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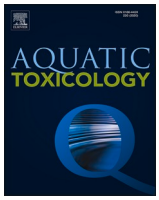

Tissue metal concentrations and antioxidant enzyme activity in western north Atlantic white sharks (*Carcharodon carcharias*)

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ABSTRACT

Anthropogenic practices have increased metal contamination in marine ecosystems. Most sharks have long lifespans, occupy an important ecological position at the top of marine food webs, and can accumulate metals. However, reference levels of metal contaminants in the tissues of sharks, particularly, apex predators such as the white shark (*Carcharodon carcharias*), are lacking. In this study, concentrations of copper (Cu), cadmium (Cd), nickel (Ni), lead (Pb), silver (Ag), and zinc (Zn) were measured in the muscle tissue of white ($n = 42$) and tiger (*Galeocerdo cuvier;* $n = 3$) sharks. Metal exposure in various species, including sharks, has been correlated with increased oxidative stress. Therefore, the main objectives of this study were to assess metal accumulation and antioxidant enzyme activity (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) in the muscle tissue of the population of white sharks and tiger sharks inhabiting the Western North Atlantic. The measured parameters were qualitatively compared between species. The small sample size of tiger sharks (collected from only one site) limited statistical analyses, therefore, white sharks were the primary focus of this study. Differences in tissue metal (Cu, Cd, Ni, and Zn) concentrations and antioxidant enzyme activities were detected based on collection site, with significant positive correlations between Cd and enzymes, SOD and CAT, and Zn and enzymes, SOD and GPx in *C. carcharias*. Differences in Ni concentration were detected based on sex, with females having higher Ni levels. Additionally, plasma osmolality was not correlated with tissue metal concentrations; however, osmolality decreased with increasing length in *C. carcharias*. This study is the first to report baseline levels of Cu, Zn, Cd, Ni, Ag, and Pb in muscle of North Atlantic white sharks and provides new insights into oxidative stress responses of these sensitive species to metal contaminants.

1. Introduction

Metals occur naturally in coastal ecosystems but are introduced to a greater extent via anthropogenic practices such as mining, urban development, wastewater treatment, agriculture, fossil fuel combustion, marine disposal of municipal solid waste, and in anti-fouling paints (Nriagu, 1996; Esslemont, 2000; Voulvoulis et al., 2000; Echols et al., 2009). Sharks as a common component of marine coastal fauna may be exposed to a variety of pollutants, including metals, particularly in stormwater runoff from more developed areas (Bielmyer et al., 2012a; Lopez et al., 2013; Somerville et al., 2020). Sharks have comparatively long lifespans, slow growth, low fecundity rates, and long gestation periods, and most occupy upper-level trophic positions (Bradford et al., 2020; Franks et al., 2021), all of which could increase their susceptibility to metal accumulation (Gelsleichter, and Walker, 2010; Bosch et al., 2015; Wosnick et al., 2021).

White sharks (*Carcharodon carcharias*) are found in temperate and sub-tropical waters of all major oceans and adults range from 3.5 to 6.4 m in length (Franks et al., 2021; Compagno, 2001; Bruce, 2008). These macropredators feed on a variety of marine mammals and fish including other elasmobranchs, are highly migratory, and exhibit residency and philopatric behaviors (Franks et al., 2021), potentially exposing them to increased pollution levels at certain locations. White sharks are categorized as Vulnerable and Moderately Depleted (Bruce, 2008), with a Decreasing Population Trend worldwide, in the International Union for Conservation of Nature (IUCN) Red List (Rigby et al., 2022). In the U.S., white sharks are protected by various coastal states and are federally managed as Prohibited Species under the Magnuson-Stevens Fishery

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Conservation and Management Act. Assessing tissue metal concentrations in this species is important for effective management strategies to rebuild their populations. Except for mercury, few studies have investigated accumulation of metals in tissues of white sharks (Mull et al., 2012; Gilbert et al., 2015; Merely et al., 2019).

Metal uptake pathways for marine animals include the intestine via ingestion, which is the dominant route, and through the gills via respiration (Webb and Wood, 2000; Bury et al., 2003; Bielmyer et al., 2005, 2006; Mathews and Fisher, 2009). When uptake exceeds detoxification and excretion, metals may bioaccumulate in various tissues including gills, intestine, liver, muscle, and kidneys (Webb and Wood, 2000; Bielmyer et al., 2005, 2006; De Boeck et al., 2007; Núñez-Nogueira, 2005; Eyckmans et al., 2013). At elevated concentrations, metals may exert toxic effects in fish such as mortality, reduced growth, decreased reproduction, and impaired respiration and osmoregulation, as well as others (Bielmyer et al., 2005, 2006; De Boeck et al., 2007; Pane et al., 2003).

Metals can exert oxidative stress in sharks and many other aquatic animals (Main et al., 2010; Brock and Bielmyer, 2013; Blewett and Wood, 2014; Patel and Bielmyer-Fraser, 2015; Bielmyer-Fraser et al., 2018a; Somerville et al., 2020). Metal exposure to aquatic organisms can lead to the formation of reactive oxygen species (ROS), which interact with biological molecules to cause protein denaturing, lipid peroxidation, and DNA mutations (Valko et al., 2005; Close and Hagerman, 2006; Ray et al., 2012). Specifically, transition metals react with hydrogen peroxide and induce oxidative stress primarily through the Fenton reaction, resulting in hydroxyl radical formation (Fenton, 1876, 1894; Imlay et al., 1988; Valko et al., 2005; Close and Hagerman, 2006). Enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) help to convert ROS to harmless forms (Martínez-Álvarez et al., 2005; Close and Hagerman, 2006; Birben et al., 2012). SOD catalyzes the conversion of superoxide anion radical to hydrogen peroxide, and via the enzymatic action of either CAT or GPx, hydrogen peroxide is converted to water (Valko et al., 2005; Close and Hagerman, 2006; Ray et al., 2012). Metal accumulation in sharks has been correlated with changes in activities of these antioxidant enzymes (Vélez-Alavez et al., 2013; Alves et al., 2016; Somerville et al., 2020). Toxicity and cellular dysfunction may result when antioxidant defenses become overburdened (Martínez-Álvarez et al., 2005; Close and Hagerman, 2006; López-Cruz et al., 2010; Birben et al., 2012; Pham et al., 2013; Vélez-Alavez et al., 2013; Marreiro et al., 2017). Continuous exposure to metals and other pollutants can be energetically costly, in producing defenses for detoxification and excretion. Further, identifying a nonlethal bioindicator of metal-induced stress in white sharks can be useful in assessing population health. Therefore, the main objectives of this study were to assess metal accumulation and enzyme activity in

Table 1
Summary data for sampled white sharks (Carcharodon carcharias) and tiger sharks (Galeocerdo cuvier). Tiger sharks are indicated with an asterisk before the shark ID.

Shark ID	Expedition	Date	Sex	Maturity	TL (cm)	Girth (cm)	EST. Weight (kg)	Latitude (dec deg)	Longitude (dec deg)
SE19–02	Southeast 2019	02/15/2019	Female	Juvenile	311	189	383.7	30.36	−80.84
SE19–03	Southeast 2019	02/22/2019	Female	Subadult	379	272	874.4	32.06	−80.42
SE19–04	Southeast 2019	02/26/2019	Male	Juvenile	266	148	200.1	32.00	−80.59
SE19–05	Southeast 2019	02/26/2019	Female	Subadult	388	233	650.7	32.00	−80.59
*SE19–01	Southeast 2019	02/15/2019	Male	Adult	306	128	211.1	30.46	−80.95
*SE19–07	Southeast 2019	02/27/2019	Female	Adult	352	N/A	317.3	32.00	−80.58
*SE19–08	Southeast 2019	02/27/2019	Female	Juvenile/ subadult	N/A	N/A	N/A	32.00	−80.58
NS19–01	Nova Scotia 2019	09/15/2019	Male	Adult	371	229	591.8	46.02	−59.68
NS19–02	Nova Scotia 2019	09/16/2019	Male	Adult	383	211	543.0	46.02	−59.68
NS19–03	Nova Scotia 2019	09/20/2019	Female	Adult	433	273	995.7	46.02	−59.68
NS19–04	Nova Scotia 2019	09/26/2019	Female	Juvenile	250	140	166.2	46.04	−59.69
NS19–05	Nova Scotia 2019	09/29/2019	Female	Subadult	347	183	357.8	44.23	−64.28
NS19–06	Nova Scotia 2019	09/30/2019	Male	Subadult	332	266	735.3	44.23	−64.29
NS19–07	Nova Scotia 2019	10/01/2019	Male	Subadult	288	173	288.3	44.23	−64.29
NS19–08	Nova Scotia 2019	10/01/2019	Female	Juvenile	313	183	348.1	44.22	−64.28
NS19–09	Nova Scotia 2019	10/03/2019	Male	Adult	346	220	539.2	44.23	−64.29
NS19–10	Nova Scotia 2019	10/03/2019	Male	Subadult	313	185	346.5	44.23	−64.29
NS19–11	Nova Scotia 2019	10/04/2019	Male	Adult	363	255	742.5	44.23	−64.28
MA20–01	Massachusetts 2020	08/09/2020	Female	Juvenile	313	120	154.7	41.48	−69.95
MA20–02	Massachusetts 2020	08/11/2020	Male	Juvenile	200	132	121.1	41.48	−69.95
MA20–03	Massachusetts 2020	08/13/2020	Male	Juvenile	246	140	163.2	41.48	−69.95
MA20–04	Massachusetts 2020	08/13/2020	Female	Juvenile	200	107	85.3	41.48	−69.95
NS20–01	Nova Scotia 2020	09/12/2020	Male	Adult	389	229	651.9	46.02	−59.68
NS20–02	Nova Scotia 2020	09/29/2020	Female	Subadult	370	250	726.4	44.23	−64.28
NS20–03	Nova Scotia 2020	10/01/2020	Male	Subadult	315	210	438.3	44.24	−64.27
NS20–04	Nova Scotia 2020	10/01/2020	Male	Adult	392	250	771.4	44.24	−64.27
NS20–05	Nova Scotia 2020	10/01/2020	Female	Juvenile	248	145	179.8	44.24	−64.27
NS20–06	Nova Scotia 2020	10/02/2020	Female	Adult	501	332	1606.0	44.24	−64.27
NS20–07	Nova Scotia 2020	10/04/2020	Female	Juvenile	304	165	272.2	44.23	−64.27
NS20–08	Nova Scotia 2020	10/04/2020	Female	Subadult	336	225	537.3	44.23	−64.27
CA21–01	North Carolina 2021	03/25/2021	Female	Subadult	342	192	400.4	34.50	−76.90
CA21–02	North Carolina 2021	03/29/2021	Female	Juvenile	230	137	153.1	34.50	−76.90
NE21–01	New England 2021	08/02/2021	Female	Juvenile	213	108	84.9	41.48	−69.95
NE21–02	New England 2021	08/04/2021	Male	Juvenile	248	121	128.6	41.48	−69.94
NE21–03	New England 2021	08/08/2021	Male	Juvenile	182	96	63.3	41.56	−69.90
NS21–01	Nova Scotia 2021	09/08/2021	Male	Adult	359	259	745.8	44.24	−64.27
NS21–02	Nova Scotia 2021	09/12/2021	Female	Juvenile	300	181	315.9	44.24	−64.27
NS21–03	Nova Scotia 2021	09/13/2021	Female	Juvenile	320	186	366.3	44.24	−64.27
NS21–04	Nova Scotia 2021	09/14/2021	Female	Juvenile	299	171	286.5	44.24	−64.27
NS21–05	Nova Scotia 2021	09/14/2021	Female	Subadult	335	233	573.3	44.24	−64.27
NS21–06	Nova Scotia 2021	09/14/2021	Male	Adult	363	199	449.1	44.24	−64.27
NS21–07	Nova Scotia 2021	09/20/2021	Male	Adult	396	217	593.3	44.23	−64.28
NS21–08	Nova Scotia 2021	09/22/2021	Male	Subadult	289	187	324.1	44.24	−64.27
NS21–09	Nova Scotia 2021	09/22/2021	Male	Juvenile	274	169	262.3	44.24	−64.27
NS21–10	Nova Scotia 2021	09/25/2021	Female	Juvenile	289	155	239.7	44.24	−64.27

muscle tissue of the population of white sharks inhabiting the Western North Atlantic.

2. Materials and methods

2.1. Field sampling

Blood and muscle tissue samples were obtained from a total of 42 white sharks during seven OCEARCH expeditions from 2019 to 2022 (Fig. 1; Table 1). For comparison, samples also were collected opportunistically from three tiger sharks (*Galeocerdo cuvier*). Most sharks were caught using modified, breakaway drumlines that allowed the captured sharks to be led to the M/V OCEARCH research ship, where the sharks were placed on a movable platform on which researchers collected data and samples for more than 20 projects. A wet towel was placed over each shark's head and eyes and a hose with fresh seawater provided oxygen over gills. After approximately 15 min of measuring and sampling, the hydraulic platform was lowered into water and the sharks were allowed to swim free. Further details of this methodology can be found in Franks et al. (2021). One tiger shark was measured and sampled boatside next to a tender vessel without use of the OCEARCH lift. These animals were secured between a tail rope a sling around the pectoral girdle for similar data collection over the same time period (15 min). The hook, tail rope and sling were removed, and the animal released at the completion of sampling. Handling and procedures were approved by Jacksonville University's Institutional Animal Care and Use Committee (Projects #2018–008 and 2021–005).

For the current project, shark total length (TL) was measured, blood was collected from the caudal vein at the ventral precaudal pit, and a muscle sample was collected from the epaxial musculature adjacent to the first or second dorsal fin. Blood was collected via an 18 gage, 1.5- or 2.0-inch needle (based on animal size) attached directly or with a 7-inch extension set to a 12 ml syringe and immediately transferred to dry lithium heparin coated vacutainers. Muscle samples were collected through a 2.0 cm long skin incision using a benzalkonium chloride cold-sterilized 1.0 cm diameter surgical scoop and were placed temporarily in a 95% ethanol sterilized glass vial.

Blood was centrifuged (LW Scientific fixed speed; Lawrenceville, GA) in a field laboratory on the MV OCEARCH immediately following animal workup at 3500 g for 10 min for plasma separation. Plasma was frozen in cryovials as 0.5 to 1.0 ml aliquots and muscle was transferred to cryovials as 1.0 g aliquots. Both were preserved frozen in the field at −20 °C. After returning to the laboratory onshore, samples were stored in a −80 °C freezer until future use.

2.2. Plasma osmolality

Plasma osmolality was measured in each sample in duplicate using a Vapor Pressure Osmometer (Model 5600 VAPRO).

2.3. Metal analyses

Frozen muscle samples were thawed and cut into two halves. One half was massed to determine wet weight (ww), dried in an oven at 80 °C for 24–48 h, and then massed again to determine dry weight (dw). The mean mass ± standard deviation of the samples was 0.25 ± 0.19 g ww and 0.05 ± 0.04 g dw. Mean moisture content in the shark muscle samples was 80.0 ± 2.6%. Dried samples were digested in trace metal grade nitric acid and heated in a water bath to 70 °C for 1–2 h. Sample digests were then diluted in ultrapure Milli-Q® water (with different volumes depending on the metal) and measured for cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn) using atomic absorption spectrometry (AAS; Perkin Elmer AAnalysts 800, Norwalk, CT) with graphite furnace detection. This method has been extensively used (Lockhart et al., 2016; Hough et al., 2020; Somerville et al., 2020). Values are presented in μg g^{-1} dw. Following the same procedure, nine replicates each of two certified reference materials from National Research Council of Canada, DOLT-5 (Dogfish liver) and DORM-4 (fish protein), were processed and analyzed for metals to determine extraction efficiencies for each metal.

Instrument calibration was performed with certified standards (Perkin Elmer, Waltham, MA) with recalibration every 40 samples and all standards and samples were analyzed in duplicate or triplicate, depending on sample volume. Blanks and QA/QC samples (± 10% of nominal values) were analyzed throughout each analysis. Limits of detection (LOD) for each metal were calculated using the following equations: $S_{DL} = S_b + 3s_b$, where S_{DL} is the signal at detection limit, S_b is the average signal of the blanks, and s_b is the standard deviation of the replicate blank measurements. The LODs were then calculated by substituting the S_{DL} for the y value in the linear regression equation ($y = mx + b$) for the standard curve and solving for x. The LOD for each metal were as follows: 0.50 μg L^{-1} Ag, 0.20 μg L^{-1} Cd, 1.06 μg L^{-1} Cu, 2.60 μg L^{-1} Ni, 1.49 μg L^{-1} Pb, and 0.52 μg L^{-1} Zn. Mean metal extraction efficiencies for DOLT-5 and DORM-4 were 99 and 103% Cd, 100 and 114% Cu, 100 and 93% Ni, 99 and 99% Pb, 98 and 103.4% Ag, and 100 and 115% Zn, respectively.

2.4. Enzyme analyses

The other half of each sample was thawed and homogenized in 50 mM mono + dibasic potassium phosphate buffer using a mortar and pestle, and then homogenate was centrifuged at 300 rpm for 10 min at 20 °C. The supernatant was measured for protein using a Pierce BCA Protein Assay Kit (Thermo Scientific). Protein standards were prepared with bovine serum albumin at concentrations ranging from 20 to 2000 μg m L^{-1}, and absorbance values of standards and samples were analyzed using a Perkin Elmer Lambda 35 UV/Vis spectrometer. Protein concentrations in the samples were determined using the linear regression equation from the standards.

Supernatants from the shark muscle samples were measured for SOD activity in duplicate using a Sigma-Aldrich 19,160 SOD kit (Sigma 1999). A colorimetric method was utilized where O_2^- is reduced to H_2O_2 and O_2, forming a formazan dye. Absorbance at 450 nm was measured in samples and standards using a Bio-Rad iMark Microplate Reader, and SOD 50% inhibition rate was calculated from the reduction in color.

The samples were analyzed for CAT activity using Sigma protocol EC 1.11.1.6 (Sigma 1994a) and an assay kit. A 0.036% H_2O_2 solution was mixed with shark homogenate. CAT activity in the samples was quantified by a decrease absorbance of H_2O_2 at 240 nm over 90 s using a Perkin Elmer Lambda 35 UV/Vis spectrometer. Similarly, GPx activity in the supernatant was quantified using a Sigma-Aldrich Assay Kit (Sigma 1994b). In brief, shark supernatant (10 μl) was mixed with 90 μl of an assay buffer (50 mM Tris, pH 8.0, and 0.5 mM ethylenediaminetetraacetic acid), 50 μl of nicotinamide adenine dinucleotide phosphate, and 10 μl of 30 mM tert-butyl hydroperoxide in a BRAND semi-micro (PMMA) disposable cuvette. After inversion for 20 s, the decrease in absorbance was measured over 4 min at 340 nm using a Perkin Elmer Lambda 35 UV/Vis spectrometer. The activity of all enzymes was normalized by protein content.

2.5. Statistics

Sigma-Plot 14.5 was used to statistically analyze all data. For each parameter (metal or enzyme), data were tested for normality and equality of variance using a Shapiro-Wilk and Brown-Forsythe test, respectively. A One-Way Analysis of Variance (ANOVA) and a pairwise multiple comparison procedure (e.g. Holm-Sidak method, Dunn's Method, Tukey's test) were performed to determine statistical differences among age class, sex, and collection sites ($p \leq 0.05$). If data were not normally distributed, then a nonparametric Kruskal-Wallis One-Way ANOVA on Ranks was performed. In this study, the locations of the Massachusetts 2020 expedition and those in the New England 2021

expedition overlapped (Fig. 1), and no statistical differences were observed between the tissue metal concentrations in the sharks collected from those two sites. Therefore, the metal values from these expeditions were grouped for comparison with other sites.

Significant correlations among all parameters were detected using Pearson Product Moment Correlation ($p \leq 0.05$). If the correlation coefficient was above zero and $p \leq 0.05$ then the relationship between parameters was designated a positive correlation. If the correlation coefficient was below zero and $p \leq 0.05$ then the relationship between parameters was designated a negative correlation (i.e., one parameter increases, and one tends to decrease).

3. Results and discussion

A total of 22 female and 20 male white sharks were sampled, ranging from 182 to 501 cm TL (Table 1). Three tiger sharks (two female and one male) ranging from 306 to 352 cm TL (TL of the juvenile was not measured) were also sampled (Table 1). Sharks were classified by age group (juvenile, subadult, or adult) using length measurements and other characteristics (Franks et al., 2021). Not all the measurements were feasible for all the collected samples due to various limitations (e.g., sample size).

3.1. Plasma osmolality

Mean plasma osmolality was 996±36, ranging from 936 to 1062 mOsm L^{-1} (Table 2). These values are within the range of those reported for other shark species (Cliff and Thurman, 1984; Pillans and Franklin, 2004; Taylor and Grosell, 2006; Wood et al., 2007, 2008; Haman et al., 2012; Hoffmayer et al., 2012; Cramp et al., 2015; Morash et al., 2016; Tunnah et al., 2016; Barragán-Méndez et al., 2019; Dwyer et al., 2020).

Table 2
Mean (±standard deviation) concentration of metal (cadmium, copper, nickel, zinc, lead, and silver) and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx) in muscle and blood plasma osmolality in white sharks (Carcharodon carcharias) and tiger sharks (Galeocerdo cuvier). Bottom numbers represent sample sizes. Values for lead and silver are solely from the Southeast 2019 expedition. All other values were below detection.

Measured parameter	*Carcharodon carcharias*	*Galeocerdo cuvier*
Cadmium (μg g^{-1} dw)	0.03 (±0.03) 39	0.07 (±0.02) 3
Copper (μg g^{-1} dw)	3.30 (±3.40) 38	7.63 (±3.79) 3
Nickel (μg g^{-1} dw)	1.65 (±2.67) 41	4.88 (±4.27) 3
Zinc (μg g^{-1} dw)	32.9 (±21.5) 37	70.5 (±397) 3
Lead (μg g^{-1} dw)	0.09 (±0.06) 4	0.16 (±0.18) 3
Silver (μg g^{-1} dw)	0.13 (±0.13) 4	0.85 (±1.28) 3
SOD (units mg^{-1} protein)	34.3 (±15.8) 31	53.3 (±14.5) 3
CAT (units mg^{-1} protein)	1.42 (±0.72) 31	2.25 (±0.21) 3
GPx (units mg^{-1} protein)	0.15 (±0.08) 31	0.18 (±0.09) 3
Osmolality (mOsm L^{-1})	996 (±36.0) 34	NM

NM = not measured.

For example, Wells et al. (1986), reported osmolality values of 1063 and 1090 mOsm L^{-1} for the shortfin mako (*Isurus oxyrinchus*) and blue shark (*Prionace glauca*), respectively. Dusky sharks (*Carcharhinus obscurus*) had a reported mean osmolality of 1027 mOsm L^{-1} (Cliff and Thurman, 1984).

Most shark species are active osmoconformers, with their plasma osmolality reflecting the environmental salinity to some extent (Cramp et al., 2015; Dwyer et al., 2020). Plasma osmolality of sharks in saltwater is typically hypertonic to the surrounding water resulting in the slight influx of water and net gain of sodium and chloride ions (Pillans and Franklin, 2004; Pillans et al., 2005). The rectal gland in sharks helps to

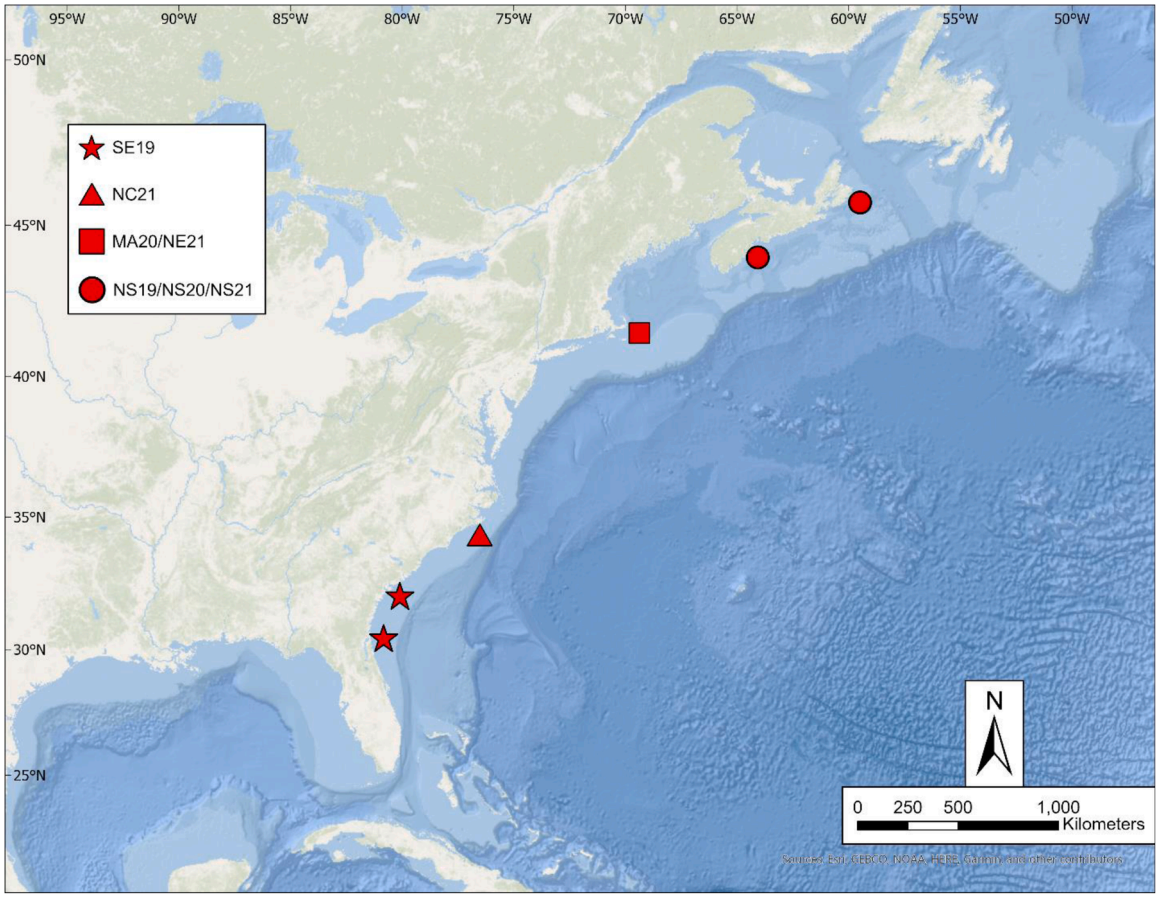

Fig. 1. GIS map showing the locations of the seven expeditions. See Table 1 for more information about the collection sites.

secrete excess salts and the high internal osmotic pressure is maintained by urea and trimethylamine N-oxide accumulation in cells and extracellular fluids (Pillans et al., 2005, 2008). The ability of sharks to osmoconform allows them to occupy a variety of aquatic environments, albeit at an energetic cost.

In the present study, white shark plasma osmolality was negatively correlated with shark TL (r=−0.505, P = 0.002, n = 34), with adults having a lower plasma osmolality than juveniles (Fig. 2). Although the salinity values at the capture sites were not measured and recorded, it is possible that the shark plasma osmolality values are somewhat indicative of the environmental salinities. Observed differences in plasma osmolality may reflect habitat and dietary preferences and/or efforts to reduce physiological challenges and energy expenditure associated with ionoregulation (Bielmyer and Grosell, 2011). More research is needed to investigate this occurrence in white sharks. Metals are known to cause both ionoregulatory and osmoregulatory disruption in fish, by binding to and disabling enzymes (e.g., Na^+/K^+ ATPase) on the gill and intestine of fish (Stag and Shuttleworth 1982; Grosell et al., 2004a; 2004b; Bielmyer et al., 2006). However, no correlations between white shark osmolality and metal accumulation in muscle tissue were observed in this study.

3.2. Metal concentrations

White sharks muscle metal concentrations ranged from non-detectable to 0.12 μg g^{-1} Cd, 0.46 to 15.0 μg g^{-1} Cu, 0.06 to 14.3 μg g^{-1} Ni, 5.44 to 107 μg g^{-1} Zn, 0.03 to 0.32 μg g^{-1} Ag, and 0.03 to 0.15 μg g^{-1} Pb. Muscle concentrations of Cd, Zn, and Cu in white sharks did not differ among age class or sex (See Fig. S1, Supporting Information). Alternatively, when grouping all age classes, Ni concentrations were significantly higher in females (2.44±0.73 μg g^{-1} Ni) as compared to males (0.74±0.17 μg g^{-1} Ni). Female, adult white sharks are known to have a broader dispersal and occupy more time offshore (Bradford et al., 2020), potentially resulting in differences in Ni exposure between the sexes. Most of the females in the present study were juvenile and sub-adult. The females of all age classes may uptake Ni to a greater extent or may have a greater biological Ni requirement, as compared to males. The essentiality of Ni in aquatic animals is still uncertain (Pyle and Couture, 2012; Blewett and Leonard, 2017). However, Ni is essential in plants, microorganisms, and to some extent mammals, serving as a vital constituent of many enzymes (Eisler 1988a; Ragsdale 1998; Hausinger 1993). Fish can regulate their nickel body burden, although currently there are no data showing Ni deficiency (Chowdhury et al., 2008). More research is needed about Ni essentiality and homeostasis in sharks.

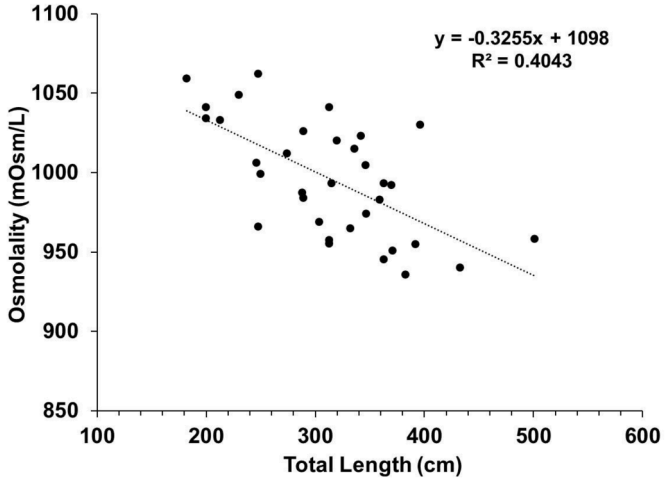

Fig. 2. Plasma osmolality versus total length in north Atlantic white shark (Carcharodon carcharias) collected from seven expeditions during 2019–2021 (Table 1).

Site-specific differences in Cd, Zn, Cu, and Ni in muscle of white sharks were observed (Fig. 3). White sharks captured in the Southeast 2019 expedition contained the highest concentrations of Zn, Cu, and Ni (Fig. 3). Relative to other collection sites, Cd concentrations were also elevated in muscle of sharks collected in the Southeast 2019, as well as

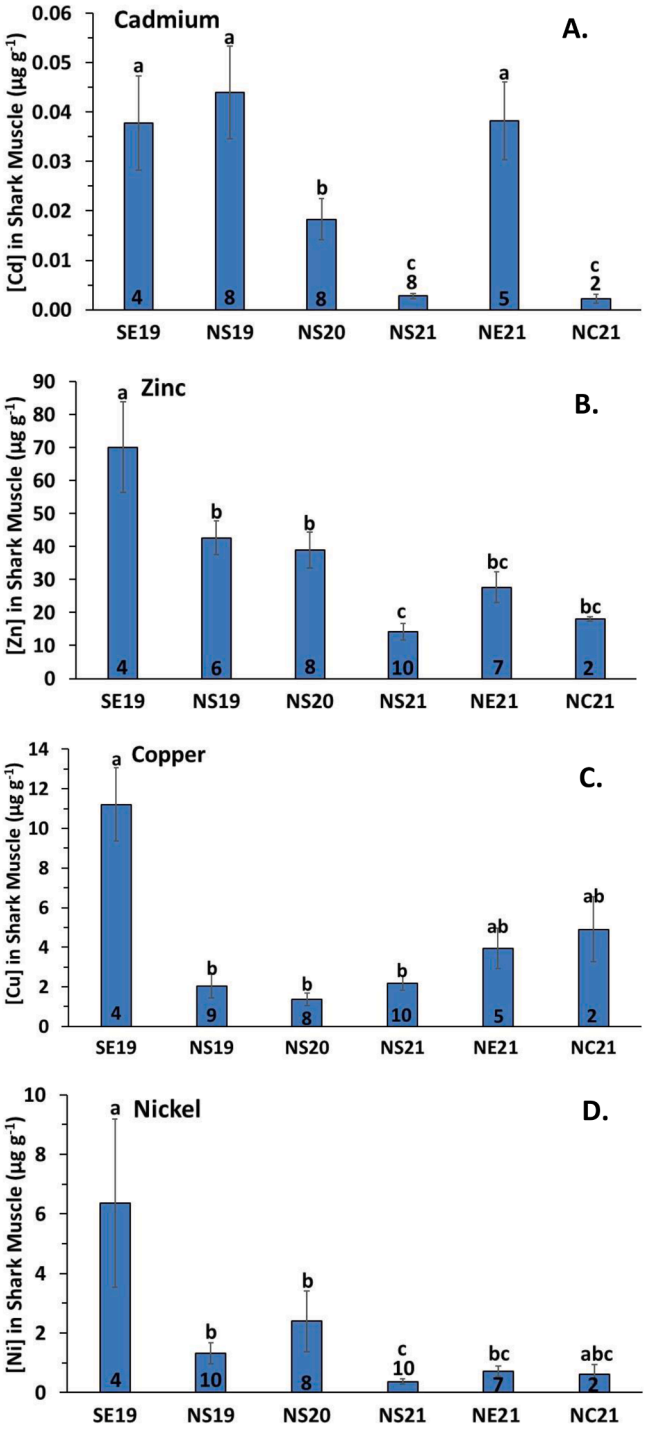

Fig. 3. Cadmium (A), zinc (B), copper (C), and nickel (D) concentrations (μg g^{-1} dw) in muscle tissue of white sharks (Carcharodon carcharias) collected from seven expeditions (Table 1). Samples from the MA20 and NE21 expeditions were collected from the same location (Fig. 1) and values were not significantly different. Therefore, the values were combined (NE21) for comparisons to other sites. Numbers inside (or above) columns represent sample size for each group. Columns with different lower-case letters indicate a statistical difference between treatments ($p \leq 0.05$).

the Nova Scotia 2019 and New England 2021 expeditions (Fig. 3A). White sharks collected during the Nova Scotia 2021 expedition typically had lower muscle metal concentrations than the Nova Scotia 2019 and 2020 expeditions. The sources for this variation are still under investigation.

Metal concentrations in muscle tissue of white sharks were comparable or slightly lower than those measured in tiger sharks ($n = 3$, collected during Southeast 2019 expedition), particularly those collected from the same site (Table 1). *C. carcharias* and *G. cuvier* are known to have similar diets to some extent, both feeding on large marine vertebrates. Dudley et al. (2000) reported concurrent scavenging by both shark species. Differences in tissue metal concentrations among species are known to be largely affected by trophic level and associated diet, as well as ecology (Vas and Gordon, 1993; Turoczy et al., 2000).

Gilbert et al. (2015) reported similar concentrations of Cd, Cu, and Zn in muscle tissue of dusky, sandbar (*Carcharhinus plumbeus*), and white sharks from south-eastern Australian waters. The metal values observed in the present study were also within the range of those reported in other shark species, including blue sharks, milk sharks (*Rhizoprionodon acutus*), starry smoothhounds (*Mustelus asterias*), shortfin makos, and Atlantic sharpnose sharks (*R. terraenovae*), among others (Domi et al., 2005; Barrera-García et al., 2012; Vélez-Alavez et al., 2013; Alves et al., 2016; Gaion et al., 2016; Adel et al., 2018; Somerville et al., 2020; Wosnick et al., 2021). In general, Ag and Pb concentrations in white shark muscle tissue were relatively low and only detected in samples collected in the Southeast 2019 expedition ($n = 4$). The Pb concentrations in the blood of *C. carcharias* were reported to be comparatively low in a recent study on white sharks collected in the same areas (Merely et al., 2019). This may indicate low levels of Pb exposure or minimal Pb uptake in these sharks. Uptake of Ag has been shown to vary among fish species (Bielmyer et al., 2008; Eisler, 1996). It is also possible that these metals preferentially accumulate in other organs (Eisler, 1988b; Luoma et al., 1999; Mager 2012). The liver is the main detoxification organ in fish and several metals have been shown to accumulate in higher proportions in the liver as compared to muscle (Mull et al., 2012; Eyckmans et al., 2013; Gilbert et al., 2015). However, this may be dependent on the life stage of the individual (Endo et al., 2008).

Concentrations of Zn in white shark muscle tissue were positively correlated with Cd, Cu, and Ni (Table 3). Additionally, Cu and Ni muscle concentrations were positively correlated (Table 3). These correlations likely reflect co-occurrence of these metals in the environment (Kay, 1985; Bielmyer-Fraser et al., 2017; Pinto et al., 2022). For example, Cd often occurs in the same environments as Zn and Ni, due to their uses in electroplating, alloys, plastic stabilizers, batteries, semiconductors, and photocells (Eisler, 1985; Kay, 1985, 1988a; Eisler, 1993; Barrera-García et al., 2012). Barrera-García et al. (2012) reported a positive correlation of Zn and Cd in blue shark tissues, consistent with the findings of the present study. Presence of multiple metals in the same environment may change uptake rates of each metal and change the severity of metal-induced effects (e.g., synergistic/antagonistic interactions) in aquatic organisms (Di Toro et al., 2001; Bielmyer et al., 2012b; 2013; Bielmyer-Fraser et al., 2018b). For example, Zn can reduce Cd uptake in fish (Wicklund et al., 1988). However, the extent of antagonistic and synergistic interactions observed among metals is complex, dependent on many factors, such as molar ratios of the metals, organism physiology, route of uptake, etc.

Table 3
Results of correlation analysis for metals (cadmium, Cd; copper, Cu; nickel, Ni, zinc, Zn) and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx) in the muscle tissue of white sharks (Carcharodon carcharias).

Measured Parameter	Cu	Ni	Zn	SOD	CAT	GPx
Cd	0.297 0.0784 36	0.129 0.432 39	0.489 ***0.00249*** 36	0.453 ***0.0119*** 30	0.365 ***0.047*** 30	0.196 0.298 30
Cu		0.6 ***6.9E-05*** 38	0.532 ***0.001*** 35	−0.00822 0.965 31	0.0701 0.708 31	−0.218 0.239 31
Ni			0.42 ***0.00973*** 37	0.172 0.354 31	0.178 0.339 31	0.164 0.377 31
Zn				0.368 ***0.0415*** 31	0.249 0.177 31	0.477 ***0.0066*** 31
SOD					0.65 ***7.6E-05*** 31	0.363 ***0.045*** 31
CAT						0.225 0.224 31

The correlation coefficient is listed as the top number; p-value is listed as the middle number; and sample size is listed as the bottom number. Significant correlations are shown in bold italics.

3.3. Enzyme activity

SOD ranged from 7.56 to 72.2 units/mg protein, GPx ranged from 0.03 to 0.36 units mg protein^{-1}, and CAT ranged from 0.29 to 3.59 units mg protein^{-1} protein in white shark muscle tissue. No differences in activity of these enzymes were detected based on sex (See Fig. S2, Supporting Information), consistent with studies of other shark species (Domi et al., 2005; Alves et al., 2016). It appeared that enzyme activity increased with age class; however, no significant differences were detected (See Fig. S2, Supporting Information). Consistent with our general findings, no differences in oxidative stress were detected due to age class in blue sharks (Barrera-García et al., 2012). Alternatively, spatial and temporal differences in enzyme activity were observed in white sharks in this study (Fig. 4). In general, activities of SOD, CAT, and GPx were higher in sharks collected from the Southeast 2019, Nova Scotia 2019, and Nova Scotia 2020 expeditions, as compared to the Nova Scotia 2021, New England 2021, and North Carolina 2021 expeditions (Fig. 4).

Mean SOD, GPx, and CAT activities in white sharks were similar to those in tiger sharks in this study (Table 2). Values of all enzymes measured were higher in white and tiger sharks, as compared to Atlantic sharpnose sharks collected from the southeastern U.S., as reported in a recent study using the same methodology (Somerville et al., 2020). Atlantic sharpnose sharks are a much smaller shark species and occupy a different ecological niche than white sharks (Compagno, 1984; Gelsleichter et al., 1999; Carlson et al., 2008). Vélez-Alavez et al. (2013) reported similar SOD activity, but higher CAT and GPx in shortfin makos. López-Cruz et al. (2010) reported similar CAT activity and lower GPx activity in the silky shark (*Carcharhinus falciformis*), shortfin mako, and smooth hammerhead (*Sphyra zygaena*), as compared to the white and tiger sharks in the present study. Differences in antioxidant enzyme concentration among species may occur due to differences in shark activity level. ROS are produced during exercise due to increased flow of oxygen to mitochondria in muscle cells and inefficiencies in electron transport (Clanton et al., 1999; Leeuwenburgh and Heinecke, 2001; Cooper et al., 2002). Therefore, more active sharks may have higher baseline values of antioxidant enzymes for protection against oxygen damage during exercise (López-Cruz et al., 2010; Vélez-Alavez et al., 2013), which inadvertently defends them against metal exposure. Other factors such as metabolism, environmental conditions, age, sex, physiology, behavior, and eating habits of the organism can also influence the production of ROS and antioxidant capacity (López-Cruz et al., 2010; Vélez-Alavez et al., 2013).

A positive correlation was observed between muscle Cd concentration and SOD and CAT activity (Table 3). Muscle Zn concentration was positively correlated with SOD and GPx activity (Table 3). SOD was also highly correlated with CAT and GPx (Table 3). Similar metal

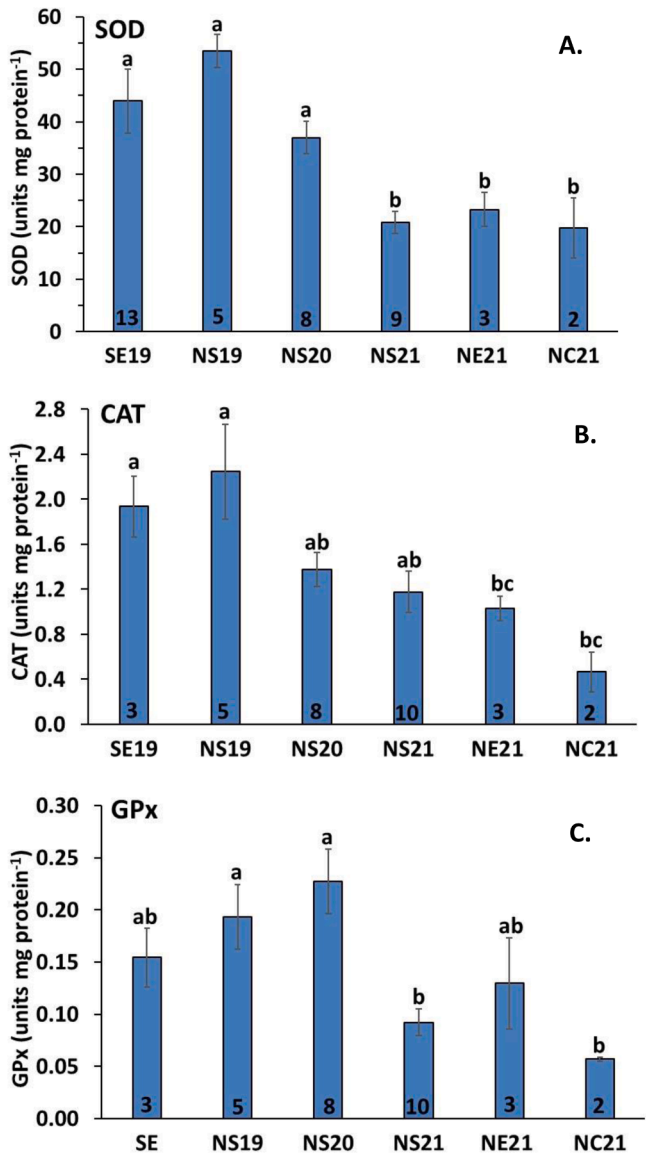

Fig. 4. Activity of superoxide dismutase (SOD; A), catalase (CAT; B), and glutathione peroxidase (GPx; C) in muscle tissue of white sharks (Carcharodon carcharias) collected from six OCEARCH expeditions (Table 1). Samples were not available for these parameters from the Massachusetts 2020 expedition. Numbers inside columns represent the sample size for each group. Columns with different lower-case letters indicate a statistical difference between treatments ($p \leq 0.05$).

concentrations in muscle tissue and activity of antioxidant enzymes (especially SOD) were observed between white sharks in this study and those reported for shortfin makos (López-Cruz et al., 2010; Vélez-Alavez et al., 2013). Tamburin et al. (2019) reported shared resource use among white sharks and shortfin makos, which likely contributes to the similarities in the measured parameters in the present study. However, it should be noted that while a positive correlation was observed between metals (Cd, Zn) and antioxidant enzyme activity in white sharks in the present study, Tamburin et al. (2019) reported a negative correlation between Cd and SOD activity in shortfin makos. This could indicate a difference in metal tolerance between the shark species and/or a difference in metal pollution levels. Antioxidant enzyme activity has been shown to increase with low levels of metal exposure and then can decrease (when defenses are overwhelmed) with higher metal exposure levels (Main et al., 2010; Patel and Bielmyer-Fraser, 2015; Duckworth et al., 2017). Barrera-García et al. (2012) also reported correlations between oxidative stress indicators and trace elements (Zn, Cd, and arsenic [As]) in the liver and kidney of blue sharks.

4. Conclusions

To our knowledge this is the first study to report baseline levels of the metals, Cd, Cu, Pb, Ni, Ag, and Zn, and antioxidant enzymes, SOD, CAT, and GPx in muscle of free ranging North Atlantic white sharks. Metal-specific differences in tissue metal concentrations were detected based on collection site for Cu, Zn, and Ni, and sex for Ni, with females having higher Ni levels. Determining contaminant load and antioxidant enzyme activity in muscle tissue of white sharks provides a nonlethal assessment of metal-induced stress in this organism. The metal values in muscle tissue of white sharks in this study were strongly correlated with oxidative stress enzymes indicating efficient detoxification strategies in this population. SOD activity appears to be a good nonlethal indicator of muscle metal concentration in white sharks with CAT and GPx activities possibly serving as good indicators of nonessential and essential element accumulation, respectively. More research is needed for the latter assertion. White shark health can be indicative of ecosystem health, as they serve as an important upper trophic level species in oceanic systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106641.

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