Organic and inorganic contamination impacts on metabolic capacities in American and European yellow eels

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Abstract

American (*Anguilla rostrata*) and European (*Anguilla anguilla*) eel populations are declining since the 1980s, and contamination is thought to play a role. To determine the influence of organic (OCPs, PBDEs, PCBs) and inorganic (Zn, As, Cd, Cu, Pb, Cr, Ni, Ag, Se, Hg) contaminants on wild yellow eels liver and muscle metabolic capacities, enzymatic assays were performed. In *A. rostrata* liver, G6PDH moderate negative correlations with Ag, Pb and As suggest impacts on lipid metabolism, and correlations between Cd and age (positive) and between Cd and Kn (negative) indicate impacts on older eels health. *A. anguilla* liver proteins, PK and LDH were positively linked to Zn, Pb and Cu, suggesting effects on glycolytic and anaerobic capacities. In *A. anguilla* muscle, absence of correlation between age and lipids plus strong positive correlations between age and OCPs, PBDEs, PCBs and Hg suggest lipid storage impairment in older contaminated eels. Overall, our study indicates contamination impacts on both species metabolic capacities, but the broader range of contaminants found in *A. anguilla* bring greater impacts compared to *A. rostrata*.

Keywords: American eel (*Anguilla rostrata*), European eel (*Anguilla anguilla*), enzyme activity, metals, organochlorinated pesticides (OCP), pollution, polychlorinated biphenyls (PCB), polybrominated diphenyl ethers (PBDE)

1 Introduction

Since the early 1980s, both American eel (*Anguilla rostrata*) and European eel (*Anguilla anguilla*) are facing severe declines in their respective populations: 40 to 80% decline for *A. rostrata* (de Lafontaine et al. 2010; Haro et al. 2000), and 90-99% for *A. anguilla*, depending on the location (ICES 2014; Stone 2003; van Ginneken and Maes 2005). Among the possible causes for these declines, many studies are pointing towards the effects of various inorganic and organic contaminants released and accumulated in the environment (Castonguay et al. 1994a; Dutil et al. 1985; Geeraerts and Belpaire 2010; Hodson et al. 1994; van Ginneken and Maes 2005). As an example, dioxin-like PCBs are thought to play a key role in the declines: Palstra et al. (2006) reported a negative relationship between TEQ-level of these contaminants and the survival of the fertilized eggs of European silver eels (adult life stage), suggesting an impairment of its reproductive capacity. As they are accumulated along with other contaminants in different tissues during the yellow stage of the eels, the somatic growth phase that can last for up to 40 years in continental waters (COSEWIC 2012; Tesch 2003), these contaminants would cause a reduction in lipid storage efficiency (Belpaire et al. 2009) and could also affect the reproductive migration of silver eels to the Sargasso Sea after their remobilization from the lipid reserves (Castonguay et al. 1994b).

Very few studies have examined the impacts of contaminants on the metabolic capacities of eels (Boldsen et al. 2013; Pelster et al. 1997). The study of Baillon et al. (2015) on the hepatic transcriptome of wild Atlantic eels revealed however that Cd affected genes related to energy metabolism, while lindane affected lipid metabolism

related genes. Otherwise, most studies are mainly restricted to experimental investigations. Among the studies found on the subject, Pierron et al. (2007a) have shown that exposing European eels at the yellow stage to a concentration of 5 µg/L of cadmium (Cd) for 30 days caused an increase in the activity of enzymes involved in liver lipolysis (triglyceride lipase, TGL) and lipogenesis (isocitrate dehydrogenase, IDH; malic enzyme, ME), as well as two enzymes involved in the production of NADPH needed for lipogenesis (glucose-6-phosphate dehydrogenase, G6PDH; 6-phosphogluconate dehydrogenase, 6-PGDH). At the same time, the authors reported lower Fulton condition factor indices (FCF) as well as lower lipid contents in the muscle of the contaminated eels, indicating a clear effect of Cd on lipid metabolism. In another study, following an exposition of young yellow eels to propanil (herbicide), Sancho et al. (2009) have reported changes in the activity of different enzymes (alkaline phosphatase, gamma-glutamyl transpeptidase, alanine aminopeptidase), and more specifically an increase in lactate dehydrogenase (LDH) activity, coupled to a decrease of protein concentrations in both liver and muscle, suggesting an enhanced use of the anaerobic energy pathway through an hypoxia-like effect, and/or a greater use of energy needed for detoxification.

Since eels from the St. Lawrence River system are mostly females, especially in the upper part of the river (COSEWIC 2012) where the contaminated sites are located, the aim of this study was to compare females of both systems. Exception made of four *A. anguilla* males captured in 2012, we sampled female American and European yellow eels in four different sites in each of the St. Lawrence River system (Quebec, Canada) and the Garonne-Gironde fluvial-estuarine system (France), which presented various

contamination levels. We measured the activities of key enzymes in liver and muscle indicative of four metabolic capacities: glycolytic capacities (pyruvate kinase, PK), aerobic capacities (citrate synthase, CS; cytochrome c oxidase, CCO), anaerobic capacities (lactate dehydrogenase, LDH) and lipid metabolism (glucose-6-phosphate dehydrogenase, G6PDH). We also measured the concentration of major inorganic (Zn, As, Cd, Cu, Pb, Cr, Ni, Ag, Se and Hg) and organic contaminants (polychlorinated biphenyls, PCBs; organochlorinated pesticides, OCPs; polybrominated diethyl ether, PBDEs) in these tissues.

The first objective of this study was to investigate, for each eel species, the relationships between metabolic capacities and contamination in both liver and muscle. The second objective was to perform an interspecific comparison of the response of tissue metabolic capacities to contamination. Our last objective was to identify contaminants most likely to interfere with accumulation of energy stores in both eel species.

2 Materials and Methods

2.1 Study sites and fish collection

For each species, four sites were selected according to the metal and organic contamination concentrations found in water, sediments, and biota in the St. Lawrence River system in Québec (Couillard et al. 1997; Desrosiers et al. 2008; Hodson et al. 1994; Pelletier 2010; Richman 1997) and in the Garonne-Gironde fluvial-estuarine system in France (Durrieu et al. 2005; Pierron et al. 2007c) (Fig. 1). In Québec these were classified as two clean sites, Saint Jean River (RSJ; 48° 51' 40" N; 64° 28' 47" W)

and Sud-Ouest River (RSO; 48° 22' 27" N; 68° 43' 02" W), and two contaminated sites, Lake Saint-François (LSF; 46° 19' 50" N; 72° 32' 06" W) and Lake Saint-Pierre (LSP; 45° 09' 18" N; 74° 23' 04" W). In France, the four sites were the Certes salt marshes (44° 41' 18" N; 1° 1' 39" W), the Dordogne River (44° 54' 30" N; 0° 15' 01" W), the Garonne River (45° 12' 07" N; 0° 43' 35" W), and the Gironde estuary (44° 43' 51" N; 0° 28' 05" W), and must be seen as a gradient of increasing contamination (Certes < Dordogne < Garonne < Gironde).

Between the end of May and the beginning of July of both 2011 and 2012 field campaigns, we collected 15 yellow eels in each site using either electrofishing or fyke nets (120 eels total for each species), preferably in a length range of 300-500 mm. Live fish were held in a portable water basin with aerated water until they were weighed, measured and sacrificed by decapitation as soon as possible in a mobile laboratory onshore. Weights and lengths for each site are reported in Table S1, together with age, relative condition factor (Kn) and Fulton condition factor. Pieces of white muscle (in a standardized area situated at 2 cm posterior to the anus, and above the lateral line) and liver were removed and put in 2 mL, acid-washed (15% v/v; Omnitrace grade HNO₃) polypropylene cryo-vials that were flash frozen in liquid nitrogen and stored at -80°C back in the laboratory.

Especially in freshwater, American eels can move from one site to another frequently during the yellow stage (COSEWIC 2012). For that reason, salinity, oxygen and temperature at the capture site are unlikely to be representative of the eels' experience and hence were not considered in the analyses.

2.2 Age determination

Both sagittal otoliths were removed from each individual for age determination, embedded in epoxy and covered with an adhesive. The convex surface was ground along the sagittal plane and polished using silicone carbide sandpapers to enhance the resolution of annular rings (ICES 2009). Otoliths were then photographed under a dissecting microscope for subsequent back-calculation of length-at-age and estimation of growth increments. All age measurements were performed by the Ministère des Forêts, de la Faune et des Parcs (MFFP, Rivière-du-Loup, QC Canada).

2.3 Enzymatic assays and protein quantification

On the day of the measurement, liver or muscle samples were cut frozen on ice and 10 mg of tissue were placed in round-bottom polypropylene culture tubes. These were then diluted in a homogenization buffer (pH 7.5; 20 mM HEPES; 1 mM EDTA; 0.1% Triton® X-100), in a ratio of 1:25 (w/v). Samples were then homogenized on ice with a rotor-stator homogenizer (Janke & Junkel Ultra-Turrax® T25, IKA-Labortechnik), by three periods of 10 sec at maximal speed (24000 rpm), with a 30 sec pause between each period. Samples were kept on ice and tested in a maximum of 7 hours after homogenization.

Enzymatic activity was measured on a 96 well microplate with a UV/visible spectrophotometer (Cary 50, Varian inc.), following experimental protocols of Lapointe et al. (2011), exception made for PK and G6PDH activities that were measured following the protocols of Gauthier et al. (2008). Each sample was tested in triplicates. For each tissue and enzyme, an optimization of reagent concentrations and the sample

dilution was performed in order to get a maximal linear reaction slope over a minimum of 3 minutes. Assays were done at a mean temperature of 24.1°C. Prior to each assay, final sample dilution and reagent solutions were prepared on ice and as follows:

- *Pyruvate kinase* (PK; E.C. 2.7.1.40): Sample dilution in homogenization buffer of 1:25 and 1:2000 (w/v; liver and muscle respectively). Imidazole buffer (imidazole 50 mM, MgCl₂ 20 mM, KCl 70 mM), pH 7.5, β-nicotinamide adenine dinucleotide (β-NADH) 0.16 mM, adenosine-5-diphosphate (ADP) 10 and 15 mM (liver and muscle respectively), lactate dehydrogenase (LDH) in excess, phospho(enol) pyruvate (PEP) 200 and 75 mM (liver and muscle respect.; omitted in controls).

- *Citrate synthase* (CS; E.C. 2.3.3.1): Sample dilution in homogenization buffer of 1:100 and 1:25 (w/v; liver and muscle respect.). Phosphate buffer 100 mM (pH 8), Tris-HCl 100 mM, dithiobis (2-nitrobenzoic) acid (DTNB) 0.1 mM, acetyl CoA 0.2 and 0.4 mM (liver and muscle respect.), oxaloacetate 3 and 4.5 mM (liver and muscle respect.; omitted in controls).

- *Cytochrome c oxidase* (CCO; 1.9.3.1): Muscle sample dilution in homogenization buffer of 1:100 (w/v). Phosphate buffer 100 mM (pH, 7.0), cytochrome C 0.07 mM, 0.33% potassium ferricyanide (in controls).

- *Glucose 6-phosphate dehydrogenase* (G6PDH; EC 1.1.1.49): Liver sample dilution in homogenization buffer of 1:100 (w/v). Imidazole buffer (imidazole 50 mM, MgCl₂ 25 mM, KCl 70 mM, pH 7.5), β-nicotinamide adenine dinucleotide phosphate (NADP) 0.3 mM, glucose-6-phosphate 200 mM (omitted in controls).

- *Lactate dehydrogenase* (LDH; EC 1.1.1.27): Sample dilution in homogenization buffer of 1:50 and 1:2000 (w/v; liver and muscle respect.). Phosphate buffer 100 mM (pH 7.0), β -NADH 0.16 mM, and sodium pyruvate 20 mM (omitted in controls).

Protein quantification was done following the method developed by Marion M. Bradford (Bradford), and by using the protocol elaborated for microplates by Bio-Rad (Bio-Rad Protein Assay). Leftovers of the samples diluted 1:25 (w/v) in homogenization buffer were frozen at -80°C after the enzymatic assays and thawed another day specifically for the protein quantification.

2.4 Metal analysis

All liver and muscle samples were freeze-dried in previously trace metal cleaned 1.5 mL microcentrifuge tubes (Fisherbrand) and kept in these vials during the whole digestion process. A 50 mg sub-sample was taken after the freeze-drying step to perform the mercury analysis. These sub-samples were processed in a mercury analyzer (Milestone DMA-80) and MESS-3 certified reference material (National Research Council Canada) was used, with a recovery rate of $103.0 \pm 0.7\%$ (mean $\pm$ SEM). For the remainder of the samples (in microcentrifuge tubes), a first digestion was done overnight at room temperature in pure trace metal grade nitric acid (HNO_3). The following day, sample digestion was completed by autoclave (All American 25X-1) for 2 hours at 120°C and 15 psi. Samples were then diluted in Milli-Q water to obtain a final concentration of 10% HNO_3 . Concentrations for Zn, As, Cd, Cu, Pb, Cr, Ni, Ag and Se were measured by inductively coupled plasma - mass spectrometry (ICP-MS; Thermo

Scientific XSeries 2), inductively coupled plasma - atomic emission spectrometry (ICP-AES; Varian Vista AX) or both methods. Analysis blanks and standards were added after each 15 samples. Method detection limits (LoD, $\mu\text{g}\cdot\text{g dw}^{-1}$) of the ICP-MS were: Zn = 0.033; As = 0.038; Cd = 0.001; Cu = 0.089; Pb = 0.007; Cr = 0.357; Ni = 0.010; Ag = 0.006; Se = 0.129. TORT-2 and DOLT-4 (for Ag and Se) certified biological reference materials (National Research Council Canada) were submitted to the same digestion protocol as the samples to monitor the efficiency of the digestion procedure. Recovery rates were: Zn = $116.7 \pm 3.0\%$; As = $102.8 \pm 1.5\%$; Cd = $106.4 \pm 1.2\%$; Cu = $107.0 \pm 5.3\%$; Pb = $80.3 \pm 4.2\%$; Cr = $104.4 \pm 3.2\%$; Ni = $93.1 \pm 2.5\%$; Ag = $88.6 \pm 5.5\%$; Se = $105.0 \pm 4.7\%$ (mean $\pm$ SEM). All metal analyses were performed at the INRS-ETE (Québec QC Canada).

2.5 Organic contamination analysis and lipid determination

Analysis of the 7 indicator PCBs (CB50 + 28, CB52, CB101, CB118, CB138, CB153, and CB180), 8 OCPs (hexachlorobenzene or HCB, lindane, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT and 4,4'-DDT), and 2 PBDEs (BDE47 and BDE100) were performed on freeze dried muscle samples following the procedures described by Tapie et al. (2008) and Tapie et al. (2011). Lipids were determined by gravimetry after filtration and evaporation of an aliquot of the DCM extract. PCB, PBDE and OCP analyses were carried out on an HP 5890 series II gas chromatograph from Hewlett-Packard (Avondale, CA, USA) coupled to a ^{63}Ni electron capture detector (ECD). A capillary column HP5-MS (Agilent Technologies, Massy, France) was used

(30 m x 0.25 mm x 0.25 μ m). These analyses were performed at the Université de Bordeaux (France).

For all the analyses, POPs were quantified relative to internal standards. Quality control consisted of the analysis of solvent procedural blanks, reproducibility and repeatability tests, injection of standard solutions as unknowns and analysis of certified reference material SRM 2974a (National Institute of Standards and Technology) for PCBs, OCPs, and PBDEs. The recoveries for five replicates on this SRM were between 70% and 110% with reproducibility ranging from 10% to 20% depending on the compounds. As described in Labadie et al. (2010), POPs levels were blank corrected and the method detection limit (LoD) was derived from the blank value variability. For PCBs, LoQs were comprised between 0.1 ng·g dw⁻¹ and 2.7 ng·g dw⁻¹; for OCPs they were between 0.1 ng·g dw⁻¹ and 0.4 ng·g dw⁻¹; for PBDEs they were between 0.1 ng·g dw⁻¹ and 0.2 ng·g dw⁻¹.

2.6 Relative condition factor calculation (Kn)

Fish relative condition factor, Kn, was estimated using the equation described in LeCren (1951), $Kn = W/W'$, where W is the individual fish weight (g), and W' is the predicted length-specific mean weight (g) for each eel species population under study. Following observations about Kn calculation summarized by Blackwell et al. (2000), especially to detect pollution or physiological stress on a segment of population, since both species are panmictic, all eels of a same species collected were considered as coming from a same population. Power-law graphs of eel weights (g) in function of their

respective lengths (L; cm) were drawn to calculate predicted length-specific weight for each individual, and gave the following formulas:

$$A. \textit{rostrata}: W' = 0.0005 \times L^{3.3108} \text{ (with } R^2 = 0.97772)$$

$$A. \textit{anguilla}: W' = 0.0007 \times L^{3.2207} \text{ (with } R^2 = 0.92912)$$

2.7 Statistical analysis

Canonical correlation analysis (CCA) is a multivariate exploratory statistical method in the same vein as Principal Components Analysis (PCA): both methods lie on the same mathematical background and results can be illustrated through similar graphical representations. Proposed by Hotelling (1936), CCA is a method used for exploring the relationships between two multivariate sets of quantitative variables observed on the same experimental units, whereas PCA deals with one data set in order to reduce dimensionality through linear combination of initial variables.

In this study, CCA was performed using XLSTAT-ADA (Addinsoft, USA) to explore the relationships between a first set of variables considered as "dependent" (weight, age, lipid content, Kn, enzymatic activities, protein concentration) and a second set of variables considered as "independent" variables (organic and inorganic contaminants). For each species, liver and muscle variables were analysed in separate CCAs on the assumption that liver contamination would not have an impact on muscle enzymatic activity, and the other way around. This way, a maximum of non-causal correlations are avoided. However, lipids were not measured in the liver. Consequently, muscle lipids have been included in the liver CCAs since the lipid metabolism indicator enzyme G6PDH is not expressed in the muscle.

Note that the sampling of eels for the global France-Canada project to which this study belongs (15 fish per site per year for a total of 120 fish per species) included a wide range of other analyses conducted in several laboratories. Consequently, not all liver and muscle samples were available for enzyme assays. Furthermore, several of the organic contaminants could not be detected in the eels for which enzyme assays were performed and this missing data prevented the use of some individuals in the CCA analyses. As a result, the sample size for the CCA analyses varied between 52 and 82.

3 Results

3.1 Biometric measurements and contamination data

Clean and contaminated sites were selected in order to maximize the gradient of contamination observed among the eels from each system. Eels captured in the contaminated sites were generally older and larger compared to fish from the clean sites, and this was especially true for the American eels (Table S1). For this reason, in 2012 an effort was made to catch smaller fish in the contaminated sites, but eels were still older and larger than in the clean sites. Although significant inter-annual variations in the tissue concentrations of some contaminants in some sites were detected (Tables S2 and S3), we determined that pooling data from eels of both years within each species in the CCA analysis provided a stronger analysis of the links between age, weight, contamination and enzyme activity. Nevertheless, this overrepresentation of older and larger contaminated eels was taken into account in the data interpretation below. Moreover, and as explained in the Materials and Methods section, the limited number of eels with complete data sets for all parameters selected prevented us from

performing statistical analyses among sites and years of sampling. Nevertheless, the data allows clearly establishing that both eel species from the contaminated sites presented higher levels of several contaminants in their liver and muscle compared to fish from the clean sites. Furthermore, *A. anguilla* liver and muscle presented higher levels of a broad range of contaminants compared to *A. rostrata*, in particular for organic contaminants in the muscle.

3.2 *A. rostrata* liver and muscle CCAs

In the CCA of *A. rostrata* liver variables, the first two factors explained 55.17% of the variability observed (Fig. 2). F1 was mainly composed of weight, Se, lipids, Cu, Pb and Zn, while the variables that correlated the most with F2 were age, Cd and Kn. On the other hand, Cr was the contaminant that showed the weakest correlation with both F1 and F2 (Table 1). For the dependent variables, all four liver enzyme activities and protein concentrations poorly correlated with F1 and F2. Moreover, the correlations of these variables with F3 were also quite weak, even if this factor was significantly explaining 18.17% of the variability (eigenvalue = 1.117).

The first two factors of the American eel muscle variables CCA explained 51.87% of the variability observed (Fig. 3), which is a level of explanation comparable to the liver CCA. F1 was mainly composed of Hg, weight, As, PBDE-47, sum PCB and sum OCP, while F2 was essentially constituted of Lipids, CCO, sum OCP, PBDE-47, Kn, CS, weight and sum PCB. An interesting point to raise here is that these factors were sharing 4 variables (weight, PBDE-47, sum PCB and sum OCP), which explains the shape of the graph showing two groups of variables pointing in opposite directions,

respectively towards the middle of quadrants 1 (top right) and 3 (bottom left). For the muscle contaminants that were the least correlated to the factors, we can note Cr, Ni, Cu, Zn, Se, Cd and Pb, while muscle proteins and LDH were the two dependant variables that were the least correlated to the factors (Table 1).

3.3 *A. anguilla* liver and muscle CCAs

For *A. anguilla* liver variables, the first two factors of the CCA explained 56.20% of the variability (Fig. 4). F1 was mainly composed of LDH, proteins, Pb, PK, CS and Zn, while the variables showing the most significant correlations with F2 were Cd and Pb. Otherwise, Cr and Ag were the only contaminants poorly correlating with both F1 and F2 (Table 1). For the dependent variables, weight, lipids, Kn and G6PDH showed the weakest correlations with the factors. However, together with age, strong correlations between weight, lipids and Ag were observed with F3 (18.74% of the variability), even if it was not a significant factor (eigenvalue = 0.9, data not shown).

The first two factors of the European eel muscle CCA explained 45.50% of the variability observed (Fig. 5). However, analysis of the significant factor F3 (eigenvalue = 1.575; Table 1) shows that it only explained 17.20% of the variability, and did not bring more valuable information. F1 was mainly composed of lipids and LDH, while F2 was mainly composed of sum OCP, sum PCB, age, CCO, PBDE-47, PBDE-100, CS and Hg. Muscle contaminants that poorly correlated with the factors were Cr, Ni, Cu and Ag, while Kn, proteins and PK were the dependent variables poorly correlated with the factors.

4 Discussion

4.1 Weight, Age, Lipids and Kn

As exposed in the Results section, weight, age and lipids were associated with various contaminants in the four CCAs. With the exception of a moderate correlation between age and muscle lipids in the muscle CCA due to the variability in the age of same-sized eels, *A. rostrata* weight, age and muscle lipids showed strong positive correlations among themselves (Tables S4 and S5), while for *A. anguilla* there was no significant correlation between age and lipids, and the relationship between weight and lipids was significant but moderate (Tables S6 and S7). These contrasting results seem to be principally linked to the strong correlations of the three variables with specific muscle contaminants: in *A. rostrata*, muscle concentrations of sum OCP, PBDE-47, PBDE-100, and sum PCB were positively correlated with weight and lipids, while in *A. anguilla* the same muscle contaminants (and also Hg) only correlated positively with age (Tables S5 and S7). Since organic contaminants such as PCBs and PBDEs are lipophilic and preferentially accumulated in fat tissues (Tapie et al. 2011), the positive correlations observed in *A. rostrata* between muscle lipid content and concentrations of organic contaminants was expected. However, except for PBDEs, concentrations of organic contaminants reached much higher concentrations in European than in American eels (Table S3). Together with the absence of correlation between age and lipids, this suggests an impairment of muscle lipid storage in *A. anguilla* induced by these compounds, since lipid reserves should normally increase with age (COSEWIC 2012; Larsson et al. 1990). Our hypothesis is supported by studies showing the negative relationships between the lipid metabolism and exposure to OCPs, PCB and

other organic contaminants (mainly herbicides and pesticides) listed in Geeraerts and Belpaire (2010); inhibition of acetyl cholinesterase activity by diazinon insecticide (Ceron et al. 1996) or thiobencarb (Fernandez-Vega et al. 1999), increased fat consumption in presence of fenitrothion insecticide (Sancho et al. 1998), and lipid metabolism affected through alterations in cell membrane properties of fish by perfluorinated compounds (PFCs) (Hu et al. 2003). As well, recent studies about the gene transcription profiles of eels did show a link between high concentrations of organic contaminants and genes related to lipolysis and cell growth (Baillon et al. 2015), and with a dysfunction of detoxification genes, correlated with low energy reserves and condition (Maes et al. 2013). Nevertheless, the influence of confounding factors (other contaminants, water temperature, growth rate, etc) cannot be totally ruled out in this and other field studies.

Another interesting point is that both American and European eels coming from the contaminated sites were generally older than individuals collected in the clean sites (Table S1). Also, muscle organic contamination levels in *A. anguilla* were clearly higher than in *A. rostrata* (Table S3). As an example, the highest sum PCB concentration for a 10 years old American eel coming from the contaminated site LSF was 200 ng·g dry weight⁻¹, two-fold lower than concentrations measured in a 10 years old European eel from Dordogne (416 ng·g d.w.⁻¹), and five-fold lower than in another 10 years old European eel from Gironde (1111 ng·g d.w.⁻¹). Taken together, these results support our hypothesis of muscle lipid impairment in those older European eels in which the highest concentrations of organic contaminants were measured. This impairment could impact negatively their reproductive success, since sufficient lipid storage is essential to

support the reproductive migration and gonad maturation of eels (Boëtius and Boëtius 1980; 1985).

Given the influence of contaminants on lipid accumulation observed in our study, the absence of strong correlations between contaminants and the relative condition factor Kn is somewhat surprising. Nevertheless, in *A. rostrata*, muscle Cd concentration, which increased with age, was negatively correlated with the Kn (Table S5), supporting that in older American eels this contaminant may interfere with energy accumulation, as reported for the yellow stage of European eels (Pierron et al. 2007a). For *A. anguilla*, given our observations discussed above of correlations between total weight, muscle lipids and contaminants, we expected lower Kn for the most contaminated fish. We did observe significant negative correlations between Kn and muscle Hg and organic contaminants (Table S7), however these were rather weak. Since only Certes can be really considered as a clean site, it is likely that the absence of strong correlations between condition and contamination in European eels is due to an underrepresentation of non-contaminated European eels of various ages and sizes in our dataset. As a result, "normal" Kn values for non-contaminated older and larger fish are probably underestimated.

In contrast to our study, Durif et al. (2006) reported a positive correlation between age and condition factor of European silver eels, although the authors mention that contamination levels (that they did not measure) could explain a part of the high individual variability that was observed (e.g. liver weight especially). A complementary explanation could be that contamination, combined to natural factors, can delay eels silvering (i.e. the transformation of yellow eels into silver eels for reproduction) when

lipid storage is not sufficient. Eels need to accumulate more than 20% of their body mass in lipids, in order to have sufficient energy reserves for their reproductive migration (Boëtius and Boëtius 1980). If they don't store more than 28% of lipids, silvering would possibly not even be initiated (Belpaire et al. 2009; Larsson et al. 1990), or, in other cases, migration would simply lead to spawning failure due to premature lipid content depletion (Svedäng and Wickström 1997). In cleaner sites, as eels are able to store lipids more efficiently, silvering can occur while they are younger and smaller, reinforcing the underrepresentation of the less contaminated older and larger yellow eels in our dataset.

4.2 Proteins and enzyme activities

In our study, the strong correlation of protein concentrations with F1 in *A. anguilla* liver CCA (Table S6) indicates an impact of contaminants on this parameter, and can be partly explained by its moderate positive correlations with Zn, Pb and Cu. Past studies on wild yellow perch (*Perca flavescens*) reported that metal contamination could be linked to fluctuations in liver protein concentrations (Audet and Couture 2003; Couture and Kumar 2003; Pierron et al. 2009). Moreover, the presence of PK and LDH in the composition of F1 highlights the positive correlations of the activity of these enzymes with liver proteins, Zn, Pb and Cu. These results could point towards a global stimulating effect of Zn, Pb and Cu on liver glycolytic and anaerobic capacities of *A. anguilla*. In support of this hypothesis, in wild yellow perch inhabiting Zn, Cd and Cu contaminated lakes, Levesque et al. (2002) observed an increase in liver PK activity and a decrease in liver glycogen concentration for fish sampled in the fall compared to

summer-caught fish, a pattern that was not observed in reference lakes. In a study on the early ontogenic stages of the American catfish (*Clarias gariepinus*), Osman et al. (2007) also observed significant variations in PK and LDH activities following exposure to various lead nitrate concentrations.

Unlike glycolytic and anaerobic capacities, aerobic capacities of eel tissues appear to be negatively affected by contamination, at least in the liver of *A. anguilla*. The correlation of liver CS with the F1 in European eel seems mostly linked to the negative correlation of this enzyme with Pb, a metal that, as discussed above, positively influenced anaerobic capacities (Table S4). In muscle however, our data does not provide clear evidence that contaminants could affect the aerobic capacities of *A. anguilla* since neither CS nor CCO activities were influenced by contaminants. Nevertheless, recent studies on the gene transcription profiles of European eels revealed that in parallel with increased transcription level of detoxification genes, elevated concentrations of various contaminants were linked to a decreased transcription level of genes involved in energy metabolism, including CCO, ultimately suggesting decreased aerobic capacities and energy production (Pujolar et al. 2012; Pujolar et al. 2013).

In contrast to our observations for European eel, in *A. rostrata*, our data do not support a contaminant-induced impairment of liver metabolic capacities. In muscle, the strong negative correlations between CS and CCO activities and concentrations of OCPs, PBDE-47 and PCBs (Table S5) appear at first sight as evidence of metabolic impairment. However, as discussed above, in *A. rostrata*, there were also strong positive correlations between these contaminants in muscle and fish weight, age and

muscle lipid concentrations. There is a well-established negative allometric scaling of muscle aerobic capacities in fish. For example, Pelletier et al. (1993) demonstrated that in Atlantic cod (*Gadus morhua*), CS and CCO activities are strongly and negatively correlated to the weight of the individuals. In *A. rostrata*, we also observed in this study a strong negative scaling of these enzymes with weight (Table S5), our dataset providing a wide range of fish weight for this species, in contrast to *A. anguilla*. Therefore, the covariance of CS and CCO activities in the muscle of American eels with the organic contaminants is likely simply due to their mutual relationships with fish size, especially since our data provide no other evidence of contaminant-induced disturbances of aerobic capacities in *A. rostrata*. This observation is clearly a consequence of the overrepresentation of the larger fish in the contaminated sites, particularly in 2011 (Table S1). Again, a better control of fish size coming from clean and contaminated sites would be necessary in future studies to facilitate examination of contaminant effects on eel metabolic capacities.

The activity of G6PDH is an indicator of fatty acid biosynthesis. Even though eels store lipids in their muscle, G6PDH activity was too low to be measurable in our eel muscle samples, in contrast to liver. The significant differences observed in our study for liver G6PDH activity among the capture sites of both eel species (Tables S8 and S9) were not clearly related to contamination. As with all indicators of condition and physiological capacities, natural factors (meteorological events preceding the eel sampling, water temperature, food availability, etc.) exert a significant influence on enzyme activities that would to some extent blur the effects of contaminants. Indeed, for *A. anguilla*, our study did not reveal any relationship between liver G6PDH activity and

tissue contamination levels (Table S6). Nevertheless, for *A. rostrata*, the negative correlations of liver G6PDH with Ag, Pb and As (Table S4) support an effect of metal contamination on lipid metabolism for this species. With the same liver samples used in the present study, Rosabal et al. (2015) did show that higher levels of these three contaminants do correlate with higher concentrations as well in "sensitive" subcellular fractions, including the heat-stable proteins, and could suggest parallel impact on cytosolic enzymes including the G6PDH. Overall though, the contaminants that we measured in liver do not appear to have a strong influence on liver G6PDH activity, given the weak correlations, if any, observed with the factors of the two liver CCAs (Table 1). However, this conclusion deserves caution, since we could not measure Hg and organic contamination in liver. Furthermore, in *A. anguilla*, the significant correlation between liver G6PDH activity and Kn, and the near significant relationship with lipids at the stringent 0.01 significance threshold chosen ($p = 0.013$), agree with our hypothesis of an impairment in muscle lipid storage in contaminated European eels. The study of Maes et al. (2013) on transcription profiles of eels supports this hypothesis: a negative relationship between muscular levels of various inorganic and organic contaminants and the health condition (condition factor and lipid reserves) was observed.

Overall, given that larger eels were found in the most contaminated sites for both species, it is likely that accumulation of contaminants does not lead to severe toxicity on eel metabolic capacities. This conclusion is supported by the absence of evidence in our study of strong relationships between the activity of enzyme indicators of aerobic, anaerobic and glycolytic capacities and tissue concentrations of contaminants. However, our study suggests that accumulation of metals such as Zn, Pb and Cu in the

liver of European eels may stimulate glycolytic capacities, perhaps as a metabolic adjustment compensating for the inhibition of aerobic capacities. Nevertheless, effects of contaminants are more obvious on metabolic processes leading to lipid accumulation, as supported by our data on tissue lipid concentrations and liver G6PDH activity. Overall, our data suggest that if contaminants play a role in the decline of Atlantic eels, *A. anguilla* is more at risk than *A. rostrata* and that a disruption of the normal pattern of lipid accumulation, essential for migration and reproduction, may be involved. Our study identified Cd, Pb, Hg and the complex mixture of organic contaminants found in Atlantic eels as contaminants of potential concern. However, the complex life cycle of eels, involving sedentary and migratory phases, makes it extremely difficult and probably impossible in many cases to eliminate the bias of fish size and age among study sites varying in contamination, since contamination is also narrowly associated with eutrophication and water temperature in the systems studied, leading to faster growth rates and lipid accumulation.

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Table 1: Eigenvalues¹ and explained variance (%) for the significant factors of the four different CCA, followed by the correlations observed between the contaminants and the factors, and between the dependent variables and the factors. Sample size for each CCA is indicated in brackets with respective eel tissue.

	<i>Anguilla rostrata</i>						<i>Anguilla anguilla</i>					
	Liver (82)			Muscle (52)			Liver (78)		Muscle (68)			
	F1	F2	F3	F1	F2	F3	F1	F2	F1	F2	F3	F4
Eigenvalues	1.827	1.691	1.117	3.170	2.329	1.526	2.073	1.350	2.890	2.121	1.575	1.078
Explained variance (%)	32.128	23.043	18.170	26.516	25.350	18.126	34.898	21.304	26.620	18.879	17.203	12.995
<i>Contaminants vs factors</i>												
Cr	-0.136	0.055	-0.589	0.107	-0.021	-0.345	0.184	0.0002	-0.137	-0.120	-0.514	0.157
Ni	0.473	-0.167	-0.252	0.160	-0.174	0.267	-0.239	-0.490	-0.327	0.189	-0.528	0.280
Cu	-0.779	0.306	-0.180	-0.047	0.103	-0.559	-0.420	-0.231	-0.269	0.130	0.013	0.458
Zn	-0.502	0.056	0.018	-0.253	-0.296	0.127	-0.550	-0.356	-0.410	-0.269	0.060	0.181
As	0.294	-0.437	-0.047	-0.704	-0.057	0.011	-0.302	-0.433	0.482	-0.191	-0.487	0.008
Se	-0.854	0.278	0.159	-0.079	-0.145	-0.005	-0.079	0.422	-0.439	0.222	-0.064	0.286
Ag	0.290	0.414	-0.593	-0.401	-0.050	0.387	0.184	0.267	-0.024	-0.196	-0.298	-0.160
Cd	0.229	0.727	0.463	-0.177	0.116	0.276	-0.088	0.779	-0.475	0.220	0.105	0.098
Pb	0.583	0.301	0.101	-0.078	-0.193	0.406	-0.670	0.603	-0.433	0.010	0.197	-0.162
Hg				0.831	-0.132	-0.132			-0.577	0.520	0.173	-0.381
Sum OCP				0.501	0.651	0.259			-0.261	0.768	0.416	-0.255
PBDE 47				0.529	0.635	0.151			-0.357	0.669	0.305	-0.224
PBDE 100				0.488	0.283	-0.028			-0.470	0.622	0.218	-0.291
Sum PCB				0.527	0.545	-0.013			-0.200	0.711	0.221	-0.432
<i>Dependent variables vs factors</i>												
Weight	-0.963	0.162	0.044	0.721	0.562	0.269	0.161	-0.205	0.151	0.410	0.084	-0.362
Age	-0.394	0.749	0.244	0.302	0.111	0.612	0.177	0.474	-0.448	0.706	0.172	-0.357
Lipids	-0.837	0.275	-0.330	0.466	0.781	0.264	-0.163	0.008	0.714	0.391	0.366	-0.141
Kn	-0.310	-0.664	-0.109	-0.396	0.623	-0.159	0.010	-0.133	0.233	-0.226	-0.085	0.060
Proteins	0.138	-0.042	-0.247	-0.258	-0.246	0.464	-0.785	-0.081	-0.071	-0.041	-0.048	0.838
PK	-0.304	-0.106	0.287	0.454	-0.458	-0.021	-0.657	0.284	0.010	0.143	0.543	0.553
CS	0.137	-0.097	-0.081	-0.275	-0.602	-0.367	0.585	-0.166	-0.067	-0.542	0.039	-0.015
CCO				-0.441	-0.707	-0.201	0.100	-0.188	-0.307	-0.673	0.515	0.106
G6PDH	-0.366	-0.250	0.323									
LDH	-0.246	0.160	-0.127	0.150	-0.004	0.224	-0.786	-0.262	0.503	-0.118	0.241	0.102

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¹ Eigenvalues were calculated using the mathematical formula $v^{-1} \cdot Av = \text{eigenvalue}$, where Av is the variable (dependent and independent) matrix and v is the canonical factor. Factors with eigenvalues equal or higher than 1 were retained following Kaiser criterion.

Figure legends

Figure 1: Maps of the sampling sites in Québec and in France. The white frames surround the "clean" sites, the grey frames surround the "intermediate" site, and the black frames surround the "contaminated" sites.

Figure 2: Canonical correlation analysis ($n = 82$) showing the relationships existing between different biometric measurements and contaminant concentrations in *A. rostrata* liver. Analysis has been conducted on $\log_{10}$ -transformed data. PK = pyruvate kinase; G6PDH = glucose-6-phosphate dehydrogenase; CS = citrate synthase; LDH = lactate dehydrogenase.

Figure 3: Canonical correlation analysis ($n = 52$) showing the relationships existing between different biometric measurements and contaminant concentration in *A. rostrata* muscle. Analysis has been conducted on $\log_{10}$ -transformed data. PK = pyruvate kinase; CS = citrate synthase; CCO = cytochrome C oxidase; LDH = lactate dehydrogenase.

Figure 4: Canonical correlation analysis ($n = 78$) showing the relationships existing between different biometric measurements and contaminant concentrations in *A. anguilla* liver. Analysis has been conducted on $\log_{10}$ -transformed data. PK = pyruvate kinase; G6PDH = glucose-6-phosphate dehydrogenase; CS = citrate synthase; LDH = lactate dehydrogenase.

Figure 5: Canonical correlation analysis ($n = 68$) showing the relationships existing between different biometric measurements and contaminant concentrations in *A. anguilla* muscle. Analysis has been conducted on $\log_{10}$ -transformed data. PK = pyruvate kinase; CS = citrate synthase; CCO = cytochrome C oxidase; LDH = lactate dehydrogenase.

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Figure 1

Figure 2

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Figure 4

Figure 5

1 **Table S1:** Biometric measurements (mean ± sd, *n* = 15 unless indicated differently in brackets) of American and European eels, for each capture site and sampling year.

Parameter	Year	American eel (<i>Anguilla rostrata</i>)				European eel (<i>Anguilla anguilla</i>)			
		RSJ	RSO	LSP	LSF	Certes	Dordogne	Gironde	Garonne
Size (mm)	2011	530 ± 87 ^B	348 ± 72 ^C	<u>891 ± 41</u> ^A	828 ± 55 ^A	434 ± 74 ^B	<u>338 ± 50</u> ^C	<u>579 ± 58</u> ^A	<u>443 ± 69</u> ^B
	2012	501 ± 109 ^C	386 ± 96 ^D	686 ± 96 ^B	815 ± 80 ^A	455 ± 71 ^{AB}	397 ± 57 ^B	443 ± 51 ^{AB}	522 ± 133 ^A
Weight (g)	2011	290 ± 146 ^C	78 ± 72 ^D	<u>1435 ± 209</u> ^A	<u>1222 ± 125</u> ^B	157 ± 121 ^B	63 ± 41 ^B (14)	<u>350 ± 124</u> ^A	<u>156 ± 87</u> ^B
	2012	278 ± 197 ^C	93 ± 71 ^C	623 ± 243 ^B	1013 ± 221 ^A	180 ± 95 ^{AB}	91 ± 42 ^B	145 ± 45 ^B	281 ± 201 ^A
Age (years)	2011	9.3 ± 2.6 ^B	7.7 ± 2.0 ^B	<u>22.4 ± 10.2</u> ^B	10.8 ± 2.0 ^A	4.1 ± 1.6 ^C (14)	<u>4.3 ± 0.8</u> ^{BC}	<u>9.3 ± 2.6</u> ^A	<u>6.0 ± 2.2</u> ^B
	2012	9.9 ± 3.0 ^{AB}	8.9 ± 2.5 ^B	10.0 ± 5.9 ^{AB}	14.6 ± 7.1 ^A	4.1 ± 1.0 ^B	6.5 ± 3.6 ^B (14)	6.9 ± 3.3 ^B	11.0 ± 3.5 ^A (14)
Muscle lipids (% w.w.)	2011	18.6 ± 10.0 (5)	5.5 (1)	28.7 ± 5.1 (5)	24.9 ± 7.1 (4)	30.6 ± 11.7 ^B (7)	24.6 ± 16.3 ^B (9)	53.1 ± 17.1 ^A (6)	<u>44.5 ± 12.2</u> ^{AB} (7)
	2012	20.2 ± 9.4 ^B (10)	6.6 ± 4.0 ^C (8)	25.1 ± 12.8 ^{AB} (10)	34.5 ± 8.6 ^{AB} (9)	40.2 ± 10.9 (10)	32.5 ± 15.0 (9)	40.1 ± 19.2 (10)	23.7 ± 14.0 (10)
Fulton	2011	0.18 ± 0.03 ^{BC}	0.15 ± 0.03 ^C	0.20 ± 0.03 ^{AB}	<u>0.22 ± 0.03</u> ^A	0.17 ± 0.02 ^A	0.15 ± 0.02 ^B	0.17 ± 0.02 ^A	0.16 ± 0.02 ^{AB}
	2012	0.18 ± 0.04 ^A	0.14 ± 0.02 ^B	0.19 ± 0.02 ^A	0.19 ± 0.02 ^A	0.18 ± 0.02 ^A	0.14 ± 0.02 ^B	0.17 ± 0.02 ^A	0.17 ± 0.02 ^A
Kn	2011	1.03 ± 0.12	0.99 ± 0.16	1.01 ± 0.15	<u>1.11 ± 0.18</u>	1.07 ± 0.13	<u>0.98 ± 0.13</u>	1.02 ± 0.08	1.02 ± 0.10
	2012	1.06 ± 0.17 ^A	0.92 ± 0.14 ^B	0.99 ± 0.11 ^{AB}	0.95 ± 0.12 ^{AB}	1.07 ± 0.12 ^A	0.90 ± 0.11 ^B	1.02 ± 0.15 ^{AB}	1.03 ± 0.14 ^A

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3 **Note:** Different letters indicate significant differences within a row (*p* < 0.05, Tukey-Kramer HSD test). An underlined value indicates a significant difference between the two sampling

4 years (*p* < 0.05 Tukey-Kramer HSD test). RSJ = Saint-Jean River; RSO = Sud-Ouest River; LSP = Lake Saint-Pierre; LSF = Lake Saint-François. Kn was calculated following the

5 methods of Blackwell et al. (2000) and LeCren (1951).

Table S2: Inorganic contamination concentrations ($\mu\text{g}\cdot\text{g dw}^{-1}$, mean $\pm$ sd, $n = 2\text{--}13$) in the liver of American and European eels, for each capture site and sampling year.

Contaminant	Year	American eel (<i>Anguilla rostrata</i>)				European eel (<i>Anguilla anguilla</i>)			
		RSJ	RSO	LSP	LSF	Certes	Dordogne	Gironde	Garonne
Cr	2011	1.65 $\pm$ 0.81	0.61 $\pm$ 0.11	<u>0.69 $\pm$ 0.37</u>	1.13 $\pm$ 1.10	2.94 $\pm$ 6.58	1.44 $\pm$ 1.36	0.76 $\pm$ 0.76	0.99 $\pm$ 0.76
	2012	3.27 $\pm$ 7.44	0.65 $\pm$ 0.17	1.12 $\pm$ 0.36	2.33 $\pm$ 2.04	2.42 $\pm$ 2.18 ^{AB}	2.82 $\pm$ 1.28 ^A	0.81 $\pm$ 0.08 ^B	1.06 $\pm$ 0.81 ^{AB}
Ni	2011	0.41 $\pm$ 0.36 ^A	0.16 $\pm$ 0.06 ^{AB}	<u>0.09 $\pm$ 0.05</u> ^B	0.19 $\pm$ 0.17 ^{AB}	0.92 $\pm$ 1.09 ^A	0.22 $\pm$ 0.33 ^{AB}	0.22 $\pm$ 0.28 ^{AB}	0.14 $\pm$ 0.07 ^B
	2012	0.51 $\pm$ 0.58 ^A	0.19 $\pm$ 0.05 ^B	0.19 $\pm$ 0.12 ^B	0.11 $\pm$ 0.05 ^B	0.26 $\pm$ 0.14	0.41 $\pm$ 0.47	0.11 $\pm$ 0.03	0.25 $\pm$ 0.23
Cu	2011	69.5 $\pm$ 39.3	33.6 $\pm$ 11.7	<u>239.8 $\pm$ 242.9</u>	<u>131.0 $\pm$ 43.9</u>	66.0 $\pm$ 17.0 ^B	<u>42.9 $\pm$ 9.7</u> ^B	<u>122.1 $\pm$ 32.9</u> ^A	51.0 $\pm$ 27.3 ^B
	2012	59.8 $\pm$ 30.9 ^B	30.1 $\pm$ 10.6 ^B	93.9 $\pm$ 34.5 ^A	95.1 $\pm$ 37.9 ^A	59.6 $\pm$ 29.8	65.0 $\pm$ 30.1	66.9 $\pm$ 31.5	34.7 $\pm$ 15.0
Zn	2011	260.0 $\pm$ 212.6	121.7 $\pm$ 27.7	288.0 $\pm$ 161.0	<u>271.3 $\pm$ 93.1</u>	<u>208.9 $\pm$ 47.3</u> ^{AB}	142.3 $\pm$ 24.2 ^C	<u>237.4 $\pm$ 68.2</u> ^A	<u>162.0 $\pm$ 47.5</u> ^{BC}
	2012	154.8 $\pm$ 37.4 ^{AB}	107.9 $\pm$ 22.7 ^B	191.7 $\pm$ 74.9 ^A	156.1 $\pm$ 56.1 ^{AB}	159.9 $\pm$ 35.0 ^{AB}	159.0 $\pm$ 45.0 ^{AB}	169.7 $\pm$ 38.6 ^A	118.3 $\pm$ 36.5 ^B
As	2011	4.00 $\pm$ 0.94 ^A	2.22 $\pm$ 0.95 ^{AB}	2.68 $\pm$ 2.39 ^{AB}	<u>1.78 $\pm$ 0.48</u> ^B	5.96 $\pm$ 1.54 ^A	2.24 $\pm$ 0.78 ^B	<u>7.79 $\pm$ 3.29</u> ^A	1.88 $\pm$ 1.71 ^B
	2012	3.23 $\pm$ 0.84 ^A	2.61 $\pm$ 1.55 ^{AB}	1.84 $\pm$ 1.44 ^{BC}	0.77 $\pm$ 0.68 ^C	7.88 $\pm$ 3.91 ^A	2.05 $\pm$ 1.34 ^B	2.69 $\pm$ 1.09 ^B	1.80 $\pm$ 3.65 ^B
Se	2011	14.8 $\pm$ 3.8 ^B	12.1 $\pm$ 1.9 ^B	<u>35.0 $\pm$ 12.4</u> ^A	<u>36.6 $\pm$ 8.5</u> ^A	15.3 $\pm$ 8.2 ^C	<u>32.7 $\pm$ 9.6</u> ^{AB}	<u>45.5 $\pm$ 13.7</u> ^A	20.7 $\pm$ 11.1 ^{BC}
	2012	11.8 $\pm$ 5.0 ^B	13.3 $\pm$ 5.7 ^B	22.2 $\pm$ 9.4 ^A	27.1 $\pm$ 5.7 ^A	15.3 $\pm$ 4.5 ^B	16.4 $\pm$ 5.1 ^{AB}	25.3 $\pm$ 7.8 ^A	22.5 $\pm$ 9.9 ^{AB}
Ag	2011	<u>0.69 $\pm$ 0.43</u> ^{AB}	0.35 $\pm$ 0.10 ^{AB}	1.36 $\pm$ 1.23 ^A	<u>0.22 $\pm$ 0.12</u> ^B	<u>0.16 $\pm$ 0.07</u> ^B	<u>0.15 $\pm$ 0.06</u> ^B	1.46 $\pm$ 0.74 ^A	0.55 $\pm$ 0.69 ^B
	2012	1.21 $\pm$ 0.56 ^A	0.69 $\pm$ 0.30 ^{AB}	0.96 $\pm$ 0.78 ^{AB}	0.48 $\pm$ 0.19 ^B	0.60 $\pm$ 0.16 ^B	0.90 $\pm$ 0.37 ^{AB}	1.35 $\pm$ 1.02 ^A	0.90 $\pm$ 0.07 ^B
Cd	2011	<u>0.44 $\pm$ 0.30</u> ^{AB}	0.63 $\pm$ 0.19 ^{AB}	<u>2.72 $\pm$ 2.98</u> ^A	0.38 $\pm$ 0.20 ^B	0.06 $\pm$ 0.04 ^C	<u>4.34 $\pm$ 1.63</u> ^B	<u>10.83 $\pm$ 6.14</u> ^A	2.40 $\pm$ 3.60 ^{BC}
	2012	0.23 $\pm$ 0.10 ^B	1.44 $\pm$ 0.85 ^A	0.84 $\pm$ 0.98 ^{AB}	0.45 $\pm$ 0.36 ^B	0.70 $\pm$ 2.14 ^B	2.31 $\pm$ 0.78 ^{AB}	3.32 $\pm$ 1.53 ^A	2.33 $\pm$ 1.19 ^{AB}
Pb	2011	0.34 $\pm$ 0.23	0.18 $\pm$ 0.02	<u>0.44 $\pm$ 0.48</u>	0.06 $\pm$ 0.03	<u>0.33 $\pm$ 0.23</u> ^C	0.52 $\pm$ 0.22 ^{BC}	<u>1.59 $\pm$ 0.62</u> ^A	<u>0.81 $\pm$ 0.43</u> ^B
	2012	0.26 $\pm$ 0.09	1.79 $\pm$ 5.58	0.14 $\pm$ 0.11	0.10 $\pm$ 0.12	0.12 $\pm$ 0.04 ^B	0.47 $\pm$ 0.26 ^{AB}	0.73 $\pm$ 0.50 ^A	0.11 $\pm$ 0.05 ^B

Note: Different letters indicate significant differences within a row ($p < 0.05$, Tukey-Kramer HSD test). An underlined value indicates a significant difference between the two sampling years ($p < 0.05$ Tukey-Kramer HSD test). RSJ = Saint-Jean River; RSO = Sud-Ouest River; LSP = Lake Saint-Pierre; LSF = Lake Saint-François.

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Table S3: Organic (ng·g dw⁻¹) and inorganic (µg·g dw⁻¹) contamination concentrations (mean ± sd, n = 1-10) in the muscle of American and European eels, for each capture site and sampling year.

Contaminant	Year	American eel (<i>Anguilla rostrata</i>)				European eel (<i>Anguilla anguilla</i>)			
		RSJ	RSO	LSP	LSF	Certes	Dordogne	Gironde	Garonne
Cr	2011	0.44 ± 0.08	<u>1.667</u>	0.47 ± 0.17	1.07 ± 1.40	0.95 ± 1.38	3.83 ± 6.77	0.36 ± 0.03	0.39 ± 0.08
	2012	0.83 ± 0.69	0.59 ± 0.35	0.68 ± 0.69	0.43 ± 0.18	0.59 ± 0.48	0.38 ± 0.02	1.01 ± 1.25	1.09 ± 1.75
Ni	2011	0.37 ± 0.46	0.14	0.23 ± 0.15	0.04 ± 0.04	0.68 ± 0.65	0.64 ± 0.97	0.71 ± 1.17	0.16 ± 0.11
	2012	0.14 ± 0.11	0.28 ± 0.40	0.15 ± 0.15	0.94 ± 1.71	0.32 ± 0.24	0.16 ± 0.06	0.17 ± 0.13	0.44 ± 0.56
Cu	2011	<u>1.35 ± 0.39</u>	1.23	0.73 ± 0.19	0.66 ± 0.22	0.63 ± 0.23	1.09 ± 0.54	1.00 ± 0.81	0.85 ± 0.15
	2012	0.84 ± 0.36	0.87 ± 0.42	1.08 ± 0.60	1.01 ± 0.69	0.89 ± 0.71	1.00 ± 0.48	0.77 ± 0.67	3.39 ± 7.03
Zn	2011	134.2 ± 100.8	90.7	<u>97.2 ± 27.9</u>	69.6 ± 15.3	66.9 ± 24.2 ^{AB}	91.9 ± 45.9 ^{AB}	45.8 ± 21.4 ^B	<u>49.2 ± 18.7</u> ^{AB}
	2012	86.9 ± 19.5 ^{AB}	123.3 ± 74.6 ^A	69.2 ± 14.8 ^B	86.9 ± 35.9 ^{AB}	80.3 ± 88.5	74.0 ± 40.4	55.3 ± 17.9	88.7 ± 29.7
As	2011	3.37 ± 1.22	1.38	0.75 ± 1.01	0.27 ± 0.03	8.65 ± 5.45 ^A	1.17 ± 0.79 ^B	4.38 ± 2.21 ^{AB}	1.17 ± 0.78 ^B
	2012	3.09 ± 1.00 ^A	1.59 ± 0.59 ^B	0.37 ± 0.14 ^C	0.84 ± 1.01 ^{BC}	16.0 ± 10.2 ^A	1.24 ± 0.75 ^B	2.54 ± 1.35 ^B	2.14 ± 4.50 ^B
Se	2011	1.95 ± 1.37	4.18	1.96 ± 0.71	3.40 ± 1.03	2.37 ± 1.57 ^B	6.23 ± 2.30 ^A	3.97 ± 1.79 ^{AB}	2.96 ± 2.28 ^B
	2012	3.67 ± 0.56	3.50 ± 2.49	3.56 ± 2.13	3.45 ± 1.57	2.39 ± 1.63	4.35 ± 1.89	4.14 ± 1.82	4.27 ± 2.57
Ag	2011	0.16 ± 0.26	0.05	0.10 ± 0.09	0.01 ± 0.01	0.15 ± 0.27	0.12 ± 0.21	0.04 ± 0.02	0.05 ± 0.06
	2012	0.12 ± 0.12	0.32 ± 0.55	0.05 ± 0.05	0.10 ± 0.16	0.43 ± 0.59	0.04 ± 0.04	0.20 ± 0.33	0.18 ± 0.32
Cd	2011	0.011 ± 0.009	0.004	0.016 ± 0.010	0.004 ± 0.003	0.061 ± 0.002 ^B	<u>0.033 ± 0.021</u> ^A	0.022 ± 0.020 ^{AB}	0.012 ± 0.004 ^B
	2012	0.055 ± 0.105	0.015 ± 0.013	0.011 ± 0.013	0.013 ± 0.010	0.012 ± 0.009	0.018 ± 0.006	0.019 ± 0.010	1.04 ± 2.02
Pb	2011	0.035 ± 0.040	0.007	0.033 ± 0.037	0.006 ± 0.004	0.024 ± 0.013	0.066 ± 0.049	0.051 ± 0.049	0.054 ± 0.040
	2012	0.045 ± 0.100	0.115 ± 0.274	0.014 ± 0.012	0.021 ± 0.020	0.032 ± 0.024	0.061 ± 0.075	0.019 ± 0.007	0.063 ± 0.057
Hg	2011	0.27 ± 0.09	0.57	0.73 ± 0.34	0.96 ± 0.13	<u>0.17 ± 0.03</u> ^C	0.38 ± 0.06 ^A	0.46 ± 0.11 ^A	<u>0.28 ± 0.06</u> ^B
	2012	0.25 ± 0.08 ^A	0.57 ± 17 ^B	0.61 ± 0.19 ^B	1.10 ± 0.41 ^C	0.11 ± 0.02 ^B	0.44 ± 0.15 ^A	0.38 ± 0.12 ^A	0.45 ± 0.10 ^A
Sum OCP	2011	22.9 ± 21.3	20.3	68.6 ± 49.0	33.5 ± 8.6	128.8 ± 82.0 ^B	58.1 ± 28.3 ^B	<u>209.0 ± 48.3</u> ^A	159.3 ± 82.3 ^A
	2012	14.3 ± 4.6 ^B	7.28 ± 8.13 ^B	34.2 ± 15.0 ^A	34.3 ± 9.5 ^A	5.98 ± 1.63 ^B	98.9 ± 53.8 ^A	102.8 ± 68.1 ^A	132.3 ± 69.9 ^A
Sum PCB	2011	19.0 ± 20.6	43.2	75.0 ± 43.2	89.1 ± 29.9	44.1 ± 21.8 ^B	<u>292.1 ± 126.3</u> ^B	<u>1761 ± 338</u> ^A	1337 ± 1846 ^{AB}
	2012	24.8 ± 7.8 ^C	15.7 ± 5.0 ^C	82.4 ± 33.0 ^B	118.4 ± 45.0 ^A	44.6 ± 16.2 ^B	815.2 ± 519.1 ^A	840.0 ± 478.0 ^A	549.1 ± 270.7 ^A

<i>PBDE-47</i>	2011	1.63 ± 1.20	1.17	21.2 ± 25.1	7.26 ± 3.88	0.10 ± 1.5e-17 ^B	3.62 ± 2.01 ^{AB}	6.82 ± 2.29 ^A	8.21 ± 7.98 ^A
	2012	2.64 ± 1.40 ^B	0.84 ± 0.62 ^B	32.9 ± 21.1 ^A	11.6 ± 3.8 ^B	0.12 ± 0.07 ^B	4.27 ± 2.66 ^{AB}	5.19 ± 3.06 ^A	7.29 ± 6.00 ^A
<i>PBDE-100</i>	2011	0.33 ± 0.19	1.37	4.09 ± 4.63	2.10 ± 1.28	0.07 ± 0.00 ^B	1.65 ± 0.91 ^{AB}	2.99 ± 0.82 ^A	3.35 ± 3.16 ^A
	2012	0.67 ± 0.32 ^B	1.57 ± 1.97 ^B	9.29 ± 5.72 ^A	3.83 ± 1.60 ^B	0.08 ± 1.46e-17 ^C	2.69 ± 1.48 ^B	3.70 ± 2.42 ^{AB}	5.31 ± 2.96 ^A

Note: Different letters indicate significant differences within a row ($p < 0.05$, Tukey-Kramer HSD test). An underlined value indicates a significant difference between the two sampling years ($p < 0.05$ Tukey-Kramer HSD test). RSJ = Saint-Jean River; RSO = Sud-Ouest River; LSP = Lake Saint-Pierre; LSF = Lake Saint-François.

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15 **Table S4:** Pearson correlation matrix ($n = 82$) of the variables used in the CCA for the liver of *Anguilla rostrata*.

Variables	Weight	Age	Lipids	Kn	Proteins	PK	CS	G6PDH	LDH	Cr	Ni	Cu	Zn	As	Se	Ag	Cd	Pb
Weight	1	0,542	0,845	0,204	-0,283	0,270	-0,194	0,165	0,154	0,093	-0,421	0,736	0,492	-0,255	0,783	-0,181	-0,077	-0,432
Age	0,542	1	0,563	-0,143	-0,314	0,152	-0,130	-0,107	0,020	-0,059	-0,234	0,464	0,299	-0,265	0,515	0,091	0,432	0,115
Lipids	0,845	0,563	1	0,208	-0,273	0,171	-0,202	0,138	0,158	0,211	-0,343	0,669	0,352	-0,336	0,657	-0,014	-0,143	-0,391
Kn	0,204	-0,143	0,208	1	-0,102	0,046	-0,052	0,090	-0,015	0,026	0,047	0,094	0,154	0,237	0,135	-0,202	-0,431	-0,164
Proteins	-0,283	-0,314	-0,273	-0,102	1	0,076	0,436	0,220	0,531	0,165	0,181	-0,052	0,011	0,101	-0,129	0,122	-0,045	0,072
PK	0,270	0,152	0,171	0,046	0,076	1	0,032	0,161	0,358	-0,097	-0,101	0,273	0,358	0,013	0,199	-0,161	-0,036	-0,131
CS	-0,194	-0,130	-0,202	-0,052	0,436	0,032	1	0,017	0,103	0,009	0,116	-0,030	0,009	0,180	-0,159	0,075	-0,059	0,196
G6PDH	0,165	-0,107	0,138	0,090	0,220	0,161	0,017	1	0,174	-0,051	-0,272	-0,013	-0,121	-0,299	0,227	-0,477	-0,198	-0,404
LDH	0,154	0,020	0,158	-0,015	0,531	0,358	0,103	0,174	1	0,148	-0,141	0,207	0,063	-0,182	0,159	-0,023	-0,039	-0,167
Cr	0,093	-0,059	0,211	0,026	0,165	-0,097	0,009	-0,051	0,148	1	0,333	0,131	0,060	-0,123	0,026	0,064	-0,261	-0,088
Ni	-0,421	-0,234	-0,343	0,047	0,181	-0,101	0,116	-0,272	-0,141	0,333	1	-0,290	0,001	0,285	-0,430	0,114	-0,018	0,346
Cu	0,736	0,464	0,669	0,094	-0,052	0,273	-0,030	-0,013	0,207	0,131	-0,290	1	0,751	-0,035	0,692	0,223	0,044	-0,264
Zn	0,492	0,299	0,352	0,154	0,011	0,358	0,009	-0,121	0,063	0,060	0,001	0,751	1	0,282	0,522	0,211	0,116	-0,081
As	-0,255	-0,265	-0,336	0,237	0,101	0,013	0,180	-0,299	-0,182	-0,123	0,285	-0,035	0,282	1	-0,223	0,376	0,019	0,354
Se	0,783	0,515	0,657	0,135	-0,129	0,199	-0,159	0,227	0,159	0,026	-0,430	0,692	0,522	-0,223	1	-0,108	0,227	-0,340
Ag	-0,181	0,091	-0,014	-0,202	0,122	-0,161	0,075	-0,477	-0,023	0,064	0,114	0,223	0,211	0,376	-0,108	1	0,279	0,340
Cd	-0,077	0,432	-0,143	-0,431	-0,045	-0,036	-0,059	-0,198	-0,039	-0,261	-0,018	0,044	0,116	0,019	0,227	0,279	1	0,423
Pb	-0,432	0,115	-0,391	-0,164	0,072	-0,131	0,196	-0,404	-0,167	-0,088	0,346	-0,264	-0,081	0,354	-0,340	0,340	0,423	1

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17 **Note:** Significant correlation at $p < 0,01$ is indicated in bold. PK = pyruvate kinase; CS = citrate synthase; G6PDH = glucose-6-phosphate

18 dehydrogenase; LDH = lactate dehydrogenase.

19 **Table S5:** Pearson correlation matrix ($n = 52$) of the variables used in the CCA for the muscle of *Anguilla rostrata*.

Variables	Weight	Age	Lipids	Kn	Proteins	PK	CS	CCO	LDH	Cr	Ni	Cu	Zn	As	Se	Ag	Cd	Pb	Hg	OCP	BDE47	BDE100	PCB
Weight	1	0,537	0,870	0,173	-0,196	0,154	-0,674	-0,836	0,227	-0,059	-0,002	-0,056	-0,217	-0,502	-0,063	-0,183	-0,034	-0,069	0,486	0,752	0,713	0,480	0,636
Age	0,537	1	0,491	-0,096	-0,173	0,060	-0,314	-0,437	0,103	-0,114	0,117	-0,113	0,122	-0,170	-0,110	0,045	0,113	0,226	0,103	0,468	0,266	0,146	0,209
Lipids	0,870	0,491	1	0,324	-0,093	-0,024	-0,564	-0,750	0,262	-0,032	0,004	-0,062	-0,270	-0,336	-0,100	-0,137	0,077	-0,057	0,245	0,757	0,716	0,432	0,646
Kn	0,173	-0,096	0,324	1	-0,011	-0,230	-0,284	-0,216	0,037	-0,094	-0,318	0,146	0,016	0,176	0,117	0,155	0,050	-0,167	-0,272	0,224	0,203	0,086	0,207
Proteins	-0,196	-0,173	-0,093	-0,011	1	0,414	0,141	0,314	0,537	-0,214	0,110	-0,354	0,075	0,163	0,251	0,239	0,078	0,185	-0,118	-0,236	-0,150	-0,099	-0,179
PK	0,154	0,060	-0,024	-0,230	0,414	1	0,052	0,093	0,531	-0,069	0,125	-0,091	0,062	-0,288	0,204	-0,169	-0,188	0,060	0,479	0,010	0,048	0,233	0,116
CS	-0,674	-0,314	-0,564	-0,284	0,141	0,052	1	0,834	-0,009	0,180	0,001	0,070	0,122	0,208	0,079	0,008	-0,030	0,045	-0,119	-0,585	-0,551	-0,312	-0,426
CCO	-0,836	-0,437	-0,750	-0,216	0,314	0,093	0,834	1	0,064	0,085	0,032	-0,064	0,155	0,270	0,106	0,143	-0,019	0,067	-0,231	-0,695	-0,631	-0,350	-0,504
LDH	0,227	0,103	0,262	0,037	0,537	0,531	-0,009	0,064	1	-0,142	0,003	-0,242	-0,135	-0,223	0,074	-0,010	-0,106	0,027	0,148	0,103	0,163	0,167	0,195
Cr	-0,059	-0,114	-0,032	-0,094	-0,214	-0,069	0,180	0,085	-0,142	1	-0,068	0,218	-0,176	0,077	-0,132	-0,131	0,019	-0,174	-0,004	-0,075	-0,076	-0,050	-0,039
Ni	-0,002	0,117	0,004	-0,318	0,110	0,125	0,001	0,032	0,003	-0,068	1	0,139	-0,093	0,175	-0,111	0,216	0,336	0,589	0,011	-0,006	0,033	0,031	-0,055
Cu	-0,056	-0,113	-0,062	0,146	-0,354	-0,091	0,070	-0,064	-0,242	0,218	0,139	1	0,282	0,123	-0,144	0,108	-0,073	0,106	-0,102	0,044	0,035	0,066	-0,132
Zn	-0,217	0,122	-0,270	0,016	0,075	0,062	0,122	0,155	-0,135	-0,176	-0,093	0,282	1	0,205	0,086	0,354	0,135	0,246	-0,142	-0,057	-0,288	-0,242	-0,387
As	-0,502	-0,170	-0,336	0,176	0,163	-0,288	0,208	0,270	-0,223	0,077	0,175	0,123	0,205	1	0,008	0,215	0,365	0,165	-0,561	-0,422	-0,606	-0,589	-0,574
Se	-0,063	-0,110	-0,100	0,117	0,251	0,204	0,079	0,106	0,074	-0,132	-0,111	-0,144	0,086	0,008	1	0,031	0,155	0,031	0,006	-0,031	0,046	0,134	0,063
Ag	-0,183	0,045	-0,137	0,155	0,239	-0,169	0,008	0,143	-0,010	-0,131	0,216	0,108	0,354	0,215	0,031	1	0,142	0,158	-0,297	-0,107	-0,113	-0,136	-0,194
Cd	-0,034	0,113	0,077	0,050	0,078	-0,188	-0,030	-0,019	-0,106	0,019	0,336	-0,073	0,135	0,365	0,155	0,142	1	0,359	-0,220	0,046	-0,003	-0,027	-0,067
Pb	-0,069	0,226	-0,057	-0,167	0,185	0,060	0,045	0,067	0,027	-0,174	0,589	0,106	0,246	0,165	0,031	0,158	0,359	1	-0,091	0,039	-0,022	0,018	-0,074
Hg	0,486	0,103	0,245	-0,272	-0,118	0,479	-0,119	-0,231	0,148	-0,004	0,011	-0,102	-0,142	-0,561	0,006	-0,297	-0,220	-0,091	1	0,287	0,371	0,517	0,526
OCP	0,752	0,468	0,757	0,224	-0,236	0,010	-0,585	-0,695	0,103	-0,075	-0,006	0,044	-0,057	-0,422	-0,031	-0,107	0,046	0,039	0,287	1	0,817	0,590	0,786
BDE47	0,713	0,266	0,716	0,203	-0,150	0,048	-0,551	-0,631	0,163	-0,076	0,033	0,035	-0,288	-0,606	0,046	-0,113	-0,003	-0,022	0,371	0,817	1	0,853	0,804
BDE100	0,480	0,146	0,432	0,086	-0,099	0,233	-0,312	-0,350	0,167	-0,050	0,031	0,066	-0,242	-0,589	0,134	-0,136	-0,027	0,018	0,517	0,590	0,853	1	0,772
PCB	0,636	0,209	0,646	0,207	-0,179	0,116	-0,426	-0,504	0,195	-0,039	-0,055	-0,132	-0,387	-0,574	0,063	-0,194	-0,067	-0,074	0,526	0,786	0,804	0,772	1

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21 **Note:** Significant correlation at $p < 0.01$ is indicated in bold. PK = pyruvate kinase; CS = citrate synthase; CCO = cytochrome C oxidase; LDH =

22 lactate dehydrogenase.

23 **Table S6:** Pearson correlation matrix ($n = 78$) of the variables used in the CCA for the liver of *Anguilla anguilla*.

Variables	Weight	Age	Lipids	Kn	Proteins	PK	CS	G6PDH	LDH	Cr	Ni	Cu	Zn	As	Se	Ag	Cd	Pb
Weight	1	0,603	0,410	0,065	-0,055	-0,044	0,064	0,152	-0,069	-0,218	-0,007	0,391	0,305	0,118	0,230	0,418	0,007	-0,026
Age	0,603	1	0,188	-0,046	-0,169	0,121	0,137	-0,019	0,014	-0,138	-0,135	0,127	0,048	-0,192	0,354	0,416	0,396	0,166
Lipids	0,410	0,188	1	0,102	0,165	0,163	-0,058	0,282	0,046	-0,090	-0,078	0,424	0,331	0,300	0,158	0,360	-0,013	0,245
Kn	0,065	-0,046	0,102	1	-0,039	-0,077	0,017	0,314	0,009	-0,097	0,100	0,047	0,076	0,188	0,056	0,021	-0,102	0,046
Proteins	-0,055	-0,169	0,165	-0,039	1	0,595	-0,087	0,054	0,648	-0,149	0,124	0,338	0,465	0,239	0,115	-0,094	0,080	0,435
PK	-0,044	0,121	0,163	-0,077	0,595	1	-0,109	0,047	0,557	-0,126	0,111	0,292	0,248	0,031	0,138	0,052	0,249	0,471
CS	0,064	0,137	-0,058	0,017	-0,087	-0,109	1	0,336	-0,158	0,112	-0,026	-0,173	-0,201	-0,162	-0,046	0,100	-0,033	-0,425
G6PDH	0,152	-0,019	0,282	0,314	0,054	0,047	0,336	1	0,032	-0,046	0,087	0,017	0,051	-0,008	-0,108	0,070	-0,153	-0,120
LDH	-0,069	0,014	0,046	0,009	0,648	0,557	-0,158	0,032	1	-0,113	0,345	0,294	0,434	0,178	-0,006	-0,158	0,007	0,307
Cr	-0,218	-0,138	-0,090	-0,097	-0,149	-0,126	0,112	-0,046	-0,113	1	0,192	-0,164	-0,136	0,151	-0,203	-0,055	-0,231	-0,227
Ni	-0,007	-0,135	-0,078	0,100	0,124	0,111	-0,026	0,087	0,345	0,192	1	0,076	0,199	0,268	-0,188	-0,208	-0,383	-0,136
Cu	0,391	0,127	0,424	0,047	0,338	0,292	-0,173	0,017	0,294	-0,164	0,076	1	0,772	0,519	0,378	0,565	0,056	0,333
Zn	0,305	0,048	0,331	0,076	0,465	0,248	-0,201	0,051	0,434	-0,136	0,199	0,772	1	0,586	0,355	0,309	-0,074	0,371
As	0,118	-0,192	0,300	0,188	0,239	0,031	-0,162	-0,008	0,178	0,151	0,268	0,519	0,586	1	0,113	0,166	-0,360	0,227
Se	0,230	0,354	0,158	0,056	0,115	0,138	-0,046	-0,108	-0,006	-0,203	-0,188	0,378	0,355	0,113	1	0,356	0,666	0,581
Ag	0,418	0,416	0,360	0,021	-0,094	0,052	0,100	0,070	-0,158	-0,055	-0,208	0,565	0,309	0,166	0,356	1	0,393	0,231
Cd	0,007	0,396	-0,013	-0,102	0,080	0,249	-0,033	-0,153	0,007	-0,231	-0,383	0,056	-0,074	-0,360	0,666	0,393	1	0,562
Pb	-0,026	0,166	0,245	0,046	0,435	0,471	-0,425	-0,120	0,307	-0,227	-0,136	0,333	0,371	0,227	0,581	0,231	0,562	1

24

25 **Note:** Significant correlation at $p < 0.01$ is indicated in bold. PK = pyruvate kinase; CS = citrate synthase; G6PDH = glucose-6-phosphate

26 dehydrogenase; LDH = lactate dehydrogenase.

27 **Table S7:** Pearson correlation matrix ($n = 68$) of the variables used in the CCA for the muscle of *Anguilla anguilla*.

Variables	Weight	Age	Lipids	Kn	Proteins	PK	CS	CCO	LDH	Cr	Ni	Cu	Zn	As	Se	Ag	Cd	Pb	Hg	OCP	BDE47	BDE100	PCB
Weight	1	0,584	0,331	0,000	-0,089	0,024	-0,229	-0,306	0,100	-0,203	-0,022	-0,065	-0,163	0,009	-0,286	0,128	0,024	-0,035	0,102	0,212	0,123	0,122	0,189
Age	0,584	1	0,119	-0,149	-0,155	0,059	-0,208	-0,213	-0,217	-0,143	0,067	0,054	-0,014	-0,350	0,139	-0,066	0,344	0,168	0,583	0,594	0,563	0,600	0,549
Lipids	0,331	0,119	1	0,108	-0,155	0,176	-0,061	-0,247	0,157	-0,329	-0,368	-0,205	-0,313	0,131	-0,189	-0,137	-0,201	-0,259	-0,133	0,202	0,100	0,008	0,210
Kn	0,000	-0,149	0,108	1	0,050	0,172	-0,040	-0,100	0,119	0,001	-0,212	-0,119	-0,019	0,097	-0,180	0,168	0,024	-0,313	-0,197	-0,204	-0,221	-0,210	-0,226
Proteins	-0,089	-0,155	-0,155	0,050	1	0,294	0,073	0,227	0,102	0,075	0,147	0,229	0,063	0,029	0,105	-0,118	0,058	-0,110	-0,207	-0,156	-0,097	-0,104	-0,221
PK	0,024	0,059	0,176	0,172	0,294	1	0,151	0,239	0,341	-0,176	-0,087	0,169	0,146	-0,250	0,000	-0,043	0,213	-0,023	-0,022	0,113	0,022	-0,039	-0,079
CS	-0,229	-0,208	-0,061	-0,040	0,073	0,151	1	0,706	0,171	-0,014	-0,118	-0,095	0,198	0,016	-0,058	0,030	0,025	-0,020	-0,180	-0,293	-0,230	-0,203	-0,255
CCO	-0,306	-0,213	-0,247	-0,100	0,227	0,239	0,706	1	0,178	-0,093	-0,199	0,017	0,271	-0,207	0,003	-0,090	0,075	0,158	-0,075	-0,179	-0,117	-0,096	-0,225
LDH	0,100	-0,217	0,157	0,119	0,102	0,341	0,171	0,178	1	-0,038	-0,210	-0,090	-0,165	0,098	-0,366	-0,103	-0,134	-0,165	-0,300	-0,158	-0,222	-0,291	-0,199
Cr	-0,203	-0,143	-0,329	0,001	0,075	-0,176	-0,014	-0,093	-0,038	1	0,251	0,319	0,064	0,008	0,166	-0,083	0,063	0,041	0,029	-0,152	-0,080	-0,063	-0,138
Ni	-0,022	0,067	-0,368	-0,212	0,147	-0,087	-0,118	-0,199	-0,210	0,251	1	0,366	0,167	0,346	0,169	0,019	0,099	0,426	-0,052	-0,196	-0,231	-0,176	-0,199
Cu	-0,065	0,054	-0,205	-0,119	0,229	0,169	-0,095	0,017	-0,090	0,319	0,366	1	0,220	0,094	0,407	-0,103	0,420	0,258	0,132	0,079	0,132	0,118	0,039
Zn	-0,163	-0,014	-0,313	-0,019	0,063	0,146	0,198	0,271	-0,165	0,064	0,167	0,220	1	-0,130	0,370	0,022	0,375	0,204	0,020	-0,130	-0,111	-0,078	-0,196
As	0,009	-0,350	0,131	0,097	0,029	-0,250	0,016	-0,207	0,098	0,008	0,346	0,094	-0,130	1	-0,135	0,061	-0,203	-0,004	-0,554	-0,522	-0,490	-0,464	-0,345
Se	-0,286	0,139	-0,189	-0,180	0,105	0,000	-0,058	0,003	-0,366	0,166	0,169	0,407	0,370	-0,135	1	-0,266	0,333	0,207	0,411	0,342	0,465	0,460	0,383
Ag	0,128	-0,066	-0,137	0,168	-0,118	-0,043	0,030	-0,090	-0,103	-0,083	0,019	-0,103	0,022	0,061	-0,266	1	-0,034	-0,106	-0,181	-0,298	-0,311	-0,260	-0,321
Cd	0,024	0,344	-0,201	0,024	0,058	0,213	0,025	0,075	-0,134	0,063	0,099	0,420	0,375	-0,203	0,333	-0,034	1	0,332	0,349	0,299	0,333	0,367	0,210
Pb	-0,035	0,168	-0,259	-0,313	-0,110	-0,023	-0,020	0,158	-0,165	0,041	0,426	0,258	0,204	-0,004	0,207	-0,106	0,332	1	0,181	0,202	0,207	0,202	0,163
Hg	0,102	0,583	-0,133	-0,197	-0,207	-0,022	-0,180	-0,075	-0,300	0,029	-0,052	0,132	0,020	-0,554	0,411	-0,181	0,349	0,181	1	0,800	0,788	0,804	0,747
OCP	0,212	0,594	0,202	-0,204	-0,156	0,113	-0,293	-0,179	-0,158	-0,152	-0,196	0,079	-0,130	-0,522	0,342	-0,298	0,299	0,202	0,800	1	0,925	0,895	0,908
BDE47	0,123	0,563	0,100	-0,221	-0,097	0,022	-0,230	-0,117	-0,222	-0,080	-0,231	0,132	-0,111	-0,490	0,465	-0,311	0,333	0,207	0,788	0,925	1	0,971	0,915
BDE100	0,122	0,600	0,008	-0,210	-0,104	-0,039	-0,203	-0,096	-0,291	-0,063	-0,176	0,118	-0,078	-0,464	0,460	-0,260	0,367	0,202	0,804	0,895	0,971	1	0,919
PCB	0,189	0,549	0,210	-0,226	-0,221	-0,079	-0,255	-0,225	-0,199	-0,138	-0,199	0,039	-0,196	-0,345	0,383	-0,321	0,210	0,163	0,747	0,908	0,915	0,919	1

28

29 **Note:** Significant correlation at $p < 0.01$ is indicated in bold. PK = pyruvate kinase; CS = citrate synthase; CCO = cytochrome C oxidase; LDH =

30 lactate dehydrogenase.

Table S8: Mean protein (mg·g⁻¹ ± standard deviation) concentration and enzymatic activity (μmol·min⁻¹·g⁻¹ ± standard deviation) for *A. rostrata* following sampling site and year.

Tissue	Parameter	2011				2012			
		RSJ	RSO	LSP	LSF	RSJ	RSO	LSP	LSF
Liver	Proteins	99.7 ± 14.3 ^{AB} (14)	<u>94.0 ± 15.3</u> ^{AB} (13)	<u>87.9 ± 16.7</u> ^B (15)	106.8 ± 13.3 ^A (15)	108.8 ± 22.5 (15)	115.2 ± 27.6 (15)	108.6 ± 23.5 (15)	105.1 ± 22.8 (15)
	PK	<u>1.3 ± 0.4</u> (14)	<u>1.2 ± 0.2</u> (12)	<u>1.3 ± 0.4</u> (13)	<u>1.4 ± 0.2</u> (15)	1.0 ± 0.3 (14)	1.0 ± 0.2 (15)	1.1 ± 0.3 (15)	1.1 ± 0.4 (15)
	CS	4.6 ± 0.7 (9)	4.5 ± 0.9 (8)	4.0 ± 1.0 (10)	4.7 ± 1.0 (10)	4.6 ± 1.0 (15)	4.6 ± 1.3 (15)	4.2 ± 0.9 (15)	4.0 ± 1.3 (15)
	G6PDH	5.6 ± 3.5 ^B (13)	6.0 ± 4.2 ^B (12)	<u>4.2 ± 2.6</u> ^B (11)	12.8 ± 3.6 ^A (15)	4.0 ± 2.6 ^B (14)	8.8 ± 5.6 ^A (15)	7.7 ± 3.2 ^{AB} (15)	10.8 ± 3.8 ^A (15)
	LDH	2.0 ± 1.2 (14)	1.8 ± 0.2 (12)	2.2 ± 0.7 (15)	2.1 ± 0.3 (15)	1.9 ± 0.5 (15)	2.1 ± 0.4 (15)	2.1 ± 0.5 (15)	2.3 ± 0.5 (15)
Muscle	Proteins	132.1 ± 25.1 (15)	<u>129.5 ± 22.7</u> (15)	<u>112.8 ± 25.4</u> (15)	<u>120.5 ± 24.8</u> (15)	144.8 ± 28.4 (10)	156.4 ± 31.1 (10)	143.8 ± 41.4 (10)	150.7 ± 46.4 (10)
	PK	111.1 ± 20.4 ^B (5)	<u>156.9 ± 17.4</u> ^A (5)	119.0 ± 27.8 ^{AB} (5)	126.7 ± 21.1 ^{AB} (5)	90.5 ± 20.4 ^B (10)	127.6 ± 22.3 ^A (10)	115.5 ± 22.8 ^{AB} (10)	130.1 ± 22.1 ^A (10)
	CS	1.1 ± 0.2 ^B (15)	1.8 ± 0.4 ^A (13)	1.0 ± 0.3 ^B (10)	0.9 ± 0.2 ^B (10)	1.3 ± 0.6 ^{AB} (10)	1.8 ± 0.7 ^A (10)	1.0 ± 0.2 ^B (10)	1.1 ± 0.1 ^B (10)
	CCO	1.8 ± 0.4 ^B (10)	3.6 ± 1.3 ^A (10)	1.3 ± 0.2 ^B (10)	1.2 ± 0.3 ^B (10)	2.0 ± 1.0 ^B (10)	4.0 ± 1.7 ^A (10)	1.5 ± 0.4 ^B (10)	1.4 ± 0.4 ^B (10)
	LDH	198.2 ± 58.4 (10)	196.6 ± 39.6 (10)	151.8 ± 52.6 (9)	208.4 ± 66.4 (10)	161.8 ± 49.0 (10)	165.0 ± 52.0 (10)	189.0 ± 56.5 (10)	195.2 ± 50.5 (10)

Note: Values in brackets indicate sample size. Different letters indicate significant differences within a row (p < 0.05, Tukey-Kramer HSD test). An underlined value indicates a significant difference between the two sampling years (p < 0.05 Tukey-Kramer HSD test). RSJ = Saint-Jean River; RSO = Sud-Ouest River; LSP = Lake Saint-Pierre; LSF = Lake Saint-François.

Table S9: Mean protein (mg·g⁻¹ ± standard deviation) concentration and enzymatic activity (μmol·min⁻¹·g⁻¹ ± standard deviation) for *A. anguilla* following sampling site and year.

Tissue	Parameter	2011				2012			
		Certes	Dordogne	Gironde	Garonne	Certes	Dordogne	Gironde	Garonne
Liver	Proteins	<u>131.5 ± 19.7</u> (15)	120.7 ± 17.3 (14)	127.7 ± 16.7 (15)	<u>120.5 ± 15.6</u> (15)	99.7 ± 11.3 ^B (10)	121.2 ± 14.9 ^A (10)	120.2 ± 20.2 ^A (10)	91.9 ± 19.0 ^B (10)
	PK	<u>1.3 ± 0.2^{AB}</u> (14)	<u>1.2 ± 0.2^B</u> (14)	1.4 ± 0.2 ^A (15)	<u>1.3 ± 0.3^{AB}</u> (14)	0.9 ± 0.2 ^B (10)	1.5 ± 0.2 ^A (10)	1.3 ± 0.2 ^A (10)	1.0 ± 0.2 ^B (10)
	CS	<u>3.8 ± 0.9^A</u> (15)	4.3 ± 1.4 ^A (14)	<u>3.5 ± 0.8^{AB}</u> (15)	<u>2.8 ± 0.7^B</u> (15)	4.8 ± 0.8 (10)	5.0 ± 0.7 (10)	4.9 ± 1.0 (10)	5.6 ± 0.8 (10)
	G6PDH	<u>8.8 ± 3.5^A</u> (15)	4.9 ± 3.9 ^B (10)	<u>4.4 ± 2.9^B</u> (10)	5.4 ± 3.4 ^{AB} (9)	4.7 ± 1.5 ^B (10)	5.7 ± 2.8 ^B (7)	13.8 ± 4.7 ^A (10)	6.5 ± 2.6 ^B (9)
	LDH	<u>2.8 ± 0.6</u> (14)	2.3 ± 0.6 (14)	2.3 ± 0.6 (15)	<u>2.4 ± 0.4</u> (14)	1.7 ± 0.4 ^B (10)	2.7 ± 0.4 ^A (10)	1.9 ± 0.3 ^B (10)	1.8 ± 0.5 ^B (10)
Muscle	Proteins	149.9 ± 30.3 (10)	144.9 ± 31.1 (10)	113.2 ± 33.7 (10)	<u>139.7 ± 37.0</u> (10)	156.4 ± 31.3 (10)	134.4 ± 32.4 (10)	142.9 ± 44.1 (10)	162.4 ± 35.8 (10)
	PK	74.7 ± 12.7 (8)	86.8 ± 21.2 (9)	77.4 ± 23.4 (6)	87.0 ± 28.6 (8)	82.5 ± 11.9 ^{AB} (10)	86.0 ± 15.6 ^{AB} (10)	70.0 ± 11.7 ^B (10)	94.2 ± 17.5 ^A (10)
	CS	1.2 ± 0.3 ^A (10)	1.1 ± 0.3 ^A (10)	<u>0.8 ± 0.1^B</u> (10)	<u>0.8 ± 0.1^B</u> (10)	1.3 ± 0.3 (10)	1.4 ± 0.4 (10)	1.1 ± 0.3 (10)	1.2 ± 0.4 (10)
	CCO	1.6 ± 0.4 ^A (10)	1.8 ± 0.4 ^A (10)	<u>1.0 ± 0.2^B</u> (9)	1.4 ± 0.4 ^{AB} (10)	1.8 ± 0.4 ^{AB} (10)	2.4 ± 0.8 ^A (10)	1.5 ± 0.5 ^B (10)	1.9 ± 0.6 ^{AB} (10)
	LDH	171.1 ± 47.5 (10)	140.9 ± 48.0 (10)	157.0 ± 28.1 (10)	185.4 ± 60.6 (10)	191.4 ± 57.9 (10)	172.6 ± 55.5 (10)	154.1 ± 53.3 (10)	157.9 ± 32.2 (10)

Note: Values in brackets indicate sample size. Different letters indicate significant differences within a row ($p < 0.05$, Tukey-Kramer HSD test). An underlined value indicates a significant difference between the two sampling years ($p < 0.05$ Tukey-Kramer HSD test). RSJ = Saint-Jean River; RSO = Sud-Ouest River; LSP = Lake Saint-Pierre; LSF = Lake Saint-François.