Rare earth element organotropism in European eel (Anguilla anguilla)

Marjorie Lortholarie *, Laurence Poirier, Abderrahmane Kamari, Christine Herrenknecht, Aurore Zalouk-Vergnoux

Laboratory Mer, Molécules, Santé (MMS, EA 2160), University of Nantes, Nantes F-44322, France

HIGHLIGHTS

• Organotropism determined in muscles, skin, gills, spleen, US, liver and gonads
• REE accumulation and organotropism were influenced by gender and life stages.
• Yellow eels accumulated more in gills, female and male silver eels in livers.
• Parasitized yellow eels accumulated more REEs than non-parasitized.

GRAPHICAL ABSTRACT

Abstract

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ABSTRACT

Rare earth elements (REEs) are metallic elements with electronic, magnetic, optical and catalytic properties which make them essential in many industrial and medical fields. REEs are therefore becoming emerging pollutants and it is important to understand their implications for ecosystem health. However, little knowledge of REE bioaccumulation in aquatic organisms is available and especially on their internal distribution in fish. In the present study, REE organotropism was determined in Anguilla anguilla from the Loire estuary (France) by determining burdens in a wide set of tissues, organs and biological fluids. Differences have been observed between life stages and genders. For yellow eels, the most accumulating organ was the gills (126.90 ± 50.78 μg/kg dw) and for silver eels, it was the liver (181.78 ± 62.04 μg/kg dw for males; 203.79 ± 111.86 μg/kg dw for females). The comparison between female silver and yellow eels shown that female silver individuals accumulated significantly more REEs in the urinary system (US), muscles, gonads, spleen and liver, while yellow individuals accumulated more in gills. The comparison between male and female silver eels also highlighted differences, indeed the females accumulated significantly more REEs in the US, gonads, skin and spleen, compared to males which accumulated significantly more in muscles and gills. REEs abundances are also different between organs, life stages and genders. The gonads of female silver eels exhibited a particular profile with the dominance of gadolinium (Gd) (up to 74.2% of ∑REEs). Moreover, the presence of Anguillicola 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 non-parasitized individuals. Regarding glass eels, REE contribution profiles in the whole body were close to those of yellow and silver eel skin.

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* Corresponding author.
E-mail address: marjorie.Lortholarie@univ-nantes.fr (M. Lortholarie).

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1. Introduction

*Anguilla anguilla* is an euryhaline and catadromous species with a reall special life cycle. After a transatlantic migration of nearly 6000 km from east to west traveled from 4 to 6 months, silver eels arrive in the Sargasso Sea for the reproduction where they die after. Laid eggs turn into leptocephalus larvae which migrate using the Gulf Stream to European coasts. Arriving at the level of the continental slope about 100 km from the coast, leptocephalus larvae become glass eels. In freshwater, they begin to feed and after some time, glass eels gradually pig-ment in black then yellow to become yellow eel. Yellow eels live in freshwater. They are benthic and sedentary for several years waiting for the silvering. This metamorphosis changes yellow eels into silver eels upon sexual maturity (Adam et al., 2008; Corolla et al., 2016). This transition phase leads to many morphological and physiological changes (Adam et al., 2008; Fontaine, 1994; Durif et al., 2015). During the downstream migrating period, the silver eels are sexually differentiated but they are not yet completely mature.

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

Among the threats to eels, the aquatic ecosystems contamination is very worrying. In fact, the European eel are able to accumulate a wide variety of environmental contaminants and some studies have highlighted the accumulation of polycyclic aromatic hydrocarbons (Ribeiro et al., 2005), pesticides and persistent organic pollutants (Couderc et al., 2015; van der Oost et al., 1996). They are also able to accumulate essential metals (Ni, Zn, Cu, Mn, Co and Fe), non-essential metals and metalloids (Hg, Al, Cr, Pb, Cd and As) (Barak and Mason, 1990; Bordajandi et al., 2003; Durrieu et al., 2005; Mansouri et al., 2018; Neto et al., 2011; Ribeiro et al., 2005; Storelli et al., 2007; Tabouret et al., 2011). The metal bioaccumulation can cause sublethal biological effects. For example, yellow eels exposed during one month to Cd (5 μg/L) presented an increase of fat consumption in their reserve (Gony, 1990). A one-month exposure to 300 μg/L of Pb, increased the number of lymphocytes and the plasma lactate levels in yellow eels (Santos and Hall, 1990). After a 7 day exposure of eels to Cu 0.2 μmol/L, significant decreases of free triiodothyronine and cortisol as well as a significant increase of glucose were observed in the plasma. In addition, a lactate decrease and an increase of nuclear abnormality frequency were measured in blood, gills, liver and urinary system (Oliveira et al., 2008). The ecotoxicology of essential and non-essential metals is now relatively well documented for eels, but other types of metallic elements, such as REEs, are becoming emerging contaminants and should be better considered.

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

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

In aquatic environments, REEs are ubiquitous contaminants, their presence has already been measured in waters, suspended particles and sediments in different environmental compartments in the world-wide such as rivers (Keasler and Loveland, 1982; Négrel et al., 2000), estuaries (Elbaz-Pouliquet and Dupuy, 1999; Vrel, 2012) and oceans (Akagi and Edanami, 2017; Dahlqvist et al., 2005; Hirata et al., 2002; Johannesson et al., 2017; Li et al., 2015). Off the Loire estuary, the area concerned by the present study, sediments of the Bay of Biscay showed mean concentrations ranging from 0.11 (Lu) to 47.79 (Ce) mg/kg (Chaillou et al., 2006). With the proximity of the Loire estuary, the mean concentrations in sediments reached 41 and 92 mg/kg for La and Ce respectively. In the suspended particulate matter of the Loire es-tuary, mean values of total REEs (Σ REEs) reached 137 mg/kg upstream of Nantes, 164 to 489 mg/kg downstream of Nantes and 89 mg/kg near the coast (Thibault de Chanvalon et al., 2018). Levels between 195 and 199 mg/kg were also measured in sediments downstream of Nantes. In the Garonne River (La Réole), Lerat-Hardy et al. (2019) measured concentrations in waters from 0.469 ng/L (Tm) to 37 ng/L (Ce) in 2017. Environmental concentrations normalized to reference reservoirs makes possible to highlight anomalies. When these anomalies are positive, that could point out an anthropic enrichment. The Sm and Gd often appeared as positive anomalies, as shown in the Garonne estuary (France) by Lerat-Hardy et al. (2019). These aquatic environmental contami-nations are worrying because REEs were shown to be able to accumu-late in aquatic organisms. In the European eel, REE accumulation has already been highlighted in different life stages, from glass eels (Figueiredo et al., 2018) to yellow and silver eels (Lortholarie et al., 2019).

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

2. Materials and methods

2.1. Chemicals

Stock solutions of 15 REEs (yttrium; Y; lanthanum; La; cerium; Ce; praseodymium; Pr; neodymium; Nd; samarium; Sm; europium; Eu; gadolinium; Gd; terbium; Tb; dysprosium; Dy; holmium; Ho; erbium; Er; thulium; Tm; ytterbium; Yb and lutetium; Lu) and Rhenium (inter-nal standard) at 1000 mg/L in 2% HNO₃, were provided by Roth (single-element ICP standard solution). REEs were oxide forms in solution (YₑO₃, La₂O₃, Ce (NO₃)₃, Pr₂O₃, Nd₂O₃, Sm₂O₃, Eu₂O₃, Gd₂O₃, Tb₂O₇, Dy₂O₃, Ho₂O₃, Er₂O₃, Tm₂O₃, Yb₂O₃, Lu₂O₃). In this work, 15 out of the
17 REEs were studied. Pm was not included because it is not naturally present in the environment and neither Sc because of many interferences during the ICP-MS analysis, which led to overestimation of concentrations. Mineralization and solutions were made with HNO₃ 65% from Fisher Scientific. Mineralization protocol was validated using the international certified material BCR-668 consisted of muscle of mussels from LGC standards.

2.2. Sampling

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

2.3. Preparation of fish tissue and parasites for REE analyses

After euthanasia, eels were weighed (g) and measured (cm) to get respectively the body weight (BW) and the body length (BL). During the dissection, the observation of the gonads allowed to visually identify the gender of individuals. Interest organs for the organotropism study were removed and weighed: muscles, skin, gills, spleen, urinary system, liver, and gonads. Two different biological fluids were also collected: blood and bile. When present, A. crassus parasites were also collected from the swim bladder of eels. A. crassus is a nematode feeding on blood and causing a disease called Anguillicosis (Adam et al., 2008). The parasites were counted, weighed and analyzed as fish tissue. The parasite index (PI) was determined using:

\[
PI = \left(\frac{\text{weight}_{\text{Anguillicolacrassus}}}{\text{weight eel body}}\right) \times 100.
\]

The otoliths were recovered to determine the age of each individual according to ICES 2009 (International Council for the Exploration of the Sea) methodology. Glass eels were observed with a binocular and trilled into three groups VI A0, V A1 and VI A2, depending on their pigmentation stage (Grellier et al., 1991). The gonads were recovered and weighed to determine the gonadosomatic index (GSI). The GSI was calculated as follows:

\[
GSI = \frac{\text{gonad weight (g)}}{\text{whole body weight (g)}} \times 100.
\]

The Fulton condition index (K) was determined as following:

\[
K = \left(\frac{\text{whole body weight (g)} \times 10^3}{\text{whole body length}^3 (cm)}\right).
\]

K allows evaluating the overall global eel health in front of environmental stresses such as pollution or feeding conditions.

At the beginning of the experiments, all the material was cleaned by immersion in HNO₃ 10% for one night and rinsed with Milli-Q water before use. The low REE concentrations expected in the muscles required a preconcentration step by calcination allowed due to sufficient muscle masses. About 1 g of muscles were calcined in a muffle furnace (Nobtherm) at 300 °C during 1 h followed by 500 °C during 2 h. The muscle samples were therefore concentrated about 10 times. For the mineralization, 250 mg of ashes of muscles, of the other organs or of A. crassus were collected and weighted. Then, the mineralization relied on a predigestion with a mix of HNO₃/HCl (2,1, v/v) for one night at room temperature and a following step using microwaves (Anton Paar, MW GO 50 Hz) (Lortholarie et al., 2019). The solutions were then transferred into Pyrex tubes and evaporated to dryness in hot-plate at 130 °C. Samples were resuspended with 1.8 mL of HNO₃ 2% and then vortexed, sonicated (20 min) and centrifuged (5 min,
2.0 rpm, 15 °C) to eliminate particles in suspension. Only the supernatants were analyzed.

2.4. Rare earth element analyses

Concentrations of REEs were determined by Inductively Coupled Plasma coupled to Mass Spectrometry (ICP-MS, Nexion 350× PerkinElmer). All samples, blanks and standards were diluted using HNO₃ 2%. Rhenium was used as internal standard (187Re isotope), with a final concentration of 1 μg/L in the solutions. Matrix effect determination was performed using a matrix matched calibration. Sample supernatant solutions were used as the matrix to prepare calibration solutions with increasing concentrations of REEs. REE isotopes were selected according to a compromise between the maximum of natural abundance and the minimum of interferences. They corresponded to: 89Y, 129La, 140Ce, 141Pr, 146Nd, 147Sm, 149Eu, 150Gd, 159Tb, 163Dy, 165Ho, 166Er, 167Tm, 171Yb and 175Lu. Limits of detection (LOD) and quantification (LOQ) were determined for each REE. Analytical and procedural blanks were prepared and analyzed using the same analytical procedure as for the samples. Finally, the blank values were subtracted from the values of the samples. The accuracy of the whole analytical protocol was validated using an international certified material containing REEs (BCR-668, muscle of mussels).

2.5. Data processing

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

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

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

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

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 + 0.8962 (R² = 0.947). Moreover, BL and BW were also significantly correlated with age. The linear regressions were respectively: age = 0.226 + 5.115 (R² = 0.565) and age = 0.015 + 3.889 (R² = 0.697).

In female silver eels, BL and BW were also positively correlated: BW = 15.256 + 5.435 (R² = 0.978). However, BW and GSI were negatively correlated, with a linear regression of GSI = −0.002 + 0.282 (R² = 0.848).

In male silver eels, BL and BW were positively correlated: BW = 6.078 + 0.1391 (R² = 0.749). Moreover, for male silver eels, BL and GSI were also positively correlated with a linear regression GSI = 0.112 + 3.701 (R² = 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-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 ± 2.73 cm for BL (vs 61.75 ± 8.99 cm) and of 92.0 ± 20.13 g for BW (vs 387.5 ± 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.

3.2. REE levels

3.2.1. In yellow eels

All the yellow eels caught bioaccumulated REEs and their presence were observed and quantified in the urinary system, muscles, gills, gonads, skin, spleen, liver and bile (Fig. 2A). A similar pattern among all the organs was observed, with higher concentration of LREEs followed by HREEs and then MREEs (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.

3.2.2. According to the life stage

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

3.2.3. According to the gender

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

### Table 1

<table>
<thead>
<tr>
<th>More concentrated</th>
<th>Less concentrated</th>
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<tr>
<td>$\sum$REEs</td>
<td>Gills</td>
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<tr>
<td>LREEs</td>
<td>Gills</td>
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<td>MREEs</td>
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<td>HREEs</td>
<td>Gills</td>
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Fig. 2. Median and Median Absolute Deviation for $\sum$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 ($\mu$g/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-value ≤ 0.05). *: significant differences between values of female and male silver eels with Kruskal Wallis test (p-value ≤ 0.05).
3.3. REE Organotropism

3.3.1. Yellow eels

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

3.3.2. Female silver eels

For female silver eels (Table 2), the organotropisms were very different considering ∑REEs, LREEs, MREEs and HREEs. The ∑REE highest concentrations were found in liver, then in the urinary system. The liver accumulated significantly more ∑REEs than the gills, gonads, skins, muscles and bile. The urinary system accumulated significantly more ∑REEs than the skin, muscles 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 but not in the same order depending on the group of REEs considered. Gills, skin and gonads (green and yellow colored) were organs intermediate concentrated. Particular attention should be paid to the gonads, and their highest particular MREE concentrations leading to the gonads as the most concentrated organ for this REE category.

3.3.3. Male silver eels

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

3.3.4. Life stage and gender influences

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

3.4. REE pattern in organs

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

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

The gonds of female silver eels displayed a very particular profile which was completely different from the gonads of other individuals (yellow eels and males silver eels) and even from the trends of all the other organs. This profile was dominated by Gd which represented up to 74.2% of ∑REEs with a median concentration of 27.94 ± 38.45 μg/kg dw. For comparison purpose, this value was greater than Gd concentration in the liver (9.76 ± 7.38 μg/kg dw) which was the most REE accumulating organ for female silver eels. However, it remained lower than Ce concentration (69.98 ± 36.69 μg/kg dw) which was the predominant element in the liver. In female silver eel gonads, Nd (9.3%) and Ce (4.7%) represented the second and third most abundant elements, after Gd.

The male silver eel muscles showed similar patterns compared to female silver eels. These patterns were different from yellow eel muscles due to a greater contribution of Pr (13.9–16.9% in silver eels vs 3.0% in yellow ones). This great contribution of Pr in silver eel muscles, leading it to the 2nd position among REEs after Ce, made the pattern also different from yellow eel muscles which was the predominant element in the liver. In male silver eel gonads, Nd (9.3%) and Ce (4.7%) represented the second and third most abundant elements, after Gd.

3.5. Correlations between biological parameters and REE concentrations in organs

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

3.5.1. Yellow eels

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

3.5.2. Silver eels

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

In male silver eels, the concentrations in gonads were positively correlated with concentrations measured in liver for all REEs (except Tm, Yb and Lu) and with concentrations measured in spleen for Sm, Gd, Tb, Dy, Ho and Er. Moreover, REE concentrations in gills were positively correlated with the age for La, Ce and Nd. There were also positive correlations between spleen and liver concentrations for Pr, Sm and Tb.

3.6. Parasitism influence

3.6.1. Yellow eels

A. crassus appeared to influence the REE contents in yellow eel organs. In fact, the levels of La, Ce and Pr were significantly higher in muscles of parasitized individuals compared to non-parasitized ones. The concentrations in parasitized vs non-parasitized yellow eels were: 0.30 ± 0.08 vs 0.150 ± 0.04 μg/kg dw for La, 2.28 ± 0.19 vs 1.31 ± 0.38 μg/kg dw for Ce and 0.09 ± 0.03 vs 0.07 ± 0.02 μg/kg dw for Pr.

The same trend was observed in the gills, since the concentrations of all REEs, except Tm and Lu, were significantly higher in parasitized individuals compared to non-parasitized ones. For example, ∑REE concentrations in gills reached 152.36 ± 63.69 and 78.44 ± 39.92 μg/kg dw, for parasitized and non-parasitized individuals respectively.

Regarding the gonads, parasitized individuals displayed concentrations of Y, La, Ce, Pr, Nd and Sm significantly higher than those of non-parasitized ones. To illustrate this observation, concentrations in parasitized vs non-parasitized yellow eels were: 1.25 ± 0.97 vs 0.55 ± 0.24 μg/kg dw, 2.68 ± 2.49 vs 0.53 ± 0.2 μg/kg dw, 5.80 ± 4.92 vs 1.22 ± 0.16 μg/kg dw, 0.73 ± 0.54 vs 0.18 ± 0.07 μg/kg dw, 2.32 ± 2.06 vs 0.45 ± 0.27 μg/kg dw and 0.46 ± 0.38 vs 0.09 ± 0.04 μg/kg dw for Y, La, Ce, Pr, Nd and Sm respectively.

The same result could be noticed for liver, where La concentrations were significantly higher in parasitized yellow eels (31.44 ± 11.73 μg/kg) compared to non-parasitized ones (16.15 ± 7.4 μg/kg dw). REE concentrations in the urinary system, skin, spleen and bile were not significantly affected by the A. crassus presence.

The ∑REE concentrations measured in the A. crassus collected in the swim bladder of yellow eels were below the LOQ.

3.6.2. Silver eels

The female silver eels showed no significant difference of REE concentrations between parasitized and non-parasitized individuals for all organs. A. crassus collected in the swim bladder of female silver eels presented ∑REE concentrations of 12.56 ± 13.18 μg/kg dw. The effect of the parasitism on REE concentrations on organs of male silver eels was not achieved because only two males among the 15 sampled individuals were not parasitized by A. crassus. The ∑REE concentration measured in A. crassus in the swim bladder of male silver eels was 19.78 ± 24.83 μg/kg.

3.7. REE bioaccumulation in glass eels

Fig. 4A presents the bioaccumulation of ∑REEs and the different groups (LREEs, MREEs and HREEs) in the whole body of glass eels at different pigmentation stages. The ∑REE median concentrations were 8.68 ± 0.8, 6.74 ± 1.95 and 9.63 ± 3.92 μg/kg dw for VI A0, VI A1 and VI A2 stages respectively. All REE concentrations are presented in Table 2 in the supplementary material. No significant difference of
concentrations between the 3 stages was highlighted. Moreover, there was no significant difference between the three stages for LREEs, MREEs and HREEs as well. The same accumulation trends were observed in glass eels than for the other life stages (yellow and silver) presented in Fig. 2. LREEs were accumulated in higher concentrations, followed by HREEs and then by MREEs. The profiles of REE abundances in glass eels are shown in Fig. 4B. Whatever the stage of pigmentation, the predominant REEs in the whole body of glass eels were Ce (27–32%) followed by Y (23%) and then La (14–16%).

4. Discussion

In this study, significant positive correlations between BL and BW were observed regardless of the life stage or the gender of the fish. In addition, the males were significantly smaller and lighter than the females. This sexual dimorphism was expected when the European eel is at the silver stage (Amérand et al., 2010; Durif, 2003). The ages between yellow eels and female silver eels or between female and male silver eels were not significantly different. This could be attributed to intraspecific variations (the yellow phase can last between 3 and 15 years) (Adam et al., 2008) as well as environmental effects which could lead to sedentary lifestyle of organisms for example (Feunteun et al., 2000). This allowed the comparison of REE bioaccumulation between the different groups and to investigate the influence of other variables such as physiological and morphological differences related to life stage or gender.

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

Regarding the life stage influence, the comparison between yellow and female silver eels revealed that the silver eels accumulated significantly more in the urinary system, muscles, gonads, spleen and liver, while yellow eels accumulated more in gills. These two life stages presented REE level differences but also differences in the element distribution throughout the organism. Indeed, the strongest REE bioaccumulation was observed in gills for yellow eels and in liver for female silver eels. To go further, the REE organotropism in yellow eels was gills > liver > kidney > skin > spleen > gonad > bile > muscles and in females silver eels was liver > kidney > spleen > gills > gonads > skin > muscles > bile. Moreover, by observing REEs in each individual organ, differences had been observed in the REE contribution within certain organs: i.e. muscles, gonads, gills and bile. Only urinary system, skin, liver and spleen presented similar profiles each other between yellow eels and female silver eels. The yellow eel muscles had a specific profile too, relatively different from the female silver eel muscles. The female silver eel gonads displayed a special profile, with a very important contribution of Gd, followed by Nd, then by Ce which was usually the REE predominant in the other gonads and organs in general. In other estuarine field studies (Amorim et al., 2007; Moermond et al., 2001; Nozaki et al., 2000), Gd was not the predominant REE measured in sediment and water. High concentrations of Gd in female silver eel gonads suggests a particular affinity of this element for this organ. An in-depth study of this phenomenon is still needed, this observation being not previously reported in the relatively rare studies about REE bioaccumulation in the gonads and particularly in fish gonads. Thus, further research must be performed to explain this specific Gd accumulation and to evaluate if it could be related to Gd anthropogenic uses which could therefore have a greater impact in estuarine and coastal areas than expected. Indeed, the Gd is a toxic ion with very interesting paramagnetic properties, so it is often used as a contrast agent for MRI (Magnetic Resonance Imaging) after being made inert by chelating it with a ligand. Nowadays, 25% of MRI use complexed Gd, this is worrying because the Gd is not removed in wastewater treatment plants and leads to aquatic environmental contamination (Parant et al., 2019). The REE bioaccumulation differences observed between the two life stages, could be caused by the silvering. Indeed, this metamorphosis leads to many external changes such as: coloration, thickening of the skin, lengthening of the pectoral fins, increased mucus secretion and increased size of the sensory organs. There is also an increase of gonad weight, a change of swim bladder wall, lipid accumulation, liver structural and metabolic changes, discontinuation and digestive tract retraction (Adam et al., 2008; Fontaine, 1994; Durif et al., 2015). The digestive tract retraction which takes place during this phase can be an

Fig. 4. (A) Median and Median Absolute Deviation (error bars) for \( \sum \) 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 (µg/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.
explanation of the higher REE concentrations in female silver eels (urinary system, muscles, gonads, spleen and liver) than in yellow eels. Indeed, the intestines are an important excretion route. The suppression of this excretion pathway may increase the pollutant accumulation in silver eels. Moreover, during the metamorphosis into silver eel, the liver undergoes morphological and physiological changes, these liver changes can cause higher accumulation of REEs. Finally, in order to successfully migrate to the Sargasso Sea, the silver eels undergo a change in their muscles. This may explain that the silver females accumulated more REEs than yellow females. Since yellow eels were only future silver females, this conclusion cannot be drawn for males.

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

REE organotropism study, i.e. the determination of the organs where REEs were accumulated, is important in the ecotoxicity understanding of their fate and potentially their mode of toxic action. Indeed, depending on the preferential accumulation organ, REEs will not have the same toxicity or detoxification way. In this work, by ordering the organs from the most to the least contaminated, some divergences were underscored between yellow and female silver eels as well as between female and male silver eels. These results can be compared with other studies carried out on fish caught in the wild or exposed in the laboratory. After a laboratory exposure of carps, Cyprinus 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) and Sm (0.25 mg/L), the organotropism determined for LREEs was: organs > gills > skeleton > muscles (Hao et al., 1996). The results were less detailed but similar to the results of the present study since the LREE organotropism from La to Nd was: liver > gills > spleen > kidney > muscles > skin > gonads > bile for male silver eels. Other publications exist on REE organotropism. For example, considering carps (Cyprinus carpio) exposed during 45 days at concentrations of 0.50 mg/L of La (as a model of LREEs), Gd (for MREEs) and Y (for HREEs), the organotropism was: internal organs > gills > skeleton > muscles > gills (Qiang et al., 1994). The study of Cardon et al., in Cardon et al., 2020, conducted on rainbow trout exposed to different Y concentrations by waterborne or by food obtained a completely different organotropism: intestine > gills > liver > muscles. The lionfish from Cuba accumulated more \( \sum \) REEs in kidney with a concentration of 0.053 mg/kg ww, higher than liver (0.018 mg/kg ww) or muscles (0.016 mg/kg ww) (Squadrone et al., 2019). The comparison of results from literature and from the present study remained complex because there was a lack of precision on the life stage or gender of fish. However, the main observations made through this present work were the differences of results between yellow eels and female silver eels as well as female and male silver eels. Moreover, the organs investigated in this study were more numerous and individually studied which is not the case of some studies in which the samples were pooled. The more detailed organotropism of the present work allowed to highlight that liver, urinary system and spleen were the most accumulating organs of REEs. In fish, the liver and the kidneys represent the main sites of metal sequestration and elimination (Pannetier et al., 2016). These organs have an important REE accumulation which can be explained by their role in the production of metallothionein and the metal detoxification. Finally, the results from the literature mentioned above concerned in vivo controlled experiments with one up to five elements in mixture. These conditions were greatly different to those of the present in situ study. In natural environment, REEs are more or less all present in the medium. An interaction can occur each other and with other contaminants or natural constituents. REE speciation and distribution in natural environments can also be different from those found in controlled experiments.

In the present work, gills were often positioned among the organs with the highest REE bioaccumulation. On one hand, this could be explained by the fact that they are in direct contact with water therefore with the metal dissolved forms. They represent the organ leading to metal penetration into the fish organism during the water filtration by the gill epithelium during the respiration. On the other hand, the gills for freshwater, marine and euryhaline fish species are the main route of calcium absorption before the intestines (Baldisserruto, 2019; Flik et al., 1995). REEs are antagonists of calcium absorption. In fact they are able to mimic Ca\(^{2+}\) due to their similar ionic radius (Figueiredo et al., 2018; Martin and Richardson, 1979). Indeed, ionic radius for the calcium is 0.09 nm and range between 0.085 and 0.106 nm for Lu and Gd, or 0.089 nm for yttrium. In addition, REEs have a higher valence (3+) than calcium, so they are able to take the Ca\(^{2+}\) place (Cui et al., 2012). In fact, their +III charge allows them to form stronger complexes (Switzer, 1978) and gives them a higher charge / volume ratio so they generally present higher affinities than Ca\(^{2+}\) for giving binding sites (Evans, 1983). In addition, their charge can also promote a higher coordination number, this is particularly true for smaller REEs which, by increasing their coordination number, can reach an ion radius closer to Ca\(^{2+}\) (Evans, 1983). However, the REE size is more important than their charge in the calcium replacement (Switzer, 1978). The similarities between REEs and calcium can cause: higher REE assimilation by the organisms, an alteration of the calcium cycle which can lead to a calcium deficiency, a decrease in the essential element assimilation depending on calcium channels, an inhibition of skeleton muscles, smooth muscle and heart muscle contractions. Moreover, REEs are able to replace the calcium in proteins leading to a change of their function. In addition, the gills play a role in the calcium excretion also. These potential characteristics of REEs could explain their high concentrations particularly found in gills of yellow eels and male silver eels. The high REE concentrations determined in the eel urinary system can also be linked to their similarity with calcium. In fact, kidneys are the main site for divalent ion excretion like calcium (Flik et al., 1995). Finally, during sexual maturation of female teleosts as eels, the vitellogenin production lead to an increase of the calcium demand. This demand is satisfied by an increase of environmental contributions or by internal remobilization (Persson et al., 1998). It had also been shown that during this maturation period there is the incorporation of calcium in the oocytes (Persson et al., 1998) for female organisms. Considering REE and Ca\(^{2+}\) similarities, this could also explain why silver female gonads accumulated more REEs, and especially Gd, than those of yellow females and silver males.

Concerning parasitism, in female yellow eels, non-parasitized individuals displayed significantly lower REE concentrations compared to parasitized individuals in muscles (La, Ce and Pr), in gills (all REEs, except Tb and Lu), in gonads (Y, La, Ce, Pr, Nd, and Sm) and in liver.
On one hand, the presence of A. crassus in the swim bladder is able to lead to numerous pathologies at macroscopic (change in thickness and opacity of the swim bladder), immunological (induction of non-specific and humoral responses) and physiological (alteration of gas excretion in the swim bladder, decrease in blood pressure, swimming ability, increased stress) levels (Habbechi et al., 2012; Kirk, 2003). Yellow parasitized eels could be more sensitive than yellow non-parasitized ones to their environment and to the contaminants, less able to detoxification, which could lead to a greater REE bioaccumulation. On the other hand, another hypothesis could be that more sensitive individuals, for other reasons than parasitism like REE contamination, could be more parasitized. Finally, it is impossible to conclude in favor of one of these two hypothesis. The reality was perhaps a mix of these explanations but both could explain the link between parasitism and higher REE concentrations in several studied tissues of female yellow eels. The results and corresponding hypothesis for female yellow eels are not appropriated for silver ones because no significant difference of REE contents was found between parasitized and non-parasitized individuals for all organs. This result highlighted that the life stage of eels could have an influence on the link between parasitism and REE concentration. In literature, some results exist about parasitism and its influence on metal bioaccumulation but without considering different life stages and gender. The publication of Palíkóvá and Baruš (2003) did not show link between A. crassus and mercury bioaccumulation in the European eel. Another study including different metals (Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn) did not show significant differences in metal accumulations in European eel tissues according to the parasitism factor (Genc et al., 2008). In this latter study, Genc et al. (2008) found Fe accumulation in A. crassus up to 25.52 times than the muscle of A. anguilla caught in the Asi River (Hatay-Turkey). In the present study, the same trend was observed for female silver eels with REE concentrations in the parasites (12.56 ± 13.18 μg/kg dw) about 2 times higher than in muscles (6.66 ± 2.13 μg/kg dw) and more than 9 times than in bile (1.29 ± 0.88 μg/kg dw). Parasites could accumulate REEs in favor of female silver eels. Nevertheless, the greater concentration of REEs in parasites compared to muscle and bile of female silver eels was not observed for female yellow eels, as REE were not detected in the A. crassus of yellow individuals. The life stage seemed to have an influence on the parasite REE contents also, probably by affecting mechanisms in parasites or in parasitized organisms. In the study of Schneebeauer et al. (2017), infected yellow eels presented in the swim bladder 1675 genes differentially transcribed compared to uninfected yellow eels, but silver eels only exhibited 291 differentially transcribed genes compared to uninfected silver eels. In 2016, Schneebeauer et al., have shown that silver eels have poorer detoxification of ROS when they are parasitized, while yellow eels do not show significant differences. It seems that the two life stages do not react in the same way to parasitism, but the result obtained in this study remained difficult to explain since knowledge on the sensitivity of life stage to parasitism are limited.

Regarding the glass eels, the pigmentation stage (VI A0, VI A1 and VI A2) did not seem to influence the REE bioaccumulation. Glass eels had median REE concentrations ranging from 6.74 to 9.63 μg/kg dw respectively for VI A1 and VI A2 stages. After the metamorphosis of the leptcephalus into the glass eels, individuals stop feeding and start eating again at the VI A3 stage. The glass eels studied in this work were caught during this fasting period. Their REE concentrations could be the result of external contamination reflecting the environmental concentrations. In another study, glass eels exposed during 3 days at 120 ng/L of lanthanum chloride in water accumulated 1.6–4.7, 1.1–6.7 and 4.8–13.0 ng/g dw in head, skinless body and viscera respectively (so with an organotropism of viscera > skinless body > head) (Figueiredo et al., 2018). These results and the concentrations found in the present work were in the same order of magnitude. The REE predominance in the whole body of glass eels showed a profile closer to the skin of yellow and silver eels than female gonads dominated by Gd. The high abundance of Gd observed in female silver eel gonads were not present in glass eels, suggesting no or weak potential maternal transfer.

5. Conclusion

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

CRediT authorship contribution statement

Marjorie Lortholari: Conceptualization, Methodology, Experimentation, Statistic, Writing, Reviewing and Editing
Laurence Poirier: Methodology, Experimentation, Writing, Reviewing and Editing
Abderrahmane Kamari: Experimentation, Christine Herrenknecht: Supervision
Aurore Zalouk-Vergnoux: Methodology, Experimentation, Writing, Reviewing and Editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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