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Tracing Cr, Pb, Fe and Mn occurrence in the Bahía Blanca estuary through commercial fish species

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HIGHLIGHTS

- Accumulation of Cr, Pb, Fe and Mn in six fish species from the Bahía Blanca estuary.
- Concentrations of metals within fish tissues showed a maximum in the gill tissues.
- *Cynoscion guatucupa* accumulated the highest Cr and Fe mean levels in the study period.
- Cr and Mn in the muscle tissues exceeded, at times, the allowable levels for consumption.

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ABSTRACT

Over the last decades the anthropogenic contamination impact has substantially increased in the Bahía Blanca estuarine area, and scarce information exists regarding metals in the biotic compartment of this estuary. Thus, fish tissues were used to evaluate metal accumulation within this aquatic environment. The study focused on the determination of Cr, Pb, Fe and Mn in the gills, liver and muscle tissues of six commercial fish species (*Brevoortia aurea, Odontesthes argentinensis, Micropogonias furnieri, Cynoscion guatucupa, Mustelus schmitti* and *Paralichthys orbignyanus*).

From the results it can be summarized that *C. guatucupa* tends to accumulate higher metal levels in the liver tissues, mostly Cr and Fe, than the other studied species. *O. argentinensis* and *P. orbignyanus*, both permanent inhabitants of the BBE, achieved the highest metal values in the gill tissues, mostly in comparison to *M. schmitti*. The gill tissues were found to be the main organ of Mn and Ni accumulation for most species, whereas in general, minimum concentrations were found for all the analyzed metals in the muscle tissues. Nevertheless, and according to the guidelines, all fish species showed at least one sample with concentrations of Mn and/or Cr above the permissible levels for human consumption.

Finally, it was highlighted the usefulness of selecting these fish species as bioindicators of metal pollution, since they are either permanent inhabitants of the estuary or, according to the sizes under analyses, spend much of their time in this coastal waters.

1. Introduction

Metals occur in the aquatic environment as a result of both natural processes and human being activities (FranÇa et al., 2005). Within anthropogenic activities, metal concentrations could be increased by means of the rapid industrialization and urbanization, massive land use changes and also could be associated to enhance terrestrial runoff, between others (Rahman et al., 2012). Contamination of aquatic ecosystems with metals has seriously increased worldwide attention, and a lot of studies have been published about the accumulation of these elements in the marine biota (Karadede and Ünlü, 2000; Yılmaz et al., 2007).

To assess the environmental condition of coastal zones such as estuaries, the study of metals in aquatic organisms, especially fishes, has been widely promoted (Borja et al., 2004; Breine et al., 2007; Harrison and Whitfield, 2006; Whitfield and Elliott, 2002).

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Fish species inhabiting polluted water bodies are exposed to a wide range of contaminants that eventually accumulate in its tissues. Moreover, metals might not only reach to harmful levels on the fish themselves but also on the top-level organisms that consume contaminated fish (*e.g.* Al-Yousuf et al., 2000; Avigliano et al., 2015). The advantages of using fish species include the ability to accumulate elements in the bioavailable and, potentially, more toxic form (Fernándes et al., 2007). Also, it is important to assess the metal contents in edible fish species in order to study potential risks to final consumers, including the human population.

According to their biochemical properties, many metals are required by living organisms, like fishes, in little but critical concentrations in order to achieve a normal growth (essential metal), but they can produce toxic effects in excessive levels (Merciai et al., 2014). Chromium (Cr), iron (Fe) and manganese (Mn) are essential metals with a significant biological role in aquatic organisms. On the other hand, lead (Pb) has unknown functions in biological systems (non-essential metal), being a toxic element that causes carcinogenic effects in marine biota (Velusamy et al., 2014). Metal accumulation in fishes also depends on other characteristics such as the tissue under analysis, fish sizes, trophic level, feeding habits, between others (Mohammadi et al., 2011).

The estuary of Bahía Blanca (BBE) is a coastal environment located in Argentina, being a mesotidal system characterized by turbid and shallow waters towards the inner zone (Guinder et al., 2009). The BBE is exposed to metals, mainly as a consequence of agricultural activities along with the urban expansion and anthropogenic waste discharges from the surrounding areas. It has a great economic value due to the presence of important industries, cities and port complexes that are in continuous development.

The BBE has one of the biggest petrochemical centers of Argentina, resulting in large amounts of effluent discharges. They mainly consist of heavy hydrocarbon fractions and particulate urea, oil derivatives, particulate polyvinyl, smoke particles, brines, chlorinated organic compounds, metals, between others (Limbozzi and Leitào, 2008). The BBE is also considered to be polluted by untreated sewage discharges (Biancalana et al., 2012; Dutto et al., 2014).

The assessment of the environmental condition of the BBE is essential to consider the association between levels of metals found in the abiotic compartments (Botté et al., 2007, 2010; La Colla et al., 2015) and metal concentrations accumulated in fishes as bioindicators of the biotic compartment. Among the fish communities inhabiting the BBE, six species were selected for metal analyses: the menhaden (*Brevoortia aurea*), the silverside *(Odontesthes argentinensis)*, the whitemouth croaker *(Micropogonias furnieri)*, the striped weakfish *(Cynoscion guatucupa)*, the smooth-hound (*Mustelus schmitti)* and the flounder (*Paralichthys orbignyanus*). These species were selected upon their different feeding characteristics and were believed to be representative of the area of analysis. Metal accumulation was analyzed in three different organs, one being a site of uptake (*i.e.* gills), another of storage and/or excretion (*i.e.* liver) and the third one the tissues used for human consumption (*i.e.* skeletal muscle).

Many studies had been conducted all around the world trying to identify differential metal accumulation processes in fish species (*e.g.* Karadede and Ünlü, 2000; Kwok et al., 2014; Wei et al., 2014). This study is an attempt to report the concentration of metals in commercial fish species from a less studied coastal environment recognized as anthropogenically impacted (Botté et al., 2007). Most literature with reference to metal levels on the coasts of the BBE is related to sediment, seawater or suspended particulate samples (*e.g.* Botté et al., 2007, 2010; Marcovecchio et al., 2010). Meanwhile, available information on metals in fishes is mostly related to data recorded more than 20 years ago (*i.e.* Marcovecchio et al., 1986, 1988a, b) or to technical reports conducted by the local government.

The anthropogenic impact has substantially increased in the BBE over the last decades, not only due the expansion in the amount of industries, factories and port activities, but also due to the increase in their productivity. Thus, the aim of this study is to determine the concentration of Cr, Pb, Fe and Mn in fish, discussing their accumulation as regards the different species and tissues under analysis. Possible bioaccumulation patterns and the usefulness of these fish species as bioindicators of pollution processes in the BBE are also under study. Levels of metals found in the muscle tissues are compared with the certified human consumption safety guidelines recommended by both international as well as national legislations.

2. Materials and methods

2.1. Study area

The BBE (Fig. 1) is a mesotidal system formed by a series of NW-SE tidal channels, separated by flats, marshes and islands (Melo, 2004). It is a coastal environment with two main cities located in the northern margins, Bahía Blanca (300,000 inhab.) and Punta Alta (60,000 inhab.) (INDEC, 2010). Both cities generate waste discharges of about 84,000 m^3/day (CTE, 2003), reaching the estuary with an incomplete pre-treatment. The main freshwater tributaries to the BBE are the Sauce Chico River (drainage area of 1600 km^2) and the Napostá Grande Creak (drainage area of 920 km^2) (Perillo et al., 2001). On the coastlines of these two tributaries there are important areas of cattle breeding and agriculture (Limbozzi and Leitào, 2008) adding different quantities of substances to the water courses without any further treatment.

The wetlands of the BBE are dominated by halophyte vegetation, principally *Spartina alterniflora* and *Sarcocornia perennis* (Negrin et al., 2016). Within estuaries, saltmarshes are widely recognized as important nursery grounds that support valuable coastal fisheries (Valiñas et al., 2012). *C. guatucupa,* together with *M. furnieri,* support the traditional fisheries of the Argentinean, Southern Brazilian and Uruguayan coastal regions (Jaureguizar et al., 2006; Ruarte et al., 2000). They are the most important fishing resources in the area of the BBE, in both social and economic terms (Carozza and Fernández Araoz, 2009; López Cazorla, 2004).

The estuary undergoes intense human-induced disturbances related to urban and industrial developments on its northern boundary, with the most important deep-water port system of Argentina located in the area. The port system contributes to the rapid resuspension of great volumes of cohesive sediment by means of the maintenance and deepening dredging activities. These activities promote the abrupt transfer of immobilized substances into bioavailable compounds that then are disseminated throughout the estuarine environment (Grecco et al., 2011). Several other industries taking part of a petrochemical center are also located in this harbor area (Limbozzi and Leitào, 2008).

2.2. Sample collection and preparation

Fish samples were caught with nets by local fishermen from the middle inner zone of the BBE (Fig. 1), consecutively during the spring season of 2011, 2012 and 2013. In total, 147 individuals were collected, corresponding to six fish species: *Brevoortia aurea*, *Odontesthes argentinensis*, *Micropogonias furnieri*, *Cynoscion guatucupa*, *Mustelus schmitti* and *Paralichthys orbignyanus*.

After being caught, fish samples were transported to the laboratory with ice. Body weight (in kg) and total body length (measured to the nearest cm) were recorded for each fish. Dissection was performed with a stainless steel knife in order to obtain

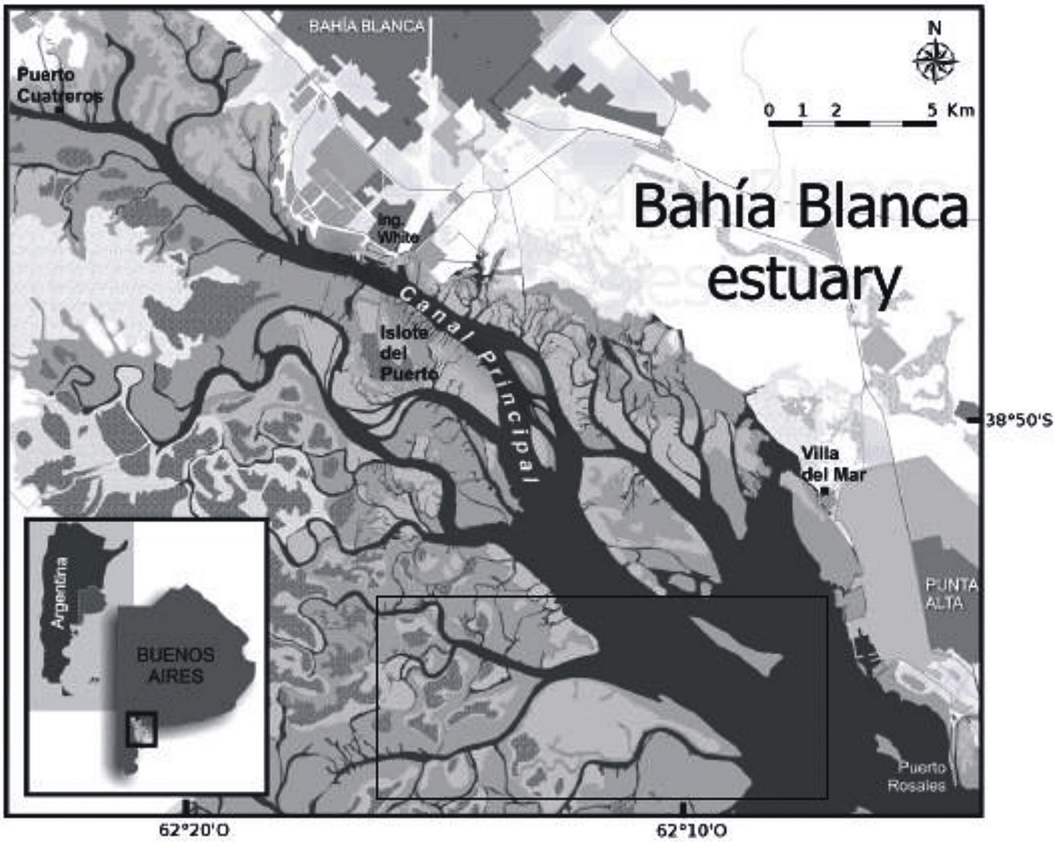

Fig. 1. The location of the Bahía Blanca estuary, with the sampling area outlined.

tissue sub samples from the gills, liver and dorsal muscle. After that, each sample was homogenized, put in polyethylene bags and cooled at −20 °C until analysis.

2.3. Analytical procedure

For metal analyses, sub samples of muscle (0.60 ± 0.01 g, wet weight), liver and gills (0.40 ± 0.01 g, wet weight) were used. Tissue portions from individual samples were subjected to an acid predigestion with 5 ml of HNO_3 (65%) for at least 3 h. Then, 1 ml of $HClO_4$ was added and then the samples were put in a glycerin bath at 110 ± 10 °C for 72 h or until the volume was reduced to less than 1 ml. The acidic extracts were transferred to centrifuge tubes and 0.7% HNO_3 was added up to 10 ml of final dissolution. Metals were analyzed on a Perkin-Elmer DV 1200 inductively coupled plasma-optical emission spectrometry (ICP OES).

2.4. Cleaning procedures

All material used during dissection and in laboratory analyses was cleaned according to international recommended protocols (APHA, 1998). The cleaning procedure included washing the material with non ionic detergent, rinsing them three times with tap water and then three times with deionized water. The material was then soaked for 24 h in a diluted acid nitric solution (5% HNO_3) and finally rinsed three times with deionized water.

2.5. Quality assurance and quality control

The following wavelength lines were used for the ICP OES analyses: Cr 205.560 nm in axial view, Pb 220.353 nm in axial view, Fe 302.107 nm in radial view and Mn 257.610 nm in radial view. Blanks of reagents were used simultaneously in each batch of analysis to corroborate the analytical quality. All analyses were done in duplicate, and the uncertainty based on one relative standard deviation of replicates was <15%. The analytical method detection limit (MDL) for each metal (μg/g) was: 0.03 for Cr, 1.2 for Pb, 0.12 for Fe and 0.015 for Mn. The analytical quality was tested against reference materials (mussel tissue flour R.M. Nº6) provided by the National Institute for Environmental Studies (NIES) from Tsukuba (Japan). The obtained values from the analysis of the reference materials were within the range of the certified ones. Recovery percentages for the four metals were >90%.

2.6. Statistical analyses

All statistical analyses were carried out using STATISTICA 7.0 (StatSoft, Inc.), following Zar (1996). As the data analyzed did not meet the assumptions of the parametric statistics and there were no possible transformations, the non-parametric test Kruskal-Wallis ANOVA was used throughout the results section. The acceptable level of statistical significance used in the study was $p < 0.05$. Metal concentrations reported as below analytical MDL were substituted by one half the MDL for statistical analyses (Jones and Clarke, 2005) and no analyses were performed when 40% or more of the concentrations of the metal evaluated were below the MDL (Federal Register, 1984). Statistical analyses were also used to evaluate metal differences in fish species between sampling years only when the total sample size was 30 or more fish individuals. Error values represent standard deviation. Graphics were performed using software R in version 3.2.0 from the R-project (Wickham, 2009).

3. Results and discussion

A total of 147 individual fish samples were analyzed, with total weight and length varying from 0.020 to 1.2 kg and from 12 to 57 cm, respectively (Table 1). Mean concentrations of Cr, Pb, Fe and Mn in fish species inhabiting the BBE estuary are presented in Table 2. Generally, minimum concentrations were found in the muscle tissues of the different species and were mostly below the MDL for all the analyzed metals. Fe and Mn were the most abundant as expected, achieving the highest mean values in all the three organs under analysis. Concerning Fe concentrations, a mean maximum in the gill tissues of *O. argentinensis* was detected (1600 μg/g) and for Mn, the highest mean value was also found in the gill tissues but corresponding to *B. aurea (*21 μg/g*).*

The mean maximum concentrations found for Cr and Pb were achieved in *P. orbignyanus* tissues, with values far below the ones obtained for the aforementioned elements. The highest mean Cr value was found in the gill tissues (0.80 μg/g), while a maximum of 2.7 μg/g for Pb was achieved in liver tissues. Nevertheless, most Pb values found during the sampling period were below the MDL for all the species under analysis.

3.1. Metal accumulation considering fish species and migratory behaviors

Although the fish species under analysis belong to the same coastal environment, metal levels and distribution within fish tissues could be distinctive, as indicated in studies from other estuaries (*e.g.* Canli and Atli, 2003; Marcovecchio, 2004). Many characteristics are of concern as regards metal distribution in fish species, including the differential aptitudes of juveniles and adults, the size distribution found in the different sampling sites, the metabolic rate of fishes *(e.g.* Akan et al., 2012; Canli and Atli, 2003; Oronsaye, 1989) or the versatility of fishes to adapt to metal burdens (Mohammadi et al., 2011; Shah and Altindag, 2005). Comparatively, data achieved in this study revealed that there were some consistent differences in metal accumulation among fish species.

It can be summarized that *C. guatucupa* tends to accumulate higher metal levels, mostly Cr and Fe, in the liver tissues and in comparison to the rest of the species analyzed. A highest mean Cr concentration in *C. guatucupa* was achieved comparing the total period of analysis against all the other fish species (0.23 ± 0.17 μg/g, $p < 0.05$). It was also evident during the first sampling date (2011), when Cr data of this fish species was compared against *M. schmitti* and *P. orbignyanus* liver tissues ($p < 0.05$ both). Also, in this same sampling date maximum mean values of Fe were obtained in the liver tissues compared to *M. schmitti* and *O. argentinensis* ($p < 0.001$ both).

Table 1
Mean body weight and mean total body length of fish collected in the Bahía Blanca estuary. n: number of fish samples analyzed; SD: standard deviation.

Scientific name	n	Mean body weight (kg) (±SD)	Mean body length (cm) (±SD)
Brevoortia aurea	26	0.34 ± 0.18	30 ± 4.8
Odontesthes argentinensis	37	0.18 ± 0.096	28 ± 4.6
Micropogonias furnieri	36	0.30 ± 0.24	27 ± 9.1
Cynoscion guatucupa	17	0.50 ± 0.36	34 ± 10
Mustelus schmitti	20	0.38 ± 0.16	47 ± 6.6
Paralichthys orbignyanus	11	0.22 ± 0.087	27 ± 3.5

From the study it was aimed to assess the environmental condition of the BBE. Nevertheless, given the migratory behaviour of *C. guatucupa* in the sizes analyzed in this study (Blasina et al., 2015; Lopez Cazorla, 1987, 1996, 2000), metal accumulation in its tissues could be linked to metals that belong from both the Bahía Blanca estuarine system as well as from the open seawaters nearby.

O. argentinensis and *P. orbignyanus* are two important species for studying metal accumulation since, as permanent inhabitants of the BBE (Lopez Cazorla, 2004, 2005; Valiñas et al., 2012), they show more accurately what happens within the estuarine system. In contrast to *C. guatucupa* results, these species both achieved the highest metal values in the gill tissues.

O. argentinensis achieved higher mean values of Cr, Fe and Mn in the gill tissues compared exclusively to *M. schmitti* ($p < 0.01$). Also, significant differences were found between the years of the sampling catches for this fish species, with higher values of Cr, Fe and Mn in the gill tissues from fish sampled in 2011 compared to the ones captured in 2012 ($p < 0.05$). As regards the liver tissues, Fe concentrations reached the higher values in 2012 compared to 2013 ($p < 0.05$) and higher Cr values in 2011 compared to 2012 and 2013 ($p < 0.001$). As for the muscle tissues, Fe also achieved higher values in 2012 compared to 2011 ($p < 0.05$).

As for *P. orbignyanus,* even though no statistical differences could be observed towards differential metal accumulation, there was a trend of greater concentrations of Mn in the gills compared to values found in *C. guatucupa* and *M. schmitti* tissues. Also, this fish species achieved a maximum mean value of Cr in the gills considering the entire period of sampling (0.80 ± 1.1).

M. furnieri showed, in sampling dates 2012 and 2013, higher mean values of Mn in the gills of juveniles of the species when compared to *M. schmitti* ($p < 0.05$). During the sampling date 2011, with almost all samples being exclusive residents of the BBE due to their sizes (see Lopez Cazorla, 2004), maximum levels of Mn in the muscle tissues were also achieved in comparison to *C. guatucupa* and *O. argentinensis* ($p < 0.01$). Additionally, Cr found in the gill tissues of *M. furnieri* reached greater values than those found in *M. schmitti* ($p < 0.05$). Significant differences were also found for Mn according to the time of the samples catches. In 2011, Mn achieved higher concentration in both the gill and muscle tissues in comparison to the other sampling dates ($p < 0.01$).

As regards *B. aurea,* metal accumulation in its tissues found maximum values compared exclusively to *M. schmitti*. During the sampling date 2012, statistically higher levels of Cr and Mn in the gill samples ($p < 0.05$ both) and Mn in muscular tissues ($p < 0.01$) were achieved with respect to *M. schmitti*. Also, in 2013, hepatic concentrations of Fe and Mn in *B. aurea* were higher than those found in the liver tissues of *M. schmitti, O. argentinensis* and *M. furnieri* ($p < 0.05$, all of them).

For the elements Cr, Pb and Fe, analyzed in both the gill and liver tissues, *M. schmitti* displayed the lowest concentrations. Only the concentrations of Mn found in the muscle tissues during the sampling date 2013 were higher than *M. furnieri* ($p < 0.01$).

Amongst the selected fish species were evaluated those inhabiting the upper water column, including B*. aurea, O. argentinensis, C. guatucupa* and *M. furnieri,* and the benthonic species *M. schmitti* and *P. orbignyanus*. Many publications have previously indicated that benthic fishes could be further exposed to metal accumulation than those fishes from the upper water column. This could be as a result of the closer contact of benthic species to sediments and their greater uptake of benthic organisms (*e.g.* Çoğun et al., 2006; El-Moselhy et al., 2014; Wei et al., 2014; Yilmaz et al., 2010). Nevertheless, neither in this study nor in other publications (Bustamante et al., 2003; El-Moselhy et al., 2014; Yi et al., 2008), significant differences were found between upper water column and benthic species with respect to their metal concentrations. Due to the

Table 2
Mean concentrations and standard deviation of Cr, Pb, Fe and Mn (expressed in μg/g) in the gills, liver and muscle tissues of *Brevoortia aurea, Odontesthes argentinensis, Micropogonias furnieri, Cynoscion guatucupa, Mustelus schmitti* and *Paralichthys orbignyanus*. The concentrations are expressed according to the year of the sampling catches. <MDL: metal concentration below the method detection limits. ±SD: standard deviation. n: number of samples.

Fish species	Period	Gill tissues (mean value in μg/g ± SD)					Liver tissues (mean value in μg/g ± SD)					Muscle tissues (mean value in μg/g ± SD)				
		n°	Cr	Fe	Mn	Pb	n°	Cr	Fe	Mn	Pb	n°	Cr	Fe	Mn	Pb
Brevoortia aurea	2011	6	1,4 ± 0,84	2700 ± 2070	43 ± 25	0,78 ± 0,30	5	0,032 ± 0043	38 ± 44	0,98 ± 1,6	<MDL	6	0,10 ± 0,024	9,9 ± 7,8	0,28 ± 0,20	<MDL
	2012	12	0,30 ± 0,22	690 ± 360	17 ± 7,7	<MDL	9	0,21 ± 0,35	960 ± 1880	22 ± 47	<MDL	12	0,048 ± 0039	18 ± 17	0,31 ± 0,34	<MDL
	2013	8	0,44 ± 0,28	560 ± 290	12 ± 4,3	<MDL	8	0,11 ± 0,10	260 ± 90	2,7 ± 1,1	<MDL	8	0,10 ± 0,11	11 ± 3,2	0,16 ± 0,15	<MDL
Odontesthes argentinensis	2011	16	1,1 ± 0,81	2840 ± 3180	27 ± 29	0,71 ± 0,33	13	0,19 ± 0,11	190 ± 71	0,72 ± 0,58	<MDL	16	0,25 ± 0,40	8,02 ± 11	0,052 ± 0,18	<MDL
	2012	11	0,18 ± 0,093	280 ± 140	4,7 ± 2,3	<MDL	10	0,057 ± 0040	205 ± 130	1,0 ± 0,52	<MDL	11	0,044 ± 0021	25 ± 24	0,077 ± 0069	<MDL
	2013	10	0,55 ± 0,48	740 ± 630	13 ± 9,1	<MDL	10	0,051 ± 0026	105 ± 26	1,3 ± 0,44	<MDL	10	0,032 ± 0018	15 ± 13	0,20 ± 0,26	<MDL
Micropogonias furnieri	2011	5	0,63 ± 0,46	910 ± 670	20 ± 7,1	<MDL	2	0,078 ± 0033	69 ± 15	1,7 ± 1,7	<MDL	10	0,095 ± 0,16	10 ± 8,5	0,60 ± 0,65	<MDL
	2012	12	0,31 ± 0,17	510 ± 330	11 ± 5,3	0,72 ± 0,31	12	0,060 ± 0043	260 ± 310	2,4 ± 1,8	<MDL	12	0,025 ± 0019	620 ± 2090	0,12 ± 0,22	<MDL
	2013	13	0,33 ± 0,15	430 ± 240	9,7 ± 4,4	<MDL	14	0,092 ± 0050	120 ± 33	1,5 ± 0,62	<MDL	14	0,052 ± 0020	11 ± 13	0,029 ± 0043	<MDL
Cynoscionguatucupa	2011	12	0,97 ± 0,96	1560 ± 1440	21 ± 14	0,79 ± 0,72	11	0,29 ± 0,15	340 ± 120	1,0 ± 0,59	<MDL	12	0,45 ± 0,65	13 ± 14	<MDL	<MDL
	2013	5	0,31 ± 0,96	290 ± 95	3,06 ± 1,28	<MDL	4	0,059 ± 0054	210 ± 88	1,5 ± 0,43	<MDL	5	0,049 ± 0034	8,5 ± 7,1	<MDL	<MDL
Mustelusschmitti	2012	9	0,065 ± 0048	129 ± 152	1,0 ± 1,86	<MDL	10	0,042 ± 0023	140 ± 62	0,97 ± 0,87	<MDL	10	0,047 ± 0052	22 ± 14	<MDL	<MDL
	2013	10	0,076 ± 0032	76 ± 34	1,6 ± 1,2	0,66 ± 0,23	10	0,059 ± 0082	49 ± 11	1,03 ± 0,27	<MDL	10	0,046 ± 0032	18 ± 9,9	0,24 ± 0,17	<MDL
Paralichthys orbignyanus	2011	6	0,80 ± 1,1	1120 ± 1830	17 ± 29	0,81 ± 0,55	7	0,073 ± 0060	220 ± 340	5,3 ± 11	2,7 ± 5,7	11	0,030 ± 0028	4,7 ± 4,8	0,31 ± 0,31	<MDL

turbidity as well as the shallow depths of the BBE (Guinder et al., 2009), benthonic as well as upper water column fishes might probably be exposed to the same quantity and quality of sediments.

From this study, it is important to highlight that the maximum ages recorded for the benthonic species *P. orbignyanus* correspond to 1 and 2 years (according to Lopez Cazorla, 2005). This fish species reaches a maximum age of 7 years and medium length of 80 cm in the BBE (Lopez Cazorla, 2005). Thus, the reduced variability in the sampling data, together with the small number of individual catches could be responsible for the lack of suitability in using *P. orbignyanus* as indicator of metal accumulation. Still, maximum concentrations found in the gill tissues of *P. orbignyanus* could be a hint of the metal levels in the surrounding waters and in the re-suspended sediments.

Differences in metal accumulation according to the trophic position of fish species had already been reported in many previous publications (*e.g.* Gu et al., 2015; Wei et al., 2014). Nonetheless, such differences were not appreciated in the present research. Moreover, *M. schmitti*, though it occupied the highest trophic position amongst the species under study, achieved the lowest metal concentrations. It is worth to highlight that anatomical, physiological and life-history characteristics distinguish chondrichthyan fishes (*i.e. M. schmitti*) from teleosts (*i.e.* the rest of the species from this study). Characteristics such as the cartilaginous skeleton, placoid scales, a spiral valve intestine, slow growth rates, late sexual maturity and low fecundity, between others, might potentially affect the uptake and retention of certain metals (Mathews et al., 2008).

Even though fish analyzed are mostly migratory species, metal accumulation in fish organs provides, to some extent, evidence of exposure to a contaminate aquatic environment (Qadir and Malik, 2011). It is important to stress that most of the species from this study are either permanent inhabitants of the estuary or, according to their sizes, spend most of their time in the coastal waters. Thus, they could be used to assess the health condition of the area from which they were collected.

3.2. Relationships between metal levels and fish sizes

The relationships between fish size and metal concentrations were analyzed in this study. Correlations found between these two parameters were mainly negative and the fish species showed differences in the relationships and also according to the year of the sample catches. *B. aurea* found decreasing concentrations of Fe and Mn in the gill tissues as the fish sizes increased during 2013 (r^2:-0.73 y r^2:-0.81, $p < 0.05$). Similarly for *C. guatucupa*, a negative relationship between Fe values in the gill tissues and length was achieved in 2011 (r^2:-0.71, $p < 0.05$). Particularly, that year was the only one that involved both juveniles and adult fish samples of *C. guatucupa*. Moreover, *P. orbignyanus* achieved negative relationships between Cr, Fe and Mn concentrations in the gill tissues and the length (r^2:-0.85, r^2: −0.83 and r^2: −0.84, respectively).

Several international articles have previously showed negative accumulation patterns between Cr, Fe and Mn and fish size (*e.g.* Canli and Atli, 2003; Nussey et al., 2000). This trend could be caused by tissue growing more rapidly than trace metal intake (Merciai et al., 2014). The negative correlations found in the gill tissues might indicate an increased in the essential metal levels up to certain values and then a decrease in the concentrations due to detoxification mechanisms (Alvarado et al., 2006; Marcovecchio and Moreno, 1993). Metabolic rate of organisms is size-specific and is higher in smaller fish, with a subsequent higher quantity of respiratory water passing through the gills per time unit. These could indicate a higher potential uptake of metals in the gill tissues of small fish individuals (Merciai et al., 2014).

O. argentinensis was the only fish species that achieved a significant increase in metal concentrations with fish size. The concentrations of Mn in the liver tissues showed higher values at the same time as the fish size increase in 2011 (r^2:0.66). On the other hand, no negative or positive relationships were found for the fish species *M. Furnieri* and *M. schmitti.*

In this study, it is important to highlight that fish size ranges were deliberately narrow, including juveniles larger than 10 cm and adults, since one of the aims of the research was to study fish that were fit for human consumption. Thus, these narrow size ranges could have influenced the achieved relationships. Also, metal accumulation is species dependant and is influenced by the sex of the individuals, age and size. All these variables together could affect the relationships between metal levels and fish sizes (Has-Schön et al., 2015).

3.3. Distribution of Cr, Pb, Fe and Mn in fish tissues

Mean metal concentrations and distribution within each of the three analyzed tissues, considering the six fish species, are presented in Fig. 2.

The comparison of metal accumulation in the analyzed tissues showed that the differences in distribution were statistically significant for Cr, Fe and Mn ($p < 0.05$). On the other hand, Pb concentrations were found to be below the MDL in more than 90% of all the analyzed samples. These could be mainly due to the fact that Pb is found in seawater in the form of complexes or bounded to microparticles, and thus not usually bioavailable. Moreover, Pb is not efficiently transported along the trophic web in the marine environment (Neff, 2002).

The gill tissues were found to be the main organ of accumulation of Cr, Fe and Mn ($p < 0.001$) for most fish species. Maximum values were found in *C. guatucupa* for Cr (3.9 μg/g) and in *O. argentinensis* for both Fe (13,600 μg/g) and Mn (130 μg/g). Nevertheless, there were also exceptions to these distributions. For instance, lack of difference between tissues was achieved for Cr in *M. schmitti*, for Fe and Mn in *P. orbignyanus* and for Fe in *M. furnieri*. Moreover, regardless the fish species, all metals displayed the lowest concentrations in the muscle tissues.

Accumulation of Cr, Fe and Mn in the gill tissues could be related to some extent to the bioavailability of these metals in the aquatic

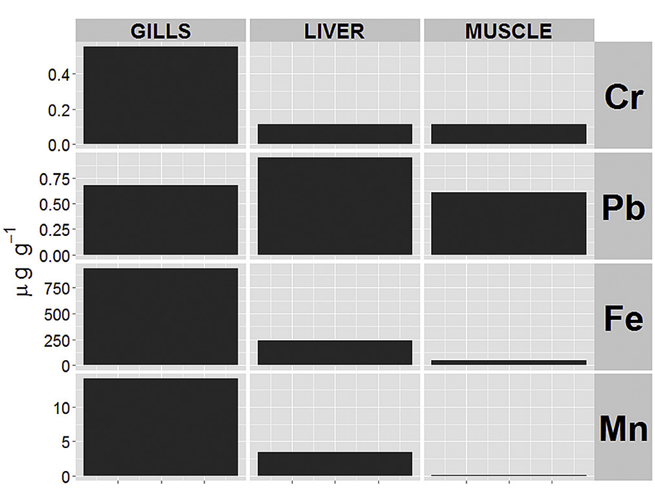

Fig. 2. Average metal concentrations corresponding to all sampling data and fish species. Data is grouped together according to the tissue of analysis.

media nearby (Karadede et al., 2004; Reynders et al., 2008; Tkatcheva et al., 2004), since water is filtered through the gills and metals are absorbed (Garnier-Laplace et al., 2000). It is suggested then that metals found in the gills are mostly accumulated from water (El-Moselhy et al., 2014). The gills are the first site of absorption from the water current, and thus are the first site of gaseous exchange, of acid-base regulation (Gorur et al., 2012) and could become significant sites of interaction with metal ions (Karadede et al., 2004; Reynders et al., 2008; Tkatcheva et al., 2004). Metal concentration in the gills might possibly be higher towards the beginning of the metal contamination, even before other tissues are exposed.

Specifically, these findings are in good concordance with values found for Cr and Fe in dissolved seawater from the same study area and the same sampling period (La Colla, *in preparation*; La Colla et al., 2015). Both dissolved metal values were found to be the maximum when compared to the concentrations found for other metals such as Cd, Cu, Ni, Zn, Hg and Pb among the elements under analysis.

3.4. Estimation of potential public health risk for fish consumption

With regards to the muscle tissues, metal concentrations were considerably lower than those found in the gills and liver tissues, as shown in Fig. 2. Concordantly, the muscular tissues are not acknowledged for being sites of metals accumulation (Alcorlo et al., 2006; Karadede and Ünlü, 2000). Nevertheless, in aquatic biota, the muscle is indeed important for being the main link to human health risks. The muscle tissues are usually evaluated as metal concentrations may exceed the permissible levels for human consumption and involved hazards (non-cancer effects) to human health (Henry et al., 2004; Marcovecchio et al., 1991; Pourang, 1995).

Although Cr, Fe and Mn are essential elements and thus necessary for human health, exposure to high levels might result in adverse effects. For instance, high doses of Mn could possibly cause neurological effects (Squadrone et al., 2016). Fe has been associated to increase risks of chronic diseases such as cardiovascular disease (EFSA, 2004). Pb is a non-essential element and is known to be a metabolic poison (Pan and Wang, 2012). Cumulative Pb could cause neurotoxicity, nephrotoxicity and many other adverse health effects (Rahman et al., 2012).

Metal values in the muscle tissues, using wet weight, were compared with both national and international guidelines, in order to establish the maximum content of metals in fish muscle tissues above which, human consumption is not allowed. Maximum permitted values provided by the international guidelines are: 0.15 μg/g for Cr (WHO, 1985), 2 μg/g for Pb (WHO, 1985), 100 μg/g for Fe (FAO, 1989) and 0.5 μg/g for Mn (WHO, 1985). Meanwhile, a maximum of 2 μg/g for Pb is provided by the Argentinean food legislation according to the ANMAT (Resolutions 116/2012 and 356/2012).

Cr and Mn levels occasionally exceeded the concentration guidelines above mentioned. 35%, 16% and 12% of the samples belonging to *C. guatucupa*, *O. argentinensis* and *B. aurea*, respectively, exhibited values of Cr above of those recommended for human consumption. As for the Mn values, 36%, 20% and 14% of samples belonging to *P. orbignyanus*, *B. aurea* and *M. furnieri*, respectively, exhibited values that could pose a public health threat if consumed. One only tissue sample from *M. furnieri* achieved concentrations of Fe above those recommended by guidelines. On the other hand, none of the fish samples analyzed presented Pb concentrations exceeding the proposed limits; in fact all muscle samples were below the MDL.

Although such metal levels were detected in the fish species analyzed, it cannot be sustained that the species are not fit for human consumption, owing to the large quantities of fish that must be eaten daily to be harmful to human health.

4. Conclusion

The current study reports updated information on Cr, Pb, Fe and Mn occurrence in commercial fish species from the Bahía Blanca coastal environment, a system under anthropogenic pressure.

Overall, the results suggest that metal burden in fishes varies with the species and metal elements, where the gill tissues were found to be the main organ of accumulation of Cr, Fe and Mn for most species.

Considering the species of analyses, *Cynoscion guatucupa* stood up as a good biondicator of Cr and Fe concentrations in the liver tissues. *Odontesthes argentinensis* and *Paralichthys orbignyanus* were important species for biomonitoring as they reflected more accurately metal concentrations in the studied estuarine environment, by being resident species for their entire life cycle. Further studies are then needed to analyze the role that the trophic position, the feeding behaviour and the characteristics of the skeletons play in metal distribution and accumulation.

As regards human health, Cr and Mn levels occasionally exceeded the maximum contents in fish muscle tissues above which, human consumption is not allowed. Care must be taken considering that edible tissues of both *C. guatucupa* and *P. orbignyanus* are not only locally consumed but they are also important fish species involved in international trades, mainly with Spain and African countries. Thus, it is recommended to conduct continuous monitoring for commercial fish species in the BBE.

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