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Assessment of Total Mercury Levels in *Clarias gariepinus* from the Sagua la Grande River, Cuba

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Abstract Total mercury levels (Thg) were quantified in *Clarias gariepinus* captured from the Sagua la Grande River (Cuba) in the vicinity of an active chlor–alkali plant, and relationships among place of capture; fish size, weight, and sex; and THg levels were assessed. THg levels ranged from 67 to 375 ng/g ww in collected fish, never exceeding the Cuban recommended maximum limit for fish consumption of 500 ng/g ww. No significant correlation was observed between mercury levels and fish allometric characteristics ($p < 0.05$); however, levels were significantly higher in fish captured below the chlor–alkali facility, suggesting a connection between mercury bioaccumulation and plant discharges.

Keywords Tropical rivers · Mercury · *Clarias gariepinus* · Bioaccumulation

Chlor–alkali plants (CAP) that use mercury (Hg) in electrolytic cells are known as sources of environmental contamination (Nriagu 1979). Although the amounts of Hg released from such sources are not significant on a global scale, such releases can have considerable local negative impacts, particularly to sensitive aquatic ecosystems. An example of such a scenario is the mercury-cell CAP facility near Sagua la Grande City, Villa Clara, Cuba. This facility has been in operation since the early 1980s and directly discharges preliminary treated effluents into the Sagua la Grande River upstream of the city. Early studies provided evidence of local Hg pollution, including elevated Hg levels in the roots of resident *Eichornia crassipes* (water hyacinths), and in the gonads of sea urchins (*Lytechinus variegates*) in the river estuary (Gonzalez 1991). However, little recent data exists on Hg conditions in the river; therefore, this study was undertaken to gather such information.

As background, it is well established that Hg released to aquatic systems can be readily transformed to methylmercury (MeHg) and biomagnification in the food web can result in elevated total Hg (THg) levels in exposed fish (Environment Canada 2002). Given that the majority of THg found in fish muscle tissue is as MeHg (Bloom 1992) and MeHg is a neurotoxin that can cause birth defects, learning and muscular disabilities, and other problems (World Health Organization 1990), there is always concern about the public consumption of Hg-exposed fish (Inskip and Piotrowski 1985). This concern is greatest in the consumption of top predator fish because biomagnification

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results in the highest levels of Hg in species at the top of the food chain (Boudou and Ribeyre 1997; Campbell et al. 2003).

The top predator in many Cuban rivers is *Clarias gariepinus* (*C. gariepinus*). Although this fish was only introduced to Cuba in 1999 via fish farming, it is a versatile omnivore and predator and has rapidly spread across Cuban rivers (Garcia and del Valle 2006), including the Sagua la Grande River. Furthermore, *C. gariepinus* is a common food source of many residents of Sagua la Grande City and, because of the CAP facility, it was decided to quantify THg levels in resident *C. gariepinus* as an indicator of possible Hg contamination. The practical goal of the study was to generally assess possible health risks from fish consumption, but then also guide public health officials in establishing regulations on the consumption of *C. gariepinus* from the Sagua la Grande River.

Materials and Methods

Twenty-seven *C. gariepinus* individuals were collected at three sample stations (nine fish per station) in January 2006 from Sagua la Grande River system for determining THg levels in fish muscle tissue (Fig. 1). Station 1 was located just below the CAP effluent discharge point upstream of the city; Station 2 was approximately 7 km downstream of the city; and Station 3 was an irrigation channel, which was supplied water from the river above the CAP discharge point.

The fish were collected overnight using set gill nets and, after capture, were measured, weighed, and classified by sex (via a distinct sexual papilla on males located just behind the anus; Clay 1979). The fish were then dissected on a polyethylene dissection board using clean stainless steel tools, and muscle tissues were removed, placed in pre-washed glass bottles, and frozen at −20°C prior to laboratory analysis. Tissue samples were then thawed, homogenised, and lyophilised, being weighed before and after lyophilisation to determine the water content of each sample. Lyophilised samples (300 mg fractions) were then digested in a pressure vessel in the presence of 4-mL of nitric acid and 0.5-mL of hydrogen peroxide by slowly raising sample temperature from room temperature to 180°C over 120 min (European Committee for Standardization 2001). THg analysis was performed on the digested samples using cold-vapour atomic absorption spectrometry (Buck Scientific 210VGP) with sodium borohydride as the reduction solution. Flow injection was use for sample introduction.

For quality assurance, Certified Reference Materials were used to assess analytical accuracy, including: IAEA-407 (containing 222 ± 24 ng/g of THg), DOLT-2 (2,140 ± 280 ng/g of THg), and DORM-2 (4,640 ± 260 ng/g of THg). Resulting extraction efficiencies for THg were 85–118% relative to IAEA-407, 97–120% with DOLT-2, and 110–125% for DORM-2. Three separate digestions were performed per sample and analytical precision was always <10%. The detection limit of the method was 38 ng of THg per g of wet weight.

The Wilcoxon Ranked-Sum test was used to determine the statistical significance of differences between individual means of detected THg ($p < 0.05$). All data were tested for normality using the Shapiro–Wilk test prior to analysis. Data also were examined using principal components analysis (PCA) to reduce the measured variables into clumped factors to potentially explain variation in THg levels in the tissues of individual fish (Miller and Miller 2001).

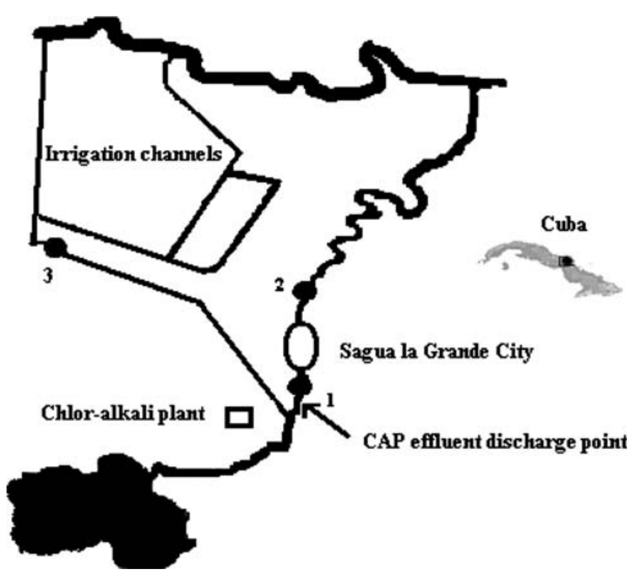

Fig. 1 Sample stations within Sagua la Grande River watershed. Sampling Stations are indicated as numbers

Results and Discussion

Synoptic data describing the collected fish are presented in Table 1. All captured *C. gariepinus* were adults of commercial size between 36 and 74 cm in length and 390 and

Table 1 Summary data and total mercury level (THg) in ng/g wet weight (ww) in *C. gariepinus* collected from the Sagua la Grande River system

Stations	N	Length (cm)	Weight (g)	Sex		THg (ng/g ww)
				Female	Male	
1	9	36–57	425–1,900	5	4	164–325
2	9	38–66	390–2,420	–	9	67–255
3	9	52–77	1,380–2,775	4	5	91–375
Total	27	36–74	390–2,775	9	18	67–375

2,780 g in weight. Figure 2 shows that detected THg levels among all fish ranged from 67 to 375 ng/g wet weight (ww), and Fig. 3 indicates that THg levels were always below the maximum recommended limit of 500 ng/g ww for fish consumption in most countries (United States Environmental Protection Agency 1997; European Economic Community 2001; Canadian Council of Ministers of the Environment 1999) and also the Cuban Food Sanitary Standard (Cuban National Bureau of Standards 2006). However, 44% of fish samples had THg levels higher than 200 ng/g ww, which is the recommended upper limit by the World Health Organization (WHO) for fish consumption by vulnerable people, such as pregnant women, individuals under 15 years, or frequent fish consumers (World Health Organization 1990).

Overall, THg levels found in *C. gariepinus* from the Sagua la Grande River were similar to those observed in other sampling programs. For example, THg levels ranged from 50 to 510 ng/g ww in *C. gariepinus* from various rivers in Ghana and South Africa (Campbell et al. 2003); although, THg levels in fish from Lake Victoria were comparatively low, ranging from 5.7 to 29.8 ng/g ww (Campbell et al. 2003). Although such comparisons are broadly useful, directly comparing THg levels in fish from different locations (even in the same species) can be tenuous because absolute THg levels in fish from different sites can vary broadly due to differing dietary patterns and trophic relationships. For example, top predators in longer food webs tend to accumulate more contaminants than the same species in shorter food webs (Boudou and Ribeyre 1997). In the Sagua la Grande River, *C. gariepinus* is an active predator that takes advantage of the small size of most native species, which partially explains relatively high THg levels observed in fish in this study.

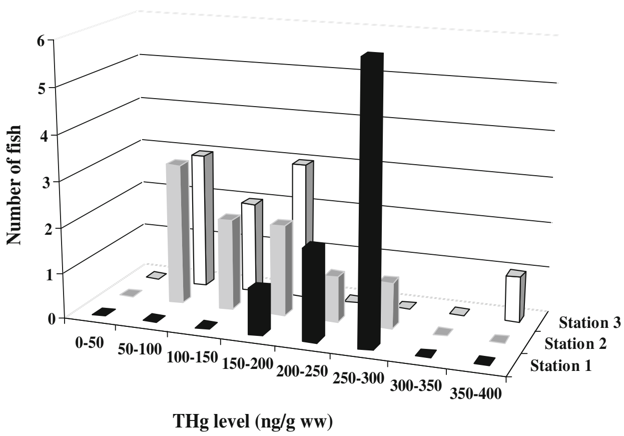

Fig. 2 Frequency distributions of THg levels per sample station in *C. Gariepinus* collected from Sagua la Grande River

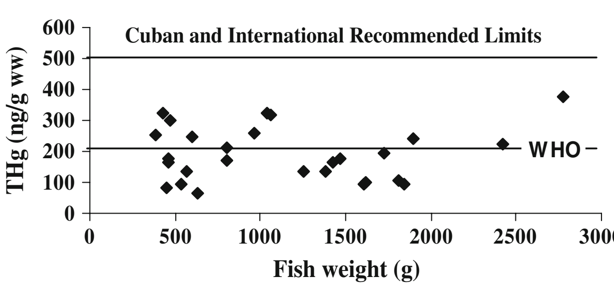

Fig. 3 THg concentrations versus fish weight relative to various international standards for consumption of fish exposed to mercury. WHO refers to the World Health Organization recommended threshold level for vulnerable people

Given that direct comparison with other studies is difficult, a PCA was performed on the data in Table 1 to better understand allometric and other factors relative to detected THg levels. The resulting PCA separated the variables into two factors: Factor 1, which includes place of capture, weight, and size, explains 51% of the data variance, whereas Factor 2, THg level and sex, explains 29% of variance in the data. Figure 4 presents the synoptic factor plot, where the place of capture is the labelling variable. Here we can see that smaller size-weight female fish from Station 1 generally have the highest THg levels of all captured fish.

In order to better understand synoptic observations from the PCA, a more detailed examination of data from Table 1 was performed. First, fish captured at Stations 1 and 2 (i.e., below the CAP facility) were significantly smaller and weighed less than fish collected at Station 3 upstream of the CAP facility ($p < 0.05$). Whether this is caused by impacts of the CAP facility or from secondary impacts of being close to Sagua la Grande City is unclear (possible impacts include reduced water quality or the selective removal of larger fish due to greater fishing activity). Alternately, *C. gariepinus* might simply be comparatively large in the irrigation channels (i.e., at Station 3) because levels of organic matter and micro fauna are likely higher and habitat is generally better for all organisms in the food chain.

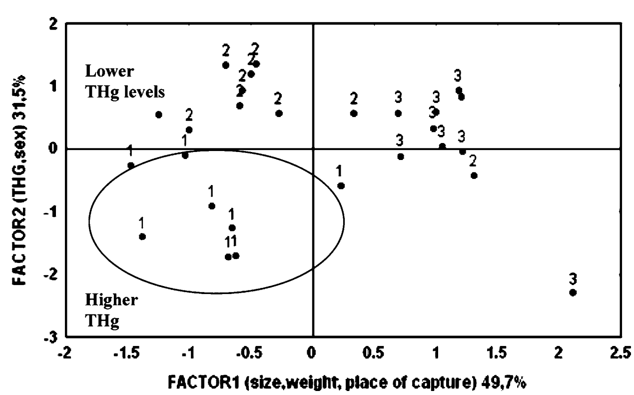

Fig. 4 Projection of different fish samples in a factor plane with place of capture as the labelling variable. Note that smaller female fish from Station 1 have the highest THg levels

Second, median THg levels in fish from Station 1 were significantly higher than levels in fish from the other two stations ($p < 0.05$), which is not surprising given that Station 1 was near the CAP facility effluent discharge point. This result may be significant to public health because 88% of the fish collected at Station 1 had THg levels above the WHO threshold concentration for at-risk groups (200 ng/g ww). For comparison, 22% of fish collected at Station 2, ~7 km downstream of Station 1, exceeded this threshold, and only 11% exceeded the threshold at Station 3. Although higher THg levels near Station 1 make sense based on proximity to the CAP facility, more data are needed to make absolute conclusions about the cause; i.e., many other chemical and physical factors can affect biomagnification and bioaccumulation at a site (Beckvar et al. 2000). However, PCA also shows that elevated THg levels are associated with Station 1 fish, which further implies a connection between the CAP facility and THg accumulation in fish.

Interestingly, median THg levels in fish from Station 3 were not significantly different than fish from Station 2 ($p < 0.05$) even though Station 2 is below the CAP facility. This might be because effects of the CAP facility do extend down to Station 2 (e.g., Hg from the facility is taken up or immobilised upstream) or the generally larger fish at Station 3 have different feeding habits than the smaller fish at Station 2, which would result in locally different levels of THg biomagnification. Alternately, since both locations are well away from the CAP facility and subject to the same meteorological events, such as hurricanes, floods, and draughts, detected THg levels in *C. gariepinus* at Stations 2 and 3 might be more influenced by regional phenomena than conditions at each site.

Finally, although the PCA suggests that small female *C. gariepinus* from Station 1 have the highest THg levels, there is no statistically significant correlation among fish between weight, size, or sex, and tissue THg levels (F-test, $p < 0.05$). As suggested earlier, this lack of correlation might be due to differing feeding habits in different locations or other factors that affect THg bioaccumulation. For example, *C. gariepinus* is known to conditionally feed on microscopic zooplankton, terrestrial insects, molluscs, fruit, and even fish half its length (Bruton 1979). Therefore, subtly different local conditions might result in significantly different feeding habits that directly impact THg incorporation, which are not necessarily related to fish size or sex.

Overall, results from this investigation show that Hg in the Sagua la Grande River is bioacumulating in resident *C. gariepinus*, particularly below the effluent discharge point of the local CAP facility. Although THg tissue levels do not exceed general Cuban standards for fish consumption, they frequently exceed recommended levels for safe consumption by vulnerable people. Therefore, we suggest that precautionary guidelines be established on fish consumption from the river until more data are available on Hg contamination in near shore zones.

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