Patterns of mercury and methylmercury bioaccumulation in fish species downstream of a long-term mercury-contaminated site in the lower Ebro River (NE Spain)

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Abstract
Since the 19th century, large amounts of industrial waste were dumped in a reservoir adjacent to a chlor-alkali plant in the lower Ebro River (NE Spain). Previous toxicological analysis of carp populations inhabiting the surveyed area have shown that the highest biological impact attributable to mercury pollution occurred downstream of the discharge site. However, mercury speciation in fish from this polluted area has not been addressed yet. Thus, in the present study, piscivorous European catfish (Silurus glanis) and non-piscivorous common carp (Cyprinus carpio) were selected, to investigate the bioavailability and bioaccumulation capacities of both total mercury (THg) and methylmercury (MeHg) at the discharge site and downstream points. Multiple Correspondence Analysis (MCA) was applied to reduce the dimensionality of the data set, and Multiple Linear Regression (MLR) models were fitted in order to assess the relationship between both Hg species in fish and different variables of interest. Mercury levels in fish inhabiting the dam at the discharge site were found to be approximately 2-fold higher than those from an upstream site; while mercury pollution progressively increased downstream of the hot spot. In fact, both THg and MeHg levels at the farthest downstream point were 3 times greater than those close to the waste dump. This result clearly indicates downstream transport and increased mercury bioavailability as a function of distance downstream from the contamination source. A number of factors may affect both the downstream transport and increased Hg bioavailability associated with suspended particulate matter (SPM) and dissolved organic carbon (DOC).

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1. Introduction
The majority of human exposure to mercury (Hg) occurs through consumption of marine and freshwater biota (Sunderland, 2007), mainly fish. Methylmercury (MeHg) has attracted special attention, both because it is the most toxic alkylmercury species and because it is highly bioaccumulable and biomagnificable throughout the aquatic food chain. As a result, higher trophic level organisms tend to have larger levels of MeHg, which may adversely affect human beings (Clarkson et al., 2003). Accordingly, to safeguard human health, numerous agencies and governments, such as the World Health Organisation (FAO/WHO, 2006), the US Environmental Protection Agency (USEPA, 2005), and the European Commission (EC, 2001), have issued public health warnings and guidelines to limit or avoid consumption of certain fish taken from Hg-contaminated waters. The concentration limit for total Hg (THg) in fish for human consumption was set at 1 µg g⁻¹ ww for predatory fish and 0.5 µg g⁻¹ ww for non-predatory species (FAO/WHO, 1991; EC, 2001). The WHO (1990) recommended a safety guideline value of 0.5 µg MeHg kg⁻¹ ww and a limit of 0.2 µg g⁻¹ ww for vulnerable groups, such as pregnant women, individuals under 15 years old, and frequent fish consumers.

The chlor-alkali industry is one of the most important sources of mercury pollution in the aquatic environment. In fact, the traditional method employed by the chlor-alkali industry to produce chlorinated solvents, relies on mercury cell technology, which releases inorganic Hg into the aquatic environment. Therefore, the environmental impact from chlor-alkali plants has been studied worldwide (Biester et al., 2002; Landis et al., 2004; Montuori et al., 2006). Although, according to both OSPAR recommendations (OSPAR, 2008) and IPPC directive (EC, 1996), the mercury cell method should be phased out, the Industry Self-Commitment scenario suggests that, in practice, such technology will continue to exist in Spain until 2020 (EC, 2004). Moreover, 3 out of 8 Spanish chlor-alkali plants which use this mercury process are located in the Ebro River basin (NE Spain) (Carrasco et al., 2010), which is the largest river basin in Spain, covering an area of 85 362 km²,
and has the highest annual waterfall (annual mean, 255–424 m² s⁻¹) of any river in the Iberian Peninsula (http://www.chembro.es). Furthermore, the Ebro River receives water from several tributaries, and all together has the potential to influence approximately 3 million people.

The present study focuses on the lower Ebro River, a heavily industrialised area in which the presence of high levels of Hg has been reported (Grimalt et al., 2003). This mercury pollution has been attributed to industrial waste discharge from an electrochemical factory, which has been producing chlorinated industrial solvents since the end of the 19th century, on one bank of the Flix reservoir. In addition, there is a $35 \times 10^4$ ton hazardous industrial solid waste deposit, containing high concentrations of Hg (up to 436 µg g⁻¹) in front of the factory. Despite the long-term Hg pollution present in the Flix dam area, its impact on the local population of aquatic organisms has been evaluated only recently (Carrasco et al., 2008; Faría et al., 2008; Navarro et al., 2009). Unfortunately, non-negligible amounts of Hg are suspected to have been transported downstream of the Flix reservoir, toward the Delta area where a wildlife reserve and Ramsar sites are located. In support of this, the maximum levels of THg in carp muscle, and the highest biological impact on feral carp populations, were not observed in Flix (hot spot), but rather several km downstream (Navarro et al., 2009). In fact, in our previous study (Navarro et al., 2009) THg in muscle, kidney and liver in common carp were measured with the final aim to correlate the biological effects on fish populations, with mercury exposure at the low Ebro River. Therefore, quantitative analyses of mRNA and biochemical biomarkers were used to assess toxic effects of chronic mercury on fish.

In the present work, we aimed to investigate the downstream transport of the mercury pollution originated at the Flix industrial dumping site by the assessment of mercury speciation in high–trophic level organisms such as benthivorous and piscivorous fish. Moreover, there is much interest in the speciation of bioaccumulated mercury in isolated downstream fish populations, and how both trophic position and distance from the source of contamination influence mercury speciation and bioaccumulation.

Within this context, the present study aims to evaluate the bioaccumulation of Hg species in 2 species of feral fish populations inhabiting the high Hg-contamination site (i.e. the hot spot) and 3 downstream points. European catfish (Silurus glanis) and common carp (Cyprinus carpio) were collected and analysed for THg and MeHg content; and variations in mercury concentration with sampling site, fish species, gender and length were investigated. Finally, a comprehensive data evaluation and exploratory multivariate statistics of the data was performed. Therefore, Multiple Correspondence Analysis (MCA) was used to reduce the dimensionality of the resulting data set, followed by application of Multiple Linear Regression models (MRL), in order to quantify the strength of the associations between both THg and MeHg concentrations and the other variables tested.

## 2. Materials and methods

### 2.1. Study area

In order to cover the entire lower Ebro River (NE Spain) (Fig. 1), from Riba-roja dam to the Ebro Delta (approximately 90 km), 5 sampling sites were selected, based on accessibility, bioavailability of fish, and mutual isolation of fish populations. The entire monitored area has vast ecological, agricultural and recreational values.

The Riba-roja dam (RB), which we used as a reference site, is located 13 km upstream of Flix, and forms a large water reservoir (210 hm³). The Flix reservoir (41°23’N, 0°55’E) (FR) is comparatively smaller (area = 320 km², volume = 11 hm³), and has a very short water residence time (0.15 d). The Meander (MD) was located immediately downstream of the Flix dam; while Ascó (AS) and Xerta (XT) sites were in consecutive sections of the river, separated by an overflow dam, located 6 and 37 km downstream of Flix, respectively (Fig. 1). To the best of our knowledge, none of the dams have specific channels which would allow fish passage between them; therefore, these 5 fish populations should be essentially isolated from each other. Nevertheless, it is possible that some fish may be carried downstream by occasional high-flow episodes, particularly for overflow dams. However, because the Riba-roja dam is 60 m tall, such downstream fish transport is highly unlikely, and can be assumed to be negligible for this dam.

### 2.2. Fish sampling

European catfish (Silurus glanis) (SGL) (n = 32) and common carp (Cyprinus carpio) (CCA) (n = 68) were caught in the 5 sampling sites previously described: RB, FR, MD, AS, and XT. Fish were captured during daylight using an electrofishing boat, equipped with a 5.0-GPP Smith-Root Inc. engine (Vancouver, WA, USA), providing up to 1000 V and 16 A. Captured fish were preserved on ice and transported to the laboratory, where fork length (to the nearest millimeter) and other life history traits were measured (Benejam et al., 2010). Dorsal muscle samples were dissected, immediately frozen, and stored at −20 °C for total- and methyl- mercury determination.

### 2.3. Analytical methods

MeHg was measured as previously described elsewhere (Carrasco et al., 2007, 2009). Briefly, 200 mg (wet weight, ww) (hereafter, all concentrations are reported as wet weight unless
CRMs DORM-2 and DORM-3 were found to be 4.66 ± 0.11 l during the course of the study. The THg concentration in the samples (usually 10), ensuring that the instrument remained calibrated during the entire analytical procedure was validated by analyzing CRM DORM-2 and Nickel boat and automatically introduced into the AMA. The entire combustion of the sample, preconcentration by gold amalgamation, thermal desorption and atomic absorption spectrometry (AAS).

The National Research Council of Canada (NRCC, DORM-2 and DORM-3) were used to validate the method (Carrasco et al., 2007, 2009). The MeHg concentration in the CRM DORM-2 was found to be 4.15 ± 0.19 µg g⁻¹ (n = 5), in good agreement with the certified value (4.47 ± 0.31 µg g⁻¹). In the CRM DORM-3, the MeHg value was 0.337 ± 0.053 µg g⁻¹ (n = 5) agreeing the certified value of 0.355 ± 0.056 µg g⁻¹. Repeatability was tested by analysing 6 replicates of MeHgCl (5.396 ng) standard on the same day, and reproducibility was evaluated by analysing 6 replicates of this standard on 6 different days. Relative standard deviations (RSDs) were found to be 9% and 1% for repeatability and reproducibility, respectively, showing the robustness of the analytical procedure.

Quantification and recovery calculations were performed based on the internal standard procedure, using i-Bu₂Hg as an internal standard. External calibration linearity ranged from 0.007 to 1 ng introduced mass in the detector (R² > 0.99), which is equivalent to 0.01–3 µg g⁻¹ Hg in the samples for MeHg. Procedural blanks below 0.01 µg g⁻¹ Hg were obtained for every batch of samples. The limit of detection (LOD) of the method, defined as the mean background noise in a procedural blank triplicate plus three times the standard deviation of the background, was 0.04 ng g⁻¹ ww. The limit of quantitation (LOQ), defined as the mean background noise in a blank triplicate plus ten times the standard deviation of the background, was 0.13 ng g⁻¹ ww.

THg was determined using an advanced mercury analyser (AMA-254, Altec, Prague, Czech Republic), based on catalytic combustion of the sample, preconcentration by gold amalgamation, thermal desorption and atomic absorption spectrometry (AAS). Approximately 100 mg of fish tissue were precisely weighed in a nickel boat and automatically introduced into the AMA. The entire analytical procedure was validated by analyzing CRM DORM-2 and DORM-3 samples at the beginning and end of each set of tissue samples (usually 10), ensuring that the instrument remained calibrated during the course of the study. The THg concentration in the CRMs DORM-2 and DORM-3 were found to be 4.66 ± 0.11 µg g⁻¹ (n = 5) and 0.387 ± 0.005 µg g⁻¹ (n = 10), respectively, which are in good agreement to the certified values (4.64 ± 0.26 µg g⁻¹ and 0.382 ± 0.060 µg g⁻¹). The LOD (0.2 ng g⁻¹ of Hg) and the LOQ (0.7 ng g⁻¹ of Hg) were calculated from blank measurements (Díez et al., 2007).

2.4. Statistical methods

Neither THg nor MeHg concentrations in fish were normally distributed based on Kolmogorov–Smirnov normality test (p < 0.05). Thus, data logarithms were used to enable further statistical analysis, and log-transformed data were normally distributed. All statistical analyses were conducted with SPSS, version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between groups were tested using one way ANOVA followed by post hoc Tukey’s multiple comparison tests. Values above or below 1.5 times the interquartile range were not considered for statistical analysis, as defined by the Tukey method. All reported means are arithmetic means unless otherwise stated.

MCA (Abdi and Valentin, 2007) is an extension of Correspondence Analysis (CA), which enables analysis of the pattern of relationships between several categorical dependent variables. In addition, MCA can be considered to be a generalisation of principal component analysis, if the variables to be analysed are categorical instead of quantitative. MLR were used to quantify the strength of associations between both THg and MeHg concentrations and the different variables studied.

3. Results

3.1. Total mercury

The median THg concentration in all tissue samples (n = 100) was 0.47 ± 0.71 µg g⁻¹, ranging from 0.07 to 3.99 µg g⁻¹ (Table 1). In both fish species, the geometric mean of the THg concentration progressively increased downstream, from site RB (0.19 ± 0.30 µg g⁻¹) to site XT (1.09 ± 0.99 µg g⁻¹) (Fig. 2). Levels at site XT (the farthest downstream site), were approximately 5.9 and 3.3-fold higher than at sites RB (upstream) and FR (hot spot), respectively. THg concentrations in fish populations from sites RB and FR were significantly different from the 3 sampling sites located downstream of the reservoir: MD, AS and XT.

Differences in THg concentrations between fish species were further studied. Considering the 5 sampling points as a population, THg levels in SGL (1.27 ± 0.90 µg g⁻¹) were significantly higher than in CCA (0.35 ± 0.31 µg g⁻¹). A ratio of 3.6:1 was found between the two species, which is similar to the 3.4:1 ratio reported for carnivorous and non-carnivorous fish species.

### Table 1

<table>
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<th>Mean Value (µg/g ww)</th>
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<tr>
<td>MeHg</td>
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in an aquatic ecosystem impacted by gold mining in Northern Colombia (Marrugo-Negrete et al., 2008). In addition, comparison of intraspecies THg levels revealed significant differences among the different sampling sites.

3.2. Methylmercury

The median MeHg concentration in all fish analysed (n = 56) was 0.62 ± 0.66 µg g⁻¹, ranging from 0.05 to 3.14 µg g⁻¹ (Table 1). Similar to the pattern observed for THg, the geometric mean of MeHg concentrations increased from site RB (0.32 ± 0.25 µg g⁻¹) to site XT (1.01 ± 0.74 µg g⁻¹) (Fig. 2). In fact, levels at sites MD, AS and XT were 2.2, 2.2 and 3.0-fold higher than at site FR (hot spot), respectively. Indeed, statistically significant differences in MeHg concentrations were observed between specimens from sites RB and FR, and specimens from site XT. In contrast, MeHg levels in individuals caught at downstream sites (sites MD, AS and XT) were not significantly different.

With respect to differences between fish species, SGL exhibited statistically significantly higher MeHg concentrations (0.91 ± 0.74 µg g⁻¹) than CCA (0.30 ± 0.26 µg g⁻¹) at all 5 sampling points. A ratio of 3.0:1 was observed between the two species.

3.3. MeHg/THg ratios

The mean value of THg occurring as MeHg was 72%. Among the 5 populations tested, the variability in %MeHg variance was substantial (range: 50–98%). Percentages of MeHg in each isolated population were further examined. The highest %MeHg was found in the population from site FR (80%), followed by sites RB (78%), AS (75%), XT (71%) and MD (67%). In addition, the European catfish exhibited, as expected, higher %MeHg values than common carp, in fish taken from sites RB (SGL: 84%; CCA: 72%), MD (SGL: 70%; CCA: 64%), AS (SGL: 77%; CCA: 64%), and XT (SGL: 72%; CCA: 68%). In contrast, MeHg percentages found in FR (hot spot) were higher in the benthivorous CCA (89%) than in the piscivorous SGL (77%). When all individuals within the lower Ebro River were considered as a single population, no significant differences between %MeHg for SGL and CCA were found. In addition, no significant intraspecies differences in %MeHg were found among the different sites.

3.4. MCA and MLR models

MCA was applied in order to investigate the concentrations of both THg and MeHg in individual fish as a function of sampling site, species, gender and length. Because the first two dimensions were able to explain 71% of the inertia contained in the original data set, they were the only ones retained. The first dimension (D1) accounted for 55% of the total original data inertia, and grouped specimens according to THg and MeHg concentrations. Negative loadings represent individuals with high MeHg and THg values (specimens within quartiles 3 and 4 for MeHg concentration and quartile 4 for THg), while positive loadings represent individuals with low MeHg and THg values.

The absolute value of the loadings is an indicator of the participation of the original variables in each dimension. However, when a complex system such as this is studied, it is hard to identify the underlying variables in the final dimensions. Therefore, even though the second dimension (D2) accounted for 16% of the total inertia, it was hard to interpret its environmental meaning.

Fig. 3 depicts projections for each variable on the first 2 dimensions. The analysis was essentially uni-dimensional, with XT and Meander sites clustered on the negative side of D1, and sites AS, RB and FR on the positive side. In addition, catfish also appeared on the negative side of D1, while carp were on the positive one. As described previously, individuals which appear on the negative side of D1 have higher THg and MeHg values; thus catfish exhibited higher values of both Hg species than carp at all sampling points. Furthermore, those individuals inhabiting sites XT and MD were the most contaminated. In addition, specimens with higher fork lengths also appeared on the negative side of D1. This result is in good agreement with the fact that mercury levels are usually higher in the muscles of older and larger fish, than in those of younger specimens, as a consequence of longer exposure time and bioaccumulation (Mirlean et al., 2005).

Finally, it should be stressed that D1 does not appear to exert any influence on fish gender, indicating...
that there were no differences in both THg and MeHg concentrations between males and females.

In order to assess the relationship between the THg and MeHg concentrations measured in fish and the scalar variables previously described (sampling site, fish species, gender and length), MLR models were applied. Regression coefficients ($\beta$) for all scalar variables, their corresponding standard errors, and $p$-values are displayed in Table 2. As can be seen, fish species were significantly related to THg ($p < 0.001$) and MeHg ($p = 0.004$) concentrations. Note that SGL exhibited a 64% ($\beta = 0.64$) higher THg concentration than CCA, and a 71% ($\beta = 0.71$) higher MeHg concentration. All sampling stations surveyed were significantly related to THg concentrations: THg concentrations were 70%, 140%, 140% and 170% higher in fish populations inhabiting FR, MD, AS and XT, respectively, than those inhabiting site RB. Similar to the pattern observed for THg, MeHg concentrations were significantly related to all sampling stations studied: MeHg concentrations increased 60%, 140%, 130% and 160% at sites FR, MD, AS and XT, respectively in comparison with site RB. Fish length was also significantly related to both THg and MeHg; however, the signs were opposite because the relationship was clear and positive only for the piscivorous SGL, but not for the benthivorous CCA, indicating increased bioaccumulation with increasing size in the former fish species. Finally, it is interesting to note that although fish gender was not related to either THg or MeHg concentrations (as concluded by MCA analysis), female specimens displayed approximately 10% higher concentrations of THg and 25% higher concentrations of MeHg than males.

4. Discussion

A review of the literature suggests that in our study, mean THg concentrations are lower than those reported for carp (0.70 $\mu$g g$^{-1}$) and catfish (1.53 $\mu$g g$^{-1}$) in the Nitra River in Slovakia (Andreji et al., 2006). In contrast, mean THg levels in CCA in the present study are much higher than those described for Ya-Er Lake in China (0.08 $\mu$g g$^{-1}$) (Jin et al., 2006) and the Záhlinice Reservoir in the Czech Republic (0.07 $\mu$g g$^{-1}$, dry weight) (Houserova et al., 2007). For MeHg values, our results for SGL and CCA were higher than the values of 0.73 $\mu$g g$^{-1}$ (SGL) and 0.22 $\mu$g g$^{-1}$ (CCA) reported for the same species in the Nitra River (Andreji et al., 2006).

Both MCA and MLR analysis suggest quite marked differences in mercury accumulation in individual fish living downstream of the source of contamination (Fig. 2). This result is in agreement with data obtained in studies of different polluted rivers impacted by Hg emitted from chemical plants: Nero River (Argentina) (Arribere et al., 2003) and Nura River (Kazakhstan) (Ullrich et al., 2007).
Physiological and life-history differences related to THg levels in feral carp inhabiting the lower Ebro River basin have been previously reported (Navarro et al., 2009; Benejam et al. 2010), with more severe biological impact several km downstream of the discharge site; although MeHg levels were not determined. Results from the present study show a downstream increase in THg and MeHg levels, confirming marked physiological effects, such as significant increases in the concentration of reduced glutathione (GSH) in the liver, and mRNA expression of two metallothionein genes (MT1 and MT2) in the kidney; which were previously described for individuals caught downstream. In fact, GSH and MT are known to be involved in the detoxification of inorganic Hg and MeHg.

Surface sediments from the lower Ebro River exhibited the highest values at the discharge point at site FR (15–170 μg g⁻¹ of Hg). Upstream of the Flix factory, mercury levels in sediments were typically below 0.5 μg g⁻¹, whereas sediments sampled downstream of the Flix dam, such as sites AS and XT, showed mercury concentrations ranging from 0.5 to 2 μg g⁻¹ (Grimalt et al., 2003; Bosch et al., 2009). The fate and transport of Hg within an aquatic system and buried in sediment is widely believed to depend on several factors, including redox chemistry, pH, and/or the local activity of methylmercury producing bacteria (Hammerschmidt and Fitzgerald, 2004); hence, the long-term deposition of Hg in the Ebro River sediment bed at site FR may provide continuous input of Hg downstream. Indeed, it is important to stress that reservoirs are known to have downstream effects on Hg concentrations in fish, by exporting MeHg in both the water and invertebrates (Schetagne et al., 2000).

In addition, the elevated mercury concentrations found in fish collected from downstream sites could reflect either fluvial transport of MeHg from upstream sites, or increased in situ production of MeHg at less impacted sites. Interestingly, a recent study of zebra mussels demonstrated that Hg levels at the MD site were higher than those found on the river bank opposite the factory (Carrasco et al., 2008), supporting the idea of river flow transport to downstream sites. Furthermore, this transport may be enhanced during floods (Vericat and Batalla, 2006). Moreover, Cid et al. (2010) described high THg concentrations (1.7 μg g⁻¹, dry weight) in SPM (suspended particulate matter) downstream of site FR, corresponding to transport of Hg approximately 60 km downstream of the source of contamination.

Net in situ production of methylmercury is controlled by the bioavailability of inorganic Hg (II) for methylation, microbial and abiotic MeHg degradation, and the activity of resident methylmercury-producing bacteria (Pasquale et al., 2009). The water type of the Ebro River has been defined as CaSO₄ (Négrel et al., 2007), implying an abundance of sulphate, thus increasing the activity of these bacteria. However, it is important to consider that microbiological studies have revealed that bacterial communities in the Ebro River change seasonally and spatially, implying that turnover of pollutants varies locally, depending on temperature, pH and other key environmental parameters (Grathwohl and Finkel, 2009). Higher temperatures also favour the activity of methylating bacteria; and river water temperatures are usually approximately 2 °C higher at site XT than site FR (http://www.chebro.es).

The fraction of total Hg (II) bioavailable to Hg (II) methylating bacteria includes pore-water dissolved Hg (II), which is not strongly complexed with DOC, and particle-associated Hg (II), which is weakly surface-bound (Pasquale et al., 2009). All approaches (Drot et al., 2007; Miller et al., 2007) that have been used to assess the fraction of total Hg(II) bioavailable to Hg (II) methylating bacteria suggest that only a small fraction of THg is bioavailable for methylation. Further research is needed to measure the actual Hg (II) pool bioavailable at the lower Ebro River.

In addition to the above, Hg transport by flooding events could also transfer “fresh” Hg to downstream sites, which may increase the pool of Hg bioavailable for methylation. In fact, a number of recent studies have reported that newly deposited or freshly added Hg is more readily methylated than ambient Hg (Branfireun et al., 2005; Orihel et al., 2006).

The mean concentration of MeHg found in fish as THg from the lower Ebro River (74%) was lower than typically reported values, which range from 90 to almost 100% (Dominique et al., 2007; Senn et al., 2010). Based on an early study in the nineties (Bloom, 1992), it is generally assumed that practically all Hg in the fish muscle tissue of upper food level consumers is MeHg. However, recent publications have reported MeHg percentages ranging from 50% to 72%, which question this assumption (Riget et al., 2000; Jin et al., 2006; Mason et al., 2006). Furthermore, it is noteworthy that highly contaminated environments may not have as elevated MeHg concentrations in fish, due to nonlinearities between total Hg input, MeHg formation, and MeHg bioaccumulation (Heyes et al., 2006); and between MeHg concentrations in the water and sediment and MeHg levels in the biota (Lawrence and Mason, 2001).

In fish, muscle is the main target for organic mercury, while the liver and kidney are targeted by inorganic and metallic mercury (Navarro et al., 2009). In the lower Ebro River, high liver/muscle ratios of THg in feral carp downstream of the Flix factory have been previously described (Navarro et al., 2009), indicating that effluents from the Flix plant contain large quantities of inorganic Hg. This result may explain why the %MeHg was found to be lower in fish populations downstream of the hot spot.

On the other hand, benthivorous CCA exhibited higher %MeHg than piscivorous SGL at the FR site. However, as we previously discussed (Carrasco, unpublished results) MeHg contamination in fish from a highly Hg polluted environment, such as Flix, may be explained by the species’ ecology, such as food, and habitat. Hence, the THg/MeHg ratio is dominated by the amount of bioavailable MeHg, rather than by differences in the trophic level.

Overall, in the lower Ebro River, mercury pollution increased progressively downstream of the industrial dumping site. A ratio between 2.5 and 3.3 to 1 was found for THg concentrations among individuals collected from downstream sites, with respect to those close to the source of contamination. Regarding MeHg levels, a ratio between 2.2 and 3.0 to 1 was obtained between individuals collected from downstream versus hot spot sites. Thus, considering the vast ecological and agricultural value of the lower Ebro River basin and the Ebro Delta, speciation of mercury is of primary importance. Furthermore, the low fraction of MeHg found in the fish species tested, and the unexpected %MeHg exhibited by SGL at the FR site, clearly indicate that single measurements of THg could lead to an under- or overestimation of pollution levels.

5. Conclusions

In the present study, mercury speciation was conducted on two feral fish species, the European catfish and common carp, in a long-term mercury-contaminated site and several downstream points in the lower Ebro River basin (NE Spain). In order to explain underlying factors influencing fish contamination levels and Hg distribution within the river basin, MCA and MLR were applied. One principal dimension, which grouped individuals according to THg and MeHg concentrations, explained more than 50% of the total inertia. Fish species and the different sampling sites were related to both THg and MeHg concentrations. Piscivorous European catfish exhibited levels of THg and MeHg 3.6 and 3.0-fold higher, respectively, than the non-piscivorous common carp. Moreover, mercury was found to be more bioaccumulated in specimens col-
lected downstream of the hot spot (the Flix dam), than in those inhabiting sites closer to the waste dumping site. Specifically, THg levels were approximately 2.5 times higher in specimens caught at sites MD and AS, and 3.3 times higher in specimens collected from site XT (the farthest downstream site) versus those at site FR. With respect to MeHg, a 2.2:1 ratio was found between individuals inhabiting sites MD and AS versus specimens inhabiting site FR, and a 3.0:1 ratio was obtained between individuals from XT and FR. This result is in good agreement with the high biological impact reported for feral carp downstream of the Flix dam. Both fluvial transport of MeHg from upstream sites, which is reflected by the high mercury levels in SPM and bivalves, and the increased fraction of Hg (II) bioavailable for methylation at downstream sites; can be regarded as key parameters which ultimately explain the higher MeHg levels found in specimens inhabiting downstream sites.

The fact that the %MeHg found in fish from the surveyed area appears to be lower than predicted values, based on worldwide studies, and piscivorous fish displayed lower %MeHg than non-piscivorous fish in the Flix reservoir (hot spot), clearly demonstrates that mercury speciation is of paramount importance to understand the trophic dynamics of mercury and its long-distance pollution transport.

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