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Tracing mercury pathways in Augusta Bay (southern Italy) by total concentration and isotope determination

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ABSTRACT

The mercury (Hg) pollution of sediments is the main carrier of Hg for the biota and, subsequently, for the local fish consumers in Augusta Bay area (SE Sicily, Italy), a coastal marine system affected by relevant sewage from an important chlor-alkali factory. This relationship was revealed by the determination of Mass Dependent (MDF) and Mass Independent Fractionation (MIF) of Hg isotopes in sediment, fish and human hair samples. Sediments showed MDF but no MIF, while fish showed MIF, possibly due to photochemical reduction in the water column and depending on the feeding habitat of the species. Benthic and demersal fish exhibited MDF similar to that of sediments in which anthropogenic Hg was deposited, while pelagic organisms evidenced higher MDF and MIF due to photoreduction. Human hair showed high values of δ^{202}Hg (offset of +2.2‰ with respect to the consumed fish) and Δ^{199}Hg, both associated to fish consumption.

1. Introduction

Mercury (Hg) is a toxic metal which, primarily in its neurotoxic form (methylmercury, MeHg), bioaccumulates through the food web and determines health risks for humans (particularly in developing fetus), like cerebral palsy, cortical blindness, deafness and severe developmental impairment (Davis et al., 1994). The biogeochemistry of Hg is highly complex due to exchanges among atmospheric, terrestrial and marine reservoirs (Fitzgerald et al., 1991). These transfers are mainly controlled by microbial activity, dark abiotic and photochemical reactions which dramatically affect speciation, mobility and bioaccumulation (Morel et al., 1998). Mercury isotopic analysis represents an effective tool to trace such transformation pathways, as well as to distinguish among different sources of mercury where detectable isotope fractionations occur. Mercury undergoes mass-dependent isotope fractionation (MDF) during transformation or exchange reactions such as microbially-mediated methylation (Rodriguez-Gonzalez et al., 2009), reduction (Kritee et al., 2007, 2008, 2009), demethylation (Bergquist and Blum, 2007; Kritee et al., 2009) and trophic transfer (Perrot et al., 2010). On the other hand, Hg also exhibits mass-independent fractionation (MIF) of the odd isotopes (^{199}Hg and ^{201}Hg) during specific physical and biogeochemical reactions such as photochemical reaction of inorganic Hg (IHg) and MeHg in the water column. This signature is generally well mirrored in marine organisms (Zheng and Hintelmann, 2009; Perrot et al., 2013) and retained in the food web (Senn et al., 2010; Blum et al., 2014). Positive MIFs were also observed in human hair (Point et al., 2011; Day et al., 2012) and they are generally related to seafood consumption (Laffont et al., 2011; Blum et al., 2014). On the other hand, MDF has been revealed in human hair, resulting in a ~+2‰ δ^{202}Hg offset with respect to the consumed seafood (Laffont et al., 2009; Sherman et al., 2013). The Augusta Bay (AB; SE Sicily) is a well-known case study where the Hg sewages from the largest Italian chlor-alkali plant (1958–2005) (Le Donne and Ciafani, 2008) caused alarming environmental concerns (ICRAM, 2005, 2008; Sprovieri et al., 2011), strongly impact on the trophic web (Ausili et al., 2008; Tomasello et al., 2012; Bonsignore et al., 2013) and evasional flux of Hg into atmosphere (Bagnato et al., 2013). Moreover, epidemiological studies carried out in the area have denounced alarming increase of congenital malformations, abortions and mortality rates (Madeddu et al., 2001, 2003). This study offers an unprecedented opportunity to simultaneously explore the Hg content and the Hg isotopic signatures of sediments, fish and

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human hair, in order to assess whether variations in Hg isotopic composition can reliably document sources and processes acting in the environment and transfer mechanisms to human population.

2. Materials and methods

2.1. Sample collection

Three box-core sediments were collected in AB during June 2012 (Fig. 1) by a scuba diver who manually inserted a Plexiglass tube (30 cm length, 6 cm i.d.) into the bottom sediment. Samples were carefully recovered on board and stored at −20 °C. At the geochemical lab of the IAMC-CNR (Capo Granitola, Italy), each core was defrost and sectioned at 1–2 cm intervals with a stainless steel saw. Twenty-eight slices were selected for total Hg concentration [Hg] and isotopic determination. Detailed information about fish sampling has been previously reported (Bonsignore et al., 2013). Briefly, fish were harvested in AB from both open sea region (mid-water sampling; bottom depth 50–100 m) (C1, C2; Fig. 1) and shallow-water region (bottom-water sampling; mean depth ~15 m) (C3, C4; Fig. 1). Six pelagic specimens collected outside the bay, as well as nine demersal and five benthic specimens collected inside the bay were randomly selected for Hg isotope analyses. Finally, 21 indigenous individuals (30–40 years old) and habitual consumers of local fish and shellfish, namely seafood bought in local markets (see details reported in the informative questionnaire, Supplementary Material), were selected for human hair collection. For each subject ~300 mg of hair (1–2 cm long) were cut with stainless steel scissors from the nape of the neck and stored in sterile bags.

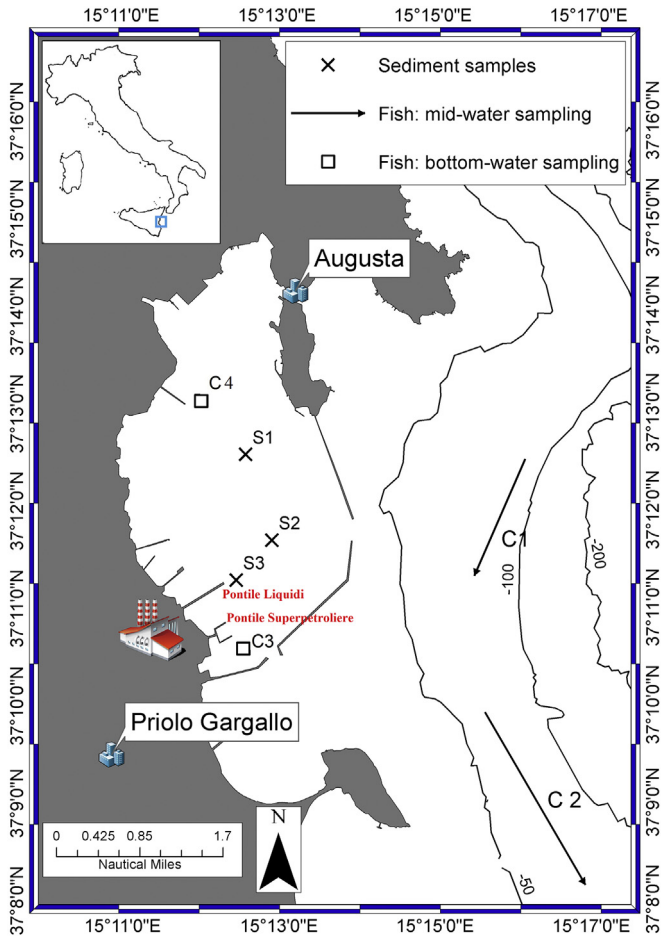

Fig. 1. Map of the Augusta Bay (AB) and sampling sites of pelagic (→), benthic and demersal fish (□) and sediments (×). The Pontile Superpetroliere and the Pontile Liquidi dams delimit the area mostly affected by industrial discharges from the chlor-alkali plant.

2.2. Analytical methods

In order to minimize contamination, acid-cleaned materials and ultrapure grade reagents were used (details are reported in the Supplementary Material). [Hg] was determined on wet muscle tissues, dry sediments and untreated human hair by the DMA80 atomic absorption spectrophotometer (Milestone, CT, USA) according to US-EPA 7473 method. For Hg isotope determinations, samples and certified reference materials (TORT-2, PACS-2 and NCS ZC 81002b) were digested (EPA Method 3052) using a MARS 5.0 microwave oven (CEM Corp., Matthews, NC, U.S.A.) in 45 ml closed Teflon vessels with an ultra-grade acid mixture. Specifically, ~0.5 g of dry sediments were digested at 180 °C for 45 min in 9 mL of HNO_3 and 3 mL of HCl (final volume ~50 mL). Meanwhile ~0.3 g of dry muscles were digested by adding 2 mL of HNO_3 and 1 mL of H_2O for 2 h, reaching 160 °C (final volume ~15 mL). The same microwave condition was used to digest ~0.15 g of untreated human hair, by adding 2 mL of HNO_3 and 0.5 mL of H_2O_2 (final volume ~10 mL). After digestion all samples were passed through syringe filters with 0.2 μm cellulose acetate membrane (WVR Intl.).

2.2.1. Hg isotope ratio (IR) measurements

Measurements were performed by cold-vapor-generation multi-collector ICP/MS spectrometry, CVG-MC-ICP/MS (Neptune, ThermoFinnigan, Bremen, Germany) at the University of Modena and Reggio Emilia, Italy. Tab. S1 and S2 (Supplementary Material) show the cup configuration and a summary of instrumental parameters for the Neptune and the sample introduction system. This method has been previously optimized in order to obtain a suitable data precision while consuming a small sample volume (~4 mL per run) fitting the sample loading loop. Each sample solution was carefully diluted with HNO_3 4% (v/v) to a final Hg concentration as close as possible to 20 μg kg^{-1}, while Hg standard concentration was matched to samples within ±10%. Samples were acquired along with the NIST SRM 3133 Hg standard in a sample-standard bracketing (SSB) sequence. Mercury isotopic ratios (IRs) are herein expressed as δ^{202}Hg, which accounts for MDF and Δ^{199}Hg and Δ^{201}Hg, indicators of MIF fractionation. Indeed δ^{202}Hg indicate the relative per-mil difference of $^{202}Hg/^{198}Hg$ between a given sample and the SRM 3133 standard, according to Eq. 1

$$\delta^{x}Hg(‰) = \left(\frac{R^{x/198}_{sample}}{\frac{1}{2}\left(R^{x/198}_{stdA} + R^{x/198}_{stdB}\right)} - 1\right) \times 1000 \quad (1)$$

where $R^{x/198}$ is the mass bias corrected data for the sample and the two SRM 3133 runs (*stdA* and *stdB*, respectively) acquired next to the sample run in the SSB sequence. Δ^{199}Hg and Δ^{201}Hg, on the other hand, express the difference between the δ^{199}Hg and δ^{201}Hg and the theoretical mass-dependent values, calculated from δ^{202}Hg, according to the kinetic mass-dependent fractionation law. The following equations were used:

$$\Delta^{199}Hg\ (‰) = \delta^{199}Hg - \left(0.2520 \times \delta^{202}Hg\right) \quad (2)$$

$$\Delta^{201}Hg\ (‰) = \delta^{201}Hg - \left(0.7520 \times \delta^{202}Hg\right) \quad (3)$$

2.2.2. Mass bias correction

Mass bias on Hg absolute IRs of each standard run was corrected following the Yang and Sturgeon (2003) approach. Conversely, mass bias on samples ratios was accounted by interpolating the biases of the two standard measurements next to each sample in the SSB sequence. Data about method, precision and accuracy are reported in the Supplementary Material.

2.3. Statistical analysis

The assumptions of normality of data and homogeneity of variance were tested using one-way Kolmogorov–Smirnov (K–S) and Bartlet's test, respectively. Differences among groups of samples with non-normal data distribution were verified by the non-parametric statistical methods Wilconox rank sum test and Kruskal–Wallis (KW) (for more than two groups). When KW showed significant differences, a pair-wise test was used in order to assess which couples contributed to the significance. A Spearman's rho correlation coefficient was performed to estimate the relationship between Hg content and the frequency of fish consumption. The relationship between fish Δ^{199}Hg and Δ^{201}Hg values was analyzed using a York regression method (York, 1966) to calculate the best-fit line with errors in both X and Y variables. Statistical significance was defined as $p < 0.05$. All the statistical analysis were carried out using statistical software R (R 3.0.1).

3. Results and discussion

3.1. Sediments as source of Hg for fish

Sprovieri et al. (2011) had already hypothesized the role of the sediments as still active source of Hg for the surrounding marine environment in AB. A further investigation (Bonsignore et al., 2013) supported this hypothesis by measuring alarming and potentially dangerous Hg contents in fish tissues of the same area. Here, we address the question whether the relationship between sediments and fish can be firmly assessed by the investigation of their Hg isotopic composition.

3.1.1. Mass Dependent fractionation

The AB sediment samples displayed negative MDF (mean δ^{202}Hg −0.39 ± 0.21‰, [1SD; n = 28]; median −0.35‰), but no MIF (mean Δ^{199}Hg: −0.02 ± 0.03‰ [1SD; n = 28]; median −0.02‰) (Table 1; Fig. 2). The δ^{202}Hg values measured in the core sediments (range: −0.91÷−0.11‰; median −0.35‰) are within the range measured in other marine areas affected by Hg pollution from chlor-alkali factories. Specifically, δ^{202}Hg values measured in surface sediments of Lake Baikal (Russia) averaged −0.64 ± 0.35‰ (2SD) (Perrot et al., 2010), while Wiederhold et al. (2015) reported a δ^{202}Hg range of −1.40÷−0.60‰ from sediments collected at Skutskär, a site near a Sweden chlor-alkali plant. No statistically significant difference in δ^{202}Hg ($p = 0.14$; KW) emerged among the northern S1 (mean −0.34 ± 0.16‰ [1SD; n = 10]; median −0.31‰), central S2 (mean −0.34 ± 0.17‰ [1SD; n = 9]; median −0.35‰) and southern S3 stations (mean: −0.53 ± 0.25‰ [1SD; n = 9]; median: −0.64‰), although the latter shows the lowest δ^{202}Hg values. Interestingly, sediments revealed an increasing [Hg] moving from the northern S1 (mean 6.54 ± 1.36 mg kg^{-1} [1SD; n = 10]) to the central S2 (mean 11.88 ± 2.62 mg kg^{-1} [1SD; n = 9]) and southern S3 sites (mean 26.70 ± 15.80 mg kg^{-1} [1SD; n = 9]) ($p = 0.03$; KW) (Table 1), in agreement with previously investigations of the area (ICRAM, 2005, 2008; Sprovieri et al., 2011). This is attributed to the effect of the uncontrolled sewage from the chlor-alkali industry particularly in the southern part of the bay (between the Pontile Superpetroliere and the Pontile Liquidi; Fig. 1) and occurring since 1950s at least until 1978, when restrictions

Table 1
Mercury concentrations and isotopic composition in sediment cores from Augusta Bay.

Sediments					
Uncertainty (2σ) = ±0.19 for Hg; ± 0.24 for δ^{202}Hg; ± 0.08 for Δ^{199}Hg; 0.14 for Δ^{201}Hg					
Sample	Depth (cm)	Hg (mg Kg^{-1})	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{201}Hg (‰)
S1 (North)	0–1	8.34	−0.26	−0.10	−0.02
	2–3	6.79	−0.66	0.00	0.00
	4–5	6.42	−0.30	−0.04	−0.02
	13–14	8.66	−0.17	−0.02	−0.01
	14–16	6.84	−0.55	0.00	0.00
	18–20	7.23	−0.32	−0.03	0.02
	24–26	5.74	−0.27	−0.06	−0.02
	26–28	4.12	−0.13	−0.04	0.01
	30–32	5.59	−0.38	−0.03	−0.03
	36–37	5.64	−0.36	−0.02	−0.06
S2 (Central)	0–1	14.3	−0.35	−0.05	−0.07
	2–4	16.0	−0.35	−0.05	−0.07
	6–8	13.4	−0.24	−0.01	0.01
	8–10	12.2	−0.24	−0.01	−0.01
	10–12	12.8	−0.11	−0.01	0.03
	14–16	9.33	−0.52	−0.05	−0.05
	18–20	11.8	−0.50	−0.05	−0.05
	20–22	8.01	−0.14	0.04	−0.01
	22–24	9.12	−0.37	−0.01	−0.03
S3 (South)	0–2	17.1	−0.64	−0.04	−0.03
	4–6	19.0	−0.68	0.00	0.00
	10–12	28.1	−0.16	0.00	−0.06
	12–14	38.4	−0.28	0.03	0.02
	14–16	55.3	−0.61	−0.01	0.04
	18–20	40.0	−0.65	−0.01	−0.04
	24–26	18.5	−0.65	0.00	−0.03
	28–30	22.1	−0.91	−0.03	−0.03
	34–36	1.78	−0.23	−0.04	0.01

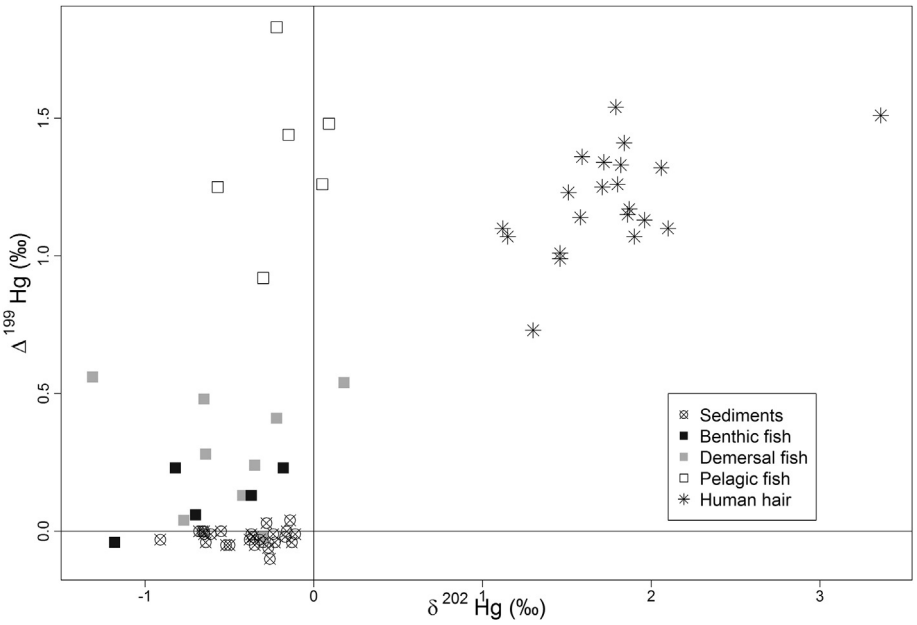

Fig. 2. Plot of Δ^{199}Hg vs. δ^{202}Hg for sediments, fish (benthic, demersal and pelagic species) and human hair.

were imposed by the Italian legislation. It is worth to note that significant correlations between Hg and Barium were reported by ICRAM (2008), especially in the southern part of AB and interpreted as chemical fingerprint of the chlor-alkali plant industrial activities.

MDF in fish samples vary widely between −1.31 and 0.18‰, with δ^{202}Hg mean value of δ^{202}Hg −0.44 ± 0.39‰ (1SD; n = 20) (Table 2; Fig. 2). The median δ^{202}Hg values resulted higher in pelagic (−0.18‰), with respect to demersal (−0.42‰) and benthic (median −0.70‰) specimens, and a 0.5‰ offset in δ^{202}Hg occurred between pelagic and benthic samples (Fig. 2). Variations do not appear correlated with the [Hg] which is higher in benthic (mean: 1.02 ± 0.38 mg kg^{-1} [1SD; n = 5]) than in demersal (mean: 0.44 ± 0.38 mg kg^{-1} [1SD; n = 9]) and pelagic fish samples (mean 0.20 ± 0.12 mg kg^{-1}[1SD; n = 5]) (Table 2). Only the pelagic top predator *Sphyraena sphyraena* showed very high [Hg] of 2.27 mg kg^{-1}. As previously reported by Bonsignore et al. (2013), the highest levels of Hg in fish muscles of the benthic species well-reflect habitat depths and suggest active mechanisms of Hg release from the polluted sediments as primary driver of bioaccumulation in the trophic web.

Furthermore, the absence of any statistical difference between the δ^{202}Hg measured in benthic and demersal specimens and sediments (p = 0.21; KW), confirms the assumption of sediments as main source of Hg to the AB fish compartment. Similarly, Perrot et al. (2010) demonstrated that the most Hg enriched fish samples from Bratsk Water Reservoir have the same isotopic composition of sediments in which anthropogenic Hg was deposited. On the other hand, and in agreement with other recent studies

Table 2
Mercury concentrations and isotopic composition in fish from Augusta Bay.

Fish							
Uncertainty (2σ) = ±0.04 for Hg ± 0.32 for δ^{202}Hg; ± 0.22 for Δ^{199}Hg; 0.10 for Δ^{201}Hg							
Species	Trawl	Habitat	Length (cm)	Hg (mg Kg^{-1})	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{201}Hg (‰)
Sardina pilchardus	→	*Pelagic*	12.3	0.09	0.09	1.48	1.42
Sardina pilchardus	→	*Pelagic*	14.7	0.11	−0.3	0.92	1.06
Boops boops	→	*Pelagic*	14.9	0.13	−0.22	1.83	1.52
Trachurus trachurus	→	*Pelagic*	22.2	0.33	−0.57	1.25	1.09
Trachurus trachurus	→	*Pelagic*	20.4	0.31	0.05	1.26	1.10
Sphyraena sphyraena	→	*Pelagic*	119	2.27	−0.15	1.44	1.09
Diplodus annularis	□	*Demersal*	11.4	0.33	−0.65	0.48	0.43
Diplodus annularis	□	*Demersal*	17.6	1.42	−0.22	0.41	0.36
Pagellus acarne	□	*Demersal*	15.2	0.26	−1.31	0.56	0.44
Pagellus acarne	□	*Demersal*	15.5	0.20	−0.35	0.24	0.40
Pagellus acarne	□	*Demersal*	15.0	0.26	0.18	0.54	0.63
Pagellus acarne	□	*Demersal*	15.4	0.26	−0.42	0.13	0.36
Pagellus erythrinus	□	*Demersal*	19.5	0.35	−0.64	0.28	0.29
Pagellus erythrinus	□	*Demersal*	20.5	0.42	−0.30	−0.02	0.10
Pagellus erythrinus	□	*Demersal*	18.8	0.47	−0.77	0.04	0.11
Mullus barbatus	□	*Benthic*	16.8	0.71	−0.82	0.23	0.16
Mullus barbatus	□	*Benthic*	16.4	0.71	−0.70	0.06	0.11
Mullus surmuletus	□	*Benthic*	20.7	0.60	−0.18	0.23	0.14
Scorphaena notata	□	*Benthic*	13.3	1.65	−0.37	0.13	0.09
Scorphaena scrofa	□	*Benthic*	13.9	1.42	−1.18	−0.04	−0.04

(Jackson et al., 2008; Gantner et al., 2009; Gehrke et al., 2011), substantial difference ($p = 0.04$; Wilcoxon-test) has been observed between the MDF of sediments and pelagic fish, testifying the occurrence of isotope fractionations during Hg transfer between the two compartments. Among the several reaction pattern (e.g., microbial methylation of Hg(II), trophic transfer of MeHg, MeHg degradation) which fractionate Hg isotopes according to mass-dependence, also photoreduction of IHg and MeHg can produce MDF (Bergquist and Blum, 2007; Zheng and Hintelmann, 2009). Given that pelagic fish caught from the external AB area display the highest MIF (see below), we suppose that the observed offset in δ^{202}Hg values was driven by mercury photoreduction in open seawater prior to be incorporated into the food web.

3.1.2. Mass Independent Fractionation

Δ^{199}Hg in the analyzed fish samples vary widely (−0.04÷1.83‰; mean 0.57 ± 0.58‰ (1SD, n = 20); median 0.34‰) (Table 2). As expected, MIF was observed in fish but not in sediments (mean Δ^{199}Hg −0.02 ± 0.03‰ [1SD, n = 28]; median −0.02‰). Indeed, MIF in fish is mainly driven by photoreduction of IHg and/or MeHg in the water column, which determines enrichment of the odd isotopes in the residual Hg-reactant compound (Bergquist and Blum, 2007). Additionally, the Δ^{199}Hg/Δ^{201}Hg ratio can identify the mercury species (MeHg or IHg) affected by photoreduction. Specifically, a slope of 1.00 indicates processes that exclusively involve IHg photoreduction, while higher slopes (~1.30) have been documented for in-vitro photochemical MeHg demethylation (Bergquist and Blum, 2007, 2009). On the basis of a York regression (York, 1966), the Δ^{199}Hg/Δ^{201}Hg ratio in the studied fish resulted 1.21 ± 0.08 (1 s.e.; n = 20) (Fig. 3) thus suggesting that photoreduction of both MeHg and IHg takes place in the water column prior to the intake in the marine food web. Significantly, the magnitude of MIFs in fish increased moving from the coastal to the open sea. MIF is close to zero in benthic (mean Δ^{199}Hg: 0.12 ± 0.12‰ [1SD; n = 5]), slightly higher in demersal (Δ^{199}Hg 0.30 ± 0.22‰ [1SD; n = 9]) and much greater in pelagic fish samples (mean Δ^{199}Hg 1.36 ± 0.30‰ [1SD; n = 6]) (Table 2; Fig. 3). This primarily suggests that, in AB, photoreduction has a crucial role, in terms of isotope fractionation, mainly in the pelagic fish compartment. This is also verified by the increase of MDF in pelagic fish with respect to the benthic and demersal specimens. Noteworthy, the clear gradient of MIF observed in fish from AB seems also well correlated with the habitat feeding. This is consistent with Blum et al. (2013), who have related the Δ^{199}Hg values of oceanic fish to the feeding habitat and the magnitude of MeHg photoreduction in the water column. In fact, pelagic fish principally feed on plankton (Frimodt, 1995) where Hg is mostly present in inorganic form (MeHg/THg ≈ 0.10) and have shown the highest positive MIF. Among the pelagic fish, the specimen of *Sphyraena sphyraena*, a pelagic predator with a specific diet based on small fishes, cephalopods and crustaceans (Ben-Tuvia, 1986) shows high MIF (Δ^{199} = 1.44‰; Table 2) probably inherited the by consumption of smaller fish. On the other hand, benthic and demersal fish usually feed on small benthic crustaceans, worms and mollusks (Hureau, 1986) containing a high percentage of MeHg (MeHg/THg ≥ 0.90) (Perrot et al., 2010, 2012). As a consequence, pelagic fish from AB showed the larger positive MIF, and the highest MDF, probably because they take up highly fractionated MeHg directly from the water column, while benthic organisms are exposed to sedimentary Hg, containing less fractionated Hg. The peculiar similarity between the Hg isotopic signature of sediments and benthic/demersal fish (Fig. 2) certainly attributes to the sediment the role of primary source of Hg for the deeper fish compartment.

3.2. Fish as source of Hg for human

The [Hg] measured in human hair samples was extremely elevated (mean: 2.32 ± 1.40 mg kg^{-1} [1SD, n = 21]) (Table 3) and exceeded, in most cases, the US EPA reference dose of 1.0 μg g^{-1} of Hg per kg body weight per day (US EPA, 2001, 2010). The highest [Hg] (mean 5.05 ± 0.23 mg kg^{-1}) correspond to individuals who consume local seafood more than three times per week (~14% of the total). A mean [Hg] of 1.97 ± 0.85 mg kg^{-1} were found in hair of individuals who consume local seafood once or twice per week, whereas people who rarely do it (~14% of the total) showed moderate Hg content in their hair (mean [Hg] 1.32 ± 0.94 mg kg^{-1}) (Fig. 4; Table 3). Spearman coefficient calculation demonstrated a positive correlation between the Hg contents and the weekly

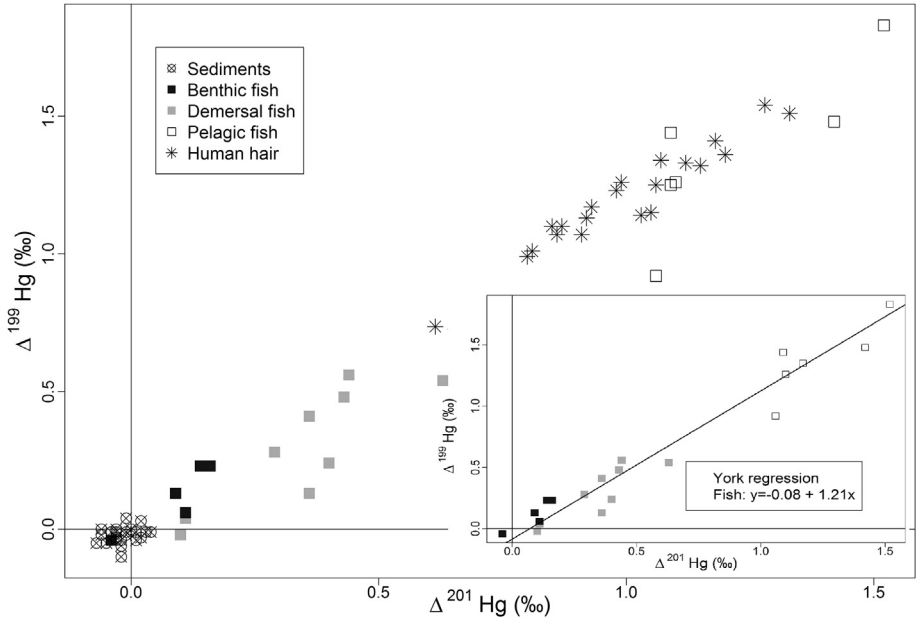

Fig. 3. Plot of Δ^{199}Hg vs. Δ^{201}Hg in sediments, fish and human hair. Calculated Δ^{199}Hg/Δ^{201}Hg York regression is 1.21 ± 0.8 (s.e.a = 0.10; s.e.b = 0.05, n = 20).

Table 3
Mercury concentrations and isotopic composition of human hair from Augusta area.

Human hair						
Uncertainty (2σ) = ± 0.11 for Hg ± 0.22 for δ^{202}Hg; ± 0.09 for Δ^{199}Hg; 0.16 for Δ^{201}Hg						
Gender	Local seafood consumption*(times a week)*	Age	Hg (mg Kg^{-1})	δ^{202}Hg (‰)	Δ^{199}Hg (‰)	Δ^{201}Hg (‰)
Female	Rarely	35	0.45	1.96	1.13	0.92
Female	Rarely	40	2.32	1.79	1.54	1.28
Female	Rarely	40	1.20	1.84	1.41	1.18
Female	1–2	35	0.72	1.12	1.10	0.85
Male	1–2	40	2.60	1.82	1.33	1.12
Female	1–2	40	1.37	1.46	0.99	0.80
Female	1–2	30	1.34	1.90	1.07	0.91
Female	1–2	30	1.46	2.10	1.10	0.87
Male	1–2	35	3.06	1.72	1.34	1.07
Female	1–2	40	3.43	2.06	1.32	1.15
Female	1–2	40	3.42	1.15	1.07	0.86
Female	1–2	40	1.49	1.46	1.01	0.81
Female	1–2	40	1.47	1.86	1.15	1.05
Male	1–2	40	1.83	1.71	1.25	1.06
Male	1–2	40	1.42	1.59	1.36	1.20
Male	1–2	40	1.97	1.30	0.73	0.64
Male	1–2	40	2.74	1.51	1.23	0.98
Male	1–2	40	1.28	1.58	1.14	1.03
Male	>3	40	5.07	1.80	1.26	0.99
Male	>3	40	5.28	1.87	1.17	0.93
Male	>3	40	4.81	3.36	1.51	1.33

frequency of seafood consumption ($\rho = 0.63$) definitively supporting the hypothesis that seafood consumption from AB represents the main source of Hg for human.

3.2.1. Isotopic composition of human hair

Human hair samples showed positive MDF (mean δ^{202}Hg 1.76 ± 0.46‰; [1SD; n = 21]) (Table 3; Fig. 2) without any evident difference among age and gender ($p > 0.05$; KW). Interestingly, the average δ^{202}Hg of native individuals' hair resulted ~2.2‰ higher with respect to the consumed fish. The enrichment of heavier Hg isotopes in human hair has been generally ascribed to the MeHg metabolism within the human body, where lighter isotopes are preferentially demethylated in the gastrointestinal tract (Rowland et al., 1988) or in other tissues such as hair follicles, blood cells (Berglund et al., 2005), liver and kidney (Suda and Hirayama, 1992) and excreted in urine and feces (Sherman and Blum, 2013). The δ^{202}Hg offset between fish and consumers is in good agreement with those reported for a Bolivian indigenous population (offset 2.0‰) (Laffont et al., 2009), a French urban population (offset 2.2‰) (Laffont et al., 2011), a North American dentists group

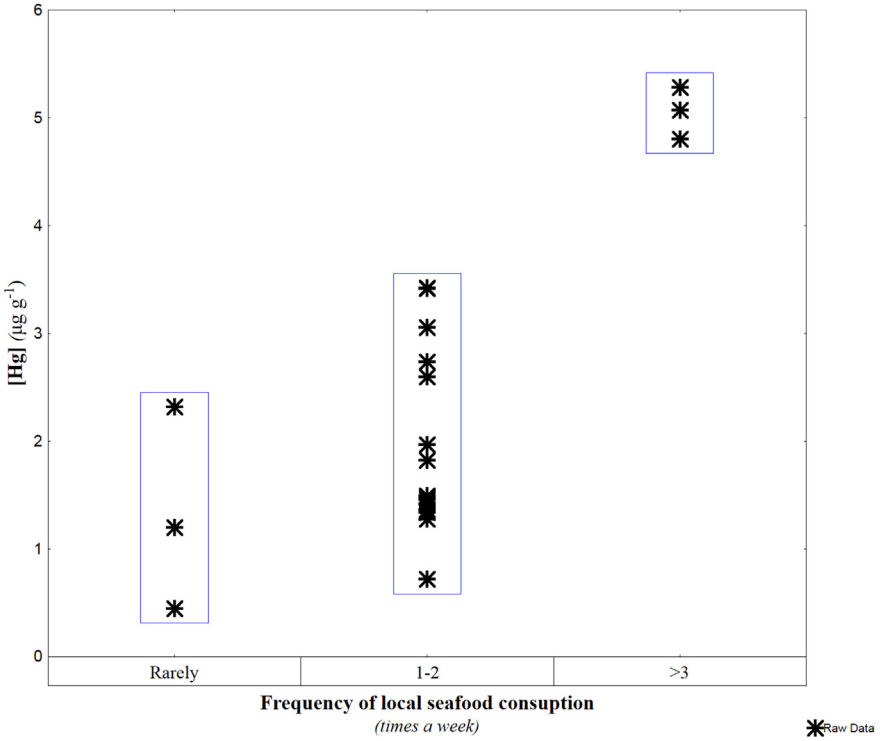

Fig. 4. Total mercury concentration in human hair of the Augusta population as a function of the weekly frequency of local fish consumption.

(offset = 1.9‰) (Sherman et al., 2013) and the Faroese whalers (offset 1.7‰) (Li et al., 2014). Hence, our results would seem to imply that consumption of seafood from AB represents the primary Hg exposure pathway for local population. In addition, results encourage the application of a +2.0 ± 0.2‰ correction to human hair δ^{202}Hg values to find the average dietary mercury isotopic signature in future studies of human exposure to MeHg, since MDF offset variations in hair likely depend only on the efficiencies of demethylation pathways in the human body (Li et al., 2014). Furthermore human hair samples from AB area exhibited high positive MIF values (mean Δ^{199}Hg 1.20 ± 0.19‰ [1SD; n = 21]; median 1.17‰) (Fig. 3) that are similar to those previously measured in hair samples from Northern Europeans (Laffont et al., 2011) and North American dental professionals (Sherman et al., 2013). Since MIF is not expected to occur in the absence of sunlight (e.g., within organisms) or during microbial processes (Kritee et al., 2007, 2009, 2010, 2013), the high MIF observed in human hair can be been interpreted as directly inherited by seafood consumption. Crucial differences were observed between Δ^{199}Hg values of human hair and benthic/demersal fish ($p > 0.05$; Wilcoxon) and interesting similarity emerged with respect to pelagic fish ($p = 0.11$; KW). This suggests that MIF was essentially preserved between human and pelagic compartments. Taking into account that nowadays the area is submitted to a ban that prevents fishing in the inner AB, it is not surprising that the majority of the local fish consumed comes from the external area, thus justifying the similarity in MIF with species collected outside the bay although consumption of fish from inside the AB cannot be excluded.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.05.033.

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