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Differential tissue accumulation of arsenic and heavy metals from diets in three edible fish species

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Abstract

Three different commercial fish species *Odontesthes bonariensis*, *Rhamdia quelen* and *Oreochromis niloticus* and fish feed were collected from four aquaculture farms. Heavy metal (Cd, Cr, Cu, Fe, Mn, Pb and Zn) and arsenic concentration were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES) in muscle, liver, gonad, skin, scale and fat from fish and in feed diets. Arsenic concentration was found in different tissues differing between species and within *O. bonariensis*. Cd was differentially accumulated in liver in *O. bonariensis* and *R. quelen;* however, in *O. niloticus* Cd was found in muscle and scales. Higher concentrations of Cr were determined in skin and scales of *O. bonariensis* and *O. niloticus*. Cu, Fe, Mn and Zn were found in all tissues being Cu and Fe concentrations higher in liver. Mn was differentially accumulated in *O. bonariensis* scales, however in *R. quelen* no significant differences were found and in *O. niloticus* liver was the main accumulation tissue. Zn concentration was higher in gonad, skin and liver from *R. quelen* and *O. bonariensis,* and in *O. niloticus* the highest concentration was found in scales. All the results were below the international limits for food safety except for the concentration of Cd in muscle and scales of *O. niloticus*.

KEY WORDS: accumulation, arsenic, fish tissue, heavy metals

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Introduction

Arsenic and heavy metals constitute a central group of aquatic contaminants due to the toxicity, high persistence and bioaccumulative properties in the food chain (Ikem & Egiebor 2005). Heavy metal concentration in fish has been studied in many countries around the world and becomes of particular interest due to the intimate relation with human health through consumption (Dural *et al.* 2007; Castro-González & Méndez-Armenta 2008; Herreros *et al.* 2008; Uysal *et al.* 2008). Bioaccumulation of heavy metals occurs in several tissues of aquatic organisms and may become toxic for fish and also for people when it reaches a substantially high level (Dural *et al.* 2007). The uptake of these pollutants is commonly related to its concentration in water and the trophic chain in natural aquatic environments; however, in confined farming environment the feed stuff becomes a key factor as a point source. The biomagnification of metals and trace elements results from the combination of gastrointestinal absorption efficiency and the transference rate to different tissues, among other factors (Kelly *et al.* 2008). Fish consumption is associated with a healthy diet due to the high contents of essential polyunsaturated fatty acids of the omega-3 family; however, fish constitute an important source of heavy metal and other contaminants (Gladyshev *et al.* 2009). The presence of these contaminants in fish feed may lead to an increase in fish products, thus a risk for production and for human consumption. Arsenic presence was reported in fish feed products to limit levels close to be excluded from the market (Sloth *et al.* 2005).

The nutrients, as well as contaminants, found in fish flesh are derived largely from the feed and, thus, farmed fish can be tailored to provide optimal levels of fatty acids, and selected vitamins and minerals for human consumption (Gordon Bell & Waagbø 2008).

Arsenic is a natural pollutant in Argentina affecting soils and surface and groundwater in natural environments (Rosso *et al*. 2011). In previous studies, trace elements were determined in source and effluent water from fish farms showing an increase of some elements which were not present in source water and may come from fish feed (Schenone *et al*. 2011). For these reasons, determination of chemical quality on fish feed and fish from aquaculture has become an emerging concern. However, the information available regarding this matter is scarce.

The aim of this study was to investigate the distribution of trace elements in fish feed and in different tissues of farmed fish. For this purpose, fish feed and three different fish species from four aquaculture sites, three in Buenos Aires province and one in Corrientes province (Fig. 1) were collected. Corrientes province is located in the northeast region of Argentina and has less agricultural and anthropic influence. However natural arsenic contamination in water and heavy metals in fish diets may contribute to the concentration of these elements in fish tissues. As, Cd, Cr, Cu, Fe, Mn, Pb and Zn were measured in muscle, liver, gonad, skin, scale and fat from fish samples and in fish feed due to the presence of these elements in water (Schenone *et al*. 2011). Catfish, *Rhamdia quelen* (Quoy & Gaimard) and Silverside, *Odontesthes bonariensis* (Valenciennes) are two of the native species studied in this study and Tilapia, *Oreochromis niloticus* (Quoy & Gaimard) as non-native specie.

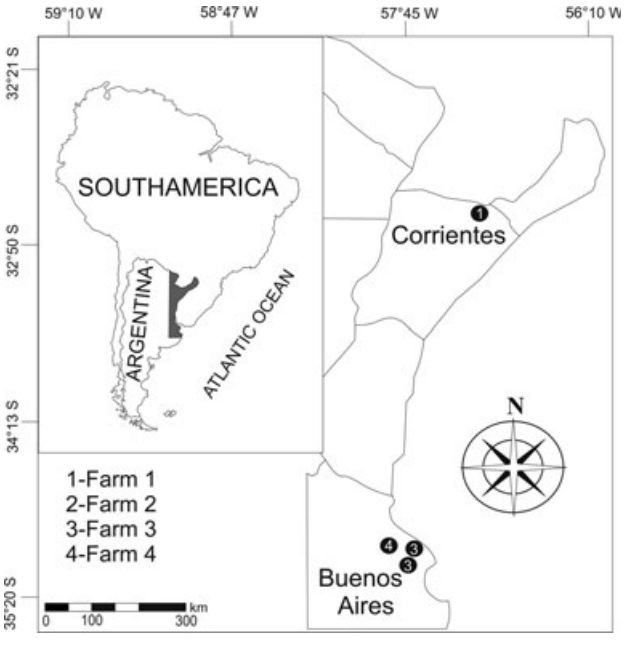

Figure 1 Map with the respective farms location.

This research on heavy metals and arsenic in aquaculture diets and products reports for the first time the differential concentration in tissues and the feed effect on the production in this region.

Materials and methods

Study area and fish samples

Fish samples from three different commercial species and fish feeds were obtained from local production farms in Buenos Aires and Corrientes province (Fig. 1). Due to the climatic conditions Corrientes province is more suitable for *R. quelen* production (Farm 1) and Buenos Aires for *O. bonariensis* (Farms 2 and 3). *Oreochromis niloticus* from farm 4 correspond to an intensive indoor production system to reach optimum culture temperatures (Table 1).

A total of three fish from each site were randomly captured from production ponds using hand nets, placed on ice and transported to the laboratory the same day. Fish were killed with percussive stunning (Van de Vis *et al*. 2003). Each sample collected from the farms was dissected for its muscle, liver, gonad, skin, scale and fat. To prevent metal contamination of the samples by the laboratory equipments, special care was taken and tissues were dissected by plastic knife and all laboratory ware was soaked in 10% HNO_3 for 48 h, and rinsed five times with distiled water, and then five times with ultra pure Milli-Q water prior to use (Turkmen & Ciminli, 2007). Each tissue was stored in plastic bags at −20 °C prior to preparation. Feed sample were collected in Ziploc^R bags from each farm, approximately 1 kg.

Sample preparation

During fish dissection, muscle, skin and gonad tissue were cut in small slices to improve digestion. Scale and fat were directly digested. The tissue samples and feed samples were digested with concentrated nitric acid. A portion of

Table 1 Size and weight of fish from the sampled farms

Location	Species	Scientific	Size range (cm)	Weight rage (g)
Farm 1	Catfish	*Rhamdia quelen*	22–24	276–290
Farm 2	Silverside	*Odontesthes bonariensis*	25–27	240–263
Farm 3	Silverside	*Odontesthes bonariensis*	24–26	250–255
Farm 4	Tilapia	*Oreochromis niloticus*	16–18	190–200

the dissected sample (2 g) was oven dry till constant weight and then transferred to a 100 mL beaker. Thereafter, 10 mL of concentrated HNO_3 was added to the sample. The beaker was covered with a watch glass and the sample heated at 200 °C on a hot plate for 3 h. The solution was evaporated slowly to near dryness. By repeating the digestion twice, all organic matter in every sample was completely digested. After cooling, a further 1 mL of concentrated HNO_3 was added to dissolve inorganic matter and the beaker rinsed with Milli-Q water. The solution was quantitatively transferred to a final volume of 50 mL in polyethylene tube. Before analysis, the samples were filtered through a 0.45 μm nitrocellulose membrane filter (Alam *et al*. 2002). Analytical blanks were run in the same way as the samples and determined using standard solutions (Merck^R, multi element standard) prepared in the same acid matrix. Certify reference material DORM-2 (National Research Council Canada, Ottowa, ON, Canada) was analysed to assure accuracy and recovery rates. All chemical used in this study were obtained from Merck^R analytical grade (Table 2).

Sample analysis

All samples were analysed by Perking Elmer (Waltham, MA, USA) Optima 2000 DV inductively coupled plasma–optical emission spectrometry (ICP-OES). The concentrations of As, Cd, Cr, Cu, Fe, Mn, Pb and Zn were determined. The concentrations were calculated on a wet weight basis for fish tissue.

Data analysis

Each reported result was the average of three analyses. The results were shown as mean ± SD. One-way ANOVA was utilized to compare the concentrations among tissues with Tukey's contrast using InfoStat statistic program. Results were considered significant at $P < 0.05$.

Table 2 Concentrations of elements (means ± SE, in mg kg^{-1} dry wt, $n = 3$) found in certified reference material DORM-2 (dogfish muscle, NRC, Canada)

Element	Certified values	Measured values	Recovery (%)
As	18.0 ± 1.1	16.4 ± 1.3	91
Cd	0.043 ± 0.008	0.046 ± 0.012	109
Cr	34.7 ± 5.5	33.5 ± 1.48	97
Pb	0.065 ± 0.007	0.067 ± 0.009	103
Cu	2.34 ± 0.16	2.32 ± 0.21	99
Fe	142 ± 10	137 ± 11.5	96
Mn	3.66 ± 0.34	3.46 ± 0.46	95
Zn	25.6 ± 2.3	23.9 ± 1.85	93

Results

As and heavy metal concentration in catfish (*R. quelen*) and feed from farm 1 are shown in Table 3. The presence of As was detected in muscle, liver and gonad but there were no significant differences, although the concentration in feed was below the detection limit. Arsenic concentration in muscle should be of particular interest due to human consumption. Cd was detected in fish feed and liver, however in the other tissues was below the detection limit. Cr was detected in fish feed and in all the tissues. The highest Cr concentration was found in gonad. Pb concentrations were below detection limit in all tissues and fish feed. Cu, Fe, Mn and Zn can be considered as micronutrients; however, in high concentrations they can be toxic. The Cu accumulation was higher in liver and lower in the other tissues. The highest Fe value was found in liver followed by the concentration in gonad; muscle, skin and fat did not show significant differences. Mn concentration did not show significant difference; however, liver showed a higher accumulation tendency. Zn highest value was observed in gonad, the other tissues had lower concentrations with no significant differences.

As and heavy metal concentrations in *O. bonariensis* and fish feed from farm 2 are shown in Table 4. Arsenic was found in muscle and liver and also in fish feed. Cd was detected in fish feed and showed a differential accumulation in liver and in the other tissues was below the detection limit, the same pattern was observed in *R. quelen*. Fish feed showed a high Cr concentration. The higher Cr concentration was found in scales and also was observed in muscle and gonad in lower concentration. Pb was below the detection limit in all tissues; however, it was detected in fish feed. Cu was observed in all tissues but there were no significant differences. Fe was detected in all tissue samples and was higher in liver and gonad, and the lower value was found in muscle. Mn concentration showed a significantly higher concentration in scale than in the other tissues. Zn was differentially accumulated in skin while muscle showed the lower value.

As and heavy metal concentrations in *O. bonariensis* and fish feed from farm 3 are shown in Table 5. Arsenic concentration was only detected in skin even though a high concentration was observed in feed a different pattern was observed in farm 2. Cd showed a differential accumulation in liver, and it was detected in fish feed. The same pattern

Table 3 Trace elements concentration (mg kg^{-1} ww) (mean ± SD) in different tissues of *Rhamdia quelen* from farm 1

	Muscle	Liver	Fat	Gonad	Skin	scale	Feed
Rhamdia quelen (Farm 1)							
As	0.55 ± 0.18[a]	0.22 ± 0.08[a]	bdl	0.38 ± 0.13[a]	bdl	nd	bdl
Cd	bdl	0.31 ± 0.23	bdl	bdl	bdl	nd	0.24 ± 0.08
Cr	0.10 ± 0.01[b]	0.14 ± 0.03[b]	0.08 ± 0.03[b]	0.23 ± 0.02[a]	0.11 ± 0.03[b]	nd	1.57 ± 0.22
Pb	bdl	bdl	bdl	bdl	bdl	nd	bdl
Cu	0.42 ± 0.03[b]	16.64 ± 7.50[a]	0.40 ± 0.16[b]	1.58 ± 0.46[b]	2.55 ± 0.71[b]	nd	77.38 ± 17.35
Fe	8.92 ± 3.68[c]	357.72 ± 82.56[a]	19.25 ± 5.10[c]	134.51 ± 24.82[b]	28.93 ± 5.65[c]	nd	223.82 ± 19.77
Mn	0.85 ± 0.61[a]	1.88 ± 0.40[a]	0.91 ± 0.90[a]	0.99 ± 0.57[a]	0.61 ± 0.14[a]	nd	434.53 ± 24.92
Zn	8.39 ± 1.26[b]	27.00 ± 8.91[b]	2.37 ± 0.47[b]	129.06 ± 29.31[a]	28.94 ± 4.09[b]	nd	625.98 ± 2.88

bdl, below detection limit; nd, not determined.
Values with non-common letter superscript are significantly different ($P < 0.05$).

Table 4 Trace elements concentration (mg kg^{-1} ww) (mean ± SD) in different tissues of *Odondetesthes bonariensis* from farm 2

	Muscle	Liver	Fat	Gonad	Skin	Scale	Feed
Odontesthes bonariensis (Farm 2)							
As	0.47 ± 0.05[a]	0.67 ± 0.29[a]	nd	bdl	bdl	bdl	2.65 ± 0.35
Cd	bdl	1.70 ± 0.56	nd	bdl	bdl	bdl	2.07 ± 0.21
Cr	0.19 ± 0.03[b]	bdl	nd	0.37 ± 0.06[b]	bdl	0.93 ± 0.24[a]	1.52 ± 0.25
Pb	bdl	bdl	nd	bdl	bdl	bdl	0.39 ± 0.08
Cu	1.10 ± 0.07[a]	1.74 ± 0.12[a]	nd	1.04 ± 0.38[a]	1.16 ± 0.27[a]	1.32 ± 0.47[a]	Nd
Fe	6.92 ± 0.78[c]	20.09 ± 5.44[ab]	nd	24.19 ± 2.99[a]	17.02 ± 2.62[ab]	9.40 ± 3.15[bc]	Nd
Mn	0.44 ± 0.03[b]	1.196 ± 0.27[b]	nd	1.10 ± 0.51[b]	1.538 ± 0.21[b]	24.03 ± 4.12[a]	Nd
Zn	8.75 ± 1.28[c]	18.42 ± 2.49[b]	nd	14.50 ± 2.65[bc]	40.13 ± 7.27[a]	14.21 ± 2.46[bc]	Nd

bdl, below detection limit; nd, not determined.
Values with non-common letter superscript are significantly different ($P < 0.05$).

Table 5 Trace elements concentration (mg kg^{-1} ww) (mean ± SD) in different tissues of *Odondetesthes bonariensis* from farm 3

	Muscle	Liver	Fat	Gonad	Skin	Scale	Feed
Odontesthes bonariensis (Farm 3)							
As	bdl	bdl	nd	bdl	0.61 ± 0.01	bdl	2.49 ± 0.15
Cd	bdl	0.28 ± 0.01	nd	bdl	bdl	bdl	1.95 ± 0.17
Cr	0.12 ± 0.01[b]	bdl	nd	0.24 ± 0.01[b]	0.27 ± 0.02[b]	1.02 ± 0.24[a]	1.21 ± 0.37
Pb	bdl	bdl	nd	bdl	bdl	bdl	0.60 ± 0.28
Cu	0.32 ± 0.01[d]	1.35 ± 0.11[b]	nd	1.08 ± 0.20[bc]	2.09 ± 0.24[a]	0.93 ± 0.03[c]	nd
Fe	4.76 ± 0.26[d]	62.78 ± 5.33[a]	nd	32.00 ± 2.43[d]	10.96 ± 1.65 [cd]	15.64 ± 3.44[c]	nd
Mn	0.25 ± 0.04[b]	1.14 ± 0.19[b]	nd	0.93 ± 0.13[b]	0.67 ± 0.07[b]	16.89 ± 2.16[a]	nd
Zn	6.04 ± 0.35[c]	14.30 ± 0.82[b]	nd	25.28 ± 4.09[a]	21.26 ± 4.65[ab]	16.74 ± 1.14[b]	nd

bdl, below detection limit; nd, not determined.
Values with non-common letter superscript are significantly different ($P < 0.05$).

was observed in *R. quelen* (farm 1) and *O. bonariensis* (farm 2). With regard to Cr, there was a significantly higher concentration in scale than in muscle, gonads and skin. In liver, the concentration was below the detection limit. Cr concentration in feed was similar to farm 2 feed. This result is similar to that obtained in *O. bonariensis* (farm 2), where the highest accumulation occurs in scales. It is known that some fish species accumulate metals in mucus and thus, its relation with scales (Maunder *et al.* 2011). The highest concentration of Cu was observed in skin, differing significantly from other tissues. The lowest Cu concentration was observed in muscle. Fe concentration was significantly higher in liver than in other tissues. The lower Fe concentration was observed in muscle. This differential accumulation in the liver is consistent with that observed in farm 1 and 2. With respect to Mn, the highest concentration was observed in scales as in *O. bonariensis* from farm 2. The other tissues showed lower concentra-

Table 6 Trace elements concentration (mg kg^{-1} ww) (mean ± SD) in different tissues of *Oreochromis niloticus* from farm 4

	Muscle	Liver	Fat	Gonad	Skin	Scale	Feed
Oreochromis niloticus (Farm 4)							
As	bld	bdl	bdl	nd	nd	bdl	bdl
Cd	0.24 ± 0.11[b]	bdl	bdl	nd	nd	20.34 ± 1.61[a]	0.65 ± 0.08
Cr	0.31 ± 0.01[b]	bdl	1.23 ± 0.30[b]	nd	nd	9.73 ± 0.66[a]	0.67 ± 0.10
Pb	bdl	bdl	bdl	nd	nd	bdl	bdl
Cu	1.77 ± 0.15[b]	52.51 ± 3.40[a]	2.35 ± 0.18[b]	nd	nd	5.84 ± 0.55[b]	6.41 ± 0.68
Fe	15.37 ± 2.27[b]	76.96 ± 6.30[a]	15.70 ± 2.26[b]	nd	nd	7.90 ± 0.29[b]	115.34 ± 13.34
Mn	0.34 ± 0.02[c]	2.61 ± 0.26[a]	2.02 ± 0.09[a]	nd	nd	1.09 ± 0.23[b]	30.62 ± 2.78
Zn	8.74 ± 0.61[b]	18.31 ± 3.89[a]	5.61 ± 0.33[b]	nd	nd	40.86 ± 3.64[a]	65.92 ± 5.98

bdl, below detection limit; nd, not determined.
Values with non-common letter superscript are significantly different ($P < 0.05$).

tions of Mn, with no differences. There was a higher concentration of Zn in gonad than in other tissues but no significant difference was found with skin. The values obtained are similar to those obtained in *O. bonariensis* from farm 2.

As and heavy metal concentrations in *O. niloticus* and fish feed from farm 4 are shown in Table 6. As and Pb concentrations in all tissues were below the detection limit and also in fish feed. The highest Cd concentration was found in scales, differing to the species above. In muscle, there was a lower Cd concentration and in the other tissues, values were below the detection limit. It is interesting to remark that Cd in muscle was only detected in *O. niloticus*. The presence of Cd was also detected in fish feed. The highest Cr concentration was observed in scales. Muscle and fat showed lower Cr concentrations and in liver was below detection limit. Fish feed showed Cr presence. It is interesting to remark that Cr was also accumulated in the scales of *O. bonariensis* from farm 2 and farm 3. Cu concentration showed differential accumulation in liver. The other tissues did not showed differences. This Cu distribution was also observed in *R. quelen* from farm 1. The Fe concentration showed a similar pattern as Cu with higher concentration in liver, differing from the other tissues. The higher Mn concentration was observed in liver and fat and the lower in scale and muscle. Zn concentration was higher in scale and liver and lower in muscle and fat.

Discussion

According to the results obtained in this study, the different accumulation patterns may differ between species and also within species (*O. bonariensis*) due to farming practices and other factors (e.g. genetic, age and physiological state). The values obtained are similar to those found in available bibliography (Papagiannis *et al*. 2004; Lin *et al*. 2008; Uysal *et al*. 2008; Partridge & Lymbery 2009; Yilmaz 2009; Minganti *et al*. 2010; Martins *et al*. 2011). However, it is important to remark that the values among these studies are highly variable because of many factors (e.g. species, age, feeding and water quality).

Arsenic

In this study, the As concentration showed a differential accumulation pattern; however, this pattern was different in each species. *Rhamdia quelen* showed higher values in muscle and lower in gonad and in liver while in *O. bonariensis* (farm 2), the higher concentration was found in liver and in *O. bonariensis* (farm 3) in skin.

Argentinean legislation limit for arsenic in food is 1 mg Kg^{-1} (National Administration of Drugs, Food & Medical Technology, ANMAT 2012), also UK Food Standards Agency (FSA 2012) proposed 1 mg Kg^{-1} as the statutory limit for commercial foods and the Canadian Guidelines for Chemical Contaminants and Toxins in Fish and Fish Products (Fish Products Standards & Methods Manual 2005) establish a maximum of 3.5 mg kg^{-1}. According to theses limits, the values obtained for *R. quelen* are below and should not cause any negative health effect.

The Joint Expert Committee on Food Additives (JECFA FAO/WHO 2010) determined a benchmark dose limit ($BMDL_{0.5}$) from epidemiological studies to be 3.0 μg kg^{-1} bw per day. In turn, the European Food Safety Authority Panel on Contaminants in the Food Chain (EFSA, CONTAM 2009), established a benchmark dose limit ($BMDL_{01}$) between 0.3 and 8 μg kg^{-1} bw per day for inorganic arsenic. For a 70 kg person, the weekly intake would be between 0.147 mg $week^{-1}$ and 3.920 mg $week^{-1}$ for the lower and higher value, respectively. The highest value found in muscle in this study corresponds to *R. quelen* (0.55 mg kg^{-1} ww) so, a 70 kg person would have to

consume 0.267–7.127 kg of fish muscle a week to pass the proposed limit. However, only a percentage of the total arsenic in fish is inorganic (1–5%) (Brooke & Evans 1981). Also, recently published articles are considering arsenic speciation in freshwater fish due to high amount of organic arsenic species when compared with inorganic arsenic. In some freshwater fish, it was observed that oxo-arsenosugar-phosphate were the mayor arsenic compound (Schaeffer *et al*. 2006). In many salmonids, the main arsenic compounds found were arsenobetaine (AsB), which accounted for 92–100% of extractable arsenic (Slejkovec *et al*. 2004). If so, the values observed would be far below the BMDL. It is very important to highlight the fact that not taking into account the different species but considering total arsenic as being present exclusively as inorganic arsenic would lead to a considerable overestimation of the health risk related to dietary arsenic exposure (EFSA 2009). Arsenic speciation in freshwater fish species is becoming an interesting field for research when considering food safety. It is interesting to remark the high arsenic levels in the feed from farms 2 and 3; however, the effect over tissues of *O. bonariensis* is not the same. This could be the effect of different metabolic pathways related to the genetic origins of the specie. The *O. bonariensis* is found in wide variety of environments with different concentrations of natural arsenic up to 500 μg L^{-1}.

Cadmium

Cd showed a differential accumulation pattern in liver in *R. quelen* and *O. bonariensis* from both farms (2 and 3) with similar concentration values. However, in *O. niloticus,* the Cd higher accumulation occurred in scales with a much higher value than the other species and was also detected in muscle. The scale accumulation pattern was observed for other species as an important processes for Cd excretion (Varanasi & Markey 1978; Faucher *et al*. 2008). Nogami *et al*. (2000) found in commercial fish diets Cd concentrations of 0.3 mg kg^{-1} and 1.4 mg kg^{-1}. These values resulted in a muscle concentration of 0.46 ± 0.1 mg kg^{-1} and 0.6 ± 0.1 mg kg^{-1} in viscera (Nogami *et al*. 2000). These values are within the range of the values obtained in this study. According to Zhou *et al*. (1998), metals are generally found in smaller amounts in muscle than in skin, gills and viscera in a study of *O. niloticus* in different natural environments. The maximum value allowed by the European Community (EC 2006) of Cd in muscle meat of fish is 0.05 mg kg^{-1} ww (wet weight). Moreover, the tolerable weekly intake (TWI) established by the EFSA (2009) is 2.5 μg kg^{-1} bw (body weight) for Cd (150 μg $week^{-1}$ for a 60 kg person). Considering these recommendations, we found that the value in scales and in muscle exceeds the limit set by the European Community (0.05 mg kg^{-1} ww), and so consumption might lead to a risk for human health. When considering the TWI, more than 0.625 kg of fish muscle a week for a 60 kg person would have adverse effects. According to the Public Health Regulation (Hong Kong Government 1989) and the Hong Kong Center for Food Safety, the recommended Cd limit for consumption in muscle is 2.0 mg kg^{-1} ww. Given this value, the recorded values of Cd in muscle in this study do not exceed proposed limit.

Chromium

Cr accumulation in scales was observed in *O. bonariensis* (farm 2 and 3) and *O. niloticus*; however, in *R. quelen* the highest concentration was found in gonad. The Cr concentration in *O. niloticus* muscle (0.30 mg kg^{-1} ww) did not exceed the limit proposed by the Public Health Regulation (1.0 mg kg^{-1} ww) (Hong Kong Government 1989). However, the value in fat (1.32 mg kg^{-1} ww) is over the proposed limit.

Copper, iron, manganese and zinc

Cu was observed in the analysed tissues from the three species. In *O. bonariensis* from farm 2 and 3, the accumulation distribution was variable. However, in *R. quelen* and *O. niloticus,* the higher values were found in liver. Although Cu is essential for human nutrition and health, high intake levels may cause problems (Demirezen & Uruc 2006).

Fe was also observed in all tissues from the three species with higher values in liver due to the presence of transferrin and ferritin (Neves *et al*. 2009). The highest value was observed in liver of *R. quelen*.

Mn concentrations showed similar distribution in *O. niloticus* and *R. quelen* with the highest concentration in liver. *Odontesthes bonariensis* showed accumulation of Mn in scales with higher values than the other species. It should be noted that concentrations of Mn in the livers of *O. bonariensis* were similar to those found in *O. niloticus* and *R. quelen*.

Zn concentration showed a high variability between species; however, the concentration in muscle was similar. *Rhamdia quelen* and *O. bonariensis* from farm 3 showed higher concentration of Zn in gonad than in the other tissues. Papagiannis *et al*. (2004) observed a higher concentration of Cu and Zn in gonad than in muscle in four freshwater fish species. Also Sindayigaya *et al*. (1994)

found higher levels of Cu and Zn in gonad than in muscle in two freshwater species.

Differential tissue accumulation of heavy metal is a very important factor in emerging aquaculture due to the implication on diet formulation. To reduce costs, small producers uses viscera and other parts of the fish of non-commercial value to prepare silage which is included in fish diets. This process should be of particular interest due to the bioaccumulation of heavy metals within the production.

Conclusion

This study was carried out as a first approach to understand trace metal accumulation from feed in farmed fish. All the results were below the limits proposed by EC (2001); FAO/WHO (2010) except for the concentration of Cd in muscle and scales of *O. niloticus*. However, the limit proposed by the Public Health Regulation (Hong Kong Government 1989) is higher, and if this limit is considered, the values obtained are below.

The variation of heavy metal concentration in tissues depends on the eating habits, time of exposure to different elements and metabolism of species. Although there is a wide range of international data of heavy metal values in fish, few are the relationships with accumulation patterns. Given the importance in food safety, further investigation should be necessary to establish guidelines and limits for human consumption.

The arsenic presence in fish edible tissue should be of particular interest due to the occurrence as a natural water pollutant in many regions of the world; however, the concentrations in this study were below the legal values. Furthermore, arsenic speciation should be of particular interest due to the lack of scientific information respect freshwater fish. At a regional level, the information in this emerging topic is scarce and so, further investigation should be emphasized. Research on nutrition and fish diet formulation will be the key for a responsible emerging aquaculture.

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