Accepted Manuscript

Biotransformation, antioxidant and histopathological biomarker responses to contaminants in European and American yellow eels from the Gironde and St. Lawrence estuaries

Géraldine Patey, Catherine M. Couillard, Fabien Pierron, Magalie Beaudrimont, Patrice Couture

PII: S0045-6535(17)31378-4

DOI: 10.1016/j.chemosphere.2017.08.139

Reference: CHEM 19837

To appear in: *ECSN*

Received Date: 25 May 2017

Revised Date: 6 August 2017

Accepted Date: 27 August 2017

Please cite this article as: Patey, Gé., Couillard, C.M., Pierron, F., Beaudrimont, M., Couture, P., Biotransformation, antioxidant and histopathological biomarker responses to contaminants in European and American yellow eels from the Gironde and St. Lawrence estuaries, *Chemosphere* (2017), doi: 10.1016/j.chemosphere.2017.08.139.

Biotransformation, antioxidant and histopathological biomarker responses to contaminants in European and American Yellow Eels from the Gironde and St. Lawrence Estuaries

Géraldine Patey[1,2], Catherine M. Couillard[2], Fabien Pierron[3], Magalie Beaudrimont[4] and Patrice Couture[1*]

[1] Institut National De La Recherche Scientifique (INRS), Centre Eau Terre Environnement, 490 De La Couronne, Québec (Québec), G1K 9A9, Canada. Email: geraldine.patey@ete.inrs.ca

[2] Fisheries and Oceans Canada, Maurice Lamontagne Institute, 850 Route de la Mer, Mont-Joli (Québec), G5H 3Z4, Québec, Canada. Email : catherine.couillard@dfo-mpo.gc.ca

[3] CNRS, UMR EPOC CNRS 5805, F-33400 Talence, France. Email: f.pierron@epoc.u-bordeaux1.fr

[4] Univ. Bordeaux, UMR EPOC CNRS 5805, F-33400 Talence, France. Email: magalie.baudrimont@u-bordeaux.fr

* Corresponding author. Email: patrice.couture@ete.inrs.ca; Phone: +1 418 654 3825

Abstract

Since the early 1980s, populations of American (*Anguilla rostrata*) and European eels (*Anguilla anguilla*) have suffered a sharp decline. The causes of their decline are likely multifactorial and include chemical pollution. A field study was conducted in eight sites varying in organic and metal contamination along the St. Lawrence (Eastern Canada) and Gironde (France) systems to investigate the relationships among contaminants, biological characteristics and biotransformation, antioxidant and histopathological biomarkers in eels from both species. For *A. rostrata*, no major influences of persistent organic contaminants on biomarkers were identified. For *A. anguilla*, eels from the most contaminated site expressed higher surface of MelanoMacrophage Centers (MMCs) and eels from another contaminated site expressed higher amount of lipofuscin pigment. These two histopathological biomarkers were also associated with aging. Compared to eels from the cleanest French site, higher hepatic catalase activity and density of MMC in eels from contaminated sites was related to higher concentration of organic (DDT and metabolites, sum of PCBs, sum of PBDEs) and inorganic (Hg and Cd) contaminants. In both species, a higher deposition of spleen hemosiderin pigment was measured in eels from the most brackish sites compared to eels living in freshwater environments. Our results suggest an association between higher hemosiderin pigment and metal contamination (As for *A. anguilla* and Pb for *A. rostrata*). Parasitism by *A. crassus* was observed in European eels from freshwater sites but not in eels from brackish habitats. Overall, contamination may pose a greater risk for the health of European compared to American eels.

Keywords: EROD activity, Antioxidant enzymes, Histopathological markers, Pollution, Atlantic Eels

1. Introduction

In the Northern hemisphere, the abundance of European eel (*Anguilla anguilla*) and American eel (*Anguilla rostrata*), here called "Atlantic eels", has severely declined since the early 1980's (FAO/ICES, 2009; Cosewic, 2012). The causes of their decline are likely multifactorial, including variables such as oceanographic and climate changes, overfishing, barriers to migration, habitat degradation, parasites and chemical pollution (Castonguay et al., 1994; Geeraerts and Belpaire, 2010). Although management and restoration programs have been conducted in several hydrosystems in Europe and North America, eel stocks have not recovered to levels of the past (ICES 2016). An increasing number of recent reports support that pollutants might be among the possible synergistic causes contributing to the collapse of eel stocks (Belpaire et al. 2016). Due to their unique ecological and physiological traits, eels are particularly prone to bioaccumulation of contaminants through gills, skin and contaminated food during their feeding and growth stage (yellow eel). Eels are facultative catadromous fish that can be in contact with contaminated sediments for extended periods, leading to the accumulation of high concentrations of organic contaminants due to their high lipid content (Robinet and Feunteun, 2002; Daverat and Tomás, 2006; Thibault et al., 2007). Finally, eels are considered good bio-indicators of contaminated habitats and are expected to reflect the impacts of local pollutants (Belpaire and Goemans, 2007).

Recently, decreasing muscle concentrations of organic contaminants such as PolyChlorinated Byphenyls (PCBs) and OrganoChlorine Pesticides (OCPs) have been reported in eels from some historically contaminated habitats since their ban 40 years ago (Belpaire et al., 2016). From 1988 to 2008, a decreasing trend in PCB and OCP concentrations has been reported in the muscle of *A. rostrata* from the historically contaminated Lake Ontario (Byer et al. 2013). This trend was confirmed by a significant decrease in the embryotoxic potential of organic mixtures extracted from Lake Ontario eels captured in 1988, 1998 and 2008 for developing *Fundulus heteroclitus* (Rigaud et al. 2016). However, these contaminants are persistent in sediments and levels of PCBs, OCPs and PolyBrominated Diphenyl-Ethers (PBDEs) still remain high and preoccupying in yellow eels, for example in eels from the Gironde system (Tapie et al., 2011; Guhl et al., 2014). In addition to organic contaminants, metals such as mercury (Hg) and cadmium (Cd) show no trend of decrease and persist in contaminated areas (Maes et al., 2008).

Consequently, eels inhabiting contaminated areas could be still affected by a combination of contaminants acting synergistically.

Several field and laboratory studies have reported effects of contaminants in eels at several levels of biological organization from the cellular, molecular and tissue levels to the level of whole organisms using various biological biomarkers to investigate their health status. They have shown that contaminants can delay growth, reduce lipid storage efficiency, cause oxidative stress, induce DNA damage and histopathological lesions, which can ultimately negatively affect migratory and reproductive capacities (Couillard et al., 1997; Robinet and Feunteun, 2002; Palstra et al., 2006; Pierron et al., 2007; Geeraerts and Belpaire, 2010, Gravato et al. 2006). Others effects such as disturbances of the immune and endocrine systems and a reduction of migratory and reproductive capacities have been also reported (Belpaire et al. 2007). In addition, ecological gradients such as latitudinal cline, longer distances to the spawning site and differences in salinity directly or indirectly affect growth rate, body condition, size at metamorphosis and lipid storage, which in turn could affect contaminant uptake (Vøllestad, 1992; Edeline et al., 2007; Thibault et al., 2007; Belpaire et al., 2009; Jessop, 2010; Daverat et al., 2012). For *A. anguilla*, studies have suggested that the nematode *Anguillicola crassus* in the swimbladder of yellow eels could affect their swimming performance, reduce their resistance to other stressors such as contamination and increase spleen size, macrophage and lymphocyte production to combat parasites (Kirk et al., 2000; Lefebvre et al., 2004).

In a series of related studies performed by our team along the St. Lawrence and Gironde hydrosystems (Eastern Canada and Southwest France, respectively), we have identified that certain metals and organic contaminants could disturb physiological functions in yellow Atlantic eels (Baillon et al., 2015a, 2015b, 2016; Caron et al., 2016; Pannetier et al., 2016). In Baillon et al. (2015a, 2015b, 2016), we conducted a large scale and without *a priori* transcriptomic based approach. The global hepatic transcriptome of animals was determined by RNA-Seq. Genes that most likely responded to single factors were identified. We detected transcriptomic responses typical of PCB-170 and Cd exposure in wild *A. anguilla* collected in the most contaminated site in the Gironde Estuary, associated with changes in the transcription levels of genes involved in hepatic energy metabolism. In another paper (Caron et al. 2016), we focused on biomarkers involved in glycolytic, aerobic and anaerobic capacities and lipid metabolism. We reported an association between silver (Ag), lead (Pb) and arsenic (As) contamination in wild *A. rostrata* from the St. Lawrence River system and inhibition of the enzyme G6PDH, involved in lipid

103 metabolism in liver and which also plays a role in oxidative stress response. This study has also
104 shown an association between zinc (Zn), copper (Cu) and Pb contamination and altered
105 glycolytic and anaerobic capacities in wild *A. anguilla* from the Gironde system. More recently,
106 we have demonstrated a significant difference in early size-at-age (a proxy of early growth rate)
107 between yellow eels captured upstream compared to downstream sites of the St. Lawrence and
108 the Gironde systems (Patey et al., submitted). These differences were present as early as 1 year
109 old and could influence contaminant accumulation.

110 In the present study, we investigated the relationships among tissue concentrations of
111 inorganic and organic contaminants, biological characteristics such as size, age, condition factor
112 and muscle lipids and a set of complementary biomarkers such as hepatic biotransformation
113 enzymes, antioxidant enzymes and splenic histopathological measurements in Atlantic eels. The
114 selected biomarkers have been successfully used, alone or in combination, in previous
115 biomonitoring studies on eels (Couillard et Hodson, 1996; Pacheco et Santos, 2002; Van der
116 Oost et al., 2003; Buet et al., 2006) and are among the list of recommended biomarkers in the
117 Report of the Workshop of the Working Group on Eels and the Working Group on Biological
118 Effects of Contaminants (ICES 2016). First, we examined the induction of Cytochrome P450-
119 dependent monooxygenase, measured as ethoxyresorufin *O*-deethylase (EROD) in eel livers.
120 This biotransformation marker is involved in a well-documented biochemical reaction of fish to
121 organic planar contaminants such as PCBs, polycyclic aromatic hydrocarbons (PAHs), dioxins
122 and furans and may be related to other effects such as oxidative stress (Van der Oost et al., 2003;
123 Whyte et al., 2008). Secondly, we measured hepatic activities of two antioxidant enzymes,
124 catalase (CAT) and superoxide dismutase (SOD), commonly used as biomarkers of antioxidant
125 capacities in fish and induced by several contaminants such as PCBs, PAHs, organochlorine
126 pesticides (OCPs) and metals (Van der Oost et al., 1996; Livingstone, 2001). In addition, we
127 examined a suite of histopathological biomarkers induced by chronic exposure to contaminants,
128 including density and surface of melano-macrophage centers (MMCs) and deposition of
129 lipofuscin and hemosiderin pigments in spleen (Wolke, 1992; Couillard et al., 1997; Fournie et
130 al., 2001; Au, 2004). In general, biomolecular biomarkers such as EROD, CAT and SOD are
131 more sensitive and specific short-term responses (days to weeks), whereas histopathological
132 biomarkers are responses to chronic exposure to contaminants and reveal more persistent
133 damage.

In this study, yellow eels were sampled in four sites varying in anthropogenic pressure and contamination level in each of the Saint Lawrence System (SLS, Quebec, Canada) and the Gironde System (GS, France), for two consecutive years. The objectives of this study were: (1) to examine variations in responses of biomarkers in wild American and European eels among sampling sites varying in contamination levels and between sampling years within a species; (2) to investigate the relationships between biological characteristics, contaminants and biomarker responses; and (3) finally, to generate a database to better target and prioritize future studies on the impacts of contaminants on eel health in the Gironde and St. Lawrence River basins.

2. Materials and methods

2.1 Sampling sites

A total of 240 yellow eels (15 yellow eels/site/year/species) were captured in eight different sites selected according to salinity, metal and organic contamination gradients found in sediments and biota along the SLS and the GS in May and June 2011 and 2012 as previously described by Baillon et al. (2015b) and Laporte et al. (2016). For *A. rostrata*, each year, 60 yellow eels were sampled in two references sites, St Jean River (SJR, freshwater tributary of the Gulf of the SLS) and Sud-Ouest River (SOR, freshwater tributary of the estuary of the SLS) and two fluvial contaminated sites, St. Pierre Lake (SPL) and St. François Lake (SFL). Yellow eels from SOR and SJR were captured in running freshwater habitats, which are tributaries opening into a brackish estuary suggesting an influence of marine water (Thibault et al., 2007).

For *A. anguilla*, each year, 60 yellow eels were sampled in two fluvial sites, the Dordogne river (DOR, considered as the least contaminated site in the watershed of the Gironde) and the Garonne river (GAR, located near the city of Bordeaux and contaminated), one estuarine site, Gironde (GIR, contaminated by metals and organic contaminants, (Kessaci et al., 2014)) and finally, one salt marsh site in the Arcachon Bay, Certes (CER) considered as a reference site.

2.2 Wild eels sample collection

All yellow eels were processed using the same standardized protocols in France and in Québec. European and American yellow eels were captured using fyke nets (mesh size of 6.4 mm), trawl (mesh size of 31 mm) and electro-fishing by professional fishers or technical staff. Given the substantial variability in the size of eels growing in different habitats, it was not

166 possible to collect same-sized eels in each sampling site. Body Length (BL) (± 1 mm) and Body
167 Mass (BM) (± 0.1 g) were measured for each fish in order to estimate the condition factor K
168 ($K=[(BM\ (g)/(BL\ (cm))^3]$). We also determined the ocular index (OI) expressed in % which
169 reflects changes in eye diameter, a useful criterion for assessing the degree of sexual
170 maturation/silvering in eels. Ocular enlargement occurs early in the sequence of sexual
171 maturation (Acou et al., 2005; Durif et al., 2005) and is thought to be a requirement for migration
172 at depth in the ocean. Then, eels were killed as soon as possible by decapitation and rapidly
173 dissected to collect parts of liver, caudal muscle and spleen for biochemical and histological
174 analysis. Samples of muscle were dissected in a standardized area situated at 2 cm posterior to
175 the anus and above the lateral line and kept in aluminium foil at -20°C. Samples of liver were
176 stored in cryogenic tubes at -80°C and samples of spleen were fixed in bottles containing 10%
177 phosphate-buffered formalin. Age was determined from transverse sections of sagittal otoliths as
178 described previously by Verreault et al. (2009). Muscle lipid content was determined by
179 gravimetry after filtration and evaporation of an aliquot of the DiChloroMethane (DCM) extract
180 from the same standardized area of muscle described above and was expressed in percentage of
181 dry-basis lipid content. The moisture content was determined as described by Tapie et al., (2011)
182 and expressed in percentage of wet-basis moisture content. Finally, each swimbladder was
183 dissected and examined macroscopically to determine the presence of the parasite *A. crassus*
184 (Girard and Elie, 2007). Parasite identification followed descriptions of Moravec and
185 Taraschewski (1988).
186 Metal analyses (Ag, As, Cd, Cr, Cu, Ni, Pb, Se, Zn, Hg) were carried out in liver and
187 muscle samples as previously described by Pannetier et al. (2016). The concentrations of seven
188 indicators of PCBs (CB50+28, CB 52, CB 101, CB 118, CB 138, CB 153 and CB 180), eight
189 OCPs (2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT and 4,4'-DDT,
190 hexachlorobenzene (HCB) and lindane) and eight PBDEs (BDE 28, 47, 49, 99, 100, 153, 154,
191 183, 209), were performed on muscle samples following the procedures described by Caron et al.
192 (2016).
193
194 2.3 EROD activity
195 Supplies and chemicals for EROD assay were purchased from Sigma–Aldrich (St-Louis,
196 MO, USA). EROD activity was measured using a spectrofluorimetric method adapted for
197 microplates by Fragoso et al. (1998). This measure is based on the increase of the fluorescence in

198 the reaction medium due to the transformation of 7-ethoxyresorufin (7-ER) into resorufin. EROD
199 activity was measured in the post mitochondrial supernatant prepared from homogenates of 50
200 mg crushed liver in 1 ml of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (0.1
201 M, pH = 7.8) grinding buffer and centrifuged at 10 000 g during 20 min at 4°C. Reaction
202 mixtures contained 50 µl of supernatant in HEPES buffer, 10 µl of nicotinamide adenine
203 dinucleotide phosphate (NADPH, 20 mg/ml), and 50 µl of 7-ethoxyresorufin (0.024 mg/ml) in
204 DiMethylSulfOxide (DMSO). The activity was quantified in 96-well microplates using a
205 Cytofluor II® plate reader (excitation 530 nm, emission 590 nm, PerSeptive Biosystems,
206 Framingham, MA, USA). The fluorescence of resorufin was measured at 60 s intervals during 13
207 min. Fluorescence readings were compared to a resorufin standard curve. All samples were
208 assayed in triplicate. Total protein concentration of the supernatant was measured with Bradford
209 reagent, using bovine serum albumin as a protein standard (BIO-RAD Laboratories, Hercules,
210 CA, USA). EROD activity was expressed as pmoles resorufin/min/mg protein. In addition to
211 randomized samples from different sites on each microplate, a positive control was added to
212 assess the quality and repeatability of measurements. Liver homogenates from pooled livers of
213 15 Atlantic tomcod which were injected 20 mg/kg benzo[a]pyrene (10 mg/ml in corn oil)
214 intraperitoneally and sampled 48 h after treatment were used as positive controls (Couillard et
215 al., 2004). Coefficients of variation among samples and positive control replicates were < 20%,
216 and the coefficient of variation of the sample means was < 25%.
217
218 2.4 Antioxidant enzymes
219 Catalase (CAT) and superoxide dismutase (SOD) enzymes were measured in cytosolic
220 supernatant prepared from homogenates of 50 mg crushed liver in HEPES buffer. Liver samples
221 were constantly kept on ice during homogenization. An aliquot of the homogenate was
222 centrifuged at 1500 g for 5 min at 4°C and the supernatant was stored at – 80°C for SOD assays
223 whereas a second aliquot was centrifuged at 10 000 g for 15 min at 4°C and the supernatant was
224 stored at – 80°C for CAT assays. Catalase and SOD activities were measured using commercial
225 kits from Cayman Chemical Company Inc. (Ann Arbor, MI, USA) in 96 well microplates with a
226 UV/visible spectrophotometer (Cary 50, Varian inc.). The CAT assay is based on the reaction of
227 the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The generated
228 formaldehyde is allowed to react with 4-amino-3-hydrazine-5-mercapto-1,2,4-triazole (as the
229 chromogen). The aldehydes, upon oxidation, change from colorless to a purple color that is

measured spectrophotometrically at 540 nm. One unit (U) of CAT activity is defined as the amount of enzyme that causes the formation of 1 nmol of formaldehyde per minute at 25°C. The SOD assay utilizes a tetrazolium salt for detection of superoxide anion radicals generated by xanthine oxidase and hypoxanthine measured spectrophotometrically at 450 nm. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. All samples were tested in triplicate and each absorbance reading was compared to a standard curve and a positive control. Total protein concentration of the supernatant was determined using the Bradford (1976) method using bovine serum albumin as standard and CAT and SOD activities were expressed as μmol/min/mg protein.

2.5 Histopathological biomarkers

The spleen was selected as a tissue for the examination of the impacts of contaminants on histopathological biomarkers in fish (Au, 2004). A transversal section from the central part of spleen was fixed in 10% phosphate-buffered formalin within minutes after death. Samples were then embedded in paraffin and 6 μm thick sections were stained with Hematoxylin and Eosin (HE) (Luna, 1968). Two additional sections of spleen were stained with Perls' Prussian blue staining for ferric iron associated with hemosiderin deposits or with Schmorl staining for lipofuscin deposits (Luna, 1968).

To be categorised as a MMC, a MMC had to be composed of three or more macrophages containing yellow to dark brown pigments within their cytoplasm (Couillard and Hodson, 1996). In sections of spleen stained with HE, the density of MMCs was evaluated by counting the number of MMCs at 100X magnification in three randomly selected microscopic fields and by dividing this number by the area of the fields examined (2.86 mm^2). The surface areas of 10 randomly selected MMC were measured with an image analysis system (Image-Pro Express software, version 6.0) and mean of surface area of MMCs were calculated for each fish. The proportion of 25 randomly selected MMCs that stained positively with Perls' Prussian Blue for hemosiderin and Schmorl for lipofuscin was evaluated at 100X magnification. The intensity of staining was graded from 1 to 5 (1: no macrophage stained; 2: 0-25% stained; 3: 25-50% stained; 4: 50-75% stained and 5: > 75%). The reproducibility of the methods was assessed by repeated measurements of a subsample (≈ 10%) of slides. The coefficient of variation among the mean of the third quartile (Q3) for the density and for the surface of MMCs separately in twelve eels were < 25% (repeated 3 times by two different observers for each eel). All tissues were examined

blindly and randomly without the observer knowing the site of origin of eels. Density was expressed as number of MMC/mm^2 and surface as mm^2/MMC.

2.6 Statistical analyses

Analyses of variance were conducted using Prism v. 7.0 (GraphPad Software Inc., San Diego, CA, USA) and multivariate analyses were conducted using XLSTAT-ADA (Addinsoft, USA). Male eels were removed due to a small sample size (N=3 in the GS and N=0 in the SLS). Eels ≤ 2 years old and ≥ 20 years old were also removed, because they were restricted to a small number of sites/sampling year. The total number of eels retained for statistical analyses of variance were 105 in *A. rostrata* and 111 in *A. anguilla* (Table 2).

Before performing the tests, homoscedasticity and normality of data were verified using the Bartlett and the Shapiro-Wilk tests respectively. Biological characteristics, contaminant concentrations and biomarker data were first compared among sampling years within sites using the student t-test ($p \leq 0.05$). Then, data were compared among sampling sites within species using a one-way ANOVA test followed by Tukey-Kramer HSD test to identify significant differences between pairs ($p \leq 0.05$). A non-parametric Kruskal-Wallis test (KW) followed by Dunn's test was performed when the ANOVA's assumptions were not met.

Principal Component Analyses (PCAs) and Kendall rank correlation were performed to explore the relationships among biomarker responses, contaminants and biological characteristics for the two years combined in each species. Prior to PCA analyses, correlation matrices were performed by category of contaminants for PCBs, DDTs, and for PBDEs in order to group contaminant variables correlated with each other from the same category and improve the quality of PCA (data not shown). Due to very strong correlations among all PCB congeners, the sum of 7 PCBs was used. For contaminants of the DDT family, only the muscle concentrations of 4,4'-DDD, 2,4'-DDE and 4,4'-DDE were retained. For contaminants of the PBDE family, only PBDEs 47, 100, 153 and 154 were included. Finally, essential metals (Cu, Se and Zn) in liver and muscle were removed from the analysis since their concentrations were unlikely to pose a risk to the health of eels (Pannetier et al., 2016). Consequently, the non-essential metals Ag, Cd, Pb, Hg and As in muscle and in liver (except for liver Hg due to high proportion of missing values) were kept for PCAs. Only individuals for which measurements were available for each variable were retained for the PCAs (N=82 for *A. rostrata* and N=87 for *A. anguilla*).

In summary, a set of 17 variables including 9 categories of contaminants [sum of PCBs, sum of DDT and metabolites, hexachlorobenzene, lindane, sum of PBDEs, muscle Ag, Cd, Pb, Hg and As, liver Ag, Cd, Pb and As], 5 biological variables (BL, BM, lipids, age, number of *A. crassus*) and 7 biomarker variables (EROD, CAT, SOD, density and surface of MMCs, lipofuscin and hemosiderin pigments) were considered in a total of 82 yellow eels for *A. rostrata* and 87 yellow eels for *A. anguilla* captured in both years. For contaminants, non-detectable data were replaced by ½ of their Limits of Detection (LoDs). Kendall rank correlations were used to assess the strength relationships among biomarkers, contaminants and biological characteristics. The significance level (p value) of the Kendall's correlations was fixed at 0.05.

3. Results and discussion

3.1 Morphometry and level of contamination in *A. rostrata* and *A. anguilla*

Since contamination and biological characteristics of eels from this study have been reported in companion studies (Baillon et al., 2015b; Baillon et al., 2016; Caron et al., 2016; Laporte et al., 2016; Pannetier et al., 2016), they will only be briefly summarized here.

For *A. rostrata*, fish from the fluvial lakes SPL and SFL were generally larger, older and fatter than fish from SOR and SJR (significantly in 2011 but not in 2012) and no *A. crassus* was detected in American eels (Tables 2 and S.1). As reported by Baillon et al. (2015b), eels from the fluvial lakes SPL and SFL were more contaminated with organic chemicals such as PCBs, PBDEs and DDT and metabolites than fish from the downstream sites SOR and SJR (Table S.2). For non-essential metals, significantly higher concentrations of Cd and Pb were observed in eels sampled in the downstream site SOR compared to the upstream site SFL (Table S.3 and S.4). Muscle Hg was significantly higher in eels from upstream sites SPL and SFL compared to SJR (Pannetier et al., 2016). For *A. anguilla*, in 2011, fish from GIR were significantly longer, heavier and fatter than eels from all other sites and in 2012, the size of eels from GIR was comparable to that of fish from GAR and CER (Table S.1). In both years, eels from GAR were significantly older than eels from DOR and CER and eels from DOR were significantly smaller and lighter than eels from the other sites. A concentration gradient of increasing organic contamination (PCBs, PBDEs and DDT and metabolites) was observed along the French sites in the order CER-DOR-GAR-GIR (Table S.2). High tissue concentrations of Cd (muscle and liver) and Pb (liver) were detected in eels from the most contaminated sites GIR and GAR and

surprisingly also in eels from DOR, a site originally considered as clean (Pannetier et al., 2016). In both species, higher As concentrations were found in fish from the reference sites CER and SJR and from the contaminated site GIR which had in common an elevated salinity (Pannetier et al., 2016). For *A. anguilla* from the most contaminated site GIR, muscle PCBs, DDT and metabolites and Cd concentrations were 10 and 11-folds higher than in *A. rostrata* from the contaminated sites SPL (Tables S.2 and S.4). Eels from SPL had two-fold higher muscle PBDE concentrations and three-fold higher muscle Hg concentrations than eels from GIR (Tables S.2 and S.3). Eels from CER had three-fold higher concentrations of muscle As than eels from SJR (Tables S.2 and S.4). Finally, the ocular index (OI) reached 2.7 ± (2.0, 3.8) (median ± (Q1, Q3); n=108) and 3.9 ± (3.3, 4.8) (median ± (Q1, Q3); n=111) for *A. rostrata* and for *A. anguilla* respectively. In pre-migrating silver eels, this index should be > 6.5 (Cottrill, 2002; Pankhurst, 1982). Hence, our eels were not pre-migrating and were all yellow eels.

3.2 Patterns of biological characteristics, contaminants and biomarker responses in *A. rostrata*

The first 3 principal components of the PCA (PC1, PC2 and PC3) covered 37 % of the total variability of the overall data (Table 1). Principal component 1 was mainly composed of biological variables and organic and inorganic contaminants and it separated in two distinct clusters eels from the contaminated sites SFL and SPL from the reference sites SOR and SJR (Figs. 2 a and b). The previously described associations between high concentrations of persistent organic contaminants, higher fat content and older age (Larsson et al, 1990) are illustrated on this axis. No significant association between muscle lipids and biomarker responses were observed although muscle lipids were positively correlated to concentrations of lipophilic organic contaminants (Table S.6). Thus, there is no evidence of a detrimental effect of these compounds on lipid accumulation in yellow eels. Principal component 2 was mainly composed of metal contaminants (Figs. 2 a and b). The majority of eels from SJR were associated with muscle and liver As in agreement with higher As levels (although non-significant, Tables S.3 and S.4) compared to the group of eels from SOR which was associated with liver Cd. Higher As concentrations in fish from SJR are typical of fish living in marine environments (Pannetier et al., 2016) and could be explained by differences in migratory behavior, spatial distribution and feeding. Further studies combining chemical analyses of otoliths with biomarker and contaminant measurements are recommended to verify this

hypothesis. No biomarker variables were distributed along the PC1-axis or the PC2-axis (Table 1 and Fig. 2 a). Since PC1 and PC2 discriminated sampling sites, our results are consistent with the absence of significant differences for most biomarkers (EROD activity, density, surface and accumulation of pigments in splenic MMC) among sampling sites and between years presented in Table 2.

The density and surface of MMCs and the deposition of hemosiderin pigments were distributed along the PC3-axis (Table 1). Hemosiderin pigments in spleen MMC were associated to a group of eels from SOR and SJR sites (Fig. 2 a). Moreover, they were negatively correlated with size and organic contaminants and weakly positively correlated with muscle and liver Pb (Table S.5). An experimental study using common carp (*Cyprinus carpio* L.) has already demonstrated hemosiderin deposition in fish exposed to a mixture of metals (Cd, Pb, Cr and Ni) compared to controls (Vinodhini and Narayanan, 2008). However, given the weak correlation and the lack of significant difference among sites (Fig. 2 a), it seems difficult to conclude for an effect of Pb on deposition of hemosiderin pigment. Note however that in a companion study from the same research project, Caron et al. (2016) identified an association between Ag, Pb and As contamination and an inhibition of the hepatic enzyme G6PDH in the same eels as those used in this study, suggesting an effect of metal contamination in these eels. This enzyme is involved in lipid metabolism and is closely related to antioxidant response (Martinez-Alvarez et al., 2005). Differences in environmental conditions in estuarine compared to fluvial sites could contribute to increase hemosiderin deposition in SJR and SOR sites through exposure to an unknown biological or chemical hemolytic agent.

Catalase and SOD activities were not significantly correlated with PC1, PC2 or PC3 axes and their explained variance (%) was low (Table 1). Although CAT activity was not correlated with biological characteristics or contaminants (Table S.5), eels from SOR expressed significantly higher activities of hepatic CAT than fish from other sites in 2011 (but not in 2012) and a significant inter-annual difference was observed, with higher CAT activity in 2011 in SOR (Table 2). SOD activity was negatively correlated with size, muscle lipid content and PCBs, more weakly with age, PBDEs and DDT and metabolites and was associated with a group of eels from SOR and with some fish from SPL (Fig. 2 and Table S.5). This observation is consistent with significantly higher liver SOD activity found in eels from SOR in both years and with the significant inter-annual difference observed for SOD, higher in 2012 in SPL fish (Table 2). SOD activity was weakly positively correlated to liver Cd which could possibly explain the high level

of SOD activity in SOR and SPL fish (Table S.5). As shown in Fig. 2, a majority of eels from SOR and some eels from SFL were associated with the liver Cd vector in agreement with the significantly higher liver Cd observed in eels from SOR compared to eels from SJR and SFL (Table S.4). Similar results were observed by Martinez-Alvarez et al. (2005), who reported that Cd exposure could cause an increase in antioxidant enzymes by competing with essential metals on protein-binding sites. Further experimental studies are needed to confirm the potential cause-effect relationship behind this weak association.

Although EROD activity did not vary significantly among sites and years, in 2011 however, EROD activity was highly variable in eels from SPL compared to the other sites (with a high 75^{th} percentile of 31.6 compared to 15.7 and 10.3 for SOR and SJR sites respectively, Table 2).

Previous studies conducted in the Gironde and St. Lawrence River systems have reported that some eels during their growth phase migrate from the upstream to the downstream sections and inversely (Daverat and Tomás, 2006; Thibault et al., 2007). Inter-individual variations in migratory behaviour and growth rates (Patey et al., submitted), in addition to variations in diet selection (Pegg et al., 2015) could affect contaminant uptake. Although a wide range of persistent contaminants (PCBs, PBDEs, pesticides and metals) were measured in eels in this study, other contaminants could influence the biomarker responses. PAHs are known to induce both EROD and antioxidant enzyme activities (Martinez-Alvarez et al., 2005; Whyte et al., 2008). For example, in field and experimental studies, Gravato et al. (2006, 2010) reported significant increases in liver EROD activity and antioxidant capacities in European eels contaminated by PAHs. Seasonal or inter-annual variations in natural factors such as temperature and salinity could also influence antioxidant defense and biotransformation enzyme activities in fish, even if exposure remains constant (Martinez-Alvarez et al., 2005; Whyte et al., 2008; Chainy et al., 2016). Eels sampled in tributaries from the downstream St. Lawrence system (SOR and SJR), exposed to a strong salinity gradient over a short distance, are possibly more prone to variations in salinity, metal contaminants and temperature which may possibly cause inter-annual variations in antioxidant enzymes (Thibault et al., 2007; Pannetier et al., 2016).

3.3 Patterns of biological characteristics, contaminants and biomarker responses in *A. anguilla*

The first 3 principal components of the PCA covered 39 % of the variability of the overall data (Table 1). PC1 was mainly composed of age, organic and inorganic contaminants and mainly contributed to separate the most contaminated sites GIR and GAR from the reference site CER (Fig. 3 and Table 1). Like for *A. rostrata*, *A. anguilla* muscle lipids were significantly correlated with morphometric and organic contaminants and no strong correlations were observed among biomarker responses and muscle lipids (Kendall rank correlation < 0.200, Table S.6) suggesting no impairment of contaminants on fat reserves.

Hemosiderin pigment deposition in splenic MMC was distributed along the PC2-axis (Table 1 and Fig. 3 a). Hemosiderin pigment, muscle and liver As scored positively on PC2 and were significantly and positively correlated with each other (Fig. 3 a and Table S.6). Moreover, vectors of these variables are oriented between eels from CER and GIR (Fig. 3 b). This observation is consistent with significantly higher values in fish from the salt marsh site CER and intermediate values in fish from the estuarine site GIR observed in both years (Fig. 1 a). Moreover, it was in agreement with the observation that eels living in brackish or saltwater environments accumulate more As than their freshwater counterparts (Pannetier et al., 2016). Sorensen and Smith (1981) demonstrated the presence of As in hemosiderin granules in the liver of adult channel catfish (*Ictalurus punctatus*) exposed experimentally to As and suggested that this deposit constituted a mechanism for storage and detoxification. The presence of hemosiderin pigments is linked to a breakdown of red blood cells which could cause hemosiderosis, especially in the spleen, and increase the size and the number of MMCs in fish from contaminated areas (Khan and Kiceniuk, 1984). Further studies are needed to determine if eels living in saltwater environments are exposed, in addition to As, to other chemical or biological hemolytic agents leading to increased deposition of hemosiderin in their spleen.

Although other biomarkers were not significantly correlated with PC1, PC2 and PC3 axes and their explained variance (%) were low (Table 1), contrary to what was observed for *A. rostrata*, in *A. anguilla* most biomarkers displayed significant spatial and inter-annual variations (Table 2). Variations in lipofuscin pigments showed significant differences among sites in 2011, with higher values in fish from GAR compared to fish from DOR (Fig. 1 b). Lipofuscin deposition in splenic MMC was more associated with age and GAR fish (Table S.6 and Fig. 3 b). Moreover, eels from GAR were older than fish from other French sampling sites (Table S.1). Lipofuscin is known as an “aging pigment” and an increase in lipofuscin pigments in MMCs is generally found in older fish (Wolke, 1992; Agius and Roberts, 2003). Therefore, in *A. anguilla*,

452 lipofuscin pigments globally increased in older and more contaminated fish, mostly found in
453 GAR, in contrast with *A. rostrata* in which lipofuscin deposition was not significantly correlated
454 to either of these variables.

455 Values of density of MMCs were significantly higher in GAR compared to DOR, with
456 intermediate values for fish from GIR and CER in 2012 (Table 2). In 2011, the surface of MMCs
457 expressed significantly higher values in eels from GIR compared to DOR. The PCA analysis
458 showed that the strongest correlations were between density of MMC and muscle Hg and Cd
459 (Kendall's tau = 0.281 and 0.235 respectively) and between surface of MMC and age, BL and
460 BM (Kendall's tau = 0.384, 0.337 and 0.317, respectively, Table S.6). Several studies have
461 reported on correlations between increased number of MMC in fish exposed to Hg and other
462 metals (Meinelt et al., 1997; Agius and Roberts, 2003; Schwindt et al., 2008). Khan et al. (1994)
463 observed an increase in the size of splenic MMCs with increasing length and weight of winter
464 flounder living in a contaminated area and Couillard and Hodson (1996) also reported an
465 increase of surface of MMCs in the spleen of old wild Atlantic tomcod collected from estuarine
466 sites receiving pulp and paper mill effluents. Supported by these studies, the increase in the
467 density of MMCs in eels from GAR with Hg and Cd contamination and the increase in the
468 surface of spleen MMCs in eels from GIR was possibly associated with aging. Since the
469 Kendall's tau of these correlations was weak, we do not exclude that other potential
470 contaminants and/or natural factors not measured could influence the response of these
471 biomarkers.

472 Catalase activity was more strongly correlated with higher organic contaminants such as
473 DDT and metabolites, PBDEs and PCBs and associated to eels from GAR and GIR (Fig. 3 and
474 Table S.6). In addition, fish from GIR, GAR and DOR expressed significantly higher liver CAT
475 activities compared to CER fish in 2011 (Table 2). In 2012, only fish from GIR had significantly
476 higher CAT activities compared to GAR fish (Table 2). Liver CAT activity was significantly
477 higher in 2012 than in 2011 but only in fish from CER, in contrast to liver SOD which remained
478 unchanged for these fish, but which decreased between 2011 and 2012 in GAR and GIR. To
479 support the possible effect of organic contaminants on hepatic CAT enzyme in GAR and GIR,
480 other field studies have reported significant increases in CAT activity in yellow *A. anguilla*
481 exposed to organic contaminants (Regoli et al., 2003; Ahmad et al., 2006; Buet et al., 2006).

482 No significant relationships between EROD activity and tissue contaminant
483 concentrations were observed indicating that this biomarker response was not associated with

484 exposure to the measured persistent contaminants in the Gironde system (Tables S.6). Although
485 concentrations of organic contaminants in eels from GAR and GIR were higher in 2011, EROD
486 activity was twice higher in 2012 in fish from GIR compared to fish from DOR and CER and
487 was also higher in fish from GAR compared to DOR (Table 2). We hypothesize that other
488 chemical factors not measured, such as PAHs, may have contributed to the higher EROD activity
489 found in eels from GAR and GIR in 2012. Elevated concentrations of PAHs and pyrene were
490 detected in the muscle tissue of European flounder (*Platichthys flesus*) in the Gironde Estuary
491 (Laroche et al. 2012). Further studies are needed to confirm the link between PAH exposure and
492 EROD induction in eels from GAR and GIR.

493 Finally, the parasite *A. crassus* was detected only in *A. anguilla* (Table 2). The number of
494 *A. crassus* was not correlated with any biological, contaminant or biomarker variables (Table
495 S.6). In 2011, fish from the freshwater sites DOR and GAR presented a significantly higher rate
496 of infection compared to samples from the brackish water site GIR and the salt marsh site CER,
497 consistent with field and experimental studies having demonstrated that infection levels of *A.*
498 *crassus* decrease with an increase in salinity (Kirk et al., 2000; Morrison et al., 2003; Lefebvre
499 and Crivelli, 2012).

500

501 4. Conclusions

502 Our study revealed similarities in the biomarkers examined between *A. anguilla* and *A.*
503 *rostrata*, supporting their use in comparative ecotoxicological studies. The basal levels of EROD
504 activity and of biomarkers of histopathology were similar in eels of both species from reference
505 sites. A high deposition of hemosiderin was observed in eels from the most brackish sites in both
506 species. However, the cause of this deposition is uncertain. In *A. anguilla*, it was strongly
507 associated to As accumulation whereas in *A. rostrata*, it was weakly associated to Pb. Further
508 studies are needed on the relationship between environmental variations, migration and feeding
509 patterns and accumulation of potentially hemolytic agents in Atlantic eels growing in saline
510 water, and on the potential impact of this accumulation on blood cells integrity and on the
511 immune function.

512 In comparison to its American cousin from the St. Lawrence system, *A. anguilla* from
513 Southwest France may be more affected by contaminants. Higher CAT activity, density of MMC
514 and hemosiderin pigment were observed in eels from the contaminated sites GAR and GIR and
515 were related to organic and metal contaminants such as DDTs, PCBs, PBDEs and muscle Hg and

Cd. This, in association with the higher concentrations of PCBs, DDTs and metals observed in the tissues of *A. anguilla* from GAR and GIR compared to *A. rostrata*, suggests a more important risk of impacts of these contaminants in these eels compared to American eels from the more contaminated sites SPL and SFL. For *A. rostrata*, in both study years, neither the PCA nor ANOVA identified major influences of persistent organic or inorganic contaminants on EROD, catalase or histopathological biomarkers.

Field studies have already reported that the sensitivity of biomarkers could vary from one habitat to another in response to the complex mixtures of contaminants and other environmental conditions (Wolke, 1992; Van der Oost et al., 2003). Contaminants such as PAHs, dioxins and furans, not measured in our study, are known to induce biotransformation and antioxidant enzymes in the short-term and histopathological biomarkers over longer periods of exposure (Van der Oost et al., 2003). The yellow stage of both species are not strictly catadromous and perform short migrations in order to forage food in more productive environments (Daverat and Tomás, 2006; Thibault et al., 2007). Even if these migrations are short (≈ 10 km) compared to the reproductive migration of silver eels, they are of considerable importance because they occur during the prolonged growth stage and involve switching between environments varying widely in contaminants and other abiotic factors such as temperature and salinity (Thibault et al., 2007). Finally, the small number of eels that could be collected in this study and the wide range of estimated age (2 to 18 years old) may constitute biases and impose a requirement to be prudent regarding the overall conclusions. The peculiar biology of these species, which grow at widely different rates depending on their habitat (Patey et al, submitted), makes it impossible to eliminate biases such as size and age among study sites. For that purpose, complementary studies with larger sample size and a greater number of sites are recommended. Nevertheless, this study provides a database to better target and prioritize future studies on the impacts of contaminants on eel health in the Gironde and St. Lawrence River basins. Combined with others (Pierron et al., 2013; Baillon et al., 2015a; Baillon et al., 2015b; Baillon et al., 2016; Caron et al., 2016) it supports that contamination represents a risk to the health of European eels from the Gironde system that could be contributing to the lack of recovery of these fish.

5. Acknowledgments

We wish to acknowledge all the personnel of the INRS-ETE, UMR EPOC (university of Bordeaux) and IRSTEA (Bordeaux-Cestas) who participated in the field sampling. We are also

grateful to A. Caron, P. Pannetier and H. Budzinski for contaminant concentration measurements and to B. Légaré for his expert technical work on EROD assay. We would like to thank G. Verreault and the technical staff from the Ministère des Forêts, de la Faune et des Parcs for otolith expertise and for providing us with age data. This work was supported by the joint ANR Blanc (ANR-10-INTB-1704)-NSERC SGP grant IMMORTEEL and by the Aquitaine region (GAGILAU and MICANG projects). The laboratory work of Géraldine Patey was in part supported by Fisheries and Oceans Canada.

6. References

Acou, A., Boury, P., Laffaille, P., Crivelli, A. J., Feunteun, E., 2005. Towards a standardized characterization of the potentially migrating silver European eel (*Anguilla anguilla*, L.). Archiv für Hydrobiologie 164: 237-255

Agius, C., Roberts, R., 2003. Melano-macrophage centres and their role in fish pathology. J. of Fish Dis. 26, 499-509.

Ahmad, I., Pacheco, M., Santos, M.A., 2006. *Anguilla anguilla* L. oxidative stress biomarkers: an in situ study of freshwater wetland ecosystem (Pateira de Fermentelos, Portugal). Chemosphere 65, 952-962.

Au, D., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Mar. Pollut. Bull. 48, 817-834.

Baillon, L., Oses, J., Pierron, F., du Colombier, S.B., Caron, A., Normandeau, E., Lambert, P., Couture, P., Labadie, P., Budzinski, H., 2015a. Gonadal transcriptome analysis of wild contaminated female European eels during artificial gonad maturation. Chemosphere 139, 303-309.

Baillon, L., Pierron, F., Coudret, R., Normendeau, E., Caron, A., Peluhet, L., Labadie, P., Budzinski, H., Durrieu, G., Sarraco, J., 2015b. Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels. Ecotoxicology 24, 71-84.

Baillon, L., Pierron, F., Oses, J., Pannetier, P., Normandeau, E., Couture, P., Labadie, P., Budzinski, H., Lambert, P., Bernatchez, L., 2016. Detecting the exposure to Cd and PCBs by means of a non-invasive transcriptomic approach in laboratory and wild contaminated European eels (*Anguilla anguilla*). Environ. Sci. Pollut. R. 23, 5431-5441.

Belpaire, C., Goemans, G., 2007. Eels: contaminant cocktails pinpointing environmental contamination. ICES J. of Mar. Sci. 67, 1423-1436.

579 Belpaire, C.G.J., Goemans, G., Geeraerts, C., Quataert, P., Parmentier, K., Hagel, P., De Boer, J.,
580 2009. Decreasing eel stocks: survival of the fattest? Ecol. Freshw. Fish 18, 197-214.
581 Belpaire, C., Pujolar, J.M., Geeraerts, C., Maes, G.E., 2016. Contaminants in eels and their role
582 in the collapse of the eel stocks. Biol. Ecol. Anguillid Eels. 225
583 Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities
584 of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.
585 Buet, A., Banas, D., Vollaire, Y., Coulet, E., Roche, H., 2006. Biomarker responses in European
586 eel (*Anguilla anguilla*) exposed to persistent organic pollutants. A field study in the Vaccarès
587 lagoon (Camargue, France). Chemosphere 65, 1846-1858.
588 Byer, J.D., Lebeuf, M., Alaee, M., Brown, R.S., Trottier, S., Backus, S., Keir, M., Casselman, J.,
589 Hodson, P.V., 2013.Spatial trends of organochlorinated pesticides, polychlorinated biphenyls,
590 and polybrominated diphenyl ethers in Atlantic anguillid eels. Chemosphere 90,1719–1728.
591 Caron, A., Pannetier, P., Rosabal-Rodriguez, M., Budzinski, H., Lauzent, M., Labadie, P., Nasri,
592 B., Pierron, F., Baudrimont, M., Couture, P., 2016. Organic and inorganic contamination
593 impacts on metabolic capacities in American and European yellow eels. Can. J. Fish. Aquat.
594 Sci. 73 (10), 1557-1566.
595 Castonguay, M., Hodson, P., Couillard, C., Eckersley, M., Dutil, J.-D., Verreault, G., 1994. Why
596 is recruitment of the American eel, *Anguilla rostrata*, declining in the St. Lawrence River and
597 Gulf? Can. J. Fish. Aquat. Sci. 51, 479-488.
598 Chainy, G.B.N., Paital, B., Dandapat, J., 2016. An overview of seasonal changes in oxidative
599 stress and antioxidant defence parameters in some invertebrate and vertebrate species.
600 Scientifica 2016.
601 Cosewic, 2012. COSEWIC assessment and status report on the American eel *Anguilla rostrata*
602 in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 109pp.
603 Cottrill, R. A., McKinley, R. S., Van Der Kraak, G., 2002. An examination of utilizing external
604 measures to identify sexually maturing female American eels, *Anguilla rostrata*, in the St.
605 Lawrence River. Environ. Biol. Fish. 65, 271-287.
606 Couillard, C., Wirgin, I., Lebeuf, M., Legare, B., 2004. Reduction of cytochrome P4501A with
607 age in Atlantic tomcod from the St. Lawrence Estuary, Canada: relationship with emaciation
608 and possible effect of contamination. Aquat. Toxicol. 68, 233-247.

609 Couillard, C., Hodson, P., Castonguay, M., 1997. Correlations between pathological changes and
610 chemical contamination in American eels, *Anguilla rostrata*, from the St. Lawrence River.
611 Can. J. Fish. Aquat. Sci. 54, 1916-1927.
612 Couillard, C.M., Hodson, P.V., 1996. Pigmented macrophage aggregates: A toxic response in
613 fish exposed to bleached-kraft mill effluent? Environ. Toxicol. and Chem. 15, 1844-1854.
614 Daverat, F., Beaulaton, L., Poole, R., Lambert, P., Wickström, H., Andersson, J., Aprahamian,
615 M., Hizem, B., Elie, P., Yalçın-Özdilek, S., 2012. One century of eel growth: changes and
616 implications. Ecol. Freshw. Fish 21, 325-336.
617 Daverat, F., Tomás, J., 2006. Tactics and demographic attributes in the European eel *Anguilla*
618 *anguilla* in the Gironde watershed, SW France. Mar. Ecol. Prog. Ser. 307, p. 247-p. 257.
619 Durif, C., Dufour, S., Elie, P., 2005. The silvering process of *Anguilla anguilla*: a new
620 classification from the yellow resident to the silver migrating stage. J. Fish Biol. 66, 1025–
621 1043.
622 Edeline, E., Beaulaton, L., Le Barh, R., Elie, P., 2007. Dispersal in metamorphosing juvenile eel
623 *Anguilla anguilla*. Mar. Ecol. Prog. Ser. 344, 213-218.
624 FAO/ICES, 2009. FAO European Inland Fisheries Advisory Commission; International Council
625 for the Exploration of the Sea. Report of the 2008 session of the Joint EIFAC/ICES Working
626 Group on Eels. EIFAC Occasional paper(No. 43): 192pp.
627 Fournie, J.W., Summers, J.K., Courtney, L.A., Engle, V.D., Blazer, V.S., 2001. Utility of splenic
628 macrophage aggregates as an indicator of fish exposure to degraded environments. J. Aquat.
629 Anim. Health 13, 105-116.
630 Fragoso, N.M., Parrott, J.L., Hahn, M.E., Hodson, P.V., 1998. Chronic retene exposure causes
631 sustained induction of CYP1A activity and protein in rainbow trout (*Oncorhynchus mykiss*).
632 Environ. Toxicol. and Chem. 17, 2347-2353.
633 Geeraerts, C., Belpaire, C., 2010. The effects of contaminants in European eel: a review.
634 Ecotoxicology 19, 239-266.
635 Girard, P., Elie, P., 2007. Manuel d'identification des principales lésions
636 anatomomorphologiques et des principaux parasites externes des anguilles.
637 CEMAGREF/Association Santé Poissons Sauvages. p. 81
638 Gravato, C., Guimarães, L., Santos, J., Faria, M., Alves, A., Guilhermino, L., 2010. Comparative
639 study about the effects of pollution on glass and yellow eels (Anguilla anguilla) from the

estuaries of Minho, Lima and Douro Rivers (NW Portugal). Ecotoxicol. Environ. Saf. 73, 524–533

Gravato, C., Teles, M., Oliveira, M., Santos, M.A., 2006. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla L*. -the influence of pre-exposure to b-naphthoflavone. Chemosphere 65, 1821-1830

Gravato, C., Santos, M.A., 2002. Juvenile sea bass liver biotransformation and erythrocytic genotoxic responses to pulp mill contaminants. Ecotoxicol. Environ. Saf. 53, 104-112

Guhl, B., Stürenberg, FJ., Santora, G., 2014. Contaminant levels in the European eel (*Anguilla anguilla*) in North Rhine-Westphalian rivers. Environ. Sci. Eur. 26, 26.

ICES. 2016. Report of the Workshop of the Working Group on Eel and the Working Group on Biological Effects of Contaminants (WKBECEEL), 25–27 January 2016, Os, Norway. ICES CM 2015/SSGEPD:20. 98 pp.

Jessop, B.M., 2010. Geographic effects on American eel (*Anguilla rostrata*) life history characteristics and strategies. Can. J. Fish. Aquat. Sci. 67, 326-346.

Kessaci, K., Coynel, A., Blanc, G., Deycard, V.N., Derriennic, H., Schäfer, J., 2014. Impacts of anthropogenic pressures on the water quality of the Gironde Estuary (SW France) from the Urban Agglomeration of Bordeaux: spatial characterization and inputs of trace metal elements (Ag, As, Cd, Cu, Pb and Zn). EGU General Assembly Conference Abstracts, p. 10196.

Khan, R., Barker, D., Hooper, R., Lee, E., Ryan, K., Nag, K., 1994. Histopathology in winter flounder (Pleuronectes americanus) living adjacent to a pulp and paper mill. Arch. Environ. Contam. and Toxicol. 26, 95-102.

Khan, R., Kiceniuk, J., 1984. Histopathological effects of crude oil on Atlantic cod following chronic exposure. Can. J. Zool. 62, 2038-2043.

Kirk, R., Kennedy, C., Lewis, J., 2000. Effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae. Dis. Aquat. Organ. 40, 211-218.

Laporte, M., Pavey, S.A., Rougeux, C., Pierron, F., Lauzent, M., Budzinski, H., Labadie, P., Geneste, E., Couture, P., Baudrimont, M., 2016. RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. Mol. Ecol. 25, 219-237.

Larsson, P., Hamrin, S., Okla, L., (1990) Fat content as a factor inducing migratory behaviour in the eel (*Anguilla anguilla L.*) to the Sargasso Sea. Naturwissenschaften, 77, 488-490.

671 Lefebvre, F., Crivelli, A.J., 2012. Salinity effects on anguillicolosis in Atlantic eels: a natural
672 tool for disease control. Mar. Ecol. Prog. Ser. 471, 193-202.
673 Lefebvre, F., Mounaix, B., Poizat, G., Crivelli, A.J., 2004. Impact of the swimbladder nematode
674 *Anguillicola crassus* on *Anguilla anguilla*: variation in liver and spleen masses. J. Fish Biol.
675 435-447.
676 Livingstone, D., 2001. Contaminant-stimulated reactive oxygen species production and oxidative
677 damage in aquatic organisms. Mar. pollut. bull. 42, 656-666.
678 Luna, L.G., 1968. Manual of histologic staining methods of the Armed Forces Institute of
679 Pathology. Graw-Hill Book Company, New York. 3rd edition. 258 pp.
680 Maes, J., Belpaire, C., Goemans, G., 2007. Spatial variations and temporal trends between 1994
681 and 2005 in polychlorinated biphenyls, Organochlorine pesticides and heavy metals in
682 European eel (*Anguilla anguilla L.*) in Flanders, Belgium. Environ. Pollut. 153, 223–237
683 Martinez-Alvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and
684 abiotic factors. Fish Biol. Fisher. 15, 75-88.
685 Meinelt, T., Krüger, R., Pietrock, M., Osten, R., Steinberg, C., 1997. Mercury pollution and
686 macrophage centres in pike (*Esox lucius*) tissues. Environ. Sci. and Pollut. Res. 4, 32-36.
687 Moravec, F., Taraschewski, H., 1988. Revision of the genus *Anguillicola Yamaguti*, 1935
688 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptions of two new
689 species, *A. novaezelandiae sp. n.* and *A. papernai sp. n.* Folia Parasitol. 35, 125-146.
690 Morrison, W.E., Secor, D.H., Piccoli, P.M., 2003. Estuarine habitat use by Hudson River
691 American eels as determined by otolith strontium: calcium ratios. American Fisheries Society
692 Symposium, pp. 87-99.
693 Palstra, A.P., van Ginneken, V.J., Murk, A.J., van den Thillart, G.E., 2006. Are dioxin-like
694 contaminants responsible for the eel (*Anguilla anguilla*) drama? Naturwissenschaften 93, 145-
695 148.
696 Pankhurst N. W., 1982. Relation of visual change to the onset of sexual maturation in the
697 European eel Anguilla anguilla (L.). J. Fish Biol., 21, 279-296.
698 Pannetier, P., Caron, A., Campbell, P.G., Pierron, F., Baudrimont, M., Couture, P., 2016. A
699 comparison of metal concentrations in the tissues of yellow American eel (*Anguilla rostrata*)
700 and European eel (*Anguilla anguilla*). Scien. Total Environ. 569, 1435-1445.
701 Patey, G., Couillard, C.M., Drouineau, H., Verreault, G., Pierron, F., Lambert, P., Baudrimont,
702 M., Couture, P. Early back-calculated size-at-age of Atlantic yellow eels sampled along

ecological gradients in the Gironde and St. Lawrence hydrographical systems. Submitted to Can. J. Fish. Aquat. Sci. (MS# CJFAS-2017-0025).

Pegg, J., Andreou, D., Williams, C., Britton, J., 2015. Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite. Parasitology 142, 1579-1587.

Pierron, F., Baillon, L., Sow, M., Gotreau, S., Gonzalez, P., 2013. Effect of low-dose cadmium exposure on DNA methylation in the endangered European eel. Environ. Sci. Technol. 48, 797-803.

Pierron, F., Baudrimont, M., Bossy, A., Bourdineaud, J.-P., Brèthes, D., Elie, P., Massabuau, J.-C., 2007. Impairment of lipid storage by cadmium in the European eel (*Anguilla anguilla*). Aquat. Toxicol. 81, 304-311.

Regoli, F., Winston, G.W., Gorbi, S., Frenzilli, G., Nigro, M., Corsi, I., Focardi, S., 2003. Integrating enzymatic responses to organic chemical exposure with total oxyradical absorbing capacity and DNA damage in the European eel *Anguilla anguilla*. Environ. Toxicol. and Chem. 22, 2120-2129.

Rigaud, C., Couillard, C.M., Pellerin, J., Légaré, B., Byer, J.D., Alaee, M., Lebeuf, M., Casselman, J.M., Hodson, 2016. Temporal vatiations in embryotoxicity of Lake Ontario American eel (*Anguilla rostrata*) extracts to developing *Fundulus heteroclitus*. Sci. Tot. Environ. 541, 765-775.

Robinet, T.T., Feunteun, E.E., 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels? Ecotoxicology 11, 265-277.

Schwindt, A.R., Fournie, J.W., Landers, D.H., Schreck, C.B., Kent, M.L., 2008. Mercury concentrations in salmonids from western US national parks and relationships with age and macrophage aggregates. Environ. Sci. Technol. 42, 1365-1370.

Sorensen, E.M., Smith, N.K., 1981. Hemosiderin granules: cytotoxic response to arsenic exposure in channel catfish. Bull. Environ. Contam. Toxicol. 27, 645-653.

Tapie, N., Le Menach, K., Pasquaud, S., Elie, P., Devier, M.H., Budzinski, H., 2011. PBDE and PCB contamination of eels from the Gironde estuary: from glass eels to silver eels. Chemosphere 83, 175-185.

Thibault, I., Dodson, J.J., Caron, F., Tzeng, W., Iizuka, Y., Shiao, J., 2007. Facultative catadromy in American eels: testing the conditional strategy hypothesis. Mar. Ecol. Prog. Ser. 344, 219-229.

735 Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in
736 environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57-149.
737 Van der Oost, R., Goksøyr, A., Celander, M., Heida, H., Vermeulen, N.P., 1996. Biomonitoring
738 of aquatic pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: pollution-induced
739 biochemical responses. Aquat. Toxicol. 36, 189-222.
740 Verreault, G., Dargere, W., Tardif, R., 2009. American eel movements, growth, and sex ratio
741 following translocation. Eels at the edges: Science, status and conservation concerns.
742 American Fisheries Society, Symposium, pp. 129-136.
743 Vinodhini, R., Narayanan, M., 2008. Bioaccumulation of heavy metals in organs of fresh water
744 fish *Cyprinus carpio* (Common carp). Int. J. Environ. Res. 5, 179-182.
745 Vøllestad, L.A., 1992. Geographic variation in age and length at metamorphosis of maturing
746 European eel: environmental effects and phenotypic plasticity. J. Anim. Ecol., 41-48.
747 Whyte, J.J., Jung, R., Schmitt, C., Tillitt, D., 2008. Ethoxyresorufin-O-deethylase (EROD)
748 activity in fish as a biomarker of chemical exposure. Crit. Rev. Toxicol. 30, 357-570.
749 Wolke, R., 1992. Piscine macrophage aggregates: a review. Annu. Rev. Fish Dis. 2, 91-108.
750

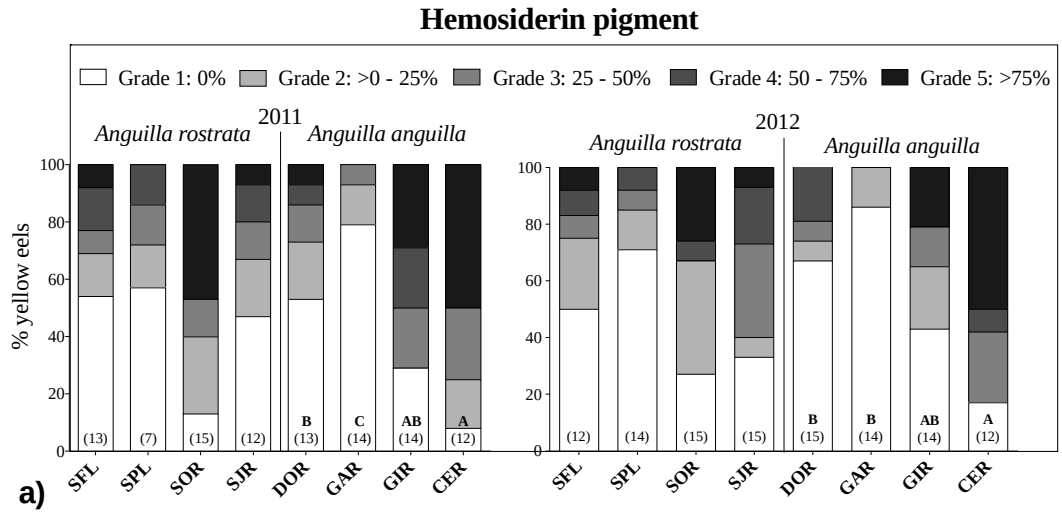
Table 1: Explained variance (%) for each significant principal component (PC) of the three different PCA followed by the significant variables in each PC. Variables significantly correlated with the principal components (PC1, PC2, PC3) in each PC are noted in bold.

Species	***Anguilla rostrata***			***Anguilla anguilla***		
Years	**2011-2012**			**2011-2012**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Explained variance (%)	22.21	8.07	6.63	19.72	11.05	8.01
Variables						
Body Length (mm)	**11.39**	2.64	0.09	5.37	**9.82**	3.36
Body Mass (g)	**11.56**	3.67	0.15	4.67	**11.39**	3.81
Lipid content (%)	**8.36**	5.48	0.01	4.44	5.01	2.22
Age (year)	2.29	6.38	**12.09**	**8.61**	0.02	7.45
Number *A. crassus*	0.00	0.00	0.00	0.07	9.26	1.02
EROD (pmol/min/mg protein)	0.67	0.18	4.88	0.36	0.03	**9.66**
Catalase (µmol/min/mg protein)	0.07	1.66	1.71	3.58	0.01	1.00
Superoxide dismutase (µmol/min/mg protein)	2.45	0.72	1.23	0.09	0.11	4.19
Density of MMCs (number/mm^2)	0.04	0.38	**16.08**	1.37	1.06	3.28
Surface of MMCs (MMC/mm^2)	0.08	1.95	**21.72**	3.54	2.09	5.45
Hemosiderin pigment (median of grades)	1.68	1.58	**13.77**	0.00	**11.29**	5.24
Lipofuscin pigment (median of grades)	0.02	0.01	0.01	1.91	0.00	0.59
Hexachlorobenzene (ng/g dw)	6.64	6.51	2.11	5.72	0.32	6.12
Lindane (ng/g dw)	0.04	0.10	0.14	0.18	7.67	**9.81**
Sum of DDT and metabolites (ng/g dw)	**10.57**	1.93	0.00	**12.08**	1.68	1.12
Sum of PBDEs (ng/g dw)	**8.27**	0.35	0.35	**9.33**	3.60	1.07
Sum of PCBs (ng/g dw)	**11.30**	0.14	0.01	**10.77**	1.10	3.59
Muscle As (µg/g dw)	4.87	**14.33**	0.00	0.39	**12.52**	4.64
Muscle Ag (µg/g dw)	0.32	7.54	0.02	0.03	0.84	2.60
Muscle Cd (µg/g dw)	0.65	0.79	3.71	0.19	2.04	5.95
Muscle Pb (µg/g dw)	0.40	2.27	0.01	0.07	2.32	0.44
Muscle Hg (µg/g dw)	4.76	8.01	4.39	**7.57**	1.89	2.59
Liver As (µg/g dw)	3.96	**13.44**	0.71	0.17	**12.50**	3.98
Liver Ag (µg/g dw)	3.13	7.67	0.00	**6.08**	0.65	0.13
Liver Cd (µg/g dw)	0.50	8.49	**13.33**	**7.03**	2.05	0.36
Liver Pb (µg/g dw)	**5.99**	3.77	3.50	**6.39**	0.74	**10.34**

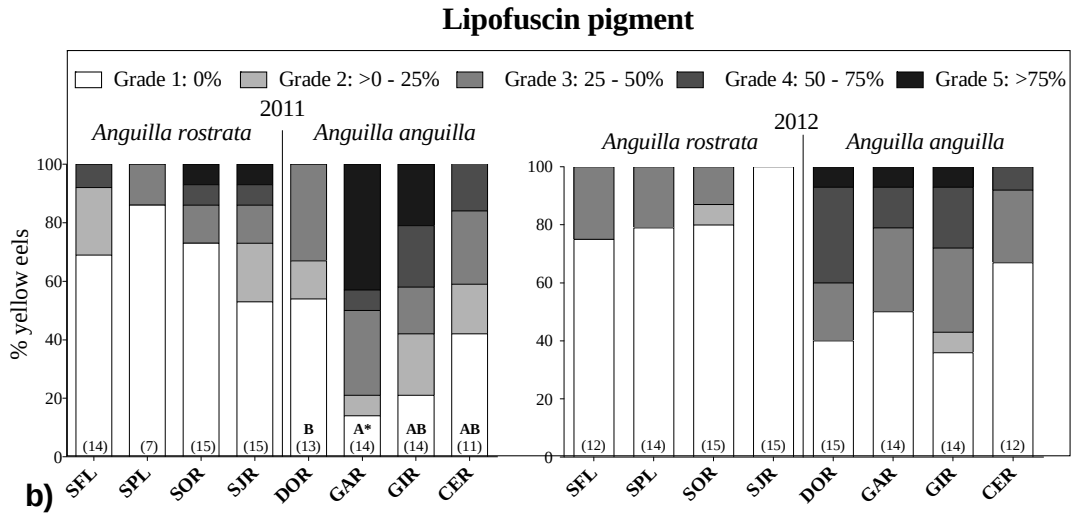
Table 2: Spatial and temporal variations of hepatic EROD activity, antioxidant enzymes, splenic melanomacrophage centers (MMCs) and number of *A. crassus* (median (Q1; Q3) (n)) of yellow *A. rostrata* in the St. Lawrence system and *A. anguilla* in the Gironde system in 2011 and 2012. Different letters indicate significant differences among sites within species and within sampling years (ANOVA or KW, $p \leq 0.05$). An asterisk * indicates a significant difference between 2011 and 2012 (T-test, $p < 0.05$). DOR= Dordogne; GAR= Garonne; GIR= Gironde; CER= Certes; SFL = Saint-François Lake; SPL = Saint-Pierre Lake; SOR = Sud-Ouest River; SJR = Saint-Jean River; NA= Absent.

Species		***Anguilla rostrata***				***Anguilla anguilla***			
Biomarkers	**Years**	SFL	SPL	SOR	SJR	DOR	GAR	GIR	CER
Liver EROD (pmol/min/mg protein)	2011	7.1 (5.7; 8.8) (15)	8.9 (5.2; 31.6) (7)	10.5 (7.9; 15.7) (15)	8.7 (7.6; 10.3) (12)	11.9 (6.3; 13.9) (13)	7.2 (6.1; 14.2) (14)	10.4 (6.9; 12.4) (15)	8.8 (5.1; 11.2) (11)
	2012	5.1 (3.7; 11.7) (12)	7.1 (6.1; 9.9) (14)	11.6 (8.9; 15.9) (15)	11.4 (8.5; 22.5) (15)	9.9 (6.2; 11.0) **C** (15)	18.6 (13.9; 25.7) **AB** (15)	19.7 (16.9; 40.2) **A** (14)	13.3 (8.9; 16.3) **BC** (14)
Liver Catalase (µmol/min/mg protein)	2011	2368 (1827; 2896) **BC** (14)	4078 (2657; 5638) **AB** (7)	3991 (3102; 4312) **A*** (12)	2246 (1971; 2647**)** **C** (15)	2319 (1902; 2581) **A** (8)	2263 (1455; 2904) **A** (14)	3515 (2495; 4931) **A** (14)	893(631; 984) **B** (10)
	2012	2168 (1323; 3319) (12)	2260 (1534; 3598) (14)	1953 (1663; 2641) (13)	2350 (1523; 2887) (15)	1961 (1567; 2408) **AB** (12)	1834 (1606; 2039) **B** (13)	2768 (2296; 3589) **A** (14)	1919 (1544; 3148) **AB*** (12)
Liver superoxide dismutase (µmol/min/ mg protein)	2011	5322 (2635; 7629) **A** (12)	962 (619; 2044) **B** (5)	5404 (4627; 7261) **A** (12)	5058 (3793; 5850) **A** (14)	3910 (3061; 4694) (8)	4281 (2933; 4901) * (14)	3651 (3161; 5261) * (14)	3857 (2762; 4482) (10)
	2012	3320 (2092; 5498**)** **B** **(**12)	3851 (3346; 5305) **AB*** **(**14)	6275 (4605; 7646) **A** **(**14)	4345 (3008; 6620) **AB** (15)	3203 (2592; 4175) * (12)	2766 (1775; 3623) (12)	2398 (1827; 3155) (14)	3037 (1957; 3838) (12)
Density of MMCs (number/mm^2)	*2011*	2.1 (1.4; 2.8) (15)	2.8 (1.7; 4.5) (7)	2.4 (1.7; 3.1) (15)	2.4 (1.7; 3.1) (15)	4.2 (2.8; 5.1) (13)	2.8 (2.1; 3.9) (14)	3.8 (2.4; 4.5) (15)	2.8 (2.4; 4.5) (11)
	2012	2.8 (2.4; 3) (12)	2.6 (1.7; 3.2) (14)	3.5 (2.4; 3.8) (15)	2.8 (2.1; 3.5) (15)	2.8 (2.1; 4.2) **AB** (15)	4.5 (2.4; 6.3) **A** (15)	2.8 (2.3; 5.8) **AB** (14)	1.9 (1.7; 2.3) **B** (12)
Surface of MMCs (MMC/mm^2)	2011	0.003 (0.002; 0.005) (15)	0.005 (0.003; 0.006) (7)	0.005 (0.003; 0.007) (15)	0.002 (0.002; 0.004) (15)	0.003 (0.002; 0.005) **B** (13)	0.004 (0.003; 0.007) **AB** (14)	0.007 (0.006; 0.01) **A*** (15)	0.003 (0.002; 0.007) **AB** (11)
	2012	0.004 (0.004; 0.006) (12)	0.003 (0.002; 0.004) (14)	0.005 (0.003; 0.007) (15)	0.003 (0.001; 0.006) (15)	0.005 (0.004; 0.008) * (15)	0.006 (0.004; 0.008) (15)	0.005 (0.003; 0.005) (14)	0.005 (0.003; 0.008) (12)
Number of *A. crassus*	2011	NA	NA	NA	NA	1 (0.5; 3) **A*** (13)	2.5 (0; 4.3) **A** (14)	0 **B** (15)	0 **B** (11)
	2012	NA	NA	NA	NA	0 (0; 0) (15)	0 (0; 5) (15)	0 (0; 1) (14)	0(0; 0.3) (14)

1

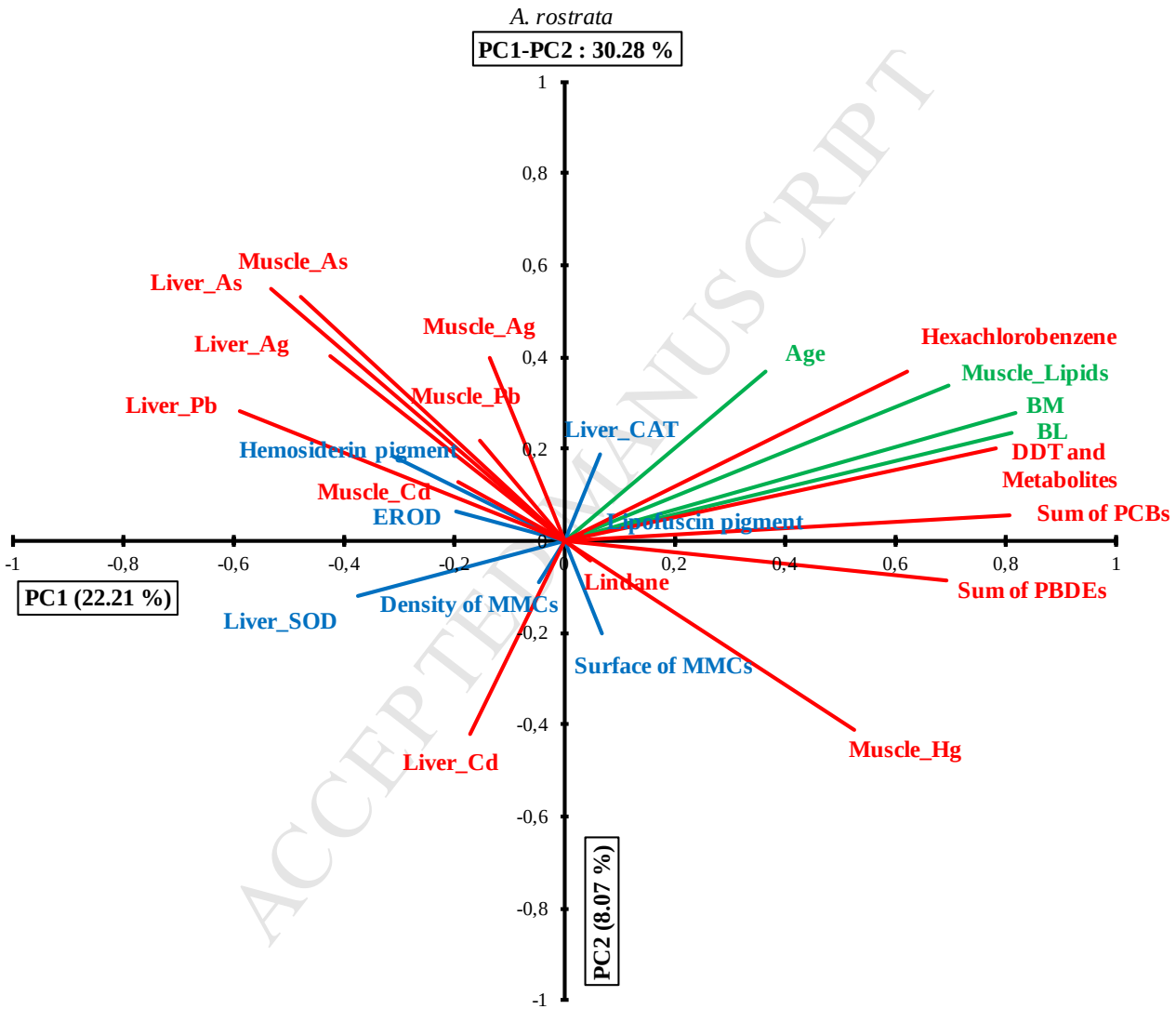


2

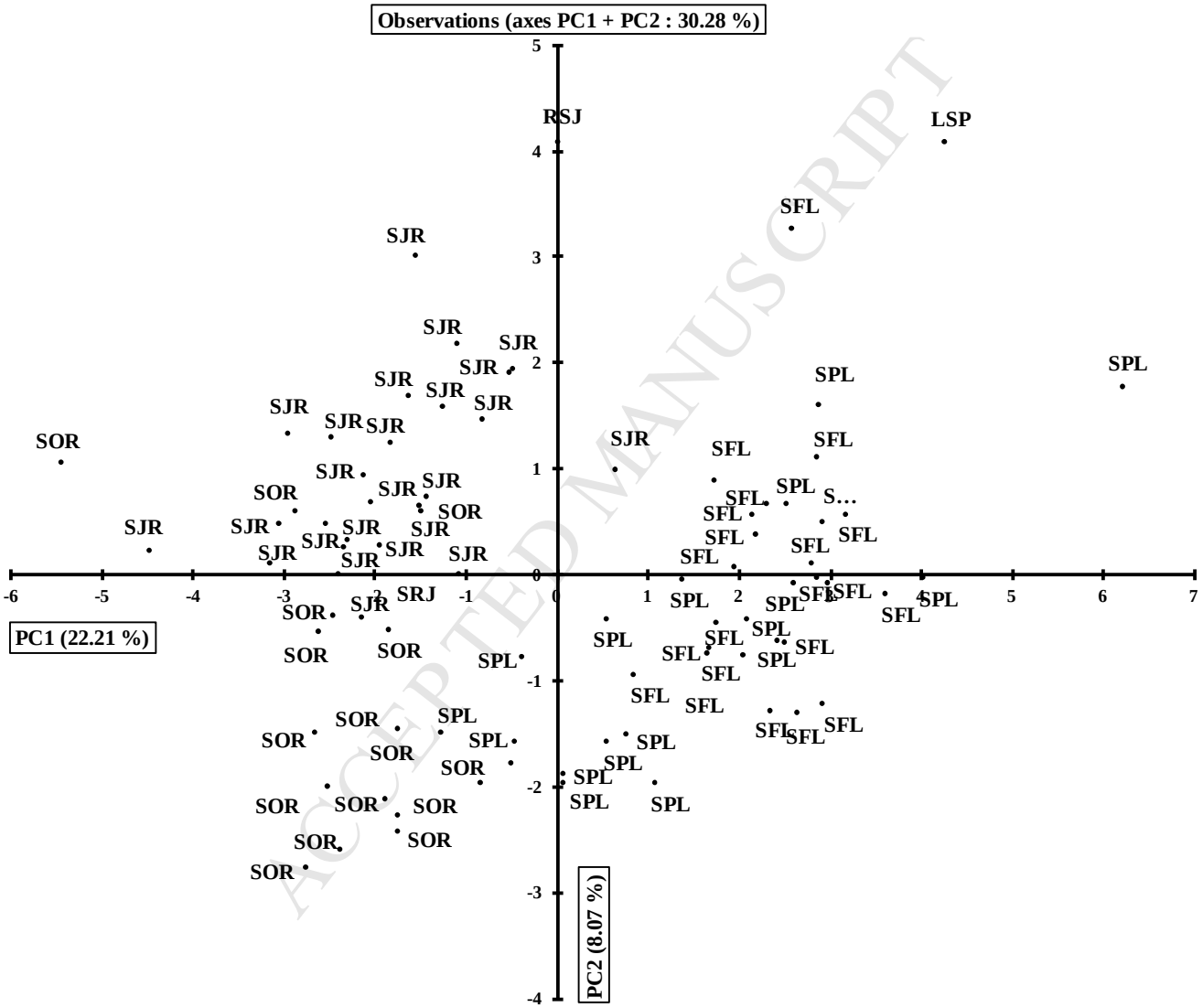


3

4 **Figure 1.** Spatio-temporal variations of the relative proportions of *A. rostrata* in the St.
5 Lawrence System and *A. Anguilla* in the Gironde System with different grades of **a)**
6 hemosiderin and **b)** lipofuscin pigment depositions in spleen MMCs in 2011 and 2012.
7 Sample size (n) is indicated in parentheses. Different letters indicate significant differences
8 among sites within species and within a sampling year (Kruskal-Wallis test, $p \leq 0.05$).

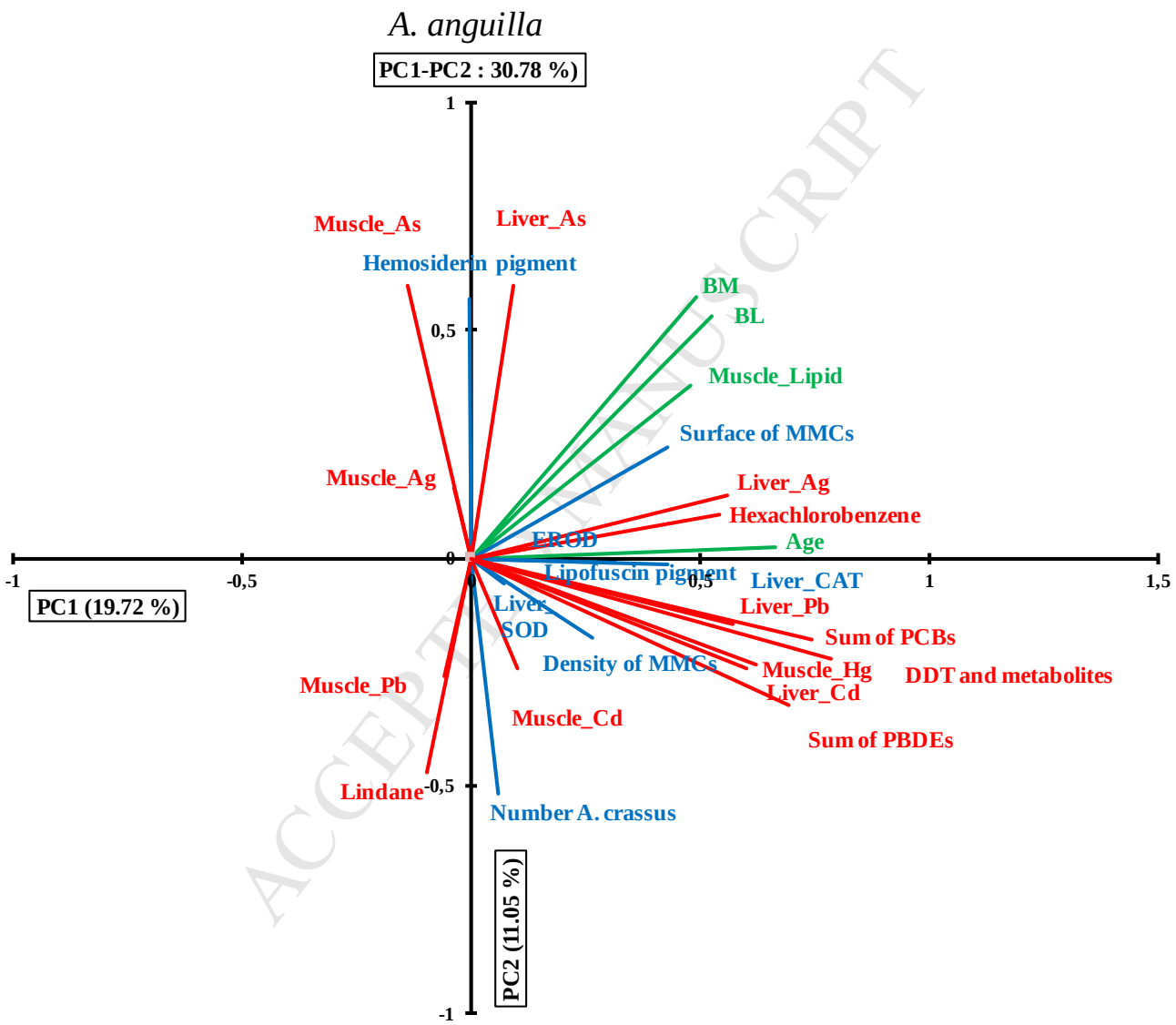

A. rostrata
PC1-PC2 : 30.28 %
PC1 (22.21 %)
PC2 (8.07 %)
Muscle_As
Liver_As
Liver_Ag
Muscle_Ag
Muscle_Pb
Liver_Pb
Hemosiderin_pigment
Muscle_Cd
EROD
Liver_SOD
Density of MMCs
Liver_CAT
Age
Hexachlorobenzene
Muscle_Lipids
BM
BL
DDT and Metabolites
Sum of PCBs
Lipofuscin pigment
Lindane
Sum of PBDEs
Surface of MMCs
Muscle_Hg
Liver_Cd

9 a)



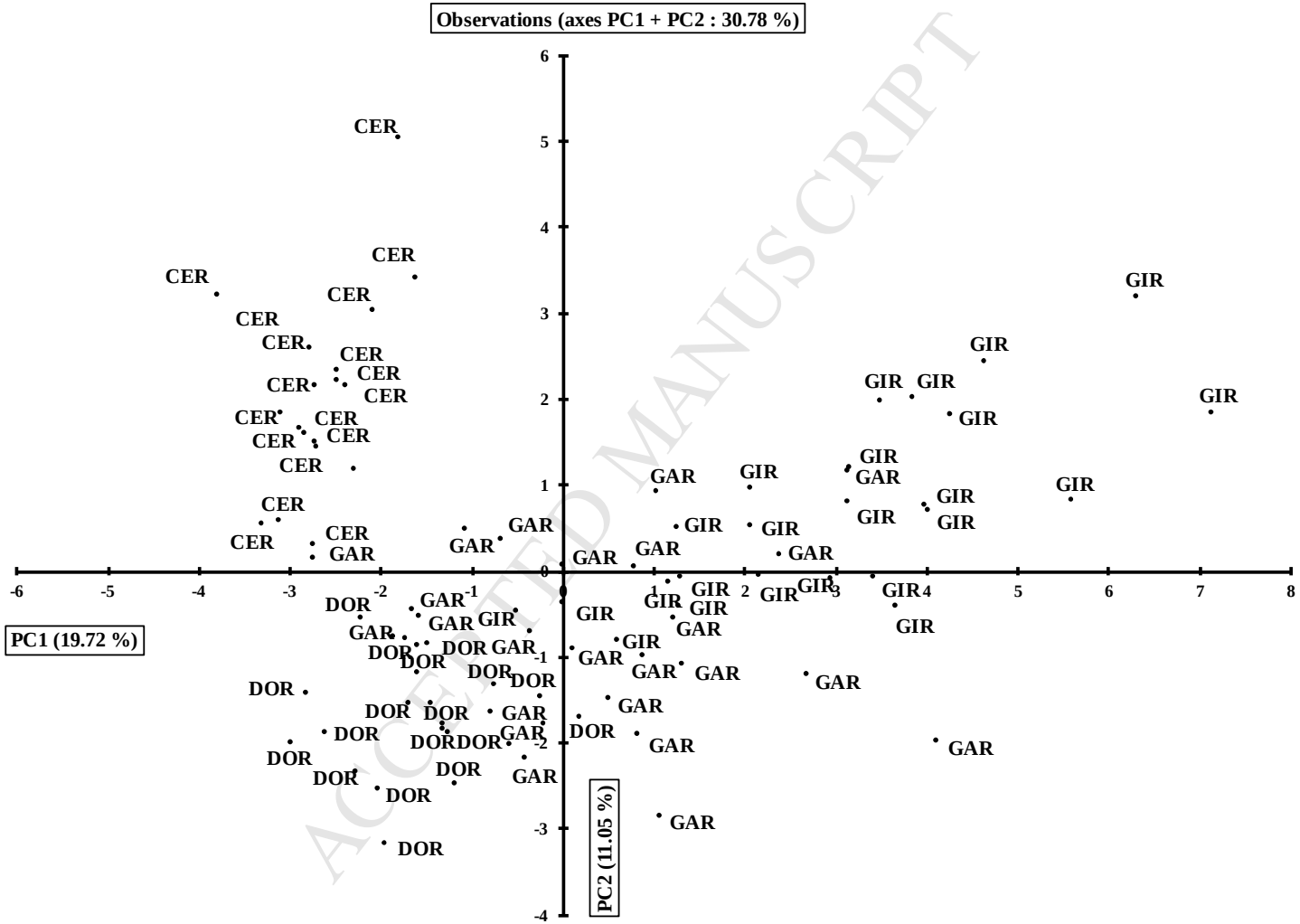
10 b)

Figure 2. Principal component analysis (PCA) (n=82) illustrating: a) Relationships between patterns of contamination (red), biomarker response (blue) and biological characteristics (green) in *A. rostrata* in 2011 and 2012 and b) Screening of eels from two fluvial contaminated sites, Lake St. Pierre (LSP) and Lake St. François, (LSF) and two references sites, St Jean River (SJR) and Sud-Ouest River (SOR). Scores for the principal components 1 and 2 were 22.21% and 8.07%, respectively. Sites of capture are identified for each individual. BM: Body Mass, BL: Body Length, MMCs: Melano-Macrophage centers, SOD: superoxide dismutase and CAT: catalase.



17 a)

18



19 b)

Figure 3. Principal component analysis (PCA) (n=87) illustrating: a) Relationships existing between the pattern of contamination (red), biomarker responses (green) and biological characteristics (blue) in *A. anguilla* in 2011 and 2012 and b) Screening of eels from contaminated sites, Gironde estuary (GIR) and Garonne River (GAR) and two references sites, Dordogne River (DOR) and the salt marsh Certes (CER). Scores for the principal components 1 and 2 were 19.72% and 11.05%, respectively. Sites of capture are identified for each individual. BM: Body Mass, BL: Body Length, MMCs: Melano-Macrophage centers, SOD: superoxide dismutase and CAT: catalase

Highlights

- Patterns of contaminants and biological characteristics differed among sites and species.
- In American eels, biomarker responses did not differ clearly among sites and years.
- In European eels, biomarker responses were higher in contaminated sites.
- High spleen hemosiderin deposition was observed in eels from the most brackish sites.
- European eels may be more affected by contaminants than American eels.