

# Delineation of Pb contamination pathways in two Pectinidae: the variegated scallop Chlamys varia and the king scallop Pecten maximus

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| 1  | Delineation of Pb contamination pathways in two Pectinidae: the variegated scallop   |  |  |  |  |
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| 2  | Chlamys varia and the king scallop Pecten maximus  |  |  |  |  |
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**Abstract:** Bioaccumulation of Pb was determined in *Chlamys varia* and *Pecten maximus* exposed to  $^{210}$ Pb via seawater, food and sediment. Both scallops readily concentrated dissolved Pb with whole-body 7-d concentration factors of  $250 \pm 40$  and  $170 \pm 70$ , respectively. In both species, more than 70% of Pb taken up from seawater was strongly retained within tissues (biological half-life > 1.5 month) whereas Pb ingested with phytoplankton was poorly assimilated (< 20%). As *P. maximus* lives buried in the sediment, this exposure pathway was assessed and showed low bioaccumulation efficiency for sediment-bound Pb (transfer factor < 0.015). Despite the poor transfer efficiency of Pb from food and sediment, the use of a global bioaccumulation model indicated that the particulate pathway (food and/or sediment) constituted the major bioaccumulation route of Pb in both scallops. Whatever the exposure pathway, the digestive gland and kidneys always played a major role in Pb accumulation. In scallop tissues, Pb was predominantly associated with the insoluble subcellular fraction, suggesting a low bioavailability of Pb for scallop consumers.

**Keywords:** Metal; Uptake kinetics; Retention; Bioavailability; Mollusc

#### 1. Introduction

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Lead (Pb) is widely reported as a contaminant of concern in the marine environment (Laws, 46 47 2000; Clark, 2001). Marine contamination by this non-essential metal is mainly due to 48 releases from anthropogenic activities and the atmosphere constitutes the principal transport 49 vector towards the Oceans (Laws, 2000). Pb is bioaccumulated by living organisms and more 50 particularly by marine invertebrates (e.g., Temara et al., 1998; Neff, 2002). 51 Among filter-feeders, scallops have demonstrated their ability to concentrate elevated levels 52 of various metals in their tissues (e.g., Bryan, 1973; Bustamante and Miramand 2005a,b; 53 Metian et al., 2007, 2008a,b), even in areas far from anthropogenic sources such as the 54 Antarctic Ocean (e.g., Berkman and Nigro, 1992). In particular, scallops were reported to 55 display higher bioaccumulation capacity for Pb than other filter-feeders such as oysters and 56 mussels occurring in the same areas (Brooks and Rumsby, 1965; Segar et al., 1971). 57 Moreover, Pb concentrations in Chlamys varia tissues were shown to reflect the 58 contamination level of their environment (Bustamante and Miramand, 2005a). However, little 59 is known about the metabolism of Pb in pectinids and available information is mainly restricted to baseline Pb concentrations in tissues and organs of scallops from different 60 61 geographic areas, viz. the Antarctic Ocean (Berkman and Nigro, 1992), the Greenland waters 62 (Johansen et al., 2000) and the coastal waters of France, United Kingdom and Canada (Bryan, 63 1973; Ray et al., 1984; Bustamante and Miramand, 2005b). Therefore, the aim of the present work was to investigate the pathway-specific 64 bioaccumulation of Pb in pectinids (viz., water, food and/or sediment pathways). Two 65 pectinid species were considered (the variegated scallop *Chlamys varia* and the king scallop 66 67 Pecten maximus) and bioaccumulation was investigated using highly sensitive radiotracer techniques (<sup>210</sup>Pb) (Warnau and Bustamante, 2007). The bioaccumulation of <sup>210</sup>Pb was 68

studied at the levels of 1) whole individual, 2) tissues and organs and 3) cells of the different tissues, in order to determine the biokinetic parameters of Pb uptake and retention, and the body and subcellular distribution of Pb. In addition, the relative contribution of each exposure pathway to the global Pb bioaccumulation was assessed for both species using a dynamic bioaccumulation model (Landrum et al., 1992; Thomann et al., 1995; Wang et al., 1996; Metian et al., 2008b).

### 2. Materials and methods

# 78 2.1. Sampling

During spring 2005, one hundred variegated scallops, *Chlamys varia*, and seventy king scallops, *Pecten maximus*, were collected on the French Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA diving. They were cautiously transported to IAEA-MEL premises in Monaco and were acclimated to laboratory conditions (constantly aerated open circuit aquarium; flux:  $50 \text{ l h}^{-1}$ , salinity: 36 p.s.u.; temperature:  $17 \pm 0.5 \text{ °C}$ ; pH:  $8.0 \pm 0.1$ ; light/dark cycle: 12 h/12 h) for 6 weeks prior to experiments. During this period, scallops were fed daily an algal mixed diet ( $5 \cdot 10^4 \text{ cell ml}^{-1}$ ) composed of *Isochrysis galbana* and *Skeletonema costatum*.

# 2.2. Radiotracer and counting

The  $^{210}$ Pb radiotracer of high specific activity was purchased from CERCA LEA, France ( $^{210}$ Pb as Pb(NO<sub>3</sub>)<sub>2</sub> in 1M HNO<sub>3</sub>,  $T_{\frac{1}{2}} = 22.3$  years). The tracer was  $\gamma$ -counted (46.5 KeV; 4.05% intensity) using two well-type NaI detectors connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The absolute detection efficiency was  $3.4 \pm 0.2\%$  under the conditions selected. The radioactivity was determined by

comparison with standards of known activity and of appropriate geometries. Measurements were corrected for counting efficiency and physical radioactive decay. Activities of <sup>210</sup>Pb in the samples were much over the detection limits but the counting time was adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al., 2006a).

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#### 2.3. Seawater exposure

Fifteen C. varia and 15 P. maximus (average weight  $\pm$  SD: 29  $\pm$  6 g and 73  $\pm$  6 g, respectively) were placed in a 70-l glass aquarium (0.45-µm filtered seawater, constantly aerated closed circuit aquarium; salinity: 36 p.s.u.; temperature:  $17 \pm 0.5$  °C; pH:  $8.0 \pm 0.1$ ; light/dark cycle: 12 h/12 h) and exposed for 7 d to <sup>210</sup>Pb dissolved in seawater (0.5 kBq l<sup>-1</sup>). No change in pH was detectable after the tracer addition. <sup>210</sup>Pb spike and seawater were renewed twice a day the first two days and then daily in order to keep radioactivity constant in seawater. During each renewal of seawater and spike, the scallops were fed briefly (30 min) S. costatum and I. galbana (5 10<sup>4</sup> cells ml<sup>-1</sup>) in clean seawater. <sup>210</sup>Pb activity in seawater was checked before and after each spike renewal, yielding a time-integrated activity of <sup>210</sup>Pb (0.33)  $\pm 0.14$  kBq l<sup>-1</sup>) (Rodriguez y Baena et al., 2006b). Nine tag-identified scallops of each species were removed at different time intervals to be radioanalyzed alive and then returned to their aquarium. At the end of the 7-d exposure period, 5 individuals of *C. varia* and *P. maximus* (not belonging to the tag-identified batches) were sacrificed and dissected. Shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated and radioanalyzed to assess <sup>210</sup>Pb body distribution. The 10 remaining scallops were placed in non-contaminating conditions (constantly aerated open circuit; flux: 50 l h<sup>-1</sup>; salinity: 36 p.s.u.; temperature: 17 ± 0.5 °C; pH:  $8.0 \pm 0.1$ ; light/dark cycle: 12 h/12 h; daily feeding on S. costatum and I. galbana, 5 10<sup>4</sup> cells ml<sup>-1</sup>) for 36 d (*P. maximus*) or 91 d (*C. varia*). The 9 tag-identified individuals of each species were regularly radioanalyzed to determine the depuration kinetics of <sup>210</sup>Pb. At the end of the depuration period, 4 individuals of *C. varia* and *P. maximus* were collected and dissected into several body compartments as previously described.

When dissections were carried out, subcellular distribution of  $^{210}$ Pb between soluble and insoluble fractions was also investigated, according to the method described in Bustamante and Miramand (2005b). Briefly, a part of digestive gland, gills and adductor muscle 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 and in presence of dithiothreitol as antioxidant agent. The homogenates were centrifuged at 80,000 G for 1 h at 5°C in a Sorvall RC28S ultracentrifuge to separate particle-free supernatant (cytosol; soluble fraction) from the pellet (insoluble fraction). Homogenate aliquots, cytosols, and pellets were then  $\gamma$ -counted.

# 2.4. Food exposure

The Haptophyceae *Isochrysis galbana* was used to study <sup>210</sup>Pb dietary transfer to scallops. Phytoplankton cells were exposed to 1.5 kBq  $I^{-1}$  of <sup>210</sup>Pb during their exponential growth phase (7 d). The cells were then filtrated (1- $\mu$ m mesh size Osmonic filters) when haemotocymeter counting determined a concentration of  $10^7$  cell m $I^{-1}$ . Phytoplankton cells were then resuspended in a 70-1 aquarium where 6 individuals of both *C. varia* and *P. maximus* (average weight  $\pm$  SD:  $22 \pm 4$  g and  $91 \pm 4$  g, respectively) had been placed previously for one week. The <sup>210</sup>Pb content of the radiolabelled *I. galbana* and exposure medium was  $\gamma$ -counted before and after the filtration. Scallops were allowed feeding for 2 h on the radiolabelled *I. galbana* (average concentration of 5 104 cell m $I^{-1}$  among this time). After the feeding period, all scallops were  $\gamma$ -counted and flowing seawater conditions (50 l h $^{-1}$ ) were restored in the aquarium, with daily feeding on *S. costatum* and *I. galbana* (5  $10^4$  cells

ml<sup>-1</sup>). Over the next 8 d, all individuals were regularly radioanalyzed to follow the whole-body depuration kinetics of <sup>210</sup>Pb. All individuals were collected after 8 d of depuration and sacrificed to determine the body distribution of the remaining <sup>210</sup>Pb (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues).

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#### 2.5. Sediment exposure

Sediment was collected in Wimereux (North-Atlantic coast of France). Sediment grain size distribution was determined using a Mastersizer micro (Malvern) and the dry/wet wt ratio was calculated after freeze-drying using a LABCONCO Freezone18. Aerated sediment (8 kg) was dispatched in two 5-1 plastic bottles, spiked with <sup>210</sup>Pb (275 kBq in each bottle) and then constantly agitated for 6 d according to the method described in Danis et al. (2003) and adapted by Metian et al. (2007). After this period, part of the sediment was laid in a 20-1 aquarium to form a continuous, 4-cm height layer. Weakly bound <sup>210</sup>Pb was allowed to leach overnight under flowing seawater (50 l h<sup>-1</sup>). Twelve *P. maximus* (average weight ± SD: 143 ± 3 g) were then placed in the aquarium for 7 d (open circuit, seawater flux: 50 l h<sup>-1</sup>), during which 8 tag-identified individuals were regularly radioanalyzed alive. During the exposure period, sediment samples were regularly collected and γ-counted to verify that the <sup>210</sup>Pb activity in sediment remained constant. At the end of the uptake period, 4 P. maximus were collected, weighed and dissected. Separated tissues and organs (shell, digestive gland, kidneys, gills, gonad, adductor muscle and remaining soft tissues) were radioanalyzed to determine the body distribution of <sup>210</sup>Pb. The remaining individuals were transferred for 16 d to a new aquarium (non contaminating conditions; open circuit, seawater flux: 50 l h<sup>-1</sup>) to follow the depuration kinetics of <sup>210</sup>Pb. At the end of the depuration period, 4 scallops were collected and dissected as described above to determine the <sup>210</sup>Pb body distribution. The 4

remaining organisms were dissected grossly (separation of the whole soft parts from the shells) and γ-counted.

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- 171 2.6. Data analysis
- 172 Uptake and depuration kinetics for all exposure pathways were fitted using kinetic models and
- statistical methods as described by Warnau et al. (1996a,b) and Metian et al. (2007). The level
- of significance for statistics and modelling was always set at  $\alpha = 0.05$ .

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# 3. Results

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- 178 3.1. Seawater exposure
- Uptake of <sup>210</sup>Pb in whole-body C. varia and P. maximus followed linear kinetics ( $R^2 = 0.81$
- and 0.93, respectively; Fig. 1A). <sup>210</sup>Pb was accumulated more rapidly in *C. varia* than in *P.*
- 181 maximus ( $k_u = 36$  vs. 24  $d^{-1}$ , respectively; Fig. 1A). Consequently, the whole-body
- 182 concentration factor (CF) of <sup>210</sup>Pb measured at the end of the uptake period (CF<sub>7d</sub>) was higher
- in C. varia (250  $\pm$  40) than in P. maximus (170  $\pm$  70). The CF<sub>7d</sub> calculated for the different
- organs and tissues (Table 1) clearly show that <sup>210</sup>Pb was concentrated differently by the body
- compartments of each species. For C. varia, the highest CF7d were found in the digestive
- gland and kidneys reached (1,490  $\pm$  240 and 1,780  $\pm$  770, respectively). These CF<sub>7d</sub> were not
- significantly different from each other (p > 0.05) but were higher than those of the other body
- 188 compartments. In the case of P. maximus, the kidneys displayed the highest  $CF_{7d}$  (1,890  $\pm$
- 189 1,050).
- 190 In terms of body load, <sup>210</sup>Pb distribution among soft tissues also differed between the two
- species (Table 1). In C. varia, <sup>210</sup>Pb was mainly distributed in the digestive gland (50%) and
- only 15% of the total <sup>210</sup>Pb was found in the kidneys. In contrast, <sup>210</sup>Pb was distributed

similarly ( $p_{\text{Tukey after arcsin transformation}} > 0.05$ ) among the different organs of *P. maximus* at the 193 end of the exposure period: digestive gland, kidneys, gills, gonad, mantle, intestine, adductor 194 muscle all accounted for ~12 to ~30% of the total <sup>210</sup>Pb body load. 195 After the 7-d exposure experiment, non-contaminating conditions were restored and 196 197 depuration kinetics were followed for 91 and 36 d for C. varia and P. maximus, respectively. The whole-body depuration kinetics of <sup>210</sup>Pb were best described by a double exponential 198 model ( $R^2 \ge 0.85$ ; Fig. 1) displaying similar <sup>210</sup>Pb depuration kinetic parameters for both 199 species. The major part of  $^{210}$ Pb ( $A_{01} > 70\%$ ) was efficiently retained ( $T_{b1/2} > 1.5$  month; Fig. 200 201 1A). At the end of the depuration period, the <sup>210</sup>Pb body distribution differed from that observed at 202 203 the end of the exposure period (Table 1). This difference was limited in C. varia: the digestive gland remained the organ containing the highest  $^{210}$ Pb proportion (54 ± 14% of the total body 204 205 load), and the only slight but significant difference (p = 0.028) was found in the gonad (7% at the end of exposure period vs. 4% at the end of the depuration period). In contrast, difference 206 in <sup>210</sup>Pb body distribution between exposure and depuration periods was more pronounced in 207 208 *P. maximus*: the percentage in the kidney increased from  $15 \pm 5\%$  at the end of the uptake 209 period to  $70 \pm 19\%$  at the end the depuration period. This increase was due to (1) a significant increase in  $^{210}$ Pb activity in the kidneys between the two periods (from 620 ± 350 up to 1,390 210  $\pm$  1,110 Bq g<sup>-1</sup> wet wt); and (2) a simultaneous decrease in <sup>210</sup>Pb activity in the other tissues 211 212 (i.e., 76 to 96% according to the body compartment; data not shown). In Figure 2, we show the subcellular distribution of <sup>210</sup>Pb in the digestive gland, gills and 213 214 adductor muscle of both species at the end of the uptake and depuration periods of the seawater experiment. <sup>210</sup>Pb was always mainly present in the insoluble fraction of the cells of 215 216 the considered tissues (> 50%) and increased between the two sampling times (end of uptake and end depuration period), except in the adductor muscle of *C. varia*. 217

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- 219 3.2. Dietary exposure
- The depuration kinetics of <sup>210</sup>Pb ingested with food (the Haptophyceae phytoplankton
- 221 Isochrysis galbana) were best fitted using a double exponential model in both C. varia and P.
- maximus ( $R^2 = 0.94$  and 0.99, respectively; Fig. 1 B). Both species were characterized by a
- rather low assimilation efficiency for  $^{210}$ Pb ingested with food (AE = 6 18%). However,
- once incorporated, <sup>210</sup>Pb was very strongly retained in the tissues of both species, with
- biological half-lives that were virtually infinite (depuration rate constants k<sub>el</sub> not significantly
- different from 0; Fig 1B).
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- 228 3.3. Sediment exposure
- Sediment used in our experiment was mainly composed (95.8%) of grains with a diameter
- 230 ranging from 76 to 302 µm and was characterized by a dry/wet wt ratio of approximately
- 231 0.80. The <sup>210</sup>Pb activity in spiked sediment remained constant all along the exposure period
- 232 (viz.,  $47 \pm 3 \text{ Bq g}^{-1} \text{ wet wt, n} = 21$ ).
- The whole-body uptake kinetics of sediment-bound <sup>210</sup>Pb in *P. maximus* was best fitted by a
- first-order saturation model ( $R^2 = 0.62$ ; Fig. 3) that rapidly reached a plateau (estimated  $TF_{ss} =$
- 235  $0.011 \pm 0.001$ ).
- 236 Among the different body compartments of *P. maximus*, the kidneys displayed the highest
- TF<sub>7d</sub> which values were characterized by a rather high inter-individual variation (TF<sub>7d kidneys</sub> =
- 238  $0.20 \pm 0.20$ ; Table 2). The digestive gland showed the second highest  $TF_{7d}$  (0.17  $\pm$  0.07) and
- contained the highest proportion of the total <sup>210</sup>Pb body load (i.e. 46%) at the end of the
- 240 exposure period (Table 2).
- 241 The <sup>210</sup>Pb whole-body depuration kinetics after exposure via the sediment were best described
- by a bi-exponential model ( $R^2 = 0.54$ ; Fig. 3). A large fraction of the <sup>210</sup>Pb taken up ( $A_{0s} =$

47%) was rapidly lost whereas the remaining  $^{210}$ Pb fraction (A<sub>0l</sub> = 53%) was strongly retained in *P. maximus* tissues, with an estimated T<sub>b½</sub> of 43 d (Fig. 3). When the different tissues were considered separately, the activity levels of  $^{210}$ Pb were near or under the detection limit. Nevertheless,  $^{210}$ Pb activity could be measured accurately in the whole soft tissues and compared to that determined at the end of the uptake period. A significant decrease of  $^{210}$ Pb activity was observed at the end of the depuration period (from  $0.88 \pm 0.19$  down to  $0.14 \pm 0.02$  Bq g<sup>-1</sup> wet wt). After 16 d in non contaminated conditions, only 15% of the initial  $^{210}$ Pb activity remained associated with the whole soft parts (data not shown).

### 4. Discussion

Pectinids are well known to accumulate high levels of metals in their tissues (e.g., Brooks and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2005b; Metian et al., 2007, 2008a,b). However, only few works have focused on Pb levels in scallops (see Table 3). These studies have shown that the concentrations of this metal in the whole soft parts ranged between 0.65 and 16 μg g<sup>-1</sup> dry wt, which are generally higher than those found in other filter-feeders such as oysters and mussels inhabiting the same area (e.g., Brooks and Rumsby, 1965; Segar et al., 1971). It has also been showed that Pb body concentrations in *Chlamys varia* were directly related to the degree of Pb contamination in the surrounding environment (Bustamante and Miramand, 2005b) and that, in a given area, Pb concentrations varied according to the scallop species considered (Bryan, 1973). Therefore it appeared important to investigate Pb uptake and depuration kinetics in scallops exposed via different contamination pathways in order to better understand/characterize the processes driving Pb bioaccumulation in scallops.

When exposed to dissolved Pb, both the variegated scallop C. varia and the king scallop Pecten maximus displayed efficient bioconcentration capacities. C. varia bioconcentrated Pb ca. 50% faster than P. maximus. After 7 days of exposure, uptake kinetics were still in a linear phase and reached relatively elevated concentration factors (CF) for both species ( $CF_{7d} = 250$  $\pm$  40 for C. varia and 170  $\pm$  70 for P. maximus) compared to other organisms such as the mussel Mytillus galloprovincialis (CF<sub>7d</sub>: 150  $\pm$  20; Boudjenoun et al., 2007). The high bioconcentration capacity of the scallops is probably related to their biological characteristics as they have a more efficient filtration system and/or a larger gill surface area than mussels (Beninger and St-Jean, 1997, Ward et al., 1998). Indeed, gills are generally considered as the main entry of dissolved metals in marine filter-feeder organisms (Marigómez et al., 2002). Once taken up from the dissolved phase, most of the Pb  $(A_{0l} > 70\%)$  was strongly retained within the tissues of both species ( $T_{b1/2}$ ) > 1.5 month). These observations are in accordance with previous studies that reported elevated absorption efficiencies ( $A_{0l} \ge 60\%$ ; Fisher et al., 1996) and retention ( $T_{b/d} \ge 23$  d; Boudjenoun et al., 2007) of dissolved Pb in mussels. Such efficient bioconcentration and retention of Pb in C. varia and P. maximus suggests that seawater could play an important role in the global Pb bioaccumulation in scallops. When scallops were exposed to Pb via the food pathway, the metal was poorly assimilated by the two species (AE  $\leq$  18%). The major part of the Pb ingested with phytoplankton (> 80%) was lost within less than 2 days. The Pb AE determined in scallops is much lower than in the mussel Mytilus galloprovincialis fed the phytoplankton Thalassiosira pseudonana (AE = 50%, Fisher et al., 1996). Metian et al. (2008b) have shown that the AE of Ag ingested with food was influenced substantially by the phytoplankton species used as food. However, according to Fisher et al. (1983; 1987), this should not be the case for Pb as these authors have shown that Pb bioaccumulation is rather non specific and invariant with physiological condition of the phytoplankton cells. Therefore the low Pb AE found for both scallop species

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would not be due to the particular phytoplanktonic species used as food but rather to some specificities of the scallops (e.g., differences in digestive processes). Interestingly, even though AE ranged between only 6 and 18%, we found out that dietary transfer may play a major role in the global accumulation of Pb in the scallops (see below). In contrast to C. varia which is living on rocky substrata, P. maximus lives buried into the sediments. When exposed to radiolabelled sediment, uptake of Pb by P. maximus was quite poor (TF<sub>ss</sub> = 0.011). However the assimilated fraction of the sediment-bound metal was retained with a similar biological half-life ( $T_{b/2}$ ) = 43 d) than when taken up via the food (52) d) or seawater (47 d). The granulometry of the sediment used in our study (mostly composed of grain of size from 76 to 302 µm) allows assuming that no ingestion of particles did occur, as the gills, the peribuccal system or the stomach were reported to be efficient in selecting the size of the particles that are actually ingested by the scallops (e.g., Beninger et al., 1991; Brillant et al., 2000). If this hypothesis was to be true, this would suggest that sedimentrelated contamination would have occurred through Pb bioaccumulation from porewater. However, two facts are supporting the counter hypothesis that particles could actually have been ingested. First, some studies have reported the occurrence of large particles (up to 950 µm) in the stomachs of scallops (Mikulich and Tsikhon-Lukanina, 1981; Shumway et al., 1987) and, second, the digestive gland contained 45% of the whole Pb load when scallops were exposed to contaminated sediment. As a porewater exposure pathway should be equivalent to a seawater exposure, one would have expected to find a rather more homogeneous distribution among digestive gland, gills and kidneys, as was observed at the end of the exposure period in our seawater experiment (see Table 1). Hence, although requiring confirmation, ingestion of sediment grains could actually represent a source of Pb exposure for *P. maximus*.

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In order to assess the contribution of the different routes of exposure in global Pb bioaccumulation, the biokinetic parameters determined from our experiments were used along with parameters taken from the literature (distribution coefficient in sediment, concentration factor in phytoplankton, scallops ingestion rate; IAEA, 2004; Metian et al., 2008b) to feed the global bioaccumulation model originally described Thomann (1981). This model was further developed and commonly used by several authors (e.g., Thomann et al., 1995; Wang et al., 1996; Reinfelder et al., 1998; Metian et al., 2008b). Assuming that Pb is distributed in the different compartments of the marine environment (seawater, phytoplankton and sediment) according to the distribution coefficient in sediment ( $K_{d,Pb} = 10^5$ ; ocean margin sediments) and the range of concentration factors in phytoplankton ( $CF_{phyto,Pb} = 10^4 - 10^5$ ) available from the literature (IAEA, 2004), the model computations indicated that the relative contributions of each exposure route were substantially influenced by the CF<sub>phyto,Pb</sub> values. In particular, in C. varia for which only seawater and food exposure pathways were considered, the dietary pathway was found to contribute for 86% of the global Pb bioaccumulation when considering the recommended CF<sub>phyto,Pb</sub> value (10<sup>5</sup>; IAEA, 2004), whereas the dissolved pathway was the dominant pathway (62%) when considering the lowest CF<sub>phyto,Pb</sub> value (10<sup>4</sup>). Lower food contribution when CF<sub>phyto,Pb</sub> increase could be related to a stronger retention of Pb on the phytoplanktonic cell, thus reducing the bioavailaibility of the metal. For the sediment-dweller P. maximus, three different exposure pathways were taken into account (seawater, food and sediment). Model computations showed that the particulate pathway (food and sediment) was always the major source (95 - 97%) of Pb bioaccumulation for P. maximus. However, depending on the CF<sub>phyto,Pb</sub> value, the contributions of food vs sediment pathways shifted from 5 vs 90% (for  $CF_{phyto,Pb} = 10^4$ ) to 33 vs 64% (for  $CF_{phyto,Pb} = 10^5$ ). As available values for CF<sub>phyto,Pb</sub> and K<sub>d,Pb</sub> parameters are essentially average, non-specific values published by the IAEA (2004), it is recommended to further refine these two parameters for environmental

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conditions close to those prevailing in the scallop habitats before further interpreting and extrapolating the results presented here. The kidneys and the digestive gland appeared to play an important role in the uptake and retention of Pb in the tissues of both scallop species. However, these organs were involved differently according to the exposure pathway and the species considered. In scallops collected in the field, the highest Pb concentrations are always reported in the kidneys, when those organs were analysed separately (Table 3). In addition, the kidneys contained more than 50% of the total Pb body burden in Aequipecten opercularis, C. varia and P. maximus (e.g., Bryan, 1973; Bustamante and Miramand, 2005b). As concentrations recorded from the field are supposed to represent a steady-state situation, kidneys may be reasonably considered as organs playing a key role in detoxification and storage of Pb. This is also supported by our experimental observations in *P. maximus*. Indeed, differences in body distribution observed between the end of the seawater uptake and depuration periods suggest that translocation of Pb from different tissues and organs towards the kidneys and/or its storage in these organs occurred in P. maximus (which contained 70% of total whole soft parts Pb body burden one month after the exposure period). In contrast, if any translocations of Pb in C. varia, target organ(s) of those processes would be different than in P. maximus as Pb remained mainly present in the digestive gland all along both exposure and depuration periods. It is known that bivalves take up dissolved Pb mainly through the gills and that the metal is then translocated towards the different organs and tissues via blood granulocytes and high molecular weight plasma ligands (Marigómez et al., 2002). Interspecific variations in Pb concentrations observed in scallops in the field (see Table 3) could thus be related to interspecific differences in translocation mechanisms. In this respect, waterborne Pb would be mainly translocated towards the kidneys (followed by storage) in P. maximus whereas it would be mainly translocated towards the digestive gland in C. varia. It is noteworthy that in field studies,

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renal Pb levels are always much higher in *P. maximus* than in *C. varia*, even for organisms displaying similar whole soft parts Pb concentrations (see Table 3). Nevertheless, it would be worth developing studies on translocation routes to and storage mechanisms in scallop digestive gland and kidneys as it would help greatly in better understanding how Pb as well as other metals are handled and detoxified by these organisms. The results of the subcellular fractioning, in both *C. varia* and *P. maximus*, indicated that Pb was mainly associated with the insoluble cellular fraction, which is consistent with similar fractioning carried out with *C. varia* from the field (Bustamante and Miramand, 2005b). As this insoluble subcellular fraction includes the haemocyte lysosomes which are well-known to play an important role in metal detoxification and storage in bivalves (e.g., Ballan-Dufrançais et al., 1985), further studies should not neglect the role of these free-circulating cells in addressing Pb translocation targetting.

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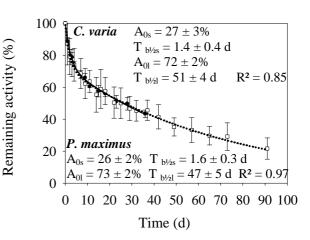
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# A. Seawater experiment

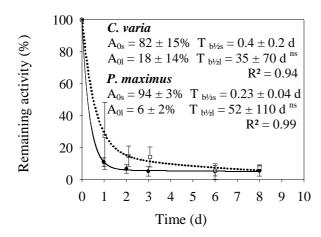
# A1. Uptake kinetics

#### 350 $k_u = 36 \pm 1 d^{-1}$ $R^2 = 0.93$ C. varia 300 Concentration Factor **P.** maximus $k_u = 24 \pm 1 d^{-1}$ $R^2 = 0.81$ 250 200 150 100 50 3 5 8 9 10 0 2 6 Time (d)

# A2. Depuration kinetics



# B. Food experiment



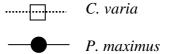
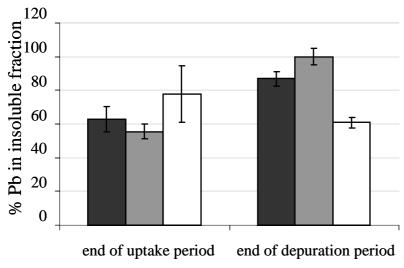


Figure 1. Uptake and depuration biokinetics of  $^{210}$ Pb in the scallops *Chlamys varia* and *Pecten maximus* (A) exposed for 7 d to the radiotracer via seawater (A1. Concentration Factors; mean  $\pm$  SD; n = 9), then maintained for 36 or 91 d in non contaminated conditions (A2. Remaining activity, %; mean  $\pm$  SD; n = 9) and (B) fed for 2 h radiolabelled *Isochrysis galbana* (Remaining activity, %; mean  $\pm$  SD; n = 6).

Estimated parameters.  $k_u$ : uptake rate constant ( $d^{-1}$ );  $A_{0s}$  and  $A_{0l}$ : activity (%) lost according to the short- and the long-lived exponential component, respectively;  $T_{b\frac{1}{2}}$ : biological half-life (d). ns: parameter not significantly different (p > 0.05) from 0 ( $k_{el}$ ) or from the infinite ( $T_{b\frac{1}{2}l}$ ).

# A. Chlamys varia



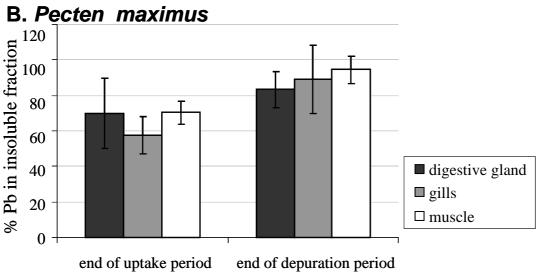
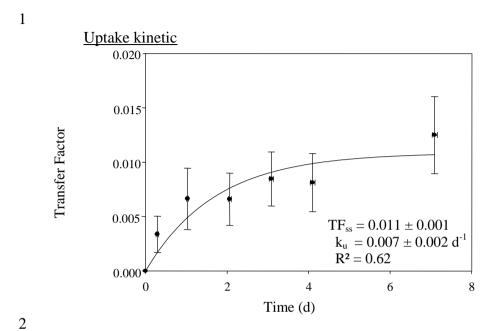


Figure 2. Subcellular distribution (%) of  $^{210}$ Pb in selected tissues (digestive gland, gills and muscle) of *Chlamys varia* (A) and *Pecten maximus* (B) at the end of the 7-d seawater exposure (n = 4 for both species) and of the depuration period (36 d, n = 4 for *P. maximus* and 91 d, n = 4 for *C. varia*) periods.



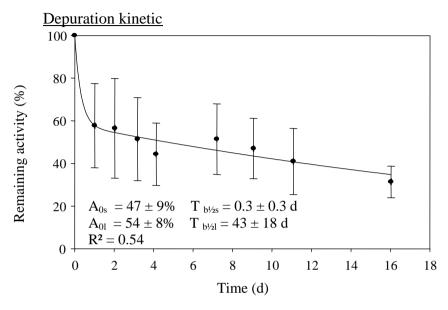


Figure 3. Uptake and depuration biokinetics of  $^{210}\text{Pb}$  in the scallop *Pecten maximus* exposed for 7 d via the sediment (Transfer Factor; mean  $\pm$  SD; n = 8), then maintained for 16 d in non contaminated conditions (Remaining activity, %; mean  $\pm$  SD; n = 8).

Estimated parameters. TF<sub>ss</sub>: transfer factor at steady state; other parameters as in Fig. 1.

Table 1. Concentration Factors (mean  $\pm$  SD) and body distribution among soft parts (mean %  $\pm$  SD) of <sup>210</sup>Pb in the scallops *Chlamys varia* and *Pecten maximus* exposed to the metal via seawater.

|                 | Seawater exposure |              |   |  |  |
|-----------------|-------------------|--------------|---|--|--|
| Compartment     | End of 7-d up     |              | End of depuration period (91 d for <i>C. varia</i> , 36 d for |  |  |
|                 | (n =              | (5)          | P. maximus, n = 4)  |  |  |
|                 | Concentration     | Distribution | Distribution  |  |  |
|                 | Factor            | (%)          | (%)   |  |  |
| Chlamys varia   |                   |              |   |  |  |
| Digestive gland | $1490 \pm 240$    | $50 \pm 6$   | $54 \pm 14$   |  |  |
| Gills           | $250 \pm 240$     | $10 \pm 3$   | $4\pm3$   |  |  |
| Kidneys         | $1780 \pm 550$    | $15 \pm 6$   | $17 \pm 16$   |  |  |
| Intestine       | $490 \pm 680$     | $1 \pm 0$    | $3 \pm 4$   |  |  |
| Gonad           | $410 \pm 140$     | $7 \pm 2$    | $4 \pm 1$   |  |  |
| Foot            | $550 \pm 360$     | $3\pm3$      | $3 \pm 4$   |  |  |
| Mantle          | $45 \pm 11$       | $5 \pm 1$    | $9 \pm 8$   |  |  |
| Adductor muscle | $56 \pm 26$       | $8 \pm 3$    | $4 \pm 2$   |  |  |
| Remaining       | $230 \pm 80$      | $3\pm2$      | $2 \pm 1$   |  |  |
| Whole body      | $250 \pm 40$      |              |   |  |  |
| Pecten maximus  |                   |              |   |  |  |
| Digestive gland | $570 \pm 250$     | $28 \pm 5$   | $12 \pm 8$  |  |  |
| Gills           | $110 \pm 30$      | $22 \pm 7$   | $7 \pm 6$   |  |  |
| Kidneys         | $1890 \pm 1050$   | $15 \pm 5$   | $70 \pm 19$   |  |  |
| Intestine       | $340 \pm 130$     | $2 \pm 1$    | < 1   |  |  |
| Gonad           | $300 \pm 230$     | $4\pm2$      | $5 \pm 4$   |  |  |
| Foot            | $150 \pm 60$      | $1 \pm 1$    | $1 \pm 0$   |  |  |
| Mantle          | $35 \pm 8$        | $14 \pm 4$   | 1 ± 1   |  |  |
| Adductor muscle | $48 \pm 35$       | $12 \pm 5$   | $4 \pm 2$   |  |  |
| Remaining       | $170 \pm 40$      | $4\pm2$      | $1 \pm 0$   |  |  |
| Whole body      | $170 \pm 70$      |              |   |  |  |

Table 2. Transfer Factors (mean  $\pm$  SD, n = 4) and body distribution (mean %  $\pm$  SD, n = 4) of  $^{210}$ Pb in *Pecten maximus* after 7 d of exposure via the sediment.

| Compartment       | Transfer Factor   | Distribution (%) |  |
|-------------------|-------------------|------------------|--|
| Digestive gland   | $0.17 \pm 0.07$   | 46 ± 4           |  |
| Gills             | $0.008 \pm 0.006$ | $5 \pm 5$        |  |
| Kidneys           | $0.20 \pm 0.20$   | $15 \pm 13$      |  |
| Gonad             | $0.05 \pm 0.06$   | $7 \pm 7$        |  |
| Adductor muscle   | $0.003 \pm 0.003$ | $8 \pm 6$        |  |
| Remaining tissues | $0.009 \pm 0.005$ | $18 \pm 5$       |  |
| Whole soft parts  | $0.017 \pm 0.004$ | 100              |  |

Table 3. Concentration of Pb (µg g<sup>-1</sup> dry wt) in different scallop species from various locations.

| Scallop species          | Location                       | Whole soft parts<br>concentration<br>(µg g <sup>-1</sup> dry wt) | Organ displaying highest concentration (µg g <sup>-1</sup> dry wt) | References                       |
|--------------------------|--------------------------------|--|--|----------------------------------|
| Adamussium colbecki      | Antarctica (Explorers cove)    | $2.6 \pm 0.4$  | -  | Berkman and Nigro, 1992          |
| A. colbecki              | Antarctica (Swoya station)     | from 1.0 to 3.2  | -  | Berkman and Nigro, 1992          |
| Aequipecten opercularis  | U.K. (English channel)         | $12.0 \pm 5.2$   | kidneys (827 $\pm$ 236)  | Bryan, 1973                      |
| Chlamys islandica        | Greenland                      | $0.65 \pm 0.12^{a}$  | -  | Johansen et al., 2000            |
| C. nobilis               | Hong Kong markets              | $2.05 \pm 1.35^{a}$  | -  | Fang et al., 2001                |
| C. nobilis               | Guangdong markets              | $1.0 \pm 0.2^{a}$  | -  | Fang et al., 2001                |
| C. varia                 | W France (Ré island)           | $1.91 \pm 0.07$  | kidneys $(28.4 \pm 7.0)$   | Bustamante and Miramand, 2005b   |
| C. varia                 | W France (La Rochelle)         | $2.66 \pm 0.21$  | kidneys $(41.6 \pm 3.7)$   | Bustamante and Miramand, 2005b   |
| Pecten alba              | Australia (Port Phillip bay)   | $14.06 \pm 0.72*$  | gonad $(18.8 \pm 3.9) **$  | Wu and Groves, 1995              |
| P. jacobaeus             | Italy (Middle Adriatic Sea)    | $0.75 \pm 0.30^{a}$  | -  | Storelli and Marcotrigiano, 2001 |
| P. novae-zelandiae       | New Zealand (Tasman bay)       | 16   | kidneys (137)  | Brooks and Rumsby, 1965          |
| P. novae-zelandiae       | New Zealand                    | 6.70 <sup>a</sup>  | stomach (7 a)  | Nielsen and Nathan, 1975         |
| P. maximus               | NW France (W. English channel) | -  | $0.57\pm0.14^{\dagger\dagger\dagger}$                              | Cossa et al., 1990               |
| P. maximus               | NW France (E. English channel) | -  | $0.69 \pm 0.38^{\dagger\dagger\dagger}$                            | Cossa et al., 1990               |
| P. maximus               | U.K. (English channel)         | $2.0 \pm 0.2$  | kidneys $(159 \pm 38)$   | Bryan, 1973                      |
| P. maximus               | Scotland (different locations) | from $< 0.5$ to $5.0^{a}$  | gonad (from $< 1.0 \text{ to } 8.5^{\text{ a}}$ ) **               | Topping, 1973                    |
| P. maximus               | Irish Sea                      | 8.3  | gonad (31) **  | Segar et al., 1971               |
| P. maximus               | Norway (Frøya)                 | $0.6 \pm 0.1^{a}$  | kidneys $(41 \pm 13^{a})$  | Julshamn et al., 2008            |
| Placopecten magellanicus | Canada (George & Browns Banks) | $2.0~^\dagger$   | gills (2.1) ††   | Ray et al., 1984                 |
| P. magellanicus          | U.S.A. (mid-Atlantic coast)    | 3.59   | -  | Pesch et al., 1977               |
| P. magellanicus          | U.S.A. (Atlantic coast)        | <u>-</u>   | gonad (< 19.5 °a) **   | Greig et al., 1978               |

<sup>&</sup>lt;sup>a</sup> Data on a wet wt basis were converted on a dry wt basis, using a conversion factor of 5 (Greig et al., 1978).

<sup>\*</sup> whole soft parts without gonad and adductor muscle.

<sup>\*\*</sup> only adductor muscle and gonad were analyzed separately.

† whole soft parts without adductor muscle, mantle and gills.

†† only adductor muscle, mantle, gills and viscera were analyzed separately.

††† only adductor muscle were analyzed separately.