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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)

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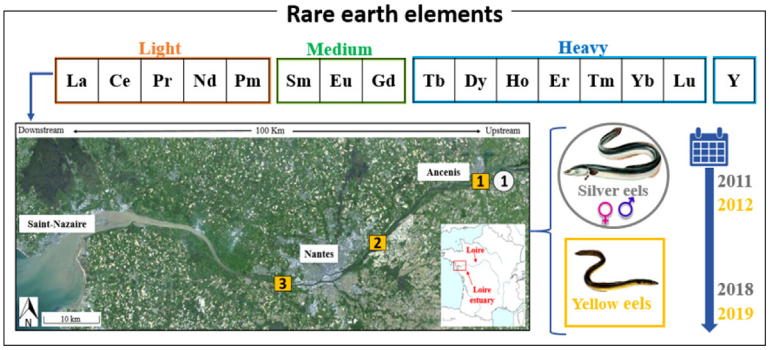
HIGHLIGHTS

- Limited information is available describing REE bioaccumulation in fish species.
- The first study of REE bioaccumulation by European eels in the wild.
- Male silver eels accumulated significantly more some REEs than females.
- Significant increases of REE muscle levels in silver eels between 2012 and 2018.
- Eel's biometry and life stage were influencing factors on REE bioaccumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present work, rare earth elements (REEs) were measured in European eel muscles (*Anguilla anguilla*) from the Loire estuary in France. This study site is characterized by a large anthropogenic pressure with potential activities releasing REEs such as oil refineries, aeronautic and naval industries, wind turbine industries, hospitals with magnetic resonance imaging and coal-fired power plants. These activities may lead to increased REE concentrations in sediments the primary habitat of European eels. In the present work, REE bioaccumulation was evaluated by determining the concentrations in yellow and silver eel muscles sampled at three different locations in the Loire estuary and at two periods (2011/2012 and 2018/2019). The aims of this study were the understanding of the spatio-temporal influences (sampling site and sampling period) and intraspecific variations (age, sex, sexual maturation, length, weight, and parasitism) on the whole REE bioaccumulation. The mean value of the sum of REE concentrations (∑REEs) was 2.91, 6.48 and 12.60 μg/kg of muscle from respectively yellow eels, female silver eels and male silver eels fished in 2018/2019. The results showed that silver males accumulated more REEs than silver females and silver eels accumulate more REEs than yellow ones. Regarding the determination of spatio-temporal variations, an increase of REE concentrations for silver eel muscles between the two periods was observed, certainly related to the increase of REE uses. Finally, a trend of higher contamination of eels sampled in the downstream of Nantes was noticed for yellow eels.

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1. Introduction

Rare earth elements (REEs) are a group of 17 metallic elements with close chemical and physical properties: scandium (Z = 21), yttrium (Z = 39) and 15 lanthanides with atomic numbers ranging from 57 (lanthanum; La) to 71 (lutetium; Lu). REE atomic mass increases gradually along with their atomic number. This feature makes it possible to classify REEs according to their atomic number into two groups: light REEs (LREE; from La to europium; Eu) and heavy ones (HREE; from gadolinium; Gd to Lu). However, in the literature, REEs are also divided into three groups: light (from La to promethium; Pr), medium (from samarium; Sm to Gd) and heavy (from terbium; Tb to Lu) (Bru et al., 2015; Kogel et al., 2006). In both cases, even if yttrium is one of the lightest REEs, it is classified among the HREEs owing to its similar physico-chemical properties and its presence in the same deposits. Scandium is not classified in any of these REE groups because of its absence in the same deposits as the others (Bru et al., 2015).

REEs are prevalently used in the industrial sector where they are employed in numerous processes such as the production of supermagnets and luminophore, petroleum catalysis, pigmentation of glasses and plastics, metallurgical alloys, nuclear and medical imaging. (Bru et al., 2015). Nowadays, these elements are particularly essential in emerging clean energy technologies (wind turbines, electric vehicles, and energy-efficient lighting), leading to a continuous increase of their demand (Alonso et al., 2012). In 2014, the consumption of REEs was evaluated at 120 Kt and it is continuously increasing by 6% each year (Bru et al., 2015). Hence, these numerous anthropic uses are causing REE releases in the environment and particularly in natural aquatic ecosystems, disturbing the natural biogeochemical cycles. Finally, REEs are becoming emergent contaminants but are rarely taken into account in the current European regulations. In fact, only wastes and treated water from mining activities are involved in three European regulations which established threshold concentrations of REEs (EIA directive/EU1452/; Directive/EU 0621/; Directive/EU 1359/).

Aquatic organisms are able to bioaccumulate REEs leading to their introduction in the food chain. Indeed, this phenomenon has previously been observed in plankton in the Mediterranean Sea (Strady et al., 2015), algae in China (Strady et al., 2015), bivalves in Japan and Germany (Cho et al., 2004; Akagi and Edanami, 2017), fish in the United States and China (Mayfield and Fairbrother, 2015; Qiang et al., 1994; Figueiredo et al., 2018) and turtles in Sicily (Censi et al., 2013). For instance, in 2015, Mayfield and Fairbrother studied 10 North American fish species (*Catostomus macrocheilus, Catostomus catostomus, Oncorhynchus mykiss, cottoidae,* Micropterus dolomieu*, Sander vitreus, Lota lota, Oncorhynchus nerka, Coregonus clupeaformis* and *Prosopium williamsoni*) showing that the sum of REE concentrations (∑REEs) in freshwater fish muscle ranged from 0.014 to more than 3 mg/kg dry weight (dw). Another study conducted in China (Yang et al., 2016) on 4 species of freshwater fish (*Ctenopharyngodon idella, Carassius carassius, Hypophthalmichthys nobilis* and *Cyprinus carpio carpio*) and 6 species of marine fish (*Platycephalus fuscus, Scophthalmus maximus, Trichiurus lepturus, Lateolabrax japonicus,* Sphyraena *barracuda* and *Larimichthys polyactis*) highlighted ∑REE means of 35.8 μg/kg dw in freshwater fish and 21.0 μg/kg dw in marine fish. Recently, the study of Nørregaard et al. (2019) showed a REE accumulation in liver, gill and muscles of *Salvelinus alpinus* after a 15 day exposure to Ce (97–243 μg/L), La (735–914 μg/L) and Y (485–552 μg/L).

REE bioaccumulation is concerning because these elements are able to cause toxic effects to the aquatic biota. For instance, after an exposure to 120 ng/L of lanthanum chloride during 3 or 7 days, glass eels showed a significant increase in AChE activity. These results highlighted the neurotoxicity of La^{3+}, inhibiting the binding of acetylcholine (Figueiredo et al., 2018). Embryotoxic and teratogenic effects were also attributed to REEs in *Danio rerio*. Hatching rate diminution, mortality rate increase and larval malformations were shown after exposure with 0.1 mmol/L of La or Yb (Cui et al., 2012; Mácová et al., 2014). At cellular scale, REEs were demonstrated as cytotoxic and causing mitotic activity inhibition, mitotic aberration and micronuclei introduction (Pagano et al., 2015). Moreover, REEs were able to induce oxidative stress on cells (Hongyan et al., 2002). These authors showed that an exposure of *Carassius auratus* at 0.05 mg/L Yb^{3+} induced changes of enzymatic activities (superoxide dismutase, chloramphenicol acetyltransferase, glutathione s-transferase, and glutathione peroxidase). A study conducted in 2017 (Hua et al., 2017) showed that the La^{3+} was able to cause necrosis in the gills and liver of *Gobiocypris rarus*.

At the European scale, only one study has been conducted on REE bioaccumulation in fish (Squadrone et al., 2019). The present study focused on the Loire River (France) which is one of the largest and the last wild river of west Europe. The Loire is characterized as a "wild river" because there is no barrier (dikes and banks) that prevent the migration of fish and it is one of the least polluted rivers in Europe. It is the longest river in France and its drainage basin spreads on a surface of 117,000 m^2. The Loire study is interesting due to its nature preserved characteristic and its estuary, which represents one of the main entry routes for glass eels in continental waters. In addition, few studies have been conducted on REE in the Loire and in particular in the Loire estuary. Its estuary is characterized by a large anthropogenic pressure with potential activities emitting REEs such as oil refineries, aeronautic and naval industries, wind turbine industries, hospitals with Magnetic Resonance Imaging (MIR) and coal-fired power plants. Estuaries are a mixing zone between freshwater and saltwater leading to physico-chemical modifications and considerable nutrient inputs from the oceans. These areas are thus very conducive for plant growth and constitute crucial habitats for numerous wild species. REE study in these ecosystems is essential to understand phenomena which could influence REE distribution in water and sediment and hence their bioaccumulation in aquatic organisms.

European eels (*Anguilla anguilla*) are a key species of the Loire estuary for different reasons. First, they are benthic species during their life, thus living in contact with the sediment which is the preferential storage compartment of REEs. Secondly, eel diet is composed of fish, crustaceans, and invertebrates so they are at the top of the trophic chain of estuarine ecosystem (Corolla et al., 2016; Don et al., 2017). Thirdly, this species is sedentary part of its life corresponding to the yellow stage. This makes it possible to correlate their bioaccumulation with environmental concentrations (Figueiredo et al., 2018). Moreover, *Anguilla anguilla* is consumed by humans, consequently, it is important to know the REE levels in the muscles to determine human exposure by fish consumption. Besides, eels are a threatened species with declining numbers of individuals, so it is important to study the pollutant impacts on this species (Adam et al., 2008). Finally, this species is of considerable socio-economic relevance because its fishing is an important employment sector and its sale is very profitable.

During its lifetime in continental water, *Anguilla anguilla* is exposed to many contaminants and it is already well known that eels particularly bioaccumulate many pollutants such as polycyclic aromatic hydrocarbons (Ribeiro et al., 2005), pesticides, persistent organic pollutants (Couderc et al., 2015; van der Oost et al., 1996) and metals (Maes et al., 2008; Redmayne et al., 2000). Regarding metals, they accumulate essential (Ni, Zn, Cu, Mn, Co and Fe) and non-essential metals (Hg, As, Cr, Pb, Cd and Al), the concentrations found in eels from different European regions were reported in

Table 1
Essential and non-essential metals bioaccumulated in European eel muscles.

Location	Units	Life Stage	Essential						Non-essential						Publication
			Ni	Zn	Cu	Mn	Co	Fe	Hg	As	Cr	Pb	Cd	Al	
Camargue France	Means mg/kg dw	–	0.13–0.83	55.4–57.1	0.19–0.43	0.11–0.32	0.02–0.06	12.5–17.7	0.16–0.61	0.17–4.67	1.46–2.48	0.21–0.79	nd	16–47.2	(Ribeiro et al., 2005)
Gironde estuary France	Means mg/kg ww	–	–	10.2	0.15	–	–	–	0.17	–	–	–	<0.02	–	(Durrieu et al., 2005)
Adour estuary France	Means mg/kg ww	Yellow eels	–	12.6–14.3	0.2–0.4	–	–	–	–	–	–	0.004–0.014	0.001–0.004	–	(Tabouret et al., 2011)
Lesina lagoon Italie	Means mg/kg ww	–	–	17.9–24.6	0.39–1.13	–	–	–	0.13–0.24	–	–	–	0.02–0.04	–	(Storelli et al., 2007)
Tagus estuary Portugal	Median mg/kg dw	Yellow eels	–	43.6–75.5	0.83–1.23	–	–	–	0.20–1.05	–	–	0.07–0.55	–	–	(Neto et al., 2011)
England	Means mg/kg ww	–	–	–	–	–	–	–	0.21–0.37	–	–	0.05–0.08	0.03–0.05	–	(Barak et Mason, 1990)
Southern Atlantic coast Spain	Means mg/kg ww	–	0.015–0.020	10.1–13.0	0.5–1.5	4.71–14.1	–	4.11–5.89	0.010–0.023	0.52–2.91	0.143–0.368	0.03–0.09	0.015–0.050	–	(Usero et al., 2004)
River Turia Spain	Means mg/kg ww			16.95	0.977					0.2279		0.1018	0.0049		(Bordajandi et al., 2003)
Sebou estuary Maroc	Means mg/kg ww	–	–	131.52	11.21	–	–	1578	–	–	–	0.89	–	–	(Mansouri et al., 2018)

-: not determined in the study.

Table 1. Compared to other estuarine fish species, the European eels, characterized by a long residence time in estuary, are among the most contaminated species (Durrieu et al., 2005) and could be considered as a good metal bioindicator (Linde et al., 1996).

In a context of demographic growth and increasing global REE uses, the aims of the present study were (i) to investigate the REE bioaccumulation in the European eel caught in the wild because it was never been done before, by determining the concentrations in yellow and silver eel muscles and (ii) to study the influence of spatio-temporal (3 sampling sites and 2 sampling periods) and intraspecific (age, sex, sexual maturation, length, weight and parasitism) parameters on the whole REE bioaccumulation.

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 (internal standard) at 1000 mg/L in 2% HNO_3, were provided by Roth (single-element ICP standard solution). REEs were oxide forms in solution (Y_2O_3, La_2O_3, Ce $(NO_3)_3$, Pr_6O_{11}, Nd_2O_3, Sm_2O, Eu_2O_3, Gd_2O_3, Tb_4O_7, Dy_2O_3, Ho_2O_3, Er_2O_3, Tm_2O_3, Yb_2O_3, Lu_2O_3). In this work, 15 out of the 17 REEs are studied. Pm is not included because it is not naturally present in the environment and Sc either because of much interference during the ICP-MS analysis, which leads to overestimation of Sc concentrations. Mineralization and solutions were made with HNO_3 65% from Fisher Scientific. Mineralization protocol was validated using the international certified material BCR-668, muscle of mussels. Otolith mounting material such as epoxy resin (EpoFix) and abrasive paper come from Struers.

2.2. Considered species

Anguilla anguilla is a catadromous species. Its life cycle is given in the Fig. 1. Yellow eels live in freshwater. They are benthic and sedentary for several years waiting for the metamorphosis called

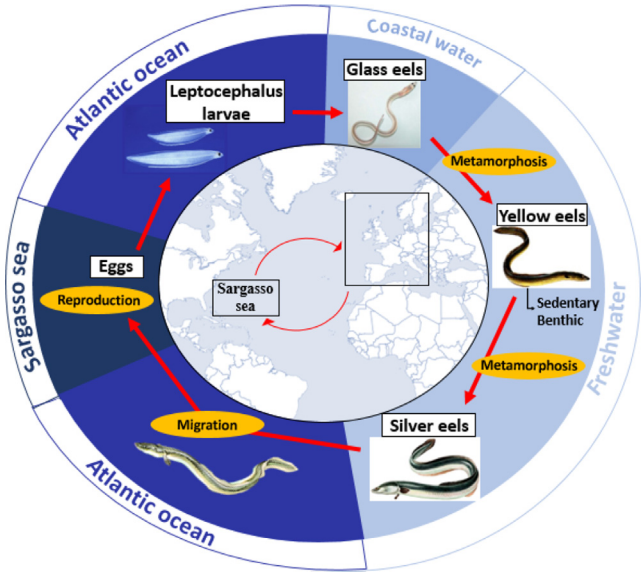

Fig. 1. European eel life cycle.

silvering. This transition phase changes yellow eels in silver eels (future genitor) when they reach sexual maturity and it is characterized by morphological and physiological changes (Adam et al., 2008; Corolla et al., 2016). Then, silver eels migrate to the marine environment, the Sargasso Sea, for the reproduction where they die after. Laid eggs turn into leptocephalus larvae which migrate to European coasts using the Gulf Stream. Arriving at the level of the continental slope (submarine zone which links the continental shelf and the abyssal plain), leptocephalus larvae become glass eels. At the level of freshwater, they begin to feed and after time, glass eels become yellow eels. Yellow eels live in streams for several years before becoming silver eel at sexual maturity and so on.

2.3. Studied area and sampling sites

The present study focused on a large area: the Loire estuary (France) which extends from Saint-Nazaire to Ancenis (100 km) (Fig. 2). This estuary is characterized by a strong demographic pressure carried mainly by 2 cities, Nantes with 306,694 inhabitants (in 2016, Nantes is the sixth most populated city in France) and Saint-Nazaire with 69,719 inhabitants. The metropolis Nantes/Saint-Nazaire has 867,130 inhabitants. In 2016, the Pays de la Loire region ranked third in France for its population growth. This increase is mainly provided by Nantes and Saint-Nazaire, with respectively 18,849 and 2,622 inhabitants more between 2011 and 2016. The high population density and access to medical care facilitated, lead to the establishment of new Magnetic Resonance Imaging (MRI). In 2019, 39 MRI are listed in the Pays de la Loire region. The Loire estuary is also exposed to strong industrial pressure, associated with potential sources of REEs. The refinery of Donges is the second most important of France with a capacity of 11 Mt/year. Nearby, there is the third European pole of Airbus Group (Saint-Nazaire) and Alstom, a wind turbine producer (Montoir-de-Bretagne). Between Saint-Nazaire and Nantes, there is the Cordemais coal thermal power plant, which produces about 25% of the electricity consumption of the Loire region. Works characterizing REE contamination in the Loire estuary are scare. The study of Negrel (1997) mentioned mean concentrations of the Loire estuary sediment reaching 41 mg/kg of La and 92 mg/kg of Ce. Another study (De Chanvalon et al., 2016) measured $\sum$REE means in suspended particulate matter and sediments of the Loire estuary. Values in suspended particulate matter reached 137 mg/kg upstream of Nantes, 164 to 489 mg/kg downstream of Nantes and 89 mg/kg in a coastal site. For sediment, levels from 195 to 199 mg/kg were measured upstream of Nantes. Nearby the Loire estuary, in the Bay of Biscay, the study of Chaillou et al. (2006) measured mean concentrations ranging from 0.11 μg/g (Lu) to 47.79 μg/g (Ce) in sediments.

Eels were captured by professional fishermen by means of fish traps. *Anguilla anguilla* was fished at both stages: yellow and silver. Yellow eels were caught in June 2012 at three sites, Varades (47°21′58″N – 1°1′31″O) located 50 km upstream from Nantes, Bellevue (47°14′15″N – 1°28′2″O) located 8 km upstream from Nantes and Haute-Indre (47°11′39″N – 1°40′4″O) located 12 km downstream from Nantes. Yellow eels were also fished in June 2019 at Haute-Indre. Silver eels were caught during the downstream period in November 2011 and December 2018 at Varades. But since they were downstream migrating, they cannot be considered as from this specific site. Bellevue and Haute-Indre being respectively upstream and downstream Nantes will help to characterize the impact of the city on the Loire River. The sampling site Varades makes it possible to characterize the inputs of REEs from the upstream of the river because this site is few influenced by the tide balance. Between the two sampling periods (2011/2012 and 2018/2019) different factors can lead to variation of the REE concentrations in the Loire estuary such as the increase of inhabitant density, the setting up of MRI and the increase in the REE uses. For each study site, year and sexual maturity, 10–15 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.

2.4. Dissection and biometric analyses

Fishes were executed by lethal dose of eugenol (1 mL/L, leaving fishes 30 min after the last respiratory movement). Then they were weighed (g) and measured (cm). After dissection, muscles were collected and frozen at −20 °C before freeze-drying. Sex identification was visually determined during the dissection according to the observation of the gonads.

Sagittal otoliths were recovered from cranial boxes on both sides of brains to determine the age of each individual according to ICES 2009 (International Council for the Exploration of the Sea) methodology. Then, they were cleaned and stored dry in Eppendorf tubes. They were put in silicon mold and included in epoxy resin with a drying time of 24 h. Epoxy resin blocks were mounted on glass slides and sanding with abrasive paper (using different grits of sandpaper) until reaching the nucleus. They were attacked with EDTA 5% for 3 min, stained with toluidine blue 5% (in EDTA 5%) for 30 min and washed with water. Age determination consisted of annulus counting after the zero band using binoculars.

During dissection, *Anguillicola crassus* parasites were collected from the swim bladder of eels. *Anguillicola crassus* is a nematode indigenous to Eastern Asia feeding on blood and causing a disease called Anguillicosis (Adam et al., 2008). The parasites were counted and weighed. The parasite index (PI) was determined as follows:

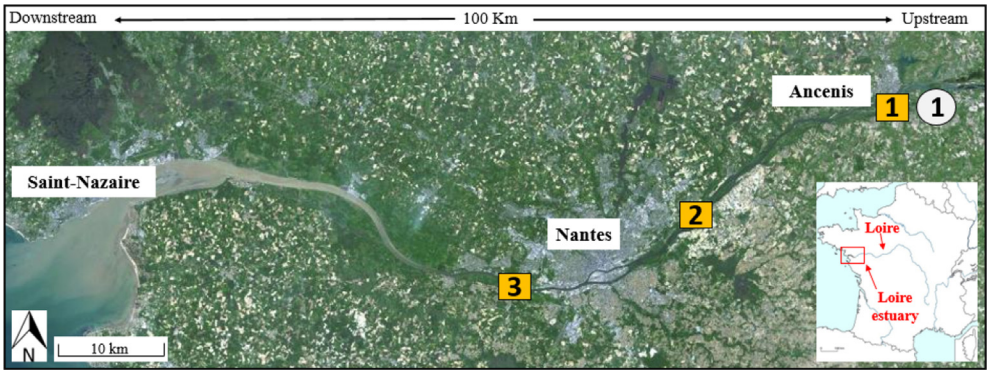

Fig. 2. Sampling sites of yellow (yellow squares) and silver (gray ring) eels captured in the Loire estuary. 1 = Varades, 2 = Bellevue and 3 = Haute-Indre. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PI = weight of *Anguillicola crassus (g)* / whole body weight (g) * 100. PI was studied to evaluate the infection level of individuals and its influence on the health of the eels (for example on Fulton condition index; K), as well as on the REE concentrations in eel muscles.

The lipid percentage of muscles was determined from 1 g lyophilized muscle tissue using a liquid-liquid extraction. The muscles were soaked in a dichlomethane-methanol (1:1, v/v) mixture during 24 h and then filtered and decanted. After that, the organic phase is recovered, dried with Na_2SO_4, evaporated to dryness and weighed. The percentage of lipids was calculated from this mass related to the mass of starting muscles (Blanchet-Letrouvé et al., 2014).

The gonads were recovered and weighed to determine the gonadosomatic index (GSI). The GSI was calculated as follows: GSI = gonad weight (g)/whole body weight (g) * 100.

The Fulton condition index (K) was determined as following: K = (whole body weight (g)*10^5) / whole body length^3 (cm), K allows evaluating the overall global eel health in front of environmental stresses such as pollution or feeding conditions.

2.5. Preparation of fish tissue for REE analyses

At the beginning of the experiments, all the material was cleaned by immersion in HNO_3 10% for one night and rinsed with Milli-Q water before use. Freeze-dried muscle tissues were homogenized before chemical analysis. As a first step, about 1 g of muscles were carbonized in a muffle furnace (Nobertherm) at 300 °C for 1 h and at 500 °C during 2 h to concentrate about 10 times the sample. Ashes were then predigested with a mix of HNO_3/HCl (2:1, v/v) for one night at room temperature. Then, they were mineralized by micro-waves (Anton Paar, MW GO 50 Hz) (5 min to reach 100 °C maintained during 10 min, then, 15 min to reach 170 °C maintained 35 min). The solutions were transferred into Pyrex tubes and evaporated to dryness in hot-plate at 130 °C. Samples were resuspended with 1.8 mL of HNO_3 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 used for analysis. The accuracy of the analytic protocol was validated using an international certified material containing REEs (BCR-668, muscle of mussels).

2.6. Rare earth element analyses

Concentrations of REEs were determined by Inductively Coupled Plasma coupled to Mass Spectrometry (ICP-MS, Nexion 350x PerkinElmer). All samples, blanks, and standards were diluted or prepared using HNO_3 2%. Rhenium was used as internal standard, using ^{187}Re 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 solutions of calibration with the addition of REEs with increasing concentrations. The calibration range was built with concentrations of 5, 10, 50, 100, 500, 1000 ng/L for REEs from La to Eu, and 50, 100, 500, 1000, 5000 ng/L for Gd to Lu and Y. This choice of calibration has been implemented using REE concentrations found in articles and the fact that REE concentrations increase with the atomic number. REE isotopes were selected according to a compromise between the maximum of natural abundance and the minimum of interferences. They corresponded to: ^{89}Y, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{171}Yb and ^{175}Lu. Limits of detection (LOD = (standard deviation of y-intercept/slope) *3.3) and quantification (LOQ = LOD*3) were determined for each REE. The LOD values determined in ng/kg of muscle dw were: 5.45 (Y), 2.76 (La), 5.05 (Ce), 3.71 (Pr), 2.23 (Nd), 2.26 (Sm), 1.53 (Eu), 2.2 (Gd), 4.56 (Tb), 2.36 (Dy), 2.92 (Ho), 3.91 (Er), 5.59 (Tm), 2.77 (Yb) and 4.26 (Lu). Analytical and procedural blanks were prepared and analyzed using the same analytical procedure as for samples. Finally, the blank values were subtracted from the values of the samples.

2.7. Data processing

The bioaccumulation results were given in nanograms per kilogram of muscle tissues dry weight (ng/kg dw). It was chosen in shaping the results to separate REEs into 3 groups: light (LREEs: La to Nd), medium (MREEs: Sm to Gd) and heavy (HREEs: Tb to Lu and Y). LREE concentrations were calculated by summing concentrations from La to Nd, MREE concentrations by summing concentrations from Sm to Gd, HREE concentrations by summing concentrations from Tb to Lu and Y. Total REE concentrations (∑REE) is the addition of LREE, MREE and HREE concentrations.

The multiplication factors (MF) were calculated between the two sampling periods as following: MF = (median REE concentration in 2018/2019) / (median REE concentration in 2011/2012). The MFs were used to examine concentration trends between the two sampling periods (2011/2012 and 2018/2019).

2.8. Statistical analyses

The statistical analysis was achieved with Statistica software (v 7, StatSoft, Inc.). The results of the Kolmogorov-Smirnov test showed the need to use non-parametric tests to work on the data. Differences between biological parameters (length, weight, PI, age, lipid content, K and GSI) were studied with Kruskal-Wallis test. Influences of biological parameters on REE bioaccumulation were studied using correlation tests (with log transformations of REE concentrations). The intraspecific variations such as life stage (yellow and silver stages) and gender (males and females) were determined using Mann-Whitney test as well as comparison of REE muscle concentrations between 2011/2012 and 2018/2019. Before the comparison of REE muscle concentrations for each gender and life stage between the two periods of sampling, a Mann-Whitney test was applied to verify that the size and age of the organisms were not significantly different which could lead to their influence on the results. REE concentrations in eel muscles fished at the three sampling sites (Varades, Bellevue and Haute-Indre) were compared by means of Kruskal-Wallis test. Significance levels for all statistical tests were set at p-value $\leq$ 0.05.

3. Results

3.1. Biological parameters

The biological parameters such as length, weight, age, lipid content, parasitism (PI), and gonadosomatic index (GSI) are presented in Table 2 according to the life stage of eels (yellow or silver), the gender, the sampling sites (Varades, Bellevue and Haute-Indre) and the fishing period (2011/2012 or 2018/2019).

Considering the fishing year 2011/2012, the body length and weight of yellow eels were significantly greater at Bellevue than both at Varades (p-value = 0.014) and at Haute-Indre (p-value = 0.041). Moreover, silver males were significantly smaller than silver females (p-value < 0.001) and yellow eels from Bellevue and Haute-Indre (p-value < 0.001 and p-value = 0.023 respectively). Silver females with a mean age of 14 were significantly older than males with a mean age of 8 (p-value < 0,001). Silver eels were less parasitized by *Anguillicola crassus* than yellow eels and this was significantly different for the female silver eels and yellow eels from Haute-Indre (p-value = 0.020). Overall, silver eels had a higher lipid contents than yellow eels. This observation is significantly marked comparing male silver eels and yellow eels from

Table 2
Median biological parameters (minimum - maximum) and muscle concentration of light REEs (LREEs: La to Nd), medium REEs (MREEs: Sm to Gd), heavy REEs (HREEs: Tb to Lu and Y) and total REEs (∑REEs) in studied eels according to the year of fishing (2011/2012 and 2018/2019), the sex maturity stage (yellow and silver) and the gender (undifferentiated, females and males).

	2011/2012						2018/2019		
	Yellow eels			Silver eels			Yellow eels	Silver eels	
Gender	Undifferentiated	Undifferentiated	Undifferentiated	Females	Males		Undifferentiated	Females	Males
n	10	10	10	14	15		15	14	15
Sites	Varades	Bellevue	Haute-Indre	-	-		Haute-Indre	-	-
Length (BL; cm)	49.5 (43.4–64.9)	69.2 (52.2–87.2)	50.6 (42.3–63.0)	58.5 (50.3–83.5)	38.3 (36.5–40.8)		56.3 (47.2–67.5)	62.0 (53.0–74.0)	38.2 (34.0–41.7)
Weight (BW; g)	169 (114–459)	604 (262–1685)	180 (123–359)	272 (181–992)	74 (65–95)		330 (203–651)	394 (261–612)	92 (138–141.3)
Age (year)	8 (5–13)	12 (7–15)	10 (6–14)	12 (9–21)	7 (6–14)		10 (6–18)	12 (7–24)	9 (6–13)
Lipids (%)	4.9 (0.7–15.2)	17.3 (2.3–35.0)	9.9 (0.6–34.8)	25.9 (18.8–34.2)	33.9 (24.8–37.2)		12.5 (5.6–24.3)	12.6 (0.2–29.3)	26.0 (6.4–40.2)
PI[a] (%)	0.0 (0.0–0.9)	0.3 (0.0–0.6)	0.6 (0.0–3.2)	0.0 (0.0–0.4)	0.1 (0.0–0.7)		0.0 (0.0–1.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)
GSI[b] (%)	0.2 (0.1–0.6)	0.5 (0.3–1.5)	0.4 (0.1–1.6)	1.9 (1.7–2.2)	0.7 (0.3–1.1)		0.5 (0.3–1.1)	1.5 (1.2–1.9)	0.7 (0.1–1.0)
K[c]	0.14 (0.12–0.18)	0.18 (0.14–0.25)	0.14 (0.12–0.18)	0.14 (0.11–0.15)	0.14 (0.13– 0.17)		0.17 (0.14–0.20)	0.17 (0.14–0.19)	0.19 (0.14–0.23)
LREEs (ng/kg dw)	2021 (29.4–1674)	1635 (29.4–1674)	2550 (45.6–2025)	4389 (31.8–3167)	5600 (104.1–4133)		2649 (88.2–2127)	5574 (195.5–3659)	10,566 (395.0–7422)
MREEs (ng/kg dw)	203.5 (<LOQ–187.9)	161.1 (<LOQ–159.7)	223.9 (2.2–205.2)	9.7 (1.6–5.7)	93.3 (6.5–56.7)		47.2 (1.5–28.8)	138.1 (34.5–62.0)	296.1 (76.4–138.0)
HREEs (ng/kg dw)	10.3 (<LOQ–7.6)	< LOQ	126.2 (2.7–74.8)	250.7 (2.6–123.6)	509.9 (2.7–330.9)		216.5 (5.5–42.3)	770.3 (9.25–408.9)	1740 (22.8–1041)
L/H[d]	196	–	20	18	11		12	7	6
L/M[e]	10	10	11	452	60		56	40	36
∑REEs (ng/kg dw)	2235 (<LOQ–1674)	1796 (<LOQ–1674)	2901 (2.2–2025)	4649 (1.6–3167)	6203 (2.7–4133)		2912 (1.5–2127)	6482 (9.25–3659)	12,602 (22.8–7422)

LOQ: Limit Of Quantification.
[a] PI: parasitism index (%).
[b] GSI: gonadosomatic index (%).
[c] K: Fulton's condition factor.
[d] L/H: LREEs/HREEs.
[e] L/M: LREEs/MREEs.

the three sites (Varades p-value < 0.001, Bellevue p-value = 0.010 and Haute-Indre p-value < 0.001). Silver females had significantly higher GSI than males (p-value = 0.005) and yellow eels (Varades p-value < 0.001, Bellevue p-value = 0.003 and Haute-Indre p-value < 0.001).

For the fishing year 2018/2019, silver males were again smaller and lighter than silver females and yellow eels (p-value < 0.001 for both). Silver females with a mean age of 14 were significantly older than males reaching a mean age of 9 (p-value = 0,046). No significant difference was observed for GSI and PI.

In 2011/2012, the body length (BL) and the body weight (BW) were significantly positively correlated each other whatever the life stage (yellow and silver) and the gender. The linear regression equations were BL = 45.28 + 0.34 * BW with R^2 = 0.923 for yellow eels, BL = 30.95 + 0.96 * BW with R^2 = 0.649 for male silver eels and BL = 46.72 + 0.39 3 *BW with R^2 = 0.973 for female silver eels. The same correlations were observed for fish sampled in 2018/2019.

Analyzing correlations between biological parameters and REE concentrations, some significant trends were observed depending on the life stage and the nature of REE. Regarding yellow eels from the three sites, in 2011/2012, BL and BW were negatively correlated with La, Ce, Pr, Nd, Sm and Eu concentrations, BW was also negatively correlated with Ho. About female and male silver eels, no correlation was found between BL/BW and REE concentrations.

In 2018/2019, for yellow eels from Haute-Indre, there were negative correlations of BL with Yb and BW with Pr, Nd, Eu, Gd and Tb concentrations. BL and BW were not correlated with REE concentrations for male silver eels. About female silver eels, there were positive correlation of BL and BW with Tb and Lu concentrations.

Regarding lipids, only yellow eels showed negative correlation of lipids with La, Ce, Sm and Eu in 2012 and with Eu and Lu in 2019. No significant correlation involving GSI, age and K with REE contents were observed whatever the year and the life stage.

Concerning PI, values obtained in this work were in the same range of magnitude than those found in previous works performed in the same area (Blanchet-Letrouvé et al., 2014; Couderc et al., 2015). PI significantly correlated neither with K, not with REE concentrations in the muscles. This result traduced no differences in REE bioaccumulation between parasitized and non-parasitized organisms as it was previously demonstrated for heavy metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) (Genc et al., 2008).

3.2. REE bioaccumulation in 2011/2012

3.2.1. Yellow eels and spatial variations

The sampling strategy adopted in 2011/2012 allowed the study of potential spatial variations of REE concentrations in yellow eel muscles captured in three different sites along the Loire estuary. Table 2 displays the total REE concentrations except Sc (∑REEs) as well as LREE, MREE and HREE concentrations. Whatever the site, LREE concentrations were the highest reaching about 2000 ng/kg dw, followed by those of MREEs (10 times less) and finally HREEs. Considering the fishing site, eels captured at Haute-Indre tend to be more contaminated than those from Varades and finally Bellevue, but this observation is only significant for two HREEs (Dy and Er) (p-value < 0.05). The L/M (LREEs/MREEs) ratios were about 10 for the three sites, but the values obtained for L/H (LREEs/HREEs) were more variable.

To go further in the analysis, Fig. 3 shows individual element concentrations in yellow eel muscles according to the studied site. The most abundant element was Ce with values reaching about 1500 ng/kg dw. Ce was followed by the other LREEs (La, Nd and Pr respectively reaching 150, 20 and 10 ng/kg dw) and the MREEs except Gd which was not detected in the muscles whatever the site. Concerning HREEs, they were not systematically detected as

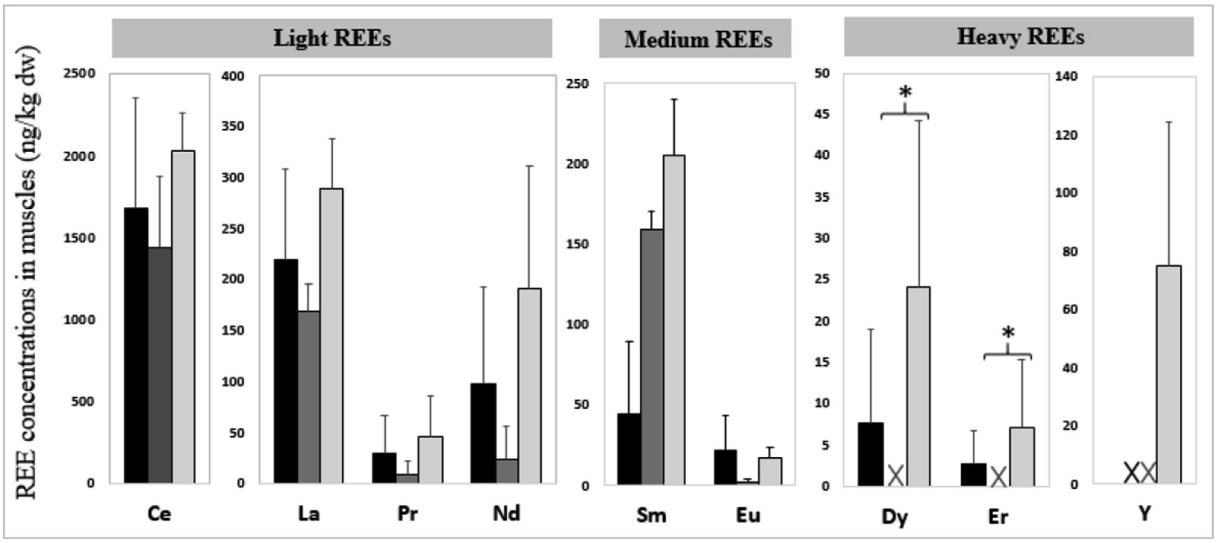

Fig. 3. Median and Median Absolute Deviation (MAD) for REE concentrations (ng/kg dw) in muscles of yellow eels collected in 2011/2012 at 3 sites: Varades (n = 10. black bars); Bellevue (n = 10. dark gray bars) and Haute-Indre (n = 10. light gray bars). X: concentrations lower than LOQ; *: significant different values with Kruskal Wallis test (p-value $\leq$ 0.05).

the other REEs (Tb, Ho, Tm, Yb and Lu lower than LOQ for the three sites). For example, Dy and Er were not detected in Bellevue whereas Y was only detected in Haute-Indre.

The homogeneity of BL and BW individuals from Haute-Indre and Varades allowed us to evaluate the influence of the site. The higher concentrations observed at Haute-Indre, mainly for HREEs, could be explained by the location of this site, being downstream Nantes in the estuary, more exposed to industries, treatment plants, discharges and hospitals present near the city. Regarding Bellevue, the higher size of individuals from this site could explain the inferiority of REE concentrations, for elements influenced by BL and BW (La, Ce, Pr, Nd, Sm, Eu, Ho).

3.2.2. Influence of life stage

The sampling strategy adopted in 2011/2012 allowed the study of potential variations of REE concentrations according to the life stage of eels, by capturing both yellow and silver eels which represent respectively undifferentiated and mature sexual stages. Table 2 shows that ∑REEs were significantly higher (p-value = 0.006) in muscles of female silver eels than yellow eels from Haute-Indre, respectively 4649 and 2901 ng/kg dw. Life stage appears to be an influencing factor on REE bioaccumulation. Regarding LREEs, MREEs and HREEs, the trend was not similar because LRREs and HREEs were higher in female silver eel muscles than yellow eel muscles and it was the contrary for MREEs. This is also observable with ratios: L/M was higher than L/H for the silver eels and the contrary was observed for yellow eels. There was an effect of the element nature on the differentiation of contamination between the two life stages. There was an enrichment of MREEs in yellow eels and of HRREs in silver eels.

To go deeper, Fig. 4 shows the median levels of each REE in the muscles of yellow eels of Haute-Indre (more contaminated site) as well as of both male and female silver eels. In order to highlight the potential influence of the life stage on muscle concentrations of each REE, the figure presents the significant differences between yellow eels and female silver eels using an asterisk. This comparison, focused on females, was chosen because yellow eels of the present study were probably mostly future females since their sizes were all higher than 42 cm which is the approximate threshold for males (Adam et al., 2008). The yellow eels bioaccumulated significantly more Nd, Sm and Eu, the last two being MREEs. Concerning silver eels, they bioaccumulated significantly more Ce and Pr which are LREEs, and HREEs, such as Tb, Ho, Er and Y but without significant differences. Due to the increase of Pr, Er and the detection of Gd, Tb and Ho in female silver eel muscles, the distribution of REEs was lightly changed with Pr before Nd among LREEs, Gd before Eu among MREEs and Er becoming the most abundant of the HREEs after Y.

3.2.3. Silver eels and gender variations

As for the study of spatial and life stage variations in 2011/2012, the comparison of muscle contamination of female and male eels can be done thanks to the sampling strategy. Table 2 shows that ∑REEs and the different groups, *i.e.* LREEs, MREEs and HREEs, were significantly higher in male silver eel muscles than those of the females (p-value < 0.001 for ∑REEs, LREEs and HREEs and p-value = 0.013 for MREEs). For example, ∑REEs reached 4649 ng/kg dw in females versus 6203 ng/kg dw for males. The L/M ratios were higher than L/H ratios both for males and females which displayed an enrichment of HREEs in silver eels.

Presenting the detail of each REE muscle concentration, Fig. 4 allows the determination of the REE responsible for the gender difference. Globally, all individual REEs presented higher concentrations in male muscles compared to those of females, except Pr equal in both gender and Tb not detected in males. Nevertheless, the differences were significant for La, Ce, Nd, Sm and Gd only. The distribution of REEs was not really impacted except for Tb which was only detected in female muscles making it the second last REE less abundant.

3.3. Evolution of the REE bioaccumulation between 2011/2012 and 2018/2019

After the description of results obtained for sampling campaigns in 2011/2012, it seemed really interesting to compare them to results found in further sampling campaigns performed 7 years after, *i.e.* 2018/2019. The advantage of the comparison of the two sampling period was to highlight a potential increase of the anthropogenic releases of REEs in the environment. The new technologies, the setting up new MRIs and the production of renewable energy increased the REE uses these last years and could cause higher releases in the environment (Lerat-Hardy et al., 2019).

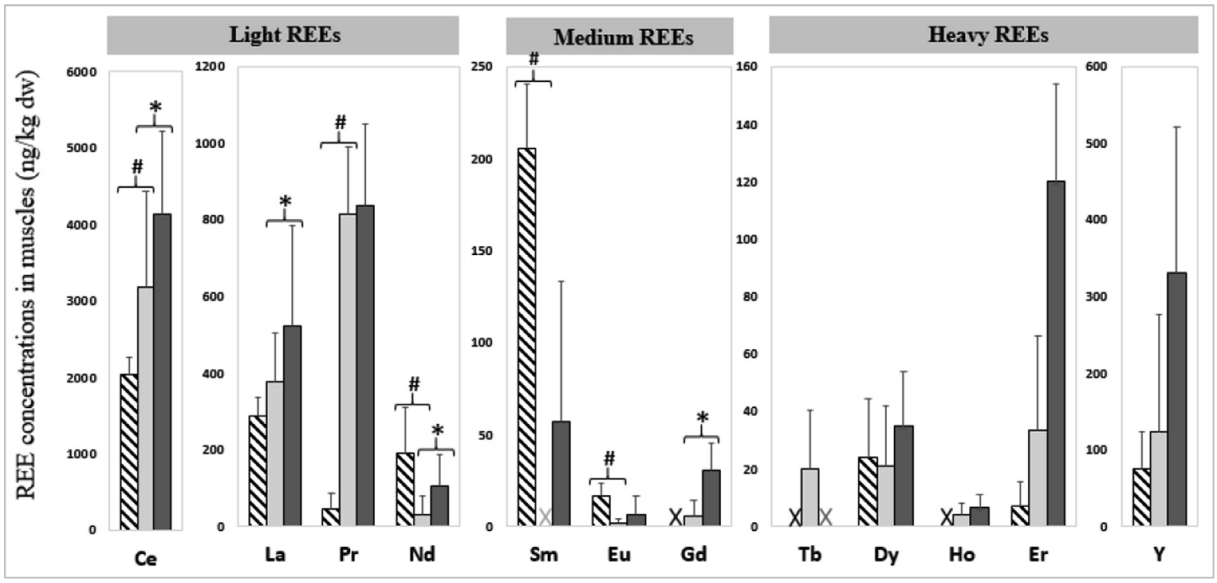

Fig. 4. Median and Median Absolute Deviation (MAD) for REE concentrations (ng/kg dw) in muscles of yellow eels (n = 10; striped bars) and female (n = 14; light gray bars) and male (n = 15; gray bars) silver eels collected in 2011/2012. X: concentrations lower than LOQ. #: significant different values between yellow eels and female silver eels with Kruskal Wallis test (p-value ≤ 0.05). *: significant different values between female and male silver eels with Kruskal Wallis test (p-value ≤ 0.05).

3.3.1. Global evolution

Table 2 shows the results of ∑REEs, LRREs, MREEs and HREEs for muscles of yellow eels from Haute-Indre (the most contaminated site in 2011/2012) as well as female and male silver eels captured in 2018/2019. Preliminary statistical tests showed that the age and the size of individuals for each gender and life stage were not significantly different between 2011/2012 and 2018/2019. Consequently, the samples of the two periods could be compared and the differences attributed to the temporal evolution and not to an influence of the age or size of the organisms.

Considering yellow eels, ∑REEs were very close between 2011/2012 and 2018/2019 with respective values reaching 2901 and 2912 ng/kg dw. Nevertheless, the notable difference was the contribution of HREEs which was higher than the contribution of MREEs in 2018/2019 whereas it was the contrary in 2011/2012. In 2018/2019, the L/H ratio was not anymore higher than L/M because of a decrease of MREEs and an increase of HREEs.

Table 3 details the multiplication factors of individual REE concentrations between 2011/2012 and 2018/2019. The results showed that the reasons for the inversion of MREEs and HREEs were: the significant decrease of Sm (MREE) as well as the detection of Tb, Ho, Yb and Lu (not detected in 2011/2012) and the significant increase of Y.

About silver eels, ∑REEs were significantly higher in 2018/2019 than in 2011/2012 for both females (p-value = 0.002) and males (p-value = 0.003). For example, the highest temporal difference was for ∑REEs twice in 2018/2019 compared to 2011/2012, reaching 12602 ng/kg dw in males. The distribution of LRREs, MREEs and HREEs was not changed. Table 3 shows that all REEs were significantly higher in 2018/2019 except Ce in female silver eel muscles, with multiplication factors ranging from 1.8 to 14.6 respectively for Ce and Tb in male muscles, and from 1.4 to 20.9 respectively for Pr and Eu in female muscles. In female muscles, MREEs, *i.e.* Sm, Eu and Gd, were more than 10 times higher in 2018/2019, this increase was also observed in males, but only for Eu and Tb. Tm, Yb and Lu, not detected in 2011/2012, were detected in 2018/2019 in all silver eels and it was also the case for Tb and Sm, respectively in female and male muscles.

3.3.2. Evolution of life stage differences

Table 2 shows that the ∑REE differences between yellow and silver eels observed in 2011/2012 was still observable in 2018/2019. Nevertheless, the higher MREE concentrations detected in yellow eels than silver ones in 2011/2012 was not observed anymore in 2018/2019 leading to L/M ratios higher than L/H ratios for both studied life stages. Fig. 5 details the median levels of each REE in the muscles of yellow eels and of both male and female silver eels captured in 2018/2019. Significant higher concentrations of Nd, Sm and Eu in yellow eels compared to silver eels observed in 2011/2012 disappeared to lead to significant higher concentrations in silver eels for most REE, except for Nd and Eu.

Table 3
Multiplication factors of REE concentrations in muscles of silver eels (males and females) and of yellow eels between 2011/2012 and 2018/2019.

	Light REEs				Medium REEs			Heavy REEs							
	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	Y
Yellow eels	1.0	1.1	2.0	0.9	0.1	1.6	1.3	+	1.1	1.3	2.0	nd	+	+	2.2
Female silver eels	1.6	1.2	1.4	6.1	18.4	20.9	10.7	+	2.7	8.3	1.9	+	+	+	3.3
Male silver eels	2.1	1.8	1.9	3.8	+	12.6	4.6	14.6	3.9	6.8	2.5	+	+	+	3.1

Box in gray = significant differences of REE concentration between 2011/2012 and 2018/2019; Mann-Whitney test with p-value < 0.05.
+: REEs not detected in 2011/2012 and detected in 2018/2019.
nd: REE detected neither in 2011/2012 nor in 2018/2019.

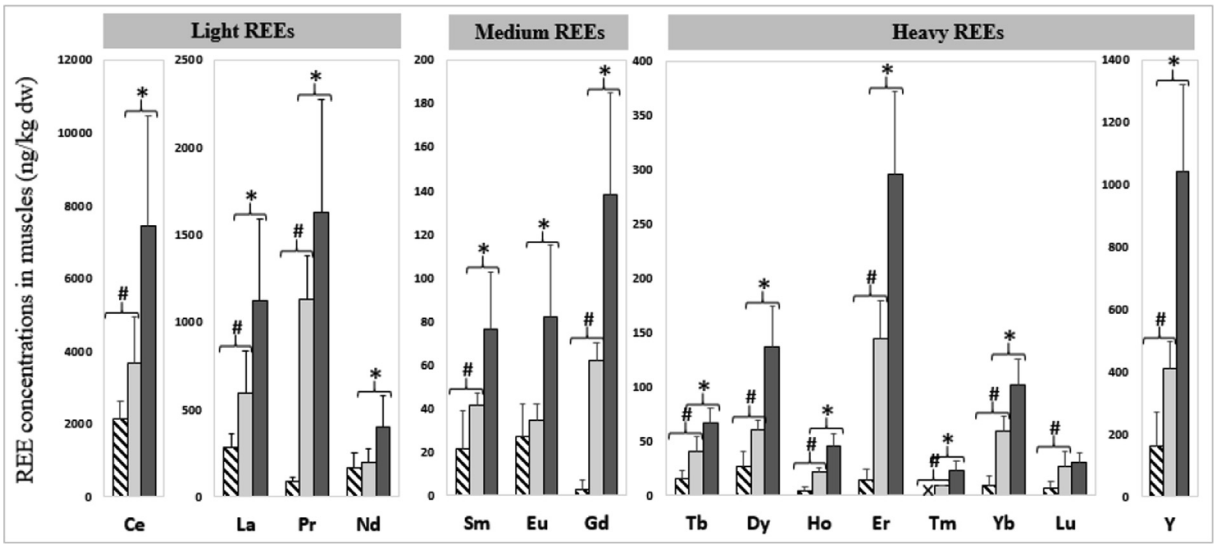

Fig. 5. Median and Median Absolute Deviation (MAD) for REE concentrations (ng/kg dw) in muscles of yellow eels (n = 15; striped bars) and female (n = 14; light gray bars) and male (n = 15; gray bars) silver eels collected in 2018/2019. X: concentrations lower than LOQ. #: significant different values between yellow eels and female silver eels with Kruskal Wallis test (p-value ≤ 0.05). *: significant different values between female and male silver eels with Kruskal Wallis test (p-value ≤ 0.05).

3.3.3. Evolution of gender differences

The ∑REEs higher in male than female muscles observed in 2011/2012 was also observed in 2018/2019 (Table 2). The difference is more accentuated in 2018/2019 with values about twice greater (1.9 versus 1.3 in 2011/2012). The L/M ratios were again higher than L/H ratios both for males and females. Ratios between concentrations in males and females of about 2 were also valuable for LREEs, MREEs and HREES whereas they were different in 2011/2012, *i.e.* respectively 1.3, 9.6 and 2.5. As depicted in the Section 3.3.1 and shown in Table 3, the reason was increases which were element dependent. Fig. 4 shows that as in 2011/2012, all the individual REE concentrations were higher in male muscle compared to females but with significant differences for all REEs except Lu (in 2011/2012, differences were significant only for La, Ce, Nd, Sm and Gd).

4. Discussion

About biological parameters, the significantly smaller size of silver males compared to females was normal because of an existing sexual dimorphism for this species. Moreover, the sexual dimorphism can also explain the significantly higher GSI in females than males. Higher GSI and lipid contents in silver eels compared to yellow eels can be attributed to the silvering metamorphosis. It is well known that this metamorphose is accompanied by an increase of gonad weight, change of swim bladder wall, lipids accumulation, liver structural and metabolic change, food discontinuation and digestive tract retraction (Adam et al., 2008; Fontaine, 1994; Durif et al., 2015).

The study of REE bioaccumulation by yellow and silver eels have not been done before. However, La bioaccumulation during laboratory experiments has been studied for *Anguilla anguilla* in glass eel stage. The results highlighted an effective bioaccumulation. In fact, after an exposure of glass eels at 120 ng/L of lanthanum chloride during 3 days, organisms accumulated 1.6–4.7 μg/kg dw in head, 1.1–6.7 μg/kg dw in skinless body and 4.8–13.0 μg/kg dw in viscera (Figueiredo et al., 2018). Few about REE bioaccumulation by fish collected from the field were already performed, but not on European eels. Two studies were performed in China. The first one was about 30 tropical fish species from the Nansha sea area (Li et al., 2016). The mean value of total REE concentrations (∑REEs) measured in fish were 13.3 μg/kg dw. It was not mentioned what organ was investigated but the value is comparable to those found in the present study. As a reminder, in 2018/2019, ∑REEs reached mean values of 2.9, 6.5 and 12.6 μg/kg dw respectively in yellow, female silver and male silver eels. The second study of China was achieved on 4 freshwater fish species and 6 marine fish species from Shandong province (Yang et al., 2016). The mean ∑REEs measured in muscles were 35.8 and 21.0 μg/kg wet weight (ww) respectively for freshwater and marine fish species. Since the percentage of humidity of the eel muscle reached 70–80% for eels of the present study, the REE concentrations in the fish muscles from China was more than 100 times higher than those of eels from Loire estuary. Much higher values were also found in two studies on 10 freshwater fish species from a reservoir in Washington State (United States) (Mayfield and Fairbrother, 2015) and on fish from Quebec lakes (Canada) (Amyot et al., 2017). The respective mean measured values were: 0.243 and 0.11–0.45 mg/kg dw. Nevertheless, the whole bodies of fish were investigated in the last two studies whereas the present study focused on the muscle. It is an important difference since the muscle is known as the organ of fish the least accumulating REEs (Mayfield and Fairbrother, 2015; Qiang et al., 1994). In 2015, Mayfield and Fairbrother also highlighted REE bioaccumulation in freshwater fish with significant negative correlations between REE concentrations (Sc, La, Ce, Nd and Y) in muscle and age, total length or weight for sucker species of fish. They also observed the same correlations for mouth bass and walleye but they were not significant for all conditions. In the present study, the same negative correlations were sometimes observed, especially for yellow eels, but not always in a significant way and not for the same elements according to the year of fishing (2011/2012: La, Ce, Pr, Nd, Sm and Eu; 2018/2019: Tb and Lu). No general trend could really be drawn on this basis. Whatever the gender, the life stage, the period or the sites, eels accumulated more LREEs than MREEs or HREEs. These results can be explained by the REEs natural abundance, indeed the LREE (Ce = 65.5 ppm) are more abundant in the Earth's crust than the HREE (Tm = 0.5 ppm) (Bru et al., 2015). The comparison of bioaccumulation of REEs in yellow eel muscles of the present study and other metals measured in yellow eel muscles from the Tagus estuary in Portugal (Neto et al., 2011) showed that the order of magnitude was completely different. Yellow eels accu-

mulated more essential or non-essential metals than REEs. For example, Zn, Cu, Hg and Pb concentrations were higher than REE concentrations, respectively 10,000 times, 1000 times, 1000 times and 100 times (Table 1).

The yellow eels fished at the three sites in 2011/2012 (Varades, Bellevue and Haute-Indre) showed some differences of REE muscle concentrations, which are significant only for Dy, Er and Y. The comparison of samples from Haute-Indre and Varades showed a possible impact of the Nantes city on the contamination of eels, as it was already demonstrated for polybrominated diphenyl ethers (PBDEs) in these individuals by Couderc et al. (2015). PBDE muscle levels were twice greater at Haute-Indre than Varades and Bellevue being intermediate. These authors also investigated other organic contaminants, *i.e.* PCB and PFAS, without highlighting the impact of Nantes on their bioaccumulation by eels. In the present study, REE concentrations tend to be higher in Haute-Indre compared to Varades, mostly for HREEs (Dy, Er, Y). The position of Bellevue in this pollution gradient was not discernable, probably due to a weight dilution effect. These two studies, carried out on the same fish sampled in 2011/2012, showed different trends related to the considered contaminants. This conclusion could be explained by different emission areas as well as different environmental fates or bioaccumulation patterns of pollutants.

In this study, intraspecific variations were observed. The life stage and the gender of *Anguilla anguilla* seemed to influence the bioaccumulation of the REEs in the muscles. As mentioned above, in 2011/2012, yellow eels accumulated significantly more Nd, Sm and Eu than silver eels. Moreover, the same year, silver eels accumulated significantly more Ce, Pr and Er. In 2018/2019, it was all REEs except Nd and Eu. The silvering metamorphose which takes place during the eel's life circle, turning yellow eels into silver eels, is a probable hypothesis to the differences observed between the life stages. The silvering leads to many external changes such as change of color, thickening of the skin, lengthening of the pectoral fins, increase in mucus secretion and increase in size of the sensory organs (eyes and nostrils). During this metamorphose, there are also an increase of gonad weight, change of swim bladder wall, lipids accumulation, liver structural and metabolic change, food discontinuation and digestive tract retraction (Adam et al., 2008; Fontaine, 1994; Durif et al., 2015). These important morphological and physiological modifications during metamorphosis could cause changes in the REE absorption, in their distribution in the eel organs and in detoxification mechanisms. Moreover, different exposures in the field could also constitute another hypothesis to explain the life stage differences. Yellow eels are known to be subservient to an area whereas silver eels were fished during their downstream to Sargasso Sea, without really know where they came from and where they spent life. Silver eels were fished upstream the living sites of yellow eels, so they could be exposed to pollution sources upstream the studied area.

The gender is an important factor in bioaccumulation, indeed it influences morphology, physiology, comportment, food preference and detoxification mechanisms (Burger, 2007). In the present work, it has been shown that male silver eels accumulate more REEs in muscles than females whatever the year considered. Results of the literature obtained studying bioaccumulation of metals in fish muscles from the field are relatively different depending on the species and the element chosen. Results presented in this work were in accordance with two other studies: males accumulated significantly more than females, this was observed in horse mackerel with As in Portugal (Vieira et al., 2011) as well as in Skipjacks with Fe and Se in Reunion and the Mozambique canal (Kojadinovic et al., 2007). In the study of Lange et al. (1994) about fish of the same size, the males accumulated more Hg than females but this result was not observable anymore for fish of the same age. Nevertheless, other studies found no influence of the gender on bioaccumulation, *e.g.* Cr, Mn, and Ni in the Mogget (Rajkowska and Protasowicki, 2013) and Fe, Mn, Zn and Cu in the bream and pike (Nussey and van Vuren, 2006). About REEs, in 2017, MacMillan et al., investigated livers of brook trout where they found 2.5 times more REEs in males than females, corroborating the results of the present study.

A significant increase of $\sum$REE concentrations between 2011/2012 and 2018/2019 was notable in silver eel muscles whereas it was stable for yellow eels from Haute-Indre. Otherwise, L/M ratios were higher than L/H ratios for all the studied eels, except yellow eels fished in 2011/2012. This result allows to conclude about a potential old enrichment in MREEs (Sm) in the Loire estuary associated with particularly weak levels of HREEs (Tb, Ho, Yb and Lu not detected at this period). With time, the levels of HREEs in muscle of yellow eels, as well as the global levels of REEs in silver eel muscles, significantly increased. For silver eels, the evolution of concentrations of Sm, Eu, Gd and Tb is particularly worrying with multiplication factors higher than 10 after 7 years and even exceeding 20 for Eu. These increases could be explained by the development of new technologies in different fields. For example, the uses of REEs in petroleum catalysis, permanent magnets, electronic device and medical applications accentuated in recent years has led to an increasingly important demand of REEs. The REE world demand has gone from 113 250 tons of Rare Earth Oxides (REO) in 2012 (Bru et al., 2015) to 123 100 tons in 2016 (Goodenough et al., 2018). The increasing uses of REEs lead to an environmental contamination, which is ubiquitous affecting: the atmosphere (Wang et al., 2001), the soils (Tyler, 2004), the lakes (Johannesson and Zhou, 1999; Sheard et al., 2012), the rivers (Keasler and Loveland, 1982) and the oceans (Alibo and Nozaki, 1999; Kuss et al., 2001; Petersen et al., 2016). As an example, Gd is commonly used as contrast agent in MRI and is not removed in Wastewater Treatment Plants (WWTP). Some publications linked the presence of Gd in rivers to the number of MRIs performed in the local hospitals (Song et al., 2017; Kulaksız and Bau, 2011). In France during the last years, the increase of MRI numbers (from 463 in 2008 to 839 MRI in 2016) (Detournay and Courouve, 2017) has led to a Gd concentration increase in the environment. The study conducted on the Garonne estuary (France) showed the average flux of anthropogenic Gd to the environment increased from 54 ± 2.3 kg/year in 2010–2013 to 75 ± 2.2 kg/year in 2013–2017 (Lerat-Hardy et al., 2019).

5. Conclusion

In the present study, REE bioaccumulation was determined in muscles of *Anguilla anguilla* in two life stages (yellow and silver) and two periods (2011/2012 and 2018/2019). A spatial study was also achieved by fishing yellow eels on 3 sites (Varades, Bellevue and Haute-Indre) of the Loire estuary in 2011/2012. This study showed variations of muscle REE concentrations attributed to biometry, life stage and gender of the organisms. For example, the fish sampled in the site downstream of Nantes urban area presented higher concentrations of some REEs, compared to Varades upstream site. Moerover, silver eel males accumulated more REEs in muscle than females and yellow eels. However, eels are fairly complex fish that undergo many morphological and physiological changes during their life circle. A better understanding of the REE bioaccumulation in eel muscles and of the intraspecific parameter effect (gender and life stage) has to go on through the determination of REE organotropism. This study also showed an increase of REE concentrations in silver eel muscles between 2011/2012 and 2018/2019, certainly related to the increasing REE uses. This hypothesis must be corroborated by the analysis of sediments and water from the Loire estuary. These data constitute the begin-

ning of a time series to monitor REE releases in estuarine systems according to human uses.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflict of interest.

References

Adam, G., Feunteun, E., Prouzet, P., Rigaud, C., 2008. L'anguille européenne: Indicateurs d'abondance et de colonisation. Éditions Quæ, Versailles.

Akagi, T., Edanami, K., 2017. Sources of rare earth elements in shells and soft-tissues of bivalves from Tokyo Bay. Mar. Chem. 194, 55–62. https://doi.org/10.1016/j.marchem.2017.02.009.

Alibo, D.S., Nozaki, Y., 1999. Rare earth elements in seawater: particle association, shale-normalization, and Ce oxidation. Geochim. Cosmochim. Acta 63, 363–372. https://doi.org/10.1016/S0016-7037(98)00279-8.

Alonso, E., Sherman, A.M., Wallington, T.J., Everson, M.P., Field, F.R., Roth, R., Kirchain, R.E., 2012. Evaluating rare earth element availability: a case with revolutionary demand from clean technologies. Environ. Sci. Technol. 46, 3406–3414. https://doi.org/10.1021/es203518d.

Amyot, M., Clayden, M.G., MacMillan, G.A., Perron, T., Arscott-Gauvin, A., 2017. Fate and trophic transfer of rare earth elements in temperate lake food webs. Environ. Sci. Technol. 51, 6009–6017. https://doi.org/10.1021/acs.est.7b00739.

Barak, N.A.-E., Mason, C.F., 1990. Mercury, cadmium and lead in eels and roach: the effects of size, season and locality on metal concentrations in flesh and liver. Sci. Total Environ. 92, 249–256. https://doi.org/10.1016/0048-9697(90)90334-Q.

Blanchet-Letrouvé, I., Zalouk-Vergnoux, A., Vénisseau, A., Couderc, M., Le Bizec, B., Elie, P., Herrenknecht, C., Mouneyrac, C., Poirier, L., 2014. Dioxin-like, non-dioxin like PCB and PCDD/F contamination in European eel (Anguilla anguilla) from the Loire estuarine continuum: Spatial and biological variabilities. Sci. Total Environ. 472, 562–571. https://doi.org/10.1016/j.scitotenv.2013.11.037.

Bordajandi, L.R., Gómez, G., Fernández, M.A., Abad, E., Rivera, J., González, M.J., 2003. Study on PCBs, PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the River Turia (Spain). Chemosphere 53, 163–171. https://doi.org/10.1016/S0045-6535(03)00417-X.

Bru, k., Christmann, P., Labbé, J.F., Lefebvre, G., 2015. Panorama 2014 du marché des Terres Rares [WWW Document]. URL http://www.mineralinfo.fr/sites/default/files/upload/documents/Panoramas_Metaux_Strateg/rp-65330-fr-terresrarespublic.pdf (accessed 6.1.18).

Burger, J., 2007. A framework and methods for incorporating gender-related issues in wildlife risk assessment: Gender-related differences in metal levels and other contaminants as a case study. Environmental Research, Scientific Group on Methodologies for the Safety Evaluation of Chemicals: Workshop 16—Gender Differences and Human and Ecological Risk 104, 153–162. https://doi.org/10.1016/j.envres.2006.08.001.

Censi, P., Randazzo, L.A., D'Angelo, S., Saiano, F., Zuddas, P., Mazzola, S., Cuttitta, A., 2013. Relationship between lanthanide contents in aquatic turtles and environmental exposures. Chemosphere 91, 1130–1135. https://doi.org/10.1016/j.chemosphere.2013.01.017.

Chaillou, G., Anschutz, P., Lavaux, G., Blanc, G., 2006. Rare earth elements in the modern sediments of the Bay of Biscay (France). Mar. Chem. 100, 39–52. https://doi.org/10.1016/j.marchem.2005.09.007.

Cho, Y.-C., Frohnhoefer, Robert C., Rhee, G.-Y., 2004. Bioconcentration and redeposition of polychlorinated biphenyls by zebra mussels (Dreissena polymorpha) in the Hudson River. Water Res. 38, 769–777. https://doi.org/10.1016/j.watres.2003.10.046.

Corolla, J.-P., Ziemski, F., Kupfer, M., 2016. Anguilla anguilla | DORIS [WWW Document]. URL http://doris.ffessm.fr/Especes/Anguilla-anguilla-Anguille-856/(rOffset)/0 (accessed 1.4.19).

Couderc, M., Poirier, L., Zalouk-Vergnoux, A., Kamari, A., Blanchet-Letrouvé, I., Marchand, P., Vénisseau, A., Veyrand, B., Mouneyrac, C., Le Bizec, B., 2015. Occurrence of POPs and other persistent organic contaminants in the European eel (Anguilla anguilla) from the Loire estuary, France. Sci. Total Environ. 505, 199–215. https://doi.org/10.1016/j.scitotenv.2014.09.053.

Cui, J., Zhang, Z., Bai, W., Zhang, L., He, X., Ma, Y., Liu, Y., Chai, Z., 2012. Effects of rare earth elements La and Yb on the morphological and functional development of zebrafish embryos. J. Environ. Sci. 24, 209–213. https://doi.org/10.1016/S1001-0742(11)60755-9.

De Chanvalon, A.T., Metzger, E., Mouret, A., Knoery, J., Chiffoleau, J.F., Brachpapa, C., 2016. Particles transformation in estuaries: Fe, Mn and REE signatures through the Loire Estuary | Elsevier Enhanced Reader [WWW Document]. https://doi.org/10.1016/j.seares.2016.11.004.

Detournay, B., Courouve, L., 2017. Les insuffisances en matiere d'equipements d'imagerie medicale en france: etude sur les delais d'attente pour un rendez-vous irm en 2017 [WWW Document]. URL https://www.fnmr.org/uploads/documents/1511540755_201709_cemkaeval_etude_delais_attente_irm.pdf (accessed 7.3.19).

Don, J., Tourenne, M., Barrabes, M., Chanet, B., 2017. Platichthys flesus | DORIS [WWW Document]. URL http://doris.ffessm.fr/Especes/Platichthys-flesus-Flet-801/(rOffset)/0 (accessed 1.4.19).

Durif, C., Élie, P., Dufour, S., Marchelidon, J., Vidal, B., 2015. Analyse des paramètres morphologiques et physiologiques lors de la préparation à la migration de dévalaison chez l'anguille européenne (Anguilla anguilla) du lac de Grand-Lieu (Loire-Atlantique) 13.

Durrieu, G., Maury-Brachet, R., Girardin, M., Rochard, E., Boudou, A., 2005. Contamination by heavy metals (Cd, Zn, Cu, and Hg) of eight fish species in the Gironde estuary (France). Estuaries 28, 581–591. https://doi.org/10.1007/BF02696069.

Figueiredo, C., Griloa, T., Lopes, C., Brito, P., Diniz, M., Caetano, M., Rosa, R., Raimundo, J., 2018. Accumulation, elimination and neuro-oxidative damage under lanthanum exposure in glass eels (Anguilla anguilla) - ScienceDirect [WWW Document]. URL https://www.sciencedirect.com/science/article/pii/S0045653518308713 (accessed 9.11.18).

Fontaine, Y.A., 1994. L'argenture de l'Anguille : métamorphose, anticipation, adaptation. Bulletin Français de la Pêche et de la Pisciculture 171–185. https://doi.org/10.1051/kmae:1994012.

Genc, E., Sangun, M.K., Dural, M., Can, M.F., Altunhan, C., 2008. Element concentrations in the swimbladder parasite Anguillicola crassus (nematoda) and its host the European eel, Anguilla anguilla from Asi River (Hatay-Turkey). Environ. Monit. Assess 141, 59–65. https://doi.org/10.1007/s10661-007-9878-9.

Goodenough, K.M., Wall, F., Merriman, D., 2018. The rare earth elements: demand, global resources, and challenges for resourcing future generations. Nat. Resour. Res. 27, 201–216. https://doi.org/10.1007/s11053-017-9336-5.

Hongyan, G., Liang, C., Xiaorong, W., Ying, C., 2002. Physiological responses of Carassius auratus to ytterbium exposure. Ecotoxicol. Environ. Saf. 53, 312–316. https://doi.org/10.1006/eesa.2002.2223.

Hua, D., Wang, J., Yu, D., Liu, J., 2017. Lanthanum exerts acute toxicity and histopathological changes in gill and liver tissue of rare minnow (Gobiocypris rarus). Ecotoxicology 26, 1207–1215. https://doi.org/10.1007/s10646-017-1846-8.

Johannesson, K.H., Zhou, X., 1999. Origin of middle rare earth element enrichments in acid waters of a Canadian High Arctic lake. Geochim. Cosmochim. Acta 63, 153–165. https://doi.org/10.1016/S0016-7037(98)00291-9.

Keasler, K.M., Loveland, W.D., 1982. Rare earth elemental concentrations in some Pacific Northwest rivers. Earth Planet. Sci. Lett. 61, 68–72. https://doi.org/10.1016/0012-821X(82)90039-5.

Kogel, J.E., Trivedi, N.C., Barker, J.M., Krukowski, S.T., 2006. Industrial minerals & rocks: commodities, markets, and uses. SME.

Kojadinovic, J., Potier, M., Le Corre, M., Cosson, R.P., Bustamante, P., 2007. Bioaccumulation of trace elements in pelagic fish from the Western Indian Ocean. Environ. Pollut. 146, 548–566. https://doi.org/10.1016/j.envpol.2006.07.015.

Kulaksız and Bau, 2011. Appl. Geochem. 26 (11), 1877–1885. https://doi.org/10.1016/j.apgeochem.2011.06.011.

Kuss, J., Garbe-Schönberg, C.-D., Kremling, K., 2001. Rare earth elements in suspended particulate material of North Atlantic surface waters. Geochim. Cosmochim. Acta 65, 187–199. https://doi.org/10.1016/S0016-7037(00)00518-4.

Lange, T.R., Royals, H.E., Connor, L.L., 1994. Mercury accumulation in largemouth bass (Micropterus salmoides) in a Florida Lake. Arch. Environ. Contam. Toxicol. 27, 466–471. https://doi.org/10.1007/BF00214837.

Lerat-Hardy, A., Coynel, A., Dutruch, L., Pereto, C., Bossy, C., Gil-Diaz, T., Capdeville, M.-J., Blanc, G., Schäfer, J., 2019. Rare earth element fluxes over 15 years into a major European Estuary (Garonne-Gironde, SW France): Hospital effluents as a source of increasing gadolinium anomalies. Sci. Total Environ. 656, 409–420. https://doi.org/10.1016/j.scitotenv.2018.11.343.

Li, J.-X., Zheng, L., Sun, C.-J., Jiang, F.-H., Yin, X.-F., Chen, J.-H., Han, B., Wang, X.-R., 2016. Study on ecological and chemical properties of rare earth elements in tropical marine organisms. Chinese J. Anal. Chem. 44, 1539–1546. https://doi.org/10.1016/S1872-2040(16)60963-5.

Linde, A.R., Arribas, P., Sanchez-Galan, S., de BiologiaFuncional, D., Oviedo, U., IngenierlaQufmica, D., 1996. Eel (Anguilla anguilla) and Brown Trout (Salmo trutta) Target Species to Assess the Biological Impact of Trace Metal Pollution in Freshwater Ecosystems 6.

Mácová, S., Babula, P., Plhalová, L., Žáčková, K., Kizek*, R., Svobodová, Z., 2014. Acute toxicity of lanthanum to fish Danio rerio and Poecilia reticulata. J. Biochem. Technol. 2, 46–47.

Maes, J., Belpaire, C., Goemans, G., 2008. Spatial variations and temporal trends between 1994 and 2005 in polychlorinated biphenyls, organochlorine pesticides and heavy metals in European eel (Anguilla anguilla L.) in Flanders, Belgium. Environ. Pollut., Chelating Agents Environ. 153, 223–237. https://doi.org/10.1016/j.envpol.2007.07.021.

Mansouri, D., Fegrouche, R., Allami, H., Fadli, M., 2018. Bioaccumulation of some metallic elements in the gills, the muscle and the liver of the eel (Anguilla anguilla L.) and the cabot mullet (Mugil cephalus L.) of the estuary of Oued Sebou (Morocco).

Mayfield, D.B., Fairbrother, A., 2015. Examination of rare earth element concentration patterns in freshwater fish tissues. Chemosphere 120, 68–74. https://doi.org/10.1016/j.chemosphere.2014.06.010.

Negrel, Ph., 1997. Multi-element chemistry of loire estuary sediments: anthropogenicvs. natural sources. Estuar. Coast. Shelf Sci. 44, 395–410. https://doi.org/10.1006/ecss.1996.0139.

Neto, A.F., Costa, J.L., Costa, M.J., Pereira, M.E., Duarte, A., Caçador, I., Domingos, I., 2011. Accumulation of metals in Anguilla anguilla from the Tagus estuary and relationship to environmental contamination. J. Appl. Ichthyol. 27, 1265–1271. https://doi.org/10.1111/j.1439-0426.2011.01814.x.

Nørregaard, R.D., Kaarsholm, H., Bach, L., Nowak, B., Geertz-Hansen, O., Søndergaard, J., Sonne, C., 2019. Bioaccumulation of rare earth elements in juvenile arctic char (Salvelinus alpinus) under field experimental conditions. Sci. Total Environ. 688, 529–535. https://doi.org/10.1016/j.scitotenv.2019.06.180.

Nussey, G., van Vuren, J., 2006. Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, Labeo umbratus (Cyprinidae), from Witbank Dam, Mpumalanga 16.

Pagano, G., Guida, M., Tommasi, F., Oral, R., 2015. Health effects and toxicity mechanisms of rare earth elements—Knowledge gaps and research prospects. Ecotoxicol. Environ. Saf. 115, 40–48. https://doi.org/10.1016/j.ecoenv.2015.01.030.

Petersen, J., Pröfrock, D., Paschke, A.C., Broekaert, J.A., Prange, A., 2016. Development and field test of a mobile continuous flow system utilizing Chemcatcher for monitoring of rare earth elements in marine environments. Environ. Sci. Water Res. Technol. 2, 146–153. https://doi.org/10.1039/C5EW00126A.

Qiang, T., Xiao-rong, W., Li-qing, T., Le-mei, D., 1994. Bioaccumulation of the rare earth elements lanthanum, gadolinium and yttrium in carp (Cyprinus carpio). Environ. Pollut. 85, 345–350. https://doi.org/10.1016/0269-7491(94)90057-4.

Rajkowska, M., Protasowicki, M., 2013. Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environ Monit Assess 185, 3493–3502. https://doi.org/10.1007/s10661-012-2805-8.

Redmayne, A.C., Kim, J.P., Closs, G.P., Hunter, K.A., 2000. Methyl mercury bioaccumulation in long-finned eels, Anguilla dieffenbachii, from three rivers in Otago, New Zealand. Sci. Total Environ. 262, 37–47. https://doi.org/10.1016/S0048-9697(00)00534-9.

Ribeiro, C.A.O., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (Anguilla anguilla) at the Camargue Nature Reserve, France. Aquat. Toxicol. 74, 53–69. https://doi.org/10.1016/j.aquatox.2005.04.008.

Sheard, E.R., Williams-Jones, A.E., Heiligmann, M., Pederson, C., Trueman, D.L., 2012. Controls on the concentration of zirconium, niobium, and the rare earth elements in the Thor lake rare metal deposit, Northwest Territories, Canada. Econ. Geol. 107, 81–104. https://doi.org/10.2113/econgeo.107.1.81.

Song, H., Shin, W.-J., Ryu, J.-S., Shin, H.S., Chung, H., Lee, K.-S., 2017. Anthropogenic rare earth elements and their spatial distributions in the Han River, South Korea. Chemosphere 172, 155–165. https://doi.org/10.1016/j.chemosphere.2016.12.135.

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Battuello, M., Nurra, N., Sartor, R.M., Orusa, R., Robetto, S., Brusa, F., Mogliotti, P., Garrone, A., Abete, M.C., 2019. Rare earth elements in marine and terrestrial matrices of Northwestern Italy: Implications for food safety and human health. Sci. Total Environ. 660, 1383–1391. https://doi.org/10.1016/j.scitotenv.2019.01.112.

Storelli, M.M., Barone, G., Garofalo, R., Marcotrigiano, G.O., 2007. Metals and organochlorine compounds in eel (Anguilla anguilla) from the Lesina lagoon, Adriatic Sea (Italy). Food Chem. 100, 1337–1341. https://doi.org/10.1016/j.foodchem.2005.10.071.

Strady, E., Kim, I., Radakovitch, O., Kim, G., 2015. Rare earth element distributions and fractionation in plankton from the northwestern Mediterranean Sea. Chemosphere 119, 72–82. https://doi.org/10.1016/j.chemosphere.2014.05.049.

Tyler, G., 2004. Rare earth elements in soil and plant systems - A review. Plant Soil 267, 191–206. https://doi.org/10.1007/s11104-005-4888-2.

Usero, J., Izquierdo, C., Morillo, J., Gracia, I., 2004. Heavy metals in fish (Solea vulgaris, Anguilla anguilla and Liza aurata) from salt marshes on the southern Atlantic coast of Spain. Environ. Int. 29, 949–956. https://doi.org/10.1016/S0160-4120(03)00061-8.

Van der Oost, R., Opperhuizen, A., Satumalay, K., Heida, H., Vermeulen, N.P.E., 1996. Biomonitoring aquatic pollution with feral eel (Anguilla anguilla) I. Bioaccumulation: biota-sediment ratios of PCBs, OCPs, PCDDs and PCDFs. Aquatic Toxicology 35, 21–46. https://doi.org/10.1016/0166-445X(96)00002-1.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., Oliveira, M.B.P.P., 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food Chem. Toxicol. 49, 923–932. https://doi.org/10.1016/j.fct.2010.12.016.

Wang, C.X., Zhu, W., Peng, A., Guichreit, R., 2001. Comparative studies on the concentration of rare earth elements and heavy metals in the atmospheric particulate matter in Beijing, China, and in Delft, the Netherlands. Environ. Int., Environ. Geochem. Tropics Subtropics 26, 309–313. https://doi.org/10.1016/S0160-4120(01)00005-8.

Yang, L., Wang, X., Nie, H., Shao, L., Wang, G., Liu, Y., 2016. Residual levels of rare earth elements in freshwater and marine fish and their health risk assessment from Shandong, China. Mar. Pollut. Bull. 107, 393–397. https://doi.org/10.1016/j.marpolbul.2016.03.034.