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Freshwater shrimps (*Macrobrachium depressimanum* and *Macrobrachium jelskii*) as biomonitors of Hg availability in the Madeira River Basin, Western Amazon

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Abstract Total mercury (THg) concentrations measured in two freshwater shrimp species (*Macrobrachium depressimanum* and *Macrobrachium jelskii*) showed a relationship with the location of artisanal and small-scale gold mining (ASGM) from the Madeira River Basin, Western Amazon. Between August 2009 and May 2010, 212 shrimp samples were collected in the confluence of the Madeira River with three of its tributaries (Western Amazon). THg concentration was quantified in the exoskeleton, hepatopancreas and muscle tissue of the shrimps by cold vapor atomic absorption spectrophotometry. There were no significant differences between the two shrimp species when samples came from the Madeira River, but Hg concentrations were significantly lower in a tributary outside the influence of the gold mining area. Average THg concentrations were higher in the hepatopancreas (up to 160.0 ng g^{-1}) and lower in the exoskeleton and muscle tissue (10.0–35.0 ng g^{-1} and < 0.9–42.0 ng g^{-1}, respectively). Freshwater shrimps from the Madeira River respond to local environmental levels of Hg and can be considered as biomonitors for environmental Hg at this spatial scale. These organisms are important for moving Hg up food webs including those that harbor economic significant fish species and thus enhancing human exposure.

Keywords Mercury · Macroinvertebrates · Gold mining · Biomonitoring · Brazilian Amazon

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Introduction

Gold mining and deforestation has resulted in large inputs of Hg into the Amazon. The Madeira River basin being one of the most affected regions (Lacerda 1995; Bastos et al. 2006). In the region's aquatic ecosystems, divalent Hg^{2+} are easily methylated by bacteria under the physicochemical conditions found in bottom sediments and floating macrophyte banks (Guimarães et al. 2000) and transferred to aquatic food chains, accumulating to high levels in top carnivorous fish (Molina et al. 2010). In fact, nearly all studies on Hg distribution in the Amazon biota focused on fish. Recently, analysis of a large database on fish Hg concentrations concluded that these organisms show higher and relatively similar Hg concentrations along more than 600 km of river way and lowest in partially isolated sites, such as marginal lakes, regardless of food habits (Bastos et al. 2015). This suggest that Hg inputs to the river are presently diffuse and distributed along the basin, rather than from gold mining point sources as suggested by earlier data from this region when gold prospecting was at a peak (Lacerda et al. 2012). In addition, fish seems to respond well to regional differences but may not be useful as biomonitors at smaller geographical scales. In this sense, the use of benthic sedentary species as biomonitors of the concentration of bioavailable Hg in a given environment seems an obvious step (Palmer and Presley 1993). Unfortunately, to our knowledge such organisms were never studied in the Madeira river basin.

The use of aquatic organisms as biological monitors is viable if bioaccumulation at environmental levels causes no significant adverse effects to them. Besides this, a good biomonitor must be abundant and adequate to the necessary spatial scale of the monitoring program, sedentary or with a limited and/or well-defined home range, easily captured and with relatively well-known biology (Rainbow 2006; Aguirre-Rubí et al. 2017). Freshwater shrimps satisfy all of these criteria; suggesting them as key aquatic organism to monitor trace element concentrations, such as Hg, in the environment, as demonstrated by Rahimi and Gheysari (2016) and Tenorio et al. (2017). Also, these shrimps are omnivorous and live in submerged leaf litter where Hg methylation is favored. The Hg intake in shrimps occurs mostly from feeding, but also through the exoskeleton and gills directly from water (Smkiss and Taylor 1989). Body distribution of the incorporated Hg can be heterogeneous, and may vary according to taxon. Within the cells, Hg can form complexes with proteins and accumulate within specific organs; segregated in cytoplasm granules; or transferred to the exoskeleton and discharged during ecdysis within the fragments of the exoskeleton and excreted in fecal pellets (Weeks et al. 1992; Soares et al. 2011). Regardless of the total Hg present in a given environment, its relative bioavailability is the principal controlling parameter of bioaccumulation. Organic Hg compounds, in particular methyl-Hg, are the most bioavailable Hg forms present in the environment and the preferred form of Hg biomagnification through food chains and eventual human exposure. The methyl-Hg content, rather than total Hg concentrations, may finally control the existing Hg concentrations in the biota, mostly when absorption from the exoskeleton and gills also occur, such as in shrimps. Therefore, shrimps may as well act as biomonitors for these Hg forms in the environment (Palmer and Presley 1993).

There are few studies that demonstrate the role of shrimp as biomonitoring of Hg levels in the environment, rather, most research evaluate the potential risk that Hg-contaminated shrimp may pose to human health. In this sense, Delgado-Alvarez et al. (2015) considered that the concentrations of Hg in the white shrimp (*Litopenaeus vannamei*) muscle, grown in the northwestern Mexico, as well as the rate of consumption by the Mexicans, do not pose hepatopancreas of marine shrimps, *Penaeus merguiensis*, of the Khuzestan coast in the Persian Gulf, however, also without representing human health risk (Hosseini et al. 2016). Similarly, Hoang et al. (2017) determined the concentrations of total Hg and methyl-Hg in the shrimp muscle tissues marketed by the Japanese industry and concluded that there is no risk to consumers. Recently, Briand et al. (2018) have shown that there are different patterns of Hg contamination in the reefs organisms in the New Caledonia, and that shrimps constitute an important step of the local benthic food chain and thus being part of the transfer route of Hg within the reefs food web.

In the Brazilian Amazon, where Hg contamination has been reported by numerous studies, shrimps have been seldom included in assessment and monitoring programs, notwithstanding being among the aquatic organisms that can be considered biomonitors of the Hg levels in the environment. In this sense, it is important to quantify Hg concentrations in these organisms, both to assess if they can represent a risk to the fish that feed on them and eventually to humans; since although freshwater shrimps are not commercialized or directly

consumed in the region, the fish that feed on them are the dominant item included in the diet of the riverside population and if these shrimps can reflect the environmental Hg concentrations in the region. Therefore, this study presents the first report of total Hg concentrations in freshwater shrimps (*Macrobrachium depressimanum* and *Macrobrachium jelskii*) from the Madeira River, Brazilian Amazon. Also, it compares shrimp Hg burdens with total and methyl-Hg concentrations in the environmental compartments (water, suspended solids, and bottom sediments) in this region.

Materials and methods

Figure 1 shows sampling sites in the higher reaches of the Madeira River Basin, about 150 km from the state capital Porto Velho and located within the limits of the gold mining reserve where between 1980 and the early 1990's gold mining released about 80 tons of Hg into the local environment (Lacerda et al. 1989). Shrimps samples were collected in the confluence of the Madeira River with three of its tributaries: Caripunas River (MDCP), Jatuarana River (MDJT), and Jaci Paraná River (JP), and in Branco River (RB), a tributary on the right margin of the Jaci Paraná River, far from the influence of the Madeira River flooding and gold mining activities (Fig. 1).

Sampling occurred in August and November 2009 and in May 2010 and consisted of trawling nets of 7 mm mesh size for a period of 10 min on bottom sediments and floating plant mats along river margins; 212 shrimp samples ($n = 118$ *M. jelskii* and $n = 94$ *M. depressimanum*) were collected. Animals were kept in ice for transport. Sub-samples were sent to the National Institute of Amazon Research (INPA, Manaus) for identification according to García-Dávila and Magalhães (2003). For THg determinations, shrimps were thoroughly washed with deionized water and had their total length (from rostrum to telson) measured to the nearest millimeter. Samples for analysis were composed of individuals of roughly the same size. Animals were separated in exoskeleton, hepatopancreas and muscle tissue for THg determination. Approximately 500 mg of shrimp tissue homogenate (wet weight) were digested using 1.0 mL of H_2O_2 (Merck®), 3.0 mL of HNO_3 (65%, Merck®), and 3.0 mL of $KMnO_4$ (5% *w*/*v*, Merck®), and taken to a microwave oven (CEM Corporation, model MDS-2000, USA) with a frequency of 2450 MHz and wavelength of 12.2 nm for 30 min. After digestion, samples were left in room temperature overnight, when a few drops of $NH_2OH.HCl$ solution (12% *w*/*v*, Merck®) were added to the tubes to eliminate the excess of oxidizing agents and the volume taken to 10 mL with ultrapure water. THg concentrations were determined by cold vapor atomic absorption spectrophotometer (CV-AAS—FIMS-400, Perkin Elmer). Simultaneously, THg determinations of certified standards IAEA-142 (mollusk tissue) and DORM-2 (fish muscle) were also analyzed in duplicate prior to every batch of samples analyzed. Average recovery of reference standards was 102 ± 5%. Detection limit of the procedure was 0.90 ng g^{-1}.

Using ultraclean techniques, sub-surface (0.30 m) water samples were collected in amber glass bottles of 500 mL in duplicate and sealed in double-bagged polyethylene (EPA 2002). An unfiltered water sample bottle was preserved with HCl (2.0 mL/L, Merck®) and the other was filtered with 0.45 μm pore diameter filters (Milli-Q Plus, Millipore, Bedford, MA, USA) for suspended solids analyses. Bottom sediments were collected with an Ekmann dredge and stored in polyethylene bags.

Total Hg determination was done in duplicate unfiltered water samples (25 mL) after treatment with BrCl (0.02 mol L^{-1}, *w*/*v*), hydroxylamine hydrochloride (30%, *w*/*v*) and SnCl (20%, *w*/*v*) in the CG-AFS (MERX, Brooks Rand). Bottom sediments and suspended solids were treated with an oxidant solution (HNO_3; HCl; $KMnO_4$) and total Hg measured by cold vapor atomic absorption spectrophotometry (Flow Injection Mercury System-FIMS-400-Perkin Elmer) (Bastos et al. 2006). Methyl-mercury (MeHg) were quantified in unfiltered water and suspended solids following EPA (2001), using 1% APDC solution (*w*/*v*, Merck) for distillation and ethylation with sodium tetraethyl borate (1%, *w*/*v*); on a MERX-TM automated MeHg system, equipped with a purge and trap unit, a packed column GC/pyrolysis unit, and a Model III atomic fluorescence spectrophotometer (CG-AFS—MERX, Brooks Rand). All water samples were analyzed in duplicate and checked the precision and accuracy by analyses of matrix spikes of THg and MeHg (recovery 92 and 98%, respectively). The detection limit of THg and MeHg were 0.148 ng L^{-1} and 0.010 ng L^{-1}, respectively. A routine intercalibration program on water THg and MeHg determinations was performed with Brooks Rand. Method accuracy of THg and MeHg in

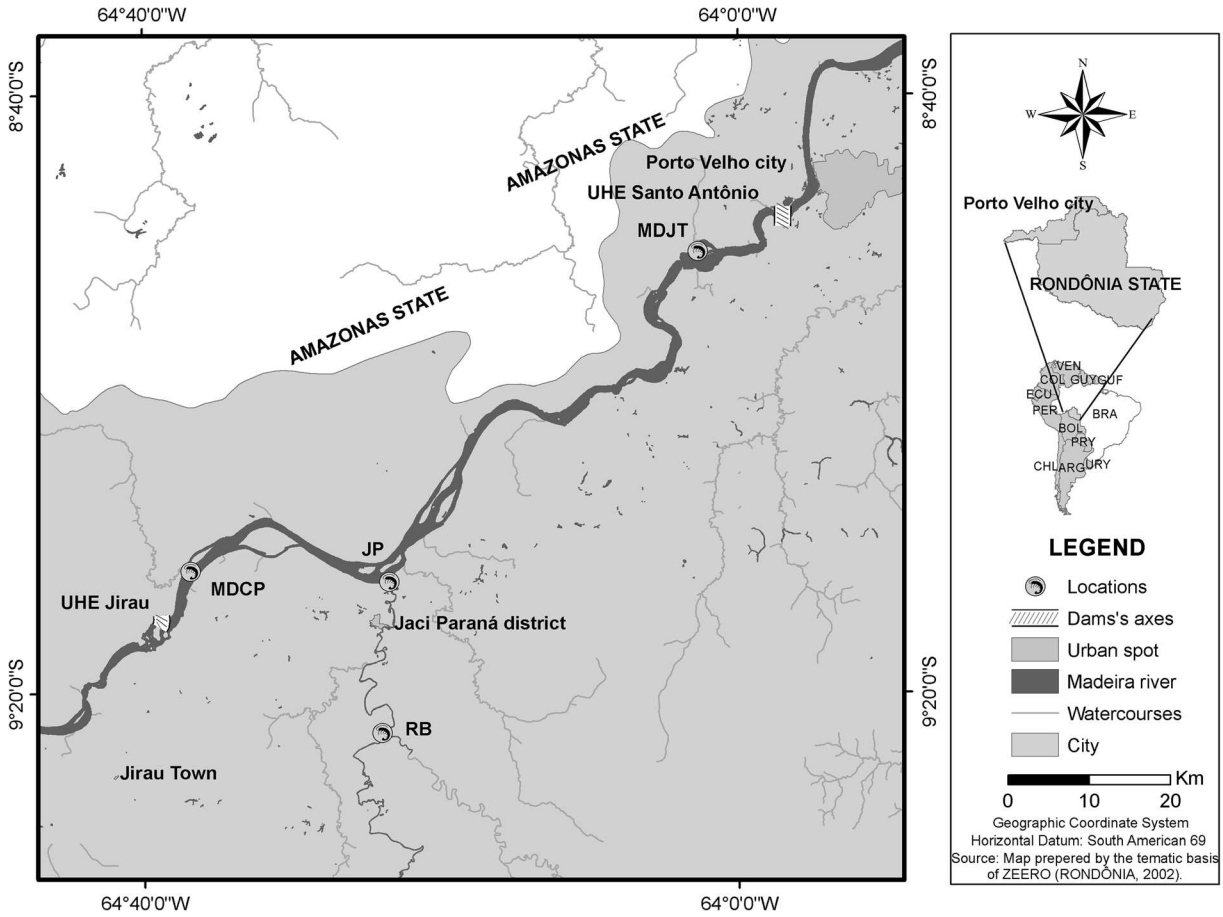

Fig. 1 The basin of the upper Madeira River showing the sampling sites: MDCP, Caripunas River; JP, Jaci-Paraná River; MDJT, Jatuarana River; and RB Branco River

sediment and suspended solids were ensured by the use of certified material with mean recovery of THg = 103% (SS-2 -*Enviro*MAT™, SPC-Science) and MeHg = 96% (IAEA-356), run with each batch of samples analyzed. Detection limit for THg was 5.00 ng g^{-1} and for MeHg was 0.060 ng g^{-1}. Results were tested for normality (Shapiro Wilks, S-W) and variance (Kruskal–Wallis, K-W ranking analysis of variance) using the XLSTAT (2010) software package. Pearson's correlation was performed utilizing the Statistica 7.0 software. All test performed considered a significance level at $\alpha = 0.05$.

Results and discussion

Table 1 shows total Hg concentrations in the different tissues of both shrimp species from the four sampling sites. Concentrations are well below the maximum limits established by the Brazilian guidelines for human consumption (500 ng g^{-1} w.w.—ANVISA 1998). There were significant differences in Hg concentrations among tissues and sites, with concentrations in the hepatopancreas being much higher than in the other two tissues, irrespective of site or species.

The observed 2.3-fold range of average THg concentrations in the hepatopancreas (70 ng g^{-1} in *M. jelskii* from the Branco River to 160 ng g^{-1} in *M. depressimanum* from the Madeira River at the Caripunas river mouth) is probably a result of the different bioavailability of Hg (Simkiss and Taylor 1989). *M. jelskii* presented significantly lower ($p < 0.05$) hepatopancreas THg concentrations in the Branco River compared to all other sites. Among the Madeira River sites and irrespective of species, THg concentrations in the hepatopancreas were not significantly different. According to Locardini and Presley (1996), the

Table 1 Total Hg concentrations (mean, standard deviation, minimum and maximum) in different tissues of freshwater shrimps from the Madeira River, Western Amazon, in ng g^{-1} wet weight

Species/tissue	*M. depressimanum*		*M. jelskii*	
	MDCP[a]	MDJT[b]	JP[c]	RB[d]
Hepatopancreas	168 ± 50 (96–217) *n* = 82	102 ± 56 (28–174) *n* = 12	141 ± 40 (86–180) *n* = 49	70 ± 62 (16–172) *n* = 69
Muscle	22 ± 14 (<0.9–40) *n* = 82	14 ± 4 (10–19) *n* = 12	16 ± 13 (<0.9–30) *n* = 49	16 ± 16 (3–42) n = 69
Exoskeleton	38 ± 17 (19–55) n = 82	22 ± 10 (7–33) n = 12	35 ± 15 (21–48) n = 49	10 ± 6 (3–19) *n* = 69
Animal length (mm)	2.09 ± 0.52 (1.5–2.1)	1.44 ± 0.19 (1.2–2.2)	3.40 ± 0.95 (2.3–5.2)	3.94 ± 0.82 (2.9–5.4)

[a] Caripunas River in November 2009 (*n* = 82)

[b] Jatuarana River in May 2010 (*n* = 12)

[c] Jaci Parana River in August 2009 (*n* = 49)

[d] Branco River in August 2009 (*n* = 69)

concentration of THg in shrimps strongly correlates with the bioaccumulation rate of Hg, which depends on the bioavailability of Hg in a given environment. As for muscle and exoskeleton, no significant difference occurred between species or sampling sites in the Madeira River. However, THg concentrations in the exoskeleton were higher than those present in muscle tissues, with the exception of the Branco River, where it was significantly lower ($p < 0.05$). Similar to the observed concentrations in the hepatopancreas, THg concentrations in the exoskeleton in the Branco River shrimps were lower than those found in the Madeira sites, but muscle concentrations in these animals were similar to those of the Madeira River.

When compared to other studies on Hg content in freshwater shrimps the concentrations found in the Madeira River animals are higher than those in *Palaemonetes kadiakensis* (grass shrimp) studied by Chumchal et al. (2008) in Lake Caddo (USA). There, average THg concentrations ranged from 57.4 to 69.5 ng g^{-1} and showed variability according to methylation efficiency in different areas of the lake. Total Hg in cray fishes *Pacifastacus leniusculus* and *Procambarus clarkia* from wildlife reserves in northern California showed similar THg concentrations to our study (178–260 ng g^{-1} and 190–254 ng g^{-1}, respectively). Nearly 100% of the THg burden being MeHg (Hothem et al. 2007). In the highly contaminated Ebro River catchment in Spain, Carrasco et al. (2011) reported THg concentration in muscle and hepatopancreas of *Procambarus clarkii* varying from 59 to 786 ng g^{-1} and 25 to 667 ng g^{-1}, respectively. Molina et al. (2010) reported Hg concentrations in freshwater shrimps in the western Amazon basin; in floodplain lakes of the Beni River, Bolivia, a tributary of the Madeira River, an area with heavy gold mining activity, they found 158 ng g^{-1} of THg in muscle tissue of *Palaemonetes invonicuos* during in the flood season and 96–308 ng g^{-1} during the low water season, an indication of seasonal varying bioavailability of Hg. These values were transformed in wet weight basis from the original results from the authors.

Table 2 shows THg concentration ratios between different organs of the two shrimp species in the four sites. For the two species and in all sampling sites, the hepatopancreas presented THg concentrations 4.0 to 8.4 times higher than the concentrations found in muscle and exoskeleton. This suggests that Hg present in food may have relatively low availability for bioaccumulation in all sites and Hg uptake directly from water may be significant. For example, under experimental conditions, Hg accumulation in muscle of shrimps feed artificially with contaminated food, are 2–3 times higher than in the hepatopancreas (Palmer and Presley 1993). The ratio between exoskeleton and muscle was > 1.0 (1.6–2.2) in the three sites in the Madeira River and suggests Hg transfer to the exoskeleton.

Table 2 Total mercury concentrations ratios found in different tissues of freshwater shrimps *M. depressimanum* and *M. jelskii* from the Madeira River basin, Rondônia, Western Amazon

Concentration ratio[a]	*M. depressimanum*		*M. jelskii*	
	MDCP	MDJT	JP	RB
EX/TM	1.7	1.6	2.2	0.6
TH/TM	8.4	7.3	8.8	4.4
TH/EX	4.8	4.6	4.0	7.0

[a] *EX* exoskeleton, *TM* muscle tissue, *TH* hepatopancreas tissue

Transfer of metals to the exoskeleton and loss by ecdysis is a known detoxification mechanism suggested for marine and freshwater shrimps (Khan et al. 1989; Smokrowski et al. 1998; Soares et al. 2011). Our results strongly suggest this is the case, at least for the animals of the Madeira River. In the Branco River, where the lowest THg concentrations in hepatopancreas and the exoskeleton occurred, the ratio between exoskeleton to muscle was lower than 1.0, suggesting that no detoxification process is occurring in this site.

Pearson correlation coefficients between Hg concentrations in the different shrimp organs of the two species showed that at the Madeira River sites, hepatopancreas THg concentrations were weakly, but significantly correlated with muscle concentrations ($r = 0.589$), but non-significant between hepatopancreas and exoskeleton ($r = 0.354$). Concentrations in muscle strongly correlate with those in the exoskeleton in the Madeira River shrimps ($r = 0.729$). This suggests that muscle THg concentrations probably reflect the overall environmental concentrations of Hg and its bioavailability in water and partially form diet. The strongest correlation found between muscle tissues and exoskeleton, supports the occurrence of a detoxification mechanism. In the Branco river animals, however, both Hg concentrations in muscle and the exoskeleton showed a highly significant correlation with hepatopancreas ($r = 0.856$; $r = 0.826$, respectively), suggesting diet as the major Hg source to these animals. Since the ratio between Hg in muscle and hepatopancreas in these animals are < 1.0, the high significant correlation between muscle and exoskeleton concentrations ($r = 0.929$) is probably derived from their correlation with hepatopancreas.

Table 3 shows THg and MeHg concentrations found in water, suspended solids, and bottom sediments from the four sites studied. Total Hg concentrations in all compartments were highest in suspended solids and showed no significant difference among the four sites. However, MeHg concentrations in waters of the Madeira River sites were one order of magnitude higher than in the Branco River. To a lesser extent, this also occurred in the suspended solids, in particular in the Jaci-Parana site. These results partially explain the differences in Hg concentrations and distribution with shrimp organs at the Branco River site relative to the Madeira River sites.

Shrimps incorporate trace metals, including Hg, into the body from solution through permeable ectodermal surfaces and across the endoderm of the gut (Rainbow 2007). MeHg accumulates more efficiently from water than inorganic Hg. For example, concentrations factors obtained from shrimp *C. crangon* for these Hg forms

Table 3 Mercury concentrations in water (ng L^{-1}), suspended solids and bottom sediments (ng g^{-1}; dry weight) from the studied rivers from the Madeira river basin, Rondônia, Western

Site	Water (ng L^{-1})		Suspended solids (ng g^{-1})		Bottom sediment (ng g^{-1})
	THg	MeHg	THg	MeHg	THg
JP	2.6 ± 1.5 (*n* = 59) (0.9–6.4)	0.10 ± 0.06 (*n* = 51) (0.01–0.30)	474 ± 558 (*n* = 66) (28–2.51)	0.20 ± 0.20 (*n* = 46) (< 0.01–0.10)	92 ± 21 (*n* = 18) (58–134)
MDJT	2.9 ± 2.1 (*n* = 58) (0.6–10.7)	0.17 ± 0.15 (*n* = 58) (0.02–0.62)	420 ± 592 (*n* = 64) (33–2.62)	0.02 ± 0.03 (*n* = 54) (< 0.01–0.13)	65 ± 38 (*n* = 7) (31–144)
MDCP	3.1 ± 2. 1 (*n* = 26) (1.0–8.5)	0.17 ± 0.12 (*n* = 29) (0.03–0.45)	417 ± 492 (*n* = 35) (31–2.23)	0.03 ± 0.03 (*n* = 22) (< 0.01–0.11)	82 ± 36 (*n* = 9) (26–136)
RB	3.2 ± 2.5 (*n* = 28) (0.9–13.1)	0.07 ± 0.03 (*n* = 29) (0.02–0.36)	429 ± 551 (*n* = 35) (29–1.95)	0.02 ± 0.01 (*n* = 21) (< 0.01–0.05)	101 ± 28 (*n* = 7) (54–142)

were 13,000 and 400 for MeHg and inorganic Hg, respectively. In addition, retention efficiency of incorporated MeHg reaches 75%, whereas for inorganic Hg, it is only 4%. In Branco River shrimps, most assimilated Hg would have come from diet, with minor contribution form water. In the Madeira River animals, the absorption of Hg, in particular of MeHg directly from water seems the major Hg pathway for assimilation. Thus, this study cannot attribute the variations in the concentrations of this metal in the shrimps of different localities to bioconcentration processes only, but to variations in the availability of THg and MeHg in water of the localities. Suspended solids and bottom sediments, which are Hg sources through diet, seem more important in the Branco River site, whereas in the Madeira River sites they represent only a minor contribution to the THg burden in shrimps. Other factors such as the stage of development of the organism, the rate of the water flow from gills, and ecological and physiological characteristics of the species may also play a role in the bioaccumulation processes, but are probably of minor importance in the present case.

It is believed that the ability of regulation of metals in Crustacea is assigned to detoxification mechanism carried out by hepatopancreas in association with ecdysis (Fialkowski and Rainbow 2006; Rainbow 2007). According to the THg concentrations found in the different tissues of *M. jelskii* and *M. depressimanum* in the Madeira River and the concentration ratios and correlations between tissues Hg concentrations, it seems that animals from the three sites in this river are already responding to environmental Hg levels sufficient to trigger detoxification processes.

Conclusion

Several impacts triggered by anthropogenic action contribute to the increase and the dispersion of Hg in the Madeira River basin, even after the cessation of major gold mining activities. Among these are the burning of the forest, conversion to agriculture and artificial flooding due to hydroeletric reservoir construction (Lacerda et al. 2012). All these activities do not occur in the river proper, but act as diffuse sources of Hg at the basin level. Diffuse emissions interacting with local biogeochemical parameters, may result in small-scale variability of Hg contamination not easily characterized. Although fish, has been successfully used to evaluate this contamination on a regional scale, this present study showed that shrimps may be a better biomonitor of the situation at a local scale. *M. jelskii* and *M. depressimanum* reflect variations in the concentrations of THg at a local scale, in response to higher bioavailability of this metal in the form of MeHg. Furthermore, the body distribution of Hg, suggests MeHg from water as the major pathways of Hg assimilation and that at least for the Madeira River, environmental levels are already triggering detoxification processes implying high exposure and eventual physiological injuries to these animals.

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