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Bioaccumulation and detoxification processes of Hg in the king scallop *Pecten maximus*: Field and laboratory investigations

M. Metian[a,b], M. Warnau[a,1], R.P. Cosson[c], F. Oberhänsli[a], P. Bustamante[b,*]

[a] *International Atomic Energy Agency – Marine Environment Laboratories (IAEA-MEL), 4 Quai Antoine Ier, MC-98000, Principality of Monaco*
[b] *Littoral, Environnement et Sociétés (LIENSs), UMR 6250, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France*
[c] *EA 2160 - MMS (Mer, Molécules, Santé), Biologie Marine – ISOMer, BP 92208, F-44322 Nantes Cedex 3, France*

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ABSTRACT

Hg bioaccumulation was investigated in the king scallop *Pecten maximus* in the laboratory and in the field. In controlled conditions, scallops were exposed to ^{203}Hg through seawater, sediment and food in order to determine its uptake and depuration kinetics. In the field, Hg and metallothionein (MT) concentrations and the metal subcellular distribution were determined in scallops from two sites of the Bay of Seine (France) differently subjected to the Seine river inputs. While Hg concentrations in the whole soft parts and kidneys (viz. the highest accumulator organ) did not differ between scallops from both sites (74–156 ng g^{-1} dry wt), they did for the digestive gland and the gills. According to the experimental results, a higher exposure to dissolved Hg might occur in the site close to the estuary whereas Hg would be mainly incorporated via the dietary pathway in the site away from the estuary. Within the cells of wild scallops, Hg was mainly associated to the cytosolic fraction in the digestive gland and gills (60–100%). However, the lack of relationship between Hg and MT levels suggests that Hg detoxification in *P. maximus* involves other, non-MT, soluble compounds. In kidneys, insoluble compounds played an important role in Hg sequestration. No effect of scallop age was observed neither on Hg and MT concentrations nor on the subcellular distribution of the metal. Finally, according to FAO/WHO recommendations (maximum weekly Hg intake), our results clearly indicate that the low Hg contents in the edible part of the king scallops from the Bay of Seine prevent any risk for human consumers.

1. Introduction

Among metals of environmental concern, mercury (Hg) is released in the marine environment from natural and anthropogenic inputs (Cossa et al., 2002), and has no known biological function. Hg bioaccumulation has been extensively studied in marine organisms. The metal is well known to be readily methylated by micro-organisms, bioaccumulated by marine biota and often biomagnified along the food chain (see e.g., Klein and Goldberg, 1970; Cappon and Smith, 1981; Eisler, 1987). Even though Hg concentrations in bivalve tissues are generally lower than those in top marine predators (Neff, 2002), the elevated toxicity of Hg for these molluscs is well known (e.g., Nelson et al., 1977; Neff, 2002). In addition, Hg-related health risks do exist for consumers of bivalves (e.g., Gutiérrez et al., 2006).

Bivalves have also been documented as relevant biomonitors of Hg contamination and are therefore used to assess the contamination status of marine coastal zones (e.g., Cossa, 1989; Claisse et al., 2001). Consequently, the average Hg concentrations in these marine sentinels, especially the Mytilidae and Ostreidae, are well characterized, as well as their bioaccumulation processes thanks to laboratory studies. In this respect, different uptake pathways have been considered in Hg bioaccumulation (e.g., Gagnon and Fisher, 1997; Blackmore and Wang, 2004), as well as different physico-chemical conditions of the media (e.g., Fowler et al., 1978; Cossa, 1989) and different chemical species of Hg (e.g., Cunningham and Tripp, 1975; Fowler et al., 1978; Blackmore and Wang, 2004).

Whereas extensive knowledge on Hg metabolism is available for Mytilidae and Ostreidae, very little is known for the Pectinidae family. These bivalves deserve interest as they are intensively fished and cultured worldwide for human consumption while they bioaccumulate many metals up to very high levels (e.g., Brooks and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2004; Metian et al., 2008).

* Corresponding author. Tel.: +33 5 46 50 76 25; fax: +33 5 46 45 82 64.
E-mail addresses: warnaumichel@yahoo.com (M. Warnau), pbustama@univ-lr.fr (P. Bustamante).
[1] Present address: LIENSs, UMR 6250, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France.

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Baseline Hg concentrations in scallop tissues are poorly documented compared to other bivalve families. Data available indicate that Hg concentrations in whole soft parts of Pectinidae generally range from 0.04 to 1.6 $\mu g\,g^{-1}$ dry wt (Klein and Goldberg, 1970; Norum et al., 2005; Chouvelon et al., 2008). However, very high concentrations of Hg (i.e., 48 $\mu g\,g^{-1}$ dry wt) were reported for *Chlamys ferrei* from the Minamata bay, Japan (Matida and Kumada, 1969), indicating that scallops are able to bioaccumulate Hg very efficiently in highly contaminated environments.

The Seine river (NW France) is one of the most metal-contaminated rivers of Europe (Chiffoleau et al., 1994; Meybeck et al., 2007). High concentrations of metals and particularly of Hg have been measured in this river (Cossa et al., 2002; Meybeck et al., 2007) and thus, the Bay of Seine (Normandy, France) into which the Seine river flows, is a typical case study for coastal Hg contamination. The Bay of Seine is also one of the most important places for the king scallop *Pecten maximus* fishery in France, which stresses the need for providing data on Hg bioaccumulation in this species.

Thus, the objective of this study was to investigate the bioaccumulation of Hg through *in situ* and laboratory approaches. First, part of this work aimed at providing baseline data on Hg concentrations in the king scallop *P. maximus* of different ages collected in the Bay of Seine from two sampling locations with different levels of contamination. Tissue and subcellular distributions of the metal as well as metallothionein concentrations were taken into consideration in order to evaluate the storage and detoxification processes that are likely to affect Hg concentrations in scallop tissues. In a second approach, we determined the Hg bioaccumulation capacities of *P. maximus* exposed via three different contamination pathways (seawater, sediment, and food), using radiotracer techniques. In order to compare the results with the field study, tissue and subcellular distributions of the radiotracer were determined for the different exposure pathways.

2. Materials and methods

2.1. Field study: sampling and sample preparation

Forty king scallops *P. maximus* were collected by dredging in July 2004 in two locations of the Bay of Seine (Normandy, French North Atlantic coast; Fig. 1). The sampling sites are situated near the Seine estuary ("close" station) and in the western part of the Bay of Seine ("away" station). These stations were reported for displaying contrasting Hg concentrations in the sediments, i.e., ranging from >1 $\mu g\,g^{-1}$ in the upper estuary to 0.2 $\mu g\,g^{-1}$ downstream (Cossa et al., 2002) and were therefore considered for their potentially different Hg bioavailabilities.

The collected individuals belonged to various age groups: 1-year old, 2-years old, 3-years old, and more than 3-years old, which were determined according to the shell yearly growth increments. Five individuals of each size class were collected at each station. Each scallop was measured, weighed and frozen on board (−20 °C). Upon arrival to the laboratory, all selected scallops ($n = 40$) were dissected to separate the digestive gland, kidneys, gills, gonad and adductor muscle. The remaining tissues were also taken into account in order to calculate the whole Hg content in the scallops. All organ and tissue samples were freeze-dried, ground to powder, and then preserved in hermetically sealed containers until further metal analyses.

2.2. Metallothionein analysis

Aliquots (approximately 100 mg) of the prepared powder of digestive gland, kidneys, gills, gonad and adductor muscle were homogenized individually in 5 volumes of buffer (100 mM Tris, pH 8.1, 10 mM β-mercaptoethanol) with a mortar and pestle on ice. The homogenates were centrifuged at 30,000 × *g* for 30 min at 4 °C in order to separate the particle-free supernatant (cytosol) from the pellet. Then, cytosol was submitted to heat-denaturation (95 °C, 15 min) and centrifuged (10,000 × *g*, 15 min) in order to separate thermostable from thermolabile proteins (e.g., Temara et al., 1997). From newly obtained supernatants, the concentrations of metallothionein (MT) were determined by differential pulse polarography through quantification of the cysteine residues (Olafson and Sim, 1979, modified by Thompson and Cosson, 1984), using a PARC EG&G Model 394 analyzer and a PARC EG&G Model 303 static mercury drop electrode (SMDE). The quantification of MTs was based on the standard addition method using rabbit liver MT (FLUKA 63995) and on the variation of height of the "B" peak which is the more electronegative peak following the reduction of cobalt wave. The method is specific for MTs after removal of contaminating proteins in tissue homogenates by heat-denaturation (Olafson and Olsson, 1991) and contribution of the mercaptoethanol and the low molar mass compounds (e.g., glutathione and free cysteins) is negligible (Olafson and Sim, 1979; Cosson unpublished results). MT concentrations in samples are reported as $\mu g\,g^{-1}$ dry wt.

2.3. Mercury analysis

All the samples generated by ultracentrifugation (see Section 2.2) were directly analyzed for Hg content. Hg analyses were carried out on the pellets (10–50 mg wet wt) and supernatants (100 µl) with an Advanced Mercury Analyzer (ALTEC AMA 254). Hg determination involved evaporation of the metal by progressive heating until 700 °C was reached and then held under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold-net. Afterwards, the net was heated to liberate the collected Hg, which was then measured by atomic absorption spectrophotometry. Hg analyses were ran with respect to a thorough quality control program including analyses of a reference material (lobster hepatopancreas TORT-2) purchased from the National Research Council, Canada. TORT-2 aliquots were treated and analyzed in the same conditions as the samples. The results were in good agreement with the certified values. Recovery was 99.6% and detection limit was 5 $ng\,g^{-1}$ dry wt. Metal concentrations in samples are reported as $ng\,g^{-1}$ dry wt.

2.4. Radiotracer experiments: sampling

In spring 2004 and 2005, seventy king scallops *P. maximus* were collected on the French Atlantic coast (Pertuis Breton, Charentes-Maritime) by SCUBA diving. They were carefully transported to IAEA-MEL premises in Monaco and were acclimated to laboratory conditions (constantly aerated open water circuit aquarium; flux: 50 l h^{-1}; salinity: 38 p.s.u; $T°$: 17 ± 0.5 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) for 8 weeks prior to the experiments. During this period, scallops were fed daily a mixed phytoplankton diet (*Isochrysis galbana* and *Skeletonema costatum*).

2.5. Radiotracer and counting

Uptake and depuration kinetics of ^{203}Hg in scallops were determined using a high specific activity radiotracer purchased from Isotope Product Lab (^{203}Hg as $HgCl_2$ in 0.1 M HCl, $T_{1/2}$ = 46.6 d). The tracer was counted using a high-resolution γ-spectrometer composed of three Germanium N or P type-detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multichannel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity of the samples was determined by comparison with standards of known activities and appropriate

geometries and was corrected for background and physical decay of the radiotracer. The counting time was adjusted to obtain propagated counting errors less than 5%.

2.6. Seawater exposure

Nine scallops (average wt ± S.D.: 78 ± 6 g) were placed in a 70-l glass aquarium (constantly aerated closed circuit aquarium; other parameters as described above) and exposed for 7 d to 1.5 kBq ^{203}Hg l^{-1} in seawater. No change in pH was detectable after the tracer addition. Spiked seawater was renewed twice a day the first 2 d and then daily in order to keep radioactivity constant in seawater. ^{203}Hg activity was checked in seawater before and after each spike renewal, yielding a time-integrated activity of 1.39 ± 0.32 kBq ^{203}Hg l^{-1} (Rodriguez y Baena et al., 2006).

The 9 scallops were collected at different time intervals and whole-body counted (same individual each time). At the end of the 7-d exposure period, 4 scallops were sacrificed and dissected. Shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated and radioanalyzed in order to assess the ^{203}Hg body distribution. The 5 remaining scallops were then placed for 21 d in non-contaminating conditions (constantly aerated open circuit) and regularly whole-body counted in order to follow the depuration of ^{203}Hg. At the end of the depuration period, 4 scallops were dissected as previously described.

2.7. Food exposure

The Haptophyceae *Isochrisis galbana* was used to study the trophic transfer of ^{203}Hg to the scallop. Phytoplankton cells were exposed for 7 d to 4.54 kBq ^{203}Hg l^{-1}. Phytoplankton media was then filtrated on 1-μm mesh size filters (Osmonic) and the cells were suspended in a 70-l aquarium (constantly aerated closed-circuit) where 6 scallops (average wt ± S.D.: 127 ± 14 g) had already been acclimated for 1 week. *I. galbana* cells were γ-counted before and after the filtration. Scallops were then allowed to feed on radiolabelled *I. galbana* for 2 h (single feeding method; Warnau et al., 1999). A cell density of 5 × 10^4 cell ml^{-1} was selected in order to avoid pseudofaeces production.

After the feeding period, all scallops were whole-body γ-counted and flowing seawater conditions (50 l h^{-1}) were restored in the aquarium. Individuals were then counted at different time intervals to follow the depuration kinetics of dietary ^{203}Hg. Four individuals were dissected after 16 d to determine the ^{203}Hg tissue distribution among the different scallop body compartments. The subcellular distribution of ^{203}Hg was also determined in the digestive gland at day 16 (see Section 2.9).

2.8. Sediment exposure

Sediment was collected in Wimereux (North-Atlantic coast of France). Sediment grain size distribution was determined using a Mastersizer micro and the ratio dry/wet weight was calculated after freeze-drying the sediment, using a LABCONCO Freezone 18. Sediments (9 kg) were spiked with 300 kBq ^{203}Hg for 6 d according to the method described in Danis et al. (2003, 2005). They were then used to form a continuous layer of 4-cm height in a 20-l glass aquarium.

Ten scallops (average wt ± S.D.: 118 ± 5 g) were then placed in the aquarium (constantly aerated open circuit) for 13 d. Six individuals were regularly whole-body radioanalyzed during the experimental period and sediment samples were regularly collected to determine any loss of activity and to calculate time-integrated activity (Rodriguez y Baena et al., 2006). ^{203}Hg activity in sediment was constant throughout the exposure period (13.7 ± 2.1 Bq g^{-1} wet wt). At the end of the exposure period, 4 scallops were dissected to determine ^{203}Hg body distribution. The remaining scallops were transferred for 31 d into a new, open circuit 20-l aquarium containing new, uncontaminated sediment and were regularly whole-body counted. ^{203}Hg was also regularly checked in the aquarium sediment; even though ^{203}Hg was never detected, the sediment was renewed after the first week. After one month of depuration, 4 scallops were dissected to determine ^{203}Hg body distribution and its subcellular distribution in the digestive gland.

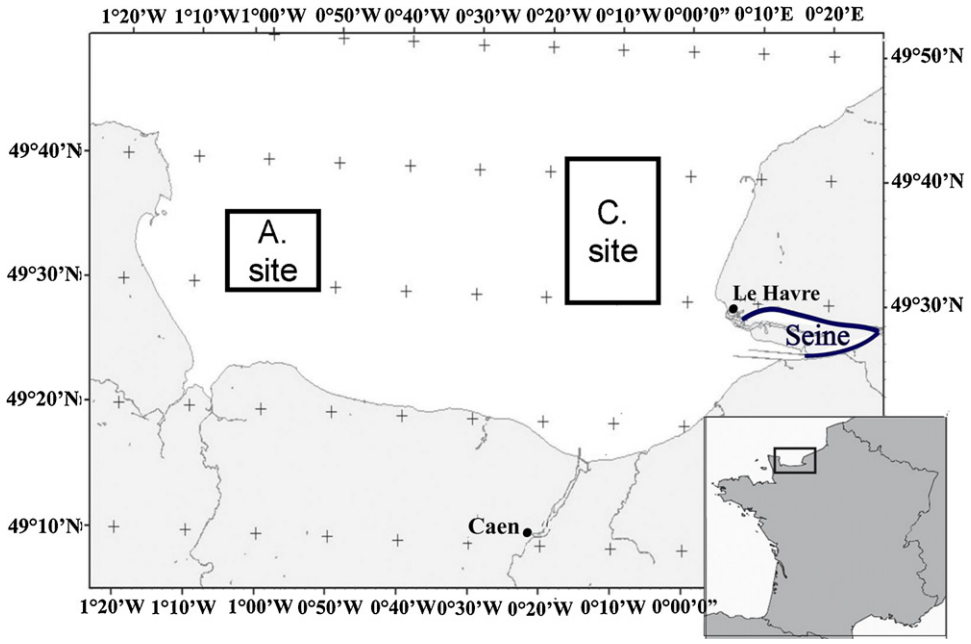

Fig. 1. Map of the Bay of Seine (Normandy, France) indicating the sampling locations of the king scallops *Pecten maximus*. A. site: away from the estuary site; C. site: close to the estuary site.

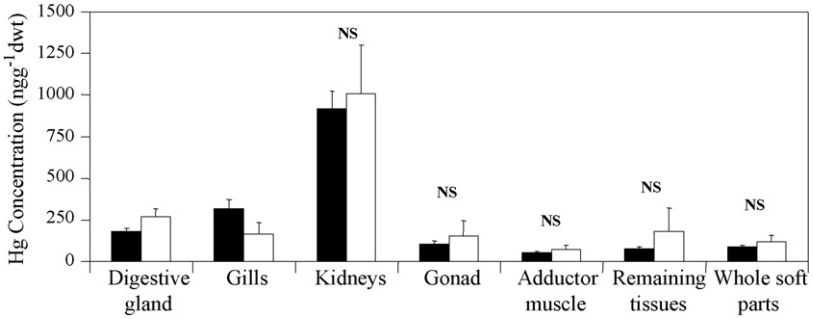

Fig. 2. Hg concentration (ng g^{-1} dry wt; mean ± S.D., $n = 5$) in the tissues and organs of 3 years-old king scallops *Pecten maximus* collected in the away (white bars) and the close to the estuary sites (black bars) in the Bay of Seine. NS: non-significant difference ($\alpha = 0.5$).

2.9. Subcellular distribution

In all experiments, scallop digestive glands were considered to assess the partitioning of ^{203}Hg between soluble and insoluble subcellular fractions as described by Bustamante and Miramand (2005). Briefly, a part of each digestive gland was homogenized individually with a mortar and pestle on ice with 10 ml of 0.02 M Tris–HCl buffer, 0.25 M sucrose, 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor), at pH 8.6. The homogenates were centrifuged at 80,000 × *g* for 1 h at 4 °C in a Sorvall RC28S ultracentrifuge to separate the particle-free supernatant (cytosol) from the pellet. Homogenate aliquots, cytosols, and pellets were then radioanalyzed.

2.10. Data analysis

Uptake of ^{203}Hg in *P. maximus* via seawater and sediments was expressed in terms of Concentration Factors (CF: ratio between ^{203}Hg activity in scallops (Bq g^{-1} wet wt) and time-integrated activity in the sea water (Bq g^{-1})) and in terms of transfer factors (TF: ratio between ^{203}Hg activity in scallops (Bq g^{-1} wet wt) and time-integrated activity in the sediment (Bq g^{-1} wet wt)) over time, respectively. Uptake kinetics of ^{203}Hg in scallops were fitted using a linear model for the seawater exposure (Eq. (1)) and a first-order exponential kinetic model (Eq. (2)) for the sediment exposure, using non-linear curve-fitting routines of Statistica® 6 software.

$$CF_t = k_u\, t \quad (1)$$

$$CF_t = CF_{ss}\,(1 - e^{-k_e\, t}) \quad (2)$$

where CF_t and CF_{ss} ($CF_{ss} = k_u/k_e$) are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and depuration rate constants (d^{-1}), respectively (Warnau et al., 1996).

Depuration of ^{203}Hg in seawater, food and sediment experiments was expressed in terms of percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in scallops at the beginning of the depuration period × 100) over time. The depuration kinetics were described by a single- (Eq. (3)) or a double- (Eq. (4)) component exponential model.

$$A_t = A_0\, e^{-k_e\, t} \quad (3)$$

$$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}\, e^{-k_{el}\, t} \quad (4)$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively (Warnau et al., 1996). For each exponential component (s and l), a biological half-life ($T_{b1/2s}$ and $T_{b1/2l}$) can be calculated (Eq. (5)) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively).

$$T_{b1/2} = {}^{\ln 2}/_{k_e} \quad (5)$$

In the context of the feeding experiment, the 'long-lived' component describes the part of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated. The corresponding A_{0l} represents the assimilation efficiency (AE) of the radiotracer (e.g., Hédouin et al., 2007). The best fitting regression modes were selected according to highest determination coefficient and examination of residuals. The level of significance for statistical analyses was always set at $\alpha < 0.05\%$.

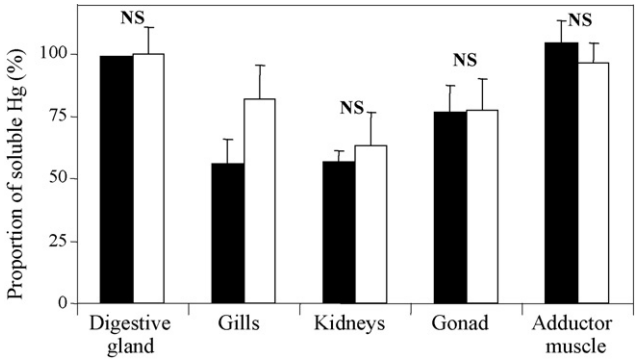

Fig. 3. Proportion of soluble Hg in cells (%; mean ± S.D., $n = 5$) from selected tissues and organs of 3 years-old king scallops *Pecten maximus* collected in the away (white bars) and the close to the estuary sites (black bars) in the Bay of Seine. NS: non-significant difference ($\alpha = 0.5$).

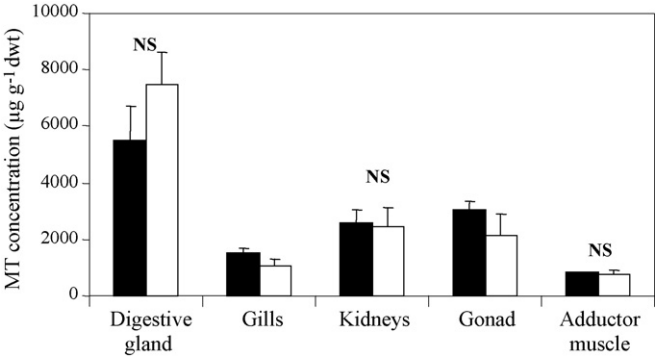

Fig. 4. Metallothionein (MT) concentration (µg g^{-1} dry wt; mean ± S.D., $n = 5$) from selected tissues and organs of king scallops *Pecten maximus* older than 3 years collected in the away (white bars) and the close to the estuary sites (black bars) in the Bay of Seine. NS: non-significant difference ($\alpha = 0.5$).

Table 1
Hg concentration (ng g^{-1} dry wt, mean ± S.D.), MT concentration (μg g^{-1} dry wt, mean ± S.D.), and subcellular distribution of Hg (cytosolic proportion, %, mean ± S.D.) in the digestive gland, the gills and kidneys of king scallops *Pecten maximus* of different ages (n = 5 for each age/site/parameter)

Location	Age	Digestive gland			Gills			Kidneys		
		Hg (ng g^{-1} dry wt)	MT (μg g^{-1} dry wt)	Hg fraction in cytosol (%)	Hg (ng g^{-1} dwt)	MT (μg g^{-1} dry wt)	Hg fraction in cytosol (%)	Hg (ng g^{-1} dry wt)	MT (μg g^{-1} dry wt)	Hg fraction in cytosol (%)
Contaminated site										
	1 yr	248 ± 95	3135 ± 936	100 ± 4	163 ± 23	2076 ± 144	92 ± 7	1764 ± 338	4377 ± 1404	59 ± 11
	2 yrs	109 ± 14	4185 ± 935	100 ± 3	151 ± 54	1494 ± 228	70 ± 7	851 ± 111	2990 ± 925	34 ± 8
	3 yrs	177 ± 28	5197 ± 604	100 ± 12	311 ± 54	1601 ± 291	77 ± 8	765 ± 95	2485 ± 187	53 ± 7
	>3 yrs	179 ± 19	5467 ± 1514	99 ± 1	316 ± 55	1495 ± 223	56 ± 10	916 ± 108	2580 ± 441	57 ± 5
Reference site										
	1 yr	93 ± 17	4101 ± 615	100 ± 9	110 ± 9	1815 ± 192	90 ± 9	*nd*	*nd*	*nd*
	2 yrs	144 ± 37	5886 ± 1328	100 ± 16	226 ± 40	1705 ± 124	90 ± 18	642 ± 100	2138 ± 243	41 ± 10
	3 yrs	90 ± 9	3248 ± 497	100 ± 6	187 ± 28	1156 ± 122	69 ± 7	666 ± 134	1735 ± 395	41 ± 4
	>3 yrs	269 ± 48	7444 ± 869	100 ± 10	162 ± 71	1104 ± 209	82 ± 13	1008 ± 296	2422 ± 673	63 ± 13

nd: Not determined.

3. Results

3.1. Field study

3.1.1. Hg in scallops of commercial size

Concentrations of Hg that were measured in the tissues and organs of *P. maximus* of commercial size (viz. scallops older than 3 years) collected from the contaminated and reference sampling sites are given in Fig. 2. The Hg concentrations in the whole soft tissues of these scallops were not significantly different between the two stations (115 ± 41 and 87 ± 12 ng g^{-1} dry wt in the sampling sites close to and away from the estuary, respectively; p = 0.18).

In both sampling site, the kidneys showed the highest Hg concentrations among the different *P. maximus* body compartments (Fig. 2) but, as in whole soft tissues, no significant difference in renal Hg concentrations was found between the two locations (site close to the estuary: 916 ± 108 ng g^{-1} dry wt; site away from the estuary: 1008 ± 296 ng g^{-1} dry wt; p = 0.53). A significant difference in Hg concentrations between the two sampling sites was found only for the digestive gland and gills (p = 0.004 and 0.005, respectively). The gills of scallops collected close to the estuary displayed significantly higher Hg concentrations than in those collected in the farther station (316 ± 55 ng g^{-1} dry wt vs. 162 ± 71 ng g^{-1} dry wt; p = 0.005). Surprisingly, an opposite trend was found for the digestive gland ("away" site: 269 ± 48 ng g^{-1} dry wt "close" site: 179 ± 19 ng g^{-1} dry wt; p = 0.004).

3.1.2. Subcellular distribution of Hg in scallops of commercial size

The subcellular distribution of Hg in the tissues and organs of the scallops from both sampling sites are compared in Fig. 3. The soluble fraction always contained the highest proportion of Hg in both areas, ranging from ~60% in the gills of scallops collected close to the estuary up to nearly 100% in the digestive gland and adductor muscle from both sampling sites (Fig. 3). The only significant difference between sampling sites was found for the gills for which scallops from the site away from the estuary showed higher soluble Hg proportions (82 ± 13% vs. 56 ± 10%, p = 0.007).

3.1.3. MT concentrations in scallops of commercial size

Fig. 4 reports MT concentrations in the digestive gland, gills, kidneys, gonad and adductor muscle of the scallops from both sampling site. The ranking of the organs according to the increasing order in their MT concentration was identical in both sampling sites: adductor muscle < gills < kidneys ≤ gonads < digestive gland. The digestive gland displayed very high MT concentrations but no significant difference was observed between the two stations (p = 0.094). Similarly, no difference was found between sites for MT neither in adductor muscle nor for kidneys (p > 0.27 and p > 0.60, respectively). In contrast, significant differences were found between the two stations for gills and gonads (p = 0.007 and 0.041, respectively), with the highest MT concentrations measured in the site close to the Seine estuary.

3.1.4. Influence of size

Table 1 shows the concentrations and subcellular distribution of Hg and MT concentrations in digestive gland, gills and kidneys in scallops according to their age. No significant trends (linear or non-linear regressions) could be established between these parameters and the age of the scallops. It appeared however that the older scallops tended to display higher MT concentrations than the younger ones in the digestive gland, whereas the inverse seemed to characterize gills and kidneys.

Table 2
Parameter estimates (mean ± ASE) of the whole-body uptake (a) and depuration (b) kinetics of ^{203}Hg in *Pecten maximus*

Experiment	(a) Uptake			(b) Depuration				
	CF_{ss} or TF_{ss} ± ASE	k_u ± ASE (d^{-1})	R^2	A_{0s} ± ASE (%)	$T_{b1/2s}$ ± ASE (d)	A_{0l} ± ASE (%)	$T_{b1/2l}$ ± ASE (d)	R^2
Seawater	–	32.8 ± 1.6	0.76		–	99 ± 2	23 ± 2	0.83
Feeding	–	–	–	75 ± 2	0.017*	25 ± 1	8.2 ± 1.2	0.99
Sediment	0.028 ± 0.001	n.d.	0.51	–	–	89 ± 3	30 ± 4	0.52

Seawater: scallops exposed to Hg for 7 d via seawater (n = 9) and then maintained for 21 d in non-contaminated conditions (n = 5); *Feeding:* scallops fed for 2 h on radiolabelled *Isochrysis galbana*, then maintained for 16 d in non-contaminated conditions (n = 6); *Sediment:* scallops exposed for 7 d via the sediments (n = 10) and then maintained for 31 d in non-contaminated conditions (n = 6). Uptake parameters: CF_{ss} and TF_{ss}: concentration and transfer factors at steady state from seawater and sediment, respectively; k_u: uptake rate constant (d^{-1}); Depuration parameters: A_{0s} and A_{0l}: activity (%) lost according to the short- and long-lived exponential component, respectively; $T_{b1/2}$: biological half-life (d). ASE: asymptotic standard error; R^2: determination coefficient.

* Not significantly different from 0 ($p > 0.05$).

3.2. Laboratory experiments

3.2.1. Seawater exposure

Whole-body uptake kinetics of ^{203}Hg in *P. maximus* were best fitted by a linear regression (R^2 = 0.76). The kinetic parameter estimates and their statistics are presented in Table 2. The whole-body concentration factor (CF) measured at the end of the exposure period ($CF_{7\,d}$) was 228 ± 92 (Table 3). $CF_{7\,d}$ measured in the different organs indicated that ^{203}Hg was especially concentrated by the gills ($CF_{7\,d}$ = 13,717 ± 4407) and to a lesser extent by the digestive gland ($CF_{7\,d}$ = 7832 ± 1213). Regarding the body distribution, ^{203}Hg was mainly found in the gills, which contained 72% of the total soft tissues load (Table 4).

At the end of the exposure period, non-contaminating conditions were restored and depuration kinetic of ^{203}Hg was followed in *P. maximus* for 21 d. The whole-body depuration kinetics of ^{203}Hg was best described by a single-component exponential model (R^2 = 0.83; Table 2). The absorption efficiency (A_{0l}) of ^{203}Hg by the scallop reached 99% and its biological half-life was 23 ± 2 d.

The body distribution of ^{203}Hg at the end of the 21-d depuration period was different from that at the end of the 7-d uptake period (Table 4). Indeed, although the gills still contained the major part of Hg in the soft tissues (47 ± 2%), the proportion of Hg in the digestive gland and the mantle of *P. maximus* increased substantially, reaching, respectively, 25 ± 6 and 17 ± 3% of the total Hg body load. This difference was mainly due to the considerable decrease in the ^{203}Hg activity in the gills between the end of uptake and depuration periods (from 19,083 ± 6131 down to 4503 ± 1976 Bq g^{-1} wet wt) while the activities in the other cited organs did not show significant difference between the two periods (10,895 ± 1688 Bq g^{-1} wet wt vs. 7571 ± 5165 Bq g^{-1} wet wt for the digestive gland and 927 ± 199 Bq g^{-1} wet wt vs. 690 ± 286 Bq g^{-1} wet wt for the mantle).

Table 3
Concentration factor (CF) and transfer factor (TF) (mean ± S.D.; n = 9 and 10, respectively) of ^{203}Hg in the different organs and tissues of *Pecten maximus* after 7 d of exposure via seawater (CF) or 13 d via sediment (TF)

Body compartments	CF	TF
Whole body	228 ± 92	0.028 ± 0.008
Digestive gland	7,832 ± 1,213	2.30 ± 1.29
Gills	13,717 ± 4,407	0.11 ± 0.01
Kidneys	3,329 ± 2,978	0.67 ± 0.41
Intestine	1,162 ± 308	0.46 ± 0.25
Gonad	1,056 ± 562	0.12 ± 0.11
Foot	783 ± 167	0.03 ± 0.01
Mantle	666 ± 143	0.02 ± 0.01
Adductor muscle	176 ± 64	0.01 ± 0.01
Remaining tissues	1,624 ± 403	0.13 ± 0.03

3.2.2. Food exposure

The depuration kinetics of ^{203}Hg ingested with phytoplankton by *P. maximus* was best fitted using a double exponential model (R^2 = 0.99, Table 2). Results indicated that 24.7 ± 1.4% of ^{203}Hg ingested with food was actually assimilated. In addition, assimilated ^{203}Hg was retained relatively weakly within the scallop tissues ($T_{b1/2l}$ = 8.2 ± 2.2 d).

In contrast to what was observed following seawater exposure, the digestive gland contained the main part of ^{203}Hg body burden (i.e., 70 ± 23%) after 16 d of depuration following the feeding (Table 4).

3.2.3. Sediment exposure

Sediment used in the experiment was mainly (95.8%) composed of grains which size ranged between 76 and 302 μm; its dry/wet wt ratio was 0.80. Whole-body uptake kinetics of ^{203}Hg bound to sediment was best fitted by a single exponential model (R^2 = 0.51; Table 2), which allowed estimating accurately the whole-body transfer factor at steady state (TF_{ss}) within the duration of the experiment. Measured $TF_{13\,d}$ and estimated TF_{ss} (0.028 ± 0.008 and 0.028 ± 0.001, respectively) were not significantly different from each other. In contrast, the uptake rate constant (k_u) was very fast and could not be determined with acceptable uncertainty due to

Table 4
^{203}Hg distribution (%, mean ± S.D.; n = 4) among the body compartments of *Pecten maximus* at the end of uptake and/or depuration phases of seawater, feeding and sediment experiments

Body compartments	Seawater		Feeding Depuration	Sediment	
	Uptake	Depuration		Uptake	Depuration
Digestive gland	14 ± 2	25 ± 6	70 ± 23	69 ± 10	43 ± 3
Gills	72 ± 2	47 ± 2	8 ± 8	12 ± 4	10 ± 1
Kidneys	2 ± 1	3 ± 1	7 ± 6	5 ± 0	22 ± 10
Intestine	< 1	1 ± 1	3 ± 1	2 ± 1	5 ± 2
Gonad	< 1	2 ± 0	1 ± 1	1 ± 1	3 ± 1
Foot	< 1	1 ± 0	1 ± 0	< 1	1 ± 0
Mantle	9 ± 1	17 ± 3	6 ± 6	7 ± 4	11 ± 3
Adductor muscle	1 ± 0	3 ± 0	2 ± 0	2 ± 2	5 ± 1
Remaining tissues	1 ± 0	2 ± 0	1 ± 0	1 ± 1	2 ± 0

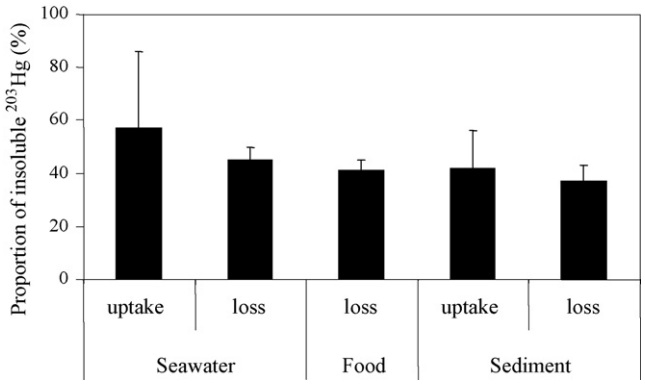

Fig. 5. Proportion of ^{203}Hg (%; mean ± S.D., $n = 5$) associated to the insoluble fraction of the digestive gland cells of the king scallop *Pecten maximus* (1) exposed for 7 d to dissolved ^{203}Hg (uptake) and then maintained for 21 d in non-contaminated conditions (loss); (2) after a 2-h feeding on ^{203}Hg-labelled *Isochrysis galbana* followed by 16 d in non-contaminated conditions (loss); (3) exposed for 7 d to ^{203}Hg via the sediments (uptake) and then maintained for 31 d in non-contaminated conditions (loss).

the 24-h time interval among sampling times. Among the different body compartments of the scallops, the highest $TF_{13\,d}$ was found for the digestive gland (2.3 ± 1.3; Table 3). Among the different body compartments, the digestive gland also contained the major part of the total ^{203}Hg body burden (i.e., 69 ± 10%; Table 4).

The whole-body depuration kinetics ^{203}Hg after exposure through sediment was best described by a single-exponential model ($R^2 = 0.52$; Table 2), which indicated that the metal was absorbed efficiently ($A_{0l} = 89 \pm 3\%$) and relatively strongly retained ($T_{b1/2l} = 30 \pm 4$ d) in scallop tissues. At the end of the 31-d depuration period, the digestive gland still contained the major fraction of ^{203}Hg, i.e., 43 ± 3% of the whole-soft tissue burden (Table 4) although the tracer activity decreased from 31.5 ± 17.6 Bq g^{-1} wet wt (end of uptake period) to 5.8 ± 1.0 Bq g^{-1} wet wt (end of depuration period).

3.2.4. Subcellular distribution

For each exposure experiment, subcellular fractioning was carried out on the scallop digestive glands. For every pathway tested (seawater, sediment and food), ^{203}Hg was mainly associated with the insoluble fraction (~60%; Fig. 5).

4. Discussion

When the whole soft tissues of *P. maximus* of commercial size (>3-years old) were considered, no significant difference in Hg concentrations was found between the two sampling sites of the Bay of Seine. This suggests either a low bioaccumulation capacity of this scallop species or/and a low bioavailability of Hg for *P. maximus* in the field. Overall, Hg concentrations in the whole soft tissues of this age class ranged from 74 to 156 ng g^{-1} dry wt, with the kidneys having the highest concentrations among the analyzed tissues (from 625 to 1433 ng g^{-1} dry wt). Therefore, although the Seine river is reported as one of the most contaminated rivers of Europe, especially regarding Hg inputs into the sea (average concentrations over the 1997–2002 period were 2.9 ± 2.1 ng l^{-1} for the dissolved phase and 1.7 ± 0.7 μg g^{-1} for the particulate phase; Cossa et al., 2002), the concentrations of Hg in the tissues of the king scallop *P. maximus* globally appeared to fall within the same range as those measured in other scallop species and/or sampling areas (Table 5).

When exposed to dissolved ^{203}Hg in seawater, *P. maximus* readily accumulated the metal in its tissues, particularly in the gills (CF > 13,000) and, to a lesser extent, the digestive gland (CF > 7000).

Table 5
Concentration of Hg (μg g^{-1} dry wt) in different scallop species from various locations around the world

Species	Origin	Whole soft-tissue Hg concentration	Highest Hg concentration	Reference
Adamussium colbecki	Antarctica	–	Gills: 0.86 ± 0.22 (M)	Bargagli et al. (1998)
Chlamys ferrei nipponensis	Japan (Minamata Bay)	48 (M)	–	Matida and Kumada (1969)
C. hastate	Canada (British Columbia)	0.1–0.2 (R)	Gills: 0.3–0.8 (R)	Norum et al. (2005)
C. varia	Spain (public market)	0.17 ± 0.08[a] (M)	–	Gutiérrez et al. (2006)
Comptopallium radula	New Caledonia	84–261 (R)	Edible parts: 76 ± 20 (M); Remainders 238 ± 96 (M)	Chouvelon et al. (2008)
Hinnites multirugosus	USA (California)	0.4–1.6 (R)	–	Klein and Goldberg (1970)
H. multirugosus	USA (California)	*Control site:* 0.30 (M), 0.25–0.35[a] (R) *Contaminated site:* 0.10 (M), 0.05–0.20[a] (R)	–	Young et al. (1981)
Mimachlamys gloriosa	New Caledonia	90–142, 107 ± 18 (M)	Edible parts: 70 ± 12 (M); Remainders 144 ± 30 (M)	Chouvelon et al. (2008)
Patinopecten sp.	Canada (British Columbia)	0.04–0.4 (M)	Post-spawning period: Remainders 1–2 (R) *Other periods:* kidneys and gonad 0.1–0.5 (R)	Norum et al. (2005)
Pecten alba	Australia (Port Phillip Bay)	–	Soft tissues without edible parts 0.20[a] (M), 0.05–0.55[a](R)	Walker et al. (1982)
P. maximus	NW France (English Channel)	–	Muscle: 0.05 ± 0.02 (M), 0.04–0.07 (R)	Cossa et al. (2002)
P. maximus	NW France (English Channel)	0.07–0.16 (R)	Kidneys: 1.0 ± 0.2 (M), 0.6–1.4 (R)	Present study
Placopecten magellanicus	USA (Atlantic coast)	*<d.l.*	*<d.l.*	Greig et al. (1978)
P. magellanicus	USA (Atlantic coast)	0.75 ± 0.15[a] (M)	–	Palmer and Rand (1977)

M: mean; R: range.
[a] Original data on a wet wt basis were converted to dry wt, using a dry/wet wt ratio of 5 (Greig et al., 1978).

These results demonstrate that *P. maximus* has actually a strong capacity to bioaccumulate Hg from its environment. Therefore, the low Hg concentrations measured in the scallops in the studied area and the lack of significant difference between both sampling sites would rather be related to the chemical speciation of the metal in the Bay of Seine. Inorganic and organic Hg readily complex with organic matter on seawater column particles and on sediment. Such a metal complexation with particulate matter decreases the metal bioavailability to marine organisms in the field (Langston, 1982, 1985), as has been confirmed in laboratory experiments (e.g., Jenne and Luoma, 1977). Complexation of Hg could actually occur in the Bay of Seine, since (1) the metal inputs from the Seine River are mainly entering the Bay as particulate Hg and (2) the difference in Hg contamination status of both sampling sites has been previously shown based on sediment analysis (Cossa et al., 2002). The low bioavailability of Hg is also supported by the analyses regularly carried out on the blue mussel *Mytilus edulis* in the framework of the French RNO (Réseau National d'Observation) programme. Indeed, no differences in Hg concentrations were found in the whole soft tissues of this recognized biodindicator species collected from sampling sites located very close to ours (e.g., Claisse et al., 2001).

In contrast to the whole-body concentrations, Hg concentrations were significantly different between sampling sites in the digestive gland and in the gills of *P. maximus*. For the gills, as expected, scallops from the site close to the estuary showed the highest values whereas, surprisingly, an opposite trend was observed for the digestive gland. These results suggest that different mechanism(s) of incorporation of the metal occur in both sites. As regards to these differences, concentrations found in the digestive gland might be influenced substantially by the metal content of the scallop food whereas the concentrations in the gills are likely rather influenced by dissolved Hg inputs. In order to better understand the results from the field study, radiotracer experiments were carried out to characterize the Hg bioaccumulation in the scallops when exposed via the dissolved and particulate pathways, the latter including food and sediment exposures. Results from the experiments were perfectly consistent with the aforementioned assumptions linking organ concentration in the field and exposure pathway. Indeed, (1) as mentioned above, scallops showed an efficient bioconcentration capacity of dissolved ^{203}Hg (this was particularly obvious in the gills which displayed very high CF and contained more than 70% of the radiotracer taken up during the exposure period); and (2) the digestive gland contained most of the ^{203}Hg following exposure either via the food or sediment (see Table 3).

The retention of the tracer in scallop soft tissues varied according to the exposure pathway, with biological half-lives ranging from 8 to 30 d (i.e., $T_{b1/2\ seawater} = 23 \pm 2$ d, $T_{b1/2\ food} = 8 \pm 1$ d and $T_{b1/2\ sediment} = 30 \pm 4$ d). Although comparable data are scarce in the literature, these $T_{b1/2}$ were shorter than those reported for mussels exposed to Hg via seawater or food (e.g., Fowler et al., 1978). Following exposure via radiolabelled phytoplankton, Hg was absorbed by the scallops with an assimilation efficiency (AE) of 25%. Such low AEs have been previously reported for inorganic Hg in several marine organisms, with values ranging from 5 to 38% (Fowler et al., 1978; Riisgaard and Hansen, 1990; Blackmore and Wang, 2004). In contrast, some studies have shown in different marine taxa, including bivalves, that organic Hg (methyl-Hg) is much more readily bioavailable that inorganic Hg. In particular, AE of methyl-Hg is higher than that of inorganic Hg, by a factor 1.4–4.1 (e.g., Cunningham and Tripp, 1975; Fowler et al., 1978; Mason et al., 1996; Blackmore and Wang, 2004). In addition these studies also showed that methyl-Hg is retained more strongly ($T_{b1/2}$ is 2.7–4.7 times longer) than the inorganic form. These differences in availability and retention of inorganic vs. methyl-Hg are very interesting facts as they could explain some discrepancies that we observed between the field study and the laboratory experiments. Indeed, while it is well known that sediment-associated bacterial flora is able to methylate part of the inorganic Hg (Compeau and Bartha, 1985), our experimental design considered only inorganic Hg (and the relatively short experiment duration prevented any significant methylation in the experimental microcosms).

The most obvious difference between the laboratory and field results was the renal distribution of Hg in the scallops and the subcellular distribution of Hg. In wild scallops of the Bay of Seine, the kidneys showed the highest Hg concentrations, reaching 1000 ng g^{-1} dry wt, i.e., ca. 5 times higher than the concentrations measured in the other organs. In contrast, in the laboratory experiments kidneys displayed a minor role in Hg metabolism, whether during exposure or depuration phases. Regarding subcellular fractioning, field study showed that Hg was generally mainly occurring in the soluble fraction of the cells (except in the kidneys) whereas in laboratory experiments, Hg tended to be mainly distributed in the insoluble subcellular fraction. Obviously, such differences between field and laboratory results could be directly related to a difference in metal speciation between field and laboratory conditions. Alternatively, they could also result from the short duration of the experiments, thereby not allowing for complete metabolisation of Hg in some organs and hence leading to the detection of incompletely equilibrated situations.

Few studies have reported Hg concentrations in scallops tissues and, to the best of our knowledge, there are no data in the open literature reporting Hg concentrations in digestive gland, gills and kidneys at the same time. In order to have a rapid overview of Hg-related information, available data have been compiled and presented in Table 5. Examination of these data indicates that, as previously mentioned, the role of gills in Hg bioaccumulation appears quite obvious for different scallop species such as *Adamussium colbecki*, *Chlamys hastaste* or *Patinopecten sp.* (Bargagli et al., 1998; Norum et al., 2005). In the Pacific scallop *Patinopecten* sp., as for *P. maximus* in our study, the highest Hg concentrations were reported for kidneys (500 ng g^{-1} dry wt), except in the post-spawning period during which the remaining tissues displayed higher values (from 1 to 2 μg g^{-1} dry wt) (Norum et al., 2005).

The subcellular partitioning of Hg in the different organs and tissues was very similar among the different age classes of scallops collected in the field, suggesting that Hg storage and detoxification are governed by similar processes all along the scallop life span. However, there was a well-marked difference in Hg levels and partitioning between soluble and insoluble subcellular fractions in the kidneys compared to other organs. Indeed, as mentioned before, *P. maximus* kidneys always contained the highest Hg concentrations independently of the site or scallop age class. In addition, whereas the soluble fraction of the cells contained almost 100% of the Hg in the digestive gland and gills, a large fraction of the Hg (i.e., 37–67%) was associated with insoluble cellular compounds in the kidneys, suggesting a difference in Hg detoxification processes occurring in these tissues. In the kidneys of *P. maximus*, several metals such as Cd, Mn or Zn have been shown to bind preferentially to mineral calcium phosphate granules (e.g., George et al., 1980). However, Hg has a stronger affinity for sulphurs than for calcareous concretions and it is therefore very likely that the high proportion of insoluble renal Hg is bound to internal or external membranar compounds rather than to mineral granules. In this respect, lysosomes have been reported to play an important role in Hg detoxification in other bivalve species (e.g., Fowler et al., 1975; Domouhtsidou and Dimitriadis, 2000; Marigómez et al., 2002) and could thus be expected to play a similar role in *P. maximus*.

Association of metals such as Ag, Cu, Cd, Hg, Zn to the soluble subcellular fraction has often been reported as metal linked to

metallothioneins (MTs), for which these elements are well known to have a high affinity (e.g., Kägi, 1991; Temara et al., 1997). To the best of our knowledge, no study was conducted specifically on MTs and Hg in Pectinidae, but several studies have reported that Cd influenced MT or MT-like protein levels in this group (e.g., Fowler and Megginson, 1986; Stone et al., 1986; Viarengo et al., 1993; Ponzano et al., 2001). However, the present study does not seem to support a similar cause–effect relationship between Hg and MT levels in *P. maximus*. Indeed, whereas the gills accumulated Hg very efficiently, they displayed relatively low MT concentrations, suggesting that Hg would not induce specifically MT synthesis in this organ. On the other hand, the MT concentration was high in the digestive gland, but its Hg content was relatively low, which indicates that the metal accumulated in this organ did not induce the production of MTs. Although our results do not preclude that MTs could be involved in Hg detoxification in *P. maximus*, they support that if any, their role would probably be a minor one. More probably, Hg could bind other cytosolic proteins or compounds such as the reduced glutathione (GSH), a thiolic tripeptide (e.g., Kägi and Hapke, 1984) which has been reported to reduce Hg^{2+} effects in the Mediterranean scallop *Pecten jacobeus* (Burlando et al., 1997).

The scallop *P. maximus* is a seafood product that is highly consumed in Europe, particularly in France (Ansell et al., 1991). The bioaccumulation of Hg in scallops could therefore represent a non-negligible risk for the consumers. However, the Hg concentrations in edible tissues (i.e., the adductor muscle and gonad, which contained 39–95 and 64–252 ng Hg g^{-1} dry wt, respectively) were much lower than the safety limits that are recommended for seafood products by the European Community market regulations (i.e., 500 ng total Hg g^{-1} wet wt; EC, 2006). Therefore, the consumption of scallops collected from the Bay of Seine is quite far from representing any risk for Human health.

Previous measurements in *P. maximus* adductor muscles were carried out in the same area 15 years ago (see Cossa et al., 2002) and reported Hg concentrations (40–70 ng g^{-1} dry wt vs. 39–95 ng g^{-1} dry wt in the adductor muscle and gonad, respectively) that matched well the concentration ranges found during our study. This former study has also shown that the metal was mainly present as methyl-Hg in *P. maximus* tissues (between 60 and 83%; Cossa et al., 2002). Based on this inorganic vs. methyl-Hg proportion and considering the Provisional Tolerable Weekly Intake (PTWI) recently reevaluated by FAO and WHO for methyl-Hg (viz. 1.6 μg kg^{-1} of body wt $week^{-1}$; FAO/WHO, 2003), it would be necessary to ingest ca. 7 kg of adductor muscles or 2.8 kg of gonads for a 70-kg adult to reach this PTWI threshold.

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