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Total mercury and selenium concentrations in *Sarpa salpa* and *Balistes capriscus* and in their respective Digenean endoparasites *Robphildollfusium fractum* and *Neoapocreadium chabaudi* from Tunisia

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Abstract

The present study reports the levels of mercury and selenium in *Sarpa salpa* and *Balistes capriscus* collected along the coast of Mahdia and Sfax (Tunisia). The systems constituted by *S. salpa* and *Robphildollfusium fractum* and by *B. capriscus* and *Neoapocreadium chabaudi* were tested as potential bioindicators to monitor environmental Hg pollution in marine ecosystems. Mercury and selenium concentrations were assessed in kidney, liver and muscle of 51 *S. salpa* and of 45 *B. capriscus* as well as in their respective endoparasites *R. fractum* and *N. chabaudi*. The Se:Hg molar ratios were evaluated for both species across the study areas. Surprisingly, the Se:Hg molar ratio in *B. capriscus* muscle from Mahdia is significantly lower than in Sfax. Our results indicate that some parasites may also be implicated in the amount of Se and Hg available in tissues and therefore contribute to oscillations of the Se:Hg molar ratios. In the model involving the carnivorous species (*B. capriscus*), the 5.1-times higher levels of mercury in *N. chabaudi* than in *B. capriscus* muscle in Sfax enable this fluke to be a sensitive biomonitoring tool for Hg pollution. The present results confirm that the habitual consumption of *S. salpa* should not suppose any potential health risk for Tunisian people. On the other hand, the consumption of *B. capriscus* may be of concern and further monitoring is advisable, since the Hg average concentration in Mahdia was above the maximum allowed Hg concentration in the edible portion of fish fixed by the European Union.

Keywords

Mercury, Selenium, Se:Hg molar ratio, *Sarpa salpa*, *Balistes capriscus*, *Robphildollfusium fractum*, *Neoapocreadium chabaudi*, Tunisia

Introduction

Several pollutants are continuously being introduced into marine ecosystems as a consequence of several sources. Heavy metals are persistent pollutants that can be accumulated by marine organisms through a variety of pathways and they are biomagnified in the food chains, becoming increasingly dangerous for human consumption. In this respect, special attention should be taken to the exogenous harmful mercury and also to selenium, an essential element acting as a protective agent against the toxicity of mercury (WHO 1987; USA/EPA 1998; Burger and Gochfeld 2011).

Adequate assess of ecosystem health by means of biomonitoring requires selecting representative species that allow compiling data in a wide range of their distribution in order to obtain more valuable and representative results. Among others, several teleostei fish species, which constitute an important part of the human diet, have been widely used to monitor marine mercury pollution (Usero *et al*. 2003; Türkmen *et al*. 2008; Aksu *et al*. 2011; Mezghani-Chaari *et al*. 2011).

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In Tunisia, fishing is an important income source throughout the country, where different levels of pollution occur according to each region's main economic activity (eg, industry, tourism). Therefore, monitoring Hg pollution in common edible fish species is necessary.

The use of helminth parasites as bioindicators for heavy metal environmental pollution in aquatic ecosystems is commonly accepted since some species can accumulate high amounts of toxic elements even with a greater extent than free living organisms commonly used for monitoring water pollution (Sures 2003, 2008). Acanthocephalans are presently recognized for their excellent metal accumulation capacities in both field and experimental conditions and adult cestodes as well as nematodes also seem to be promising bioindicators for some toxic elements (Eira *et al*. 2009; Dural *et al*. 2011). However, there is no data concerning adult trematodes parasites of fish.

The main objective of this study was to determine the levels of mercury and selenium in the edible part as well as in other tissues of the cow bream *Sarpa salpa* (Linnaeus, 1758) (Teleostei: Sparidae) and the grey triggerfish, *Balistes capriscus* Gmelin, 1789 (Teleostei: Balistidae) collected in the coast of two different Tunisian governorates (Sfax and Mahdia) with different degrees of industrialization. The present work also evaluates for the first time two fish systems involving adult trematodes *S. salpa*/*Robphildollfusium fractum* Paggi and Orecchia, 1963 (Lepocreadioidea: Gyliauchenidae) and *B. capriscus*/*Neoapocreadium chabaudi* Kohn and Fernandes, 1982 (Lepocreadioidea: Apocreadiidae) in order to determine the potential usefulness of these systems as bioindicators of mercury in the marine environment.

Materials and Methods

Tunisia has a 1,298 Km long Mediterranean coastline, where the governorates of Sfax and Mahdia are located. The coastal area off the city of Sfax, well-known for fisheries and industrial activities, receives several man made discharges enriched with organic matter nutriments and heavy metals (Serbaji *et al*. 2012). Contrarily, the touristic city of Mahdia seems to be only influenced by a minimal industrial pollution or anthropogenic effluents.

To perform the present study 51 specimens of *S. salpa* parasitized by *R. fractum* collected in 2009 using fishing nets along the coasts of Sfax (24 specimens) and Mahdia (27 specimens) and 45 specimens of *B. capriscus* parasitized by *N. chabaudi* collected during the same period using specific gill nets and hand lines (24 specimens from Sfax and 21 from Mahdia) were selected. After capture all fish specimens were immediately analysed removing their digestive tracts and scanning them for helminths by means of standard helminthological methods. Stainless steel instruments and Milli-Q water were always used. After species identification all individuals of *R. fractum* and *N. chabaudi* respectively collected from the intestine of *S. salpa* and *B. capriscus* individuals used in the present work were frozen in glass vials (–20°C) until posterior element analysis. In addition, samples of kidney, liver and muscle of all infected fishes were collected during dissection. These samples were stored in glass vials and frozen (–20°C) until posterior processing as described below.

All the analytical process was performed in the "Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB)" (certified according to ISO 9001:2008). Tissue samples were weighed (± 150mg wet weight) as well as *R. fractum* and *N. chabaudi* specimens and then digested in Teflon vessels with HNO_3 (2 ml) and H_2O_2 (1 ml) (Merck, Suprapure). Samples were left overnight at 90°C in an oven. All materials used in the process were thoroughly acid-rinsed. After digestion, samples were diluted with Milli-Q water and then they were analysed for Hg and Se by ICP-MS (Perkin Elmer Elan 6000). The analytical procedure was checked using samples of standard reference material Dogfish (*Squalus acanthias*) liver (DOLT-3) and muscle (DORM-2) from the National Research Council, Canada. Results for total mercury DOLT-3 were 0.37 ± 0.14 mg Kg^{-1} and the certified value was 0.382 ± 0.06 mg Kg^{-1} (96.8% accuracy). Results for selenium DORM-2 were 1.35 ± 0.17 mg Kg^{-1} and the certified value was 1.40 ± 0.09 mg Kg^{-1} (96.4% accuracy). Analytical blanks were also prepared and analysed along with samples in order to determine the detection limits, which were 0.11 ng ml^{-1} for mercury and 0.28 ng ml^{-1} for selenium.

The data obtained did not follow a normal distribution even after log (x+1) transformation. Therefore, the differences between element concentrations among the analysed tissues in both species and both sampling sites were assessed by the Kruskal-Wallis test and post-hoc Dunn's test. The Mann Whitney test was also used to compare concentration values in parasites across study areas and also to compare Se:Hg molar ratios among fish species and study areas. Linear regression was used to assess relationships between element concentrations in different tissues and parasites. Statistical analyses were performed in Prism 5 and JMP 9. For all tests, a significance level of $p<0.05$ was applied. All concentrations are expressed in parts per billion (ppb = ng/g) on a wet weight basis. The molar concentrations were obtained by dividing by the molecular weight (200.59 for Hg and 78.9 for Se) as proposed by Burger and Gochfeld (2011). The bioaccumulation factors were determined according to Sures *et al.* (1999), as the ratio of the element concentration in the parasites to that in different host tissues ($BF = C_{[parasite]}/C_{[host\ tissue]}$).

Results

Mercury and selenium concentrations found in kidney, liver, and muscle of *S. salpa* and *B. capriscus* from both localities as well as in their respective flukes *R. fractum* and *N. chabaudi* are shown in Table I. The Se:Hg molar ratios in the fish edible portion are also presented in Table I.

Table I. Trace element concentrations in kidney, liver and muscle of *Sarpa salpa* and *Balistes capriscus*, and in their parasites *Robphildollfusium fractum* and *Neoapocreadium chabaudi* (ng g^{-1} wet weight) from Mahdia and Sfax (Tunisia). Se:Hg, molar ratio between selenium and mercury

	Kidney		Liver		Muscle		*R. fractum*		*N. chabaudi*	
	Mahdia	**Sfax**	**Mahdia**	**Sfax**	**Mahdia**	**Sfax**	**Mahdia**	**Sfax**	**Mahdia**	**Sfax**
S. salpa	n = 27	n = 24	n = 27	n = 24	n = 27	n = 24	n = 27	n = 24		
Hg	257.9 ± 63.2 146.1–397.8	1071.7 ± 780.0 223.2–3281.1	362.8 ± 194.3 158.3–966.0	971.8 ± 684.9 216.6–2448.5	33.1 ± 24.9 4.8–82.0	74.9 ± 41.2 16.5–155.0	N.D.	N.D.		
Se	1463.4 ± 267.1 982.3–1948.9	2217.9 ± 591.3 1391.0–3718.4	2079.6 ± 332.2 1532.4–3008.1	3120.6 ± 1070.1 1933.9–6330.1	344.4 ± 130.1 134.5–858.0	455.8 ± 151.5 139.6–772.7	2463.9 ± 922.5 437.6–4421.0	1150.1 ± 933.1 125.5–3591.9		
Se:Hg					26.4	15.5				
B. capriscus	n = 21	n = 24	n = 21	n = 24	n = 21	n = 24			n = 21	n = 24
Hg	2030.3 ± 1704.9 369.9–5573.1	2226.8 ± 2905.3 294.9–12994.1	1157.8 ± 1215.8 50.9–3775.4	1397.8 ± 1715.8 127.1–6747.9	533.2 ± 226.6 185.9–894.6	212.1 ± 131.3 66.6–678.6			841.9 ± 1711.1 33.2–6496.7	1077.8 ± 1200.0 132.8–4151.0
Se	9338.9 ± 2183.2 4795.9–14062.9	9677.0 ± 4143.0 5532.4–21258.7	2466.9 ± 1507.2 759.9–6860.4	3391.3 ± 1391.9 2055.1–7947.5	858.0 ± 248.6 448.4–1488.1	1409.2 ± 433.7 717.2–2237.2			4000.1 ± 1944.7 444.0–7228.6	5977.2 ± 3009.1 2112.6–13036.6
Se:Hg					4.1	16.9				

Many significant differences were found among the concentration values obtained for each tissue in each fish species and across prospected study areas. In this study we will focus on the edible portion of the analysed fish species. With respect to fish muscle, no significant differences were found between the concentrations of Hg in *S. salpa* neither between the concentrations of Hg in *B. capriscus* from both study areas. However, in both study areas *S. salpa* muscle Hg concentrations were lower than those found for *B. capriscus* (Kruskal-Wallis, H = 75.56, p<0.0001, Dunn's test, all p<0.0001 except between *S. salpa* and *B. capriscus* from Sfax, p<0.01). Similarly, significant differences were not found between Se muscle concentrations neither in *S. salpa* nor in *B. capriscus* from both study areas. However, the Se muscle concentrations in *S. salpa* were lower than those in *B. capriscus* in both study areas (H = 72.98, p<0.0001, Dunn's test, all p<0.0001).

With respect to the parasite *R. fractum* infecting *S. salpa*, the Hg concentration was always below the detection limit. The Se concentration in *R. fractum* from Mahdia was higher than that in *R. fractum* from Sfax (Mann-Whitney, U = 92, p<0.0001). On the other hand, there was a higher concentration of Se in *N. chabaudi* infecting *B. capriscus* from Mahdia in relation to those from Sfax (U = 155, p = 0.0281). The Hg concentration in *N. chabaudi* infecting *B. capriscus* from Mahdia was lower than that in *N. chabaudi* infecting *B. capriscus* from Sfax (U = 128, p = 0.005). There was a significant negative relationship between the Se concentrations in the parasite *R. fractum* and host muscle in Mahdia ($F_{1,26}$ = 6.1856, p = 0.0199).

The Se:Hg molar ratio in *S. salpa* muscle from Mahdia was significantly higher than in *S. salpa* muscle from Sfax whereas the Se:Hg molar ratio in *B. capriscus* muscle from Mahdia was significantly lower than in Sfax (U = 159, p = 0.0019 and U = 20, p<0.0001). In Mahdia, the Se:Hg molar ratio in *S. salpa* muscle was significantly higher than in *B. capriscus* muscle whereas in Sfax, there were no significant differences between the Se:Hg molar ratio in *S. salpa* and *B. capriscus* muscle tissues (U = 1, p<0.0001 and U = 252, p<0.4642).

The bioaccumulation factors of both flukes are indicated in Table II. The highest bioaccumulation factor was found for Se, with a 7.1-times higher average concentration in *R. fractum* in comparison to muscle of *S. salpa* from Mahdia. With respect to Hg, the highest bioaccumulation factor was 5.1-times higher in *N. chabaudi* in comparison to muscle of *B. capriscus* from Sfax.

Discussion

Accumulation of pollutants in fish is of increasing concern due to food safety issues and potential human health risks, such as those related with hydrargyriasis, a disease caused by the intake of high doses of mercury producing toxic effects, which include brain, kidney and lung damage leading to a

number of other pathologies. Mercury occurs naturally in seawater, and coastal waters receive mercury runoff from land, input from rivers, and airborne deposition. Biomethylation of mercury (leading to the most toxic form of mercury) occurs in sediment, allowing for food chain biomagnification (WHO 2007). According to Scudder *et al*. (2009), about 95% of mercury in fish is methylmercury. Nowadays, fish ingestion is the only significant source of methylmercury for people in general (Rice *et al*. 2000) and therefore the mercury concentration in foodstuffs is strictly regulated by several international organisms and laws. For example, in the European Union the maximum limit of Hg in edible fish is 0.5 or 1.0 ppm (wet weight) according to fish species (Official Journal of the European Union 2006).

Unlike some edible top food web fish species (e.g. *Thunnus* spp., *Xiphias gladius*), studies focusing on mercury concentrations in teleostei fish, including Sparidae and Balistidae, are much scarcer (Türkmen *et al*. 2008; Mezghani-Chaari *et al*. 2011). As expected, the values obtained in the present study revealed that the omnivorous species (*B. capriscus*) accumulates much more mercury than the herbivorous species (*S. salpa*). Mezghani-Chaari *et al*. (2011) also analysed the mercury concentration in the edible parts of two Sparidae fish (*Diplodus annularis* and *S. salpa*) from Sidi Mansour (12 Km to the north of Sfax) and reported mean concentrations of 1100 and 80 ppb wet weight, respectively. The value obtained in *S. salpa* by the latter authors is very similar to that observed in the present study (74.9 ppb) in the muscle of cow breams from Sfax whereas the level of mercury reported in *D. annularis* was higher than the herein reported in *B. capriscus* from Sfax (212 ppb). In both studies the concentration of mercury in the herbivorous *S. salpa*, feeding at the lower levels of the food chain, is much lower than those in the omnivorous *D. annularis* and *B. capriscus*.

The present results confirm that mercury levels found in *S. salpa* from Mahdia and Sfax were well below the tolerable concentration (0.5 μg/g wet weight) fixed by the Official Journal of the European Union (2006). Therefore, the habitual consumption of *S. salpa* should not suppose any potential health risk for Tunisian people. However, the consumption of *B. capriscus* in Mahdia appears to represent a potential health risk for Tunisian people since the Hg average concentration (0.53 ppm) in *B. capriscus* muscle was slightly above the maximum limit of Hg allowed in edible fish (0.50 ppm) according to the Official Journal of the European Union (2006). The lack of statistical significance between muscular mercury concentrations in *B. capriscus* from both areas may reduce the concern associated with the potential health risk to *B. capriscus* consumers from Mahdia.

Part of the toxicity associated with mercury is related to the negative effect that inorganic mercury and methylmercury can exert on selenium-dependent enzymes (Carvalho *et al*. 2008; Pinheiro *et al*. 2009; Ralston 2009). On the other hand, selenium is though to have a protective effect against mercury toxicity (Kaneko and Ralston 2007; Ralston 2008, 2009; Ralston *et al*. 2008; Burger and Gochfeld 2012) mainly because of the high affinity of selenium to mercury. In fact, selenium is thought to sequester methylmercury and reduce its bioavailability in organisms (Sørmo *et al*. 2011). Several authors have argued that higher Se:Hg molar ratios indicate a more protective effect of selenium against mercury toxicity (Ralston 2008; Peterson *et al*. 2009a, b), even though contradictory results indicate that the effect of selenium on mercury bioaccumulation is an extremely complex process (Dang and Wang 2011). There has been a growing concern about the evaluation of Se:Hg molar ratios in fish muscle with focus on the methylmercury toxicity for humans as consumers of fish (Kaneko and Ralston 2007; Ralston 2008, 2009; Ralston *et al*. 2008; Burger and Gochfeld 2012). In the present study, the muscular Se:Hg molar ratio in *S. salpa* from Mahdia was significantly higher than in *S. salpa* from Sfax. Considering that there were no differences between Se concentrations in *S. salpa* muscle from both areas nor between Se concentrations in *B. capriscus* muscle from both areas, the differences detected between the Se:Hg molar ratio in *S. sarpa* muscle across study areas should result from the slightly higher concentration of Hg in *S. salpa* muscle from Sfax. Also, no significant differences were found between Se or between Hg muscle concentrations in *B. capriscus* from both areas. However, the Se:Hg molar ratio in *B. capriscus* muscle from Mahdia is significantly lower than in Sfax (higher Hg and lower Se in Mahdia than in Sfax). Furthermore, a higher concentration of selenium was found in *N. chabaudi* infecting *B. capriscus* from Mahdia in relation to those from Sfax. These results indicate that Se is being accumulated in *N. chabaudi* infecting *B. capriscus* from Mahdia while in Sfax Se is being used in fish tissue possibly for its protective effect against mercury toxicity.

Higher metal concentrations in fish parasites than in host fish tissues are based on the parasite reliance on host mi-

Table II. Bioaccumulation factors in *Robphildollfusium fractum* and *Neoapocreadium chabaudi* in relation to *Sarpa salpa* and *Balistes capriscus* tissues, respectively

		BF_{kidney}		BF_{liver}		BF_{muscle}	
		Mahdia	**Sfax**	**Mahdia**	**Sfax**	**Mahdia**	**Sfax**
N. chabaudi	Hg					1.6	5.1
R. fractum	Se	1.7		1.1		7.1	2.5
N. chabaudi	Se			1.6	1.8	4.7	4.2

cronutrients, mainly if they lack a gastrointestinal system. For example, acanthocephalans absorb essential elements of physiological importance from the intestine of their fish hosts (Sures 2002) and, therefore, the increased absorption of non-essential elements might be a consequence of competition between parasites and their hosts for essential elements. Furthermore, the absorption of both essential and toxic elements through the tegument can be influenced by the element itself, the size of the worm, the infected microhabitat, and the concrete location in the intestine and some particularities in the absorption process. Until now there is no information about the bioaccumulation of mercury and selenium by fluke parasites of marine fish. In *R. fractum* only selenium could be quantified probably due to the very low level of mercury in the intestine of its herbivorous fish host (*S. salpa*). The mean selenium bioaccumulation factors detected in *R. fractum* were 2.5 and 7.1-times higher than those found in cow bream muscle from Sfax and Mahdia, respectively. Furthermore, our results showed that in the case of Mahdia, increasing concentrations of selenium in *R. fractum* individuals are significantly related with its decreasing concentration in host muscle. However, no significant differences were found between Se concentrations in *S. salpa* muscle from both study areas, neither between *B. capriscus* muscle from both study areas, which is consistent with some degree of homeostatic regulation. Also, it seems that in fact when mercury pollution is higher flukes take up less selenium from their host, being probably used in mercury detoxification processes. Contrarily, in fishes with less mercury in their tissues selenium are most available to digenids increasing the bioaccumulation factors. In the model involving the carnivorous species (*B. capriscus*), larger and more mobile than *S. salpa*, it was possible to quantify the mercury concentration in *N. chabaudi* specimens but with a mean concentration lower than those in all analysed tissues and study areas except in both muscle samples (Table II). Therefore, the 5.1-times higher levels of mercury in *N. chabaudi* than in *B. capriscus* muscle in Sfax enable this fluke to be a sensitive bioindicator.

The present results indicate that some parasites may also be implicated in the amount of selenium and mercury available in different tissues of their hosts and therefore contribute to oscillations of the Se:Hg molar ratios. It is possible that the evaluation of several molar ratios should be more appropriate to test different effects, tissues and species (Burger and Gochfeld 2013). Further studies on selenium levels and mercury toxicity are necessary (Peterson *et al.* 2009a) to clarify the role of Se:Hg molar ratios in different internal tissues particularly in fish of economic importance.

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