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## Rare earth element bioaccumulation in the yellow and silver European eel (*Anguilla anguilla*): A case study in the Loire estuary (France)

Marjorie Lortholarie, Aurore Zalouk-Vergnoux, Mickaël Couderc,  
Abderrahmane Kamari, Yannick François, Christine C Herrenknecht,  
Laurence Poirier

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# 1 Rare earth element organotropism in European eel (*Anguilla anguilla*)

2

3 Marjorie Lortholarie<sup>1</sup>, Laurence Poirier<sup>1</sup>, Abderrahmane Kamari<sup>1</sup>, Christine Herrenknecht<sup>1</sup>, Aurore  
4 Zalouk-Vergnoux<sup>1</sup>

5

6 <sup>1</sup> Laboratory Mer, Molécules, Santé (MMS, EA 2160), University of Nantes, Nantes F-44322,  
7 France

8

9 Email: m.lortholarie@gmail.com

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11

## 12 Abstract

13

14 Rare earth elements (REEs) are metallic elements with electronic, magnetic, optical and  
15 catalytic properties which make them essential in many industrial and medical fields. REEs are  
16 therefore becoming emerging pollutants and it is important to understand their implications for  
17 ecosystem health. However, little knowledge of REE bioaccumulation in aquatic organisms is  
18 available and especially on their internal distribution in fish. In the present study, REE  
19 organotropism was determined in *Anguilla anguilla* from the Loire estuary (France) by determining  
20 burdens in a wide set of tissues, organs and biological fluids. Differences have been observed  
21 between life stages and genders. For yellow eels, the most accumulating organ was the gills  
22 ( $126.90 \pm 50.78 \mu\text{g/kg dw}$ ) and for silver eels, it was the liver ( $181.78 \pm 62.04 \mu\text{g/kg dw}$  for males;  
23  $203.79 \pm 111.86 \mu\text{g/kg dw}$  for females). The comparison between female silver and yellow eels  
24 shown that female silver individuals accumulated significantly more REEs in the urinary system  
25 (US), muscles, gonads, spleen and liver, while yellow individuals accumulated more in gills. The  
26 comparison between male and female silver eels also highlighted differences, indeed the females  
27 accumulated significantly more REEs in the US, gonads, skin and spleen, compared to males which  
28 accumulated significantly more in muscles and gills. REEs abundances are also different between  
29 organs, life stages and genders. The gonads of female silver eels exhibited a particular profile with  
30 the dominance of gadolinium (Gd) (up to 74.2% of  $\sum\text{REEs}$ ). Moreover, the presence of *Anguillicola*  
31 *crassus* in the swim bladder of organisms seemed to have an impact on REE bioaccumulation:  
parasitized yellow eels present higher concentrations of REEs in muscles, gills, gonads and liver than

32 non-parasitized individuals. Regarding glass eels, REE contribution profiles in the whole body were  
33 close to those of yellow and silver eel skin.

34 **Keywords:** Fish organs, Rare earth elements, Life stage, Gender, European eel  
35

36 **Highlight:**  
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- 39 • Organotropism determined in muscles, skin, gills, spleen, US, liver and gonads
- 40 • REE accumulation and organotropism were influenced by gender and life stages
- 41 • Yellow eels accumulated more in gills, female and male silver eels in livers
- 42 • Parasitized yellow eels accumulated more REEs than non-parasitized
- 43
- 44

## 45 1. Introduction 46

47 *Anguilla anguilla* is an euryhaline and catadromous species with a really special life cycle.  
48 After a transatlantic migration of nearly 6,000 km from east to west traveled from 4 to 6 months,  
49 silver eels arrive in the Sargasso Sea for the reproduction where they die after. Laid eggs turn into  
50 leptocephalus larvae which migrate using the Gulf Stream to European coasts. Arriving at the level  
51 of the continental slope about 100 km from the coast, leptocephalus larvae become glass eels. In  
52 freshwater, they begin to feed and after some time, glass eels gradually pigment in black then yellow  
53 to become yellow eel. Yellow eels live in freshwater. They are benthic and sedentary for several  
54 years waiting for the silvering. This metamorphosis changes yellow eels into silver eels upon sexual  
55 maturity (Adam et al. 2008; Corolla et al. 2016). This transition phase leads to many morphological  
56 and physiological changes (Adam et al. 2008; Fontaine 1994; Durif et al.2015). During the  
57 downstream migrating period, the silver eels are sexually differentiated but they are not yet  
58 completely mature.

59 Long considered to be a harmful species as a result of its snake-like appearance and the  
60 untrue idea that it is the cause of the noble fish species decline (salmon, trout or char), the European  
61 eel is now recognized as a species with an economic, ecological and scientific interest. However, the  
62 populations are declining since the 1980s. This decrease is not a single factor consequence but a  
63 combination of overfishing at all life stages, habitat reduction, water quality degradation, obstacle  
64 during the migration, diseases such as the Anguilliosis caused by the parasites *Anguillicola crassus*.  
65 Consequently, European eel was listed in CITES (Convention International Trade Endangered  
66 Species) Appendix II in 2009 and ranked in the red list of threatened species as a critically  
67 endangered species in 2014 by IUCN (International Union for Conservation of Nature).

68 Among the threats to eels, the aquatic ecosystems contamination is very worrying. In fact, the  
69 European eel are able to accumulate a wide variety of environmental contaminants and some studies  
70 have highlighted the accumulation of polycyclic aromatic hydrocarbons (Ribeiro et al. 2005),  
71 pesticides and persistent organic pollutants (Couderc et al., 2015; van der Oost et al., 1996). They are  
72 also able to accumulate essential metals (Ni, Zn, Cu, Mn, Co and Fe), non-essential metals and  
73 metalloids (Hg, Al, Cr, Pb, Cd and As) (Barak & Mason, 1990; Bordajandi et al., 2003; Durrieu et  
74 al., 2005; Mansouri et al., 2018; Neto et al., 2011; Ribeiro et al., 2005; Storelli et al., 2007; Tabouret  
75 et al., 2011). The metal bioaccumulation can cause sublethal biological effects. For example, yellow  
76 eels exposed during one month to Cd (5 µg/L) presented an increase of fat consumption in their  
77 reserve necessary for migration and could compromise successful reproduction (Pierron et al., 2007).  
78 Another study at the same concentration of Cd showed structural or functional perturbations in the  
79 gills of glass eels after 2 h exposure (Gony, 1990). A one-month exposure to 300 µg/L of Pb,  
80 increased the number of lymphocytes and the plasma lactate levels in yellow eels (Santos & Hall,  
81 1990). After a 7 day exposure of eels to Cu 0.2 µmol/L, significant decreases of free triiodothyronine  
82 and cortisol as well as a significant increase of glucose were observed in the plasma. In addition, a  
83 lactate decrease and an increase of nuclear abnormality frequency were measured in blood, gills,  
84 liver and urinary system (Oliveira et al., 2008). The ecotoxicology of essential and non-essential

85 metals is now relatively well documented for eels, but other types of metallic elements, such as  
86 REEs, are becoming emerging contaminants and should be better considered.

87 REEs are a set of 17 metallic elements including scandium, yttrium and 15 lanthanides from  
88 lanthanum to lutetium. They have close chemical and physical properties due to their main identical  
89 charge +III (except for some REEs may have two oxidation numbers, +III and +IV for Ce, Pr, Tb or  
90 +II and +III for Eu, Sm and Yb) and their neighboring ionic radius. REEs are usually classified  
91 according to their increasing atomic number into three groups: light (from lanthanum to  
92 promethium), medium (from samarium to gadolinium) and heavy (from terbium to lutetium,  
93 including yttrium). Due to its physico-chemical properties and its presence in the same deposits,  
94 yttrium should be among the light REEs but it is classified with the heavy REEs due to the similarity  
95 of its property and its deposits. Sc is not classified in any of these REE groups because of its absence  
96 in the same deposits (Bru et al., 2015).

97 REEs possess highly electronic, magnetic, optical and catalytic properties, leading to a  
98 prevalent use in several industrial fields such as the production of a super-magnet and luminophore,  
99 petroleum catalysis, pigmentation of glasses and plastics, metallurgical alloys, nuclear and medical  
100 imaging (Bru et al., 2015). In 2016, their global production was 126 Kt of rare earth oxides (Zhou et  
101 al., 2017). This production is set to increase for several years with the development of clean energy  
102 such as wind turbines, electric vehicles, and energy-efficient lighting (Alonso et al. 2012; Zhou et al.  
103 2017).

104 In aquatic environments, REEs are ubiquitous contaminants, their presence has already been  
105 measured in waters, suspended particles and sediments in different environmental compartments in  
106 the worldwide such as rivers (Keasler & Loveland, 1982; Négrel et al., 2000), estuaries (Elbaz-  
107 Poulichet and Dupuy, 1999; Vrel, 2012) and oceans (Akagi & Edanami, 2017; Dahlgvist et al., 2005;  
108 Hirata et al., 2002; Johannesson et al., 2017; Li et al., 2015). Off the Loire estuary, the area  
109 concerned by the present study, sediments of the Bay of Biscay showed mean concentrations ranging  
110 from 0.11 (Lu) to 47.79 (Ce) mg/kg (Chaillou et al., 2006). With the proximity of the Loire estuary,

111 the mean concentrations in sediments reached 41 and 92 mg/kg for La and Ce respectively. In the  
112 suspended particulate matter of the Loire estuary, mean values of total REEs ( $\Sigma$ REEs) reached  
113 137 mg/kg upstream of Nantes, 164 to 489 mg/kg downstream of Nantes and 89 mg/kg near the  
114 coast (De Chanvalon et al., 2016). Levels between 195 and 199 mg/kg were also measured in  
115 sediments downstream of Nantes. In the Garonne River (La Réole), Lerat-Hardy et al. (2019)  
116 measured concentrations in waters from 0.469 ng/L (Tm) to 37 ng/L (Ce) in 2017. Environmental  
117 concentrations normalized to reference reservoirs makes possible to highlight anomalies. When these  
118 anomalies are positive, that could point out an anthropic enrichment. The Sm and Gd often appeared  
119 as positive anomalies, as shown in the Garonne estuary (France) by Lerat-Hardy et al. (2019). These  
120 aquatic environmental contaminations are worrying because REEs were shown to be able to  
121 accumulate in aquatic organisms. In the European eel, REE accumulation has already been  
122 highlighted in different life stages, from glass eels (Figueiredo et al., 2018) to yellow and silver eels  
123 (Lortholarie et al., 2019).

124 The aim of this work was to explore REE internalization and distribution in a key species of  
125 the estuarine ecosystem. The European eel represent an interesting model organism due to its benthic  
126 and partial sedentary life, and several metamorphosis processes that can influence metal  
127 organotropism. The plan of the present study was i) to investigate the internal distribution of REEs in  
128 wild European eels, by determining REE concentration in muscles, skin, gills, blood, spleen, urinary  
129 system, liver and gonads, ii) to study life stage and gender influences by sampling glass, yellow and  
130 silver eels, iii) to explore the presence of parasites *A. crassus* in the swim bladder and their potential  
131 impact on bioaccumulation and organotropism.

132

## 133 2. Materials and Methods

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### 135 2.1. Chemicals

136

137 Stock solutions of 15 REEs (yttrium: Y; lanthanum: La; cerium: Ce; praseodymium: Pr,  
138 neodymium: Nd; samarium: Sm; europium: Eu; gadolinium: Gd; terbium: Tb, dysprosium: Dy;  
139 holmium: Ho; erbium: Er, thulium: Tm; ytterbium: Yb and lutetium: Lu) and Rhenium (internal  
140 standard) at 1000 mg/L in 2% HNO<sub>3</sub>, were provided by Roth (single-element ICP standard solution).  
141 REEs were oxide forms in solution (Y<sub>2</sub>O<sub>3</sub>, La<sub>2</sub>O<sub>3</sub>, Ce (NO<sub>3</sub>)<sub>3</sub>, Pr<sub>6</sub>O<sub>11</sub>, Nd<sub>2</sub>O<sub>3</sub>, Sm<sub>2</sub>O<sub>3</sub>, Eu<sub>2</sub>O<sub>3</sub>, Gd<sub>2</sub>O<sub>3</sub>,  
142 Tb<sub>4</sub>O<sub>7</sub>, Dy<sub>2</sub>O<sub>3</sub>, Ho<sub>2</sub>O<sub>3</sub>, Er<sub>2</sub>O<sub>3</sub>, Tm<sub>2</sub>O<sub>3</sub>, Yb<sub>2</sub>O<sub>3</sub>, Lu<sub>2</sub>O<sub>3</sub>). In this work, 15 out of the 17 REEs were  
143 studied. Pm was not included because it is not naturally present in the environment and neither Sc  
144 because of many interferences during the ICP-MS analysis, which led to overestimation of  
145 concentrations. Mineralization and solutions were made with HNO<sub>3</sub> 65% from Fisher Scientific.  
146 Mineralization protocol was validated using the international certified material BCR-668 consisted  
147 of muscle of mussels from LGC standards.

## 148 2.2. Sampling

151 *Anguilla anguilla* were fished in the Loire estuary (Figure 1) at three life stages: glass, yellow  
152 and silver. Glass eels were fished in December 2019 at Cordemais (47°16'58.7''N –1°53'55.1''O)  
153 located 30 km downstream of Nantes. Yellow eels were caught in June 2019 at Haute-Indre  
154 (47°11'39''N – 1°40'4''O) located 12 km downstream of Nantes. Silver eels were caught during the  
155 downstream period in December 2018 at Varades (47°21'58''N – 1°1'31''O). About silver eels,  
156 since they were downstream migrating, they cannot be considered as from this specific site. Eels  
157 were captured by professional fishermen by means of fish traps. For each study site and sexual  
158 maturity, 10 to 15 individuals were collected. After fish capture, eels were conducted to the  
159 laboratory in 200 L tanks with water from the sampling site and aeration. At the laboratory, fish were  
160 directly euthanized by lethal dose of eugenol just before their dissection.

## 161 2.3. Preparation of Fish Tissue and Parasites for REE Analyses

164 After euthanasia, eels were weighed (g) and measured (cm) to get respectively the body  
165 weight (BW) and the body length (BL). During the dissection, the observation of the gonads allowed  
166 to visually identify the gender of individuals. Interest organs for the organotropism study were  
167 removed and weighed: muscles, skin, gills, spleen, urinary system, liver, and gonads. Two different  
168 biological fluids were also collected: blood and bile. When present, *A. crassus* parasites were also  
169 collected from the swim bladder of eels. *A. crassus* is a nematode feeding on blood and causing a  
170 disease called Anguillidiosis (Adam et al., 2008). The parasites were counted, weighed and analyzed  
171 as fish tissue. The parasite index (PI) was determined using  $PI = (\text{weight } \textit{Anguillicola crassus} / \text{weight}$   
172  $\text{eel body}) * 100$ . The otoliths were recovered to determine the age of each individual according to  
173 ICES 2009 (International Council for the Exploration of the Sea) methodology. Glass eels were  
174 observed with a binocular and trilled into three groups VI A0, V A1 and VI A2, depending on their  
175 pigmentation stage (Grellier et al., 1991). The gonads were recovered and weighed to determine the  
176 gonadosomatic index (GSI). The GSI was calculated as follows:  $GSI = \text{gonad weight (g)} / \text{whole}$   
177  $\text{body weight (g)} * 100$ . The Fulton condition index (K) was determined as following:  $K = (\text{whole}$   
178  $\text{body weight (g)} * 10^5) / \text{whole body length}^3 \text{ (cm)}$ , K allows evaluating the overall global eel health in  
179 front of environmental stresses such as pollution or feeding conditions.

180 At the beginning of the experiments, all the material was cleaned by immersion in HNO<sub>3</sub> 10%  
181 for one night and rinsed with Milli-Q water before use. The low REE concentrations expected in the  
182 muscles required a preconcentration step by calcination allowed due to sufficient muscle masses.  
183 About 1 g of muscles were calcined in a muffle furnace (Nobertherm) at 300°C during 1h followed  
184 by 500°C during 2h. The muscle samples were therefore concentrated about 10 times. For the  
185 mineralization, 250 mg of ashes of muscles, of the other organs or of *A. crassus* were collected and  
186 weighted. Then, the mineralization relied on a predigestion with a mix of HNO<sub>3</sub>/HCl (2:1, v/v) for  
187 one night at room temperature and a following step using microwaves (Anton Paar, MW GO 50Hz)  
188 (Lortholarie et al., 2019). The solutions were then transferred into Pyrex tubes and evaporated to  
189 dryness in hot-plate at 130°C. Samples were resuspended with 1.8 mL of HNO<sub>3</sub> 2% and then



190 vortexed, sonicated (20 min) and centrifuged (5 min, 2.0 rpm, 15°C) to eliminate particles in  
191 suspension. Only the supernatants were analyzed.

192

#### 193 2.4. Rare Earth Element Analyses

194

195 Concentrations of REEs were determined by Inductively Coupled Plasma coupled to Mass  
196 Spectrometry (ICP-MS, Nexion 350x PerkinElmer). All samples, blanks and standards were diluted  
197 using HNO<sub>3</sub> 2%. Rhenium was used as internal standard (<sup>187</sup>Re isotope), with a final concentration of  
198 1 µg/L in the solutions. Matrix effect determination was performed using a matrix matched  
199 calibration. Sample supernatant solutions were used as the matrix to prepare calibration solutions  
200 with increasing concentrations of REEs. REE isotopes were selected according to a compromise  
201 between the maximum of natural abundance and the minimum of interferences. They corresponded  
202 to: <sup>89</sup>Y, <sup>139</sup>La, <sup>140</sup>Ce, <sup>141</sup>Pr, <sup>146</sup>Nd, <sup>147</sup>Sm, <sup>153</sup>Eu, <sup>157</sup>Gd, <sup>159</sup>Tb, <sup>163</sup>Dy, <sup>165</sup>Ho, <sup>166</sup>Er, <sup>169</sup>Tm, <sup>171</sup>Yb and  
203 <sup>175</sup>Lu. Limits of detection (LOD) and quantification (LOQ) were determined for each REE.  
204 Analytical and procedural blanks were prepared and analyzed using the same analytical procedure as  
205 for samples. Finally, the blank values were subtracted from the values of the samples. The accuracy  
206 of the whole analytical protocol was validated using an international certified material containing  
207 REEs (BCR-668, muscle of mussels).

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#### 210 2.5. Data Processing

211

212 In this work the bioaccumulation results were given in micrograms per kilogram of dry  
213 weight tissue (µg/kg dw). The results were presented using 3 groups of REEs: light (LREEs: La to  
214 Nd; Pm was not studied in this work because it is a radioactive element which is not naturally present  
215 in the environment)(*Bru et al., 2015*), medium (MREEs: Sm to Gd) and heavy (HREEs: Tb to Lu  
216 and Y).

217 The potential life stage influence on bioaccumulation was studied by the comparison of  
218 concentrations measured in different organs of the yellow eels and the female silver eels which  
219 represented sexually undifferentiated and mature sexual stages respectively. Even if yellow eels were  
220 not sexually differentiated, their size was already greater than the maximum size expected for silver  
221 males (45 cm). Indeed in this species, there is a sexual dimorphism with females larger than males  
222 (Adam et al., 2008). It was thus possible to suppose that yellow eels, sampled for this study, would  
223 become females, allowing their data comparison with those of female silver eels to study the life  
224 stage influence.

225 About parasitism, the aim was to determine if influences exist with REE concentrations in all  
226 organs of eels according to life stage or gender. Moreover, the REE contents in parasites were  
227 determined to find a potential link between REE concentrations in fish tissue and in *A. crassus*.

228 The data were statistically treated using the software Statistica software (v7, StatSoft, Inc.).  
229 The results of Kolmogorov-Smirnov test showed the need to use non-parametric tests to work on the  
230 data. The correlation of biological parameters such as: body length, body weight, gonadosomatic  
231 index, Fulton index, parasite index and age with REE concentrations in each organ was investigated  
232 using a correlation test with a casewise deletion. The casewise deletion of missing data while  
233 calculating a correlation matrix is to exclude all cases that have missing data in at least one of the  
234 selected variables. The correlations were significant when  $p\text{-value} < 0.05$ . The Kruskal-Wallis test was  
235 used for studying: (i) the influence of both sexual maturity and the gender of adult eels, by  
236 comparing respectively organ REE concentrations of yellow eels to female silver eels and male silver  
237 to female silver eels; (ii) the bioaccumulation differences between organs within a life stage or a  
238 gender; (iii) the bioaccumulation differences between the three different stages of pigmentation  
239 (VI A0, VI A1 and VI A2) of glass eels. The REE bioaccumulation differences between parasitized  
240 individuals and non-parasitized ones were studied with a Mann-Whitney U test. The results of both  
241 Mann-Whitney and Kruskal-Wallis tests were considered as significant when  $p\text{-value}$  was lower than  
242 0.05.

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### 3. Results

#### 3.1. Biometric Parameters

The correlation study between biological parameters (BL, BW, GSI, K, PI, age) was performed on 15 yellow eels, 14 female silver eels and 15 male silver eels.

In yellow eels, BL and BW were positively correlated. The linear regression equation was  $BW=22.192*BL-896.2$  ( $R^2=0.947$ ). Moreover, BL and BW were also significantly correlated with age. The linear regressions were respectively:  $age=0.226*BL-5.115$  ( $R^2=0.565$ ) and  $age=0.015*BW+3.889$  ( $R^2=0.697$ ).

In female silver eels, BL and BW were also positively correlated:  $BW=15.256*BL-543.5$  ( $R^2=0.978$ ). However, BW and GSI were negatively correlated, with a linear regression of  $GSI=-0.002*BL+0.282$  ( $R^2=0.848$ ).

In male silver eels, BL and BW were positively correlated:  $BW=6.078*BL-139.1$  ( $R^2=0.749$ ). Moreover, for male silver eels, BL and GSI were also positively correlated with a linear regression  $GSI=0.112*BL-3.701$  ( $R^2=0.749$ ). In male silver eels as in females, no correlation of BL or BW with age was observed.

The BL and BW were significantly different ( $p\text{-value}<0.01$ ) between female and male silver eels. Indeed, the males were smaller and lighter than the females, with respectively a median of  $38.2\pm 2.73$  cm for BL (*vs*  $61.75\pm 8.99$  cm) and of  $92.0\pm 20.13$  g for BW (*vs*  $387.5\pm 179.03$  g). These differences were not observed between yellow eels and female silver eels. There was no significant difference of age between yellow eels and female silver eels or between male and female silver eels (table 3 supplementary material). The same trend was observed for the PI, despite some differences observed in the rate of parasitized individuals: 66.6% of the yellow eels, 53.8% of the female and 86.6% of the males.

269

## 270 3.2. REE Levels

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### 272 3.2.1. In Yellow Eels

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274 All the yellow eels caught bioaccumulated REEs and their presence were observed and  
275 quantified in the urinary system, muscles, gills, gonads, skin, spleen, liver and bile (Figure 2A). A  
276 similar pattern among all the organs was observed, with higher concentration of LREEs followed by  
277 HREEs and then MREEs. The  $\sum$ REE median concentrations ( $\mu\text{g}/\text{kg dw}$ ) were  $46.61\pm 16.56$  for  
278 urinary system,  $2.93\pm 1.02$  for muscles,  $126.90\pm 50.78$  for gill,  $5.42\pm 3.96$  for gonads,  $26.92\pm 6.36$  for  
279 skin,  $11.21\pm 5.53$  for spleen,  $99.32\pm 61.16$  for liver and  $4.58\pm 4.23$  for bile. All REE concentrations  
280 are presented in Table 1 in the supplementary material.

281

### 282 3.2.2. According to the Life Stage

283

284 Figure 2B shows the  $\sum$ REE, LREE, MREE and HREE concentrations in the different organs  
285 of female silver eels.  $\sum$ REEs were significantly higher in the urinary system, muscles, spleen, gonads  
286 and liver of female silver eels compared to those of yellow eels. These significant differences are  
287 displayed with # between parts A and B of the Figure 2.  $\sum$ REE enrichment factors reached 2.1, 2.3,  
288 3.2, 7.0 and 9.6 in liver, muscles, urinary system, gonads and spleen respectively. The significant  
289 higher  $\sum$ REE concentrations in female silver eels were noticed among all the different groups, *i.e.*  
290 LREEs, MREEs and HREEs. Only  $\sum$ REE concentrations in gills of yellow eels were significantly  
291 higher than those of female silver eels with a factor of 2.2, due to increases of LREE and MREE  
292 concentrations.

293

### 294 3.2.3. According to the Gender

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296 Gender influence between male and female silver eels was highlighted by some differences in  
297 REE concentrations. Figures 2B and 2C show the  $\Sigma$ REE, LREE, MREE and HREE concentrations  
298 in the different organs of female and male silver eels respectively. The significant differences are  
299 displayed with \* between parts B and C of the Figure 2. Females accumulated significantly more  
300  $\Sigma$ REEs in their urinary system and skin than males, due to increases of LREE, MREE and HREE  
301 concentrations. Furthermore, females accumulated significantly more  $\Sigma$ REEs in their gonads and  
302 spleen than males because of significantly higher MREE levels. In female tissues,  $\Sigma$ REE levels were  
303 more than 1.7, 2.0, 3.1 and 6.7 times higher in spleen, urinary system, skin and gonads, respectively.  
304 On the contrary, male silver eels accumulated significantly more MREEs in gills as well as  $\Sigma$ REE in  
305 muscles due to increases of LREEs, MREEs, and HREEs.

306

### 307 3.3. REE Organotropism

308

#### 309 3.3.1. *Yellow Eels*

310

311 Table 1 presents the classification of yellow eel organs, from the more to the less  
312 concentrated in  $\Sigma$ REEs, LREEs, MREEs and HREEs. For these organisms, the organotropisms were  
313 similar considering  $\Sigma$ REEs or the categorized groups, LREEs, MREEs and HREEs. It existed only 2  
314 inversions between urinary system and liver as well as between gonads and bile for HREEs. The  
315 liver and gills accumulated significantly more than the spleen, gonads, bile and muscles.  $\Sigma$ REEs  
316 were less accumulated in gonads, bile and muscles.

317

#### 318 3.3.2. *Female Silver Eels*

319

320 For female silver eels (Table 2), the organotropisms were very different considering  $\Sigma$ REEs,  
321 LREEs, MREEs and HREEs. The  $\Sigma$ REE highest concentrations were found in liver, then in the  
322 urinary system. The liver accumulated significantly more  $\Sigma$ REEs than the gills, gonads, skins,  
323 muscles and bile. The urinary system accumulated significantly more  $\Sigma$ REEs than the skin, muscles  
324 and bile. For the other REE groups, muscles and bile were systematically the less concentrated.  
Liver, urinary system and spleen (purple and blue colored) were the three more concentrated organs

325 but not in the same order depending on the group of REEs considered. Gills, skin and gonads (green  
326 and yellow colored) were organs intermediately concentrated. Particular attention should be paid to  
327 the gonads, and their highest particular MREE concentrations leading to the gonads as the most  
328 concentrated organ for this REE category.

329

### 330 3.3.3. *Male Silver Eels*

331 For male silver eels (Table 3), as for female silver eels, the classification of the organs  
332 depended on the considered REEs, *i.e.* the accumulation of  $\Sigma$ REEs, LREEs, MREEs and HREEs  
333 was different. Considering  $\Sigma$ REEs, the liver, then the gills and urinary system were the strongest  
334 accumulator organs. The liver accumulated significantly more  $\Sigma$ REEs than muscles, skin, gonads  
335 and bile with respective enrichment factors reaching 15, 29, 33 and 192. The gills accumulated  
336 significantly more  $\Sigma$ REEs than muscle, skin, gonads and bile with respective factors of 7, 15, 16 and  
337 95. The lowest  $\Sigma$ REE concentrations measured in males were in the gonads and bile. Bile was the  
338 less concentrated for all the groups of REEs. Then, the order of the organs depending on their REE  
339 concentrations were less homogenous according to the different REE categories. For example, the  
340 muscles were in the 5<sup>th</sup> position for  $\Sigma$ REEs and LREEs while they were in the 7<sup>th</sup> position for  
341 MREEs and HREEs. In addition, for HREEs, the gills were in the first position whereas it was the  
342 liver for the other groups of REEs.

343

### 344 3.3.4. *Life Stage and Gender Influences*

345 The  $\Sigma$ REE organotropism comparison between yellow eels, female and male silver eels  
346 revealed some differences (Tables 1, 2 and 3). The order of the organs according to the different  
347 categories of REEs was homogenous for yellow eels, which was not the case for the silver  
348 individuals whatever the gender. Comparing the two genders of the silver eels, some differences  
349 existed. This observation indicated that the life stage and gender influenced the processes by which  
350 REEs were preferentially directed and stored by certain organs. In addition, it was possible to notice  
351 that there were variations in organotropism depending on the considered groups of REEs:  $\Sigma$ REEs,  
352 LREEs, MREEs and HREEs.

353

#### 354 3.4. REE Pattern in Organs

355

356 Figure 3 shows the REE distribution, in each organ of yellow eels, female and male silver  
357 eels, like patterns. This figure highlighted the predominance of some REEs according to the organs  
358 and the eels considered. It also allowed to characterize which REEs were responsible for the  
359 concentrations of  $\Sigma$ REEs, LREEs, MREEs and HREEs studied in the latter parts of the present  
360 study.

361 The urinary system, gills, skin, liver and spleen presented similar profiles whatever the life  
362 stage or the gender of eels. Indeed, Ce had the highest contribution, reaching 25.5 to 39.6 of  $\Sigma$ REEs.  
363 The following elements were La (12.6-26.1%), Nd (15.3-22.2%) and Y (4.9-24.6%).

364 The gonads of female silver eels displayed a very particular profile which was completely  
365 different from the gonads of other individuals (yellow eels and males silver eels) and even from the  
366 trends of all the other organs. This profile was dominated by Gd which represented up to 74.2 % of  
367  $\Sigma$ REEs with a median concentration of  $27.94 \pm 38.45 \mu\text{g/kg dw}$ . For comparison purpose, this value  
368 was greater than Gd concentration in the liver ( $9.76 \pm 7.38 \mu\text{g/kg dw}$ ) which was the most REE  
369 accumulating organ for female silver eels. However, it remained lower than Ce concentration  
370 ( $69.98 \pm 36.69 \mu\text{g/kg dw}$ ) which was the predominant element in the liver. In female silver eel gonads,  
371 Nd (9.3%) and Ce (4.7%) represented the second and third most abundant elements, after Gd.

372 The male silver eel muscles showed similar patterns compared to female silver eels. These  
373 patterns were different from yellow eel muscles due to a greater contribution of Pr (13.9-16.9% in  
374 silver eels *vs* 3.0% in yellow ones). This great contribution of Pr in silver eel muscles, leading it to  
375 the 2<sup>nd</sup> position among REEs after Ce, made the pattern also different from the other organs of all  
376 other eels. Regarding the yellow eel muscles, the largest contribution of Ce, reaching 72.7%, was  
377 also characteristic, compared to the other organs.

378           Regarding the bile, the REE profiles of female silver eels presented differences from those of  
379 yellow eels and male silver eels without Y and Dy, and consequently higher contributions of Ce  
380 (38.8%) and Nd (35.5%).

381           Whatever the organ, the life stage or the gender of eels considered, Tm and Lu were the least  
382 represented REEs, with <1.2% and < 0.4% respectively.

383

### 384           3.5.   Correlations between Biological Parameters and REE Concentrations in Organs

385

386           Correlations between biological parameters and REE concentrations were analyzed to  
387 investigate the influence of some living characteristics on REE bioaccumulation and internal  
388 distribution. It was also interesting to look at the correlations between REE elements to evaluate  
389 some similar bioaccumulation patterns or dissimilarity.

390

#### 391           3.5.1. *Yellow Eels*

392           In yellow eels, BW and BL were negatively correlated to REE concentrations in gonads for  
393 La, Ce, Pr, Nd, Sm and Gd. These growth parameters were positively correlated to REE  
394 concentrations in spleen for all REEs (except Tm, Yb and Lu). There were also negative correlations  
395 of BL and BW with Pr, Nd, Dy and Er concentrations in muscles. Moreover, REE concentrations in  
396 the spleen and urinary system were positively correlated for all REE (except Tm, Yb and Lu). The  
397 concentrations in gills were positively correlated with La, Ce, Pr, Nd and Tb concentrations in  
398 gonads.

#### 399           3.5.2. *Silver Eels*

400           In female silver eels, REE concentrations in the urinary system were negatively correlated  
401 with the Fulton index for all REEs. Moreover, significant positive correlations were observed  
402 between concentrations of liver for all REEs and age.

403           In male silver eels, the concentrations in gonads were positively correlated with  
404 concentrations measured in liver for all REEs (except Tm, Yb and Lu) and with concentrations  
405 measured in spleen for Sm, Gd, Tb, Dy, Ho and Er. Moreover, REE concentrations in gills were



406 positively correlated with the age for La, Ce and Nd. There were also positive correlations between  
407 spleen and liver concentrations for Pr, Sm and Tb.

408

### 409 3.6. Parasitism influence

#### 410 3.6.1. Yellow eels

411 *A. crassus* appeared to influence the REE contents in yellow eel organs. In fact, the levels of  
412 La, Ce and Pr were significantly higher in muscles of parasitized individuals compared to non-  
413 parasitized ones. The concentrations in parasitized vs non-parasitized yellow eels were:  $0.30 \pm 0.08$  vs  
414  $0.150 \pm 0.04$   $\mu\text{g/kg dw}$  for La,  $2.28 \pm 0.19$  vs  $1.31 \pm 0.38$   $\mu\text{g/kg dw}$  for Ce and  $0.09 \pm 0.03$  vs  $0.07 \pm$   
415  $0.02$   $\mu\text{g/kg dw}$  for Pr.

416 The same trend was observed in the gills, since the concentrations of all REEs, except Tm  
417 and Lu, were significantly higher in parasitized individuals compared to non-parasitized ones. For  
418 example,  $\Sigma\text{REE}$  concentrations in gills reached  $152.36 \pm 63.69$  and  $78.44 \pm 39.92$   $\mu\text{g/kg dw}$ , for  
419 parasitized and non-parasitized individuals respectively.

420 Regarding the gonads, parasitized individuals displayed concentrations of Y, La, Ce, Pr, Nd  
421 and Sm significantly higher than those of non-parasitized ones. To illustrate this observation,  
422 concentrations in parasitized vs non-parasitized yellow eels were:  $1.25 \pm 0.97$  vs  $0.55 \pm 0.24$   $\mu\text{g/kg dw}$ ,  
423  $2.68 \pm 2.49$  vs  $0.53 \pm 0.2$   $\mu\text{g/kg dw}$ ,  $5.80 \pm 4.92$  vs  $1.22 \pm 0.16$   $\mu\text{g/kg dw}$ ,  $0.73 \pm 0.54$  vs  $0.18 \pm 0.07$   $\mu\text{g/kg}$   
424  $\text{dw}$ ,  $2.32 \pm 2.06$  vs  $0.45 \pm 0.27$   $\mu\text{g/kg dw}$  and  $0.46 \pm 0.38$  vs  $0.09 \pm 0.04$   $\mu\text{g/kg dw}$  for Y, La, Ce, Pr, Nd  
425 and Sm respectively.

426 The same result could be noticed for liver, where La concentrations were significantly higher  
427 in parasitized yellow eels ( $31.44 \pm 11.73$   $\mu\text{g/kg dw}$ ) compared to non-parasitized ones  
428 ( $16.15 \pm 7.4$   $\mu\text{g/kg dw}$ ). REE concentrations in the urinary system, skin, spleen and bile were not  
429 significantly affected by the *A. crassus* presence.

430 The  $\Sigma\text{REE}$  concentrations measured in the *A. crassus* collected in the swim bladder of yellow  
431 eels were below the LOQ.

432

### 433 3.6.2. Silver eels

434 The female silver eels showed no significant difference of REE concentrations between  
435 parasitized and non-parasitized individuals for all organs. *A. crassus* collected in the swim bladder of  
436 female silver eels presented  $\sum$ REE concentrations of  $12.56 \pm 13.18 \mu\text{g/kg dw}$ . The effect of the  
437 parasitism on REE concentrations on organs of male silver eels was not achieved because only two  
438 males among the 15 sampled individuals were not parasitized by *A. crassus*. The  $\sum$ REE  
439 concentration measured in *A. crassus* in the swim bladder of male silver eels was  $19.78 \pm 24.83 \mu\text{g/kg}$ .

440

### 441 3.7. REE Bioaccumulation in Glass Eels

442

443 Figure 4A presents the bioaccumulation of  $\sum$ REEs and the different groups (LREEs, MREEs  
444 and HREEs) in the whole body of glass eels at different pigmentation stages. The  $\sum$ REE median  
445 concentrations were  $8.68 \pm 0.8$ ,  $6.74 \pm 1.95$  and  $9.63 \pm 3.92 \mu\text{g/kg dw}$  for VI A0, VI A1 and VI A2  
446 stages respectively. All REE concentrations are presented in Table 2 in the supplementary material.  
447 No significant difference of concentrations between the 3 stages was highlighted. Moreover, there  
448 was no significant difference between the three stages for LREEs, MREEs and HREEs as well. The  
449 same accumulation trends were observed in glass eels than for the other life stages (yellow and  
450 silver) presented in Figure 2. LREEs were accumulated in higher concentrations, followed by  
451 HREEs and then by MREEs. The profiles of REE abundances in glass eels are shown in Figure 4B.  
452 Whatever the stage of pigmentation, the predominant REEs in the whole body of glass eels were Ce  
453 (27-32%) followed by Y (23%) and then La (14-16%).

454

## 455 4. Discussion

456

457 In this study, significant positive correlations between BL and BW were observed regardless of  
458 the life stage or the gender of the fish. In addition, the males were significantly smaller and lighter  
459 than the females. This sexual dimorphism was expected when the European eel is at the silver stage  
460 (Amérand et al., 2010; Durif, 2003). The ages between yellow eels and female silver eels or between  
461 female and male silver eels were not significantly different. This could be attributed to intraspecific  
462 variations (the yellow phase can last between 3 and 15 years) (Adam et al., 2008) as well as

463 environmental effects which could lead to sedentary lifestyle of organisms for example (Feunteun et  
464 al., 2000). This allowed the comparison of REE bioaccumulation between the different groups and to  
465 investigate the influence of other variables such as physiological and morphological differences  
466 related to life stage or gender.

467 The metal uptake and internal distribution in aquatic organisms are influenced by many  
468 parameters linked to their living environment (compartment, pH and temperature), to the nature of  
469 the element (physico-chemical characteristics, concentration, speciation and bioavailability) and to  
470 the species (absorption, detoxification, feeding, trophic level, gender and life stage). It had already  
471 been shown that the REE bioaccumulation in European eel muscles changed with the life stage and  
472 the gender (Lortholarie et al., 2019). This observation was deepened in this work by investigating the  
473 REE concentrations in skin, gills, spleen, urinary system, liver, bile and gonads in the yellow eels,  
474 female and male silver eels.

475 Regarding the life stage influence, the comparison between yellow and female silver eels  
476 revealed that the silver eels accumulated significantly more in the urinary system, muscles, gonads,  
477 spleen and liver, while yellow eels accumulated more in gills. These two life stages presented REE  
478 level differences but also differences in the element distribution throughout the organism. Indeed, the  
479 strongest REE bioaccumulation was observed in gills for yellow eels and in liver for female silver  
480 eels. To go further, the  $\Sigma$ REE organotropism in yellow eels was gills > liver > kidney > skin >  
481 spleen > gonad > bile > muscles and in females silver eels was liver > kidney > spleen > gills >  
482 gonads > skin > muscles > bile. Moreover, by observing REEs in each individual organ, differences  
483 had been observed in the REE contribution within certain organs: i.e. muscles, gonads, gills and bile.  
484 Only urinary system, skin, liver and spleen presented similar profiles each other between yellow eels  
485 and female silver eels. The yellow eel muscles had a specific profile too, relatively different from the  
486 female silver eel muscles. The female silver eel gonads displayed a special profile, with a very  
487 important contribution of Gd, followed by Nd, then by Ce which was usually the REE predominant  
488 in the other gonads and organs in general. In other estuarine field studies (Amorim et al., 2007;

489 Moermond et al., 2001; Nozaki et al., 2000), Gd was not the predominant REE measured in sediment  
490 and water. High concentrations of Gd in female silver eel gonads suggests a particular affinity of  
491 this element for this organ. An in-depth study of this phenomenon is still needed, this observation  
492 being not previously reported in the relatively rare studies about REE bioaccumulation in the gonads  
493 and particularly in fish gonads. Thus, further research must be performed to explain this specific Gd  
494 accumulation and to evaluate if it could be related to Gd anthropogenic uses which could therefore  
495 have a greater impact in estuarine and coastal areas than expected. Indeed, the Gd is a toxic ion with  
496 very interesting paramagnetic properties, so it is often used as a contrast agent for MRI (Magnetic  
497 Resonance Imaging) after being made inert by chelating it with a ligand. Nowadays, 25% of MRI use  
498 complexed Gd, this is worrying because the Gd is not removed in wastewater treatment plants and  
499 leads to aquatic environmental contamination (Parant et al., 2019). The REE bioaccumulation  
500 differences observed between the two life stages, could be caused by the silvering. Indeed, this  
501 metamorphosis leads to many external changes such as: coloration, thickening of the skin,  
502 lengthening of the pectoral fins, increased mucus secretion and increased size of the sensory organs.  
503 There is also an increase of gonad weight, a change of swim bladder wall, lipid accumulation, liver  
504 structural and metabolic changes, discontinuation and digestive tract retraction (Adam et al. 2008;  
505 Fontaine 1994; Durif et al. 2015). The digestive tract retraction which takes place during this phase  
506 can be an explanation of the higher REE concentrations in female silver eels (urinary system,  
507 muscles, gonads, spleen and liver) than in yellow eels. Indeed, the intestines are an important  
508 excretion route. The suppression of this excretion pathway may increase the pollutant accumulation  
509 in silver eels. Moreover, during the metamorphosis into silver eel, the liver undergoes morphological  
510 and physiological changes, these liver changes can cause higher accumulation of REEs. Finally, in  
511 order to successfully migrate to the Sargasso Sea, the silver eels undergo a change in their muscles.  
512 This may explain that the silver females accumulated more REEs than yellow females. Since yellow  
513 eels were only future silver female eels, this conclusion cannot be drawn for males.

514           Regarding the gender influence, the comparison between male and female silver eels  
515 highlighted differences. The females accumulated significantly more  $\sum$ REEs in kidneys, gonads,  
516 skin and spleen, whereas the males accumulated significantly more  $\sum$ REEs in muscles and gills.  
517 Thus, it is possible to conclude that, depending on gender, the preferential REE accumulation did not  
518 occur in the same organs. The  $\sum$ REE organotropism in female was liver > kidney > spleen > gills >  
519 gonads > skin > muscles > bile and in male, liver > gills > kidney > spleen > muscles > skin >  
520 gonads > bile. As shown by the results, the gender impacted the levels of REEs but also the REE  
521 abundances. These results could be explained by differences in physiology, behavior, detoxification  
522 mechanisms and parasitism. A metabolism difference linked to sexual dimorphism could be another  
523 explanation. Because of their smaller size, males spend more energy than females on their migration  
524 (Amérand et al., 2010). The increased metabolism of males can lead to faster pollutant elimination,  
525 which perhaps explains why males accumulated less REEs in the urinary system, the gonads, the  
526 skin and the spleen. Another explanation may be due to male/female differences during the sexual  
527 maturation phase, this hypothesis will be developed later in this discussion. The study of MacMillan  
528 et al. (2017) conducted on brook trout (*Salvelinus fontinalis*) caught in Quebec lakes, showed a  
529 significant effect of gender on  $\sum$ REE concentrations in liver. The values found for liver of males  
530 were 2.5 times higher than those for females. This bioaccumulation difference between male and  
531 female livers observed for the brook trout was not observed in the present study of the European eel.

532           REE organotropism study, *i.e.* the determination of the organs where REEs were  
533 accumulated, is important in the ecotoxicity understanding of their fate and potentially their mode of  
534 toxic action. Indeed, depending on the preferential accumulation organ, REEs will not have the same  
535 toxicity or detoxification way. In this work, by ordering the organs from the most to the least  
536 contaminated, some divergences were underscored between yellow and female silver eels as well as  
537 between female and male silver eels. These results can be compared with other studies carried out on  
538 fish caught in the wild or exposed in the laboratory. After a laboratory exposure of carps, *Cyprinus*  
539 *carpio* for 43 days to a mixture of Ce (0.27 mg/L), La (0.30 mg/L), Nd (0.29 mg/L), Pr (0.06 mg/L)

540 and Sm (0.25 mg/L), the organotropism determined for LREEs was:  
541 organs >> gills > skeleton > muscles (Hao et al., 1996). The results were less detailed but similar to  
542 the results of the present study since the LREE organotropism (from La to Nd) was: liver > gills >  
543 spleen > kidney > muscles > skin > gonads > bile for male silver eels. Other publications exist on  
544 REE organotropism. For example, considering carps (*Cyprinus carpio*) exposed during 45 days at  
545 concentrations of 0.50 mg/L of La (as a model of LREEs), Gd (for MREEs) and Y (for HREEs), the  
546 organotropism was: internal organs > gills > skeleton > muscles (Qiang et al., 1994). The study of  
547 Cardon et al., in 2020, conducted on rainbow trout exposed to different Y concentrations by  
548 waterborne or by food obtained a completely different organotropism:  
549 intestine > gills > liver > muscles. The lionfish from Cuba accumulated more  $\Sigma$ REEs in kidney with a  
550 concentration of 0.053 mg/kg ww, higher than liver (0.018 mg/kg ww) or muscles (0.016 mg/kg ww)  
551 (Squadrone et al., 2019). The comparison of results from literature and from the present study  
552 remained complex because there was a lack of precision on the life stage or gender of fish. However,  
553 the main observations made through this present work were the differences of results between yellow  
554 eels and female silver eels as well as female and male silver eels. Moreover, the organs investigated  
555 in this study were more numerous and individually studied which is not the case of some studies in  
556 which the samples were pooled. The more detailed organotropism of the present work allowed to  
557 highlight that liver, urinary system and spleen were the most accumulating organs of REEs. In fish,  
558 the liver and the kidneys represent the main sites of metal sequestration and elimination (Pannetier et  
559 al., 2016). These organs have an important REE accumulation which can be explained by their role  
560 in the production of metallothionein and the metal detoxification. Finally, the results from the  
561 literature mentioned above concerned *in vivo* controlled experiments with one up to five elements in  
562 mixture. These conditions were greatly different to those of the present *in situ* study. In natural  
563 environment, REEs are more or less all present in the medium. An interaction can occur each other  
564 and with other contaminants or natural constituents. REE speciation and distribution in natural  
565 environments can also be different from those found in controlled experiments.

566 In the present work, gills were often positioned among the organs with the highest REE  
567 bioaccumulation. On one hand, this could be explained by the fact that they are in direct contact with  
568 water therefore with the metal dissolved forms. They represent the organ leading to metal penetration  
569 into the fish organism during the water filtration by the gill epithelium during the respiration. On the  
570 other hand, the gills for freshwater, marine and euryhaline fish species are the main route of calcium  
571 absorption before the intestines (Baldisserotto, 2019; Flik et al., 1995). REEs are antagonists of  
572 calcium absorption. In fact they are able to mimic  $\text{Ca}^{2+}$  due to their similar ionic radius (Figueiredo  
573 et al., 2018; Martin & Richardson, 1979)). Indeed, ionic radius for the calcium is 0.09 nm and range  
574 between 0.085 and 0.106 nm for Lu and La, or 0.089 nm for yttrium. In addition, REEs have a higher  
575 valence (3+) than calcium, so they are able to take the  $\text{Ca}^{2+}$  place (Cui et al., 2012). In fact, their +III  
576 charge allows them to form stronger complexes (Switzer, 1978) and gives them a higher charge /  
577 volume ratio so they generally present higher affinities than  $\text{Ca}^{2+}$  for given binding sites (Evans,  
578 1983). In addition, their charge can also promote a higher coordination number, this is particularly  
579 true for smaller REEs which, by increasing their coordination number, can reach an ion radius closer  
580 to  $\text{Ca}^{2+}$  (Evans, 1983). However, the REE size is more important than their charge in the calcium  
581 replacement (Switzer, 1978). The similarities between REEs and calcium can cause: higher REE  
582 assimilation by the organisms, an alteration of the calcium cycle which can lead to a calcium  
583 deficiency, a decrease in the essential element assimilation depending on calcium channels, an  
584 inhibition of skeleton muscles, smooth muscle and heart muscle contractions. Moreover, REEs are  
585 able to replace the calcium in proteins leading to a change of their function. In addition, the gills play  
586 a role in the calcium excretion also. These potential characteristics of REEs could explain their high  
587 concentrations particularly found in gills of yellow eels and male silver eels. The high REE  
588 concentrations determined in the eel urinary system can also be linked to their similarity with  
589 calcium. In fact, kidneys are the main site for divalent ion excretion like calcium (Flik et al., 1995).  
590 Finally, during sexual maturation of female teleosts as eels, the vitellogenin production lead to an  
591 increase of the calcium demand. This demand is satisfied by an increase of environmental

592 contributions or by internal remobilization (Persson et al., 1998). It had also been shown that during  
593 this maturation period there is the incorporation of calcium in the oocytes (Persson et al., 1998) for  
594 female organisms. Considering REE and Ca<sup>2+</sup> similarities, this could also explain why silver female  
595 gonads accumulated more REEs, and especially Gd, than those of yellow females and silver males.  
596 Concerning parasitism, in female yellow eels, non-parasitized individuals displayed significantly  
597 lower REE concentrations compared to parasitized individuals in muscles (La, Ce and Pr), in gills  
598 (all REEs, except Tm and Lu), in gonads (Y, La, Ce, Pr, Nd, and Sm) and in liver (La). On one hand,  
599 the presence of *A. crassus* in the swim bladder is able to lead to numerous pathologies at  
600 macroscopic (change in thickness and opacity of the swim bladder), immunological (induction of  
601 non-specific and humoral responses) and physiological (alteration of gas excretion in the swim  
602 bladder, decrease in blood pressure, swimming ability, increased stress) levels (Habbechi et al.,  
603 2012; Kirk, 2003). Yellow parasitized eels could be more sensitive than yellow non-parasitized ones  
604 to their environment and to the contaminants, less able to detoxification, which could lead to a  
605 greater REE bioaccumulation. On the other hand, another hypothesis could be that more sensitive  
606 individuals, for other reasons than parasitism like REE contamination, could be more parasitized.  
607 Finally, it is impossible to conclude in favor of one of these two hypothesis. The reality was perhaps  
608 a mix of these explanations but both could explain the link between parasitism and higher REE  
609 concentrations in several studied tissues of female yellow eels. The results and corresponding  
610 hypothesis for female yellow eels are not appropriated for silver ones because no significant  
611 difference of REE contents was found between parasitized and non-parasitized individuals for all  
612 organs. This result highlighted that the life stage of eels could have an influence on the link between  
613 parasitism and REE concentration. In literature, some results exist about parasitism and its influence  
614 on metal bioaccumulation but without considering different life stages and gender. The publication  
615 of Palíková and Baruš (2003) did not show link between *A. crassus* and mercury bioaccumulation in  
616 the European eel. Another study including different metals (Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn) did  
617 not show significant differences in metal accumulations in European eel tissues according to the



618 parasitism factor (Genc et al., 2008). In this latter study, Genc et al. (2008) found Fe accumulation in  
619 *A. crassus* up to 25.52 times than the muscle of *A. anguilla* caught in the Asi River (Hatay-Turkey).  
620 In the present study, the same trend was observed for female silver eels with REE concentrations in  
621 the parasites ( $12.56 \pm 13.18 \mu\text{g/kg dw}$ ) about 2 times higher than in muscles ( $6.66 \pm 2.13 \mu\text{g/kg dw}$ )  
622 and more than 9 times than in bile ( $1.29 \pm 0.88 \mu\text{g/kg dw}$ ). Parasites could accumulate REEs in favor  
623 of female silver eels. Nevertheless, the greater concentration of REEs in parasites compared to  
624 muscle and bile of female silver eels was not observed for female yellow eels, as REE were not  
625 detected in the *A. crassus* of yellow individuals. The life stage seemed to have an influence on the  
626 parasite REE contents also, probably by affecting mechanisms in parasites or in parasitized  
627 organisms. In the study of Schneebauer *et al.*, . (2017), infected yellow eels presented in the swim  
628 bladder 1675 genes differentially transcribed compared to uninfected yellow eels, but silver eels only  
629 exhibited 291 differentially transcribed genes compared to uninfected silver eels. In 2016,  
630 Schneebauer *et al.*, have shown that silver eels have poorer detoxification of ROS when they are  
631 parasitized, while yellow eels do not show significant differences. It seems that the two life stages do  
632 not react in the same way to parasitism, but the result obtained in this study remained difficult to  
633 explain since knowledge on the sensitivity of life stage to parasitism are limited.

634         Regarding the glass eels, the pigmentation stage (VI A0, VI A1 and VI A2) did not seem to  
635 influence the REE bioaccumulation. Glass eels had median REE concentrations ranging from 6.74 to  
636  $9.63 \mu\text{g/kg dw}$  respectively for VI A1 and VI A2 stages. After the metamorphosis of the  
637 leptocephalus into the glass eels, individuals stop feeding and start eating again at the VI A3 stage.  
638 The glass eels studied in this work were caught during this fasting period. Their REE concentrations  
639 could be the result of external contamination reflecting the environmental concentrations. In another  
640 study, glass eels exposed during 3 days at 120 ng/L of lanthanum chloride in water accumulated 1.6-  
641 4.7, 1.1-6.7 and 4.8-13.0 ng/g dw in head, skinless body and viscera respectively (so with an  
642 organotropism of viscera > skinless body > head) (Figueiredo et al. 2018). These results and the  
643 concentrations found in the present work were in the same order of magnitude. The REE

644 predominance in the whole body of glass eels showed a profile closer to the skin of yellow and silver  
645 eels than female gonads dominated by Gd. The high abundance of Gd observed in female silver eel  
646 gonads were not present in glass eels, suggesting no or weak potential maternal transfer.

647

## 648 5. Conclusion

649 This study reported data on the REE organ contents in wild organisms of European eels from  
650 the Loire estuary. The bioaccumulation examination in glass eels, yellow eels, male and female  
651 silver eels allowed to highlight the life stage and the gender influence on the REE concentrations,  
652 REE abundance in organs and hence their organotropism. This work, through a detailed  
653 organotropism, made it possible to underscore the liver, kidneys and spleen importance in the REE  
654 bioaccumulation. This can be explained by the role in the sequestration and elimination metals from  
655 these organs. In addition, the gills also appeared as organs with a high REE accumulation. This may  
656 be linked to the REE ability to mimic calcium; indeed, the gills are the main route of calcium entry in  
657 fish. To go further, the REE contribution study have shown that the REEs present in higher  
658 concentration can be different between some organs, life stages and gender. The silver female gonads  
659 attracted attention because they presented a very special profile dominated by the Gd presence.  
660 Moreover, the *Anguillicola crassus* presence seemed impact REE bioaccumulation in yellow eels  
661 because, parasitized yellow eels accumulated more in muscles, gills, gonads and liver than non-  
662 parasitized individuals. Regarding glass eels, the stage of pigmentation did not seem to have an  
663 influence on the REE bioaccumulation. In addition, the REE contribution profile in the whole body  
664 was close to the profile of the yellow and silver eel skin. In order to go deeper into the REE  
665 organotropism determination in European eels and better understand the influence of parameters  
666 such as gender or life stage it would be necessary to investigate the kinetic processes of  
667 internalization underlying this organotropism. It would also be interesting to explore reasons why Gd  
668 accumulated so much in female silver eel gonads and if it is related to Gd anthropogenic uses.

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850

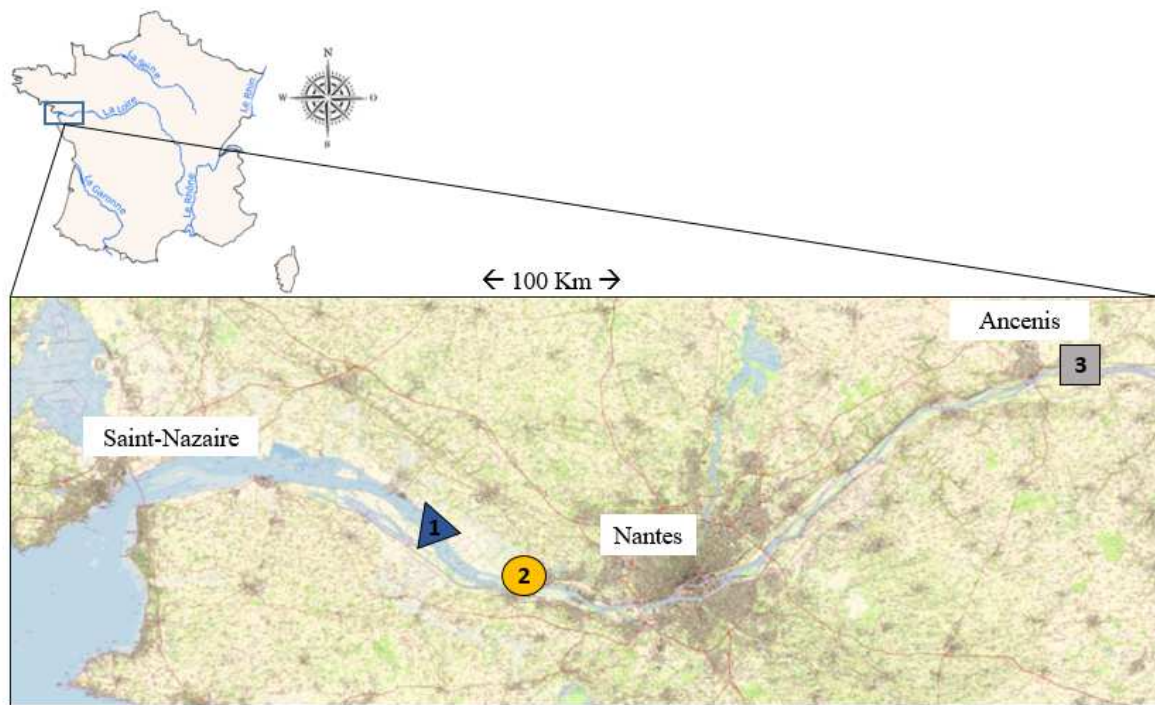
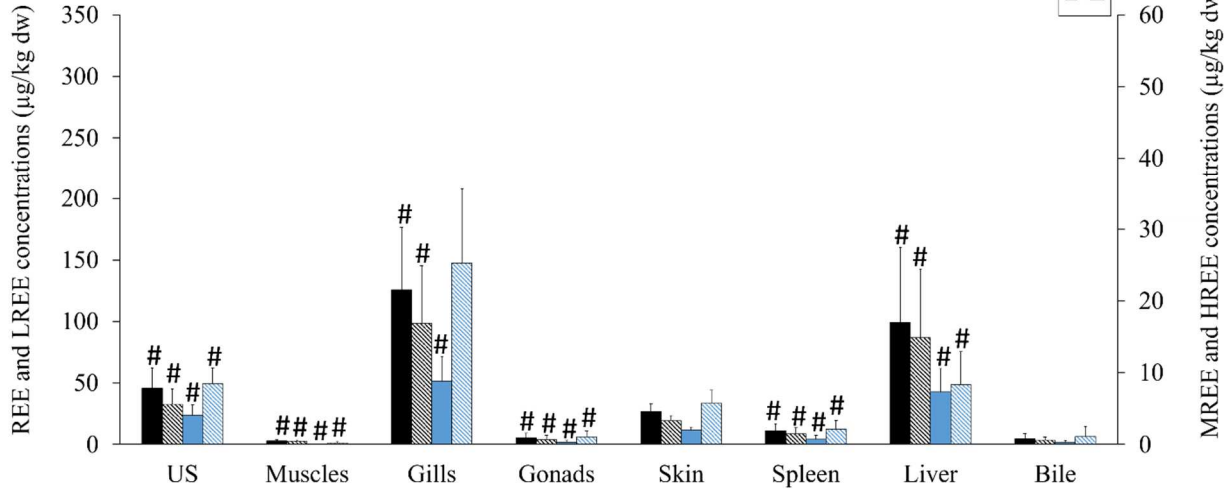


Figure 1: Sampling sites of glass eels (blue triangle), yellow (yellow ring) and silver (gray square) eels captured in the Loire estuary (1: Cordemais, 2: Haute-Indre and 3: Varades).

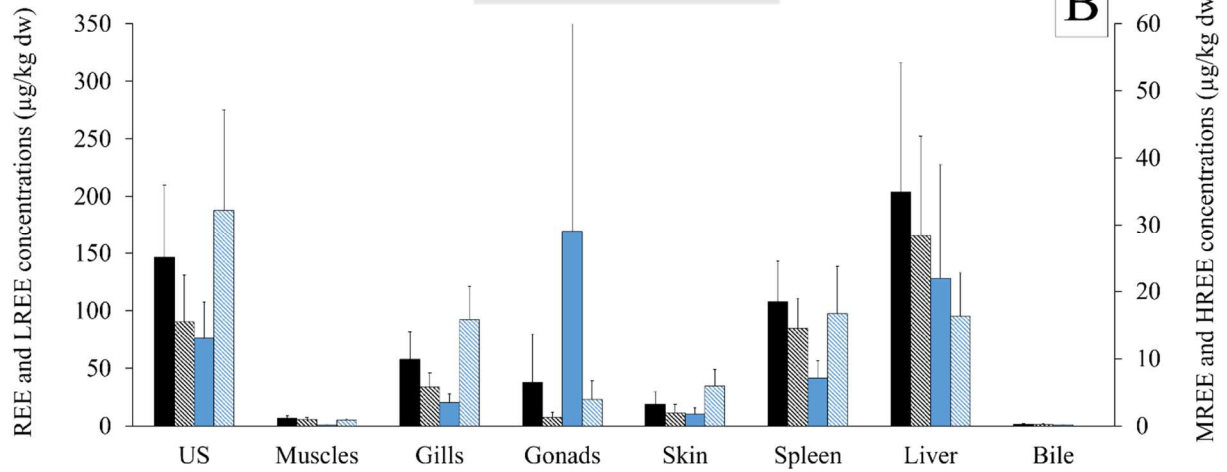
### Yellow eels

A



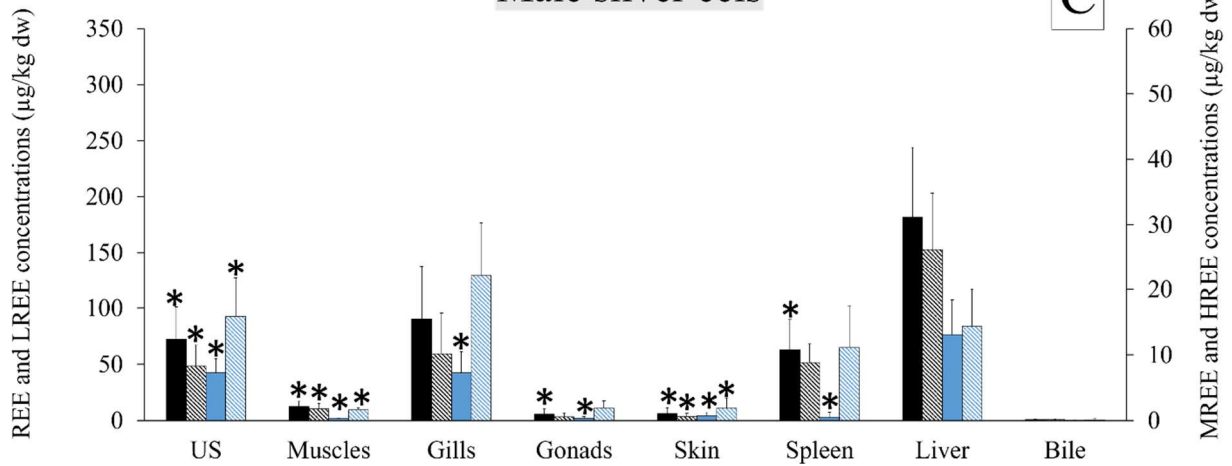
### Female silver eels

B



### Male silver eels

C



■ REEs    ▨ LREEs    ■ MREEs    ▨ HREEs

Figure 2: Median and Median Absolute Deviation for  $\Sigma$ REE (black bars), LREE (from La to Nd, black striped bars), MREE (from Sm to Gd, blue bars) and HREE (from Tb to Lu and Y, blue striped bars) concentrations ( $\mu\text{g}/\text{kg dw}$ ) in organs of (A) yellow eels (n=15), (B) female (n=14) and (C) male (n=15) silver eels.

#: significant differences between values of yellow eels and female silver eels with Kruskal Wallis test ( $p\text{-value}\leq 0.05$ )

\*: significant differences between values of female and male silver eels with Kruskal Wallis test ( $p\text{-value}\leq 0.05$ )

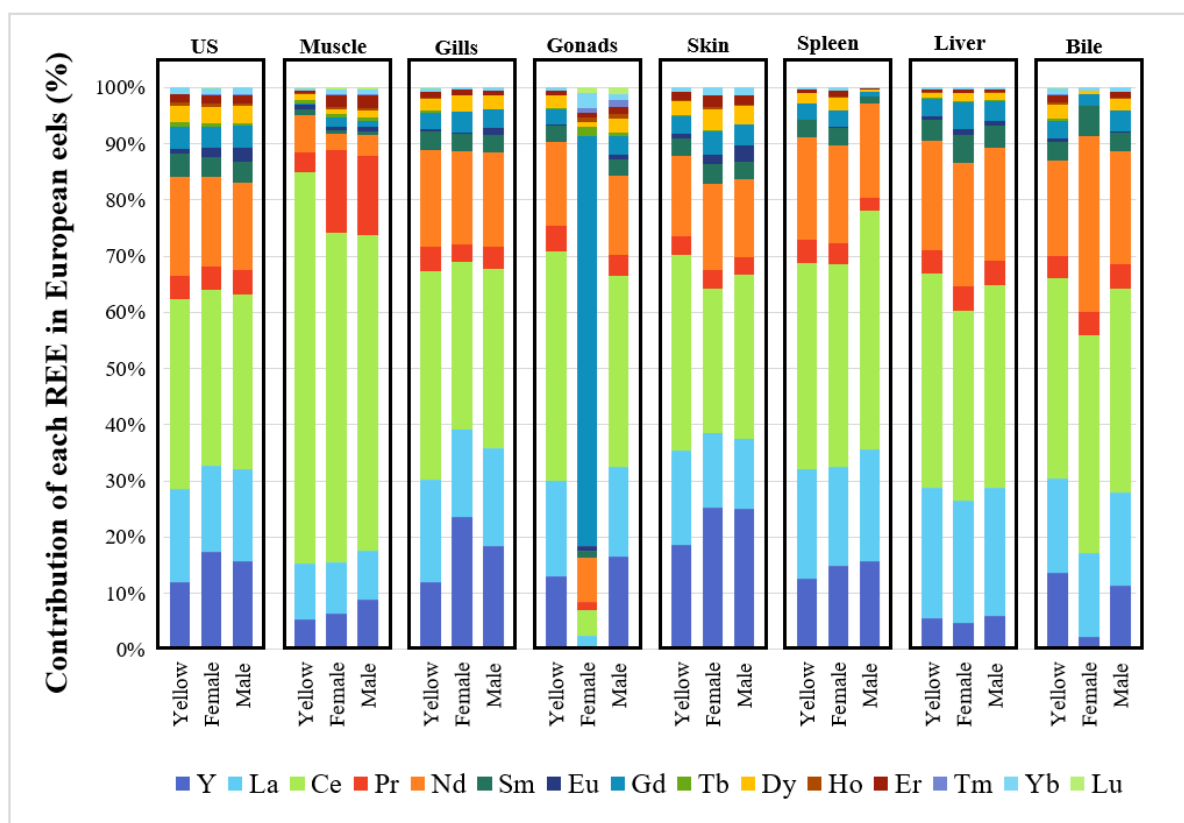


Figure 3: REE contribution (%) in each organ for yellow eels, female and male silver eels. US: Urinary system

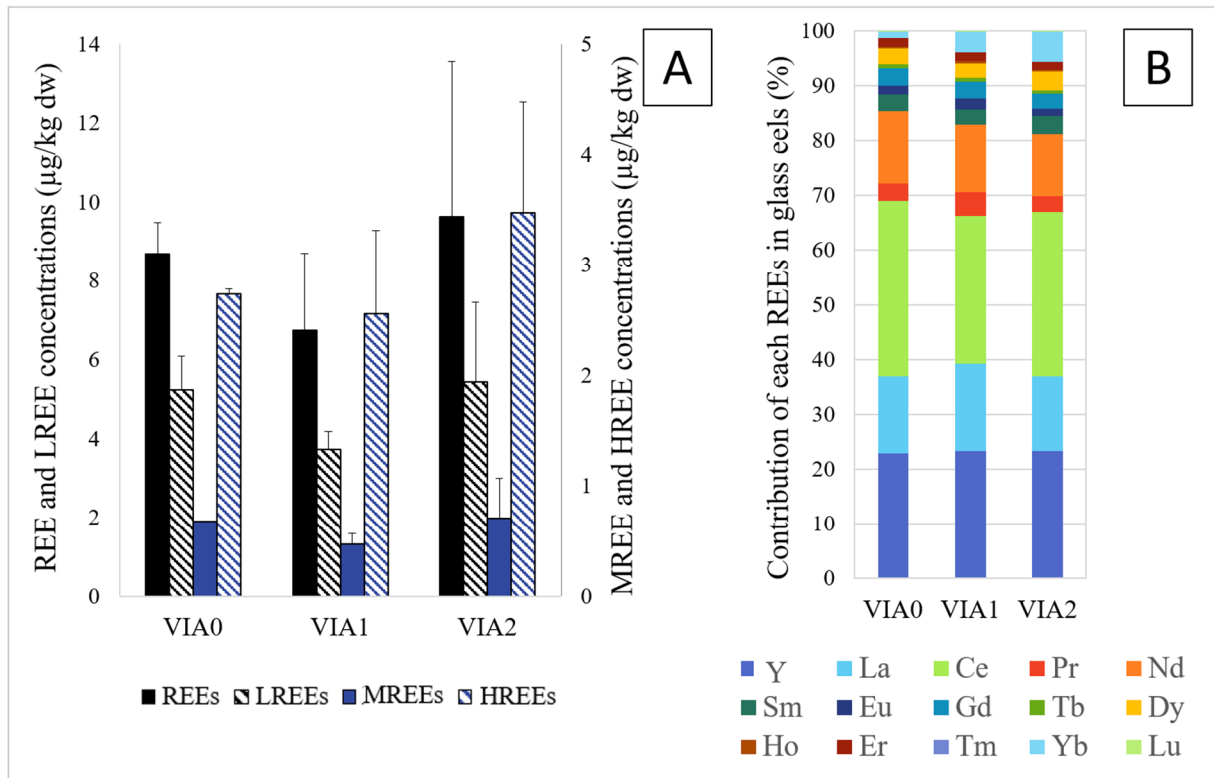


Figure 4: (A) Median and Median Absolute Deviation (error bars) for REE (back bars), LREE (from La to Nd, black striped bars), MREE (from Sm to Gd, blue bars) and HREE (from Tb to Lu and Y, blue striped bars) concentrations (ng/kg dw) in different stages of glass eels: VI A0 (n=15), VI A1 (n=15) and VI A2 (n=15). (B) REE contribution (%) in the whole body of glass eels.

Table 1: Classification of yellow eel organs from the more to the less concentrated in  $\Sigma$ REEs, LREEs, MREEs and HREEs

	More concentrated							Less concentrated	
$\Sigma$ REEs	Gills	Liver	Urinary system	Skin	Spleen	Gonads	Bile	Muscle	
LREEs	Gills	Liver	Urinary system	Skin	Spleen	Gonads	Bile	Muscle	
MREEs	Gills	Liver	Urinary system	Skin	Spleen	Gonads	Bile	Muscle	
HREEs	Gills	Urinary system	Liver	Skin	Spleen	Bile	Gonads	Muscle	

Table 2: Classification of female silver eel organs from the more to the less concentrated in  $\Sigma$ REEs, LREEs, MREEs and HREEs

	More concentrated						Less concentrated		
$\Sigma$ REEs	Liver	Urinary system	Spleen	Gills	Gonads	Skin	Muscle	Bile	
LREEs	Liver	Urinary system	Spleen	Gills	Skin	Gonads	Muscle	Bile	
MREEs	Gonads	Liver	Urinary system	Spleen	Gills	Skin	Muscle	Bile	
HREEs	Urinary system	Spleen	Liver	Gills	Skin	Gonads	Muscle	Bile	

Table 3: Classification of male silver eel organs from the more to the less concentrated in  $\Sigma$ REEs, LREEs, MREEs and HREEs

	More concentrated						Less concentrated		
$\Sigma$ REEs	Liver	Gills	Urinary system	Spleen	Muscle	Skin	Gonads	Bile	
LREEs	Liver	Gills	Spleen	Urinary system	Muscle	Skin	Gonads	Bile	
MREEs	Liver	Gills	Urinary system	Skin	Spleen	Gonads	Muscle	Bile	
HREEs	Gills	Urinary system	Liver	Spleen	Gonads	Skin	Muscle	Bile	

Graphical abstract:

