Biological assessment and metals concentration in blue shark (*Prionace glauca*) caught in the southeast-south coast of Brazil

Gabriela Vignatti [1], Vania Elisabete Schneider [2], Matheus Poletto [1]
[1] Ciências Exatas e Engenharias - Universidade de Caxias do Sul – UCS/CARVI
[2] Instituto de Saneamento Ambiental – ISAM, Universidade de Caxias do Sul – UCS
(gvignatt@ucs.br; mpolett1@ucs.br)

Abstract

Sharks are suffering from fishing industry pressure, mostly by human consumption of meat and fins, causing a great impact on its population. These top predators can bioaccumulate metals in its tissues, which brings risks to human health and indicates aquatic pollution. The objective is to determine metallic trace elements as chromium, copper, zinc and aluminium in *Prionace glauca*, while studying the biological aspects such as fork length and approximate age to indicate if there is a metal accumulation. Nine specimens were obtained through commercial fishing in the Southeast and South region of Brazil, using pelagic longline fishing. At the landing, specimens were weighed and sexed in its commercial weight (headed, finned and gutted). About 100 g of muscular flesh from dorsal region between the dorsal and caudal fin was removed. Metal determination in muscle tissue was performed through acid digestion (mixture of HNO_3 and H_2O_2). After the acid digestion, the metals concentration was determined by means of *inductively coupled plasma optical emission spectrometry* (ICP-OES). *Prionace glauca* capture during the winter in the Southeast-South region of Brazil, consists mostly by small adult males. Copper, zinc and aluminium concentration was well below de maximum limits permitted by Brazilian and international agencies. Chromium average (0.14 mg/Kg) was above the limits permitted by Brazilian legislation (0.10 mg/Kg). A significant positive correlation was found between some metals (Al, Cr and Cu) and sharks fork length. These results corroborate the bioaccumulation of some metals in *Prionace glauca*, showing the need for more studies about metal determination.

Key words: Blue Shark. Metals. South Atlantic. Bioaccumulation.

Theme Area: Environmental Impacts

1 Introduction

Metal accumulation in seafood shows great concern, mainly because it indicates the high levels of pollution in the aquatic environment (WANG & RAINBOW, 2008; KANDUČ et al. 2011) and when associated with increased worldwide production of fish in the past five decades, where fish supply as food is growing at an average annual rate of 3.2% (FAO, 2014) also raises concern about the human health.

Essentials metals as chromium, copper, zinc and aluminum in small amounts, are needed for most, if not all organisms, but in excess may be toxic (CASTRO & HUBER, 2003; SPIRO et al. 2009). Metals can often cause carcinogenic and neurotoxic effects to humans, although the real physiological consequences vary from one metal to another (SPIRO et al. 2009). Usually chromium, copper, zinc and aluminium are associated to metal plating processes (MANAHAN, 2013), reaching the aquatic ecosystem from industrial wastewater, drainage of agricultural areas, mining activities and fossil fuels (SPIRO et al. 2009; BRAGA et al. 2005; DOMENICO & SCHWARTZ, 1998).

Although copper, zinc and aluminium are essential in small doses for humans, they are known to be very toxic, even at low concentration, for some fish and plants (MANAHAN, 2013; BAIRD & CANN, 2008). Both marine and freshwater algae species are especially sensitive to copper, being adversely impacted at concentrations as low as 1-5 mg/L. Chromium toxicity in aquatic environment is increased with decreasing pH, alkalinity and hardness (EVANGELOU, 1998). An aquatic organism may present two basic types of behavior towards metal: or is sensitive to the toxic effect of a given metal or is not sensitive, but bioaccumulate increasing its harmful effects along the food chain (BRAGA et al., 2005). Sharks are susceptible to heavy metals due to the slow elimination and large capacity to incorporate metals (LOPEZ et al. 2013).

The wide-ranging, oceanic blue shark (*Prionace glauca*) is the main pelagic species captured by Brazilian commercial fishing and locally targeted species. The fins are exported to east Asia and the meat is consumed by Brazilians without real knowledge that is shark meat, since it is marketed as "cação" for better customer acceptance. In addition, Brazil is the largest importer of shark meat and possibly the major consumer of large pelagic shark meat in the world, using imported shark meat as children's meals at public schools financed by the government (BARRETO et al. 2017). United States Environmental Protection Agency - EPA (2017) advice women of childbearing age, pregnant and breastfeeding women, and young children to avoid shark meat since it has the highest mercury levels.

It is important to realize that sharks are facing intense fishing pressure, therefore its proper management and conservation are related to more biological studies associated with quantity monitoring of inorganic contaminants in shark meat, as a mean to show the risks that may come to human health by consuming it and at the same time expose marine environment pollution. Thus, the main goal of present work is to determine metallic trace elements as chromium, copper, zinc and aluminium in *Prionace glauca* using a ICP-OES equipment, while studying the biological aspects such as fork length and approximate age to indicate if there is a metal accumulation, as well as to show the need for further studies of metal concentration in sharks, since the field is currently deficient in Brazil.

2 Methods

2.1 Sampling

In July 2016, nine blue shark samples were donated by a fishing company from the port region of Itajaí, in the state of Santa Catarina, which operates in the Territorial Sea and the Exclusive Economic Zone (EEZ) of the Southeast and South region of Brazil, using pelagic longline fishing. At the landing, specimens were weighed and sexed in its commercial weight, that is, without head, viscera and dorsal, ventral and caudal fins.

Biometric parameters such as age and fork length (F_L) were obtained through equations proposed in the literature. These equations consider the weight of the carcass (commercial weight).

The age of each individual was estimated using the function proposed by von Bertalanffy:

$$Lt = L\infty \, [1 - e\text{-}k \, (t \, \text{-}t0)]$$

Where t represents age in years, the total asymptotic length, L∞ = 352.1 cm; the growth coefficient K = 0.157 and the age where the length is theoretically equal to zero, t0 = -1.01 (LESSA et al. 2004).

The fork length (Lf) was obtained from the total length through the equation proposed by Hazin et al. (1994):

$$Lf = 11.27 + 0.78 \, Lt$$

2.2 Metal determination

The metal concentration was determined taking about 100 g of skinless muscle from the dorsal region of each shark, between the dorsal and caudal fin, removed with the aid of a scalpel with stainless steel blade and then frozen in plastic bags and transported to the laboratory. The metal determination in the muscle tissue was performed through an acid digestion adapted from AOAC Official Method 999.10 (2002) and Dias et al. (2008), where a mixture of 10 mL of nitric acid (HNO_3), 0.25 mL of hydrogen peroxide (H_2O_2) and 1 g of the sample (taken with a scalpel from the central part of the muscle) kept in a bath-water at 60° C for one hour to perform acid digestion. The resulting solution was filtered and increased to 100 mL with distilled and deionized water. To avoid contamination, all glassware used was previously washed with 10% (v/v) HNO_3 solution according to the procedure recommended by AOAC Official Method 999.10 (2002). After the acid digestion, the metals concentration was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) in duplicate.

3 Results and Discussion

Among the biological aspects obtained from nine evaluated specimens consisted by 8 males and 1 female, ranging from 189 to 230 cm fork length (mean = 202.3 cm), with eight individuals classified as small adults and only one individual classified as a large adult, with a fork length greater than 230 cm, based on classification proposed by Montealegre-Quijano and Vooren (2010) with four categories: small young ($F_L \leq 129$ cm); large young (F_L 130 to 179 cm), small adults (F_L from 180 to 219 cm) and large adults ($F_L \geq 220$ cm). This result indicate that all fish specimens have reached adulthood and corroborate with the approximated age average found of 6.4 years, since sexual maturity of *Prionace glauca* is attained at approximately 6 years of age for males, while females occurs at age 7 (MONTEALEGRE-QUIJANO, 2007), although four male sharks presented ages from 5.5 to 5.8 years and the estimated age of the single female evaluated was 6.6 years.

The results for chromium (Cr), copper (Cu), zinc (Zn) and aluminium (Al) average concentrations measured in muscle of the blue shark for the present work and similar studies are presented in Table 1. The maximum allowable regulatory limits for metals in Brazil and by international agencies can be seen in Table 2.

Table 1 – Metal concentrations (mg/kg) found in muscle samples from different studies with *Prionace glauca*.

Reference	**n**	**Cr**	**Cu**	**Zn**	**Al**	**Geographic region**
Present work	9	0.14	0.98	5.38	1.70	S Atlantic
Machado, 2016	6	-	0.12	2.98	1.03	Unknown
Alves et al. 2016	20	2.58	1.15	24.61	23.77	N Atlantic
Olmedo et al. 2013	11	-	0.14	1.95	-	N Atlantic
Vas, 1991	5	-	0.24	0	-	N Atlantic
Stevens & Brown, 1974	8	-	4.40	35.00	-	N Atlantic
Barrera-García et al. 2012	44	-	1.64	6.10	-	Pacific

Table 2 – Maximum levels of metal intake permitted by Brazilian and international agencies.

Contaminant	**Brazil**	**SCF**	**IOM**	**EFSA**
Chromium	0.10 mg/kg	0.25 mg/day	-	-
Copper	30.00 mg/kg	5.00 mg/day	10.00 mg/day	-
Zinc	50.00 mg/kg	25.00 mg/day	40.00 mg/day	-
Aluminium	-	-	-	1.00 mg/Kg bw/week

Source: Brazil, 1965; SCF, 2006; IOM, 2001 and EFSA, 2008.
bw = body weight.

Average total chromium content was 0.14 mg/kg (wet weight), which exceeds maximum limit legalized by decree n. 55871 (BRAZIL, 1965) of 0.10 mg/kg for any kind of food. The European Food Safety Authority – EFSA on the Scientific Committee on Food (SCF, 2006) regulates the maximum amount of total chromium intake at 0.25 mg/day for adults in all kind of food. The samples ranged from 0.0 to 0.44 mg/kg, with 44% of samples above the Brazilian legislation and one sample almost 5 times above permitted by law. Alves et al. (2016) recently assessed 20 specimens of blue sharks from North Atlantic Ocean at the southwest coast of Portugal, finding an alarming total chromium average of 2.58 mg/kg, result that overcome seventeen times this study average concentration.

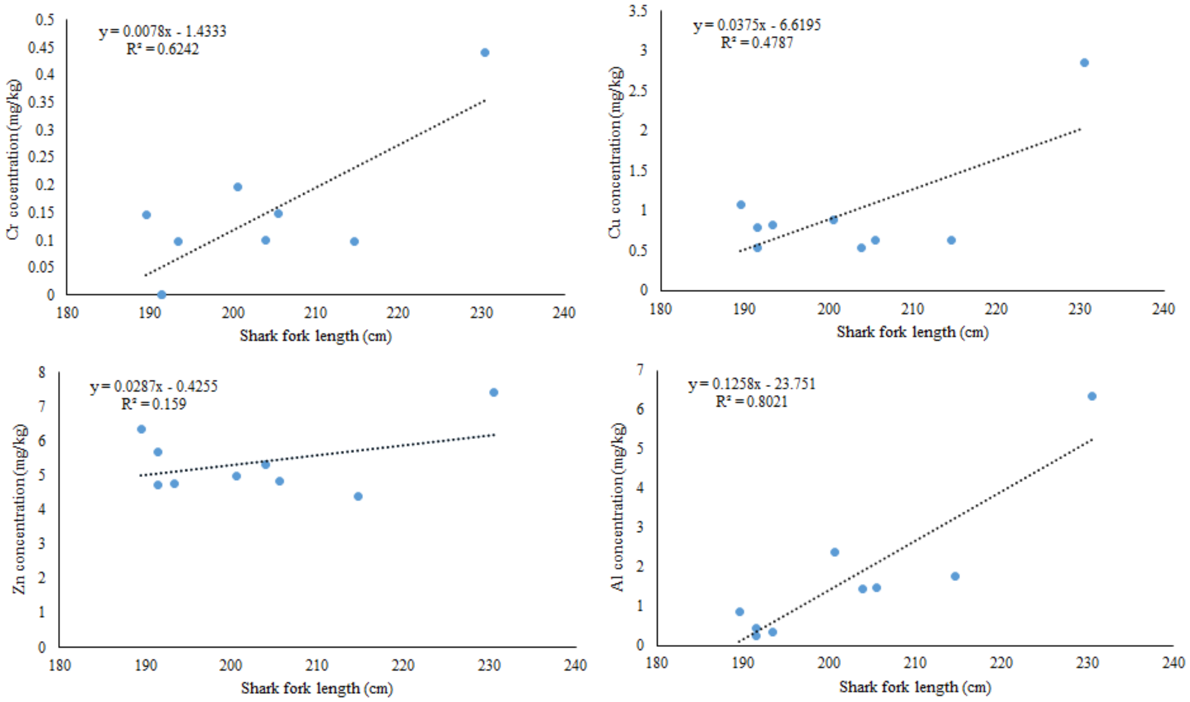
Total copper average concentration was 0.98 mg/kg (wet weight), result much lower than the Brazilian legislation limit that is 30 mg/kg for any kind of food (BRAZIL, 1965) and the United States Institute of medicine recommends 10 mg/day intake, from food and supplements (IOM, 2001). The present work results are similar with those found by Alves et al. (2016) who determined total copper average of 1.15 mg/kg and Barrera-García et al. (2012) who found average of 1.64 mg/kg in blue sharks fished from Pacific Ocean at Mexico west coast. Machado (2016) determined lower levels of copper 0.12 mg/kg (mean) in blue sharks purchased in São Paulo state (Brazil) of the municipal market, therefore, the fishing location is very difficult to determine, since shark meat may derive from many cities, states and countries.

The average of total zinc concentration determined was 5.38 mg/kg (wet weight) within the parameters of maximum limit legalized by decree n. 55871 (BRAZIL, 1965) of 50 mg/kg, pointing out that it has not been revised since 1965. International agencies like the Institute of Medicine – IOM (2001) and the European Food Safety Authority – EFSA (2006) indicate the tolerable upper intake level for adults as 40.00 mg/day and 25.00 mg/day, respectively. Barrera-García et al. (2012) obtained very similar results, determined a total zinc average concentration of 6.10 mg/kg. However, Alves et al. (2016) and Stevens & Brown (1974) who caught blue sharks from North Atlantic Ocean, had extremely high results when compared to the present study, presenting total zinc trace elements of 24.61 mg/kg and 35.00 mg/kg, respectively. Large amounts of ingested zinc can inhibit the absorption of copper in humans, causing copper deficiency.

Total aluminium average content was 1.70 mg/kg (wet weight), ranging from 0.24 to 6.34 mg/kg. Brazilian laws do not regulate aluminium intake levels in food, although the European Food Safety Authority establish a value of 1.00 mg/kg body weight per week (EFSA, 2008). Recent studies as Alves et al. (2016) and Machado (2016) determined aluminium trace elements in blue shark's muscles, finding averages of 23.77 mg/kg and 1.03 mg/kg, respectively.

As data shown in Figure 1, a significant positive correlation was found between some metals and sharks fork length. Aluminium had the most significant positive correlation (r^2 = 0.80), followed by chromium (r^2 = 0.62), copper (r^2 = 0.47) and zinc, which presented no significant correlation (r^2 = 0.15). These results corroborate the bioaccumulation of some metals in *Prionace glauca* muscle tissue, a phenomenon where toxic substances accumulate in tissues as the specimen grows and can promote biomagnification as these substances are transferred into the food chain, changing environment and its populations, destroying communities of organisms and causing effects on the ecosystem as a whole (MANAHAN, 2013).

Figure 1 - Relationships between shark fork length and metal concentrations.



The analyzed data in the present study showed that *Prionace glauca* capture during the winter in the Southeast-South region of Brazil, consists mostly by small adult males (considering fork length), which can trigger an imbalance in the species population structure. The metal determination indicated that total chromium tops the maximum limit permitted by Brazilian legislation in 44% of the tested samples and that total copper, zinc and aluminium was well below the tolerable levels by Brazilian and international agencies, who have different metals intake recommendations, mainly because there is no consensus on the information obtained in several studies and sparse data on some metals (chromium e.g.) essentiality and metabolism. Therefore, the need for more studies is essential, since metals can be toxic to aquatic biota and considered mutagenic, carcinogenic, or teratogenic to humans (EVANGELOU, 1998).

4 Conclusion

Shark fishing on the Southeast-South region of Brazil is composed mostly by small adult males during the winter. Metal assessment of total chromium, copper, zinc and aluminium showed that specimens evaluated can bioaccumulate metals in their muscle tissue and that chromium samples presented levels above the permitted limit of Brazilian legislation. Thus, information available about bioaccumulation and current levels of several important metals such as chromium, copper, zinc and aluminium in commercial shark meat in Brazil, is important in order to reduce human and environmental contamination, as well as contribute to the conservation of *Prionace glauca* in the process.

References

ALVES, L.M.; NUNES, M.; MARCHAND, P.; LE BIZEC, B.; MENDES, S.; CORREIA, J.P.; LEMOS, M.F.; NOVAIS, S.C. Blue sharks (*Prionace glauca*) as bioindicators of pollution and health in the Atlantic Ocean: Contamination levels and biochemical stress responses. **Science of the Total Environment**, v. 563, 2016, p. 282-292.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMIST - AOAC. **Official Method 999.10. Lead, cadmium, zinc, copper and iron in foods - atomic absorption spectrophotometry after microwave digestion**. Washington, DC, 2002.

BAIRD, Colin; CANN, Michael. **Environmental chemistry**. 4 ed. New York: W. H. Freeman and Company, 2008. 776p.

BARRERA-GARCÍA, A.; O'HARA, T.; GALVÁN-MAGAÑA, F.; MÉNDEZ-RODRÍGUEZ, L. C.; CASTELLINI, J. M.; ZENTENO-SAVÍN, T. Oxidative stress indicators and trace elements in the blue shark (*Prionace glauca*) off the east coast of the Mexican Pacific Ocean. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 156 (2), 2012, p. 59-66.

BARRETO, R. R.; BORNATOWSKI, H.; MOTTA, F. S.; SANTANDER-NETO, J.; VIANNA, G. M. S.; LESSA, R. Rethinking use and trade of pelagic sharks from Brazil. **Marine Policy**, v. 85, 2017, p. 114-122.

BRAGA, B.; HESPANHOL, I.; CONEJO, J.; MIERZWA, J. C.; BARROS, M.; SPENCER, M.; PORTO, M.; NUCCI, N.; NEUSA, J.; EIGER, S. **Introdução à Engenharia Ambiental: o desafio do desenvolvimento sustentável**. São Paulo: Editora Pearson, 2005. 318p.

BRASIL. Decreto n. 55871 de 26 de março de 1965. Modifica o Decreto nº 50.040, de 24 de janeiro de 1961, referente a normas reguladoras do emprego de aditivos para alimentos, alterado pelo Decreto nº 691, de 13 de março de 1962. **Diário Oficial da União**, Poder Executivo, Brasília, 09 abr. 1965.

CASTRO, P.; HUBER, M. E. **Marine Biology**. 4 ed. New York: McGraw-Hill, 2003. 456p.

DIAS, A. C. L.; GUIMARÃES, J. R. D.; MALM, O. Mercúrio total em músculo de cação *Prionace glauca* (Linnaeus, 1758) e de espadarte *Xiphias gladius* Linnaeus, 1758, na costa sul-sudeste do Brasil e suas implicações para a saúde pública. **Cad. Saúde Pública**, v. 24 (9), 2008, p. 2063-70.

DOMENICO, P. A.; SCHWARTZ, F. W. **Physical and Chemical Hydrogeology**. 2 ed. New York: John Wiley & Sons, 1998. 506p.

EUROPEAN FOOD SAFETY AUTHORITY – EFSA. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials on a request from European Commission on Safety of aluminium from dietary intake. **The EFSA Journal**, v. 754, 2008, p. 1-34.

EVANGELOU, V.P. **Environmental Soil and Water Chemistry: principles and applications**. New York: John Wiley & Sons, 1998. 564p.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO. **The State of World Fisheries and Aquaculture Opportunities and challenges**. Roma, 2014, 243p.

HAZIN, F.H.V.; KIHARA, K.; OTSUKA, K.; BOECKMAN, C.E.; LEAL, E.C. Reproduction of the blue shark *Prionace glauca* the southwest equatorial Atlantic Ocean. **Fisheries Science**, v. 60 (5), 1994, p. 487–49.

INSTITUTE OF MEDICINE – IOM. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, molybdenum, nickel, silicon, vanadium and zinc. **Food and Nutrition Board**, National Academy Press, Washington, 2001.

KANDUČ, T.; MEDAKOVIĆ, D.; HAMER, B. *Mytilus galloprovincialis* as a bioindicator of environmental conditions: the case of the eastern coast of the Adriatic Sea. **Isotopes in Environmental and Health Studies,** v. 47(1), 2011, p. 42-61.

LESSA, R.; SANTANA, F. M.; HAZIN, F. H. Age and growth of the blue shark *Prionace glauca* (Linnaeus, 1758) off northeastern Brazil. **Fisheries Research**, v. 66 (1) 2004, p. 19-30.

LOPEZ, S. A.; ABARCA, N. L.; MELÉNDEZ, R. C. Heavy metal concentrations of two highly migratory sharks (*Prionace glauca* and *Isurus oxyrinchus*) in the southeastern Pacific waters: comments on public health and conservation. **Tropical Conservation Science**, v. 6(1), 2013, p. 126-137.

MACHADO, C. S. **Quantificação do risco à saúde humana pela exposição a poluentes químicos e potencial carcinogênico às comunidades adjacentes ao Rio Pardo, Brasil**, Tese de Doutorado em Ciências, Escola de Enfermagem de Ribeirão Preto, São Paulo, 2016, 175p.

MANAHAN, Stanley E. **Fundamentals of environmental and toxicological chemistry: sustainable science.** Boca Raton: CRC Press, 2013. 576p.

MONTEALEGRE-QUIJANO, S. **Biologia populacional do tubarão-azul, *Prionace glauca* (Linnaeus, 1758), na região sudoeste do Oceano Atlântico**. Tese de Doutorado em Oceanografia Biológica, Universidade Federal do Rio Grande, Rio Grande, 2007, 214p.

MONTEALEGRE-QUIJANO, S.; VOOREN, C. M. Distribution and abundance of the life stages of the blue shark *Prionace glauca* in the Southwest Atlantic. **Fisheries Research**. v. 101 (3), 2010, p. 168-179.

OLMEDO, P.; HERNÁNDEZ, A. F.; PLA, A.; FEMIA, P.; NAVAS-ACIEN, A.; GIL, F. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury–selenium balance. **Food and chemical toxicology**, v. 62, 2013, p. 299-307.

SCIENTIFIC COMMITTE ON FOOD – SCF. Scientific Panel on Dietetic Products, Nutrition and Allergies - Tolerable upper intake levels for vitamins and minerals. **European Food Safety Authority (EFSA)**, 2006.

SPIRO, T. G.; STIGLIANI, W. M.; YAMAMOTO, S. M. **Química Ambiental**. 2 ed. São Paulo: Pearson Prentice-Hall, 2009. 334p.

STEVENS, J. D.; BROWN, B. E. Occurrence of heavy metals in the blue shark *Prionace glauca* and selected pelagic in the NE Atlantic Ocean. **Marine Biology**, v. 26(4), 1974, p. 287-293.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY – EPA. **EPA-FDA Fish Advice: Technical Information**, 2017. Available in: <https://www.epa.gov/fish-tech/epa-fda-fish-advice-technical-information#main-content>. Accessed in: 01 December 2017.

VAS, P. Trace metal levels in sharks from British and Atlantic waters. **Marine pollution bulletin**, v. 22 (2), 1991, p. 67-72.

WANG, W. X.; RAINBOW, P. S. Comparative approaches to understand metal bioaccumulation in aquatic animals. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 148(4), 2008, p. 315-323.