Evidence of contamination-associated damage in blue sharks (Prionace glauca) from the Northeast Atlantic

Luís M.F. Alves, Ariana B Moutinho, Luis J Gómez, Ana L Oropesa, Juan Muñoz-Arnanz, Begoña Jiménez, Marco F.L. Lemos, Vanessa F Fonseca, Henrique Cabral, Sara C Novais

Science of the Total Environment 872 (2023) 162095

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evidence of contamination-associated damage in blue sharks (*Prionace glauca*) from the Northeast Atlantic

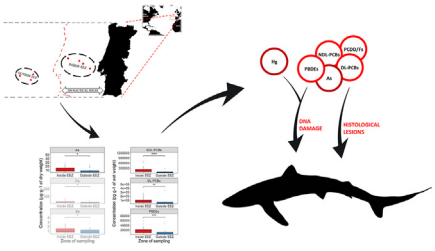
Luís M.F. Alves [a,*], Ariana B. Moutinho [a], Luis J. Gómez [b,c], Ana L. Oropesa [c,d], Juan Muñoz-Arnanz [e], Begoña Jiménez [e], Marco F.L. Lemos [a], Vanessa F. Fonseca [f], Henrique Cabral [g], Sara C. Novais [a]

[a] MARE- Marine and Environmental Sciences Centre & ARNET – Aquatic Research Infrastructure Network Associated Laboratory, ESTM, Polytechnic of Leiria, Peniche, Portugal
[b] Anatomy and Pathological Anatomy Area, School of Veterinary Medicine, University of Extremadura, Cáceres 10003, Spain
[c] BRILiC - Biotechnology Research Institute in Livestock & Cynegetic, University of Extremadura, Cáceres 10003, Spain
[d] Toxicology Area, School of Veterinary Medicine, University of Extremadura, Cáceres 10003, Spain
[e] Dept. of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, IQOG-CSIC, Madrid, Spain
[f] MARE – Marine and Environmental Sciences Centre, ARNET – Aquatic Research Infrastructure Network Associated Laboratory, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal
[g] INRAE, UR EABX, 50 Avenue de Verdun, 33612 Cestas, France

HIGHLIGHTS

- Contamination differences in blue sharks originated differences in stress responses.
- Contaminants correlated strongly and positively with stress biomarkers.
- Proximity to urbanized zones increases stress responses in blue sharks.
- DNA damage and histopathological alterations were the more responsive biomarkers.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Rafael Mateo Soria

Keywords:
Elasmobranchs
Metals
POPs
Ecotoxicology
Pelagic
Impact assessment

ABSTRACT

Top predators such as most shark species are extremely vulnerable to amassing high concentrations of contaminants, but not much is known about the effects that the contaminant body burden imparts on these animals. Species like the blue shark (*Prionace glauca*) are very relevant in this regard, as they have high ecological and socioeconomic value, and have the potential to act as bioindicators of pollution. This work aimed to assess if differences in contaminant body burden found in blue sharks from the Northeast Atlantic would translate into differences in stress responses. Biochemical responses related to detoxification and oxidative stress, and histological alterations were assessed in the liver and gills of 60 blue sharks previously found to have zone-related contamination differences. Similar zone-related differences were found in biomarker responses, with the sharks from the most contaminated zone exhibiting more pronounced responses. Additionally, strong positive correlations were found between contaminants (i.e., As, PCBs, and PBDEs) and relevant biomarkers (e.g., damaged DNA and protective histological alterations). The present results are indicative of the potential that this species and these tools have to be used to monitor pollution in different areas of the Atlantic.

* Corresponding author at: Edifício CETEMARES, Avenida do Porto de Pesca, 2520 – 630 Peniche, Portugal.
E-mail address: luis.f.alves@ipleiria.pt (L.M.F. Alves).

http://dx.doi.org/10.1016/j.scitotenv.2023.162095
Received 8 November 2022; Received in revised form 19 January 2023; Accepted 4 February 2023
Available online 13 February 2023

1. Introduction

Pollution is nowadays recognized as one of the most serious threats faced by marine ecosystems (UN, 2022). Inorganic and organic contaminants such as arsenic (Neff, 1997; Taylor et al., 2017) and polychlorinated biphenyls (PCBs) (Baeyens et al., 2007; Schlenk et al., 2008), respectively, have been shown to negatively impact both marine fauna and its human consumers, so it is essential to monitor their concentrations and effects. Since these contaminants usually have an earlier effect at lower levels of biological organization (Lemos, 2021), focusing our attention on the detection of cellular and histological alterations may allow us to detect problematic contamination events earlier in time for the implementation of remediation measures. The alterations caused by exposure to contamination can be followed by assessing various parameters, namely the presence of reactive oxygen species (ROS), the lipid peroxidation (LPO) and DNA damage they induce, the activities of enzymes involved in detoxification of contaminants such as glutathione-S-transferase (GST), the activities of antioxidant enzymes (i.e., glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)), and histological changes.

Another approach to improve the chances of a timely detection of contamination issues is to use bioindicator species (Holt, 2010), which act as a reference to their ecosystem and therefore avoid the need for the assessment of many different species. Sharks have the potential to be used as bioindicators of pollution (Alves et al., 2022), and blue sharks in particular have been extensively used as pollution sentinels (Tiktak et al., 2020). This species has also shown potential to give insight into the impacts of contamination on marine ecosystem (Alves et al., 2015, 2016; Barrera-García et al., 2013) and has shown to pose some potential risks to human consumers (Alves et al., 2023; Muñoz-Arnanz et al., 2022). In a previous work, the concentrations of contaminants in the tissues of blue sharks captured in different zones of the North Atlantic were assessed, with the sharks captured closer to continental shore exhibiting higher levels of contamination when compared with their counterparts captured in a more oceanic environment (Alves et al., 2023). The present work aims primarily at assessing if the same sharks possessed similarly location-associated differences in detoxification and oxidative stress-related responses, as well as differences in histological lesions, and if those responses could be associated with different levels of bioaccumulated organic and inorganic contaminants.

2. Methods

2.1. Organisms

The blue sharks sampled for this work were captured as by-catch by a commercial longliner operating in the North Atlantic. The sampling was opportunistic and occurred between March and December 2019, resulting in the collection of tissue samples from 60 blue sharks. The 60 sharks were characterized and previously grouped (Alves et al., 2023) according to sex (male or female), size/maturity stage [small juveniles (SJ, <130 cm FL), large juveniles (LJ, 130 cm ≥ FL <183 cm, for males, and 130 cm ≥ FL < 180 cm, for females), and adults (AD, ≥183 cm FL, for males, and ≥ 180 cm FL, for females)], and geographical zone of capture (Inside EEZ, ≤ 200 nautical miles (NM) from shore and inside Portugal's EEZ; Outside EEZ, > 200 NM from shore and outside Portugal's EEZ) (Table 1).

To meet the methodological demands of the biomarker assays, the sampling was performed only on individuals that were alive and showed no obvious signs of prolonged stress when captured. The sharks were sacrificed as soon as they were landed on the vessel, and the location, size (fork length (FL), cm) and sex were documented. The sample collection was performed immediately after landing and appropriate measures were taken to minimize cross-contamination (i.e., one animal was sampled at a time, sampling was conducted in a different location from where the animals were being landed, and all material was frequently and methodically cleaned between individuals; more details of the sampling can be seen in Alves et al., 2022). All samples destined for the analysis of biochemical responses were kept on liquid nitrogen until reaching the laboratory where they were then stored at −80 °C. Samples destined for histopathological analysis (measuring approximately 10 mm × 30 mm, with no >10 mm of thickness) were kept in Bouin's solution (75 % picric acid, 25 % formaldehyde solution, and 5 % glacial acetic acid) in a 1:4 (weight:volume) ratio for appropriate fixation until reaching the laboratory, where they were rinsed with running tap water, dehydrated in graded alcohol solutions, cleared in xylene, and embedded into paraffin blocks using an automatic tissue processor.

Table 1
Descriptive summary of the 60 sharks sampled. SJ = small juveniles; LJ = large juveniles; AD = adults; Inside EEZ = caught up to 200 nautical miles (NM) from Portugal's continental coast and inside the country's EEZ; Outside EEZ = caught >200 NM from Portugal's continental coast and outside the country's EEZ.

Sex	Size Group	Inside EEZ	Outside EEZ	Total		
Female	SJ	7	5	12	22	60
	LJ	5	5	10		
	AD	0	0	0		
Male	SJ	3	1	4	38	
	LJ	9	8	17		
	AD	16	1	17		
Total		40	20			
		60				

2.2. Contaminant chemical analysis

The concentrations of inorganic and organic contaminants in the liver and muscle of the sharks were previously determined, and the methodology for the analysis have been described in detail in Muñoz-Arnanz et al. (2022) and Alves et al. (2023). Briefly, inorganic contaminants were measured using total x-ray fluorescence (TXRF), and organic contaminants were measured using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). For the present work, data pertaining to the concentrations detected in liver samples was used, namely the concentrations of aluminium (Al), arsenic (As), calcium (Ca), copper (Cu), iron (Fe), mercury (Hg), potassium (K), sodium (Na), manganese (Mn), phosphorus (P), lead (Pb), sulphur (S), selenium (Se), strontium (Sr), titanium (Ti), zinc (Zn), dioxin-like polychlorinated biphenyls (DL-PCBs), non-dioxin-like polychlorinated biphenyls (NDL-PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs).

2.3. Histopathological analysis

All samples (gills and liver) were routinely processed, each paraffin block was sectioned in 5 μm thick serial sections that were stained with Hematoxylin-Eosin (H-E), Periodic Acid Schiff (PAS) and Prussian blue dye (Panreac Quimica, Spain) for light microscopic examination. A qualitative analysis of tissues was done with a photomicroscope (Eclipse 80, Nikon®, Tokyo, Japan) using an image analyser (Nis-elements, Nikon®, Tokyo, Japan). Observations were done in fields at 100×, 200× and 400× magnifications. In addition to the descriptive study of lesions, a severity grading scale was established to assess the degree of histologic changes: 0 (absent: structure of the organ is normal, with no lesions present), 1 (lesions present in <33 % of each studied field), 2 (lesions present between 33 % and 66 % of each studied field) and 3 (lesions present in >66 % of each studied field). Ten randomly selected and not overlaid fields were used (200×; area/field: 250,000 μm^2).

2.4. Biochemical responses

2.4.1. Tissue preparation

To perform the biochemical biomarker assays, gill and liver samples were homogenized and separated according to each assay's requirements. The homogenizing buffer used for all homogenizations consisted of potassium phosphate 0.1 mol L^{-1} (pH 7.4), phenylmethylsulfonyl fluoride (PMSF) 0.1 mmol L^{-1}, ethylenediamine tetraacetic acid (EDTA) 1 mmol

L^{-1}, dithiothreitol (DTT) 1 mM, and potassium chloride (KCl) 150 mM. Approximately 500 mg of each tissue from each organism were homogenized in a 1:10 proportion by ultrasonication (4 cycles of 5 s at 20 % amplitude, pauses of 30 s on ice). A portion of the resulting homogenized tissue was transferred to a microtube containing 2,6-dieter-butyl-4-metylphenol (BHT) 4 % in methanol for the posterior measurement of lipid peroxidation (LPO), and another portion was separated for the quantification of reactive oxygen species (ROS). The rest of the homogenized tissue was centrifuged at 10000*g* for 20 min (4 °C) to isolate the post mitochondrial supernatant (PMS), which was then separated into different microtubes and stored at −80 °C for later assessment of protein concentration and the activities of glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Additionally, for the quantification of damaged DNA, approximately 50 mg of each tissue were homogenized in a 1:10 proportion using a Precellys Dual Bead Beater with the Cryolys refrigerator unit (Bertin Instruments, Montigny-le-Bretonneux, France), and performing 2 cycles of 20 s at 6500 rpm using zirconium beads (1.4 mm). Blanks were prepared in all biochemical assays, using homogenizing buffer instead of the sample. All spectrophotometric measurements were performed in quadruplicates at 25 °C, using a Synergy H1 Hybrid Multi-Mode microplate reader (BioTek® Instruments, Vermont, USA).

2.4.2. Protein quantification

The soluble protein concentration in the PMS fraction, needed for the normalization of the measured parameters, was quantified according to the Bradford method (Bradford, 1976). The method was adapted from BioRad's Bradford microassay set up in a 96-well flat bottom plate, using bovine γ-globulin as the protein standard, and absorbance was read at 600 nm. Results were expressed in mg of protein mL^{-1}.

2.4.3. Detoxification and antioxidant mechanisms

The assessment of GST activity was performed using an adaptation of the procedure described by Habig et al. (1974), by following the formation of the thioether glutathione dinitrobenzene, a product of the reaction between GSH and 1-Chloro-2,4-Dinitrobenzene (CDNB), at 340 nm, for 3 min. The activity of GST was calculated using a molar extinction coefficient of $9.6 \times 10^3\ M^{-1}\ cm^{-1}$ and expressed in nmol $min^{-1}\ mg^{-1}$ of protein.

Selenium-dependent GPx (Se-GPx) and total GPx (T-GPx) were assessed using the method described in Flohé and Günzler (1984), adapted to a 96-well microplate. The activities of Se-GPx and T-GPx were measured using hydrogen peroxide (H_2O_2) and cumene hydroperoxide ($C_6H_5C(CH_3)_2OOH$) as substrates, respectively, and monitoring the continuous decrease in nicotinamide adenine dinucleotide phosphate (NADPH) concentration at 340 nm for 5 min. The activities of Se-GPx and T-GPx were calculated using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$ and expressed in nmol $min^{-1}\ mg^{-1}$ of protein. The SOD activity was assessed according to McCord and Fridovich (1969), and as adapted to a 96-well microplate by Lima et al. (2007). This method follows the reduction of cytochrome C mediated by xanthine/xanthine oxidase at 550 nm. Results were expressed in U mg^{-1} of protein using a SOD standard of 1.5 U mL^{-1}, with one U representing the amount of enzyme in the sample that induces a 50 % inhibition of the reduction of cytochrome C. The activity of CAT was evaluated by following the decrease in H_2O_2, adapting the method described by Claiborne (1985) to a 96-well microplate. Absorbance was read at 240 nm and the concentration of H_2O_2 was calculated using a molar extinction coefficient of 40 $M^{-1}\ cm^{-1}$. Results were expressed in μmol $min^{-1}\ mg^{-1}$ of protein.

2.4.4. Oxidative stress and oxidative damage

The amount of ROS was assessed according to Socci et al. (1999) adapted to a 96-well microplate. The assay measures the conversion of non-fluorescent 2′,7′-dichlorofluorescein diacetate (DCFDA) into 2′,7′-dichlorofluorescein (DCF), in the presence of ROS. The fluorescence of DCF was read using an excitation/emission wavelength of 485/525 nm. Results were expressed in fluorescence units (FU) mg^{-1} of wet weight (ww).

The LPO was measured by determining the content of thiobarbituric acid reactive substances (TBARS), following the method described by Ohkawa et al. (1979) and Bird and Draper (1984), adapted by Wilhelm Filho et al. (2001) and Torres et al. (2002). The TBARS were quantified colorimetrically at 535 nm using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmol of TBARS g^{-1} of ww. The DNA damage (double-strand breaks) was assessed according to the method described by Olive (1988), adapted from De Lafontaine et al. (2000). Damaged DNA was quantified by fluorescence, using an excitation/emission wavelength of 360/450 nm. Results were expressed as mg of DNA g^{-1} of ww, using calf thymus DNA as standard.

2.5. Statistical analyses

The contaminant dataset was retrieved from the works of Muñoz-Arnanz et al. (2022) and Alves et al. (2023). For the present study, given the nature of the addressed biological parameters of fast response to stress, only liver contaminant levels were used. Shapiro-Wilks tests were used to assess the normality of the data. The existence of correlations between variables was assessed using Spearman's test, and the strength of the correlations found was categorized according to the obtained absolute correlation coefficient as in Schober et al. (2018): 0.00 to 0.10 – negligible correlation; 0.10 to 0.39 – weak correlation; 0.40 to 0.69 – moderate correlation; 0.70 to 0.89 – strong correlation; 0.90 to 1.00 – very strong correlation. Statistical differences amongst the different groups of data were evaluated using Wilcoxon rank sum tests. Given the lack of adult females, statistical sex differences were assessed using sharks measuring only up to the size of the largest female (i.e., 160 cm (FL)). The significance level for all statistical tests was set at 0.05. The influence of sex, size/maturity, and zone of capture on different biomarker responses was investigated in the entire set of sharks sampled using Principal Component Analysis (PCA). All statistical analysis and data plotting were performed on R Version 4.0.3 (R Core Team, 2020), using the additional packages “Hmisc” (Harrel, 2022) and “ggplot2” (Wickham, 2016) in the integrated development environment RStudio Version 1.3.1093 (RStudio Team, 2020).

3. Results

3.1. Contaminant body burden

The concentrations of organic and inorganic contaminants measured in the blue sharks' liver samples have been previously described in the works by Muñoz-Arnanz et al. (2022) and Alves et al. (2023), respectively, and a summary can be seen in Supplementary Table 1. Since Alves et al. (2023) focused the analysis on the inorganic contaminants' accumulation mainly in the muscle tissue, accumulation data on the liver will be further explored herein. No sex-associated differences in the liver concentrations of either organic or inorganic contaminants were found for any of the assessed compounds (Wilcoxon Rank Sum, all $p > 0.05$). Positive correlations with sharks' size (FL) were found for all organic contaminant groups assessed in liver samples (Muñoz-Arnanz et al., 2022), and tmoderate and weak positive correlations were observed for the concentrations of As (rho = 0.478, *p*-value = $1.138e^{-4}$) and Fe (rho = 0.366, p-value = $4.042e^{-3}$), respectively. Additionally, a weak negative correlation between FL and Sr was detected (rho = −0.274, p-value = $3.414e\text{-}^{2}$).

When all sharks were considered, contaminants in liver samples were found to be tendentially higher in sharks from inside EEZ (Supplementary Fig. 1), with the concentrations of As, Fe, Se, NDL-PCBs, DL-PCBs, and PBDEs being significantly higher in that zone of capture (Fig. 1). To address the potential bias caused by the fact that 16 of the 17 adults were sampled at locations from the area inside EEZ, a similar analysis was performed using the group of samples pertaining to juvenile sharks, which possessed a much more balanced composition between zones of capture. The analysis of the juveniles showed the same tendency observed for all sharks, with sharks from inside EEZ presenting overall tendentially higher concentrations of inorganic and organic contaminants and significantly higher

concentrations of NDL-PCBs, DL-PCBs, PBDEs, Mn, P, and Se (Supplementary Fig. 2).

3.2. Histological lesions

The histopathological assessment indicated that the gill samples from all sharks exhibited lesions, with the samples being mainly affected by progressive alterations, observed as proliferation or/and hyperplasia of the epithelial cells of the secondary lamellae, that tended to collapse the interlamellar space leading to lamellar fusions and dilation of capillary vessels (Fig. 2A), and, ultimately, circulatory disturbance. An increase in the number of mucous cells in the interlamellar space was found in 17 % of the sharks. A light positivity to PAS staining was observed in some individuals (Fig. 2B). In addition, the proliferation of the connective tissue in the most affected areas of the secondary lamellae was registered in 24 % of the sharks (Fig. 2C). Regressive changes were also described in the liver samples of all animals. The most pronounced histological alteration in liver tissue was a massive hepatocyte degeneration, represented by steatosis phenomena (> 90 % of the cells), and normal hepatocytes around vessels (Fig. 2D). Hepatocytes with signs of mitosis (Fig. 2D) and scar connective tissue proliferation (Fig. 2E) were also remarkable lesions. The degree of severity of the histological lesions observed in both gill and liver samples was assessed (on a scale from 0 to 3, with 3 representing the most severe degree of lesion) and the results can be seen in Supplementary Table 2. In gills, dilation of capillary vessels and connective tissue proliferation were the lesions with the highest and lowest mean severity degree, respectively. In the liver, 59 of the 60 animals presented the highest degree of severity for the only lesion ranked (hepatocyte degeneration), the exception being one shark that scored the second highest value of severity for that lesion. One-sided Wilcoxon rank sum tests showed no differences between sex groups. No correlations with size were observed (Spearman's test, all $p > 0.05$), but Wilcoxon rank sum tests indicated that small juvenile sharks had significantly higher epithelial proliferation in gills than both large juveniles (p-value = $1.253e^{-2}$, W = 299.5) and adults (p-value = $3.456e^{-2}$, W = 180.5), and that adults had significantly higher levels of connective tissue proliferation in gills than large juveniles (p-value = $4.212e^{-3}$, W = 147).

Finally, animals from inside EEZ showed significantly higher epithelial proliferation in gills when both all animals (p-value = $2.302e^{-3}$, W = 566) and only juveniles were assessed (p-value = $9.668e^{-4}$, W = 346.5)

3.3. Biochemical responses

All biochemical biomarkers were successfully measured in both liver and gills, except for CAT in the latter, for which no measurable activity could be detected. The values of all parameters assessed in both tissues were compared and differences between tissues were observed for all biomarkers (Supplementary Fig. 3). Regarding oxidation sources and their effects, the results were mixed, with gills presenting higher values of ROS and Damaged DNA, while the liver presented higher levels of LPO. Most enzymatic biomarkers were higher in the liver, with the only exception being Se_GPx.

The influence of physiological and geographical factors (i.e., sex, size/maturity stage, and zone of sampling) in the different biochemical responses was assessed by performing a Principal Component Analysis (PCA). Three biplots were created with the same PCA results of the biomarker values measured in both liver and gills, each emphasizing a different factor (i.e., sex – Fig. 3A, size/maturity – Fig. 3B, and zone of capture – Fig. 3C). Axis 1 and 2 of the PCA explained 39.7 % of the variability observed, and both were influenced by a combination of biomarkers measured in each tissue.

Vector displacement indicates that biomarkers measured in the liver mostly aligned positively with their gill counterparts, with Damaged DNA being the exception and presenting negative associations between the values measured in the liver and gills. Spearman's tests confirmed weak positive correlations between tissue pairs for ROS (rho = 0.278, p-value = $3.177e^{-2}$) and T_GPx (rho = 0.274, p-value = $3.379e^{-2}$), a moderate positive correlation for SOD (rho = 0.618, p-value = $1.405e^{-7}$), and a weak negative correlation for damaged DNA (rho = −0.309, p-value = $1.640e^{-2}$) (Supplementary Fig. 4). Regarding the influence of sex in the responses, the biplots show a great deal of overlap between female and male groups (Fig. 3A), and concurringly the only differences shown by one-sided Wilcoxon rank sum tests were higher values for liver T_GPx (W = 102, p-value = $7.725e^{-3}$) and gills GST (W = 104, p-value = $9.728e^{-3}$) in females. There was also a high percentage of overlap amongst the three groups of size/maturity (Fig. 3B), but adults showed different response profiles than the two groups of juveniles, with adult sharks seeming to be characterized by higher levels of most biomarkers. Wilcoxon rank sum tests mostly confirmed this interpretation and identified some differences in biochemical biomarker responses between size/maturity groups, mainly on gills (Fig. 4). In liver, adult sharks exhibited higher levels of T_GPx than large juveniles (W = 322, p-value = $1.273e^{-2}$), higher SOD than both large juveniles (W = 319, p-value = $1.543e^{-2}$) and small juveniles (W = 204, p-value = $6.793e^{-3}$), and lower CAT than large juveniles (W = 88, p-value = $2.096e^{-4}$). In gills, adults showed higher levels of ROS than small juveniles (W = 206, p-value = $6.076e^{-3}$), higher LPO than both large juveniles (W = 350, p-value = $1.551e^{-3}$) and small juveniles (W = 226, p-value = $3.897e^{-4}$), higher Se_GPx than both large juveniles (W = 346, p-value = $2.172e^{-3}$) and small juveniles (W = 204, p-value = $6.793e^{-3}$), but lower levels of damaged DNA than both large juveniles (W = 48, p-value = $1.122e^{-6}$) and small juveniles (W = 21, p-value = $2.971e^{-6}$), and lower GST than large juveniles (W = 123, p-value = $4.776e^{-3}$) and small juveniles (W = 59, p-value = $2.376e^{-3}$). Spearman's tests confirmed the existence of correlations between the size of the sharks (FL) and the values of some biochemical biomarkers. In the liver, SOD showed a moderate positive correlation with size (rho = 0.418, p-value = $8.939e^{-4}$), and in gills there were weak positive correlations with ROS (rho = 0.291, p-value = $2.41e^{-2}$), Se_GPx (rho = 0.379, p-value = $2.8e^{-3}$), and SOD (rho = 0.255, p-value = $4.977e^{-2}$), a moderate positive correlation with LPO (rho = 0.461, p-value = $2.106e^{-4}$), and a moderate negative one with Damaged DNA (rho = −0.517, p-value = $2.371e^{-5}$).

Lastly, the PCA biplot concerning the sampling zones highlighted differences between animals caught in inside and outside EEZ (Fig. 3C), with the disposition of the dotted samples in relation to the vectors indicating that the animals from inside EEZ were characterized by overall more pronounced biochemical responses when compared to the animals from outside EEZ. One-sided Wilcoxon rank sum tests ($p < 0.05$) revealed significant differences between zones for several biomarkers measured in both tissues. DNA damage in liver (W = 597, p-value = $8.241e^{-4}$) and SOD in both tissues (W = 537, p-value = $3.148e^{-2}$ and W = 533, p-value = $1.886e^{-2}$ in liver and gills, respectively) were significantly higher in sharks from inside EEZ, while LPO (W = 197.5), p-value = $7.684e^{-4}$ and CAT (W = 253, p-value = $1.034e^{-2}$) in liver, and GST (W = 273, p-value = $2.364e^{-2}$) and DNA damage (W = 142, p-value = $2.695e^{-5}$) in gills were significantly lower in sharks from inside EEZ.

Using a similar approach to the one used when trying to minimize the bias introduced in the contaminant concentration by the skewness of the sampling (i.e., most adult sharks were sampled inside EEZ, and all adults were males), a PCA was performed using only data from juvenile sharks (SJ + LJ). Two corresponding biplots were created and can be seen in Fig. 5, each emphasizing a different factor (i.e., sex – Fig. 5A, and zone of capture – Fig. 5B). Axis 1 and 2 of the PCA explained 36.3 % of the variability observed. Contrary to what was observed in Fig. 4, the vectors in Fig. 6 clustered into two clearly distinct groups: one formed by damaged DNA (liver) and SOD (liver and gills), and another formed by the remaining biomarkers.

The biplot of the PCA highlighting different sex groups in juvenile sharks does not substantiate clear differences, with sharks from both sexes presenting homogenous distributions, and Wilcoxon Rank Sum tests only identified significant differences between sex groups for liver T_GPx, with males presenting higher levels than females (W = 130, p-value =

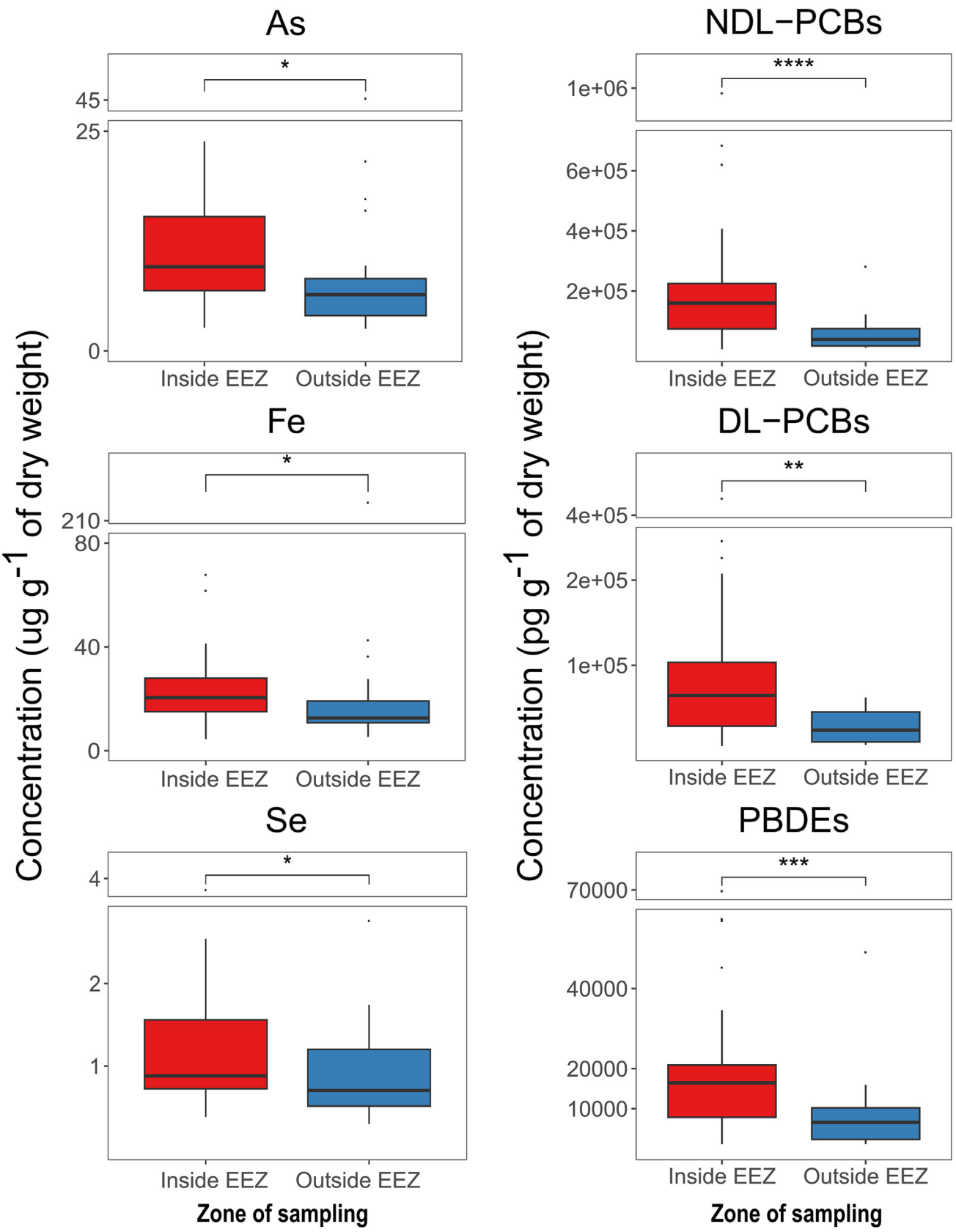


Fig. 1. Boxplots for the concentrations of contaminants measured in liver samples from blue sharks (*Prionace glauca*) that showed significant differences between zones of sampling. Inside EEZ encompasses sharks captured in locations up to 200 nautical miles (NM) from the continental coast; Outside EEZ encompasses sharks captured in locations over 200 NM from the continental coast. Differences between groups were assessed using Wilcoxon rank sum tests. * = *p*-value <0.05, ** = p-value <0.01, *** = p-value <0.001, **** = p-value <0.0001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

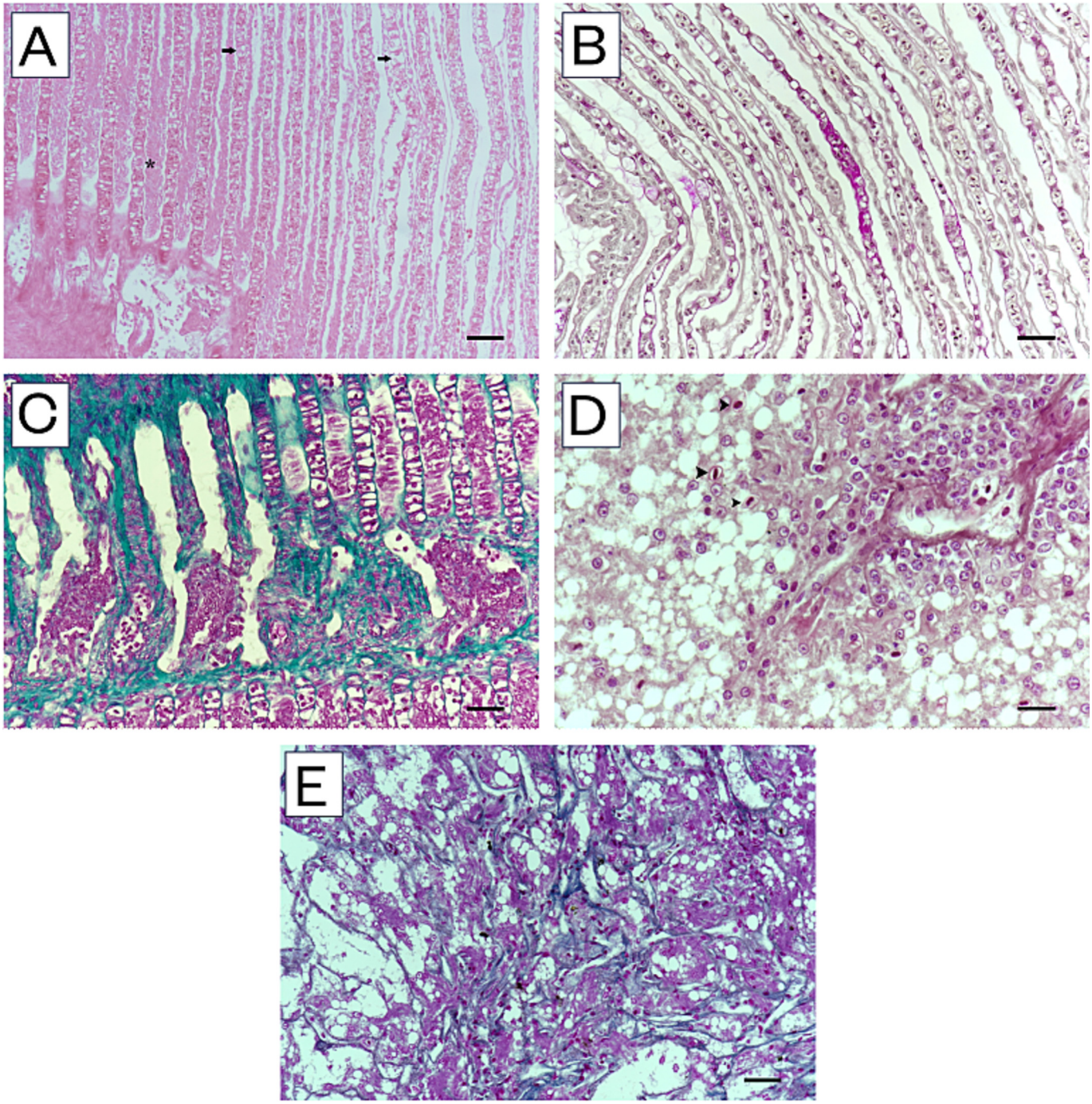


Fig. 2. Histological lesions observed in tissue samples from blue sharks (*Prionace glauca*) captured in the Northeast Atlantic. A Gills; cellular proliferation and acellular debris in some lamellae (asterisk). Dilations of vessels (arrow). H-E. Scale bar = 400 μm. B Gills; slight positivity to PAS (purple). PAS. Scale bar = 200 μm. C Gills; interlamellar connective proliferation (green). Masson's trichrome. Scale bar = 200 μm. D Liver; hepatocytes with great vacuole in its cytoplasm. Some normal hepatocytes around vessels. Cells in mitosis (arrowheads). H-E. Scale bar = 200 μm. E Liver; disrupting in the tissular architecture with scar connective tissue proliferation (green). Masson's trichrome. Scale bar = 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$1.353e^{-2}$). Akin to what was observed when all sharks were considered, highlighting the zone of capture showed the clearest segregation on the responses measured even when no adults are considered. Juveniles from inside EEZ were mostly characterized by high levels of DNA damage in liver tissue and high activity of SOD in both tissues, and juveniles from outside EEZ presented generally more diverse responses and were also associated with lower levels of the biomarkers that were elevated in animals from the other zone.

3.4. Contamination versus responses

Correlations between contaminants (liver) and histopathological and biochemical biomarkers (liver and gills) were assessed for all sharks and the results can be seen in Supplementary Fig. 5. The correlations pertaining, specifically, to the contaminants that were significantly higher in samples from inside EEZ for either all sharks or just juveniles are highlighted in Fig. 6.

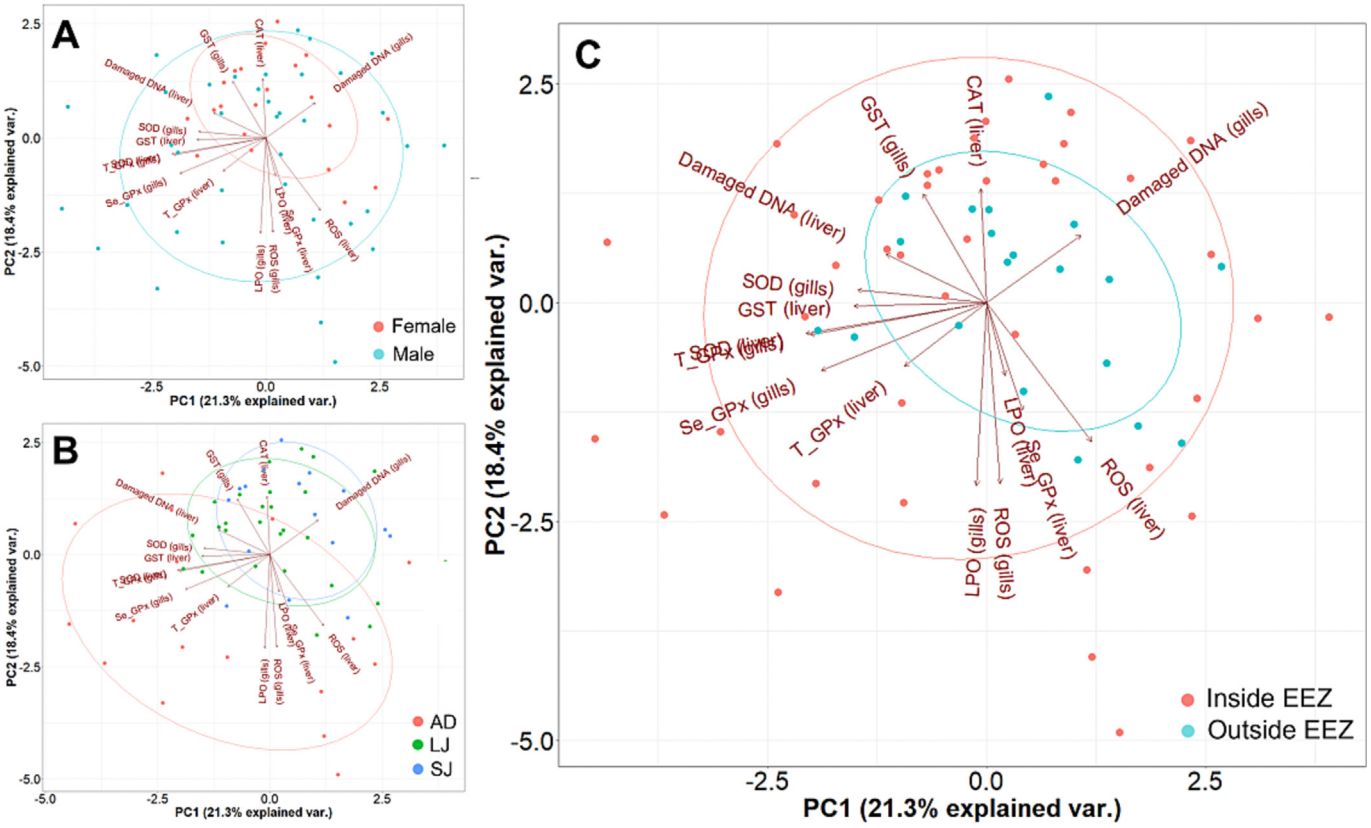


Fig. 3. Biplots created with axis 1 and 2 from the same Principal Component Analysis (PCA) performed for biochemical responses measured in liver and gill samples from blue sharks (*Prionace glauca*), highlighting: A) the different sexes – red = female samples, blue = male samples; B) the different size/maturity groups – pink = adults (AD), green = large juveniles (LJ), blue = small juveniles (SJ); C) the different zones of sampling – pink = individuals sampled at locations inside EEZ (≤ 200 nautical miles), blue = individuals sampled at locations outside EEZ (> 200 nautical miles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

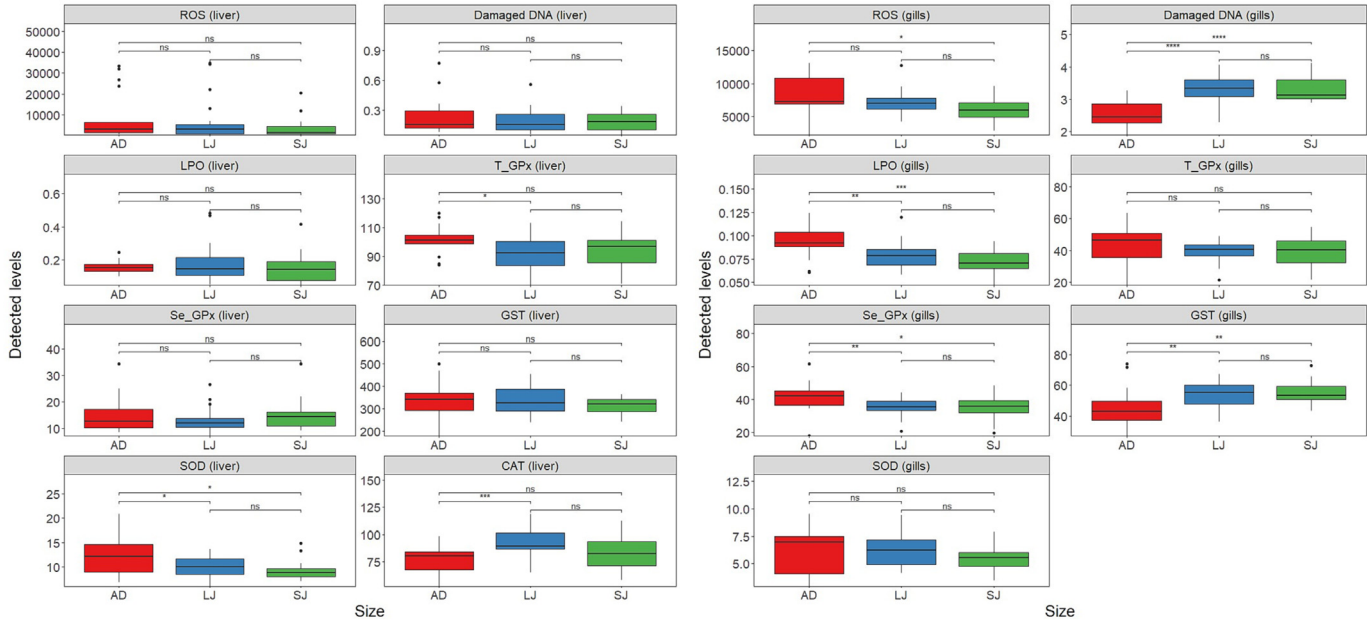


Fig. 4. Boxplots for the biochemical biomarkers measured in liver and gill samples from blue sharks (*Prionace glauca*) with different size groups captured in the Northeast Atlantic. ROS (reactive oxygen species; fluorescence units (FU) mg^{-1} of wet weight (ww), damaged DNA (units), LPO (lipid peroxidation; nmol of TBARS g^{-1} of ww), T_GPx (total glutathione peroxidase; nmol min^{-1} mg^{-1} of protein), Se_GPx (selenium-dependent GPx; nmol min^{-1} mg^{-1} of protein), GST (glutathione-S-transferase; nmol min^{-1} mg^{-1} of protein), SOD (superoxide dismutase; U mg^{-1} of protein), CAT (catalase; μmol min^{-1} mg^{-1} of protein). Differences between groups were assessed using Wilcoxon rank sum tests. ns = p-value >0.05, * = p-value <0.05, ** = p-value <0.01, *** = p-value <0.001, **** = p-value <0.0001; SJ = small juveniles; LJ = large juveniles; AD = adults. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The nature of the correlations varied depending on the pair of contaminant and biomarker being tested. Regarding the liver biomarkers, both As and DL-PCBs correlated positively with SOD, and As also correlated positively with T_GPx. Most of the contaminants represented in Fig. 6 correlated negatively with both ROS and LPO measured in liver samples, but they all correlated positively with the presence of damaged DNA in the same tissue. The trend was completely reversed when looking at damaged DNA measured in the gills, with all contaminants correlating negatively with this biomarker. Arsenic correlated positively with both Se_GPx and T_GPx measured in gills, and all the three groups of organic contaminants correlated positively with some degree of histological damage in that tissue.

4. Discussion

Large marine predators are known to accumulate higher levels of pollutants in their bodies than most of the other animals in their ecosystem (Olmedo et al., 2013; Teffer et al., 2014). This may have negative impacts on their health and pose a threat to their human consumers, but also provides an opportunity to use these animals as bioindicators of marine contamination. The blue shark is one of the best-studied shark species in this regard, with dozens of reports on its contamination levels available in the literature (Tiktak et al., 2020). However, not much is known about the effects that high concentrations of contaminants have on this species. Juvenile blue sharks caught in the North Atlantic have been found to possess elevated concentrations of both inorganic and organic contaminants, with some of those contaminants having been linked to signs of negative impacts on the health of the sharks (Alves et al., 2016). Recently, the concentrations of organic and inorganic contaminants, in liver and muscle, respectively, detected in a different and more diverse set of blue sharks caught in the same region were reported to be even more worrisome (Alves et al., 2023). The same study also identified a tendentially higher degree of contamination in sharks caught closer to Portugal's continental shore (i.e., inside EEZ), when compared with sharks caught in a more oceanic zone (i.e., outside EEZ).

The present study intended to build on the findings described in Alves et al. (2023) by assessing if the differences in levels of contaminants lead to differences in biomarker responses in liver and gill tissues, and if specific contaminants could be inducing specific responses that could be used to assess the health status of the sharks. To do so, biochemical responses related to oxidative stress were measured in the liver, along with the histopathological alterations in gills and livers, organs with important physiological and metabolic functions related to contaminant uptake and detoxification.

4.1. Histological lesions

The histopathological evaluation showed that the gills of the sampled sharks were mainly affected by progressive alterations, and these lesions were found in all studied individuals. While there were some differences in the prevalence of some lesions amongst size/maturity groups, both juveniles and adults from inside EEZ exhibited higher severity levels of epithelial proliferation when compared with sharks from outside EEZ. Given the nature of the histological alterations assessed, the higher degree of epithelial proliferation in sharks from inside EEZ may be associated with the fact that sharks from that zone had significantly higher concentrations of contaminants (further discussed in subsequent sections). Proliferative changes, which involve an increase in the respiratory diffusion distance, represent a defence mechanism to reduce the entry of exogenous agents (Kaya et al., 2013). However, this protection strategy can impair gas exchange and excretion of substances, impacting physiological and metabolic functions (Campagna et al., 2007). An increase in the number of mucous cells in the interlamellar space was found in some sharks, along with a light positivity to PAS staining (PAS staining reveals polysaccharides as well as mucoprotein). In fish, mucous is composed of glycoproteins, sialic acid (a carboxylated monosaccharide), sulphated monosaccharides and glycosaminoglycans. Goblet cells produce mucous granules that release this material by exocytosis (as reviewed by Shephard, 1994). Since mucous contains specific immunoglobulins and lysozymes (as reviewed by Shephard, 1994), the increase in its secretion is another protective response of the gills against external factors (Reverter et al., 2018), that can also hinder adequate oxygen uptake by increasing the lamellar fusion. Regarding the liver, regressive changes were described in all sharks sampled. Accumulation of fat is a reversible change due to an imbalance of lipid metabolism (increased synthesis or uptake of lipids and/or decreased lipid metabolism) or inhibition of lipid excretion impairing metabolic function, a consequence

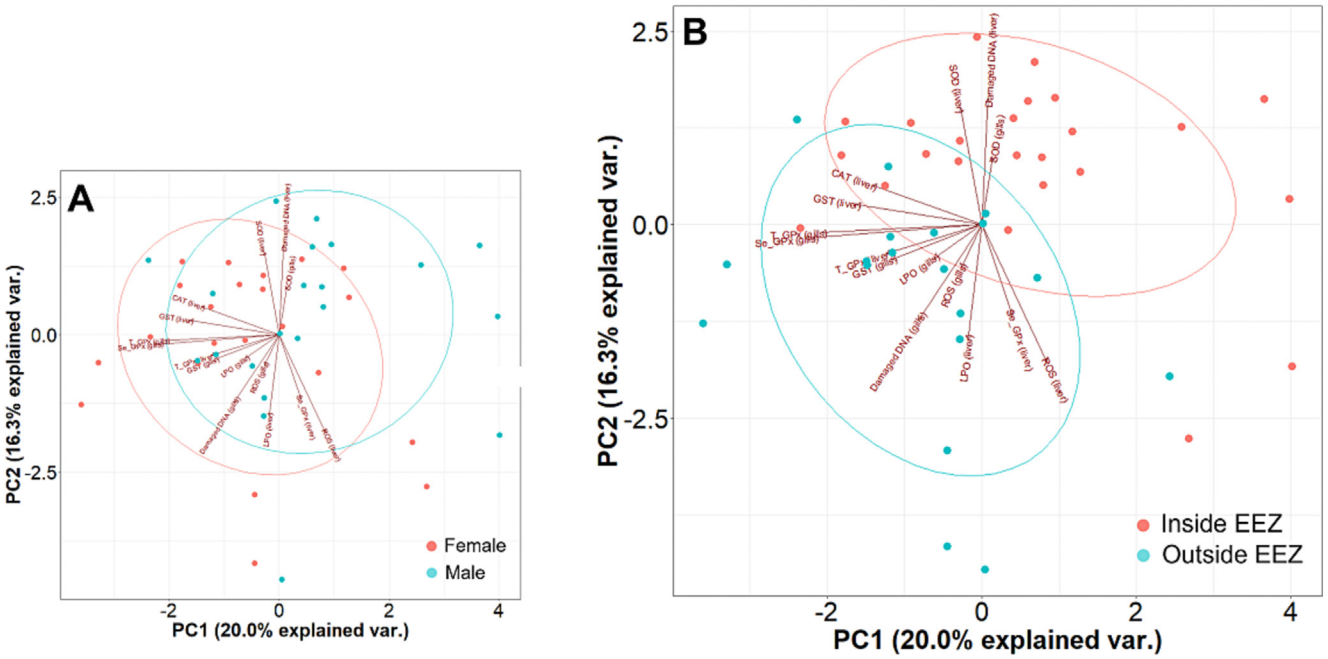


Fig. 5. Biplots with axis 1 and 2 from the same Principal Component Analysis (PCA) performed for biochemical responses measured in liver and gill samples from juvenile blue sharks (*Prionace glauca*), highlighting: A) the different sexes – pink = female samples, blue = male samples; B) the different zones of sampling – pink = individuals sampled at locations inside EEZ (≤ 200 nautical miles), blue = individuals sampled at locations outside EEZ (> 200 nautical miles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

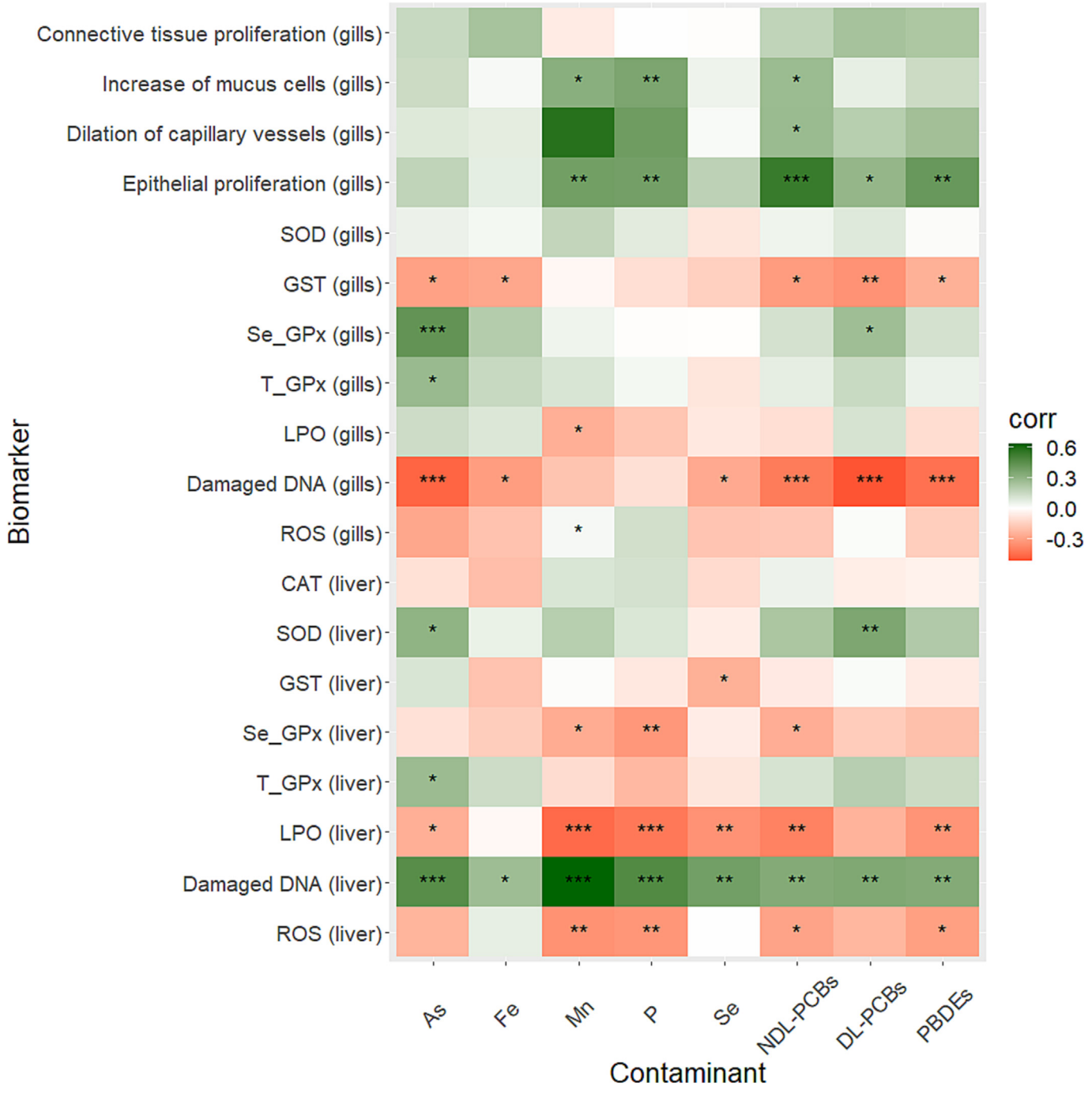


Fig. 6. Heatmaps with correlations (Spearman's test) between liver contaminants significantly more concentrated in individuals from inside EEZ, and biomarkers measured in gills and liver from blue sharks (*Prionace glauca*) captured in the Northeast Atlantic. * = 0.05 > p-value >0.01; ** = 0.01 > p-value >0.001; *** = p-value <0.001. Red represents negative associations; green represents positive associations. ROS = reactive oxygen species, LPO = lipid peroxidation, T_GPx = total glutathione peroxidase, Se_GPx = selenium-dependent GPx, GST = glutathione-S-transferase, SOD = superoxide dismutase, CAT = catalase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of reduced hepatocyte surface area (George et al., 2017). Hepatocytes with signs of attempted regeneration and scar connective tissue proliferation like repair tissue after an injury (Zachary and McGavin, 2012), were also remarkable lesions. Since all sharks scored the same value on the severity score for hepatocyte degeneration (i.e., the highest (Supplementary Table 2), this parameter was not considered further for the integration with the contamination data, being of limited use as a biomarker to assess the effects of the contamination detected on the sharks studied in this work. Overall, the histological lesions found in the liver are not pathognomonic but provide information about hepatic functionality. The liver is an essential organ in the response to contamination for most organisms, being responsible for the biotransformation and detoxification of contaminants (Grant, 1991). This organ is known to be particularly important for sharks and their relatives, as these fishes lack a swimming bladder and rely on the liver for buoyancy (Ebert and Winton, 2010). Sharks have also been shown to produce high amounts of liver oils with important antioxidant and immunological traits (Kim and Karadeniz, 2012). While blue shark muscle tends to possess higher concentrations of inorganic contaminants when compared with the liver (Alves et al., 2023; Boldrocchi et al., 2019), the latter usually assimilates contaminants faster, giving insight to

more recent exposures and therefore allowing for a better tracking of contamination. The liver is also the organ where some of the most nefarious contaminants (i.e., organic) tend to accumulate preferably (Boldrocchi et al., 2019; Muñoz-Arnanz et al., 2022) and where the first and main responses to contamination occur, making it ideal to study not only the concentrations of contaminants but also the early responses they may elicit.

4.2. Biochemical biomarkers of oxidative stress

The liver's role in the response to contamination justifies the overall more pronounced biochemical responses in this tissue when compared with gills (Supplementary Fig. 5). The failure to detect CAT activity in the gills suggests lower levels of this enzyme in this tissue, a characteristic already described in other species of sharks (Lopes et al., 2018). These lower levels of CAT could explain the higher level of Se_GPx in gills when compared with liver, likely due to Se_GPx compensating for the low CAT activity in the decomposition of H_2O_2. Nevertheless, the analysis of correlations between the biomarker pairs that were successfully analysed in both tissues indicates an overall concordance between biomarker pairs. This result could be used to argue that gills have the potential to complement and/or replace liver as a target tissue in future monitoring studies, offering the advantage of not requiring destructive sampling and allowing the use of live sharks as bioindicators. While sex seems to not induce significant differences in the contaminant accumulation in these sharks (Muñoz-Arnanz et al., 2022; Alves et al., 2023), our findings showed some occasional differences between sexes for some of the biochemical biomarkers assessed (T_GPx in liver and GST in gills). Additionally, some differences were observed between size/maturity groups, where mostly higher antioxidant enzymatic activities were seen in adults. While affecting a small percentage of the responses, something already reported by other studies targeting the same species (Barrera-García et al., 2012, 2013), these differences are a reminder that the chosen biomarker responses can be affected by factors other than the contaminants here assessed (Alves et al., 2022; van der Oost et al., 2003), and that the biological differences existing in each of the sex and size/maturity groups assessed should be considered when using these tools in pollution monitoring surveys.

4.3. Contamination versus biological responses

Using contaminant data from the same set of sharks described in Alves et al. (2023), the present work showed that the tendency for higher contamination in sharks caught closer to the Portuguese continental shore is also true for inorganic contaminants measured in liver samples, with the concentrations of As, Fe, and Se being significantly higher inside EEZ (Fig. 1). Additionally, the results indicate that despite the concentrations of some contaminants correlating positively with the size of the sharks, which leads to the known tendency of adult sharks to be more contaminated than juveniles, the same zone-associated differences remained when only the juvenile sharks were assessed. These results indicate that while size/maturity stage did affect the concentrations of contaminants in the liver of these sharks, the zone they preferentially habit exerted a higher degree of influence.

Looking at Fig. 3, it is possible to observe that most sharks from inside EEZ (Fig. 3C) are males (Fig. 3A) and that many of them are adults (Fig. 3B). Since some positive correlations between size and biochemical biomarkers were found, it can be theorized that the differences in responses observed for sharks from inside EEZ could be mostly because that zone contained almost all the adult sharks. This difference in the demographic composition between both zones, and the overall lack of adult females in our sampling, can be attributed to the combination of the opportunistic nature of our sampling and of the spatial and temporal segregations that have been described for this species in the Atlantic (Vandeperre et al., 2014a, 2014b), something already explored in Alves et al., 2023. To make sure that our interpretation of Fig. 3 was not being forced by the skewness of the sampling, a similar analysis on only juveniles was performed. The PCA (Fig. 5) helped to dismiss that hypothesis, as the sample distribution between sexes and zones is much more balanced (Fig. 5A) and it shows that juveniles from inside and outside EEZ also possess different response patterns (Fig. 5B). Juvenile sharks from inside EEZ were characterized by high levels of DNA damage in liver and high SOD activity in both liver and gills, which positively correlated with each other (DNA liver and SOD gills: rho = 0.334, p-value = $9.188e^{-3}$; DNA liver and SOD liver: rho = 0.369, p-value = $3.876e^{-3}$), suggesting higher oxidative stress levels in this group of juvenile individuals.

While the results show that some of the variability of the biomarker responses measured could be explained by factors other than the contaminants measured, some strong and significant correlations could be found between the liver contaminant levels and both biochemical responses and severity of tissue lesions. Given this lack of specificity to chemical stressors of the biological responses measured, and to increase the relevance of the relationships found with the contaminants, the present analysis was focused on the contaminants that showed to be significantly more accumulated in individuals sampled inside EEZ (Fig. 6).

Although most biomarker pairs from both tissues correlated positively, the correlations between each of the pairs' counterparts and the concentrations of contaminants varied. In both liver and gills the presence of As seems to be inducing the activity of SOD and GPx. Although this species has been reported as having mechanisms to help it deal with As and other contaminants (i.e. metallothioneins) (Hauser-Davis et al., 2021), our results are to be expected given the antioxidant role of the aforementioned enzymes (Winston and Di Giulio, 1991). Notably, organic contaminants were negatively associated with Se_GPx, which aligns with earlier observations using the same species also collected in the North Atlantic (Alves et al., 2016). These negative correlations between GPx and contaminant concentrations have also been reported for other pelagic (Vélez-Alavez et al., 2013) and coastal (Somerville et al., 2020) shark species. Most contaminants correlated negatively with ROS and LPO measured in both liver and gills. While the negative correlation between contaminants and ROS in the liver is somehow unexpected but could be potentially related to the over induction of the antioxidant system, an apparent decrease in LPO in that tissue may occur with the increase of contaminants if the exposure leads to a reduction in levels of highly unsaturated fatty acids, which are very susceptible to being oxidized (Hsieh and Kinsella, 1989). Since studies have shown that exposure to different stressors, including contaminants, can induce alterations in the fatty acid profile (FAP) of fish (Ferain et al., 2018; Gonçalves et al., 2021; Liao et al., 2019), and differences in FAP have been reported in elasmobranchs exhibiting different contamination profiles (Rosenfelder et al., 2012), it would be valuable to perform an evaluation of the fatty acid profile of these sharks' liver and assess if these more contaminated individuals would indeed show a reduction in highly unsaturated fatty acids. In the gills, the observed negative correlation between contaminants and damaged DNA may be associated with the histological lesions observed. While the pathologies described in the gills of the studied sharks are chronic alterations not specifically related to any particular agent, and could be induced by many different stressors (i.e., exposure to environmental pollutants, infectious processes, seasonal variation, etc.), a great deal of the ROS, LPO and DNA damage occurring in the gills is likely derived from their functions in gas exchanges and the absorption/excretion of contaminants (Ellis, 2003). Since the histopathologic analysis indicates that contamination is positively correlated with a protective response in the gills of the sampled sharks, which can result in reduced gas exchanges and reduced absorption and excretion of substances (a notion reinforced by the weak negative correlation existing between ROS in the gills and epithelial proliferation in the same tissue (Spearman's test, rho = -0.317, p-value = $1.369e^{-2}$), higher concentrations of contamination may lead to less ROS from both respiration and bioaccumulation processes which in turn reduce the oxidative damage to the lipids and DNA in this tissue.

Remarkably, all contaminants represented in Fig. 6 correlated positively with damaged DNA in the liver, a clear sign that the presence of these contaminants is inducing harm to the health of these sharks. Positive associations between DNA damage in the liver and the concentrations of inorganic and organic contaminants measured in that tissue have already

been reported in blue sharks from the North Atlantic (Alves et al., 2016), supporting the idea that this is a good biomarker of effect of contamination in the liver of this species. It is noteworthy that in that previous study from 2016, with a much smaller number of individuals and all being juveniles, the biomarkers that showed stronger relationships with the contaminant's levels were precisely the DNA damage (positive correlations) and Se_GPx activity (negative correlation). The fact that this trend was this time observed in with a larger and more complete sampling set reinforces the potential of these biomarkers for assessing contamination effects in this species. While the monitoring of the health of the populations of this species presents some difficulties due to the areas it chooses for reproduction, the frequency and scale of its migrations, and the unreliability of its fisheries' data, this study's results indicate that pollution is exerting damage to these animals and therefore forcing them to diverge energetic resources that could otherwise be used for growth and reproduction, and may cause cumulative impacts in an already heavily pressured species (Lemos, 2021).

5. Conclusions

The present study builds on the findings of previous works and shows that sharks caught closer to Portugal's continental shore (inside EEZ) present higher concentrations of inorganic contaminants in their livers, when compared with individuals caught in more oceanic locations (outside EEZ). This study also indicates that the higher levels of contamination detected in sharks caught inside EEZ may be eliciting more pronounced biochemical responses. Our data showed higher levels of DNA damage in the liver and histological lesions in the gills in animals from the zone where contaminants like As, PCBs, and PBDEs were found to be significantly more concentrated. Despite the contradicting results with what seems to be one of the best indicators of contamination impacts on these sharks' health (i.e., damaged DNA), the gills showed potential as a source tissue for future monitoring studies using other biomarkers. They offer the advantage of allowing for lesser-destructive sampling and, according to our data, the histological damage observed in this tissue was a good indicator of contamination with prominent groups of organic contaminants. The results of this study demonstrate the importance of integrating responses at different levels of biological organization, from molecular and biochemical to more apical endpoints, to increase the knowledge on the pathways towards adverse outcomes and to pinpoint the most valuable biomarkers to assess physiological alterations in these organisms. The fact that positive correlations with injury were found specifically for contaminants that were, for the most part, significantly higher in the more contaminated zone is indicative of the suitability of the species and these tools to be used to monitor contamination and its effects in different areas of the Atlantic.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162095.

CRediT authorship contribution statement

Luís M.F. Alves: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Ariana B. Moutinho:** Investigation. **Luis J. Gómez:** Investigation, Writing – original draft. **Ana L. Oropesa:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Juan Muñoz-Arnanz:** Investigation, Writing – review & editing. **Begoña Jiménez:** Writing – review & editing. **Marco F.L. Lemos:** Resources, Conceptualization, Writing – review & editing. **Vanessa F. Fonseca:** Supervision, Conceptualization, Formal analysis, Writing – review & editing. **Henrique Cabral:** Supervision, Writing – review & editing. **Sara C. Novais:** Supervision, Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing.

Data availability

Data will be made available on request.

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.

Acknowledgements

This study had the support of Fundação para a Ciência e a Tecnologia (FCT) through the project BLUESHARKER (PTDC/CTAAMB/29136/2017), co-financed by COMPETE2020 (POCI-01-0145-FEDER-029136), the Strategic Project granted to MARE – Marine and Environmental Sciences Centre (UIDB/04292/2020 and UIDP/04292/2020), the project granted to the Associate Laboratory ARNET (LA/P/0069/2020), and the grant awarded to Luís Alves (SFRH/BD/122082/2016). Vanessa F. Fonseca was supported by FCT, via research contract 2021.00244.CEECIND. Sara Novais is funded by national funds (OE), through FCT, in the scope of the framework contract foreseen in the numbers 4, 5, and 6 of the article 23, of the Decree-Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

References

Alves, L.M.F., Lemos, M.F.L., Correia, J.P.S., da Costa, N.A.R., Novais, S.C., 2015. The potential of cholinesterases as tools for biomonitoring studies with sharks: biochemical characterization in brain and muscle tissues of Prionace glauca. J. Exp. Mar. Bio. Ecol. 465, 49–55. https://doi.org/10.1016/j.jembe.2015.01.006.

Alves, L.M.F., Nunes, M., Marchand, P., Le Bizec, B., Mendes, S., Correia, J.P.S., Lemos, M.F.L., Novais, S.C., 2016. Blue sharks (Prionace glauca) as bioindicators of pollution and health in the Atlantic Ocean: contamination levels and biochemical stress responses. Sci. Total Environ. 563–564, 282–292. https://doi.org/10.1016/j.scitotenv.2016.04.085.

Alves, L.M.F., Lemos, M.F.L., Cabral, H., Novais, S.C., 2022. Elasmobranchs as bioindicators of pollution in the marine environment. Mar. Pollut. Bull. 176, 113418. https://doi.org/10.1016/j.marpolbul.2022.113418.

Alves, L.M.F., Lemos, M.F.L., Moutinho, A.B., Ceia, F.R., Muñoz-Arnanz, J., Jiménez, B., Cabral, H., Novais, S.C., 2023. Assessment of contaminants in blue sharks from the Northeast Atlantic: profiles, accumulation dynamics, and risks for human consumers. Environ. Pollut. 316, 120467. https://doi.org/10.1016/j.envpol.2022.120467.

Baeyens, W., Leermakers, M., Elskens, M., Van Larebeke, N., De Bont, R., Vanderperren, H., Fontaine, A., Degroodt, J.-M., Goeyens, L., Hanot, V., Windal, I., 2007. PCBs and PCDD/FS in fish and fish products and their impact on the human body burden in Belgium. Arch. Environ. Contam. Toxicol. 52, 563–571. https://doi.org/10.1007/s00244-005-0266-6.

Barrera-García, A., O'Hara, T., Galván-Magaña, F., Méndez-Rodríguez, L.C., Castellini, J.M., Zenteno-Savín, T., O'Hara, T., Galván-Magaña, F., Méndez-Rodríguez, L.C., Castellini, J.M., Zenteno-Savín, T., O'Hara, T., Galván-Magaña, F., Méndez-Rodríguez, L.C., Castellini, J.M., Zenteno-Savín, T., 2012. Oxidative stress indicators and trace elements in the blue shark (Prionace glauca) off the east coast of the Mexican Pacific Ocean. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 156, 59–66. https://doi.org/10.1016/j.cbpc.2012.04.003.

Barrera-García, A., O'Hara, T., Galván-Magaña, F., Méndez-Rodríguez, L.C., Castellini, J.M., Zenteno-Savín, T., 2013. Trace elements and oxidative stress indicators in the liver and kidney of the blue shark (Prionace glauca). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165, 483–490. https://doi.org/10.1016/j.cbpa.2013.01.024.

Bird, R.P., Draper, H.H., 1984. [35] Comparative studies on different methods of malonaldehyde determination, pp. 299–305 https://doi.org/10.1016/S0076-6879(84)05038-2.

Boldrocchi, G., Monticelli, D., Omar, Y.M., Bettinetti, R., 2019. Trace elements and POPs in two commercial shark species from Djibouti: implications for human exposure. Sci. Total Environ. 669, 637–648. https://doi.org/10.1016/j.scitotenv.2019.03.122.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Campagna, A.F., Eler, M.N., Fracácio, R., Rodrigues, B.K., Verani, N.F., 2007. The toxic potential of aldrin and heptachlor on Danio rerio juveniles (Cypriniformes, Cyprinidae). Ecotoxicology 16, 289–298. https://doi.org/10.1007/s10646-006-0127-8.

Claiborne, A., 1985. Catalase activity. (1985)CRC Handbook of Methods for Oxygen Radical Research. CRC Press, Boca Raton, FL, pp. 283–284.

Team, Rs, 2020. RStudio: Integrated Development Environment for R.

De Lafontaine, Y., Gagné, F., Blaise, C., Costan, G., Gagnon, P., Chan, H.M., 2000. Biomarkers in zebra mussels (Dreissena polymorpha) for the assessment and monitoring of water quality of the St Lawrence river (Canada). Aquat. Toxicol. 50, 51–71.

Ebert, D.A., Winton, M.V., 2010. Sharks And Their Relatives II, Sharks And Their Relatives II: Biodiversity, Adaptive Physiology, And Conservation. CRC Press https://doi.org/10.1201/9781420080483.

Ellis, M.M., 2003. Effects of Pollution on Fish, Progressive Fish-Culturist. Blackwell Science Ltd, Oxford, UK https://doi.org/10.1002/9780470999691.

Ferain, A., Bonnineau, C., Neefs, I., De Saeyer, N., Lemaire, B., Cornet, V., Larondelle, Y., De Schamphelaere, K.A.C., Debier, C., Rees, J.F., 2018. Exploring the interactions between polyunsaturated fatty acids and cadmium in rainbow trout liver cells: a genetic and

proteomic study. Aquat. Toxicol. 205, 100–113. https://doi.org/10.1016/j.aquatox.2018.09.005.
Flohé, L., Günzler, W.A., 1984. [12] Assays of glutathione peroxidase, pp. 114–120 https://doi.org/10.1016/S0076-6879(84)05015-1.
George, O.O., Amaeze, N.H., Babatunde, E., Otitoloju, A.A., 2017. Genotoxic, histopathological and oxidative stress responses in catfish, Clarias gariepinus, exposed to two antifouling paints. J. Health Pollut. 7, 71–82. https://doi.org/10.5696/2156-9614-7.16.71.
Gonçalves, A.M.M., Rocha, C.P., Marques, J.C., Gonçalves, F.J.M., 2021. Fatty acids as suitable biomarkers to assess pesticide impacts in freshwater biological scales – a review. Ecol. Indic. 122. https://doi.org/10.1016/j.ecolind.2020.107299.
Grant, D.M., 1991. Detoxification pathways in the liver. J. Inherit. Metab. Dis. 14, 421–430. https://doi.org/10.1007/BF01797915.
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8.
Harrel, F.E., 2022. Harrell Miscellaneous.
Hauser-Davis, R.A., Rocha, R.C.C., Saint'Pierre, T.D., Adams, D.H., 2021. Metal concentrations and metallothionein metal detoxification in blue sharks, Prionace glauca L. from the Western North Atlantic Ocean. J. Trace Elem. Med. Biol. 68, 126813. https://doi.org/10.1016/j.jtemb.2021.126813.
Holt, E.a., 2010. Bioindicators: using organisms to measure environmental impacts. Nat. Educ. Knowl. 3, 1–8. https://doi.org/10.5962/bhl.title.62081.
Hsieh, R.J., Kinsella, J.E., 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. Adv. Food Nutr. Res. 33, 233–341. https://doi.org/10.1016/S1043-4526(08)60129-1.
Kaya, H., Çelik, E.Ş., Gürkan, M., Yılmaz, S., Akbulut, M., 2013. Effects of subchronic exposure to phosalone on oxidative stress and histopathological alterations in common carp (Cyprinus carpio, L., 1758). J. Toxicol. Environ. Health A 76, 853–864. https://doi.org/10.1080/15287394.2013.823136.
Kim, S.K., Karadeniz, F., 2012. Biological importance and applications of squalene and squalane. Advances in Food And Nutrition Research, 1st ed. Elsevier Inc. https://doi.org/10.1016/B978-0-12-416003-3.00014-7.
Lemos, M.F.L., 2021. Biomarker studies in stress biology: from the gene to population, from the organism to the application. Biology 10, 1340. https://doi.org/10.3390/biology10121340 (Basel).
Liao, K., Ran, Z., Meng, R., Xu, J., Cao, J., Xu, X., Wang, Y., Xu, S., Yan, X., 2019. Long-chain polyunsaturated fatty acid biosynthesis and its response to cadmium exposure in silver pomfret. Aquat. Toxicol. 206, 61–71. https://doi.org/10.1016/j.aquatox.2018.11.002.
Lima, I., Moreira, S.M., Osten, J.R.-V., Soares, A.M.V.M., Guilhermino, L., 2007. Biochemical responses of the marine mussel Mytilus galloprovincialis to petrochemical environmental contamination along the North-western coast of Portugal. Chemosphere 66, 1230–1242. https://doi.org/10.1016/j.chemosphere.2006.07.057.
Lopes, A.R., Sampaio, E., Santos, C., Couto, A., Pegado, M.R., Diniz, M., Munday, P.L., Rummer, J.L., Rosa, R., 2018. Absence of cellular damage in tropical newly hatched sharks (Chiloscyllium plagiosum) under ocean acidification conditions. Cell Stress Chaperones 23, 837–846. https://doi.org/10.1007/s12192-018-0892-3.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. J. Biol. Chem. 244, 6049–6055. https://doi.org/10.1016/S0021-9258(18)63504-5.
Muñoz-Arnanz, J., Bartalini, A., Alves, L., Lemos, M.F., Novais, S.C., Jiménez, B., 2022. Occurrence and distribution of persistent organic pollutants in the liver and muscle of Atlantic blue sharks: relevance and health risks. Environ. Pollut. 309, 119750. https://doi.org/10.1016/j.envpol.2022.119750.
Neff, J.M., 1997. Ecotoxicology of arsenic in the marine environment. Environ. Toxicol. Chem. 16, 917–927. https://doi.org/10.1002/etc.5620160511.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
Olive, P.L., 1988. DNA precipitation assay: a rapid and simple method for detecting DNA damage in mammalian cells. Environ. Mol. Mutagen. 11, 487–495. https://doi.org/10.1002/em.2850110409.
Olmedo, P., Pla, A., Hernández, A.F.F., Barbier, F., Ayouni, L., Gil, F., 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ. Int. 59, 63–72. https://doi.org/10.1016/j.envint.2013.05.005.
van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.
R Core Team, 2020. R: A Language And Environment for Statistical Computing.
Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2018. Biological and ecological roles of external fish mucus: a review. Fishes 3, 40–41. https://doi.org/10.3390/fishes3040041.
Rosenfelder, N., Lehnert, K., Kaffarnik, S., Torres, J.P.M., Vianna, M., Vetter, W., 2012. Thorough analysis of polyhalogenated compounds in ray liver samples off the coast of Rio de Janeiro,Brazil. Environ. Sci. Pollut. Res. 19, 379–389. https://doi.org/10.1007/s11356-011-0569-2.
Schlenk, D., Celander, M., Gallagher, E.P., George, S., James, M., Kullman, S.W., Van Den Hurk, P., Willett, K., 2008. The Toxicology of Fishes, The Toxicology of Fishes. CRC Press https://doi.org/10.1201/9780203647295.
Schober, P., Boer, C., Schwarte, L.A., 2018. Correlation coefficients. Anesth. Analg. 126, 1763–1768. https://doi.org/10.1213/ANE.0000000000002864.
Shephard, K.L., 1994. Functions for fish mucus. Rev. Fish Biol. Fish. 4, 401–429. https://doi.org/10.1007/BF00042888.
Socci, D.J., Bjugstad, K.B., Jones, H.C., Pattisapu, J.V., Arendash, G.W., 1999. Evidence that oxidative stress is associated with the pathophysiology of inherited hydrocephalus in the H-Tx rat model. Exp. Neurol. 155, 109–117. https://doi.org/10.1006/exnr.1998.6969.
Somerville, R., Fisher, M., Persson, L., Ehnert-Russo, S., Gelsleichter, J., Bielmyer-Fraser, G., 2020. Analysis of trace element concentrations and antioxidant enzyme activity in muscle tissue of the Atlantic sharpnose shark, Rhizoprionodon terraenovae. Arch. Environ. Contam. Toxicol. 79, 371–390. https://doi.org/10.1007/s00244-020-00753-8.
Taylor, V., Goodale, B., Raab, A., Schwerdtle, T., Reimer, K., Conklin, S., Karagas, M.R., Francesconi, K.A., 2017. Human exposure to organic arsenic species from seafood. Sci. Total Environ. 580, 266–282. https://doi.org/10.1016/j.scitotenv.2016.12.113.
Teffer, A.K., Staudinger, M.D., Taylor, D.L., Juanes, F., 2014. Trophic influences on mercury accumulation in top pelagic predators from offshore New England waters of the northwest Atlantic Ocean. Mar. Environ. Res. 101, 124–134. https://doi.org/10.1016/j.marenvres.2014.09.008.
Tiktak, G.P., Butcher, D., Lawrence, P.J., Norrey, J., Bradley, L., Shaw, K., Preziosi, R., Megson, D., 2020. Are concentrations of pollutants in sharks, rays and skates (Elasmobranchii) a cause for concern?A systematic review. Mar. Pollut. Bull. 160, 111701. https://doi.org/10.1016/j.marpolbul.2020.111701.
Torres, M.A., Testa, C.P., Gaspari, C., Masutti, M.B., Panitz, C.M.N., Curi-Pedrosa, R., de Almeida, E.A., Di Mascio, P., Wilhelm Filho, D., 2002. Oxidative stress in the mussel Mytella guyanensis from polluted mangroves on Santa Catarina Island, Brazil. Mar. Pollut. Bull. 44, 923e932.
United Nations, 2022. Ocean Conference of the United Nations. Interactive Dialogue 1: Addressing Marine Pollution. GA Res 73/292, UN Doc A/CONF.230/2022/9 (April 2022).
Vandeperre, F., Aires-da-Silva, A., Fontes, J., Santos, M., Serrão Santos, R., Afonso, P., 2014a. Movements of blue sharks (Prionace glauca) across their life history. PLoS One 9, e103538. https://doi.org/10.1371/journal.pone.0103538.
Vandeperre, F., Aires-da-Silva, A., Santos, M., Ferreira, R., Bolten, A.B., Serrao Santos, R., Afonso, P., 2014b. Demography and ecology of blue shark (Prionace glauca) in the central North Atlantic. Fish. Res. 153, 89–102. https://doi.org/10.1016/j.fishres.2014.01.006.
Vélez-Alavez, M., Labrada-Martagón, V., Méndez-Rodriguez, L.C., Galván-Magaña, F., Zenteno-Savín, T., 2013. Oxidative stress indicators and trace element concentrations in tissues of mako shark (Isurus oxyrinchus). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165, 508–514. https://doi.org/10.1016/j.cbpa.2013.03.006.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Wilhelm Filho, D., Tribess, T., Gáspari, C., Claudio, F., Torres, M., Magalhães, A.R., 2001. Seasonal changes in antioxidant defenses of the digestive gland of the brown mussel (Perna perna). Aquaculture 203, 149–158. https://doi.org/10.1016/S0044-8486(01)00599-3.
Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137–161. https://doi.org/10.1016/0166-445X(91)90033-6.
Zachary, J.F., McGavin, M.D., 2012. Inflammation And Healing in Pathologic Basis of Veterinary Disease. 5th edition. Editorial Elsevier.