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1 **Investigation of Ag in the king scallop *Pecten maximus***
2 **using field and laboratory approaches**

3
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20

21

22 **Abstract**

23

24 The bioaccumulation, tissue and subcellular distributions of Ag were investigated in the
25 king scallop *Pecten maximus* from the Bay of Seine fishery area (France) in laboratory and in
26 field conditions. Experimental investigations with the radiotracer ^{110m}Ag showed that the
27 scallop readily concentrated Ag when exposed via seawater and to a much lower extent when
28 exposed via sediment. Retention of the metal incorporated via all tested contamination
29 pathways was shown to be very strong, but the assimilation efficiency of Ag ingested with
30 food was found to be tightly depending on the phytoplankton strain used to feed the scallops
31 (74 and 33% with *Skeletonema costatum* and *Isochrysis galbana*, respectively). The uptake
32 and depuration kinetic parameters determined in the laboratory experiments were used to run
33 a global bioaccumulation model. The latter indicated that the major uptake pathway of Ag in
34 *P. maximus* was strongly depending on the food quality. Indeed, when fed the diatom *S.*
35 *costatum* which was characterised by a high affinity for Ag (high distribution constant K_{df})
36 the relative contribution of the feeding pathway reached 98% of the global Ag
37 bioaccumulation. In contrast, when fed *I. galbana* which displayed a lower K_{df} than *S.*
38 *costatum*, dietary Ag was retained to a lesser extent by *P. maximus*, and seawater appeared as
39 the major contributing uptake pathway. In wild scallops collected from reference and
40 contaminated sites, Ag was mainly concentrated in the digestive gland and secondarily in the
41 gills, and was mainly found associated with the insoluble subcellular fraction in all the scallop
42 tissues.

43

44 **Keywords:** Metal; bioaccumulation; exposure pathway; relative contribution; bivalves;
45 Pectinid.

46 1. Introduction

47

48 Inputs of Ag in the marine environment through sewage sludge from the coastal cities is
49 of environmental concern (Sañudo-Wilhelmy and Flegal, 1992; Luoma et al., 1995),
50 especially as this metal is well known to be one of the most toxic elements (e.g., Warnau et
51 al., 1996b; Ratte, 1999). Once in the marine environment, Ag tends to accumulate in the
52 sediments and to concentrate in benthic deposit- and filter-feeders (Bryan, 1985; Luoma et al.,
53 1995). In bivalves, Ag concentrations were shown to be closely related to the contamination
54 degree of their environment (e.g., Young and Jan, 1979; Bustamante and Miramand, 2005)
55 and the occurrence of Ag in these organisms may therefore be used as a valuable proxy of
56 urban contamination (Sañudo-Wilhelmy and Flegal, 1992; Luoma et al., 1995).

57 Among filter-feeders, the Pectinidae are known to accumulate very high concentrations of
58 Ag (e.g., Brooks and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2005; Metian
59 et al., 2008). This accumulation is especially strong in the digestive gland, in which Ag
60 concentrations are generally above $60 \mu\text{g g}^{-1}$ dry wt, without apparent toxic effects (Brooks
61 and Rumsby, 1965; Bryan, 1973; Bustamante and Miramand, 2005). It is well documented in
62 bivalves that Ag is detoxified via binding with thiol groups and is then precipitated in the
63 connective tissues, particularly in the digestive system (e.g., Berthet et al., 1992). Ag is also
64 known to display high affinity for metallothioneins (MTs). As these proteins have been
65 reported to occur in high concentrations in Pectinidae (e.g., Viarengo et al., 1993; Ponzano et
66 al., 2001), MTs could also contribute significantly to Ag detoxification in this group.

67 Overall, although Ag bioaccumulation has been investigated in several bivalves (e.g.,
68 Wang et al., 1996; Reinfelder et al., 1997), little information is available on Ag
69 bioaccumulation and detoxification processes in Pectinidae. In particular, uptake and
70 depuration kinetics of Ag are only documented for one species (the variegated scallop
71 *Chlamys varia*) exposed via seawater (Metayer et al., 1990). In the latter study, the Ag

72 exposure concentrations were very high (1 to 1,000 $\mu\text{g l}^{-1}$) and far higher than concentrations
73 actually found in marine waters, even in heavily contaminated areas (Ratte, 1999; Gorsuch et
74 al., 2003). Furthermore, such elevated Ag concentrations resulted in toxic effects in marine
75 bivalves (e.g., Eisler, 1996). Nevertheless, *C. varia* displayed a strong retention capacity for
76 Ag in its tissues (Bustamante and Miramand 2005), where it was stored as Ag sulphide
77 (Martoja et al., 1989).

78 The present work investigated the processes governing Ag bioaccumulation in the king
79 scallop *Pecten maximus*, through both field and laboratory approaches. As a first step, Ag
80 bioaccumulation was assessed in controlled laboratory conditions during experimental
81 exposures via seawater, food or sediment, using the $^{110\text{m}}\text{Ag}$ γ -emitting radiotracer. This
82 approach allowed determining the uptake and depuration kinetic parameters of Ag as well as
83 its tissue and subcellular distributions for each contamination pathway. The parameters were
84 then used to feed a global bioaccumulation model in order to delineate the relative
85 contribution of the three different pathways of exposure. In a complementary field study, wild
86 scallops were collected from a Ag-clean site and a Ag-contaminated site and analysed for
87 their concentrations, and tissue and subcellular distributions in Ag and MTs. Results from
88 both field and laboratory studies were then combined in order to better understand the
89 bioaccumulation of Ag in *P. maximus* as well as to further assess whether *P. maximus* could
90 be used as a reliable bioindicator species of marine Ag contamination.

91

92 **2. Materials and methods**

93

94 *2.1. Laboratory experiments*

95

96 King scallops (*Pecten maximus*) were collected on the Atlantic coast of France (Bay of
97 Seine) and acclimated for 3 weeks to laboratory conditions. During this period, scallops were

98 fed daily a microalgal diet ($5 \cdot 10^4$ cells ml^{-1}) composed of the Bacillariophyceae *Skeletonema*
99 *costatum* and the Prymnesiophyceae *Isochrysis galbana*.

100 Thirteen individuals (mean weight: 208 ± 46 g) were placed in a 50-l aquarium and
101 exposed for 7 d to $0.4 \text{ kBq l}^{-1} \text{ }^{110\text{m}}\text{Ag}$ dissolved in filtered ($0.45 \mu\text{m}$) seawater, according to
102 the methodology described in Warnau et al. (1996c, 1999). At each daily renewal of seawater
103 and spike, the scallops were fed briefly (30 min) *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells ml^{-1})
104 in clean seawater. At the end of this period, 4 scallops were dissected and the remaining
105 scallops were placed in non contaminated, flowing seawater to follow Ag depuration for 36 d.
106 During the depuration period, scallops were fed daily *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells
107 ml^{-1}).

108 Fourteen scallops (mean weight: 118 ± 5 g) were placed in a 50-l aquarium and exposed
109 for 13 d under flowing seawater to sediment previously spiked with $^{110\text{m}}\text{Ag}$ ($31 \text{ Bq }^{110\text{m}}\text{Ag g}^{-1}$
110 wet wt) according to the method described in Danis et al. (2005) and adapted as in Metian et
111 al. (2007). After 13 d, 4 individuals were dissected, and the remaining individuals were placed
112 in depuration conditions for 49 d (clean sediment under flowing seawater, daily feeding on *S.*
113 *costatum* and *I. galbana* at $5 \cdot 10^4$ cells ml^{-1}). The spiked sediment was mainly composed
114 (95.8%) of grain which size ranged from 76 to 302 μm ; its dry/wet wt ratio was 0.80. At the
115 end of the 49-d depuration period, 4 scallops were dissected.

116 Eighteen scallops (mean weight: 170 ± 69 g) were placed in a 50-l aquarium and exposed
117 to radiolabelled food (phytoplankton; $5 \cdot 10^4$ cells ml^{-1}) of contrasting nature in order to
118 encompass the complexity of the phytoplankton diet of scallops (Mikulich and Tsikhon-
119 Lukamina, 1981; Shumway et al., 1987). In this respect, one culture of *S. costatum* (Si-walled
120 diatom) and one of *I. galbana* (naked flagellate) were previously exposed during their
121 exponential growth phase to 5 and 4 $\text{kBq }^{110\text{m}}\text{Ag l}^{-1}$ for 10 and 7 d, respectively, and then
122 separated from the spiked seawater ($1\text{-}\mu\text{m}$ filtration) according to the method described in

123 Metian et al. (2007). Nine scallops were fed *S. costatum* and 9 were fed *I. galbana*. After a 2-
124 h feeding on one or the other phytoplankton strain (pulse-chase feeding method, Warnau et
125 al., 1996c; Metian et al., 2007), all scallops were whole-body γ -counted (see below, end of
126 this section) and then placed in non contaminated, flowing seawater conditions, with daily
127 feeding on *S. costatum* and *I. galbana* ($5 \cdot 10^4$ cells ml^{-1}). Four scallops were dissected after
128 21 d (*S. costatum*) or 16 d (*I. galbana*).

129 Preliminary feeding experiments were also carried out in order to assess the ingestion rate
130 (IR) of both phytoplankton strains by *P. maximus*. In this respect, four batches of three
131 scallops were each placed in a 20-l aquarium (constantly aerated) supplied with $5 \cdot 10^4$ cells
132 ml^{-1} . In each aquarium, three samples of seawater (5 ml) were collected at t_0 and after 30 and
133 60 min. The cell density was counted in each sample under a light microscope, using a Thoma
134 cell, and the difference between final and initial cell densities was converted to IR ($\text{g g}^{-1} \text{d}^{-1}$).

135 During the three experiments (seawater, food and sediment), samples of water and/or
136 sediment were collected twice a day during both exposure and depuration phases and
137 radioanalysed in order to check for any possible variation in $^{110\text{m}}\text{Ag}$ activity in the
138 microcosms (exposure phases) or for any possible $^{110\text{m}}\text{Ag}$ recycling via seawater (exposure
139 phase via sediment and depuration phase for the three pathways). Data obtained during the
140 exposure periods were used to determine the time-integrated activity (Rodriguez y Baena et
141 al., 2006) and to calculate the concentration (seawater exposure) and transfer (sediment
142 exposure) factors (see Metian et al., 2007).

143 In the three experiments (seawater, sediment and food), the dissected scallops were used
144 to determine the distribution of $^{110\text{m}}\text{Ag}$ among the different body compartments (shell,
145 digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining
146 soft tissues). In all experiments, the distribution of $^{110\text{m}}\text{Ag}$ between the soluble and insoluble
147 subcellular fractions was also determined in the digestive gland, according to the method

148 described by Bustamante and Miramand (2005). Briefly, a part of the gland was homogenized
149 individually with a mortar and pestle on ice with 10 ml of 0.02M Tris-HCl buffer at pH 8.6.
150 The homogenates were then centrifuged at 30,000 g for 1 h at 4°C in a Sorvall RC28S
151 ultracentrifuge to separate particle-free supernatant (cytosol; i.e., the soluble fraction) from
152 the pellet (i.e., the insoluble fraction). Aliquots of the homogenates, cytosols, and pellets were
153 radioanalysed using a γ spectrometer (see here below).

154 Biokinetic experiments were carried out using a high-specific activity ^{110m}Ag radiotracer
155 purchased from Isotope Product Lab, Germany (^{110m}Ag as AgNO_3 , $T_{1/2} = 249.8$ d).

156 Radioactivity in live scallops that were radioanalysed daily during uptake and depuration
157 experiments and in dissected and centrifuged samples were counted using a high-resolution γ -
158 spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC
159 33-195-R, Canberra[®] and Eurysis[®]) connected to a multi-channel analyzer and a computer
160 equipped with a spectra analysis software (Interwinner[®] 6).

161 The radioactivity was determined by comparison with standards of known activity and of
162 appropriate geometry. Measurements were corrected for counting efficiency and physical
163 radioactive decay. The counting time was adjusted to obtain a propagated counting error less
164 than 5% (Warnau et al., 1999).

165

166 2.2. Field study

167

168 Ten *P. maximus* were collected by SCUBA diving in 2 different locations the Bay of
169 Seine in July 2004: a site near the estuary (49°35'0" N ; 0°10'0" W) which is known to be
170 contaminated by chemicals, and a reference site (49°33'0" N ; 1°0'0" W) located far from the
171 Seine river mouth in the western part of the Bay (Gilliers et al., 2006).

172 Scallops were frozen on board (-20°C) and dissected immediately upon return to the
173 laboratory in order to separate the digestive gland, gills, kidneys, gonad and adductor muscle;

174 the other tissues and organs constituted the remaining soft tissues. All dissected tissue
175 samples were freeze-dried and grounded to powder. Two aliquots (ca. 150 mg dry wt) of each
176 tissue were treated as described above to separate the soluble and insoluble subcellular
177 fractions according to the method of Bustamante and Miramand (2005).

178 Metallothionein-like protein (MT) concentrations were determined in heat-treated
179 supernatants by differential pulse polarography, which allows quantifying the cysteinic
180 residues (Thompson and Cosson, 1984; Temara et al., 1997). Polarographic measurements
181 were carried out with a PARC Model 174A analyzer and a PARC EG&G Model 303 static
182 mercury drop electrode (SMDE). Standard quantification of MTs was based on rabbit liver
183 metallothionein (Sigma, M-7641) by the standard addition method. MT concentrations in
184 scallop tissues were expressed as $\mu\text{g g}^{-1}$ dry wt.

185 In addition, Ag was analysed in supernatants and pellets after acidic digestion with 4:1
186 (v:v) ultrapure 65% HNO_3 and 70% HClO_4 at 80°C for 2 d. The acids were then evaporated
187 until dryness and the residues were dissolved in 0.3 N ultrapure 65% HNO_3 . Metal analyses
188 were carried out using an atomic absorption spectrophotometer Hitachi Z-5000 with Zeeman
189 background correction. Reference tissues, dogfish liver (DOLT-3, NRCC) and lobster
190 hepatopancreas (TORT-2, NRCC) were treated and analysed in the same way as the samples.
191 Results were in good agreement with the Ag certified values: recoveries ranged from 93 to
192 106% ($n = 10$). Detection limit was $0.005 \mu\text{g Ag g}^{-1}$ dry wt. Ag concentrations in scallop
193 tissues were expressed as $\mu\text{g g}^{-1}$ dry wt.

194

195 2.3. Data analysis

196

197 Uptake and depuration kinetics of the different exposure experiments were fitted using the
198 kinetic models and statistical methods as described by Warnau et al. (1996a,c) and Metian et
199 al. (2007).

200 The relative contribution of each uptake pathway was determined using the global
 201 bioaccumulation model [originally proposed by Thomann \(1981\) and Landrum et al. \(1992\),](#)
 202 [and further used and developed by other authors \(e.g., Thomann et al., 1995; Wang et al.,](#)
 203 [1996; Reinfelder et al., 1998; Metian et al., JEMBE or MEPS\),](#) in which three different uptake
 204 pathways (seawater, food and sediment) were considered. In this model, the introduction of
 205 the term A_{01} allowed considering only the fraction of the metal that was actually incorporated
 206 by the organisms exposed via seawater, sediment and food. The metal concentrations in
 207 sediment and food were calculated using their distribution coefficients (K_d and K_{df} ,
 208 respectively). The concentration in sediment, C_s ($Bq\ g^{-1}$ wet wt) was determined according to
 209 the relation:

$$210$$

$$211 \quad C_s = C_w K_d \quad (\text{eq.1})$$

$$212$$

213 where C_w is the concentration of the radiotracer in experimental spiked seawater ($Bq\ l^{-1}$) and
 214 K_d the Ag distribution coefficient in sediment according to IAEA (2004).

215 The concentration in the phytoplankton strains, C_f ($Bq\ g^{-1}$ wet wt) was determined
 216 according to the relation:

$$217$$

$$218 \quad C_f = C_w K_{df} \quad (\text{eq.2})$$

$$219$$

220 where K_{df} is the Ag distribution coefficient in the phytoplankton strain (*S. costatum* or *I.*
 221 *galbana*) that was measured in our experiments.

222 Total Ag concentration in the organisms (i.e. the sum of each concentration resulting from
 223 the uptake through the different pathways) was defined as:

$$224$$

$$225 \quad C_t = C_{w,ss} + C_{s,ss} + C_{f,ss} \quad (\text{eq.3})$$

Supprimé : developed by Landrum et al. (1992) and reviewed by Thomann et al. (1995) and Wang et al. (1996)

226
 227 where $C_{w,ss}$, $C_{s,ss}$ and $C_{f,ss}$ are the steady-state metal concentrations ($Bq\ g^{-1}$) in scallops
 228 resulting from uptake via seawater, sediment and food, respectively. The latter were computed
 229 according to the relations:

230
 231
$$C_{w,ss} = (A_{0l,w} k_{u,w} C_w) / k_{e,w} \quad (\text{eq.4})$$

232
 233
$$C_{s,ss} = (A_{0l,s} k_{u,s} C_s) / k_{e,s} \quad (\text{eq.5})$$

234
 235
$$C_{f,ss} = (AE\ IR\ C_f) / k_{e,f} \quad (\text{eq.6})$$

236
 237 where A_{0l} is the metal fraction (%) strongly retained in scallop tissues, k_u is the uptake arte
 238 constant (d^{-1}), k_e is the depuration rate constant (d^{-1}), AE is the metal assimilation efficiency
 239 from food (i.e., $A_{0l,f}$; %), and IR is the ingestion rate ($g\ g^{-1}d^{-1}$), and where w, s, and f
 240 subscripts refer to seawater, sediment and food as exposure source, respectively.

241 The relative contributions of the three pathways (dissolved, sediment and food) were then
 242 assessed according to the following relations:

243
 244
$$\%_{\text{seawater}} = C_{w,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}) \quad (\text{eq.7})$$

245
 246
$$\%_{\text{sediment}} = C_{s,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}) \quad (\text{eq.8})$$

247
 248
$$\%_{\text{food}} = C_{f,ss} / (C_{f,ss} + C_{s,ss} + C_{w,ss}). \quad (\text{eq.9})$$

249

250 In the field study, one-way ANOVA was used to test for differences among Ag and MT
251 concentration data using the corresponding routines in the software Statistica[®] 6.1. Level of
252 significance for the statistical analyses was always set at $\alpha = 0.05\%$.

253

254 3. Results

255

256 3.1. Laboratory experiments

257

258 3.1.1 Exposure via seawater

259

260 The whole-body uptake kinetics of $^{110\text{m}}\text{Ag}$ in *P. maximus* were best fitted by a first-order
261 saturation exponential equation ($R^2 = 0.71$). The kinetics and the estimates of its parameters
262 and associated statistics are shown in Figure 1a. Although the steady-state was not reached
263 during the time course of the experiment, the concentration factor at steady-state could be
264 estimated with reasonable precision ($\text{CF}_{\text{ss}} = 801 \pm 164$). After 7 d of exposure, the measured
265 $\text{CF}_{7\text{d}}$ was 620 ± 210 in the whole-body individuals, $3,740 \pm 1,020$ in the whole soft parts,
266 $56,600 \pm 13,400$ in the digestive gland and $4,440 \pm 3,110$ in the gills (data not shown). At the
267 end of the uptake period, $^{110\text{m}}\text{Ag}$ was mainly located in the soft tissues ($98 \pm 2\%$ of the total
268 $^{110\text{m}}\text{Ag}$ content), among which the digestive gland contained the major part of the tracer ($71 \pm$
269 7%), followed by the gills ($13 \pm 7\%$) (Fig. 2).

270 During the 36-d depuration period following seawater exposure, the whole-body
271 depuration kinetics of $^{110\text{m}}\text{Ag}$ was best described by a bi-exponential equation ($R^2 = 0.22$; Fig.
272 1a). The results showed that the major part of $^{110\text{m}}\text{Ag}$ was efficiently incorporated ($A_{01} = 82\%$)
273 and strongly retained in the tissues of *P. maximus* (biological half-life not significantly
274 different from infinite). At the end of the depuration period, the body distribution of $^{110\text{m}}\text{Ag}$
275 indicated that the digestive gland stored the main part of the total body $^{110\text{m}}\text{Ag}$ ($80 \pm 8\%$), as

276 previously observed at the end of the uptake phase ($71 \pm 7\%$) (Fig. 2).

277

278

279 3.1.2. Exposure via the sediment

280

281 The whole-body uptake kinetics of ^{110m}Ag bound to sediments were best fitted by a first
282 order saturation exponential equation ($R^2 = 0.81$) that reached a steady-state equilibrium after
283 ca. 10 d of exposure (Figure 1b). $\text{TF}_{13\text{d}}$ *in toto* and in the whole soft parts were 0.22 ± 0.07
284 and 0.69 ± 0.19 , respectively. Among the different organs and tissues, the highest $\text{TF}_{13\text{d}}$ was
285 found for the digestive gland (11.8 ± 4.9), which contained $65 \pm 13\%$ of the whole-body
286 ^{110m}Ag activity (Fig. 2).

287 The whole-body depuration kinetics after exposure via spiked sediment were best
288 described by a mono-exponential equation ($R^2 = 0.64$; Fig. 2). The results indicated that 90%
289 of ^{110m}Ag previously bioaccumulated were efficiently retained, with a biological half-life of
290 40 d. At the end of the 31-d depuration period, the body distribution of ^{110m}Ag clearly showed
291 that the major part of the metal (ca. $66 \pm 8\%$) was distributed in the digestive gland, the latter
292 proportion being not significantly different to that observed at the end of the exposure period
293 (Fig. 2).

294

295 3.1.3. Exposure via the food

296

297 Prior to feeding the scallops with one out of the two selected diets (*Skeletonema costatum*
298 or *Isochrysis galbana* strain), the phytoplankton cultures were exposed to ^{110m}Ag for 7 and 10
299 d, respectively. At the end of the exposure period, the distribution coefficient between
300 phytoplankton and water (K_{df}) was determined in order to assess the efficiency of their
301 affinity for Ag. K_{df} was $6.86 \cdot 10^5$ for *S. costatum* and $4.43 \cdot 10^4$ for *I. galbana*.

302 In addition, the preliminary assessment of the ingestion rate (IR) of phytoplankton
303 indicated that *P. maximus* ingested both *S. costatum* and *I. galbana* with similar ($p = 0.01$)
304 rates, i.e. $IR = 0.0404 \text{ g g}^{-1} \text{ d}^{-1}$.

305 Following the pulse-chase feeding using either *S. costatum* or *I. galbana* as food, the
306 whole-body depuration kinetics of ^{110m}Ag were best fitted using a bi-exponential equation (R^2
307 = 0.46 for *S. costatum* and 0.93 for *I. galbana*) (Fig. 1c). The assimilation efficiency (AE) of
308 ^{110m}Ag ingested with food was higher when scallops were fed *S. costatum* than *I. galbana* (i.e.
309 $AE = 72$ vs. 33%). Furthermore, when ingested with *S. costatum*, the tracer was much
310 strongly retained in *P. maximus* tissues ($T_{b/2l}$ not different from infinite; Fig. 1c1) than when
311 ingested with *I. galbana* ($T_{b/2l} = 11 \text{ d}$; Fig. 1c2). ^{110m}Ag was mainly distributed in the digestive
312 gland ($> 93\%$) with both food sources (Fig. 2).

313

314 3.1.4. Subcellular distribution

315

316 For all three exposure modes, ^{110m}Ag was always mainly associated with the insoluble
317 subcellular fraction of the digestive gland cells (84 to 98%; see Fig. 2).

318

319 3.1.5. Global bioaccumulation model

320

321 In order to assess the relative contribution of each uptake pathway to the global Ag
322 accumulation in *P. maximus*, the different kinetic parameters obtained for the three exposure
323 modes to Ag (seawater, food, and sediment) were used to feed a global bioaccumulation
324 model (see section 2.3.) along with other parameters such as the distribution coefficient of Ag
325 in sediment ($K_d = 2 \cdot 10^4$; IAEA, 2004), the Ag K_{df} in phytoplankton strains ($K_{df} = 6.86 \cdot 10^5$ for
326 *S. costatum* and $4.43 \cdot 10^4$ for *I. galbana*; present study) and the ingestion rate of phytoplankton
327 in scallops ($IR = 0.0404 \text{ g g}^{-1} \text{ d}^{-1}$ for both strains; present study).

328 Results of the computations are shown in Figure 3. Most interestingly, the phytoplankton
329 strain used as food source strongly influenced the modelling results. In fact, the latter food
330 source was actually driving the outcomes of the modelling and the computations were thus
331 performed considering one or the other phytoplankton strain. With *S. costatum*, the feeding
332 pathway was by far the major contributor (98%) to the global bioaccumulation of Ag in *P.*
333 *maximus* (Fig. 3a), whereas seawater was the main contributor (63%) followed by sediment
334 (28%) when the scallops were fed *I. galbana* (Fig. 3b).

335

336 3.2. Field study

337

338 Concentrations of Ag in the tissues and organs of *P. maximus* collected from the two
339 sampling sites are given in Table 1. Scallops from the contaminated zone showed
340 significantly higher ($p = 0.004$) Ag concentrations in their whole soft tissues than that from
341 the reference site. Such a difference was also significant for the digestive gland ($p = 0.004$),
342 the gills ($p = 0.0001$) and the remaining tissues ($p = 0.004$). In both sites, the highest Ag
343 concentrations and loads were found in the digestive gland and the lowest in the gonad and
344 the adductor muscle (Table 1).

345 In all the tissues and organs analysed, Ag was mainly associated with the insoluble
346 subcellular fraction, which contained from 77% (adductor muscle) up to 97% (digestive
347 gland) of the metal (Table 1). Interestingly, the subcellular distribution of Ag was not
348 significantly different between the two sites for all organs and tissues but the gills ($p = 0.01$)
349 (Table 1).

350 The concentrations in metallothionein-like proteins varied from $750 \mu\text{g g}^{-1}$ dry wt in the
351 adductor muscle up to $7,450 \mu\text{g g}^{-1}$ dry wt in the digestive gland (Table 1). The concentrations
352 were only significantly different between the two sites for the gills ($p = 0.007$), gonad ($p =$

353 0.041) and remaining tissues ($p = 0.013$), with the contaminated site showing the highest
354 values.

355

356

357 **4. Discussion**

358

359 Important Ag releases in the Bay of Seine occur through the Seine River which is one of
360 the most heavily contaminated rivers in Europe (Roux et al., 2001). The average Ag
361 concentrations measured in this study in the whole soft tissues of *Pecten maximus* from the
362 Bay of Seine ranged from 2.6 to 7.2 $\mu\text{g g}^{-1}$ dry wt). The digestive gland, gills and remaining
363 tissues of the scallops collected in the vicinity of the estuary (i.e. the contaminated site)
364 displayed Ag concentrations that were two to ten times higher than those from the reference
365 site (Table 1). This spatial variation of Ag concentrations in scallop tissues has already been
366 reported in other species such as *Chlamys varia*, which was therefore considered as a good
367 biomonitor species for this metal (Bustamante and Miramand, 2005) and thus for urban
368 contamination. Indeed, Ag is generally considered as a reliable proxy for domestic inputs in
369 coastal waters due to Ag-enrichment in the sewage sludge from coastal cities (e.g., Sañudo-
370 Wilhelmy and Flegal, 1992; Luoma et al., 1995; Andren and Bober, 2002).

371 Data on Ag concentrations have been reported for several scallop species such as *Hinnites*
372 *giganteus* (Young and Jan, 1979), *C. varia* (Bustamante and Miramand, 2005),
373 *Comptopallium radula* (Metian et al., 2008), and *P. maximus* (Segar et al., 1971; Bryan,
374 1973). The highest Ag concentrations were generally found in the digestive gland of these
375 scallop species.

376 The digestive gland of the scallops collected at the reference and contaminated sites of the
377 Bay of Seine displayed mean Ag concentrations of 32 and 89 $\mu\text{g g}^{-1}$ dry wet, respectively. In
378 the contaminated site, these digestive gland concentrations were rather elevated in comparison

379 to data from the literature for *P. maximus*. Indeed, mean values previously reported were 8.9
380 and 13.6 $\mu\text{g g}^{-1}$ dry wt from the Irish Sea and from the English Channel, respectively (Segar et
381 al., 1971; Bryan, 1973). However, in other scallop species, values ranged from 30 to 77 $\mu\text{g g}^{-1}$
382 dry wt (Bryan, 1973; Mauri et al., 1990; Bustamante and Miramand, 2004).

383 The gills of *P. maximus* from the contaminated site displayed very high Ag concentrations
384 viz. similar to those measured in the digestive gland from the reference site. This strongly
385 suggests that an important incorporation of the metal occurred through the dissolved pathway.
386 Indeed, this organ is well-known to play a key role in dissolved metal accumulation in marine
387 filter-feeders (Rainbow, 1990). This assumption is well supported by our laboratory
388 experiments showing that *P. maximus* efficiently concentrated waterborne Ag ($\text{CF}_{7\text{d}} > 3,700$
389 in the whole soft parts). Uptake of dissolved Ag might occur in all the soft parts in direct
390 contact with seawater, particularly at sites with high permeability such as the gills (Rainbow,
391 1990). However, in our experiments, most of the radiotracer ($> 70\%$) was found in the
392 digestive gland, which showed very high CF ($> 56,000$), even after a short exposure time of 7
393 d. This strongly suggests that translocation of the metal occurred from the tissues in direct
394 contact with seawater (gills) towards the digestive gland, where the metal would have been
395 detoxified and stored. Interestingly, although uptake of Ag bound to sediment was weak
396 ($\text{TF}_{13\text{d}}$ in whole soft parts = 0.69 ± 0.19), most of the metal was found in the digestive gland
397 after 13 d of exposure ($65 \pm 13\%$). TFs in scallop tissues were far lower (by approximately 3
398 orders of magnitude) than CFs calculated from seawater exposure, indicating that sediment-
399 bound Ag is poorly bioavailable to *P. maximus*. This is consistent with results reported for
400 other bivalve species, for example in oysters of the genus *Crassostrea* (Abbe and Sanders,
401 1990, Ettajani et al., 1992).

402 The major role of the digestive gland in Ag bioaccumulation and detoxification was
403 especially evidenced by results of the trophic transfer experiments. When scallops were fed *S.*
404 *costatum* and *I. galbana*, their digestive gland contained most of the metal remaining at the

405 end of the depuration period (90 and 70%, respectively). However, both the assimilation
406 efficiency (AE, %) and the retention capacity ($T_{b/2}$, d) of the metal strongly depended on the
407 phytoplankton strain used as food source, i.e. 72% and 268 d for *S. costatum* and 33% and 11
408 d, for *I. galbana*. Elevated AEs of Ag ingested with *S. costatum* could be due to the storage of
409 the metal under bioavailable forms in the cytoplasm of the phytoplankton cells (Reinfelder
410 and Fisher, 1991; Wang et al., 1996; Reinfelder et al. 1997). However, the gut residence time
411 that appears to be longer for *S. costatum* than for *I. galbana* (see the much faster initial
412 decrease in depuration kinetics after feeding on *I. galbana*; Fig. 1c) could also influence the
413 metal assimilation efficiency (Wang and Fisher, 1996).

414 Seawater, sediment and food exposures all resulted in important Ag bioaccumulation in
415 the digestive gland of *P. maximus*, in which the metal was always associated mainly with
416 insoluble subcellular compounds whatever the exposure pathway, i.e. from 84 to 98% of the
417 total Ag (see Fig. 2). In wild scallops from the Bay of Seine, Ag was similarly mainly
418 associated with the insoluble subcellular fraction of the digestive gland cells of *P. maximus*
419 collected in both the reference site and the contaminated one. No clear relationships were
420 observed between Ag concentrations or subcellular distribution and MT concentrations (see
421 Table 1). In particular, distribution of Ag in the subcellular soluble fraction (where MTs are
422 located; Thompson and Cosson, 1984) was much higher (2 to 7 times) in adductor muscle
423 than in digestive gland, although the muscular MT content was one order of magnitude lower
424 than in digestive gland. This would suggest that, if any, the MTs are only slightly contributing
425 to Ag sequestration and subcellular distribution in scallop tissues, which is in agreement with
426 previously observations indicating that, in the digestive gland of bivalves, Ag was associated
427 with lysosomes and partially combined with mineral or organic sulphurs within the
428 connective tissues (Bryan, 1973; Ballan-Dufrançais et al., 1985; Berthet et al., 1992).

429 In the natural environment, the three contamination pathways studied here occur and
430 contribute simultaneously to the global bioaccumulation of the metal in the organisms.

431 Through the use of the kinetic parameters that we obtained experimentally, along with
432 published K_d for sediments (IAEA, 2004) and K_{df} and IR that we measured, the delineation of
433 the relative contribution of the three exposure pathways has been assessed, using the model
434 developed by Landrum (1992) that we adapted to take into account the three uptake routes.
435 The results showed that food quality played a major role in determining the major route(s) of
436 Ag accumulation in *P. maximus* (Figure 3). With a Si-walled diatom (*S. costatum*) as food
437 source, the dietary pathway was the main pathway of Ag uptake (98%) whereas when
438 scallops were fed a naked flagellate (*I. galbana*), the major contribution of the metal came
439 from seawater (63%) and secondarily from sediment (28%). The difference in the outcomes
440 of the two runs of the model are due to the higher K_{df} , AE and $T_{b1/2}$ characterising Ag ingested
441 with *S. costatum*. The information available in the literature regarding diet composition of
442 scallops in the wild is scarce. It mainly reports that the diet of wild scallops is complex and
443 can display quite large variations both in space and time (e.g., Mikulich and Tsikhon-
444 Lukamina, 1981; Shumway et al., 1987; Grant and Cranford, 1991). Hence, without proper
445 characterisation of this diet, it still appears rather premature to use the modelling approach to
446 explain the observations from the field. Nevertheless, the bioaccumulation model that we used
447 here allowed considering the sediment pathway separately for the first time. Sediment was
448 expected to be an important pathway of contamination for *P. maximus*, since: 1) it is living at
449 the seawater-sediment interface and thus can accumulate sediment-bound metals either
450 directly by contact with the mantle and tentacles when deployed (filtering posture) or
451 indirectly after desorption and release to the seawater or porewater, 2) it filters and ingests
452 metal-rich particles from both the water column and the sediment surface (Shumway et al.,
453 1987) and 3) sediment generally displays metal concentrations that are several orders of
454 magnitude higher than those reported in seawater (e.g., Luoma, 1989). However, the model
455 clearly showed that sediment had a minor contribution in Ag bioaccumulation in *P. maximus*.

456

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458

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466

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588 **Captions to Figures**

589

590 Fig. 1. Uptake and depuration kinetics of ^{110m}Ag in *Pecten maximus*: (a) seawater pathway
 591 (mean concentration factor \pm SD, $n = 13$ and mean % remaining activity \pm SD, $n = 9$), (b)
 592 sediment pathway (mean transfer factor \pm SD, $n = 14$ and mean % remaining activity \pm SD, n
 593 = 10), and (c) food pathway (mean % remaining activity \pm SD, $n = 9$) via two phytoplankton
 594 strains, (c1) *Skeletonema costatum* and (c2) *Isochrysis galbana*.

595 Uptake parameters. CF_{ss} : concentration factor at steady state; TF_{ss} : transfer factor at steady
 596 state; k_{u} : uptake rate constant (d^{-1}).

597 Depuration parameters (long-lived component). $A_{0\text{l}}$: activity (%) lost according to the long-
 598 lived component; k_{el} : depuration rate constant (d^{-1}); $T_{\text{b}/2\text{l}}$: biological half-life (d), R^2 :
 599 determination coefficient; *: parameters not significantly different ($p > 0.05$) from 0 (k_{el}) or
 600 from the infinite ($T_{\text{b}/2\text{l}}$).

601

602 Fig. 2. ^{110m}Ag distribution (mean % \pm SD; $n = 4$) among the different body compartments of
 603 *Pecten maximus* exposed via: (a) seawater (end of uptake and depuration periods), (b)
 604 sediment (end of uptake and depuration periods) and (c) the food (end of the depuration after
 605 feeding on *Skeletonema costatum* -SKE- and *Isochrysis galbana* -ISO).

606

607 Fig. 3. Relative contribution of the different exposure pathways to global Ag bioaccumulation
 608 in *Pecten maximus* when (a) *Isochrysis galbana* or (b) *Skeletonema costatum* are considered
 609 as food source.

610 Table 1. *Pecten maximus*. Concentration (mean \pm SD; $\mu\text{g g}^{-1}$ dry wt; n = 10), tissue distribution (mean \pm SD; %; n = 10), proportion of Ag
 611 associated to insoluble compounds (mean \pm SD;% ; n = 10) and metallothionein-like protein (MT) concentrations (mean \pm SD; $\mu\text{g g}^{-1}$ dry
 612 wt; n = 10) in scallops from a contaminated and a reference sites in the Bay of Seine.

613

	Ag concentration		Proportion of Ag body		Proportion of insoluble Ag		MT concentration	
	($\mu\text{g g}^{-1}$ dry wt)		burden (%)		(%)		($\mu\text{g g}^{-1}$ dry wt)	
	Contaminated	Reference	Contaminated	Reference	Contaminated	Reference	Contaminated	Reference
Digestive gland	89 \pm 29	32 \pm 11	78 \pm 11	86 \pm 5	94 \pm 3	97 \pm 2 ^{NS}	5,450 \pm 1,200	7,450 \pm 1,200 ^{NS}
Gills	31 \pm 14	3.1 \pm 0.7	18 \pm 10	6 \pm 2	96 \pm 3	92 \pm 2	1,500 \pm 180	1,100 \pm 170
Kidneys	8.5 \pm 3.2	5.7 \pm 2.6 ^{NS}	1 \pm 0	2 \pm 1	86 \pm 4	78 \pm 10 ^{NS}	2,580 \pm 440	2,420 \pm 670 ^{NS}
Gonad	3.5 \pm 1.1	2.4 \pm 1.0 ^{NS}	1 \pm 0	1 \pm 0	87 \pm 3	88 \pm 3 ^{NS}	3,070 \pm 310	2,150 \pm 760
Adductor muscle	0.6 \pm 0.4	0.4 \pm 0.1 ^{NS}	< 1	1 \pm 0	89 \pm 5	77 \pm 13 ^{NS}	830 \pm 40	750 \pm 150 ^{NS}
Remaining tissues	1.4 \pm 0.3	0.7 \pm 0.3	3 \pm 1	4 \pm 3	92 \pm 2	89 \pm 4 ^{NS}	2,000 \pm 110	1,620 \pm 240
Whole soft parts	7.2 \pm 1.8	2.6 \pm 0.7	100	100	-	-	-	-

614 NS: non significant difference between contaminated and reference sites (p > 0.05)

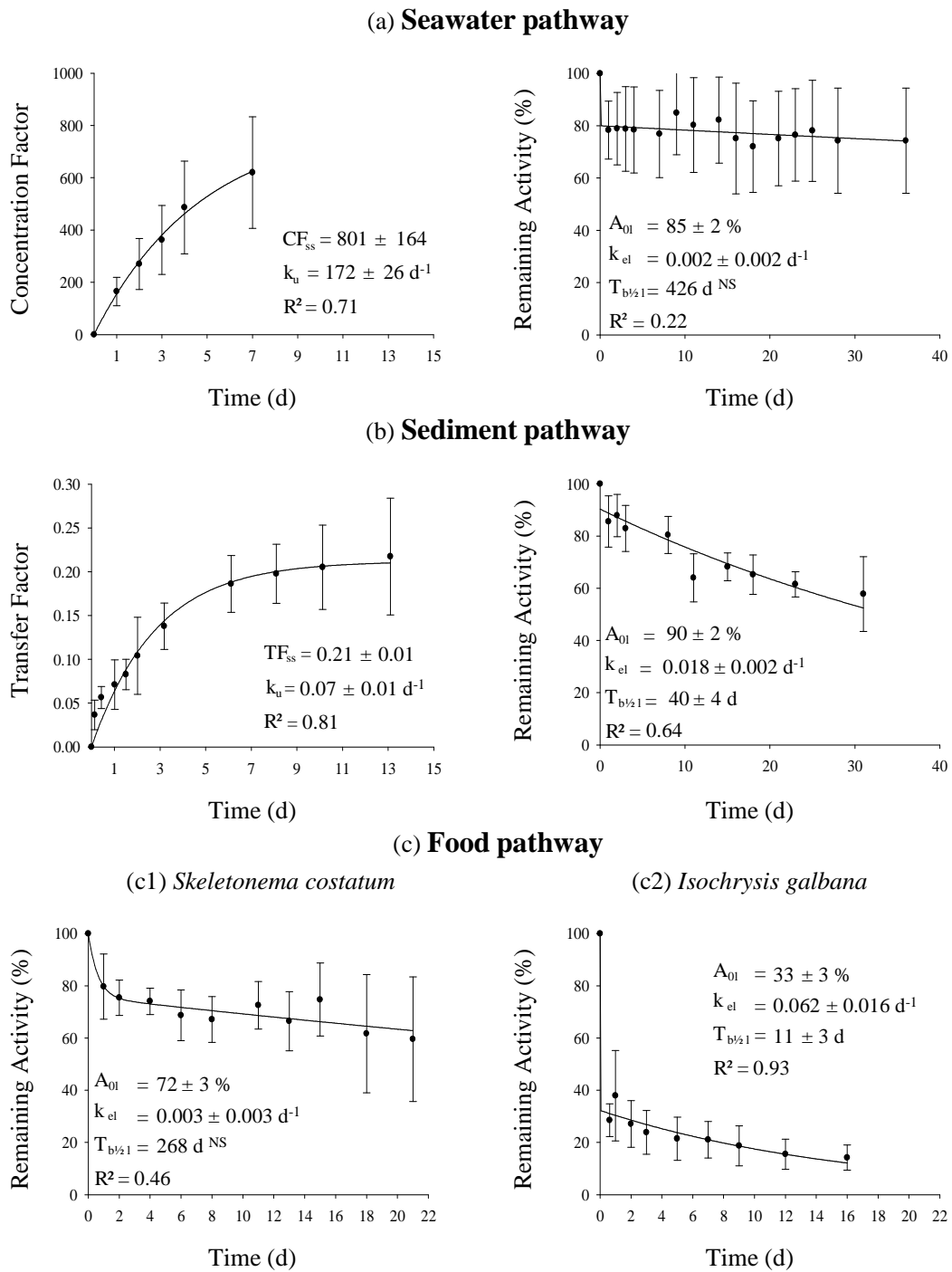
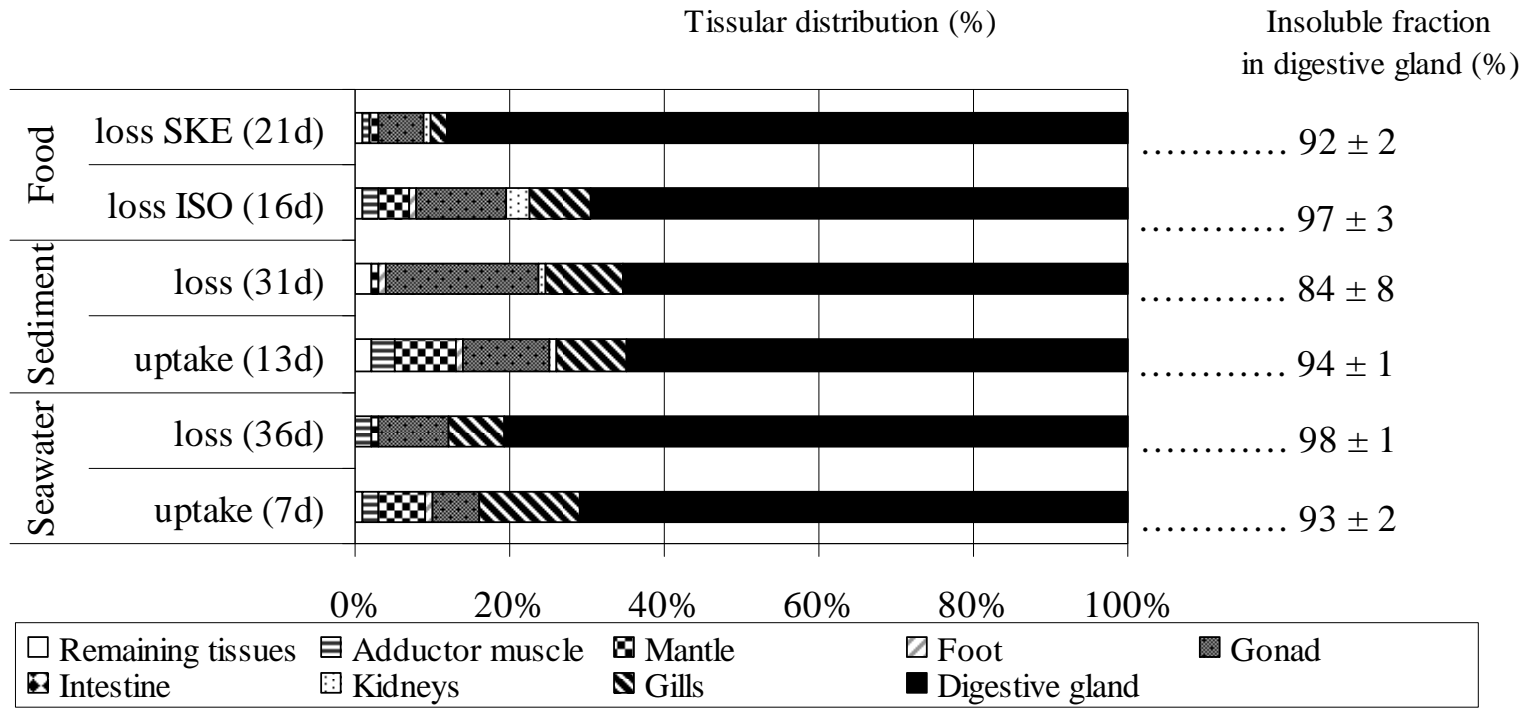
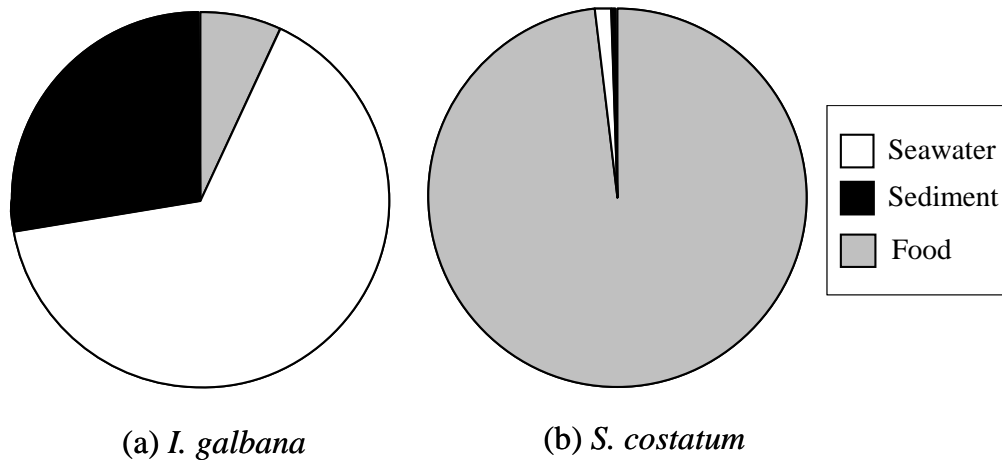


Fig. 1



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