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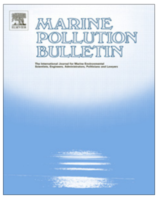

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Mercury (Hg) and methyl mercury (MeHg) concentrations in fish from the coastal lagoon of Orbetello, central Italy

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ABSTRACT

Total mercury (Hg_{tot}) and methyl mercury (MeHg) were quantified in several specimens of *Dicentrarchus labrax* and *Sparus aurata* from the east basin of the Orbetello lagoon, central Italy. The size of each specimen was recorded to estimate body burdens (BBs); $=Hg_{tot}$ and MeHg were measured in fillets of both species. Hg_{tot} and MeHg in *S. aurata* ranged between 0.355–1.58 and 0.341–1.53 µg/g wet weight (ww), respectively; in *D. labrax*, their ranges were 0.284–2.54 and 0.214–2.35 µg/g ww. Approximately 90% of the concentrations measured exceeded Hg_{tot} regulatory maximum level of 0.5 µg/g ww; however, exceedance rate was different in the two species studied. No correlations between specimen size and Hg_{tot} or MeHg BBs were detected in this study.

Mercury (Hg) contamination is still an environmental and public health concern in both developed and developing countries. International concern regarding this chemical is highlighted by the decision taken by the United Nations Environment Programme (UNEP) in 2009 to negotiate an international legally-binding instrument for the global control of Hg pollution to be signed in 2013. The European Union (EU) legislative framework focuses on two key aspects of protection of the environment and human health. Regarding the environment, Hg and Hg compounds are classified as priority hazardous substances that should be eliminated from all discharges, emissions, and losses to protect surface water bodies. Specifically, EU Directive 2008/105/CE lays down an environmental quality standard (EQS) of 20 µg/kg wet weight (ww) for Hg and Hg compounds in fish and biota, to be implemented by 2015, as a means to achieve a good chemical status for all European surface water bodies. Regarding food contamination, Regulation 1881/2006/EC defined two species-specific maximum levels (0.5 and 1.0 µg/g ww) for total mercury (Hg_{tot}) in marketed sea food.

For its toxicological properties, possible presence in food, and transfer to humans, methyl mercury (MeHg) is the most important form of the metal: in particular, fish and aquatic biota are the predominant foods involved in human exposure. The well-known adverse effects of MeHg on human health span from subtle neurological syndromes (intelligence quotient deficits, decrement in motor and attention performance, etc.) observed in toddlers exposed to low chronic levels *in utero* (National Research Council, 2000) to fatalities and devastating neurological damage at the highest exposures experienced in the Minamata and Iraqi episodes (Choi and Grandjean, 2012). The foetus is considered to be the most sensitive stage of life, although some recent studies suggested that the cardiovascular system is also a significant target (National Research Council, 2000; Guallar et al., 2002; Virtanen et al., 2005).

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Among aquatic environments, coastal lagoons may be particularly at risk for MeHg production (Rothenberg et al., 2008). Indeed, the chemical is naturally produced in lagoon sediments at a higher rate than in other aquatic environments (Holmes and Lean, 2006). Conditions that affect methylation include Hg concentration, ambient temperature, level of dissolved oxygen, amount of organic matter, sulphate concentration, and acidity (Driscoll et al., 2007). Additionally, most of these factors are linked to changes in surface water bodies caused, for example, by climate changes (Carere et al., 2011). In this context, the Orbetello lagoon in central Italy—a salted coastal formation comprising two communicating basins (east and west basins)—is of great interest on account of its use for farming fish of substantial commercial value, the European seabass and the Gilthead seabream. The basins cover areas of 10.00 and 15.25 km^2, respectively, and are classified as a "national reclamation site" by the Italian legislation (Legislative Decree of the Ministry of the Environment 152/2006) due to a prolonged presence of mining activities and industrial installations which caused substantial contamination by heavy metals and organic pollutants (ICRAM-Università di Siena, 2008). The former mining activities are of great concern as one of the main risk factors for Hg release into the environment is runoff from abandoned cinnabar mines and there is one such mine just above the east basin. Additionally, the area is not far from one of the most important global sources of cinnabar, namely Monte Amiata (Tuscany).

The principal aim of this study was to investigate the levels of Hg_{tot} and MeHg in specimens from the east basin of the two free-ranging species of significant commercial interest *Dicentrarchus labrax* (Gilthead seabream) and *Sparus aurata* (European seabass), to check their compliance with current regulatory values (0.5 μg/g ww; Regulation 1881/2006/EC) and gather enough occurrence data for a possible appraisal of the health risks for human beings.

Twenty-seven live specimens of European seabass and Gilthead seabream were collected in December 2010 and February 2011 from the Consortium managing the fishing activities in the Orbetello. Fish were collected under the supervision of the laboratories in charge of the study (Chemical Toxicology Unit, Department of the Environment and Primary Intervention, Istituto Superiore di Sanità (Roma) and Laboratory of Analytical Chemistry, Istituto Zooprofilattico Sperimentale (Roma)). Specimens were immediately frozen with dry ice and transported to the laboratory where they were allowed to thaw out at room temperature (De Felip et al., 2005); the length and weight of each specimen were recorded. Fillets were excised from fish bodies as prescribed by Regulation 333/2007/EC; the same procedure was adopted regardless of fish size. To obtain an analytical sample, only polytetrafluroethylene (PTFE) acid cleaned vessels and high quality stainless steel cutting edges were adopted. From each specimen two analytical samples were obtained for independent determinations of Hg_{tot} and MeHg. Samples were then stored separately at −20 °C until pre-treatment.

Total Hg concentrations were determined using the cold vapour direct mercury analyzer (DMA 80; Milestones SrL). This technique employs a sequence of thermal decomposition, gold amalgamation and atomic absorption spectrophotometry. To evaluate the accuracy and precision of the analytical methodology, a certified standard reference material (SRM) (1566b-Oyster tissue) was run in parallel with the samples. Measured and certified values did not differ significantly at 95% confidence level. All the results reported in this study have a relative standard deviation less than |±7%|.

MeHg concentrations were determined by a gas chromatography–mass spectrometry procedure (Ipoly et al., 2004). One hundred fifty μL of a 10-μg/mL ethyl mercury chloride methanol solution (internal standard) were added to approximately 0.5 g ww of homogenised sample. The fortified matrix was extracted with 5 mL of a 6-M hydrochloric acid solution (sonication for 30 min). After cooling, 10 mL of a 10% (w/v) sodium chloride solution was added to the sample, shaken for three minutes, and centrifuged at 2400 rpm for 10 min. The supernatant was transferred to a screw-capped conical centrifuge tube and extracted twice with 4-mL toluene. The total organic extract (≈8 mL) was subjected twice to back-extraction, each time with 2 mL of a 1% (w/v) (L)-cysteine-aqueous solution. The derivatization reaction was carried out on the collected cysteinic solution (≈4 mL): 0.2 mL of a 0.1-M sulfuric acid solution, 1.0 mL of a saturated copper sulfate solution, 0.2 mL of a 1% sodium tetrapropyl borate solution, and 1.0 mL of *n*-hexane were added and vigorously shaken for 30 min. The organic upper layer was analyzed by high resolution gas chromatography–tandem mass spectrometry (HRGC–MS/MS) in the simultaneous scan and single-ion monitoring mode (SCAN/SIM). An internal standard of ethyl mercury was used for the determination of MeHg concentrations. Aliquots of certified reference material CRM 463 (tuna fish) were run in parallel with the samples. Ten separate analysis of this CRM gave a trueness of 104% and a relative standard deviation of 2.2% (Commission Decision 2002/657/EC).

Neither Hg_{tot} nor MeHg concentrations in the samples analyzed were normally distributed (Kolmogorov–Smirnov normality test, $P < 0.05$). All statistical analyses were conducted with Statgraphics 16.1 for Windows (StatPoint Technologies, Inc.). Data were evaluated by checking their frequency distribution features and the relationship between Hg_{tot} and MeHg levels. To characterize the factors potentially influencing body burdens (BBs), the relationships between chemical concentrations and the fish organism in terms of weight and standard length were studied.

The Hg_{tot} and MeHg concentrations determined in European seabass and Gilthead seabream samples ($N = 27$ and 27, respectively) are shown in Fig. 1. The statistical descriptors of Hg_{tot} and MeHg occurrence values in the two species analyzed are reported in Table 1 while Table 2 summarizes those concerning weight and length data. The two Hg_{tot} and MeHg data sets were not normal distributed (Shapiro and Wilk's W statistics, $P < 0.05$); however, the log-transforming of the European seabass dataset confirmed a *P*-value greater than 0.05, and the hypothesis that the data were from a normally distributed population was not rejected in these cases. The Gilthead seabream did not confirm it. The same evaluations on body characteristics, on the contrary, shoved a clear normal distribution (Shapiro and Wilk's W statistics, $P > 0.05$) such as expected.

Of European seabass samples, only OR-03 exhibits a Hg_{tot} concentration below the pertinent maximum level of 0.5 μg/g ww all the other samples exceed it by a factor of 1.4–3.2, Hg_{tot} and MeHg levels ranging respectively between 0.355–1.58 and 0.341–1.53 μg/g ww. The Hg_{tot} mean of 1.14 μg/g ww is approximately 10 times higher than the European average of 0.109 μg/g ww (EFSA, 2004) and six times higher than that of fish collected from the Mediterranean area (di Domenico et al., 2011; Miniero et al., 2013). Thus, all the European seabass specimens potentially collectable from the Orbetello east lagoon at the time of sampling would be characterized by a Hg_{tot} occurrence exceeding the regulatory level with a <95% confidence level. MeHg levels exhibit the same distribution type with a mean of 0.971 μg/g ww, which is the 85% of Hg_{tot} mean estimate. As to seabass, samples SP-04, -07, -11, -13, -16, -19, -23, and -27 exhibit Hg concentrations below the pertinent maximum level of 0.5 μg/g whereas all the others exceed it by a factor of approximately 1.1–4.6 (Fig. 1). Notwithstanding its different position in the food chain—Gilthead seabream' trophic level is higher than that of European seabass—the number of samples of this species showing levels below the regulatory value is larger than that of European seabass.

As to the data density distributions, the 27 data represented in Fig. 2c and d suggest the underlying presence of two populations, the first comprising data below the regulatory level, the second including only values above it. This difference, apparently not