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Methylmercury bioconcentration in muscle tissue of the European eel (*Anguilla anguilla*) from the Adour estuary (Bay of Biscay, France)

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The life history of the European Eel (Anguilla anguilla) begins in the Sargasso Sea in the Atlantic Ocean where the Leptocephalus larvae drift with the gulf stream in order to reach European coastal waters. After their metamorphosis into transparent juveniles "glass" eels and an acclimatising phase in the estuaries, they migrate upstream into the rivers to become yellow eels (sub-adult stage). The yellow eels spend between 2 and 20 years of their lifetime in freshwater until they change into silver eels (adult stage) and finally migrate back to the Atlantic Ocean for spawning (Gomez-Mourelo, 2005). A. anguilla is thus an organism able to tolerate a wide range of environmental conditions, such as variations in oxygen availability, different ranges of salinities and exposure to many anthropogenic compounds. In addition, it is a migratory, benthic and benthivorous species at the top of the food chain and is characterised by a high fat content (>30%). For all these reasons A. anguilla is prone to bioaccumulate a wide range of contaminants and it has been widely employed throughout the last years as a bioindicator of the pollution caused by metals (Batty et al., 1996; Has-Schön et al., 2006) and organic contaminants (Storelli et al., 2007; Yamaguchi et al., 2003). The environmental and toxicological impact of Hg bioaccumulation in fish is related to the methylation of inorganic mercury to form the more toxic methylmercury (MeHg) species. In this way, fish tend to concentrate in their tissues MeHg by a factor of 105-107, leading to dangerous levels even in areas with tolerable Hg concentrations (Mason et al. 1996). It has been reported that about 98% of the Hg present in aquatic systems is immobilised in the sediments (Stein et al., 1996) and that most of the MeHg is produced at the sediment water interface as a result of biotic or abiotic transformations caused by specific redox gradients and bacterial activity (Gilmour and Henry, 1998.). According to this, A. anguilla may be an effective biomagnificator and bioaccumulator of Hg due to its longevity during the continental development phase in freshwaters (where it forages and lives upwards 15 years) and its position at the top of the food chain as a carnivorous species feeding on the benthic fauna (Mancini et al., 2005).

The River Adour (located at the South West of France) has a length of 335 Km, enters the Atlantic Ocean at 43°30' North latitude 1°32' West longitude and drains a large agricultural area of 17000 km2. The Adour estuary is affected by the dynamic macrotidal range (up to 70 km upstream) and it is under a strong anthropogenic pressure due to urban, agricultural and industrial activities such as tourism, fishery or recreational boats (Brunet and Astin, 1999). In the upstream estuarine zone, the river presents large flat man-made modified floodplains (known locally as the "Barthes") that constitute an area of 15 km2 lying up to 2 km on both sides of the river. The Barthes are flooded twice a year and play a significant hydraulic and hydrological role owing their high storage capacity. Such capacity together…

with the existence of man-made dykes affords flood protection to the region and allows a significant dilution of point discharges in the area (Brunet and Astin, 2000). The Barthes is a privileged environment for *A. Anguilla* (Gomez-Mourelo, 2005) and its exploitation constitutes the basis of the economy of the local professional fishermen and hence an important economic role in this region. However, *A. Anguilla* stock has been reported to be in dangerous decline in all its geographic life area (Dekker, 2000; Feunteun, 2002).

Mercury speciation analyses in surface sediments of macrotidal estuaries and coastal systems from the River Adour have shown a moderately contamination of MeHg and inorganic mercury (Stoichev et al., 2004). Moreover, the bioaccumulation and biomagnification tendency of MeHg in the trophic network of benthic macrofauna from the Adour Estuary and its adjacent coastal zone has been recently observed (Monperrus et al., 2005). Thus, the speciation analysis of mercury in *A. Anguilla* from the river Adour appears to be necessary in order to ascertain the risk of transfer (generated by the mercury biomagnification) to the higher levels of the food web, including human beings. *A. anguilla* has been used as a biomarker for the study of mercury contamination in many aquatic ecosystems (Batty et al., 1996; Burger et al., 2001; Edwards et al., 1999; Linde et al., 1998; Maes et al., 2005; Oliviera Ribeiro et al., 2005). However, there is only one study providing information about the MeHg levels in eels, particularly in long-fined eels *Anguilla dieffenbachii* from New Zealand (Redmayne, 2000). Therefore, this is the first environmental study reporting Hg speciation data from European eels. The aim of the present work is the determination of the inorganic mercury and MeHg levels in muscle tissues of *A. anguilla* from two different aquatic ecosystems of the Adour Estuary.

This study is part of a research program, the “Groupement de Recherche Adour” (GDR Adour), involving several laboratories that investigate possible effects of contaminants on dynamic eel populations. During this program, the sampling strategy was defined by the LRHA (Laboratoire de Ressources Halieutique d’Aquitaine)–IFREMER (Institute Français pour l’Exploration de la Mer) according to, first, various phases characterizing the annual biological cycle of *A. anguilla* - colonization-sedentarisation-downstream migration - , and second, the specific period of agricultural practice such as maize plantation, irrigation and pesticide treatments. According to this, three periods of sampling corresponding approximately to the months of April, July and October were selected.

The mercury speciation data reported in the present study come from eels caught from two sampling sites (Fig 1): the downstream estuarine zone (Redon site) and a canal located upstream in the floodplains (Barthes) at Saint Laurent de Gosse, sampled in July and October 2005, respectively. The Redon site is located in the mixing zone of Adour Estuary and therefore, it is under the influence of urban and industrial activities as well as physicochemical processes caused by mixing of riverwater and seawater. On the other hand, the sampling site located in the Barthes (Saint Laurent de Gosse) is in the freshwater tidal zone of the estuary and mostly subject to the agricultural activities developed within the Barthes catchments. It is also linked up to the estuary only by valves, permitting at some point the input of fluvial water.

A total of 22 yellow eel samples were analysed for Hg speciation. Fifteen samples were collected from the downstream estuarine zone (Redon site) and the rest from the upstream wetland of one Barthes (Saint Laurent de Gosse). The individual length of the eels ranged from 23.9 to 65 cm (mean: 43.2 ± 12.2), and the weight ranged from 22 to 607 g

(mean: 180.1 ± 163.8). The eels were transported to the laboratory in cool boxes and then dissected to remove their organs. The muscle tissues of the eels were lyophilised and homogenised before analysis. A sample of 0.1 g of the lyophilised muscle tissue was digested with 4 ml of 25% tetra methyl ammonium hydroxide (TMAH) by using a microwave assisted extraction at 70°C for 4 minutes. Then, 0.4 mL of the extract was adjusted to pH=4 with an acetic acid/sodium acetate buffer solution. The mercury species were derivatised using $NaBPr_4$ after the addition of ethyl mercury as internal standard and after five minutes of mechanical shaking they were extracted into isooctane for GC-ICP-MS (Gas Chromatography - Inductively Coupled Plasma Mass Spectrometry) or GC-MIP-AED (Gas Chromatography - Microwave Induced Plasma Atomic-Emission Detection) analysis. The analytical methodology was optimised in previous publications (Tseng et al., 1997; Moreno et al., 2006) and was validated by the analysis of the certified reference material DORM 2 (dogfish muscle tissue from the National Research Council of Canada). The results obtained in the validation of the methodology were well in agreement using both detection techniques and can be observed in **Table 1**.

The concentrations of the mercury species in muscle tissues of *A. anguilla* collected from the two different sampling sites (the estuary and the floodplains) are summarized in **Table 2**. The average concentration of total Hg was found to be 0.31 ± 0.10 and 0.18 ± 0.04 µg Hg g^{-1} (expressed as wet weight) for the estuary and the floodplains, respectively. These concentrations are always below 0.5 µg Hg g^{-1} which is the maximum level set by the European Union for total Hg in foodstuffs (Commission Regulation No 78/2005) and the admitted value set by the World Health Organisation for human consumption (International Programme on Chemical Safety, Environmental Health Criteria No. 1, Mercury). Concerning the MeHg, higher values were encountered in the downstream estuary (mean: 0.27±0.09 µg

Hg g^{-1} wet weight) compared to the floodplains which average 0.11 ± 0.03 µg Hg g^{-1}. As a result, most of the total mercury found in the samples is present as MeHg (**Table 2)**. Indeed, the average percentage of MeHg from the total mercury burden was found to be 86% in the samples from the Estuary and 65% in those from the floodplains. These numbers indicate the clear need of applying specific speciation protocols to investigate the environmental and toxicological impact of metallic contaminants.

Table 3 compares the results obtained in this work with those collected from previous publications reporting total mercury and MeHg levels in eels from different parts of the world. As it can be observed, the results obtained for total mercury in the river Adour are in the same order of magnitude than other published studies. However for the case of MeHg our results can be only compared with one single study reporting MeHg levels in eels from New Zeeland (*A. dieffenbachii*). Similar values are obtained in both studies in terms of concentration levels and percentage of MeHg in the samples.

In contrast to the values from the estuary, the MeHg concentrations in the muscle tissues from the floodplains are not changing drastically according to the individual length of the eel but show a significant correlation (**Figure 2)**. Within the large scatter observed for estuarine MeHg values, one part of the eels show MeHg concentrations closed to those found in the floodplains. This similarity can be the consequence of the eel life history as they are able to migrate from the estuarine either to river or coastal habitats. Indeed, in a recent study in the Gironde estuary, Fablet et al. (in press) have shown, according to Sr:Ca profiles in otoliths, that 72% of the eels sampled changed their habitats once or more. Thus, we cannot exclude that some of eels caught in the Adour estuary were recently coming from the adjacent

coastal or floodplain areas. Another reason to explain these results may be a large spatial variability in the MeHg content in both sediment and benthic food.

If we consider the percentages of methyl mercury (normalised to the total mercury content), there is clear linear regressions with the length of the eels exhibiting significant correlations whatever the origin of eels for both ecosystems (p value < 0.01, **Figure 3**). Such correlation remains higher in the floodplains (Figure 3). It is worth noticing that the lower initial values obtained for the small eels in the floodplains indicates a lower initial exposure to MeHg. The higher slope indicates a higher biomagnification rate versus the length of the fish than that obtained in the downstream estuary. Nevertheless, both ecosystems show the same biomagnification degree in the largest eels. These results can be explained with the different characteristics of both environmental compartments (i.e. salinity, food availability) and/or the individual physiological characteristics such as growth rate.

Although there is still no data regarding the contamination levels and the reactivity of Hg in the "Barthes", previous works in the Adour Estuary have shown that mercury species concentrations (particularly MeHg) were encountered in urban related effluents at significant higher levels compared to the rivers draining the upstream watershed (Point, 2005). Moreover, Stoichev et al. (2006) reported that the MeHg levels in surface waters from the Adour estuary were characterised by longitudinal variations observing the highest levels (in both dissolved and particulate fraction) within the downstream urban estuarine area. This was explained not only by the high methylation potential of the sediments but also by the direct anthropogenic inputs of MeHg from specific discharge points. Such methylation potential was found to be enhanced under anaerobic conditions in estuarine sediments from the Adour River (Rodriguez et al., 2004). On the other hand, the mercury species concentrations in estuary

coastal sediments from the Adour were found to be high enough to assess the impact of estuarine inputs on the nearby coastal area (Stoichev et al., 2004). Finally, in agreement with these results, the MeHg analysis in three different trophic groups (suspension feeders, predators and deposit feeders) from different sampling sites of the downstream Adour estuary showed that MeHg is also subject to biomagnification in the trophic foodchain in this ecosystem (Monperrus et al., 2005).

Taking into account those previous results, the better correlations obtained for the eels from the floodplains in both **Figures 2 and 3** can be explained by the population type and the exposure mode. Concerning the eels from the "Barthes" which is a relatively closed ecosystem, it can be assumed that the exposure is mostly based on identical trophic route provided by a specific local food chain. On the other hand, because the downstream estuary is prone to receive the additional MeHg contribution from anthropogenic sources, various mercury accumulation routes related to different trophic chains can be assumed. This research permits the establishment of the basis for a large scale of study in the Adour estuary and gives for the first time preliminary useful information about MeHg burden, variability and composition of mercury species in muscle tissues of European eels (*A. Anguilla*). In addition we have shown that *A. anguilla* is an effective bio-accumulator of MeHg even for aquatic environments moderately contaminated by mercury, demonstrating the usefulness of this species as biomarker of the impacts of mercury pollution in different aquatic ecosystems.

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Table 1. Results obtained in the analysis of the certified reference material DORM 2 (dogfish mussel tissue). Uncertainty of the results is expressed as 1s standard deviation.

Analytical Technique	Concentrations in ng Hg g^{-1} (dry weight)		
	MeHg	Hg(II)	HgT
GC-ICP-MS	4319 ± 87	70 ± 18	4389 ± 92
GC-MIP-AES	4096 ± 402	Not determined	Not determined
Certified Values	4470 ± 320	Not certified	4640 ± 260

Table 2. Mean values of the methyl mercury, inorganic mercury and total mercury concentrations (µg Hg g^{-1} wet weight) in the muscle tissues of A. anguilla collected from the two sampling sites of the Adour Estuary (uncertainty of the values is expressed as 1s standard deviation).

Sites	n	MeHg (µg Hg g^{-1})		Hg(II) (µg Hg g^{-1})		HgT (µg Hg g^{-1})	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Estuary	**15**	0.27 ± 0.09	0.12-0.45	0.04 ± 0.03	0.003-0.13	0.31 ± 0.10	0.16-0.48
Floodplains	**7**	0.11 ± 0.03	0.08-0.16	0.07 ± 0.05	0.004-0.16	0.18 ± 0.04	0.12-0.24

Table 3. Comparison of total mercury and methyl mercury concentrations in eels obtained in similar studies (µg Hg g^{-1} as wet weight).

Species	Location	Type	Total Hg	MeHg	Sample	Ref.
A. Anguilla	Vaccares (France)	Pond	0.22 (n=15)		Liver	Batty, 1996
A. Anguilla	Berre (France)	Pond	0.23 (n=15)		Liver	Batty, 1996
A. Anguilla	East Anglia (UK)	River estuary	0.26 (n=51)		Muscle	Edwards, 1997
A. Anguilla	East Anglia (UK)	River broadening	0.10 (n=51)		Muscle	Edwards, 1997
A. dieffenbachii	Leith (New Zealand)	River	0.12 (n=1)	0.08 (n=27)	Muscle	Redmayne, 2000
A. dieffenbachii	Flemming (New Zealand)	River	0.31 (n=1)	0.48 (n=34)	Muscle	Redmayne, 2000
A. dieffenbachii	Kyeburne (New Zealand)	River	0.65 (n=1)	0.50 (n=23)	Muscle	Redmayne, 2000
A. rostrata	Savannah River (USA)	River	0.15 (n=24)		Muscle	Burger, 2001
A. Anguilla	Thames River (UK)	River	0.15 (n=2)		Muscle	Yamaguchi, 2003
A. Anguilla	La Capelière (France)	Pond	0.03 (n=9)		Muscle	Ribeiro, 2005
A. Anguilla	La Capelière (France)	Pond	0.06(n=10)		Liver	Ribeiro, 2005
A. Anguilla	Fumemorte (France)	Pond	0.09 (n=9)		Muscle	Ribeiro, 2005
A. Anguilla	Fumemorte (France)	Pond	0.08 (n=9)		Liver	Ribeiro, 2005
A. Anguilla	Mornèse (France)	Pond	0.12 (n=8)		Muscle	Ribeiro, 2005
A. Anguilla	Mornèse (France)	Pond	0.15 (n=8)		Liver	Ribeiro, 2005
A. Anguilla	Yser (Belgium)	River	0.15 (n=8)		Muscle	Maes, 2005
A. Anguilla	Meuse (Belgium)	River	0.17 (n=20)		Muscle	Maes, 2005
A. Anguilla	Scheldt (Belgium)	River	0.09 (n=33)		Muscle	Maes, 2005
A. Anguilla	Tiber River (Italy)	River	0.23 (n=8)		Muscle	Mancini, 2005
A. Anguilla	Lesina (Italy)	Lagoon	0.18 (n=2)		Muscle	Storelli, 2007
A. Anguilla	Adour River (France)	River estuary	0.31 (n=15	0.27 (n=15)	Muscle	This work
A. Anguilla	Adour River(France)	River floodplain	0.18 (n=7)	0.11 (n=7)	Muscle	This work

Legends for Figures:

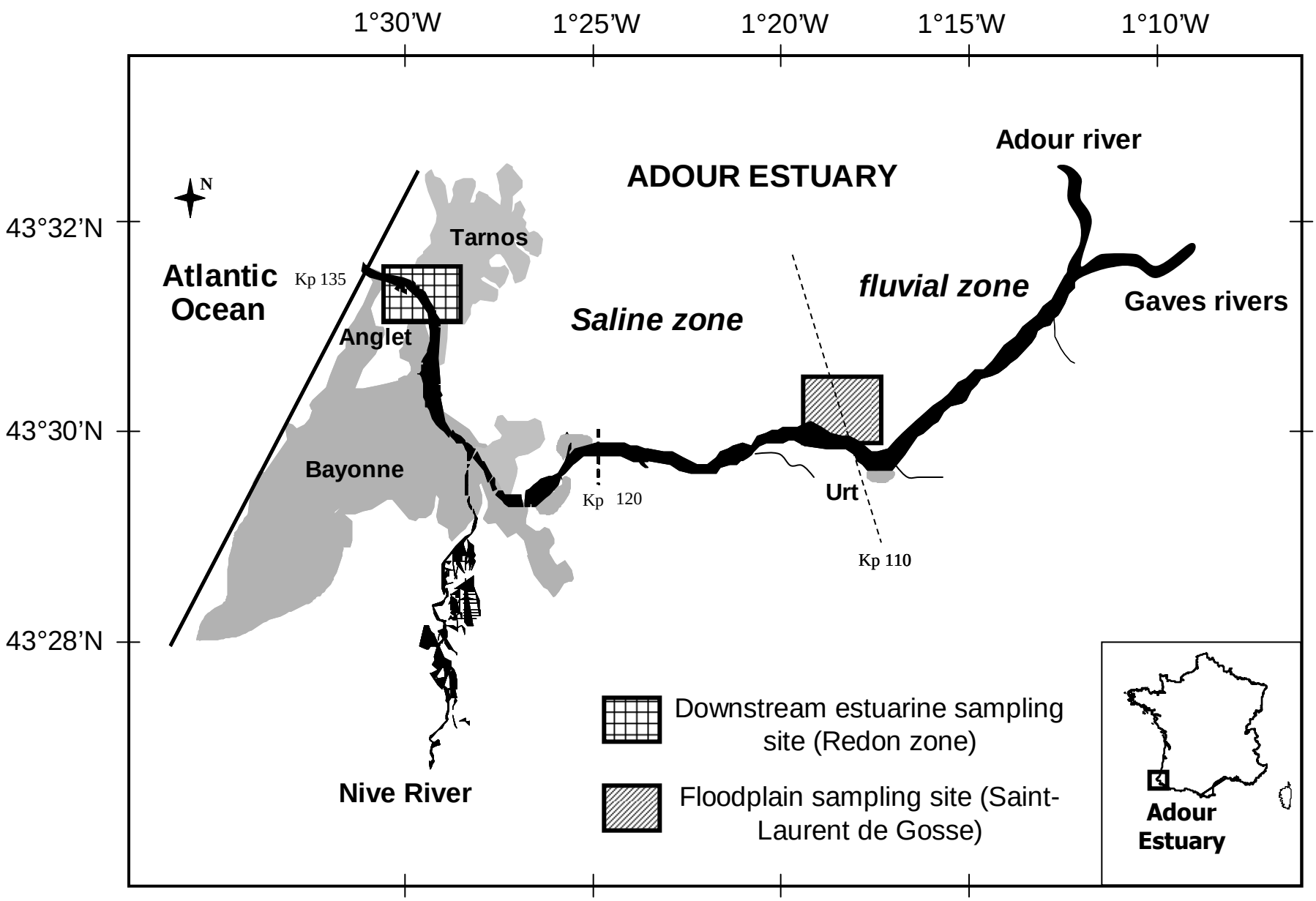
Figure 1. Sampling area locations in the lower estuary and upper estuary floodplain (Adour River, France).

Figure 2. Concentration of MeHg (µg Hg g^{-1}as wet weight) according to the length of the eels from the downstream urban estuary and from the floodplains of the Adour River.

Figure 3. Percentage of MeHg according to the length of the eels from the downstream urban estuary and from the floodplains of the Adour River.

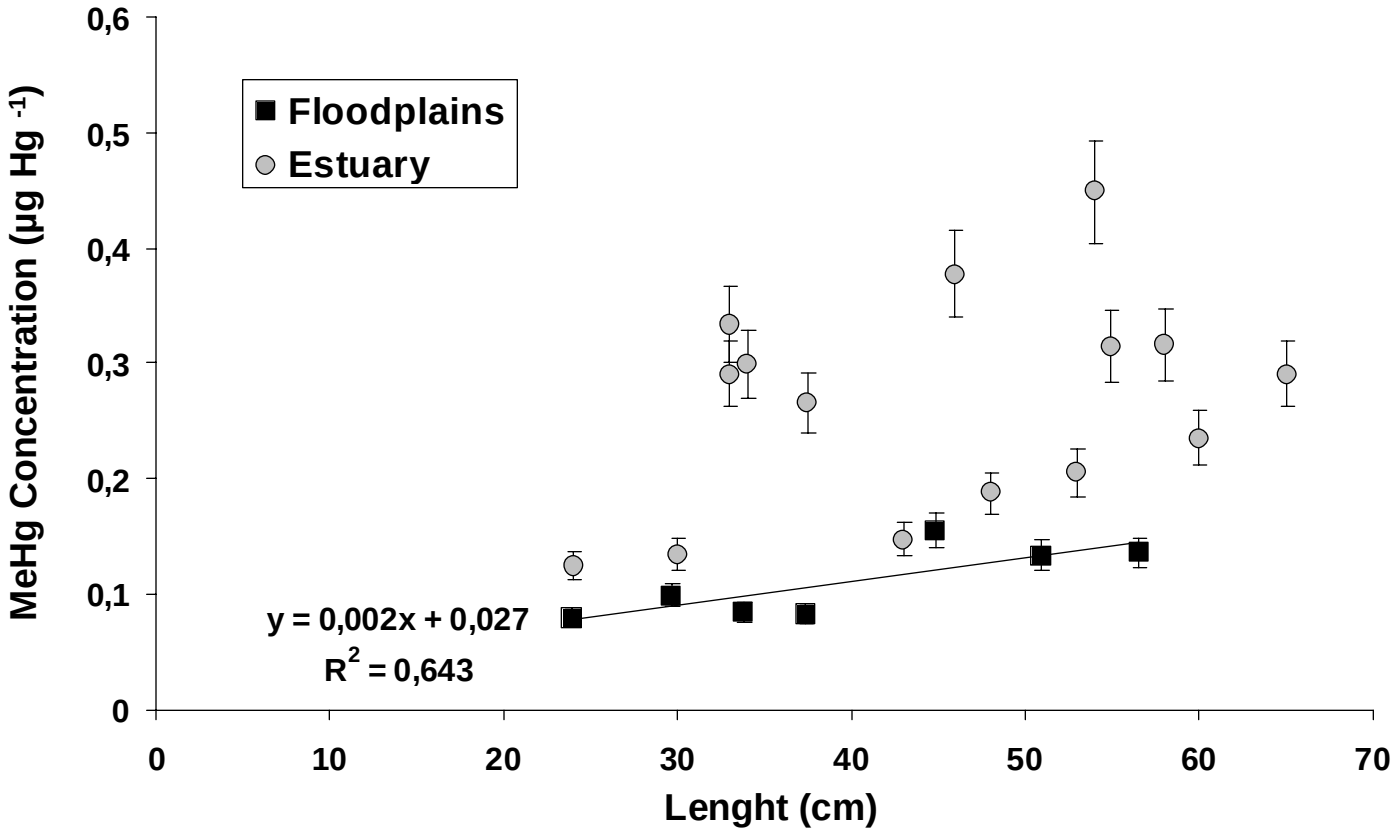
Figure(s)

Figure 1



Figure(s)

Figure 2



Figure(s)

Figure 3