



HAL
open science

Metal subcellular partitioning determines excretion pathways and sensitivity to cadmium toxicity in two marine fish species

Gaël Le Croizier, Camille Lacroix, Sébastien Artigaud, Stéphane Le Floch, Jean-Marie Munaron, Jean Raffray, Virginie Penicaud, Marie-Laure Rouget, Raymond Laë, Luis Tito de Morais

► To cite this version:

Gaël Le Croizier, Camille Lacroix, Sébastien Artigaud, Stéphane Le Floch, Jean-Marie Munaron, et al.. Metal subcellular partitioning determines excretion pathways and sensitivity to cadmium toxicity in two marine fish species. *Chemosphere*, 2019, 217, pp.754-762. 10.1016/j.chemosphere.2018.10.212 . hal-02494936

HAL Id: hal-02494936

<https://hal.science/hal-02494936v1>

Submitted on 16 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Metal subcellular partitioning determines excretion pathways and sensitivity to cadmium toxicity in two marine fish species.

Le Croizier Gaël ^{1,*}, Lacroix Camille ², Artigaud Sébastien ¹, Le Floch Stéphane ²,
Munaron Jean-Marie ¹, Raffray Jean ¹, Penicaud Virginie ¹, Rouget Marie-Laure ³, Laë Raymond ¹,
Tito De Morais Luis ⁴

¹ Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 CNRS/UBO/IRD/IFREMER, BP 70, 29280 Plouzané, France

² Centre of Documentation, Research and Experimentation on Accidental Water Pollution (CEDRE), 715 rue Alain Colas, CS 41836, Brest 29218-Cedex 2, France

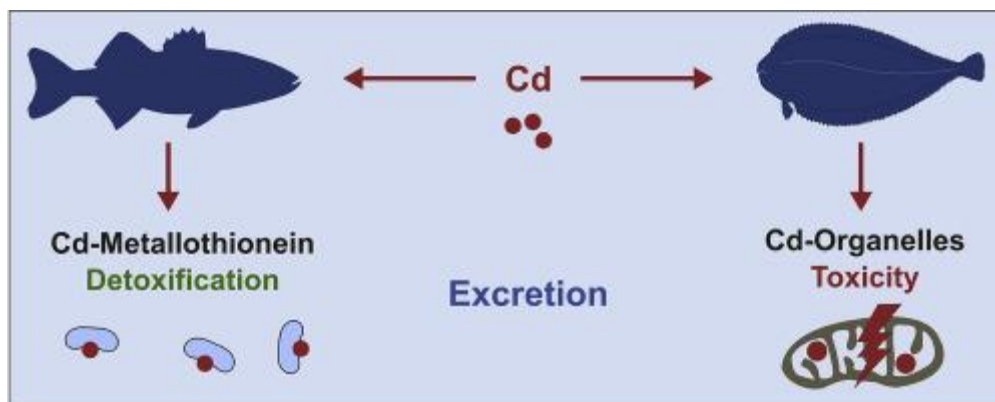
³ Institut Universitaire Européen de la Mer (IUEM), Université de Bretagne Occidentale (UBO), CNRS UMS 3113, 29280 Plouzané, France

* Corresponding author : Gaël Le Croizier, email address : gael.lecroizier@hotmail.fr

Abstract :

Subcellular cadmium (Cd) partitioning was investigated in the liver of two marine fish species, the European sea bass *Dicentrarchus labrax* and the Senegalese sole *Solea senegalensis*, dietary exposed to an environmentally realistic Cd dose for two months followed by a two-month depuration. Cd exposure did not modify Cd cellular partitioning for either species, refuting the spillover hypothesis. Both species contained most of the Cd in the detoxifying fraction but displayed different handling strategies. Cd was largely bound to heat stable proteins (HSP) including metallothioneins (MT) in sea bass while Cd was more linked to metal rich granules (MRG) in sole. Whole liver concentrations and subcellular partitioning were also determined for essential elements. The greatest impairment of essential metal homeostasis due to Cd exposure was found in sole. These elements followed the Cd partitioning pattern, suggesting that they are involved in antioxidant responses against Cd toxicity. Cd consumption diminished sole growth in terms of body weight, probably due to lipid storage impairment. During the depuration period, the two species showed contrasting partitioning patterns, implying different pathways for Cd elimination from the liver. In sea bass, MT-bound Cd would be excreted through bile or released into blood, crossing the cell membrane via a protein transporter. In sole, MRG-bound Cd would be sequestered by organelles before being released into the blood via vesicular exocytosis. These distinct strategies in cellular Cd handling in the liver might account for differential sensitivity to Cd toxicity and differential Cd excretion pathways between the two marine fish species.

Graphical abstract



Highlights

► Sea bass and sole displayed different Cd subcellular partitioning in the liver. ► Cd was largely bound to metallothionein-like proteins in the sea bass liver. ► Cd was mainly linked to metal rich granules in the sole liver. ► Essential metals followed the Cd partitioning pattern in hepatic cells. ► Handling strategies might account for sensitivity and Cd excretion.

Keywords : Sub-cellular fractionation ; *Dicentrarchus labrax* ; *Solea senegalensis* ; Essential element distribution ; Elimination ; Depuration

28 INTRODUCTION

29 Aside from natural sources, marine ecosystems can be subjected to metal contamination
30 from urban effluents and industrial activities. Cadmium (Cd) is a common by-product of the
31 mining industry and can reach high levels in some regions (World Health Organization,
32 2010). Coastal regions in West Africa, which belong to the Canary Current Large Marine
33 Ecosystem (CCLME), are thereby particularly subjected to Cd residue due to the direct
34 release of phosphogypsum into water by the phosphate industry (Auger et al., 2015;
35 Cheggour et al., 1999; Gaudry et al., 2007). Marine consumers such as fish mainly
36 accumulate metals through trophic pathway, which can lead to significant Cd levels in the
37 organs of fish from the CCLME. Moreover, fish species from this region display a wide range
38 of Cd concentrations (Afandi et al., 2018; Diop et al., 2016), resulting partially from
39 differences in foraging habitats and dietary habits (Borrell et al., 2016; Goutte et al., 2015; Le
40 Croizier et al., 2016; Metian et al., 2013). In addition to ecological traits, the observed
41 interspecific variability in terms of Cd bioaccumulation results from different physiological
42 characteristics, including the presence of metal binding proteins like metallothioneins (MT)
43 (Le Croizier et al., 2018; Moulis et al., 2014; Zalups and Ahmad, 2003). Cd is a toxic element
44 responsible for numerous impairments in fish, such as oxidative damage, disruption of
45 essential metal homeostasis, endocrine and ionoregulatory disruption, histopathology and

46 depression of the immune system, which can finally affect growth and survival (McGeer et
47 al., 2011).

48 Recently, attention has been focused on fish species ability to cope with metal toxicity,
49 depending on subcellular partitioning of the element (Eyckmans et al., 2012; Leonard et al.,
50 2014). At the cellular level, Cd can take different toxic chemical forms including: free or
51 complexed ion forms (*e.g.* Cd^{2+} , CdCl_2), bound to enzymes (*e.g.* cytochromes), bound to
52 organic acids (*e.g.* citrates), or bound to cellular constituents causing damages (*e.g.* DNA)
53 (Vijver et al., 2004). Today, only a few forms of Cd speciation are considered as detoxified:
54 Cd complexed to peptides (*e.g.* glutathione) or functional, transport or sequestration
55 proteins (*e.g.* metallothioneins), and Cd trapped in vesicles of the lysosomal system or
56 precipitated in mineral granules (Wang and Rainbow, 2006). Very little is however known
57 about the intracellular mechanisms leading to Cd elimination (Moulis et al., 2014; Zalups and
58 Ahmad, 2003).

59 While one of the adverse effects of toxic metals is their interference with essential elements
60 (Martelli et al., 2006; Moulis, 2010a), some of them confer a protective role against metal
61 toxicity, directly through formation of detoxifying complexes (Sasakura and T. Suzuki, 1998)
62 or by indirectly preventing oxidative stress due to their association with antioxidant enzymes
63 (Martínez-Álvarez et al., 2005; Talas et al., 2008). The significance of essential element
64 subcellular partitioning in their protective role against toxic metals has never yet been
65 considered. Moreover, despite evidence of the influence of cellular components like MT in
66 metal accumulation kinetics in marine species (Wang and Rainbow, 2010), most previous
67 studies only emphasize on the influence of metal intracellular handling on sensitivity of
68 organisms to toxic elements (Campbell et al., 2008; Eyckmans et al., 2012; Giguère et al.,
69 2006; Leonard et al., 2014; Wang and Rainbow, 2006). Very few studies have therefore

70 investigated the link between metal subcellular partitioning and kinetics in fish (Glynn, 1991)
71 and information about the relationships between Cd intracellular handling and excretion
72 pathways is lacking for marine fish.

73 In order to fill these gaps, the present study aimed to investigate Cd intracellular distribution
74 in the liver of two different marine fish species which are naturally present in the CCLME, a
75 region particularly prone to Cd contamination. For this purpose, European sea bass
76 *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis*, were exposed for two months
77 to an environmentally realistic dietary cadmium (Cd) dose followed by a depuration period
78 of a further two months. Analyses were conducted at the end of the uptake period and at
79 the end of the depuration period and Cd partitioning was examined in six major subcellular
80 fractions: cellular debris, metal-rich granules, mitochondria, organelles, cytosolic enzymes,
81 cytosolic proteins and peptides, as well as in the storage lipid fraction of the liver when
82 present. Sensitivity of the two species was assessed according to: (1) Cd subcellular
83 partitioning during accumulation and depuration periods, (2) impairments to essential metal
84 subcellular distribution, (3) essential metal concentrations in the whole liver and their
85 partitioning within hepatocytes, (4) growth and hepatosomatic index calculation. Finally,
86 hypotheses were made regarding the influence of Cd cellular speciation in the liver and Cd
87 biliary excretion or transport to other tissues.

88

89 **MATERIALS AND METHODS**

90 All animal procedures were in accordance with the French and EU guidelines for animal
91 research (project approval number: 03266.03).

92 **Fish and experimental procedures**

93 All details about animal procedures and experimental design can be found in Le Croizier et
94 al., 2018. Briefly, immature sea bass *Dicentrarchus labrax* (length: 14.1 ± 0.7 cm; weight 29.8
95 ± 4.5 g) used in this experiment were obtained from a commercial hatchery (Aquastream,
96 Ploemeur, France), whereas immature Senegalese sole *Solea senegalensis* (length: 14.9 ± 1.1
97 cm; weight 36.4 ± 7.9 g) were provided from a marine farm (Ferme marine de l'Adour,
98 Anglet, France). The fish were transported to the Cedre (Centre of Documentation, Research
99 and Experimentation on Accidental Water Pollution, Brest, France). After receiving
100 anaesthesia by bathing in a $0.05 \text{ mL}\cdot\text{L}^{-1}$ solution of tricaine methanesulfonate (MS-222)
101 (Ackerman et al., 2005), each fish was randomly assigned to one of twelve high density
102 polyethylene tanks that had a 300 L volume (six tanks for each species, 40 sole and 50 sea
103 bass per tank to ensure equivalent biomass) at the Cedre's marine animal facility. This facility
104 is an independent greenhouse that is submitted to a natural photoperiod and supplied with
105 a continuous seawater flow from the bay of Brest. The temperatures in the experimental
106 tanks followed the outdoor temperature. Fish were first acclimated to the experimental
107 conditions for one month, during which they were fed daily with dried commercial pellets
108 (Turbot label rouge 1.4 mm, Le Gouessant Aquaculture).

109 To obtain an environmental relevant Cd concentration in the fish food, a Cd-enriched diet
110 was prepared in order to reach a Cd level around $25 \mu\text{g}\cdot\text{g}^{-1}$, which corresponds to the Cd
111 level reported in potential prey within the natural distribution of the two fish species (Bodin
112 et al., 2013; Maanan, 2008). For this purpose, the commercial pellets were spiked with a 100
113 $\text{mg}\cdot\text{L}^{-1}$ Cd (as CdCl_2 in milliQ water) solution for 30 min. The diet was then placed at -20°C ,
114 freeze dried and broken into small pellets before usage. This preparation led to a Cd
115 concentration of $22.9 \pm 0.3 \mu\text{g}\cdot\text{g}^{-1}$ dw in the Cd-enriched food. The control diet was prepared
116 in the same way, but with the addition of milliQ water only, where the measured

117 background Cd level was $0.71 \pm 0.00 \mu\text{g}\cdot\text{g}^{-1}$ dw. For each of the four conditions (*i.e.*, Cd-
118 exposed and control fish, for both species) there were three replicate tanks. Dietary Cd
119 exposure was initiated by randomly assigning three of the six tanks per species to the Cd-
120 enriched food. The six remaining tanks were fed the control diet. Cd exposure was
121 conducted for 60 days, after which a depuration period was conducted that lasted for 60
122 days. During the depuration period, all of the tanks were fed the control diet. Daily food
123 distribution was performed slowly and continuously over 24 h with a clockwork feeder
124 (COFA, Paris, France) to prevent pellets from remaining in the tank and thus avoid Cd
125 desorption in water. Sea bass were fed at 1.7 % body weight while sole were fed at 1 % body
126 weight per day to meet the physiological requirements of each species (Danion et al., 2011;
127 Salas-Leiton et al., 2010). The bottom of each tank was siphoned every day to avoid Cd
128 leaching from faeces.

129 Physico-chemical parameters (O₂, pH, temperature, salinity) and water quality (nitrates,
130 nitrites) were measured every ten days. During the experiment, the water temperature
131 decreased from 20.7 to 12.8 °C, oxygen saturation increased from 81.2% to 98.2%, pH
132 increased from 8.0 to 8.2 and salinity increased from 35.2 to 35.7. The water was free of
133 nitrate and nitrite (Colorimetric test JBL) in both experiments.

134 **Biological sampling**

135 After the two months of Cd exposure, three fish per tank (nine for each treatment) were
136 anesthetized by bathing in a $0.05 \text{ mL}\cdot\text{L}^{-1}$ solution of MS-222 before being euthanized by
137 bathing in a $0.2 \text{ mL}\cdot\text{L}^{-1}$ solution of MS-222 (Ackerman et al., 2005). They were weighed,
138 measured and dissected with ceramic tools to avoid metal contamination. The liver was
139 collected, weighed and put in acid-washed (10% HNO₃) individual plastic microcentrifuge

140 tubes, flash frozen in liquid nitrogen and stored at -80°C until analysis. Another sampling was
141 made in the same way after a further two months of depuration.

142 **Subcellular partitioning procedure**

143 This procedure generally followed the protocol of Rosabal et al. (2015), adapted from that of
144 Wallace et al. (2003), which has been validated and extensively used on various fish species
145 (Campbell et al., 2008; Eyckmans et al., 2012; Giguère et al., 2006; Lapointe et al., 2009a).
146 Briefly, liver samples were fractionated into six operationally-defined subcellular fractions:
147 cell membranes (cellular debris), metal-rich granules (MRG); mitochondria, microsomes +
148 lysosomes (organelles), heat-denatured proteins (HDP) including cytosolic enzymes, and
149 heat-stable proteins and peptides (HSP) such as metallothionein (MT) and glutathione (GSH).
150 Three liver samples of fish from the same tank were pooled to obtain approximately 200 mg
151 of wet sample and homogenized in 1.5 mL of a solution containing Tris-HCl 20 mmol·L⁻¹ pH
152 8.6, 0.01% DTT (dithiothreitol) as a reducing agent and 1 % of an antiproteolytic agent
153 (Protease Inhibitor Mix, GE Healthcare). A 100 µL aliquot was removed from the liver
154 homogenate for determining total trace metal concentrations in the liver and assessing
155 metal recovery from the subcellular fractions. The remainder of the liver homogenate was
156 centrifuged at 1450×g for 15 min at 4°C. The supernatant (S1) was transferred to an acid-
157 washed 1.5-mL polypropylene microcentrifuge tube for further separations. The pellet from
158 this centrifugation was suspended in 0.5 mL of ultrapure water, heated to 100°C for 2 min,
159 digested with an additional 500 µL of 1 N NaOH at 65°C for 60 min. Centrifugation at
160 10,000×g for 10 min at ambient temperature (~20°C) was performed to separate the NaOH-
161 resistant fraction (referred to as "MRG") from the cellular debris fraction, which includes cell
162 membranes, unbroken cells and nuclei. The S1 supernatant was centrifuged at 10,000×g for

163 30 min at 4°C to produce the mitochondrial fraction. The resulting supernatant was
164 ultracentrifuged at 100,000×g for 60 min at 4°C, giving a pellet containing other organelles
165 (microsomes and lysosomes) and the cytosolic fraction in the supernatant. To separate the
166 heat-stable peptides and proteins (HSP) from the heat-denatured proteins (HDP), the
167 cytosolic fraction was held at 80°C for 10 min, left on ice for 1 h and then centrifuged at
168 50,000×g for 10 min at 10°C. The HSP fraction, which includes MT, was collected from the
169 supernatant. Each fraction was finally kept at -20°C until the metal analyses were performed.

170 **Metal analyses**

171 Subcellular fractions were digested in a mixture of 2 mL 70% HNO₃ and 0.5 mL 30% H₂O₂
172 (both of ultrapure quality) in closed Teflon vessels. Mineralization was performed on a
173 hotplate for 4 h at 100°C. A 100-μL aliquot was removed from the digestate and diluted to 15
174 mL with Milli-Q quality water (Merck Millipore). Cadmium and essential metals (Ca, Cu, Fe,
175 Mg, Mn, Se and Zn) were analysed using an ICP Q-MS (X Series 2, Thermo Scientific) at the
176 Pôle de Spectrométrie Océan (PSO, Plouzané, France) with an internal standard solution of
177 Rhodium (2 μg·L⁻¹). Reference materials (fish protein DORM-4 and dogfish liver DOLT-5,
178 NRCC) were treated and analysed in the same way as the samples. The results for reference
179 materials displayed mean metal recoveries of 85 ± 6 % for DORM-4 and 91 ± 6 % for DOLT-5.
180 Mean recoveries (± SD) of DORM-4 reference samples (n = 3) were 81 ± 2 % for Cd, 78 ± 3 %
181 for Ca, 95 ± 2 % for Mg, 89 ± 5 % for Mn, 86 ± 5 % for Fe, 81 ± 2 % for Cu, 79 ± 1 % for Zn, 92
182 ± 3 % for Se. Mean recoveries (± SD) of DOLT-5 reference samples (n = 3) were 99 ± 2 % for
183 Cd, 83 ± 1 % for Ca, 96 ± 4 % for Mg, 92 ± 4 % for Mn, 98 ± 6 % for Fe, 86 ± 3 % for Cu, 85 ± 2
184 % for Zn, 86 ± 2 % for Se. Blanks were included in each analytical batch. The limits of
185 detection (ng·g⁻¹ dry wt), corresponding to the mean concentration of the blank solutions,

186 were 0.2 (Cd), 1.3 (Ca), 0.4 (Cu), 0.1 (Fe), 0.2 (Mg), 0.01 (Mn), 0.1 (Se) and 0.4 (Zn). Total
187 metal concentrations in the liver ($\mu\text{g}\cdot\text{g}^{-1}$) are given on a dry weight basis ($\mu\text{g}\cdot\text{g}^{-1}$ dw), based
188 on a previous study involving the same fish and where the liver samples were freeze-dried
189 before analysis (Le Croizier et al., 2018). The proportion of metal in each fraction is
190 expressed as a percentage of the sum of the concentrations of all fractions. As it was not
191 present in all of the liver samples, the storage lipid fraction was not taken into account in the
192 calculation of metal partitioning. Metal proportion in this fraction was expressed as a
193 percentage of the total metal concentration in the liver. “Sensitive fractions” gathered the
194 Cd contained in the sensitive fractions (*i.e.* mitochondria + HDP + organelles, while the
195 “metal-detoxified fractions” gathered the Cd contained in the detoxifying fractions (*i.e.* MRG
196 + HSP) (Eyckmans et al., 2012; Leonard et al., 2014). The total metal concentration recovery
197 in the liver from the sum of the metal concentrations measured in all fractions, including
198 lipids, was $95 \pm 11\%$ for sea bass and $96 \pm 6\%$ for sole.

199 **Data analysis**

200 All data tested statistically were first checked for normality (Shapiro–Wilks tests) (Shapiro
201 and Wilk, 1965) and homogeneity of variances (Bartlett tests) (Bartlett, 1937). When these
202 conditions were met, raw data were used and one-way ANOVAs, followed by Tukey’s HSD
203 tests, were performed to test for differences between treatments and species. Otherwise,
204 non-parametric analogues were used, *i.e.* Kruskal-Wallis tests (KW), followed by Conover-
205 Iman multiple comparison tests with Bonferroni’s adjustment (Conover and Iman, 1979). All
206 of the statistical analyses were performed using the open source software R (version 3.4.3, R
207 Core Team, 2017).

208

209 **RESULTS AND DISCUSSION**210 **Impact of Cd elimination on Cd and essential metal partitioning**

211 After being exposed to cadmium, the two-month depuration period led to many changes in
212 metal partitioning (Table 1).

213 First, Cd proportion significantly increased in the organelle fraction (from $37.3 \pm 5.9\%$ to 51.5
214 $\pm 4.8\%$) in sole liver at the end of the depuration period. The organelle fraction includes
215 cellular components involved in the vesicular transport, which is a major pathway for
216 extracellular excretion: Golgi apparatus (GA), endoplasmic reticulum (ER) and lysosomes. ER
217 manages the sequestration of molecules and their binding to excretory proteins, which are
218 transported through the GA and cytosol by vesicles (including lysosomes) and finally
219 excreted across the plasma membrane. Increasing Cd in the organelle fraction during the
220 depuration period may reflect Cd exocytosis via vesicles, which could lead to total Cd
221 elimination from the liver tissue, as described in sole in a previous study (Le Croizier et al.,
222 2018).

223 Second, Cd exposure followed by a depuration period also led to a displacement of essential
224 elements (Fe, Mg, Mn and Zn) from metal rich granules (MRG) to organelles in sole liver (*e.g.*
225 Fe proportion significantly increased from 30.1 ± 2.4 to $41.2 \pm 1.4\%$ in organelles while it
226 significantly decreased from $16.9 \pm 3.2\%$ to $9.2 \pm 2.6\%$ in MRG). In marine organisms, MRG
227 are inorganic insoluble concretions containing mainly Ca phosphate (George et al., 1980), as
228 reflected by the high proportion of Ca in the MRG fraction in both species ($69.3 \pm 4.1\%$ and
229 $32.6 \pm 13.7\%$ for sea bass and sole, respectively, during the depuration period). These
230 structures can play an important role in accumulation and detoxification of metals in fish
231 (Lapointe et al., 2009b; Leonard et al., 2014).

232 The simultaneous increase in proportion of Cd and essential metals in organelles and
233 decrease of essential metals in MRG may be caused by sequestration of MRG by ER, which is
234 part of the organelle fraction (Table 1; Figure 2). This hypothesis seems reasonable since
235 MRG in our species contained the highest proportion of Ca, and ER is deeply involved in Ca
236 storage (Görlach et al., 2006). Supplementing fish diet has furthermore been shown to
237 increase Cd in MRG while decreasing it in organelles (Ng et al., 2009). Although the authors
238 did not draw this conclusion, our hypothesis is that increasing cellular Ca concentration will
239 reduce the probability for Cd bound to calcium granules to be sequestered by ER during Ca
240 uptake. The changes in essential metal cellular repartition would thus be a side effect of Cd
241 transport from MRG to organelles (*i.e.* ER, GA and vesicles, in that order) before final
242 exocytosis (Table 1; Figure 2).

243 Conversely, no change in Cd partitioning was observed in sea bass liver despite effective Cd
244 elimination as well as Cd biliary excretion observed in this species during the depuration
245 period (Le Croizier et al., 2018). In mammals, Cd bound to glutathione (GSH) was shown to
246 be excreted from hepatocytes to bile while Cd bound to metallothionein (MT) was released
247 into the blood before reaching other organs like kidney (Ballatori, 1991; Chan et al., 1993;
248 Klaassen, 1978; Nordberg, 1978). In fish, MT were reported in bile (Hauser-Davis et al., 2012)
249 and were shown to transport metal during biliary excretion in Cu- and Se-exposed fish
250 (Hauser-Davis et al., 2016, 2014). As more than half of the Cd (e.g. $61.8 \pm 5.6\%$ after the
251 depuration period) was bound to HSP regardless of condition in the sea bass (Table 1), this
252 may suggest that Cd complexed to components of the HSP fraction (*i.e.* MTLP or GSH) was
253 excreted directly from cytoplasm to bile or blood without passing through another cellular
254 compound (Figure 2).

255 This elimination of Cd-saturated MT may lead to a new pool of free MT, which would be
256 available for binding other metals. In accordance with the well-identified large Zn-binding
257 capacities of MT and the competition between Cd and Zn (Maret, 2011; Moulis, 2010b), MT
258 turnover during exocytosis of Cd-MT complexes may have enhanced Zn binding to newly-
259 synthesized MT, as suggested by the significant increasing proportion of Zn (from $21.1 \pm$
260 1.9% to $27.7 \pm 2.1\%$) in the HSP fraction at the end of the depuration period (Table 1).
261 Finally, it should be noted that more disturbances in metal homeostasis were observed in
262 the sole liver following Cd exposure (*i.e.* changes in Fe, Mg, Mn and Zn partitioning; Table 1),
263 indicating a greater sensitivity to Cd toxicity by interference with essential elements in this
264 species than in sea bass, which only showed a modification in the Zn distribution.

265 **Links between subcellular Cd handling and Cd toxicity in the two studied species**

266 While more than half of the Cd ($61.8 \pm 5.6\%$) was bound to HSP in the sea bass after the
267 depuration period, Cd partitioning in sole showed a significantly greater Cd pool in the
268 sensitive fractions ($74.3 \pm 6.2\%$) compared with the detoxifying fractions ($13.3 \pm 4.0\%$). The
269 different storage strategies imply that different supposed paths for Cd exocytosis, discussed
270 above (*i.e.* direct transport of MT-bound Cd to the membrane in sea bass *versus* passing of
271 MRG-bound Cd through ER and GA before excretion by vesicles in sole), may thus be
272 responsible for a greater sensitivity of sole to Cd toxicity (Figure 2; Figure 3).

273 When present, the storage lipid fraction accounted for a non-negligible part of the
274 internalized Cd in liver ($44.1 \pm 9.8\%$ in control sole and $24.9 \pm 0.5\%$ in control sea bass after
275 the depuration period) (see Supporting Information), highlighting the need to consider this
276 novel fraction in studies on metal subcellular partitioning. Cd is known to stimulate the lipid
277 peroxidation process, defined as oxidative deterioration of polyunsaturated fatty acids and

278 resulting in alteration of cell membranes (Roméo et al., 2000; Viarengo et al., 1989). Cd
279 binding to this fraction may thus cause oxidation of neutral lipids, preventing their use in
280 membrane structure. Cd was also shown to reduce lipid storage efficiency, increasing
281 utilisation of triglycerides, which finally led to a lower growth in terms of body weight in
282 exposed fish (Pierron et al., 2007). Although no growth impairment was observed according
283 to length measurement or HSI calculation, a significant weight decrease was found in Cd-
284 exposed sole compared with controls (38.8 ± 5.6 g vs. 46.0 ± 7.2 g, respectively) (Figure 5;
285 Table S2). Moreover, no storage lipid fraction was found in the livers of Cd exposed soles
286 (see Supporting Information). In addition to the disturbances in essential metal homeostasis,
287 the greater sensitivity of the sole to Cd toxicity was thus revealed by an alteration of the
288 whole-body condition due to fat consumption.

289 **Essential metal protection against Cd toxicity in the two studied species**

290 One of the main mechanisms for Cd cellular toxicity is the induction of oxidative stress by
291 production of oxygen free radicals (Almeida et al., 2002; Roméo et al., 2000). On the other
292 hand, some elements are essential for the activity of antioxidant enzymes like glutathione
293 peroxidases (GPx), catalases (CAT) and superoxide dismutases (SOD), which contain Se, Fe
294 and Mn-Cu-Zn as cofactors, respectively (Vural et al., 2010). These enzymes are highly
295 involved in preventing oxidative stress in fish and a relative higher level of essential metals
296 may thus provide a better protection against Cd toxicity through higher activity of
297 antioxidant enzymes (Basha and Rani, 2003; Janz, 2011; Martínez-Álvarez et al., 2005). For
298 instance, oxidative stress caused by Cd was reduced by Se treatment in the liver of the
299 rainbow trout (Talas et al., 2008), while a Zn-deficiency was responsible for oxidative stress
300 in the same species (Hidalgo et al., 2002). Besides the well documented role of Se in the

301 detoxification of mercury (Hg) through formation of Hg-Se complexes (Khan and Wang,
302 2009; Pelletier, 1986), it has been suggested that Se could also complex with Cd and
303 subsequently bind to selenoprotein P, thus reducing Cd availability and toxicity (Sasakura
304 and T. Suzuki, 1998; Siscar et al., 2014). The resistance of a species to Cd toxicity may thus
305 depend partially on the level of elements enhancing antioxidant response or able to complex
306 Cd. The two fish species investigated in our study presented some differences in hepatic
307 concentrations of such elements (Figure 3). While sea bass liver contained significantly more
308 Fe ($27.8 \pm 6.6 \mu\text{g}\cdot\text{g}^{-1}$ in sea bass vs. $8.3 \pm 2.5 \mu\text{g}\cdot\text{g}^{-1}$ in sole), sole showed higher
309 concentrations of Cu, Mn and Se (*e.g.* $66 \pm 35.6 \mu\text{g}\cdot\text{g}^{-1}$ of Cu in sole vs. $9.9 \pm 2.0 \mu\text{g}\cdot\text{g}^{-1}$ of Cu in
310 sea bass) (see Supporting Information and Figure 3). Regarding essential metal
311 concentrations, the two species thus seem to possess contrasting defense capabilities,
312 involving different antioxidant enzymes.

313 Despite significantly higher levels of Cd in two sensitive fractions in sea bass ($12 \pm 4.2\%$ in
314 mitochondria and $6.3 \pm 1.8\%$ in cytosolic HDP) compared with sole ($3.8 \pm 1.2\%$ in
315 mitochondria and $0.5 \pm 0.0\%$ in HDP) after Cd exposure, sea bass also contained more
316 antioxidant metals (Cu, Se and Zn) in these fractions (*e.g.* $12.3 \pm 3.7\%$ vs. $3.2 \pm 0.2\%$ of Cu in
317 mitochondria for sea bass and sole, respectively) (Figure 1). Similarly, after depuration, large
318 proportions of Cu, Mg, Mn, Se and Zn were observed alongside the significantly higher Cd
319 proportions in two sensitive fractions ($51.5 \pm 4.8\%$ in organelles and $18.8 \pm 1.1\%$ in cytosolic
320 HDP) in sole compared with sea bass ($15.4 \pm 4.7\%$ in organelles and $9.0 \pm 1.4\%$ in HDP). As
321 the three types of antioxidant enzyme (GPx, CAT and SOD) are all found in cytosol,
322 mitochondria and organelles (Bai et al., 1999; Martínez-Álvarez et al., 2005; Orbea et al.,
323 2000), the fact that essential metals followed Cd distribution in sensitive fractions may

324 reflect their mobilization to activate antioxidant defences and a role in preventing Cd
325 damage to cellular components.

326 **Link between Cd partitioning and hepatic excretion**

327 In a previous study, higher MT concentration combined with higher Cd biliary excretion and
328 relocation to muscle were found in sea bass than in sole, suggesting that MT level would
329 enhance Cd excretion from the liver (Le Croizier et al., 2018). These differences between the
330 two species were attributed to metabolism and/or phylogenetic divergences. Indeed,
331 seabass is characterized by a higher metabolism, which can increase the need for essential
332 metals and thus the need for binding sites such as MT. Moreover, the two species exhibited
333 significant variation in the MT sequence, potentially leading to difference in terms of
334 function. Finally, these evolutionary divergences have probably been accentuated by
335 contrasting ecological niches (*i.e.*, demersal for sea bass *versus* benthic for sole), leading sea
336 bass to be more adapted to manage Cd sequestration in MT compared to sole. Even if Cd
337 was also excreted from sole hepatocytes, Cd elimination seemed higher in sea bass (not
338 significant) since around 60% of the Cd in liver was eliminated in this species after two
339 months of depuration *versus* around 40% in sole (Le Croizier et al., 2018) (Figure S1,
340 Supporting Information). Regarding subcellular partitioning, two main mechanisms were
341 likely to give sea bass a greater Cd elimination from hepatocytes.

342 First, the large proportion of Cd bound to HSP may facilitate biliary excretion via transport of
343 Cd/GSH complexes through a specific transporter of GSH present in the canalicular
344 membrane (Zalups and Ahmad, 2003), or via transport of Cd/MT complexes as suggested by
345 recent observations of MT in bile of metal-exposed fish (Hauser-Davis et al., 2016, 2014,
346 2012) (Figure 2).

347 Second, transport of Cd/MT through plasma membrane in the sea bass may be faster than
348 vesicular exocytosis occurring in sole. Although MT release from various organs to the blood
349 has been proven, the mechanisms for epithelial transport of MT (Chan et al., 1993; Moltedo
350 et al., 2000) and Cd/MT complex transport (Moulis et al., 2014; Sabolić et al., 2010; Zalups
351 and Ahmad, 2003) are unknown. FIHUREby the classical vesicular secretory pathway while
352 transport across membranes must occur directly from the cytoplasm to the exterior of the
353 cell through ATP-binding cassette transporters (ABC transporters) (De Lisle et al., 1996)
354 (Figure 2). Conversely, vesicular exocytosis of MRG-derived Cd in sole must involve
355 successive steps, including fusion of vesicle and plasma membranes, which induces a slower
356 removal of Cd compared to direct excretion in sea bass.

357

358 **CONCLUSIONS**

359 The present study identified two different Cd elimination pathways in European sea bass
360 *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis*, exposed for two months to an
361 environmentally realistic dietary Cd dose. The species-specific strategies imply differences in
362 metal transport and sensitivity to Cd toxicity. In sea bass, Cd was mainly bound to
363 metallothionein-like proteins and would be excreted through bile or released into the blood.
364 In sole, granules-bound Cd would be sequestered by organelles and may thus be responsible
365 for a greater sensitivity of sole to Cd toxicity, revealed by an alteration of the whole-body
366 condition due to fat consumption. These results moreover bring significant novelties
367 demonstrating that MT and MRG are involved in metal elimination from liver, through either
368 biliary excretion or transport to other tissues, as these cell components were so far
369 considered as long term metal storage fractions (Vijver et al., 2004; Wallace et al., 2003;
370 Wang and Rainbow, 2010). Future studies should therefore focus on the membrane

371 transport mechanisms of MT and MRG, to fully understand the implications of elimination
372 pathways on metal retention and sensitivity of marine fish species to toxic elements such as
373 Cd.

374

375 **Acknowledgements**

376 The authors thank greatly Jean Raffray for the dissection of the fish. This study benefited of
377 the technical support of the Centre of Documentation, Research and Experimentation on
378 Accidental Water Pollution (CEDRE). This work was financially supported by the French
379 National Research Agency project ANR-11-CEPL-0005 EPURE.

380

381 **REFERENCES**

- 382 Ackerman, P.A., Morgan, J.D., Iwama, G.K., 2005. Les anesthésiques.
383 Afandi, I., Talba, S., Benhra, A., Benbrahim, S., Chfiri, R., Labonne, M., Masski, H., Laë, R.,
384 Morais, L.T.D., Bekkali, M., Bouthir, F.Z., 2018. Trace metal distribution in pelagic
385 fish species from the north-west African coast (Morocco). *Int Aquat Res* 10, 191–205.
386 <https://doi.org/10.1007/s40071-018-0192-7>
- 387 Almeida, J.A., Diniz, Y.S., Marques, S.F.G., Faine, L.A., Ribas, B.O., Burneiko, R.C.,
388 Novelli, E.L.B., 2002. The use of the oxidative stress responses as biomarkers in Nile
389 tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination.
390 *Environment International* 27, 673–679. [https://doi.org/10.1016/S0160-](https://doi.org/10.1016/S0160-4120(01)00127-1)
391 [4120\(01\)00127-1](https://doi.org/10.1016/S0160-4120(01)00127-1)
- 392 Auger, P.A., Machu, E., Gorgues, T., Grima, N., Waeles, M., 2015. Comparative study of
393 potential transfer of natural and anthropogenic cadmium to plankton communities in
394 the North-West African upwelling. *Science of The Total Environment* 505, 870–888.
395 <https://doi.org/10.1016/j.scitotenv.2014.10.045>
- 396 Bai, J., Rodriguez, A.M., Melendez, J.A., Cederbaum, A.I., 1999. Overexpression of Catalase
397 in Cytosolic or Mitochondrial Compartment Protects HepG2 Cells against Oxidative
398 Injury. *J. Biol. Chem.* 274, 26217–26224. <https://doi.org/10.1074/jbc.274.37.26217>
- 399 Baldisserotto, B., Chowdhury, M.J., Wood, C.M., 2005. Effects of dietary calcium and
400 cadmium on cadmium accumulation, calcium and cadmium uptake from the water,
401 and their interactions in juvenile rainbow trout. *Aquatic Toxicology, Mechanisms in*
402 *Metal Toxicology* 72, 99–117. <https://doi.org/10.1016/j.aquatox.2004.11.019>
- 403 Ballatori, N., 1991. Mechanisms of metal transport across liver cell plasma membranes. *Drug*
404 *Metab. Rev.* 23, 83–132. <https://doi.org/10.3109/03602539109029757>
- 405 Bartlett, M.S., 1937. Properties of sufficiency and statistical tests. *Proc. R. Soc. Lond. A* 160,
406 268–282. <https://doi.org/10.1098/rspa.1937.0109>

- 407 Basha, P.S., Rani, A.U., 2003. Cadmium-induced antioxidant defense mechanism in
408 freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicology and*
409 *Environmental Safety* 56, 218–221. [https://doi.org/10.1016/S0147-6513\(03\)00028-9](https://doi.org/10.1016/S0147-6513(03)00028-9)
- 410 Bodin, N., N’Gom-Kâ, R., Kâ, S., Thiaw, O.T., Tito de Morais, L., Le Loc’h, F., Rozuel-
411 Chartier, E., Auger, D., Chiffolleau, J.-F., 2013. Assessment of trace metal
412 contamination in mangrove ecosystems from Senegal, West Africa. *Chemosphere* 90,
413 150–157. <https://doi.org/10.1016/j.chemosphere.2012.06.019>
- 414 Borrell, A., Tornero, V., Bhattacharjee, D., Aguilar, A., 2016. Trace element accumulation
415 and trophic relationships in aquatic organisms of the Sundarbans mangrove ecosystem
416 (Bangladesh). *Science of The Total Environment* 545–546, 414–423.
417 <https://doi.org/10.1016/j.scitotenv.2015.12.046>
- 418 Campbell, P.G.C., Kraemer, L.D., Giguère, A., Hare, L., Hontela, A., 2008. Subcellular
419 Distribution of Cadmium and Nickel in Chronically Exposed Wild Fish: Inferences
420 Regarding Metal Detoxification Strategies and Implications for Setting Water Quality
421 Guidelines for Dissolved Metals. *Human and Ecological Risk Assessment: An*
422 *International Journal* 14, 290–316. <https://doi.org/10.1080/10807030801935009>
- 423 Chan, H.M., Zhu, L.F., Zhong, R., Grant, D., Goyer, R.A., Cherian, M.G., 1993.
424 Nephrotoxicity in Rats Following Liver Transplantation from Cadmium-Exposed
425 Rats. *Toxicology and Applied Pharmacology* 123, 89–96.
426 <https://doi.org/10.1006/taap.1993.1225>
- 427 Cheggour, M., Langston, W.J., Chafik, A., Texier, H., Idrissi, H., Boumezzough, A., 1999.
428 Phosphate industry discharges and their impact on metal contamination and intertidal
429 macrobenthos: Jorf Lasfar and Safi coastlines (Morocco). *Toxicological &*
430 *Environmental Chemistry* 70, 159–179.
- 431 Conover, W.J., Iman, R.L., 1979. Multiple-comparisons procedures. Informal report (No. LA-
432 7677-MS). Los Alamos Scientific Lab., NM (USA).
- 433 Dallinger, R., Egg, M., Köck, G., Hofer, R., 1997. The role of metallothionein in cadmium
434 accumulation of Arctic char (*Salvelinus alpinus*) from high alpine lakes. *Aquatic*
435 *Toxicology* 38, 47–66. [https://doi.org/10.1016/S0166-445X\(96\)00840-5](https://doi.org/10.1016/S0166-445X(96)00840-5)
- 436 Danion, M., Le Floch, S., Kanan, R., Lamour, F., Quentel, C., 2011. Effects of in vivo chronic
437 hydrocarbons pollution on sanitary status and immune system in sea bass
438 (*Dicentrarchus labrax* L.). *Aquatic Toxicology* 105, 300–311.
439 <https://doi.org/10.1016/j.aquatox.2011.06.022>
- 440 De Lisle, R.C., Sarras, M.P., Hidalgo, J., Andrews, G.K., 1996. Metallothionein is a
441 component of exocrine pancreas secretion: implications for zinc homeostasis. *Am. J.*
442 *Physiol.* 271, C1103-1110.
- 443 Diop, M., Howsam, M., Diop, C., Cazier, F., Goossens, J.F., Diouf, A., Amara, R., 2016.
444 Spatial and seasonal variations of trace elements concentrations in liver and muscle of
445 round Sardinelle (*Sardinella aurita*) and Senegalese sole (*Solea senegalensis*) along the
446 Senegalese coast. *Chemosphere* 144, 758–766.
447 <https://doi.org/10.1016/j.chemosphere.2015.08.085>
- 448 Eyckmans, M., Blust, R., De Boeck, G., 2012. Subcellular differences in handling Cu excess
449 in three freshwater fish species contributes greatly to their differences in sensitivity to
450 Cu. *Aquatic Toxicology* 118–119, 97–107.
451 <https://doi.org/10.1016/j.aquatox.2012.03.019>
- 452 Fan, W., Xu, Z., Wang, W.-X., 2015. Contrasting metal detoxification in polychaetes,
453 bivalves and fish from a contaminated bay. *Aquatic Toxicology* 159, 62–68.
454 <https://doi.org/10.1016/j.aquatox.2014.11.024>
- 455 Gaudry, A., Zeroual, S., Gaie-Levrel, F., Moskura, M., Boujral, F.Z., El Moursli, R.C.,
456 Guessous, A., Mouradi, A., Givernaud, T., Delmas, R., 2007. Heavy Metals Pollution

- 457 of the Atlantic Marine Environment by the Moroccan Phosphate Industry, as Observed
458 through their Bioaccumulation in *Ulva Lactuca*. *Water, Air, & Soil Pollution* 178,
459 267–285.
- 460 George, S.G., Pirie, B.J.S., Coombs, T.L., 1980. Isolation and elemental analysis of metal-rich
461 granules from the kidney of the scallop, *Pecten maximus* (L.). *Journal of Experimental*
462 *Marine Biology and Ecology* 42, 143–156. [https://doi.org/10.1016/0022-](https://doi.org/10.1016/0022-0981(80)90172-0)
463 [0981\(80\)90172-0](https://doi.org/10.1016/0022-0981(80)90172-0)
- 464 Giguère, A., Campbell, P.G.C., Hare, L., Couture, P., 2006. Sub-cellular partitioning of
465 cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*)
466 sampled along a polymetallic gradient. *Aquatic Toxicology* 77, 178–189.
467 <https://doi.org/10.1016/j.aquatox.2005.12.001>
- 468 Glynn, A.W., 1991. Cadmium and Zinc Kinetics in Fish: Studies on Water-Borne ¹⁰⁹Cd and
469 ⁶⁵Zn Turnover and Intracellular Distribution in Minnows, *Phoxinus phoxinus*.
470 *Pharmacology & Toxicology* 68, 485–491. [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-0773.1991.tb01274.x)
471 [0773.1991.tb01274.x](https://doi.org/10.1111/j.1600-0773.1991.tb01274.x)
- 472 Görlach, A., Klappa, P., Kietzmann, D.T., 2006. The Endoplasmic Reticulum: Folding,
473 Calcium Homeostasis, Signaling, and Redox Control. *Antioxidants & Redox*
474 *Signaling* 8, 1391–1418. <https://doi.org/10.1089/ars.2006.8.1391>
- 475 Goutte, A., Cherel, Y., Churlaud, C., Ponthus, J.-P., Massé, G., Bustamante, P., 2015. Trace
476 elements in Antarctic fish species and the influence of foraging habitats and dietary
477 habits on mercury levels. *Science of The Total Environment* 538, 743–749.
478 <https://doi.org/10.1016/j.scitotenv.2015.08.103>
- 479 Harrison, S.E., Curtis, P.J., 1992. Comparative accumulation efficiency of ¹⁰⁹cadmium from
480 natural food (*Hyalella azteca*) and artificial diet by rainbow trout (*Oncorhynchus*
481 *mykiss*). *Bull. Environ. Contam. Toxicol.* 49, 757–764.
482 <https://doi.org/10.1007/BF00200791>
- 483 Hauser-Davis, R.A., Bastos, F.F., Tuton, B., Chávez Rocha, R., Pierre, T.S., Ziolli, R.L.,
484 Arruda, M.A.Z., 2014. Bile and liver metallothionein behavior in copper-exposed fish.
485 *Journal of Trace Elements in Medicine and Biology* 28, 70–74.
486 <https://doi.org/10.1016/j.jtemb.2013.09.003>
- 487 Hauser-Davis, R.A., Gonçalves, R.A., Ziolli, R.L., Campos, R.C. de, 2012. A novel report of
488 metallothioneins in fish bile: SDS-PAGE analysis, spectrophotometry quantification
489 and metal speciation characterization by liquid chromatography coupled to ICP-MS.
490 *Aquatic Toxicology* 116–117, 54–60. <https://doi.org/10.1016/j.aquatox.2012.03.003>
- 491 Hauser-Davis, R.A., Silva, J.A.N., Rocha, R.C.C., Saint’Pierre, T., Ziolli, R.L., Arruda,
492 M.A.Z., 2016. Acute selenium selenite exposure effects on oxidative stress biomarkers
493 and essential metals and trace-elements in the model organism zebrafish (*Danio rerio*).
494 *Journal of Trace Elements in Medicine and Biology* 33, 68–72.
495 <https://doi.org/10.1016/j.jtemb.2015.09.001>
- 496 Hidalgo, M.C., Expósito, A., Palma, J.M., Higuera, M. de la, 2002. Oxidative stress generated
497 by dietary Zn-deficiency: studies in rainbow trout (*Oncorhynchus mykiss*). *The*
498 *International Journal of Biochemistry & Cell Biology* 34, 183–193.
499 [https://doi.org/10.1016/S1357-2725\(01\)00105-4](https://doi.org/10.1016/S1357-2725(01)00105-4)
- 500 Janz, D.M., 2011. 7 - Selenium, in: Chris M. Wood, A.P.F. and C.J.B. (Ed.), *Fish Physiology,*
501 *Homeostasis and Toxicology of Essential Metals*. Academic Press, pp. 327–374.
502 [https://doi.org/10.1016/S1546-5098\(11\)31007-2](https://doi.org/10.1016/S1546-5098(11)31007-2)
- 503 Kamunde, C., MacPhail, R., 2011a. Subcellular interactions of dietary cadmium, copper and
504 zinc in rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 105, 518–527.
505 <https://doi.org/10.1016/j.aquatox.2011.08.005>

- 506 Kamunde, C., MacPhail, R., 2011b. Metal-metal interactions of dietary cadmium, copper and
507 zinc in rainbow trout, *Oncorhynchus mykiss*. *Ecotoxicol. Environ. Saf.* 74, 658–667.
508 <https://doi.org/10.1016/j.ecoenv.2010.10.016>
- 509 Khan, M.A.K., Wang, F., 2009. Mercury-selenium compounds and their toxicological
510 significance: Toward a molecular understanding of the mercury-selenium antagonism.
511 *Environmental Toxicology and Chemistry* 28, 1567–1577. [https://doi.org/10.1897/08-](https://doi.org/10.1897/08-375.1)
512 [375.1](https://doi.org/10.1897/08-375.1)
- 513 Klaassen, C.D., 1978. Effect of metallothionein on hepatic disposition of metals. *Am. J.*
514 *Physiol.* 234, E47-53.
- 515 Kwong, R.W.M., Andrés, J.A., Niyogi, S., 2010. Molecular evidence and physiological
516 characterization of iron absorption in isolated enterocytes of rainbow trout
517 (*Oncorhynchus mykiss*): Implications for dietary cadmium and lead absorption.
518 *Aquatic Toxicology* 99, 343–350. <https://doi.org/10.1016/j.aquatox.2010.05.012>
- 519 Lapointe, D., Gentès, S., Ponton, D.E., Hare, L., Couture, P., 2009a. Influence of Prey Type
520 on Nickel and Thallium Assimilation, Subcellular Distribution and Effects in Juvenile
521 Fathead Minnows (*Pimephales promelas*). *Environ. Sci. Technol.* 43, 8665–8670.
522 <https://doi.org/10.1021/es901929m>
- 523 Lapointe, D., Gentès, S., Ponton, D.E., Hare, L., Couture, P., 2009b. Influence of Prey Type
524 on Nickel and Thallium Assimilation, Subcellular Distribution and Effects in Juvenile
525 Fathead Minnows (*Pimephales promelas*). *Environ. Sci. Technol.* 43, 8665–8670.
526 <https://doi.org/10.1021/es901929m>
- 527 Le Croizier, G., Lacroix, C., Artigaud, S., Le Floch, S., Raffray, J., Penicaud, V., Coquillé,
528 V., Autier, J., Rouget, M.-L., Le Bayon, N., Laë, R., Tito De Morais, L., 2018.
529 Significance of metallothioneins in differential cadmium accumulation kinetics
530 between two marine fish species. *Environmental Pollution* 236, 462–476.
531 <https://doi.org/10.1016/j.envpol.2018.01.002>
- 532 Le Croizier, G., Schaal, G., Gallon, R., Fall, M., Le Grand, F., Munaron, J.-M., Rouget, M.-
533 L., Machu, E., Le Loc'h, F., Laë, R., De Morais, L.T., 2016. Trophic ecology
534 influence on metal bioaccumulation in marine fish: Inference from stable isotope and
535 fatty acid analyses. *Science of The Total Environment* 573, 83–95.
536 <https://doi.org/10.1016/j.scitotenv.2016.08.035>
- 537 Leonard, E.M., Banerjee, U., D'Silva, J.J., Wood, C.M., 2014. Chronic nickel
538 bioaccumulation and sub-cellular fractionation in two freshwater teleosts, the round
539 goby and the rainbow trout, exposed simultaneously to waterborne and dietborne
540 nickel. *Aquatic Toxicology* 154, 141–153.
541 <https://doi.org/10.1016/j.aquatox.2014.04.028>
- 542 Maanan, M., 2008. Heavy metal concentrations in marine molluscs from the Moroccan
543 coastal region. *Environmental Pollution* 153, 176–183.
544 <https://doi.org/10.1016/j.envpol.2007.07.024>
- 545 Maret, W., 2011. Metals on the move: zinc ions in cellular regulation and in the coordination
546 dynamics of zinc proteins. *Biometals* 24, 411–418. [https://doi.org/10.1007/s10534-](https://doi.org/10.1007/s10534-010-9406-1)
547 [010-9406-1](https://doi.org/10.1007/s10534-010-9406-1)
- 548 Martelli, A., Rousselet, E., Dycke, C., Bouron, A., Moulis, J.-M., 2006. Cadmium toxicity
549 in animal cells by interference with essential metals. *Biochimie, Facets of*
550 *Environmental Nuclear Toxicology* 88, 1807–1814.
551 <https://doi.org/10.1016/j.biochi.2006.05.013>
- 552 Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant Defenses in Fish: Biotic
553 and Abiotic Factors. *Rev Fish Biol Fisheries* 15, 75–88.
554 <https://doi.org/10.1007/s11160-005-7846-4>

- 555 McGeer, J.C., Niyogi, S., Scott Smith, D., 2011. 3 - Cadmium, in: Chris M. Wood, A.P.F. and
556 C.J.B. (Ed.), *Fish Physiology, Homeostasis and Toxicology of Non-Essential Metals*.
557 Academic Press, pp. 125–184. [https://doi.org/10.1016/S1546-5098\(11\)31025-4](https://doi.org/10.1016/S1546-5098(11)31025-4)
- 558 Metian, M., Warnau, M., Chouvelon, T., Pedraza, F., Rodriguez y Baena, A.M., Bustamante,
559 P., 2013. Trace element bioaccumulation in reef fish from New Caledonia: Influence
560 of trophic groups and risk assessment for consumers. *Marine Environmental Research*
561 87–88, 26–36. <https://doi.org/10.1016/j.marenvres.2013.03.001>
- 562 Moltedo, O., Verde, C., Capasso, A., Parisi, E., Remondelli, P., Bonatti, S., Alvarez-
563 Hernandez, X., Glass, J., Alvino, C.G., Leone, A., 2000. Zinc Transport and
564 Metallothionein Secretion in the Intestinal Human Cell Line Caco-2. *J. Biol. Chem.*
565 275, 31819–31825. <https://doi.org/10.1074/jbc.M002907200>
- 566 Moulis, J.-M., 2010a. Cellular mechanisms of cadmium toxicity related to the homeostasis of
567 essential metals. *Biometals* 23, 877–896. <https://doi.org/10.1007/s10534-010-9336-y>
- 568 Moulis, J.-M., 2010b. Cellular mechanisms of cadmium toxicity related to the homeostasis of
569 essential metals. *Biometals* 23, 877–896. <https://doi.org/10.1007/s10534-010-9336-y>
- 570 Moulis, J.-M., Bourguignon, J., Catty, P., 2014. CHAPTER 23:Cadmium, in: *Binding,*
571 *Transport and Storage of Metal Ions in Biological Cells*. pp. 695–746.
572 <https://doi.org/10.1039/9781849739979-00695>
- 573 Ng, T.Y.-T., Klinck, J.S., Wood, C.M., 2009. Does dietary Ca protect against toxicity of a low
574 dietborne Cd exposure to the rainbow trout? *Aquatic Toxicology* 91, 75–86.
575 <https://doi.org/10.1016/j.aquatox.2008.10.008>
- 576 Nordberg, M., 1978. Studies on metallothionein and cadmium. *Environmental Research* 15,
577 381–404. [https://doi.org/10.1016/0013-9351\(78\)90120-2](https://doi.org/10.1016/0013-9351(78)90120-2)
- 578 Orbea, A., Fahimi, H.D., Cajaraville, M.P., 2000. Immunolocalization of four antioxidant
579 enzymes in digestive glands of mollusks and crustaceans and fish liver. *Histochem*
580 *Cell Biol* 114, 393–404. <https://doi.org/10.1007/s004180000207>
- 581 Pelletier, E., 1986. Mercury-selenium interactions in aquatic organisms: A review. *Marine*
582 *Environmental Research* 18, 111–132. [https://doi.org/10.1016/0141-1136\(86\)90003-6](https://doi.org/10.1016/0141-1136(86)90003-6)
- 583 Pierron, F., Baudrimont, M., Bossy, A., Bourdineaud, J.-P., Brèthes, D., Elie, P., Massabuau,
584 J.-C., 2007. Impairment of lipid storage by cadmium in the European eel (*Anguilla*
585 *anguilla*). *Aquatic Toxicology* 81, 304–311.
586 <https://doi.org/10.1016/j.aquatox.2006.12.014>
- 587 Pouil, S., Bustamante, P., Warnau, M., Metian, M., 2018. Overview of trace element trophic
588 transfer in fish through the concept of assimilation efficiency. *Marine Ecology*
589 *Progress Series* 588, 243–254. <https://doi.org/10.3354/meps12452>
- 590 Pouil, S., Warnau, M., Oberhänsli, F., Teyssié, J.-L., Metian, M., 2015. Trophic transfer of
591 ¹¹⁰Ag in the turbot *Scophthalmus maximus* through natural prey and compounded
592 feed. *Journal of Environmental Radioactivity* 150, 189–194.
593 <https://doi.org/10.1016/j.jenvrad.2015.08.016>
- 594 Roméo, M., Bennani, N., Gnassia-Barelli, M., Lafaurie, M., Girard, J.P., 2000. Cadmium and
595 copper display different responses towards oxidative stress in the kidney of the sea
596 bass *Dicentrarchus labrax*. *Aquatic Toxicology* 48, 185–194.
597 [https://doi.org/10.1016/S0166-445X\(99\)00039-9](https://doi.org/10.1016/S0166-445X(99)00039-9)
- 598 Rosabal, M., Pierron, F., Couture, P., Baudrimont, M., Hare, L., Campbell, P.G.C., 2015.
599 Subcellular partitioning of non-essential trace metals (Ag, As, Cd, Ni, Pb, and Tl) in
600 livers of American (*Anguilla rostrata*) and European (*Anguilla anguilla*) yellow eels.
601 *Aquatic Toxicology* 160, 128–141. <https://doi.org/10.1016/j.aquatox.2015.01.011>
- 602 Sabolić, I., Breljak, D., Škarica, M., Herak-Kramberger, C.M., 2010. Role of metallothionein
603 in cadmium traffic and toxicity in kidneys and other mammalian organs. *Biometals* 23,
604 897–926. <https://doi.org/10.1007/s10534-010-9351-z>

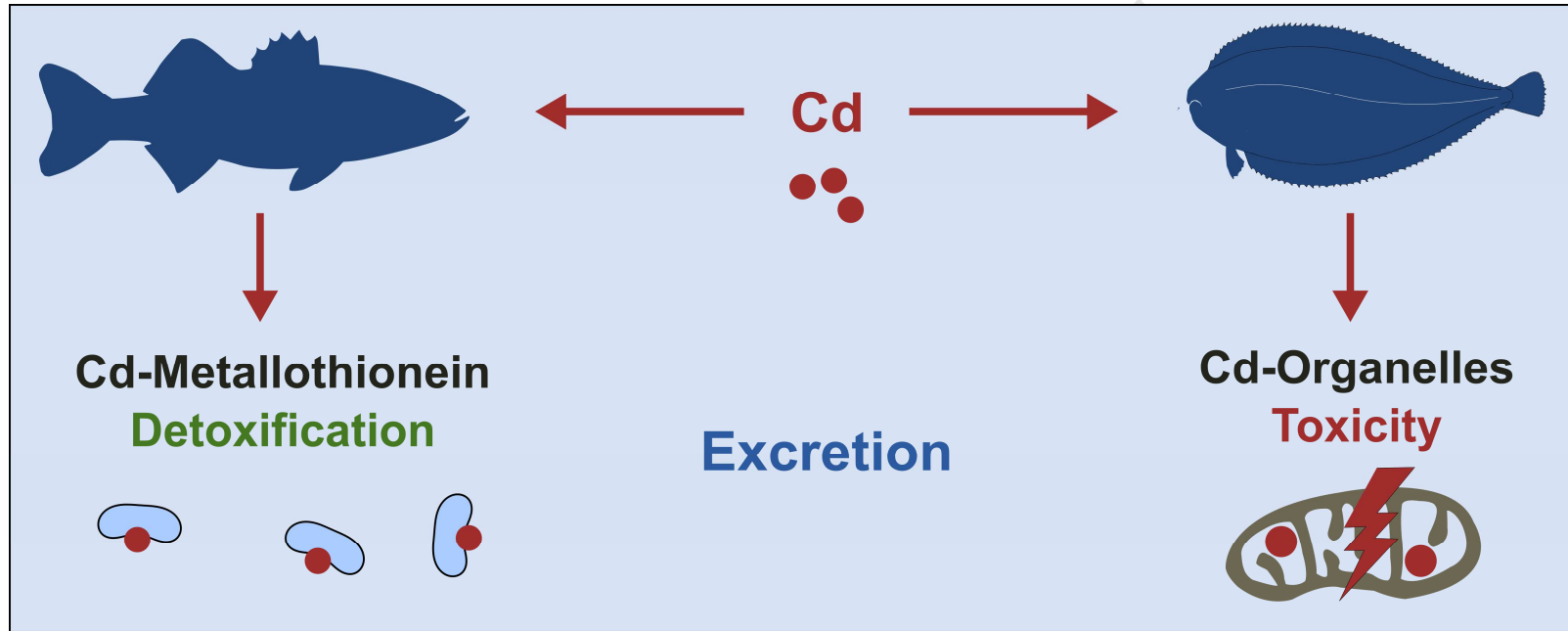
- 605 Salas-Leiton, E., Anguis, V., Martín-Antonio, B., Crespo, D., Planas, J.V., Infante, C.,
606 Cañavate, J.P., Manchado, M., 2010. Effects of stocking density and feed ration on
607 growth and gene expression in the Senegalese sole (*Solea senegalensis*): Potential
608 effects on the immune response. *Fish & Shellfish Immunology* 28, 296–302.
609 <https://doi.org/10.1016/j.fsi.2009.11.006>
- 610 Sasakura, C., T. Suzuki, K., 1998. Biological interaction between transition metals (Ag, Cd
611 and Hg), selenide/sulfide and selenoprotein P. *Journal of Inorganic Biochemistry* 71,
612 159–162. [https://doi.org/10.1016/S0162-0134\(98\)10048-X](https://doi.org/10.1016/S0162-0134(98)10048-X)
- 613 Shapiro, S.S., Wilk, M.B., 1965. An Analysis of Variance Test for Normality (Complete
614 Samples). *Biometrika* 52, 591–611. <https://doi.org/10.2307/2333709>
- 615 Siscar, R., Koenig, S., Torreblanca, A., Solé, M., 2014. The role of metallothionein and
616 selenium in metal detoxification in the liver of deep-sea fish from the NW
617 Mediterranean Sea. *Science of The Total Environment* 466–467, 898–905.
618 <https://doi.org/10.1016/j.scitotenv.2013.07.081>
- 619 Talas, Z.S., Orun, I., Ozdemir, I., Erdogan, K., Alkan, A., Yılmaz, I., 2008. Antioxidative role
620 of selenium against the toxic effect of heavy metals (Cd⁺², Cr⁺³) on liver of rainbow
621 trout (*Oncorhynchus mykiss* Walbaum 1792). *Fish Physiol Biochem* 34, 217–222.
622 <https://doi.org/10.1007/s10695-007-9179-9>
- 623 Viarengo, A., Pertica, M., Canesi, L., Accomando, R., Mancinelli, G., Orunesu, M., 1989.
624 Lipid peroxidation and level of antioxidant compounds (GSH, vitamin E) in the
625 digestive glands of mussels of three different age groups exposed to anaerobic and
626 aerobic conditions. *Marine Environmental Research, Responses of Marine Organisms
627 to Pollutants* 28, 291–295. [https://doi.org/10.1016/0141-1136\(89\)90246-8](https://doi.org/10.1016/0141-1136(89)90246-8)
- 628 Vijver, M.G., van Gestel, C.A.M., Lanno, R.P., van Straalen, N.M., Peijnenburg, W.J.G.M.,
629 2004. Internal Metal Sequestration and Its Ecotoxicological Relevance: A Review.
630 *Environ. Sci. Technol.* 38, 4705–4712. <https://doi.org/10.1021/es040354g>
- 631 Vural, H., Demirin, H., Kara, Y., Eren, I., Delibas, N., 2010. Alterations of plasma
632 magnesium, copper, zinc, iron and selenium concentrations and some related
633 erythrocyte antioxidant enzyme activities in patients with Alzheimer's disease. *Journal
634 of Trace Elements in Medicine and Biology* 24, 169–173.
635 <https://doi.org/10.1016/j.jtemb.2010.02.002>
- 636 Wallace, W.G., Lee, B., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn
637 in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically
638 detoxified metal (BDM). *Mar Ecol Prog Ser* 249, 183–197.
639 <https://doi.org/10.3354/meps249183>
- 640 Wang, W., Rainbow, P.S., 2006. Subcellular Partitioning and the Prediction of Cadmium
641 Toxicity to Aquatic Organisms. *Environ. Chem.* 3, 395–399.
- 642 Wang, W.-X., Rainbow, P.S., 2010. Significance of metallothioneins in metal accumulation
643 kinetics in marine animals. *Comparative Biochemistry and Physiology Part C:
644 Toxicology & Pharmacology* 152, 1–8. <https://doi.org/10.1016/j.cbpc.2010.02.015>
- 645 World Health Organization, 2010. Exposure to cadmium: A Major public health concern.
- 646 Zalups, R.K., Ahmad, S., 2003. Molecular handling of cadmium in transporting epithelia.
647 *Toxicology and Applied Pharmacology* 186, 163–188. [https://doi.org/10.1016/S0041-
648 008X\(02\)00021-2](https://doi.org/10.1016/S0041-008X(02)00021-2)
- 649 Zhao, W., Liu, W., Chen, X., Zhu, Y., Zhang, Z., Yao, H., Xu, S., 2014. Four Endoplasmic
650 Reticulum Resident Selenoproteins May Be Related to the Protection of Selenium
651 Against Cadmium Toxicity in Chicken Lymphocytes. *Biol Trace Elem Res* 161, 328–
652 333. <https://doi.org/10.1007/s12011-014-0135-0>
- 653

Species	Element	Subcellular partitioning (%)					
		Organelles		MRG		MTLP	
		Control	Exposed	Control	Exposed	Control	Exposed
<i>D. labrax</i>	Cd	20.1 ± 0.9	15.4 ± 4.7	1.3 ± 0.3	1.6 ± 0.7	53.7 ± 1.4	61.8 ± 5.6
	Ca	18.4 ± 16.3	12.9 ± 3.1	53.8 ± 42.8	69.3 ± 4.1	13.2 ± 12.6	9.6 ± 3.7
	Cu	20.2 ± 1.2	16.5 ± 4.8	2.4 ± 0.7	2.5 ± 1.3	53.2 ± 1.2	59.8 ± 5.9
	Fe	56.6 ± 1.2	60.6 ± 12.8	2.7 ± 0.5	3.1 ± 1.5	6.7 ± 2	8.3 ± 8.3
	Mg	42.1 ± 1	40.3 ± 5.4	3.8 ± 1.3	4.2 ± 1.1	33.7 ± 1.8	38.8 ± 5.7
	Mn	38.2 ± 2.3	37.7 ± 4.2	5.8 ± 2.6	6.1 ± 0.7	29.3 ± 1.9	33 ± 4.5
	Se	31.7 ± 1.2	28.4 ± 5.5	3.1 ± 0.3	3.9 ± 1	16.6 ± 0.1	18.6 ± 3.1
	Zn	28.1 ± 0.8	23.5 ± 5	1.5 ± 0.1	1.5 ± 0.2	21.1 ± 1.9	27.7 ± 2.1
<i>S. senegalensis</i>	Cd	37.3 ± 5.9	51.5 ± 4.8	16.4 ± 9	9.2 ± 5.1	3.9 ± 2.4	4.1 ± 2.2
	Ca	28.3 ± 10.2	39.5 ± 14.7	37.7 ± 16.9	32.6 ± 13.7	17.5 ± 2	13.5 ± 2.3
	Cu	36.6 ± 6.3	49.3 ± 7.6	16.9 ± 9.6	8.1 ± 2.8	3.9 ± 3.3	6.8 ± 3.4
	Fe	30.1 ± 2.4	41.2 ± 1.4	16.9 ± 3.2	9.2 ± 2.6	8.9 ± 1.3	6.8 ± 1.3
	Mg	36.5 ± 6.4	52.1 ± 3.4	13.6 ± 2.7	7.7 ± 1.3	28.6 ± 2.9	23 ± 4.3
	Mn	36.8 ± 3.7	49.4 ± 1.8	16.8 ± 3	10.3 ± 1.5	23 ± 4.2	20.1 ± 2.8
	Se	32.8 ± 3.9	43.6 ± 6.8	10.6 ± 6.8	5.8 ± 1.4	11.6 ± 2.9	12.3 ± 1.5
	Zn	31.4 ± 3.2	43.3 ± 4.1	10.6 ± 7	7.4 ± 2.2	5.8 ± 0.5	4.6 ± 0.9

Table 1

Total metal concentration (ppm dry weight, mean ± sd) and subcellular partitioning (% of the total metal contained in each fraction, mean ± sd) of metal elements, after 60 days of cadmium depuration, in the liver of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* for both treatments (exposed and control fish, n = 3). Values are shown only for organelles, metal rich granules (MRG) and heat stable proteins (HSP) fractions where metal partitioning significantly differed between control and exposed fish (values in bold; ANOVA, p < 0.05).

Graphical abstract



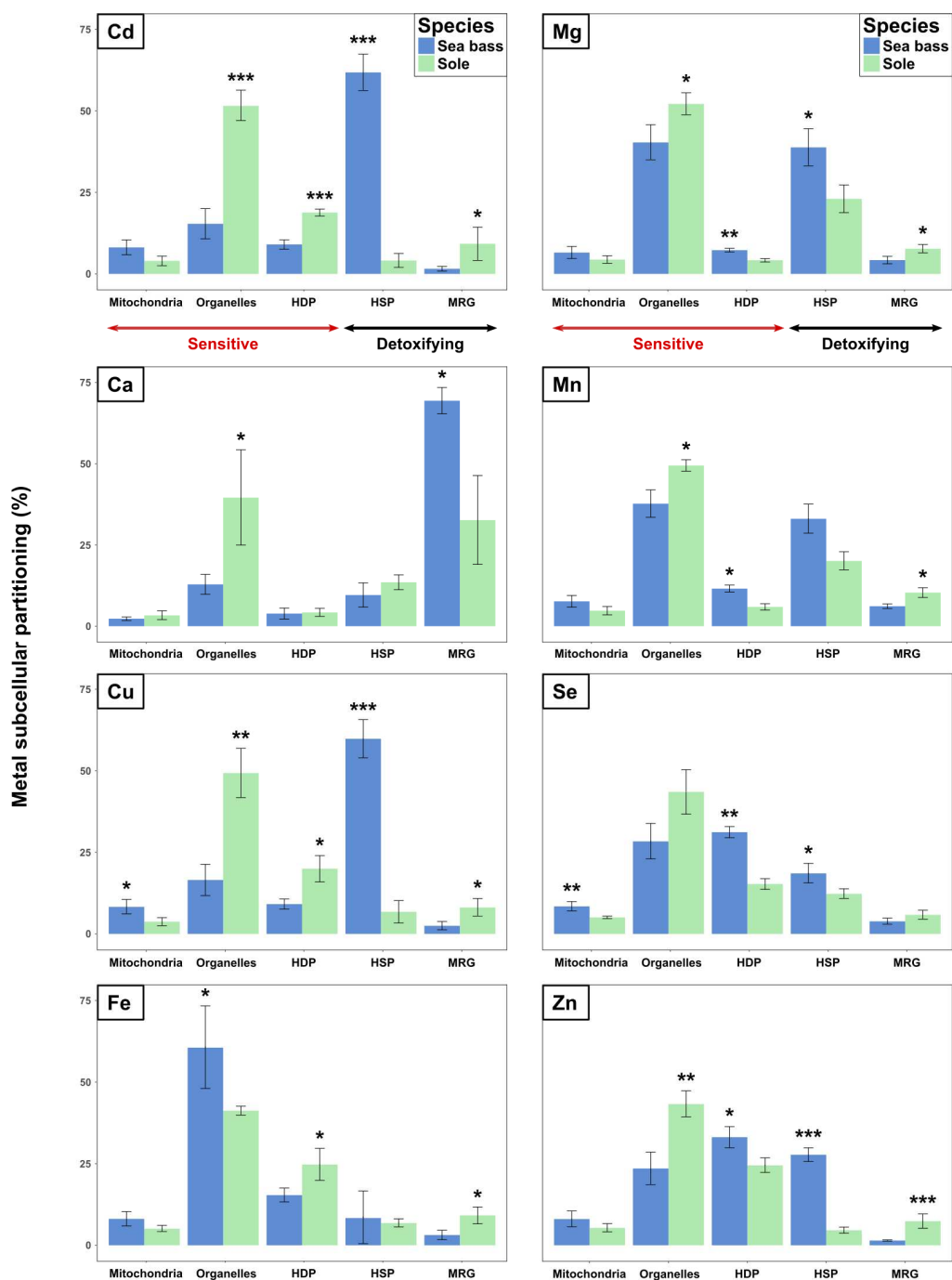


Figure 1: Subcellular partitioning of cadmium (Cd) and essential metals (% of the total metal contained in each fraction) in the liver of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* (n = 3) after 60 days of depuration. The cellular debris fraction is not shown as it is neither a sensitive nor a detoxifying fraction, but was taken into account in the calculation of the partitioning (for Cd proportions in cellular debris, see Supporting Information). Significant difference between species (ANOVA) are indicated by * p < 0.05, ** p < 0.01, *** p < 0.001).

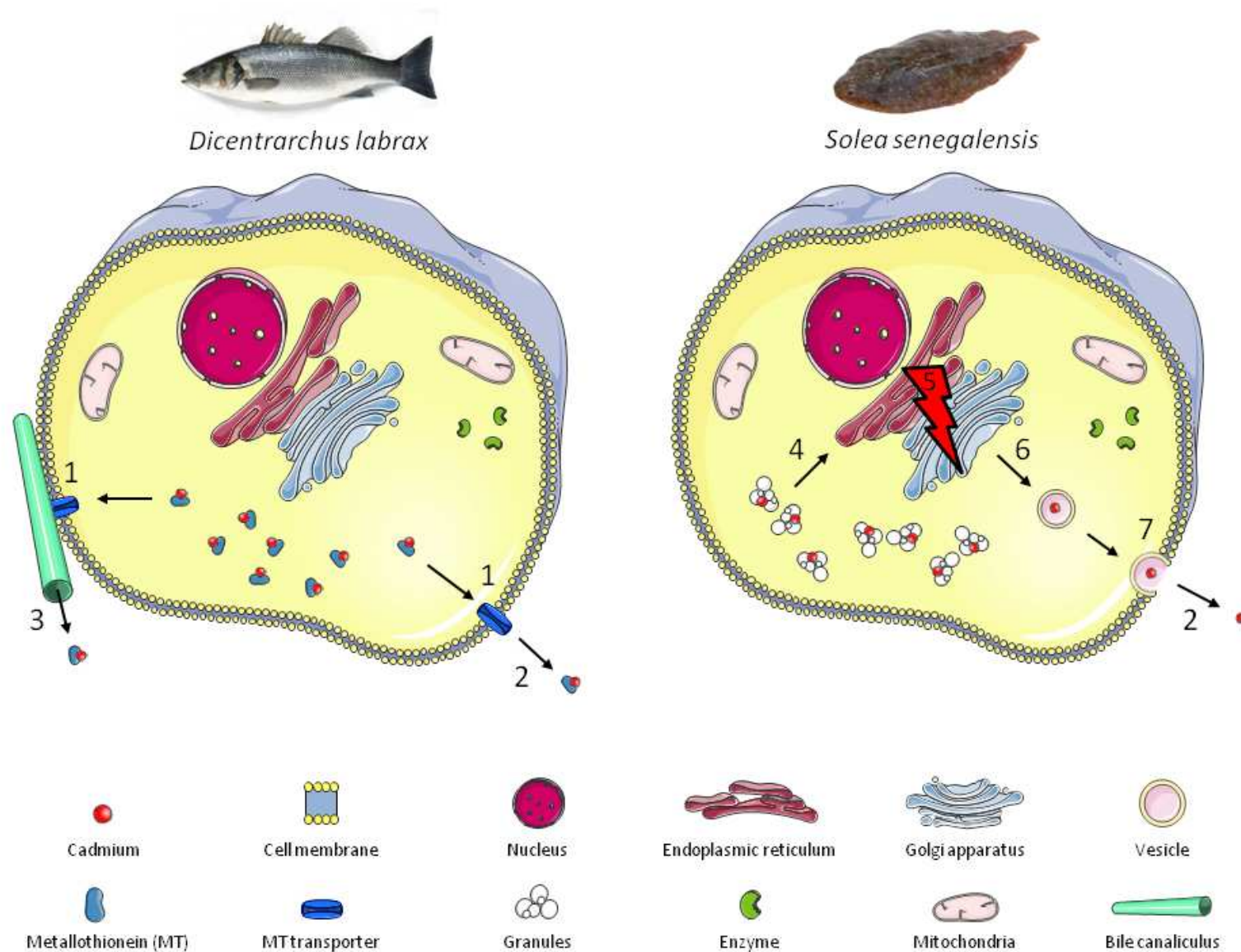


Figure 2: Subcellular Cd dynamics in hepatocytes of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis*. Only the two main mechanisms of Cd handling are represented: binding to metallothionein in sea bass and binding to granules in sole. 1: Excretion of Cd/MT complexes through MT transporters; 2: Release into the blood; 3: Release into bile; 4: sequestration of metal rich granules by the endoplasmic reticulum; 5: damage due to Cd toxicity; 6: vesicular transport; 7: vesicular exocytosis.

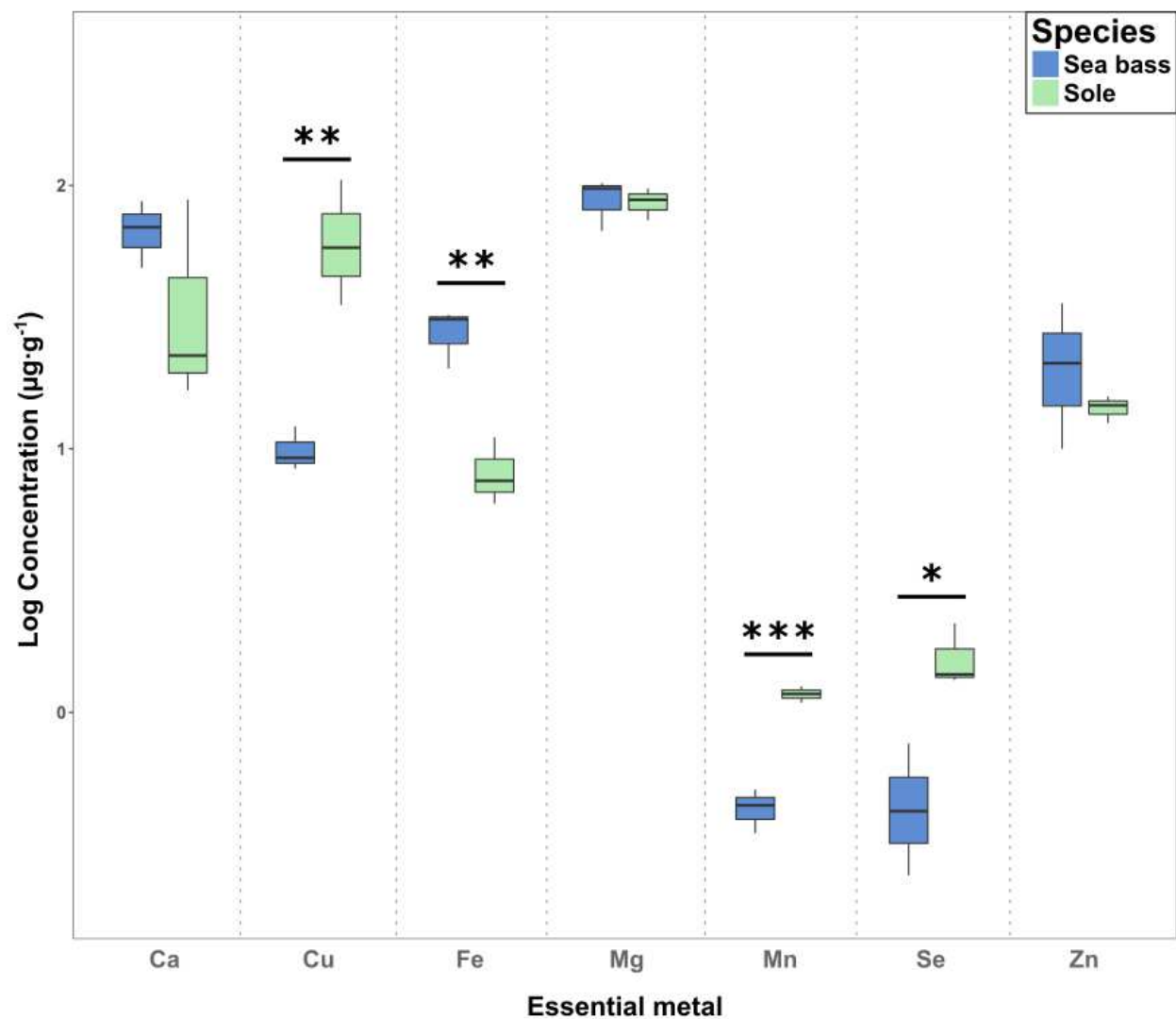


Figure 3: Total essential metal concentrations (log transformed values, $\mu\text{g}\cdot\text{g}^{-1}$ dw) in the liver of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* after 60 days of dietary Cd exposure (n = 3). Significant differences between species (ANOVA) are indicated by * p < 0.05, ** p < 0.01, *** p < 0.001. No differences were found in total essential metal concentrations between control and exposed fish of the same species (see Supporting Information).

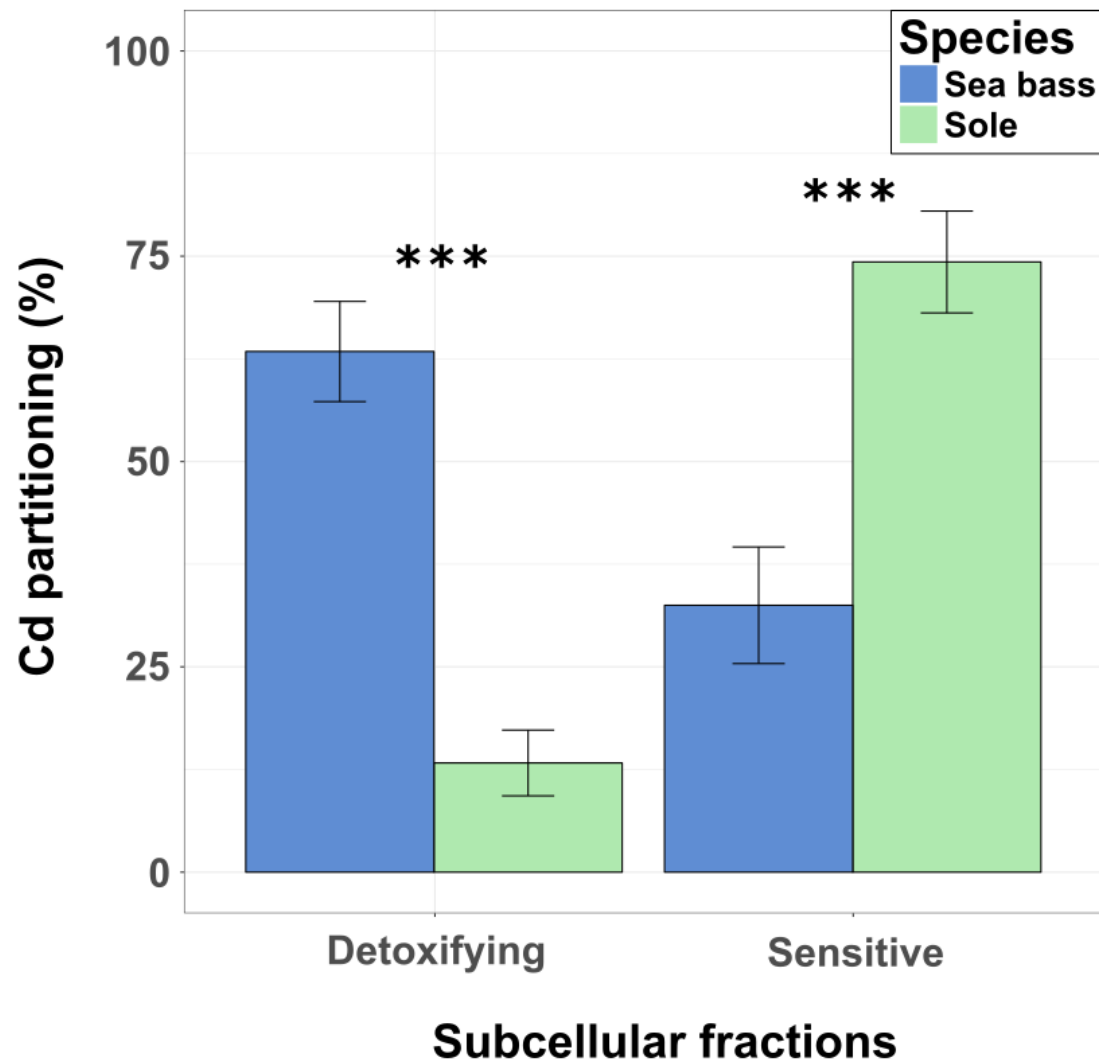


Figure 4: Subcellular partitioning (% of the Cd contained in each fraction type, mean \pm sd) of Cd after 60 days of depuration in the liver of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* (n = 3). Sensitive fractions = mitochondria + HDP + organelles; detoxifying fractions = MRG + HSP. Significant differences between species (ANOVA) are indicated by *** p < 0.001.

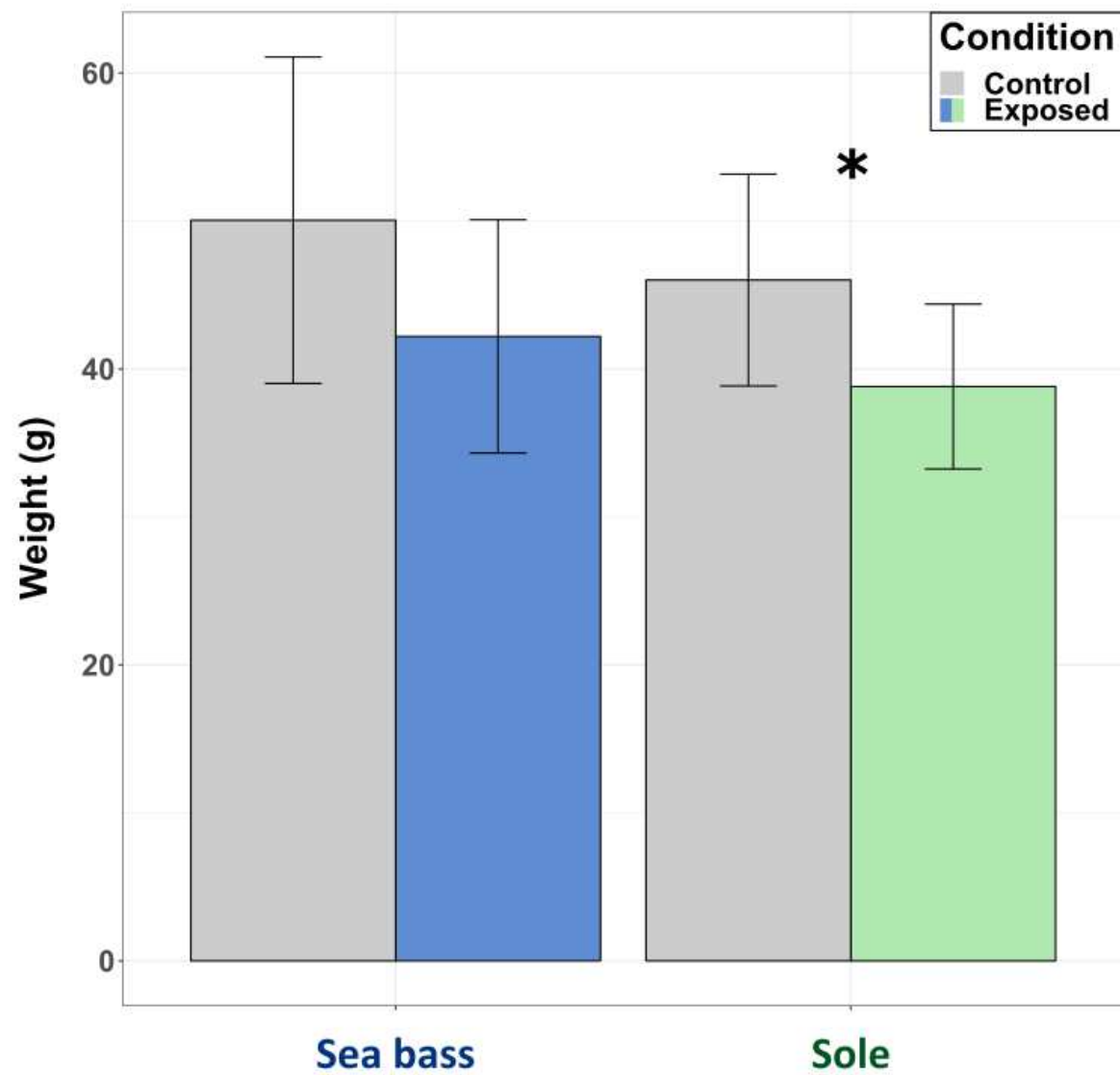


Figure 5 : Weight (g) (mean \pm sd) of sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* after 60 days of Cd dietary exposure for both treatments (exposed and control fish, n = 3). Significant differences between conditions (ANOVA) are indicated by * $p < 0.05$.

HIGHLIGHTS

- Sea bass and sole displayed different Cd subcellular partitioning in the liver
- Cd was largely bound to metallothionein-like proteins in the sea bass liver
- Cd was mainly linked to metal rich granules in the sole liver
- Essential metals followed the Cd partitioning pattern in hepatic cells
- Handling strategies might account for sensitivity and Cd excretion