Fisheries Synopsis 125; Food and Agriculture Organization of the United Nations: Rome, Italy, 1984; Volume 4, pp. 1–655.
38. Monteiro, F.; Lemos, L.S.; de Moura, J.F.; Rocha, R.C.C.; Moreira, I.; Di Beneditto, A.P.; Kehrig, H.A.; Bordon, I.C.A.C.; Siciliano, S.; Saint'Pierre, T.D.; et al. Subcellular metal distributions and metallothionein associations in rough-toothed dolphins (*Steno bredanensis*) from Southeastern Brazil. *Mar. Pollut. Bull.* **2019**, *216*, 263–273. [CrossRef] [PubMed]
39. Boush, G.M.; Thieleke, J.R. Mercury content in sharks. *B Environ. Contam. Tox.* **1983**, *30*, 284–290. [CrossRef] [PubMed]
40. MacNeil, M.A.; Skomal, G.B.; Fisk, A.T. Stable isotopes from multiple tissues reveal diet switching in sharks. *Mar. Ecol. Prog. Ser.* **2005**, *302*, 199–206. [CrossRef]
41. Bosch, A.C.; O'Neill, B.; Sigge, G.O.; Kerwath, S.E.; Hoffman, L.C. Heavy metal accumulation and toxicity in smooth hound (*Mustelus mustelus*) shark from Langebaan Lagoon, South Africa. *Food Chem.* **2016**, *190*, 871–878. [CrossRef]
42. Atli, G.; Canli, M. Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. *Environ. Toxicol. Phar.* **2008**, *25*, 33–38. [CrossRef]
43. Pethbridge, H.; Cossa, D.; Butler, E.C.V. Mercury in 16 demersal sharks from southeast Australia: Biotic and abiotic sources of variation and consumer health implications. *Mar. Environ. Res.* **2010**, *69*, 18–26. [CrossRef]
44. Branco, V.; Vale, C.; Canário, J.; dos Santos, M.N. Mercury and selenium in blue shark (*Prionace glauca*, L. 1758) and swordfish (*Xiphias gladius*, L. 1758) from two areas of the Atlantic Ocean. *Environ. Pollut.* **2007**, *150*, 373–380. [CrossRef]
45. Barghigiani, G.; Pellegrini, D.; D'ulivo, A.; De Ranieri, S. Mercury assessment and its relation to selenium levels in edible species of the Northern Tyrrhenian Sea. *Mar. Pollut. Bull.* **1991**, *22*, 406–409. [CrossRef]
46. Alves, L.M.F.; Lemos, M.F.L.; Moutinho, A.B.; Ceia, F.R.; Munoz-Arnanz, J.; Jimenez, B.; Cabral, H.; Novais, S.C. Assessment of contaminants in blue sharks from the Northeast Atlantic: Profiles, accumulation dynamics, and risks for human consumers. *Environ. Pollut.* **2023**, *316*, 120467. [CrossRef]
47. Kazama, H.; Yamaguchi, Y.; Harada, Y.; Kaneko, N.; Mizushima, H.; Tsuchiya, K.; Nemoto, M.; Takaku, Y.; Sahoo, Y.V.; Tanaka, M. Mercury concentrations in the tissues of blue shark (*Prionace glauca*) from Sagami Bay and cephalopods from East China Sea. *Environ. Pollut.* **2020**, *266*, 115192. [CrossRef] [PubMed]
48. Turoczy, N.J.; Laurenson, L.J.B.; Allinson, G.; Nishikawa, M.; Lambert, D.F.; Smith, C.; Cottier, P.E.; Irvine, S.B.; Stagnitti, F. Observations on metal concentrations in three species of shark (*Deania calcea*, *Centroscymnus crepidater* and *Centroscymnus owstoni*) from Southeastern Australian Waters. *J. Agric. Food Chem.* **2000**, *48*, 4357–4364. [CrossRef] [PubMed]
49. McMeans, B.C.; Borgå, K.; Bechtol, W.R.; Higginbotham, D.; Fisk, A.T. Essential and non-essential element concentrations in two sleeper shark species collected in arctic waters. *Environ. Pollut.* **2007**, *148*, 281–290. [CrossRef] [PubMed]
50. Núnez-Nogueira, G. Concentration of essential and non-essential metals in two shark species commonly caught in Mexican (Gulf of Mexico) coastline. In *Golfo de México, Contaminación e Impacto Ambiental: Diagnóstico y Tendencias*, 2nd ed.; Botello, A.V., Rendón von Osten, J., Gold-Bouchot, G., Agraz-Hernández, C., Eds.; Universidad Autónoma de Campeche, Universidad Nacional Autónoma de México, Instituto Nacional de Ecología: Centro EPOMEX, Universidad Autónoma de Campeche:: Campeche, Mexico, 2005; pp. 451–474.
51. Kim, G.B.; Kang, M.R.; Kim, J.W. Specific accumulation of heavy metals in squid collected from offshore Korean waters: Preliminary results for offshore biomonitoring and food safety assessment. *Fish. Sci.* **2008**, *74*, 882–888. [CrossRef]
52. Adel, M.; Copat, C.; Asl, M.R.S.; Conti, G.O.; Babazadeh, M.; Ferrante, M. Bioaccumulation of trace metals in banded Persian bamboo shark (*Chiloscyllium arabicum*) from the Persian Gulf: A food safety issue. *Food Chem. Toxicol.* **2018**, *113*, 198–203. [CrossRef] [PubMed]
53. Mathews, T.; Fisher, N.S. Dominance of dietary intake of metals in marine elasmobranch and teleost fish. *Sci. Tot. Environ.* **2009**, *407*, 5156–5161. [CrossRef]
54. Mathews, T.; Fisher, N.S.; Jeffree, R.A.; Teyssié, J.L. Assimilation and retention of metals in teleost and elasmobranch fishes following dietary exposure. *Mar. Ecol. Prog. Ser.* **2008**, *360*, 1–12. [CrossRef]
55. Pentreath, R.J. The accumulation from seawater of ^{65} Zn, ^{54}Mn, ^{58}Co, and ^{59}Fe by the thornback ray, *Raja clavata*, L. *J. Exp. Mar. Biol. Ecol.* **1973**, *12*, 327–334. [CrossRef]
56. Smith, W.D.; Miller, J.A.; Heppell, S.S. Elemental markers in elasmobranchs: Effects of environmental and growth on vertebral chemistry. *PLoS ONE* **2013**, *8*, e62423. [CrossRef]
57. De Boeck, G.; Eyckmans, M.; Lardon, I.; Bobbaers, R.; Sinha, A.K.; Blust, R. Metal accumulation and metallothionein induction in the spotted dogfish *Scyliorhinus canicula*. *Comp. Biochem. Phys. A* **2010**, *155*, 503–508. [CrossRef]

58. Mehrim, A.I. Physiological, biochemical and histometric responses of *Nile tilapia* (*Oreochromis niloticus* L.) by dietary organic chromium (*chromium picolinate*) supplementation. *J. Adv. Res.* **2014**, *5*, 303–310. [CrossRef]
59. Torres, P.; da Cunha, R.T.; Maia, R.; dos Santos Rodrigues, A. Trophic ecology and bioindicator potential of the North Atlantic tope shark. *Sci. Total Environ.* **2014**, *481*, 574–581. [CrossRef] [PubMed]
60. Lyons, K.; Carlisle, A.; Preti, A.; Mull, C.; Blasius, M.; O'Sullivan, J.; Winkler, C.; Lowe, C.G. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Mar. Environ. Res.* **2013**, *90*, 27–38. [CrossRef]
61. Riesgo, L.; Sanpera, C.; García-Barcelona, S.; Sanchez-Fortún, M.; Coll, M.; Navarro, J. Understanding the role of ecological factors affecting mercury concentrations in the blue shark (*Prionace glauca*). *Chemosphere* **2023**, *313*, 137642. [CrossRef] [PubMed]
62. Taylor, D.L.; Kutil, N.J.; Malek, A.J.; Collie, J.S. Mercury bioaccumulation in cartilaginous fishes from Southern New England coastal waters: Contamination from a trophic ecology and human health perspective. *Mar. Environ. Res.* **2014**, *99*, 20–33. [CrossRef] [PubMed]
63. de Pinho, A.P.; Guimarães, J.R.D.; Martins, A.S.; Costa, P.A.S.; Olavo, G.; Valentin, J. Total mercury in muscle tissue of five shark species from Brazilian offshore waters: Effects of feeding habit, sex, and length. *Environ. Res.* **2002**, *89*, 250–258. [CrossRef] [PubMed]
64. Bajc, Z.; Jenčič, V.; Gačnik, K.Š. The heavy metal contents (Cd, Pb, Cu, Zn, Fe and Mn) and its relationships with the size of the rudd (*Scardinius erythrophthalmus*) from lake Cerknica, Slovenia. *Slov. Vet. Res.* **2016**, *53*, 69–75.
65. Monperrus, M.; Tessier, E.; Amouroux, D.; Leynaert, A.; Huonnic, P.; Donard, O.F.X. Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea. *Mar. Chem.* **2007**, *107*, 49–63. [CrossRef]
66. Álvaro-Berlanga, S.; Calatayud-Pavía, C.E.; Cruz-Ramírez, A.; Soto-Jiménez, M.F.; Liñán-Cabello, M.A. Trace elements in muscle tissue of three commercial shark species: *Prionace glauca*, *Carcharhinus falciformis*, and *Alopias pelagicus* off the Manzanillo, Colima coast, Mexico. *Environ. Sci. Pollut. Res.* **2021**, *28*, 22679–22692. [CrossRef] [PubMed]
67. Lara, A.; Galvan-Magana, F.; Elorriaga-Verplancken, F.R.; Marmolejo-Rodríguez, A.J.; Gonzalez-Armas, R.; Arreola-Mendoza, L.; Sujitha, S.B.; Jonathan, M.P.; Pantoja-Echevarría, L.M. Mercury, selenium and cadmium in juvenile blue (*Prionace glauca*) and smooth hammerhead (*Sphyrna zygaena*) sharks from the Northwest Mexican Pacific coast. *Mar. Pollut. Bull.* **2022**, *175*, 488–491. [CrossRef] [PubMed]
68. Hornung, H.; Krom, M.D.; Cohen, Y.; Bernhard, M. Trace metal content in deep-water sharks from the eastern Mediterranean Sea. *Mar. Biol.* **1993**, *115*, 331–338. [CrossRef]
69. Asante, K.A.; Agusa, T.; Mochizuki, H.; Ramu, K.; Inoue, S.; Kubodera, T.; Takahashi, S.; Subramanian, A.; Tanabe, S. Trace elements and stable isotopes (δ^{13} C and δ^{15} N) in shallow and deep-water organisms from the East China Sea. *Environ. Pollut.* **2018**, *156*, 862–873. [CrossRef] [PubMed]
70. Olmedo, P.; Hernández, A.F.; Pla, A.; Femia, P.; Navas-Acien, A.; Gil, F. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance. *Food Chem. Toxicol.* **2013**, *62*, 299–307. [CrossRef] [PubMed]
71. LeBlanc, P.J.; Jackson, A.L. Arsenic in marine fish and invertebrates. *Mar. Pollut. Bull.* **1973**, *4*, 88–90. [CrossRef]
72. Giovos, I.; Brundo, M.V.; Doumpas, N.; Kazlari, Z.; Loukovitis, D.; Moutopoulos, D.K.; Naasan, R.; Spyridopoulou, A.; Papadopoulou, A.; Papapetrou, M.; et al. Trace elements in edible tissues of elasmobranchs from the North Aegean Sea (Eastern Mediterranean) and potential risks from consumption. *Mar. Pollut. Bull.* **2022**, *184*, 114129. [CrossRef]
73. Walker, T.I. Mercury concentrations in edible tissues of elasmobranchs, teleosts, crustaceans and molluscs from south-eastern Australian waters. *Mar. Freshwater Res.* **1988**, *39*, 39–49. [CrossRef]
74. Mársico, E.T.; Machado, M.E.S.; Knoff, M.; São Clemente, S.C. Total mercury in sharks along the southern Brazilian Coast. *Arq. Bras. Med. Vet. Zoo.* **2007**, *59*, 1593–1596. [CrossRef]
75. Vas, P. Trace metal levels in sharks from British and Atlantic waters. *Mar. Pollut. Bull.* **1991**, *22*, 67–72. [CrossRef]
76. Kim, S.J.; Lee, H.K.; Badejo, A.C.; Lee, W.C.; Moon, H.B. Species-specific accumulation of methyl and total mercury in sharks from offshore and coastal waters of Korea. *Mar. Pollut. Bull.* **2016**, *102*, 210–215. [CrossRef]
77. García-Hernández, J.; Cadena-Cárdenas, L.; Betancourt-Lozano, M.; García-De-La-Parra, L.M.; García-Rico, L.; Márquez-Farías, F. Total mercury content found in edible tissues of top predator fish from the Gulf of California, Mexico. *Toxicol. Environ. Chem.* **2007**, *89*, 507–522. [CrossRef]
78. Escobar-Sánchez, O.; Galván-Magaña, F.; Rosíles-Martínez, R. Biomagnification of mercury and selenium in blue shark *Prionace glauca* from the Pacific Ocean off Mexico. *Biol. Trace Elem. Res.* **2011**, *144*, 550–559. [CrossRef]
79. Lopez, S.A.; Abarca, N.L.; Meléndez, R.C. Heavy metal concentrations of two highly migratory sharks (*Prionace glauca* and *Isurus oxyrinchus*) in the southeastern Pacific waters: Comments on public health and conservation. *Trop. Conserv. Sci.* **2013**, *6*, 126–137. [CrossRef]
80. Maz-Courrau, A.; López-Vera, C.; Galvan-Magaña, F.; Escobar-Sánchez, O.; Rosíles-Martínez, R.; Sanjuán-Muñoz, A. Bioaccumulation and biomagnification of total mercury in four exploited shark species in the Baja California Peninsula, Mexico. *B Environ. Contam. Tox.* **2012**, *88*, 129–134. [CrossRef]
81. Branco, V.; Canario, J.; Vale, C.; Raimundo, J.; Reis, C. Total and organic mercury concentrations in muscle tissue of the blue shark (*Prionace glauca* L. 1758) from the Northeast Atlantic. *Mar. Pollut. Bull.* **2004**, *49*, 871–874. [CrossRef]

82. Stevens, J.D.; Brown, B.E. Occurrence of heavy metals in the blue shark *Prionace glauca* and selected pelagic in the NE Atlantic Ocean. *Mar. Biol.* **1974**, *26*, 287–293. [CrossRef]
83. Olmedo, P.; Pla, A.; Hernández, A.F.; Barbier, F.; Ayouni, L.; Gil, F. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environ. Int.* **2013**, *59*, 63–72. [CrossRef] [PubMed]
84. de Carvalho, G.G.A.; Degaspari, I.A.M.; Branco, V.; Canário, J.; de Amorim, A.F.; Kennedy, V.H.; Ferreira, J.R. Assessment of total and organic mercury levels in blue sharks (*Prionace glauca*) from the south and southeastern Brazilian coast. *Biol. Trace Elem. Res.* **2014**, *159*, 128–134.
85. Biton-Porsmoguer, S.; Bănaru, D.; Boudouresque, C.F.; Dekeyser, I.; Bouchoucha, M.; Marco-Miralles, F.; Lebreton, B.; Guillou, G.; Harmelin-Vivien, M. Mercury in blue shark (*Prionace glauca*) and short fin mako (*Isurus oxyrinchus*) from north-eastern Atlantic: Implication for fishery management. *Mar. Pollut. Bull.* **2018**, *127*, 131–138. [CrossRef] [PubMed]
86. Cordero-Maldonado, C.; Espinoza, P. Cadmium and lead levels in muscle tissue of blue shark (*Prionace glauca*) in the Southeastern Pacific Waters. *Mar. Pollut. Bull.* **2022**, *177*, 113523. [CrossRef] [PubMed]
87. Commission Regulation (EC) Number 1881/2006; Setting Maximum Levels for Certain Contaminants in Foodstuffs. L 364/5, 2006. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF (accessed on 10 January 2024).
88. Papp, A.; Pecze, L.; Szabó, A.; Vezér, T. Effects on the central and peripheral nervous activity in rats elicited by acute administration of lead, mercury and manganese, and their combinations. *J. Appl. Toxicol.* **2006**, *26*, 374–380. [CrossRef] [PubMed]
89. Chien, L.C.; Hung, T.C.; Choang, K.Y.; Yeh, C.Y.; Meng, P.J.; Shieh, M.J.; Ha, B.C. Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Sci. Total Environ.* **2002**, *285*, 177–185. [CrossRef] [PubMed]
90. Matos, J.; Lourenço, H.M.; Brito, P.; Maulvault, A.L.; Martins, L.L.; Afonso, C. Influence of bioaccessibility of total mercury, methyl-mercury and selenium on the risk/benefit associated to the consumption of raw and cooked blue shark (*Prionace glauca*). *Environ. Res.* **2015**, *143*, 123–129. [CrossRef]
91. Kalogeropoulos, N.; Karavoltsos, S.; Sakellari, A.; Avramidou, S.; Dassenakis, M.; Scoullos, M. Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish. *Food Chem. Toxicol.* **2012**, *50*, 3702–3708. [CrossRef]