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Bioaccumulation of mercury in reared and wild *Ruditapes philippinarum* of a Mediterranean lagoon

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ABSTRACT

The Marano and Grado lagoon, one of the largest wetlands in the Mediterranean Sea, has been subject to mercury contamination by industrial and mining activities. This must be considered a severe threat for Manila clam harvesting, which is an important fishing and commercial activity in the area. Contamination levels and potential risk for human consumption both in reared and wild clams collected from the lagoon were assessed by analyzing total mercury (THg) and methylmercury (MeHg) contents. In addition, relationships between THg and MeHg in sediments and in the bivalves were investigated. Increased bioaccumulation of THg but not of MeHg with increasing size of wild clam populations was observed at most sites. Higher concentrations both of THg (605 ± 210 ng g^{-1} ww) and MeHg (147 ± 37 ng g^{-1} ww) were detected in the eastern lagoon where the highest THg contents in sediments were observed as a consequence of the long-term supply of cinnabar rich suspended material from the Isonzo river.

The variation of Hg content in seeded Manila clams during growth was monitored over a period of 18 months at two sites of the western sector of the lagoon. Results showed that the two areas were suitable for clam farming, with THg levels in reared bivalves always lower than the 0.5 mg kg^{-1} ww European Community limit. At the same time, as clams grew bigger in size, their THg and MeHg concentrations decreased, becoming lower than in the starting seeded pool. Reared clams presented lower THg (84 ± 55 ng g^{-1} ww) and MeHg (44.1 ± 24.6 ng g^{-1} ww) content than wild clams of the same commercial size (>30 mm). Based on a precautionary approach, intake of Hg and MeHg with the estimated clam consumption does not seem to constitute a risk for human health in the studied area.

1. Introduction

Mercury contamination levels in coastal sediments of the northern Adriatic Sea represent some of the highest concentrations worldwide (Fitzgerald et al., 2007), as a direct consequence of anthropogenic inputs from very extensive industrial (Fabbri et al., 1998; Covelli et al., 2009) and mining (Horvat et al., 1999; Covelli et al., 2001) activities. The Marano and Grado lagoon, in particular, has been subject to a double source of contamination: first, the inflow of cinnabar (HgS)-rich particulate matter from the Isonzo river, draining waters from the former Idrija (Slovenia) Hg mine (Horvat et al., 1999; Hines et al., 2000), and second, uncontrolled Hg discharges from the Torviscosa chlor-alkali plant (Piani et al., 2005). The Idrija Hg mine operated for about 500 years until 1994, when the extraction activity definitely ceased (Faganeli et al., 2003). On the other hand, the Torviscosa chlor-alkali plant, located on the Aussa–Corno river drainage basin, discharged Hg from 1949 to 1984, when cleaner technologies were reportedly adopted (Piani et al., 2005).

Hg transfer to biota, especially in contaminated areas, constitutes an important environmental issue. MeHg, which forms *in situ* in Hg-contaminated marine environments when suitable conditions occur (Ullrich et al., 2001), is a potent neurotoxin that affects the development and health of wildlife and humans (Selin, 2009). Moreover, the consumption of marine fish and shellfish from the coastal zone is one of the principal routes of human exposure to MeHg (Fitzgerald et al., 2007) and poses a risk for populations where fish is consumed on a regular basis (Clarkson, 2002).

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Bivalves are known to bioaccumulate Hg and MeHg (e.g., Bryan and Langston, 1992) and are used as bioindicators in the environmental monitoring of marine coastal waters (e.g., Giordano et al., 1991; O'Connor, 2002; Kliaković-Gašpić et al., 2006). Although mussels are the most commonly used bioindicators in coastal environments, other bivalves such as oysters and clams can be employed in brackish waters (e.g., Claisse et al., 2001; Liang et al., 2003; Baudrimont et al., 2005). *Ruditapes philippinarum* (the Manila clam) is a filter-feeder organism that lives buried in the sediment (Sorokin and Giovanardi, 1995; Sfriso et al., 2008) and can represent a key indicator organism to test Hg bioaccumulation in contaminated coastal environments.

The Manila clam was introduced in the northern Adriatic in 1983 as a commercially exploitable resource in the Venice lagoon. Afterward, this species spread to nearby coastal areas such as the Marano and Grado lagoon (Zentilin et al., 2007) where rearing activities began in the period between 1985 and 1986 (Pellizzato and Da Ros, 2005); nowadays, it constitutes an important portion of the total clam production in the northern Adriatic Sea (Zentilin et al., 2008).

The aim of this paper is to assess Hg and MeHg bioaccumulation in Manila clams of both wild and *ad hoc* seeded populations in selected areas of the Marano and Grado lagoon, characterized by different levels of sediment contamination and to evaluate the potential risks for human health deriving from clam consumption.

2. Environmental setting

The Marano and Grado lagoon (Fig. 1) is one of the largest wetlands in the Mediterranean Sea and the second largest in Italy, after Venice. It spreads over an area of about 160 km^2, with a total length of 32 km, between the Isonzo and Tagliamento river deltas, and a width of ~5 km between the inner coastline and the barrier islands.

Water exchange with the sea occurs through six tidal inlets, while riverine discharge into the lagoon has been estimated to be about 100 m^3 s^{-1} of which the major part inflows in the western sector and is primarily due to the Stella river (50 m^3 s^{-1}), with minor contributions from the Corno (5–6 m^3 s^{-1}) and Aussa (7–8 m^3 s^{-1}) rivers (ARPA FVG, 2005). As a consequence, the highest input of river-borne nutrients occurs in the western sector, near the Stella river mouth (ARPA FVG, 2005), where the average salinity reported for 2003–2005 was 22.9 (range: 0.0–38.0). In the eastern sector of the lagoon, the freshwater contribution is limited and the waters have much higher salinity values (average: 29.1, range: 0.0–38.3, period: 2003–2005).

Manila clam harvesting for commercial purposes is allowed in some areas of the western sector of the lagoon (Marano lagoon). In the central part, the sediments are contaminated by industrial wastes from inland, and the area has been declared a Contaminated Site of National Relevance (Ministerial Decree 468/01). Thus, clam collection, farming and fishing activities are currently prohibited.

3. Materials and methods

3.1. Experimental sampling design

In order to evaluate total mercury (THg) and MeHg bioaccumulation in seeded Manila clams, four experimental stations were selected (Fig. 1). MA site was located in a sandy area close to the Lignano tidal inlet where commercial clam farming is currently active. MB site was also located in the western sector, near the Stella river mouth, characterized by nutrient-rich freshwater inputs, inside a candidate area for the extension of clam rearing activities. Another site, MC, was selected inside the interdicted area, close to the Aussa–Corno river mouth, where clam farming is currently forbidden. Finally, a site was chosen in the eastern Grado sector, GD, inside an area reserved for clam farming, which is currently not in use.

Manila clam seeds were supplied by a local hatchery (Marano). Individuals 18.0 ± 1.6 mm in size were seeded on May 22, 2008 in each of the four selected areas (25 m^2 at MB and MC sites; 75 m^2 at MA and GD sites), with a final population density of 200 ind. m^{-2}. A subsample of 100 individuals from each site was analyzed for biometric parameters and THg and MeHg contents at $t = 0$ after depurating for 48 h in 0.4 μm filtered lagoon water.

Seeded clams were collected manually or by means of a clam rake every 45 days, for a total period of 18 months. For each of the 10 samplings, 50 individuals were collected.

In six selected areas of the lagoon, wild clams were also collected (Fig. 1): three sites were located in the western sector (Marano lagoon: MC, M2, M4) and three sites in the eastern sector (Grado lagoon: IGC, VAS and BAR), following the general THg concentration gradient in the surface sediments (Acquavita et al., 2012). Sampling was carried out in winter (March 2009) and

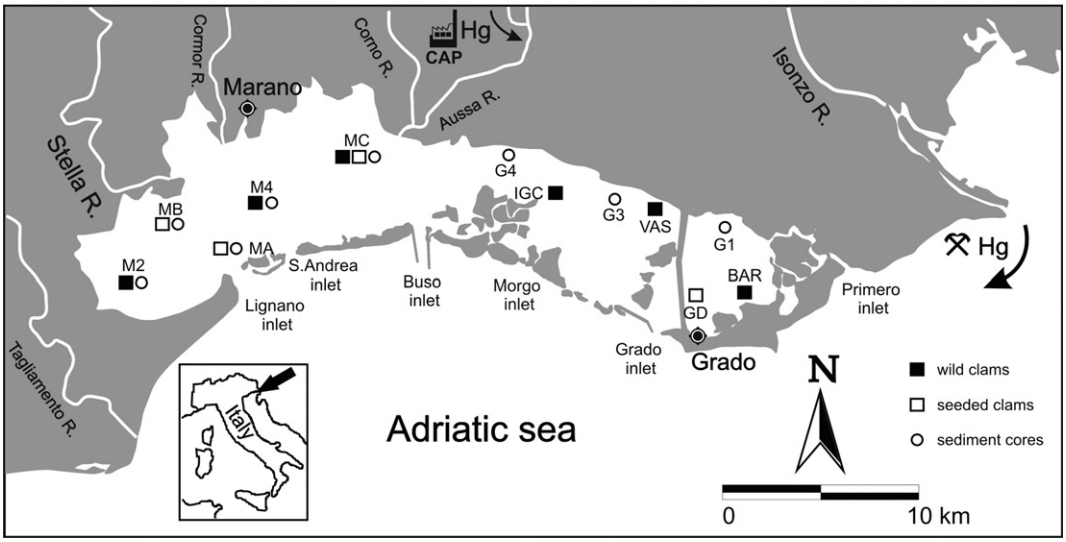

Fig. 1. Sampling sites in the Grado and Marano lagoon. Chlor-alkali plant (CAP) on the river Aussa and the Idrjia mercury mine inputs through the Isonzo river are shown.

summer (July 2008; BAR in July 2009) by trawling and/or manual collection, depending on water depth.

After collection, both seeded and wild clams were weighed and measured (length, width and thickness) with a gauge, and allowed to depurate for 48 h with 0.4 μm filtered lagoon water.

Wild clams were sorted by size, measured, opened and the intra-shell water was drained. The soft tissues of 10 individuals per class size (fewer clams were sampled and analyzed in the larger class sizes ≥45 mm) were homogenized in a HNO_3 cleaned glass container by an Ultra Turrax T 25 homogenizer. Subsamples (0.3 g ww (wet weight)) were frozen in Eppendorf HNO_3 precleaned vials for subsequent THg and MeHg determinations. The class sizes were chosen by sorting the collected clams into the following groups: 20–25, 25–30, 30–35, 35–40, 40–45, 45–50 and 50–55 mm, on the basis of the shell length. The same procedure was adopted for unsorted seeded clams collected in each sampling. The determination of THg and MeHg was carried out on three subsamples of the homogenate.

In addition, sediments were collected by hand corer (PVC liners, 10 cm diameter) in 16 sites of the lagoon, as detailed by Covelli et al. (2012). Of these, only the cores collected in correspondence to (M2, M4, MC, MA and MB) or proximity of (G1, G3, G4) the clam collection sites were considered in the present study (Fig. 1). Core subsampling was performed by cutting 1-cm-thick slices at several depths. In the present paper, only results from surface (0–1 cm) and subsurface (5–6 cm) sedimentary levels were used, as they are representative of the average depth where clams live.

3.2. Determination of total mercury in clams

Total Hg (THg) determinations were carried out with a Direct Mercury Analyser (DMA-80, Milestone srl, Italy). The sample was dried and oxidized in a continuous flow of oxygen by controlled heating. Combustion products were further decomposed in a hot catalyst bed and halogens and nitrogen/sulphur oxides were trapped. Mercury vapor was trapped on a gold amalgamator. Finally, Hg^0 was desorbed from the gold trap by heating to 700 °C and quantified by atomic emission spectroscopy using a Hg lamp ($\lambda = 253.7$ nm).

The accuracy of the method was evaluated with the Certified Reference Materials for marine bivalves SRM-2976 and SRM-2977 (National Institute of Standards & Technology). The recovery with respect to the certified values was >90%. The limit of quantification (LOQ) was 0.5 ng g^{-1} and the reproducibility better than 5%.

3.3. Determination of methylmercury in clams

For the determination of MeHg, approximately 400 mg of the sample was weighed directly into a 30 mL screw capped Teflon vial. After the addition of 2.5 mL of 4 M H_2SO_4 saturated with $CuSO_4$, 2.5 mL of 4 M KBr and 2.5 mL of toluene, the vial was closed and shaken for 10 min. The sample was then centrifuged for 10 min at 3200 rpm. The organic phase was removed with a glass Pasteur pipette and the extraction repeated with another 2.5 mL of toluene. The clean-up step was done by back-extraction with the addition of 3 mL of 1% aqueous cysteine solution in 20% Na-citrate to the collected joint toluene fractions. Samples were shaken for 10 min and then centrifuged at 3200 rpm for 10 min. 2.5 mL of cysteine solution were separated, acidified with 1 mL of 4 M H_2SO_4 saturated with $CuSO_4$ and 1 mL of 4 M KBr and MeHg extracted into 0.5 mL of toluene. 2 μl of the extracted sample was injected onto a packed GC column and detected by GC-ECD. A complete description of the method is given in Horvat et al. (1990). The recovery, based on standard addition, was estimated to be 80%, and therefore the results were corrected for this recovery factor. A calibration solution was prepared from crystalline CH_3HgCl (Merck) in toluene as the organic solvent. The limit of detection mainly depends on the sample matrix and any interfering peaks during GC-ECD. Typically the LOD for this type of sample is 0.5 ng g^{-1}. The accuracy was checked by the analysis MeHg in the reference material IAEA-142 Mussel Homogenate. The results obtained (45 ± 2) were in excellent agreement with the reference value (47 ng g^{-1} expressed as Hg, with a 95% confidence interval from 43 to 51 ng g^{-1}, Horvat et al., 1997).

3.4. Sediment analyses

The analytical determinations on sediments are described in detail elsewhere (Acquavita et al., 2012). In summary, grain size analyses were performed using a laser granulometer (Malvern Instruments LtD, UK, mod. mastersizer 2000) after removal of organic matter with H_2O_2 for 48 h.

Organic carbon and nitrogen content were determined by a Perkin Elmer 2400 CHN Elemental Analyzer, after removal of carbonates by acidification.

Total Hg was determined by CVAAS (Perkin Elmer AAnalyst 100-FIAS) after total digestion of about 200 mg of sediment samples in a closed microwave system (Milestone MLS 1200). The detection limit of the method was 0.13 μg g^{-1}. MeHg in the sediments was extracted from about 300 mg of sample with $CHCl_2$, ethylated with $NaBEt_4$ and determined after pyrolitical conversion to Hg^0 by CVAFS. The detection limit of the method was 10 pg g^{-1}.

3.5. Statistical analyses

Statistical analyses were performed with the Statistica software package (version 6.0, Statsoft, USA). In order to test if Hg forms in the clams differed during growth between sites MA and MB, the Wilcoxon non-parametric test for paired samples was used. Two-way ANOVA was used to test the effect of station and season on MeHg in wild clams. MeHg and MeHg/Hg data were transformed by the logarithm of autoscaled values and by logarithmic scaling, respectively, in order to obtain a normal distribution (χ^2 test) and homogenize the variance (Levene test). For THg in clams, as the assumption of equal variance was not met (Levene test, $p < 0.05$), the Kruskal–Wallis non-parametric test was applied. All statistical results are reported as significant at a level of $p < 0.05$.

4. Results

4.1. Growth of seeded clams

At GD and MC, the whole population died shortly after seeding. The seeding was unsuccessfully repeated at both sites but the cause of death was impossible to ascertain. The death of seeded clams was probably due to the predation by gastropods and crabs, which are the main predators of juvenile clams in the Northern Adriatic lagoons (Paesanti, 2006; Sfriso et al., 2008), but we cannot exclude, especially for site MC, the role of unsuitable environmental conditions, such as sediment pollution caused by contaminant discharges in the Aussa–Corno river (MC is located near its mouth). The settlement and survival of clam larvae and juveniles may be inhibited at polluted sites (Bryan and Langston, 1992 and references therein).

Seeded clams grew less at site MA than at site MB. The mean shell lengths after 18 months were 32.1 ± 2.9 mm and 37.1 ± 3.5 mm, at site MA and MB, respectively. The growth curves of the seeded clams (Fig. 2) showed a slowing down of clam growth during winter, when the lowest temperatures are reached. Moreover, it is evident that clams grew faster in the inner lagoon, at MB

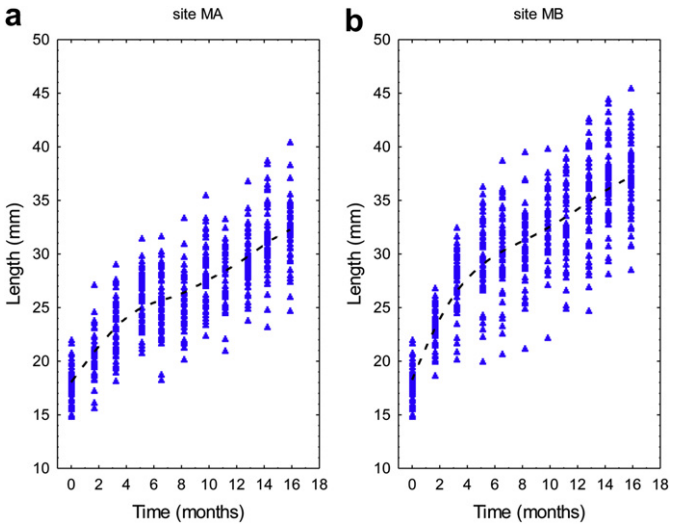

Fig. 2. Growth curves of *Ruditapes philippinarum* seeded at MA and MB sites in the Marano lagoon on May 2008.

site, near the Stella river mouth, compared to the MA site, which is close to the Lignano inlet. The Wilcoxon test for paired samples showed that the shell length at the two sites during growth was significantly different ($p \leq 0.005$).

4.2. Mercury and methylmercury in seeded clams

A decrease during clam growth for MeHg content was evident at MA site, whereas THg content was variable (Fig. 3a). THg and MeHg contents showed a decreasing trend with increasing clam size, particularly at site MB (Fig. 3b), compared to the initial concentration in the seed (114 ± 9 and 113.2 ± 7.4 ng g^{-1} ww for THg and MeHg, respectively). At both sites, the MeHg/THg ratio decreased during clam growth, from about 100% in the seed to 35–38% when the clams reached an average length of 32–33 mm (Fig. 3c,d).

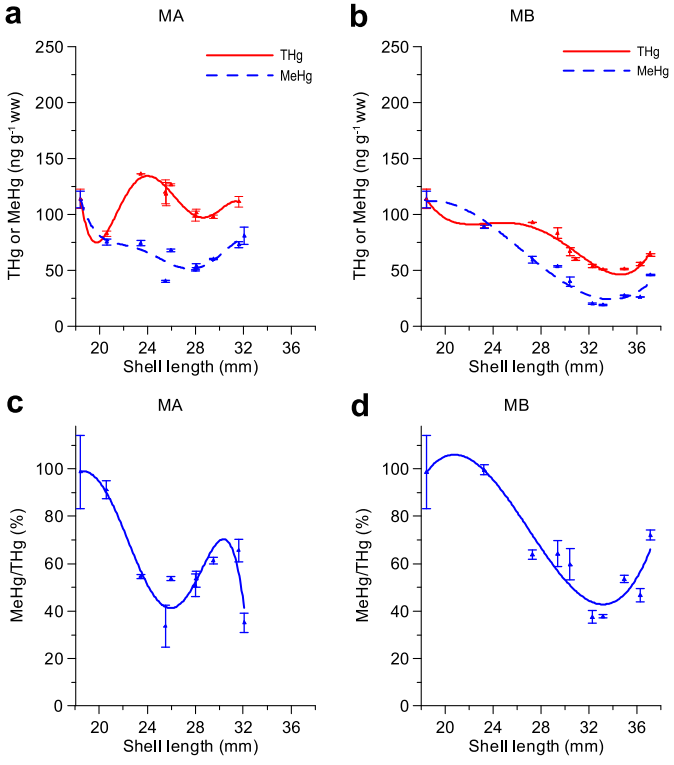

Fig. 3. Total mercury (THg) and methylmercury (MeHg) content in seeded clams as a function of size at (a) MA and (b) MB sites and MeHg/THg percentage as a function of size at (c) MA and (d) MB sites. Mean values and standard deviations are represented.

The average THg and MeHg contents in seeded clams, considering all the samplings, were higher at MA (111 ± 16 ng g^{-1} and 69.0 ± 20.1 ng g^{-1} ww for THg and MeHg, respectively) than at MB (71 ± 21 ng g^{-1} and 49.4 ± 31.0 ng g^{-1} ww for THg and MeHg, respectively). The Wilcoxon test for paired samples showed that the differences between the two stations were significant both for THg ($p = 0.011$) and MeHg ($p = 0.015$), but not for the MeHg/THg ratio ($p = 0.678$) as the concentrations of both mercury forms decreased from MA to MB site.

The mean concentration in the reared clams commercial size (>30 mm) were 84 ± 55 ng g^{-1} ww and 44.1 ± 24.6 ng g^{-1} ww for THg and MeHg, respectively.

4.3. Mercury and methylmercury in wild clams

Unlike seeded clams, Hg content in wild clams increased with increasing age. The highest Hg contents (up to 985 ng g^{-1} ww) were found in clams collected in the eastern part of the lagoon (sites: IGC, VAS and BAR, Fig. 4), where sediments are more contaminated by Hg (Acquavita et al., 2012). Here, clams of the larger size classes (>40 mm) showed average Hg contents of 605 ± 210 ng g^{-1} ww, exceeding the maximum concentration allowed by European regulation (0.5 μg g^{-1} ww; EC Reg. No 1881/2006). On the other hand, wild clams collected in the western part of the lagoon (M2, M4 and MC sites, Fig. 4) showed average THg contents of 176 ± 111 ng g^{-1} ww, markedly lower than the EC threshold.

A significant linear relationship ($p < 0.001$) was observed between THg and MeHg content in wild clams both in summer and winter (Fig. 5). However, MeHg did not show an increase with size (age) of the clams (Fig. 6), as conversely observed for THg (Fig. 4).

Moreover, the MeHg content was higher in the clams collected in summer, compared to the winter sampling (Fig. 6) as evidenced by the Wilcoxon test on paired samples ($p < 0.005$). On the other hand, THg content was generally higher (Wilcoxon test on paired samples, $p < 0.001$) in the winter sampling (Fig. 4). Thus, the average MeHg/THg ratio was higher in summer at all sites.

The percentage of Hg as MeHg in the clam soft tissue decreased with the increase of clam size at all sites, more markedly in summer (Fig. 7). The MeHg/THg ratio ranged from >90% in the 20–30 mm class size in the western lagoon to about 20% in the >40 mm class size in the eastern lagoon.

In order to investigate the possible influence of sampling site location and season on Hg contents, two-way ANOVA was applied to the dataset for the 30–45 mm size, which is the most representative class size within the clam populations collected at all stations. No effect was observed on clam length, whereas both the above-mentioned parameters influenced MeHg content and the MeHg/THg ratio (Table 1). THg concentration in wild clams was found to be influenced by location (Kruskal–Wallis test: $H(5, 64) = 42.98$, $p < 0.001$) but not by the season (Kruskal–Wallis test: $H(1, 64) = 0.8287$, $p = 0.363$).

4.4. Relationships between mercury contents in sediments and clams

Hg and MeHg contents, as well as other ancillary parameters of sediments (C_{org}, N_{tot}, grain size), were considered as average values obtained from the two levels analyzed (0–1 and 5–6 cm depth, Table 2). The top 6 cm of the sediments should reflect the average characteristics of the living environment of clams. Surface sediments in the lagoon showed an increasing gradient from west to

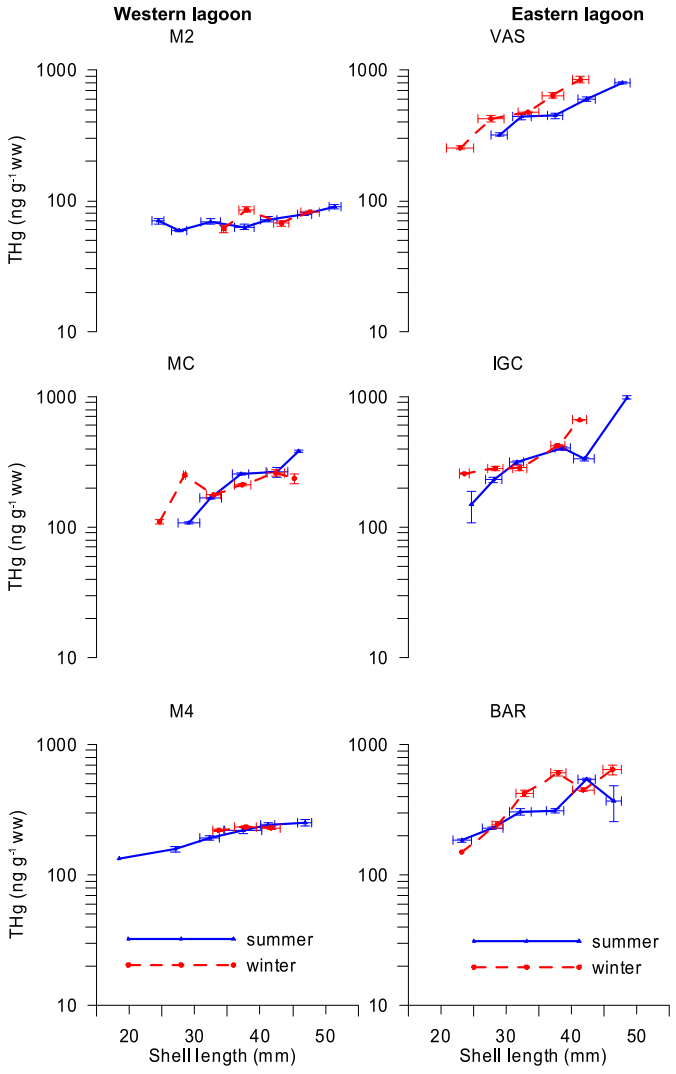

Fig. 4. Total mercury (THg) content in soft tissue of wild clams as a function of the class size (shell length) during summer and winter at 6 sites in the Marano (western) and Grado (eastern) lagoon. Mean values and standard deviations are represented.

east for Hg concentrations (ranging from 1.2 up to 10.5 $\mu g\,g^{-1}$ dry weight; Table 2). Conversely, MeHg (0.9–4.5 $ng\,g^{-1}$, dry weight) showed no discernible trend and constituted, on average, 0.07% of total Hg.

A significant correlation ($n = 8$, $r = 0.739$, $p < 0.05$) between THg content in the sediment and in clams was found only for the 30–35 mm size class. Furthermore a significant linear relationship between MeHg content in sediments and in clams was observed, both in the 30–35 mm ($n = 5$, $r = 0.957$, $p = 0.011$) and in the 35–40 mm ($n = 4$, $r = 0.983$, $p = 0.017$) size classes, if the VAS station (and G3 for the sediment) is excluded. Similar correlations between Hg and MeHg in sediments and in biota were reported by Trombini et al. (2003) in the northern Adriatic coastal lagoon near Ravenna (Pialassa Baiona).

4.5. Human health risk through clam consumption

The provisional tolerable weekly intake (PTWI) reported by the FAO/WHO Expert Committee On Food Additives (FAO/WHO, 2003) is equal to 5.0 and 1.6 $\mu g\,kg^{-1}$ body weight $week^{-1}$ for Hg and MeHg, respectively.

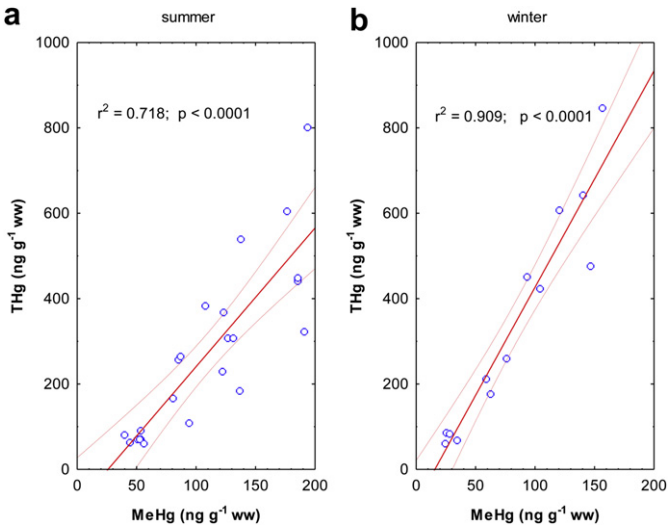

Fig. 5. Scatter plots of total mercury (THg) versus methylmercury (MeHg) content in wild clams during (a) summer and (b) winter. The data represent the average of three replicates on the homogenate pools for each class size analyzed, lines represent linear regressions and the 95% confidence bands.

In the North Eastern part of Italy the average consumption of seafood in the period 1994–1996 was 33.5 $g\,day^{-1}\,person^{-1}$ (Turrini and Sermoneta, 2005). However, considering that the general trends of fish consumption in Europe (EU-15) increased by 13.5% in the period 1994–2010 (Failler, 2007), an average fish consumption of 37.0 g ww $day^{-1}\,person^{-1}$ (Welch et al., 2002) provides a more realistic estimate of the actual seafood consumption in Italy. Consequently this value was applied in the calculations. For the purpose of this study, we assumed that all seafood

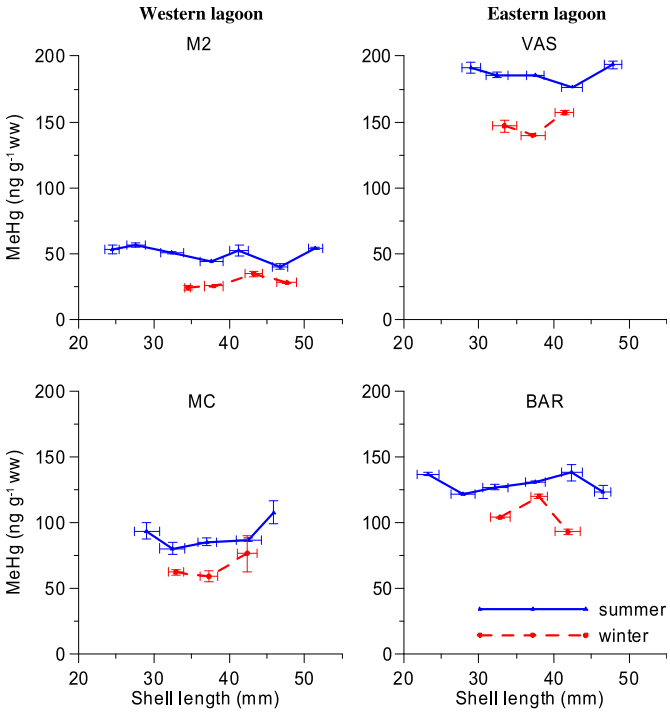

Fig. 6. Methylmercury (MeHg) content in soft tissues of wild clams as a function of class size (shell length) during summer and winter at 4 sites in the Marano (western) and Grado (eastern) lagoon. Mean values and standard deviations are represented.

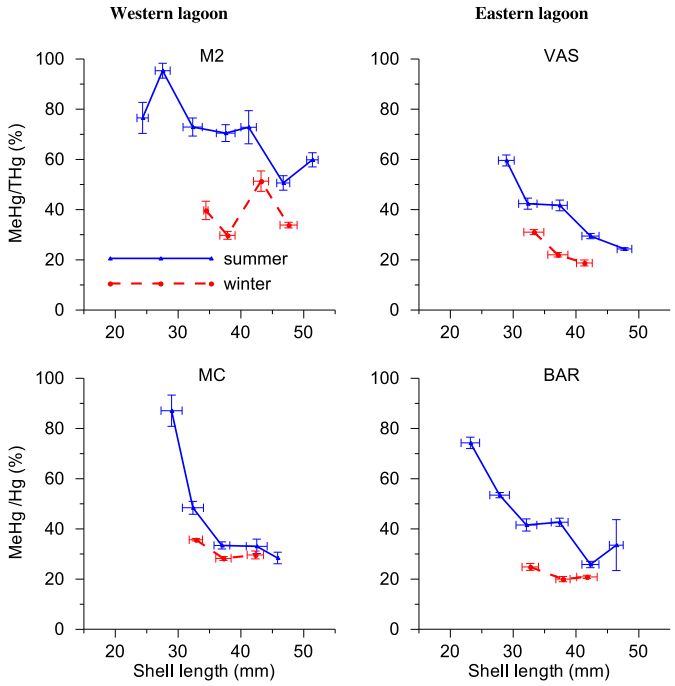

Fig. 7. Relative (%) methylmercury contents (MeHg) with respect to total mercury (THg) in soft tissues of wild clams as a function of class size (shell length) during summer and winter at four sites of the Marano (westerm) and Grado (eastern) lagoon. Mean values and standard deviations are represented.

consumed were constituted by clams. Even if this can be considered as an upper limit, this assumption provides a precautionary approach in order to estimate the risk for human consumption of clams.

The mean concentrations of THg and MeHg in clams for each size class in the most common commercial range (≥30 mm) at each sampling site were multiplied by the daily consumption per person (Table 3), to obtain the Estimated Daily Intake (EDI). Data were compared to the Tolerable Daily Intake (TDI) estimated by the Provisional Tolerable Weekly Intake (PTWI), considering an adult of 70 kg, for THg and MeHg (50 and 16 μg day^{-1} for Hg and MeHg, respectively) by means of the following Risk Quotients (Fung et al., 2004):

$$RQ = \frac{EDI}{TDI}$$

Table 1
Results of analysis of variance (two-way ANOVA).

	SS	df	MS	*F*	*p*
L					
Station	0.00100	3	0.00033	0.09	0.965
Season	0.00002	1	0.00002	0.00	0.947
Station × season	0.00488	3	0.00163	0.44	0.727
MeHg					
Station	0.04376	3	0.0146	378.1	0.000
Season	0.00289	1	0.0029	74.9	0.000
Station × season	0.00012	3	0.0000	1.0	0.401
MeHg/THg					
Station	0.3442	3	0.1147	13.662	0.000
Season	0.2223	1	0.2223	26.470	0.000
Station × season	0.0331	3	0.0110	1.313	0.299

Effects of station and season on shell length (L), MeHg concentrations and MeHg/THg ratio in wild clams (30–45 mm class size).
SS, sum of squares; MS, mean square; F, Fisher-Snedecor *F*-test value, *p*, significance value.

Table 2
Total mercury (THg) and methylmercury (MeHg) in sediments.

Station	Sand (%)	Silt (%)	Clay (%)	N_{tot} (%)	C_{org} (%)	C_{org}/N_{tot} (mol/mol)	THg (μg g^{-1} dw)	MeHg (ng g^{-1} dw)	MeHg/THg (%)
M2	16.8	75.2	8.0	0.2	1.4	7.8	2.2	1.1	0.05
M4	24.1	69.1	6.8	0.2	1.5	9.0	2.3	2.3	0.09
MC	20.3	74.0	5.7	0.1	1.0	9.4	4.5	2.2	0.05
G4	16.0	76.2	7.8	0.1	1.2	13.5	4.2	1.8	0.04
G3	12.7	77.3	10.0	0.2	1.7	14.9	5.6	0.9	0.02
G1	22.4	69.3	8.3	0.2	1.7	10.8	10.5	4.5	0.05
MA	65.5	30.9	3.6	0.1	0.6	9.5	2.6	3.0	0.14
MB	35.6	58.3	6.1	0.1	0.7	7.6	1.2	1.3	0.17

Mean values of the 0–1 and 5–6 cm levels are represented. Concentrations are expressed on dry weight (dw) basis.

The RQ values were always <1 (Table 3), meaning that the TDI was never exceeded. In a few cases, the RQ approached 1; at BAR and IGC sites, both located in the eastern part of the lagoon, but only if related to the 40–45 mm or 45–50 mm size classes. The RQ values of wild clams were 2–3-fold higher in the eastern sector of the lagoon (sites: BAR, VAS and IGC) than in the western sector (sites: MC, M4 and M2). The lowest RQ were found in reared clams.

5. Discussion

5.1. Bioaccumulation of mercury and methylmercury in wild and seeded clams

While active anthropogenic sources of Hg entering the lagoon have long ceased, studies have shown that the Idrija–Isonzo river system continues to supply Hg-rich sediments to this area (Horvat et al., 1999; Covelli et al., 2007) and, consequently, Hg contamination of biota is still a threat in the eastern part of the lagoon. Biomagnification through the trophic chain has been shown by Brambati (1996, 2001), displaying high Hg contents in the upper trophic levels, such as fish and birds. Clams in the larger size classes (>40 mm) from natural banks of the eastern basin showed elevated THg bioaccumulation (605 ± 210 ng g^{-1} ww). Similar Hg accumulation in clams of larger size (>40 mm) has been shown in Spanish coastal areas, where the EC threshold was exceeded in Manila clams (Usero et al., 1997). MeHg content is generally less than 50% of the Hg content, despite the fact that the lagoon has proven to be a favorable environment for *in situ* Hg methylation (Covelli et al., 2008; Emili et al., 2012; Hines et al., 2012).

Hg content in Manila clams determined in this study is high in comparison with other coastal areas in the Adriatic region and worldwide (Table 4). Sfriso et al. (2008), in the proximity of the MA site, found Hg contents in seeded Manila clams of commercial size (≥25 mm) within the range we obtained for samples collected in the western sector of the lagoon. Brambati (1996), in a previous study carried out in the same lagoon, reported Hg contents within a lower range, with maximum concentration values accounting for only one-third of those observed in this study, though a strict comparison is not possible as in their study the clam size was not reported (Table 4). However, it must be noted that the sampling sites of Brambati (1996) were located along the coastline in the proximity of the barrier islands, where sediments are usually richer in the sandy component and do not show high Hg contents. Compared to other transitional environments of the northern Adriatic Sea contaminated by industrial Hg, such as the Venice lagoon (Sfriso et al., 2008) and Pialassa Baiona (Trombini et al., 2003), our results for Hg contents are far higher.

Unfortunately, in the aforementioned studies, MeHg concentrations in clams were not measured, with the exception of clams collected in Pialassa Baiona (Trombini et al., 2003) where MeHg

Table 3
Estimated daily intake (DI) of mercury and risk quotients (RQ) for the different size classes of Manila clams.

Site	Type	THg_{clams} (ng g^{-1} ww)	$MeHg_{clams}$ (ng g^{-1} ww)	THg DI (μg day^{-1})	MeHg DI (μg day^{-1})	RQ_{THg}	RQ_{MeHg}
Class size	30–35 mm						
M2	Wild	65	37	2.4	1	0.05	0.09
M4	Wild	213	–	7.9	–	0.16	–
MC	Wild	217	74	8.0	3	0.16	0.17
IGC	Wild	298	–	11.0	–	0.22	–
VAS	Wild	462	166	17.1	6	0.34	0.38
BAR	Wild	275	127	10.2	5	0.20	0.29
MA	Reared	105	67	3.9	2	0.08	0.15
MB	Reared	57	26	2.1	1	0.04	0.06
Class size	35–40 mm						
M2	Wild	74	35	2.7	1	0.05	0.08
M4	Wild	225	–	8.3	–	0.17	–
MC	Wild	238	73	8.8	3	0.18	0.17
IGC	Wild	411		15.2	–	0.30	–
VAS	Wild	623	158	23.0	6	0.46	0.37
BAR	Wild	365	118	13.5	4	0.27	0.27
MB	Reared	54	27	2.0	1	0.04	0.06
Class size	40–45 mm						
M2	Wild	70	43	2.6	2	0.05	0.10
M4	Wild	283	–	10.5	–	0.21	–
MC	Wild	321	92	11.9	3	0.24	0.21
IGC	Wild	497	–	18.4	–	0.37	–
VAS	Wild	825	175	30.5	6	0.61	0.41
BAR	Wild	572	129	21.2	5	0.42	0.30
Class size	45–50 mm						
M2	Wild	81	34	3.0	1	0.06	0.08
M4	Wild	252	–	9.3	–	0.19	–
MC	Wild	235	–	8.7	–	0.17	–
IGC	Wild	985	–	36.5	–	0.73	–
VAS	Wild		–		–		–
BAR	Wild	410	108	15.2	4	0.30	0.25
Class size	50–55 mm						
M2	Wild	90	54	3.3	2	0.07	0.12

The intake is expressed as the amount of THg org MeHg in μg per person per day assuming a clam consumption of 37.0 g ww $person^{-1}$ day^{-1}. The mean total mercury (THg) and methylmercury (MeHg) content in clams are reported in the first two columns.

was, on average, similar to our average contents, whereas maximal values were twice as high for the Marano and Grado lagoon. Considering studies performed in different geographical locations such as the Atlantic and Mediterranean coasts of Spain (Usero et al., 1997), the Chinese Bohai Sea (Wang et al., 2005) and the Hong Kong coast (Pan and Wang, 2011), the average contents of both Hg and MeHg in Manila clams are undoubtedly lower than those observed in the present study.

A significant correlation ($p < 0.001$) was found between THg and MeHg, both in seeded clams at MB and in wild clams, both in summer and winter (Fig. 5). Similar correlations were observed in seawater mussels (Mikac et al., 1996), in bivalves from the Bohai Sea

Table 4
Total mercury (THg) and methylmercury (MeHg) concentrations in soft tissues of Manila clams (*Ruditapes philippinarum*) from different geographic areas.

Location	THg (ng g^{-1} ww)	MeHg (ng g^{-1} ww)	MeHg/THg (%)	Clam size (mm)
Marano and Grado lagoon, Italy (western sector)				
Av ± sd	73 ± 25	48.9 ± 26.5	49.5 ± 14.1	30–35 (reared calms)
Min–max	51–111	19.2–80.9	35.2–65.6	
Marano and Grado lagoon, Italy				
Av ± sd	261 ± 138	97.7 ± 53.8	41.9 ± 14.4	30–35 (wild clams)
Min–max	61–477	24.2–185.7	24.6–72.7	
Marano and Grado lagoon, Italy				
Av ± sd	364 ± 244	97.8 ± 53.0	36.2 ± 15.6	35–55 (wild clams)
Min–max	63–985	25.3–193.5	18.5–72.6	
Marano and Grado lagoon, Italy (western sector)[a]	250–300	–	–	30 – 35 (reared clams)
Marano and Grado lagoon, Italy[b]	100–360	–	–	n.r.
Venice lagoon, Italy[a]	30–70	–	–	30–35 (reared clams)
Atlantic and Mediterranean coasts, Spain[c]	18–389	–	–	<30 to >40 (wild clams)
Pialassa Baiona, Italy[d]	110	95	–	>25 (wild clams)
Bohai Sea, China[e]	21 ± 11	13 ± 9	59 ± 13	30–35 (wild clams)
Hong Kong coast, China[f]	47.4 ± 15.3	16.6 ± 3.5	37.6 ± 11.8	n.r. (wild clams)

n.r.: not reported; ww: wet weight.
[a] Sfriso et al. (2008).
[b] Brambati (1996).
[c] Usero et al. (1997).
[d] Trombini et al. (2003).
[e] Wang et al. (2005).
[f] Pan and Wang (2011) (data expressed as dw).

(Wang et al., 2005) and in freshwater Asiatic clams (Inza et al., 1997).

This evidence suggests a similar accumulation pathway for Hg and MeHg: when suspended particulate matter is filtered by the clams and passes through the digestive tract, it is subject to a partial extraction, which could be proportional to the THg and MeHg content in the suspended matter (Lawrence et al., 1999). Sediment-bound MeHg seems to be an important source of Hg to suspension feeding clams. The transfer of Hg and MeHg through the trophic chain is probably driven by particulate matter (Gagnon and Fisher, 1997) and/or by the association with organic matter (Bryan and Langston, 1992; Gagnon and Fisher, 1997), and the uptake of organic Hg by benthic invertebrates seems to be more efficient than the uptake of the inorganic forms (Riisgard and Hansen, 1990; Pan and Wang, 2011). A previous study carried out in the Venice lagoon showed that MeHg concentrations in particulate suspended matter are subject to marked seasonal variations, with the minima occurring during autumn–winter and maxima in the summer (Bloom et al., 2004).

Since dissolved MeHg and inorganic Hg are concentrated by unicellular organisms from the water column, due to their similar octanol–water partition coefficients (K_{ow}) and permeability through the cellular membrane (Mason et al., 1996), the bio-concentration of MeHg in clams could derive from plankton uptake. During the warm period, MeHg could accumulate in phytoplankton and be ingested and assimilated by Manila clams but could also be assumed directly from water (Pan and Wang, 2011). Then in winter/autumn, when the net MeHg production in pore-waters decreases, the bioavailability of MeHg could be limited (Hines et al., 2012). Indeed the highest net MeHg production from the Marano and Grado lagoon sediments occurs mainly in summer (Hines et al., 2012) and, therefore, it could explain the higher bioaccumulation of MeHg in clams during this season. These results are similar to those reported by Trombini et al. (2003) for a Northern Adriatic coastal lagoon (Pialassa Baiona). Besides, results from benthic chamber experiments (Emili et al., 2012) highlighted a possible link between Hg remobilization from the sediment and its bio-accumulation in benthic organisms, where the highest fluxes were recorded (MC and BAR sites).

The decrease in THg and MeHg contents observed in seeded clams of the lagoon could be due to a dilution effect, consequent upon the increase of the mass of the clam soft tissue, in the absence of a real bioaccumulation. This is more evident at MB site, in the western sector of the lagoon, where the higher availability of river-borne particulate matter and nutrients from the Stella river, which discharges about half of the total freshwater in the lagoon (ARPA FVG, 2005), is the probable reason of the faster clam growth.

The percentage of Hg as MeHg varies according to the clam size and the site where the wild clams were collected, ranging from 20% to more than 90%. The mean MeHg/THg percentage in seeded and wild clams for the 30–35 mm size were $49.5 \pm 14.1\%$ and $41.8 \pm 14.4\%$, respectively. These data are similar to those reported for *Ruditapes philippinarum* collected along the Hong Kong coast (China), $37.6 \pm 11.8\%$ (Pan and Wang, 2011), and lower than the values ($59 \pm 13\%$, Table 4) reported for clams collected in the Hong Kong area with sizes falling in a wider range: 28–43 mm (Wang et al., 2005). Other bivalves, such as mussels and oysters, were found to have similar percentages (Mikac et al., 1996; Claisse et al., 2001).

In all stations where wild clams were collected, the observed decrease of the MeHg/THg ratio with increasing clam size could be due to a higher bioaccumulation of the inorganic forms of Hg which are constantly present in much higher concentrations than MeHg both in pore-waters and sediments. A similar trend was observed in the freshwater bivalve *Dreissena polymorpha* (zebra mussel) by Carrasco et al. (2008), who found that the MeHg/THg percentage increases as the mussel size declines.

A lack of MeHg bioaccumulation with size can also be inferred by the work of Pan and Wang (2011) for *Ruditapes philippinarum* collected along the Hong Kong coast (China). These authors estimated a biological half-life of 139 days for MeHg, which could be compatible with the seasonal changes in content observed in the present study. Moreover, the MeHg content in clams could also be influenced by the activity of demethylating bacteria growing in the clams (Baldi, personal communication). Both biological processes could convert MeHg in the inorganic form, thus explaining the decrease in the MeHg/THg ratio in the larger size clams.

Another pathway, which could increase the Hg mobilization, is related to the effects of sediment resuspension on Hg bio-accumulation by clams. The re-oxygenation of anoxic sediments following resuspension events (due to storms and channel maintenance dredging) could reduce sulfide concentrations, enhancing Hg availability to bacteria and promoting methylation (Kim et al., 2004; Acquavita et al., 2012). However there are no data available to assess the spatial and seasonal relevance of resuspension processes on mercury bioaccumulation in the clams of the Marano and Grado lagoon.

5.2. Potential risks for human health due to clam consumption

As it is unknown whether the current provisional tolerable weekly intakes are sufficient to mitigate lifelong dietary exposure to heavy metals, special attention should be paid whenever concentrations in clams approach the current regulatory limits (Whyte et al., 2009).

To exceed the PTWI, an adult of 70 kg needs to consume 51 g ww day^{-1} of wild clams in the class 45–50 mm or 60 g ww day^{-1} of the 40–45 mm size. Such a clam consumption would translate into a weekly ingestion of 357–420 g ww of clams which would correspond to approximately 1.2–1.4 kg of fresh product (assuming that the edible part corresponds to 30% of the total weight, Oselladore, 2005). Considering that 0.25 kg of clams is normally used to prepare a meal, 5–6 clam-based meals should be consumed in a week to exceed the PTWI, which is highly improbable also among fishermen.

For seeded clams, the risk quotient is significantly lower compared to the larger wild clams. It seems then reasonable to assume that reared clams in the Marano and Grado lagoon, which are usually commercialized in the 30–35 mm size class, offer a guarantee that the human consumption of clams will remain well below the PTWI.

6. Conclusions

Although Hg contaminating activities (such as mining and chlor-alkali processing) have ceased, sediments of the Marano and Grado lagoon still represent an important Hg reservoir where active methylating processes take place (Emili et al., 2012; Hines et al., 2012). Contamination of benthic fauna is still ongoing and presumably will remain active in the future, as bioturbation and resuspension due to storms or to maritime traffic contribute to sediment remobilization.

Compared to other Hg contaminated coastal environments of the northern Adriatic Sea, results for the Marano and Grado lagoon show far higher Hg contents in Manila clams. The most contaminated clams were found in the eastern part of the lagoon, where sediments were affected by the largest contribution of Hg from the Idrija mine.

The percentage of Hg as MeHg varies according to the clam size and the site where the clams were collected, ranging from 20% to

more than 90% in the wild clams. MeHg contents in clams showed marked seasonal changes with a significant increase in summer, consistent with higher MeHg production in the warm season (Hines et al., 2012). This is a clear indication of the strong variability and seasonal influence on MeHg bioaccumulation. The observed decrease of the MeHg/THg ratio with increasing clam size could be due to a higher bioaccumulation of the inorganic forms of Hg, almost constantly present in pore-waters and released from the sediments, in comparison to the methylated form which is subject to marked seasonal variations of the methylation/demethylation processes (Hines et al., 2012) and of sediment-water exchanges (Emili et al., 2012).

Risk assessment based on clam consumption indicates that no detriment to human health exists. MeHg, the most toxic form of Hg, decreases during clam growth both in wild and seeded clams. Moreover, seeded clams in the least contaminated sediments of the lagoon displayed Hg levels in specimens of commercial size lower than those measured in the larger class sizes of wild clams collected in the eastern sector of the lagoon. Therefore, Manila clam farming in controlled areas represents a safer way to minimize the risks of human Hg intake with the diet, compared to uncontrolled collection of wild clams, and should therefore be highly promoted as the best practice in contaminated lagoon environments.

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