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**28 Abstract**

29 Identifying specific effects of contaminants in a multi-stress field context remain a challenge  
30 in ecotoxicology. In this context, "omics" technologies, by allowing the simultaneous  
31 measurement of numerous biological endpoints, could help unravel the *in situ* toxicity of  
32 contaminants. In this study, wild Atlantic eels were sampled in 8 sites presenting a broad  
33 contamination gradient in France and Canada. The global hepatic transcriptome of animals  
34 was determined by RNA-Seq. In parallel, the contamination level of fish to 8 metals and 25  
35 organic pollutants was determined. Factor analysis for multiple testing was used to identify  
36 genes that are most likely to be related to a single factor. Among the variables analyzed,  
37 arsenic (As), cadmium (Cd), lindane ( $\gamma$ -HCH) and the hepato-somatic index (HSI) were found  
38 to be the main factors affecting eel's transcriptome. Genes associated with As exposure were  
39 involved in the mechanisms that have been described during As vasculotoxicity in mammals.  
40 Genes correlated with Cd were involved in cell cycle and energy metabolism. For  $\gamma$ -HCH,  
41 genes were involved in lipolysis and cell growth. Genes associated with HSI were involved in  
42 protein, lipid and iron metabolisms. Our study proposes specific gene signatures of pollutants  
43 and their impacts in fish exposed to multi-stress conditions.

44 Keywords: transcriptome, multi-pollutant, wild fish populations, Atlantic eels

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## 50 **Introduction**

51 Identifying and predicting the specific impacts of contaminants on wild organisms still  
52 remains a huge challenge. Indeed, aquatic ecosystems not only present a variety of man-  
53 induced stressors but also vary spatially and temporally due to variations in natural factors  
54 (Thrush et al. 2009). Moreover, in a multi-stress context, the effects of a given factor can be  
55 modulated or amplified in interaction with another, and organisms can be more vulnerable if  
56 additive or synergistic effects occur (Lapointe et al. 2011).

57 In this context, particular emphasis was placed in recent years on the potential use of “omics”  
58 technologies in ecotoxicology. By allowing a global approach, “omics” technologies have  
59 indeed the potential to discover new mechanisms of toxicity and to classify the toxicity of  
60 contaminants. In molecular medicine, where these technologies (i.e. genomic, transcriptomic,  
61 epigenomic, proteomic or metabolomic) are already widely used, successful prospective and  
62 diagnostic cases studies were conducted with such approaches (Vlaanderen et al. 2010). In  
63 2008, the fish toxicogenomics consortium thus proposed to use “omic” technologies in the  
64 aquatic risk assessment process (Van Aggelen et al. 2010). Among the “omics” technologies,  
65 the recent development of next generation sequencing (NGS) technologies and bioinformatic  
66 tools to analyze these large datasets offers the opportunity for ecotoxicologists to investigate  
67 the effects of contaminants on genome-wide evolution or on transcriptome-wide response in  
68 non-model but environmentally relevant species (Webster and Bury 2013; Regier et al. 2013).  
69 Gene expression profiling using RNA-seq (also called whole transcriptome shotgun  
70 sequencing) could play a key role to identify new biomarkers of exposure and adverse effects  
71 and to discover new toxicity pathways.

72 In the present study, we thus used RNA-seq technology on two threatened species, the  
73 European eel (*Anguilla anguilla*) and the American eel (*Anguilla rostrata*), to test whether  
74 changes in transcriptome profiles in wild fish may be used to detect and unravel the toxicity

75 of pollutants in a context of *in situ* exposure. Atlantic eels are catadromous fish species with a  
76 complex life cycle including marine (spawning, larval phase and sexual maturation) and  
77 continental (feeding and somatic growth) environments. Historically abundant and  
78 widespread in Europe and North America, populations of Atlantic eels have suffered a sharp  
79 decline. European eel's recruitment is only one tenth of what it was in the early 1980s (Stone  
80 2003; ICES report 2009). In Canada, and more precisely in one of the most important rivers  
81 of the North American continent, the St. Lawrence River, the situation appears to be equally  
82 worrying. Available data show a decrease in recruitment of around 40 to 80% depending on  
83 the sites and years, some sites having registered a decrease of 98% since early 1980s (de  
84 Lafontaine et al. 2010). In view of these data, the European species is currently considered as  
85 critically endangered (IUCN red list) and the American eel was recently designated as  
86 threatened species by the Committee on the Status of Endangered Wildlife in Canada. As the  
87 unusual life cycle of Atlantic eels makes them particularly vulnerable to pollution, Atlantic  
88 eels are considered as sentinel species in ecotoxicology (Belpaire and Goemans 2007;  
89 Geeraerts and Belpaire 2010).

90 Thus, wild immature yellow eels (i.e. growth somatic phase) were sampled in 8 sites (4  
91 Canadian and 4 French sites) presenting a contamination gradient and also a wide range of  
92 physicochemical conditions notably in term of salinity and temperature. In France, European  
93 eels were sampled along a historically cadmium-contaminated hydrosystem, the Garonne–  
94 Gironde continuum (Durrieu et al. 2005). In addition, recent investigations have reported high  
95 concentrations of PCBs in the muscle of eels inhabiting the Gironde estuary (up to 3399 ng.g<sup>-1</sup>  
96 dw, Tapie et al. 2011). In Canada, fish were sampled along the St. Lawrence River. Past  
97 studies carried out on the muscle of American eels inhabiting this hydrosystem (Hodson et al.  
98 1994) have reported high concentrations in PCBs (up to 0.83 µg.g<sup>-1</sup> wet w), organochlorinated  
99 pesticides (up to 0.70 µg.g<sup>-1</sup> wet w) and in mercury (up to 0.26 µg.g<sup>-1</sup> wet w). The individual

100 hepatic transcriptome of 6 replicate specimens of eel per sampling site was determined by  
101 high throughput RNA sequencing using Illumina Hiseq2000 technology. In parallel, the  
102 contamination levels of fish in 25 organic pollutants and 8 metals were determined. Then, we  
103 carried out correlation analyses between the transcriptional level of a given gene and  
104 contaminant concentrations in each individual fish. Rather than perform comparisons among  
105 sampling sites, we combined fish from all sites before carrying out the statistical analyses. In  
106 addition, data from both European and American eels were combined before carrying our  
107 statistical analyses in order to (i) identify common genes and thus potential biomarkers of  
108 exposure that could be extrapolated across fish species and (ii) to work with the widest range  
109 of physic-chemical conditions and thus to remove as much as possible genes that are more  
110 likely to be affected by natural factors. Finally, condition indices such as the relative  
111 condition index (Kn), the hepato-somatic index (HSI) and the spleen somatic index (SSI)  
112 were added in the model in order to identify genes for which transcription levels could be  
113 associated to the general health status of animals.

114

115

## 116 **Materials and methods**

117

### 118 **Wild eel sample collection**

119

120 Eight sampling sites were selected in Quebec (Canada) and in France on the basis of their  
121 known gradient of contamination by metallic and organic pollutants (Lee et al. 1999; Durrieu  
122 et al. 2005). In Quebec, yellow eels (sexually undetermined fish) were collected in four sites  
123 (see table 1) located near or in the St Lawrence Estuary (St Jean Lake: 48°51'40''N  
124 64°28'47''O, Sud-Ouest River: 48°22'27''N 68°43'02''O, St Pierre Lake: 46°09'18''N

125 74°23'04''WO, St François Lake: 46°19'50''N, 74°23'04''WO). In France, immature yellow  
126 eels were collected in four sites along the Gironde aquatic system (Dordogne: 44°54'30.30"N  
127 0°15'00.61"O, Garonne: 45°12'06.62"N 0°43'34.72"O and Gironde estuary: 45°12'06.62"N  
128 0°43'34.72"O), and in the Arcachon Bay, considered as a pristine environment (Certes:  
129 44°41'18"N 1°1'39"W). For all sites, temperature, salinity and dissolved oxygen  
130 concentrations were measured.

131 All fish were collected between May 24 and June 24 of 2011, using a trawl, a fyke net or by  
132 electrical fishing. All yellow eels were size selected to minimize potential allometric bias. The  
133 total length of American and European eels ( $672.8 \pm 38.55$  mm and  $482.04 \pm 31.01$  mm,  
134 respectively; mean  $\pm$  SE, n = 24) and weight ( $811.42 \pm 118.41$  g and  $221.33 \pm 34.06$  g,  
135 respectively; mean  $\pm$  SE, n = 24) were recorded for each fish in order to estimate the relative  
136 condition factor (Kn:  $(\text{total weight (g)} / (0.0004 * (\text{total length (cm)})^{3.3828}) * 100)$ , the Hepato-  
137 Somatic Index (HSI:  $(\text{weight liver} / \text{total weight}) * 100$ ) and spleen condition index (SSI: (  
138 spleen weight / total weight) \* 100). For each sampling region, a total of 6 fish were collected  
139 and used for subsequent analyses. Fish were dissected as soon as possible and organs were  
140 divided into several samples. Samples for gene transcription analyses were stored in RNA  
141 later at -20°C until needed. For both organic and metal analyses, samples were stored at -  
142 80°C.

143

#### 144 Metal analyses

145 Metal analyses were determined from the kidneys, i.e. a main organ of metal bioaccumulation  
146 (Barbier et al. 2005). Kidney samples were freeze-dried in PFA vials (Savillex) and kept in  
147 these vials during the whole digestion process. A 50 mg sub-sample was taken after the  
148 freeze-drying step to perform the mercury analysis. These sub-samples were processed in a  
149 mercury analyser (Milestone DMA-80) and MESS-3 certified reference material (National

150 Research Council Canada) was used, with a recovery rate of  $98.8 \pm 2.5\%$ . The rests of the  
151 samples were digested overnight at room temperature in pure trace metal grade nitric acid  
152 ( $\text{HNO}_3$ ). The following day, sample digestion was completed by autoclave (All American  
153 25X-1) for 2 hours at 15 PSI. Samples were then diluted in Milli-Q water to obtain a final  
154 concentration of 10%  $\text{HNO}_3$ . Metal concentrations for Ag, As, Cd, Cr, Cu, Ni, Pb and Zn  
155 were measured by inductively coupled plasma - mass spectrometry (ICP-MS; Thermo  
156 Scientific XSeries 2), inductively coupled plasma - atomic emission spectrometry (ICP-AES;  
157 Varian Vista AX) or both methods. Blanks and standards (TORT-2 and DOLT-4, National  
158 Research Council Canada) were submitted to the same digestion protocol as kidney samples  
159 to monitor the efficiency of the procedure. Recovery rates (expressed as % of certified values)  
160 were: Ag =  $80,2 \pm 1,2\%$ ; As =  $106,5 \pm 3,8\%$ ; Cd =  $102,9 \pm 1,2\%$ ; Cr =  $90,8 \pm 5,2\%$ ; Cu =  
161  $88,8 \pm 1,9\%$ ; Ni =  $86,1 \pm 2,7\%$ ; Pb =  $82,9 \pm 26,0\%$ ; Zn =  $100,4 \pm 1,6\%$ .

162

### 163 Organic pollutants analyses

164

165 Analysis of the seven indicator PCBs (CB50+28, CB52, CB101, CB118, CB138, CB153, and  
166 CB180), 14 OCPs (hexachlorobenzene or HCB, lindane or  $\gamma$ -HCH, dieldrin, heptachlor,  
167 heptachlorepoxyde, *cis*-chlordane, *trans*-nonachlor, mirex, and DDTs), and 4 PBDEs (BDE47,  
168 BDE99, BDE119, and BDE153) were performed on muscle samples (following the  
169 procedures described by Tapie et al 2008 and 2011). PCB, PBDE and OCP analyses were  
170 carried out on an HP 5890 series II gas chromatograph from Hewlett-Packard (Avondale, CA,  
171 USA) coupled to a  $^{63}\text{Ni}$  electron capture detector (ECD). A capillary column HP5-MS  
172 (Agilent Technologies, Massy, France) was used (30 m x 0.25 mm x 0.25  $\mu\text{m}$ ).

173 As described by Labadie et al. 2010, POPs levels were blank corrected and the method  
174 detection limit (LoD) was derived from the blank value variability, For PCBs LoQs were



175 comprised between 0.2 ng/g dw and 2 ng/g dw ; for OCPs they were comprised between 0.1  
176 ng/g dw and 0.4 ng/g dw ; for PBDEs they were comprised between 0.1 ng/g dw and 0.2 ng/g  
177 dw.

178

179 Preparation of cDNA libraries and contig assembly

180

181 For the transcriptomic sequencing, the liver was selected due to its wide range of functions,  
182 including detoxification and energy metabolism. For each sampling site (Quebec and France),  
183 six biological replicates were used (n=48 eels). Briefly, total RNAs were individually  
184 extracted from fish liver using the PureLink™ RNA mini kit (Invitrogen). During this step,  
185 samples were submitted to DNaseI treatment, according to the manufacturer's instructions.  
186 Then, preparation of cDNA libraries for Illumina HiSeq 2000 sequencing was done using the  
187 Truseq™ Stranded mRNA sample prep kit, following the manufacturer's instructions. The 48  
188 individually tagged libraries were randomly pooled in equal amounts and sequenced on 4  
189 lanes at the Genome Quebec innovation Center (McGill University, Montreal, QC, Canada)  
190 using Illumina HiSeq 2000 technology (100 bp paired-ends reads).

191 Base calling was performed using PyroBayes (Quinlan et al. 2008) after trimming adapters.  
192 Each read was then renamed according to its individual barcode. This barcode and the  
193 potential primers used for cDNA amplification were subsequently removed. To construct the  
194 transcriptome, a consensus sequence of one step was used to perform the next. Therefore any  
195 sequencing errors have been ignored because they have not been included in the consensus  
196 sequence. Then the CLC Genomic Workbench 4.9 software was used to align the reads back  
197 against the assembled transcriptome. A similarity of 0.98 and a length fraction of 0.6 were  
198 used while leaving the other parameters to their default values. The consensus sequence of  
199 each *de novo* built contig was then used as a template for a reference assembly under the same

200 parameters. This second round of assembly aimed at screening for additional reads that were  
201 not included into contigs during the step of *de novo* assemblies and excluding poor-quality  
202 contigs that did not recruit any read during the reference assembly procedure. To annotate  
203 these contigs based on similarity with known proteins, they were blasted on both the swissprot  
204 and nr protein databases using BLAST program. Gene transcription was normalized by using  
205 RPKM (Mortazavi et al. 2008) (Reads Per Kilobase per Million mapped reads) before  
206 statistical analyses.

207

208 Real-time quantitative RT-PCR (RT-qPCR) validation

209

210 Eight genes that showed strong variations in their transcription levels among sampling sites  
211 were quantified by RT-qPCR on the same liver samples used for RNA-seq. For each gene,  
212 specific primer pairs were determined (see supplementary material Table S1) using the  
213 lightcycler probe design software (Roche). RNAs were extracted from 30 mg of tissue using  
214 the SV total RNA isolation system (Promega) according to the manufacturer's instruction.  
215 One  $\mu\text{g}$  of total RNAs was used for cDNA synthesis using Goscript reverse transcriptase  
216 (Promega), oligodT primer and random primers according to manufacturer's instructions.  
217 Resulting cDNAs were diluted 2 -fold for PCR reactions. RT-qPCR reactions of 20 $\mu\text{l}$  were  
218 performed in a Stratagene *Mx 3000p* system using Brilliant III Fast SYBR Green QPCR  
219 Master Mix (Stratagene-Agilent) and 1 $\mu\text{l}$  of diluted cDNAs. The reaction specificity was  
220 determined for each reaction from the dissociation curve of the PCR product and  
221 electrophoresis. Data analysis was performed using the  *$\beta$ -actin* gene as reference. Hence,  
222 during our experiment, total RNAs were quantified and 1  $\mu\text{g}$  was used to be reverse-  
223 transcribed. During the subsequent qPCR amplifications, the output cycle corresponding to

224 the *β-actin* was examined. This output cycle did not show significant differences among fish,  
225 demonstrating the relevance of the *β-actin* as reference gene in our conditions.

226

227 Statistical analyses

228

229 Concerning contaminant analyses, comparisons between fish groups were performed by  
230 analysis of variance (ANOVA), after checking assumptions of normality and  
231 homoscedasticity of the error terms. The Least Square Deviation test (LSD) was used to  
232 determine whether means between pairs of samples were significantly different from one  
233 another. Computations were performed using the statistical software Sigma Stat.

234 Due to the wide distribution area of eels and since factors unique to each site but unrelated to  
235 metal or organic contamination (e.g. temperature, salinity, ...), can also affect the gene  
236 transcription level, the FAMT approach was adopted to increase statistical power between a  
237 variation in the transcription level of an individual gene and the contamination level of fish  
238 for a given contaminant. Thus, in addition to contaminants, natural factors that showed  
239 significant variations among sites (mainly temperature and salinity) as well as some  
240 morphometric measurements (length and weight) were added to the model in order to remove,  
241 at least in part, genes for which transcription levels could be more likely related to these  
242 factors rather than with contaminants. Then, the variables such as pollutant concentrations,  
243 condition indices (i.e. the relative condition index (Kn), the hepato-somatic index (HSI) and  
244 the spleen somatic index (SSI)) or physicochemical conditions of sampling sites (i.e.  
245 temperature and salinity) were gathered in a matrix made of independent observations from a  
246 standard normal distribution. This model has been studied in Friguet et al (2009) and modified  
247 for our transcriptomic data (for more information see Coudret, 2013). By using this test, we  
248 also controlled the False Discovery Rate (FDR) over the set of contigs for a given variable.

249 The R package FAMT (Causeur, 2011) allowed us to produce a *pvalue* between a factor and  
250 contigs. For every studied variables, we then introduced two thresholds  $\delta_{i,1}$  and  $\delta_{i,2}$  and we  
251 selected a contig according to the two thresholds. In this way, the *pvalue* calculated between a  
252 given variable and one contig had to satisfy the thresholds  $\delta_{i,1}$  and *pvalue* calculated between  
253 another variable for the same contig had to satisfy the thresholds  $\delta_{i,2}$ . For each contig and each  
254 variable, the  $\beta$  represents the correlation length (see supplementary material for more details).

255

## 256 **Results and discussion**

257

258 Condition and contamination levels of eels

259

260 Morphometric measurements, conditions indices and contamination levels of all eels and their  
261 difference among sampling sites are presented in Table 1. Generally, the highest levels of  
262 contamination in both metallic and organic contaminants were observed in eels from France.  
263 Significant increases in pollutant concentrations were observed along the French gradient  
264 from Certes → Dordogne → Garonne → Gironde. To a lesser extent, we observed a  
265 contamination gradient from St. Jean → Sud-Ouest → St. François → St. Pierre for Quebec.  
266 Concerning metals, the highest variations were observed for cadmium (Cd) and silver (Ag).  
267 For Cd, animals inhabiting the Gironde estuary were 112-fold more contaminated than eels  
268 from Certes, i.e. the cleanest site in France, and 11-fold superior to Lake St. Pierre, i.e. the  
269 most contaminated site in Quebec. For Ag, eels from Gironde were 338-fold superior to eels  
270 from Certes with low concentrations of  $3.4 \pm 1.5 \text{ ng.g}^{-1}$  and  $0.01 \pm 0.001 \text{ ng.g}^{-1}$  respectively.  
271 Concerning organic pollutants, the highest variations were observed for metabolites of DDT  
272 and PCBs. For Gironde, eels were 56 and 2.3-fold more contaminated than eels from Certes  
273 and St Pierre Lake, respectively. For PCBs, eels from Gironde were 24-fold more

274 contaminated than eels from Certes and 10.6-fold more contaminated than eels from St Pierre  
275 Lake. These high contamination pressures in Gironde were associated with a poor health  
276 status of eels. Eels from Gironde presented the lowest values for Kn, HSI and SSI in  
277 comparison to eels from the other French sites.

278

279 RNA-seq data

280

281 RNA-seq generated 2.7 billion fragments averaging 75 bases in length. The assembly of these  
282 reads generated a total of 54,140 contigs, 95% of the contigs were longer than 200 bp and  
283 11.7% were longer than 1000 bp. A total of 18,115 contigs showed high homology with  
284 known sequences (BLASTX,  $E\text{value} \leq 10^{-10}$ ). A total of 11,547 unique genes of known  
285 function were identified (i.e. multiple contigs that had the same annotation were removed).

286 To validate the sequencing data, the transcriptional level of 8 genes that showed strong  
287 variations in their transcription levels among sampling sites was measured by RT-qPCR  
288 method. These two independent measures, by RNA-seq and RT-qPCR, of transcript  
289 abundance gave consistent results, i.e. transcription data obtained by these two methods were  
290 significantly correlated ( $P < 0.0001$  for all of genes tested; see supplementary material table  
291 S2).

292

293 Quantitative influence of factors on eel's transcriptome

294

295 In order to get an overview of the effects of natural and anthropogenic factors on the  
296 transcriptome profiles of eels, we first used the FAMT method with low stringency  
297 thresholds:  $\delta_{i,1} = 0.009$  and  $\delta_{i,2} = 0$ . From the 11,547 unique genes, the transcription level of  
298 1523 single genes of known function was significantly correlated with pollutants or natural

299 factors. We then counted how many contigs were linearly linked with each of the n=19  
300 variables (Figure 1 and Table S3). For biometric variables, 284 genes were identified for HSI,  
301 60 for SSI, and 55 for Kn. This corresponds to 19%, 4%, and 4% of the total number of genes,  
302 respectively. The dominant percentage of genes associated to HSI appears to be consistent  
303 with the fact that transcriptome analyses were carried out on the liver tissue.

304 Among metals, the number of genes reached 140 (9%) for As, 164 (11%) for Cd and 4% for  
305 Hg and 5% for Ag. Despite that the highest contamination gradient was observed for Ag, Cd  
306 and As seemed to have more influence on the transcriptome of eels. For POPs,  $\gamma$ -HCH  
307 (lindane) was associated with the greatest number of genes (n = 182, 12%), followed by DDT  
308 and its metabolites 4,4'DDD, 2,4'DDE, 4,4'DDE+dieldrin represented around 2% and 5% of  
309 total genes (Fig. 1). Interestingly, despite the fact that we observed very high concentrations  
310 of PCBs in eel muscle, few genes were associated with this contaminant. In contrary,  
311 numerous genes were associated with lindane whereas it was found weakly accumulated in  
312 eels. Temperature and salinity were respectively linked with 6% and 5% of genes. Since they  
313 are essential factors controlling the food intake and growth rates in fish, it is quite surprisingly  
314 that these two factors were less represented than pollutants. This could suggest that  
315 contaminants have more influence on the liver transcriptome of eels than temperature, which  
316 could be related to the fact that the liver is a major target of pollutant metabolism,  
317 accumulation and toxicity.

318

319 Genes associated with several factors

320

321 The aim of the present study was to unravel the effects engendered by anthropogenic or  
322 natural factor in wild organisms. However, it was also interesting to study genes that were  
323 associated with several factors. They could indeed represent genes that are involved in a more

324 general stress response and that could be used to assess the general health status of animals.  
325 Among genes highlighted by the FAMT analysis, 57 were significantly associated with four  
326 or more factors (Table S3). Surprisingly, no genes that are known to respond to oxidative  
327 stress or metal toxicity, such as metallothionein or heat shock proteins, were found among the  
328 57 genes. However, a significant correlation was observed for the *metalloreductase STEAP4*  
329 gene. This gene encodes for a protein located in the Golgi apparatus and that is involved in  
330  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  reduction. This gene was negatively associated with Hg, Cd, As and Ag.  
331 Moreover, three genes encoding for zinc finger proteins (*zinc finger and BTB domain-*  
332 *containing protein 12*, *zinc finger protein ZPR1*, *zinc finger MYM-type protein 2*) showed  
333 significant correlations with salinity, temperature, As, Cd or lindane. Zinc finger proteins are  
334 known to be important targets of metals like Cd, Ni or Co. Interactions of these proteins with  
335 metals can induce change in either their structure or function (Hartwig 2001). Thus, our  
336 results suggest a potential effect of as, Cd and lindane on these proteins. This could lead to  
337 disorders in gene transcription, cell growth or DNA repair (Hartwig 2001). Moreover, in the  
338 present results, numerous genes involved in gene transcription regulation and RNA  
339 processing such as the *histone-lysine N-methyltransferase SETD1B-A*, *mediator of RNA*  
340 *polymerase II transcription subunit 6* gene, *ribosomal RNA methyltransferase NOP2* and  
341 *ribosomal RNA small subunit methyltransferase NEP1* genes showed significant correlations  
342 not only with some metals and organic compounds but also with salinity and/or temperature.  
343 The fact that some metals or organic pollutants can induce changes in the transcription level  
344 of genes that are also regulated by natural factors such as temperature or salinity suggest that  
345 chronic exposure to pollutants could impair the adaptive response developed by fish to curb  
346 with variations in natural factors (Lapointe et al., 2011).

347

348 Genes associated with arsenic, cadmium, lindane and HSI

349  
350 As HSI, As, Cd and  $\gamma$ -HCH were linked with the greatest number of contigs in their  
351 respective group (i.e. condition indices, metal and organic pollutants), we then focused our  
352 investigations on these variables. To do this, we re-analyzed our data by the FAMT method  
353 but by using more restrictive thresholds (see Table 2 and Table S4 for more details). The aim  
354 of this approach was to identify genes that are most likely to be related to a single variable.

355  
356 Hepato-somatic index

357  
358 The hepato-somatic index (HSI) is a useful and robust index commonly used to estimate the  
359 energy status of fish (Chellappa et al. 1995). This parameter was incorporated in our FAMT  
360 model in order to identify genes that could represent biomarkers of the general health status of  
361 fish.

362 The transcription level of 13 genes (Table 2) showed significant correlations with the HSI of  
363 fish. Among these genes, 4 genes encode for ribosomal proteins, i.e. *60S ribosomal protein L5*  
364 (*rpl5*), *40S ribosomal protein S29* (*rps29*), *60S ribosomal protein L23* (*rpl23*) and *ubiquitin-*  
365 *60S ribosomal protein L40* (*uba52*). Interestingly, the transcription level of these genes was  
366 found to increase or decrease with increasing fish HSI. This could be linked, at least in part, to  
367 the fact that these genes are not only involved in protein translation but also in cell cycle by  
368 regulating the accumulation of the p53 protein in cell (Horn and Vousden 2008; Lee et al.  
369 2012). The p53 protein is involved in cell cycle arrest and apoptosis. In this view, it is  
370 interesting to note that increasing fish HSI were significantly correlated with a drastic and  
371 similar decrease in *rpl5* ( $\beta = -2.82$ ) and *glioma tumor suppressor candidate region gene 2*  
372 *protein* (*gltscr2*,  $\beta = -2.10$ ) transcription levels. Both *gltscr2* and *rpl5* genes are involved in the  
373 ribosomal protein-Mdm2-p53 pathway. Its activation is known to induce p53 stabilization.



374 Moreover, recent discoveries tend to show that this pathway could play a pivotal role in  
375 cellular energy homeostasis, the ribosomal protein-Mdm2-p53 pathway serving as a  
376 molecular metabolizing switch to direct nutrient utilization in an effort to promote cell  
377 survival (Deisenroth and Zhang 2011). Our results could suggest an important role of  
378 ribosomal proteins as well as the ribosomal protein-Mdm2-p53 pathway in liver development  
379 and hepatic accumulation of energy reserves. Another potential marker of cell division and  
380 hepatic growth is the gene encoding for *thioredoxin (txn)*. The transcription level of *txn* was  
381 positively associated with fish HSI ( $\beta = 1.54$ ). The TXN protein is known to play an  
382 important role in cell viability by inducing cell proliferation and increasing cell lifetime  
383 (Yoshida 2005). The transcription levels of 2 other genes involved in protein metabolism were  
384 found to be positively correlated with fish HSI. These genes, i.e. *proteasome activator*  
385 *complex subunit 2 (psme2)* and *cathepsin B (ctsb)*, encode proteins that are responsible for  
386 proteolysis (Min et al. 2013; Mohamed and Sloane 2006). Cathepsin B is known to play a key  
387 role in protein turnover (Mohamed and Sloane 2006).

388 In addition to genes involved in protein metabolism and cell division, significant correlations  
389 were observed with genes that encode proteins involved in lipid and iron metabolism. The two  
390 genes involved in lipid metabolism, i.e. *Acyl-CoA-binding protein (acbp)* and  
391 *Ethanolaminephosphotransferase 1 (ept1)*, were negatively correlated with fish HSI ( $\beta = -0.10$   
392 and  $\beta = -0.96$ , respectively). The EPT1 protein catalyzes the biosynthesis of  
393 phosphatidylethanolamine (PE), i.e. a major phospholipid (Gibellini and Smith 2010). It is  
394 somewhat surprising that an increase in fish HSI was associated with a decrease in *ept1*  
395 transcription level. This could be linked to the fact that PE is the precursor of many  
396 biologically active molecules such as pro-apoptotic substances (Gibellini and Smith 2010).  
397 The ACBP protein has been shown to act as intracellular acyl-CoA pool former, transporter  
398 and regulator of gene transcription (Oikari 2008). In a recent study, overexpression of *acbp* in

399 transgenic rats was found to trigger a down-regulation of peroxisome proliferator-activated  
400 receptor  $\gamma$  and sterol regulatory element-binding protein-1 gene transcription levels, i.e.  
401 metabolic regulators involved in lipogenesis and lipid synthesis (Oikari 2008). Thus, a  
402 decrease in *acbp* transcription level could indicate lipid storage. Finally, an increase in fish  
403 HSI was associated with an up-regulation of genes involved in both iron secretion and influx,  
404 i.e. *hephaestin-like protein 1 (hephl1)* and *putative ferric-chelate reductase 1(frsl1)*,  
405 respectively. Such results appear consistent with the fact that iron plays a key role in cellular  
406 respiration and energy metabolism. This is also consistent with the fact that fish liver plays a  
407 central role in iron metabolism and hemoglobin turn-over (Graham and Chua 2007).

408 In summary, our results showed that an increase in fish HSI is associated with changes in the  
409 transcription level of genes involved in several biological pathways including cell division,  
410 protein turnover, lipid metabolism and iron transport.

411

412 Arsenic

413

414 The FAMT method with highly restrictive thresholds allowed us to identify 9 genes for which  
415 transcription levels were significantly correlated with the As content of eels. The majority of  
416 these genes, i.e. 7 genes (Table 2), were found to be down-regulated and 2 genes were up-  
417 regulated. These 2 up-regulated genes, i.e. *E3 ubiquitin-protein ligase RNF115 (Rnf115)* and  
418 *15kDA selenoprotein (sep15)*, are involved in protein metabolism. RNF115 is an ubiquitin-  
419 protein involved in the ubiquitination pathway and proteolysis (Burger et al. 2006). SEP15 is  
420 involved in the quality control of protein folding and in the endoplasmic reticulum stress  
421 response (Labunskyy et al. 2007). Such a pattern could be linked to the fact that As is well  
422 known to interfere with protein folding, leading to accumulation of protein aggregates in cell  
423 (Martin-Pardillos et al. 2013). The up-regulation of these genes could aim to limit the

424 accumulation of unfolded proteins in cell in response to As exposure. Interestingly, among the  
425 down-regulated genes, numerous genes are involved in biological functions that are known to  
426 be involved in the vasculotoxicity of As in mammals. The various mechanisms that have been  
427 observed during As vasculotoxicity include endoplasmic reticulum stress (as described  
428 above), apoptosis, oxidative stress, inflammatory mediators and vascular smooth muscle cells  
429 (VSMC) calcification (Martin-Pardillos et al. 2013). Thus, a down-regulation of the  
430 *heterogeneous nuclear ribonucleoprotein A0 (HNRPA0)* gene that encodes a protein involved  
431 in the inflammatory response by controlling cytokine synthesis was observed in As-  
432 contaminated fish. The *zinc finger protein 36 (zfp3611)* that is known to be involved in  
433 vasculogenesis (Bell et al. 2006) was also found to be down-regulated. The *metalloprotease*  
434 *TIKI2 (trabd2)* gene was down-regulated in eels exposed to As. *Trabd2* encodes for a protein  
435 that antagonizes the Wnt signaling pathway (Clevers and Nusse 2012). Recent works have  
436 shown that Wnt signaling pathway activation can trigger calcification of VSMCs (Martinez-  
437 Moreno et al. 2012). We also observed a decrease of *threhalase* gene transcription level. This  
438 gene encodes for an enzyme that hydrolyses trehalose. Trehalose is a disaccharide known to  
439 protect membranes in case of extreme environmental conditions (Higashiyama 2002).  
440 Moreover, in osteoporosis mice model, trehalose has been shown to decrease the development  
441 of osteoporosis (Higashiyama 2002). In the same way, it is interesting to note that we  
442 observed a strong effect of As on the transcription of *25-hydroxyvitamin D-1 alpha*  
443 *hydroxylase* gene (*cyp27b1*). This gene encodes an enzyme that synthesizes the active form of  
444 vitamin D<sub>3</sub> (Rowling et al. 2007). Vitamin D<sub>3</sub> under its active form acts as a hormone that  
445 controls growth and bone remodeling. In this context, we must note that VSMCs calcification  
446 during As exposure in rats was found to be accelerated by intraperitoneal injection of vitamin  
447 D<sub>3</sub> (Martin-Pardillos et al. 2013). We could thus hypothesize that the down-regulation of  
448 *cyp27b1* could aim to protect the hepatic vasculatory system against calcification. Two last

449 genes involved in ion transport were found down-regulated in response to As exposure, the  
450 *zinc transporter ZIP11 (slc39a11)* and *transient receptor potential cation channel subfamily*  
451 *M member 5 (trpm5)*, suggesting an effect of As on cellular homeostasis. To conclude, it is  
452 interesting to pinpoint that many mechanisms that have been reported in mammals during As  
453 vasculotoxicity are affected in fish chronically exposed to As, thus suggesting that the hepatic  
454 vascular system could be a main target of As toxicity in wild fish.

455

456 Cadmium

457

458 Among genes that showed significant correlations with Cd concentrations in fish, 5 genes  
459 were found to be down-regulated and 5 were found to be up-regulated.

460 Among down-regulated genes (Table 2), two genes involved in acetylcholine metabolism  
461 were identified. First, our results showed that *Acetylcholinesterase collagenic tail peptide*  
462 (*colq*) was down-regulated by Cd exposure. This gene encodes for a protein that anchors the  
463 catalytic subunits of asymmetric acetylcholinesterase (AChE) to the synaptic basal lamina  
464 (Deprez et al. 2003). This down-regulation of *colq* could be linked to the down-regulation of  
465 another gene, i.e. utrophin (*utrn*;  $\beta = -0.003$ ). UTRN is indeed found in postsynaptic cell  
466 membrane where it is involved in the clustering of acetylcholine receptors (Campanelli et al.  
467 1994). As a down-regulation or a decreased activity of AChE is a well-known biomarker of  
468 liver damage (Xing et al. 2012), our results could suggest that chronic Cd exposure is  
469 responsible for liver damage in wild eels.

470 Interestingly, such biomarkers of tissue damage were associated with changes in the  
471 transcription level of genes involved in cell cycle regulation and apoptosis. First, the *activin*  
472 *receptor type-2A (acvr2a)* gene that encodes a receptor complex for Activin A protein was  
473 found to be up-regulated in Cd-contaminated fish ( $\beta = 0.03$ ). Activin A protein is a member of

474 the transforming growth factor- $\beta$  super family (Letterio and Roberts 1998). These factors are  
475 known to be important mediators of apoptosis in liver (Rodgarkia-Dara et al. 2006). Such a  
476 result is consistent with the up-regulation ( $\beta = 0.003$ ) of the *Band 4.1-like protein 3 (epb41l3)*  
477 gene. This gene encodes a protein that inhibits cell proliferation and induces apoptosis (Li et  
478 al. 2011). In addition, the *pericentriolar material 1 protein* gene (*pcm1*) was found to be  
479 down-regulated in Cd-contaminated fish. PCM1 is known to be responsible for the correct  
480 localization of several centrosomal proteins that are required for regulation of centrosome  
481 structure at the G2/M transition and thus, for cell division (Hames and Crookes 2005). Cd  
482 contamination seems to affect genes involved in cell division and particularly mechanisms  
483 that are responsible to cell cycle arrest. Our results could indicate that chronic Cd exposure  
484 represses hepatocyte division. This hypothesis is further supported by the fact that Cd  
485 contamination was also associated with an up-regulation of the *C-terminal binding protein 2*  
486 gene (*ctbp2*). CtBPs are transcriptional co-repressors acting *via* epigenetic mechanisms and  
487 more precisely by recruiting histone deacetylase and histone methyltransferase (Kim et al.  
488 2005). It has been shown that these proteins serve as energy sensing-repressor. Their  
489 activation by low energy or NADH levels leads to repression of gene transcription and  
490 particularly to the down-regulation of genes involved in cell division. In this view, it is  
491 interesting to note that our results also showed that Cd exposure affects the transcription of  
492 genes involved in lipid transport and protein metabolism. Cd contamination was associated  
493 with an increase of *sortilin-related receptor (sor11)* gene transcription level, a multifunctional  
494 endocytic receptor that is involved in the uptake of lipoproteins and proteases from plasma.  
495 This enzyme notably presents a high affinity for apoE-rich lipoproteins and  $\beta$ -VLDL (Taira et  
496 al. 2011). Such an increase could appear in accordance with previous results obtained by  
497 Pierron et al. 2007 who reported a significant increase in lipolysis and energy needs in the  
498 liver of eels experimentally exposed to dissolved Cd at  $5\mu\text{g}\cdot\text{L}^{-1}$  during 30 days. Finally, two

499 genes encoding enzymes involved in ubiquitin conjugation pathway were also affected by Cd  
500 contamination. The gene *E3 ubiquitin-protein ligase UBR5 (ubr5)* which encodes a protein  
501 responsible for protein ubiquitination and thus to their degradation by the proteasome (Ernst  
502 et al. 2011), was found to be down-regulated in response to metal contamination. In contrast,  
503 the *ubiquitin carboxyl terminal hydrolase 20 gene (usp20)* that encodes an enzyme that leads  
504 to protein deubiquitination was found to be up-regulated. This could potentially limit protein  
505 turnover and thus limit energy expenditure. It is interesting to note that a similar pattern was  
506 reported in wild yellow perch (*Perca flavescens*) chronically exposed to Cd pollution (Pierron  
507 et al. 2011). Using RNA-seq, authors hypothesized that chronic Cd exposure leads to energy  
508 restriction and to the activation of epigenetic mechanisms responsible for gene silencing,  
509 notably on genes involved in lipid and protein metabolism.

510

511 Lindane

512

513 First, two genes involved in transcription (Table 2), i.e. *mediator of RNA polymerase II*  
514 *transcription subunit 13 (med13)* and *RNA-binding protein 4B (rbm4b)*, were both up-  
515 regulated at the same level ( $\beta = 0.06$ ) in  $\gamma$ -HCH contaminated-fish. *Med13* encodes a  
516 transcriptional co-activator that is required for the expression of all RNA polymerase II-  
517 dependent genes (Zhang et al. 2005). RBM4B is involved in mRNA processing (Lin et al.  
518 2012). Up-regulation of these two genes could indicate a general increase in cell  
519 transcriptional activity.

520 This potential increase in transcriptional activity was coupled with an up-regulation of several  
521 genes involved in lipid catabolism. Indeed, our results showed an up-regulation of the  
522 *carnitine-O-palmitoyltransferase 2 (cpt2)* and *mitochondrial coenzyme A transporter SLC*  
523 *25A42 (slc25a42)* genes. These genes encode for enzymes involved in mitochondrial fatty

524 acid  $\beta$ -oxidation (Kang et al. 2007). Moreover, these up-regulations were associated to an up-  
525 regulation of the *peroxisomal membrane protein PEX13* gene (*pex13*;  $\beta = 0.06$ ). This gene  
526 encodes for a protein that is involved in the peroxisomal degradation of long chain fatty acids  
527 (Mannaerts 2000). Because mitochondrial  $\beta$ -oxidization can only use short and median fatty  
528 acids to generate energy (Mannaerts 2000), the up-regulation of *pex13* could contribute to  
529 provide to mitochondria short fatty acids *via* degradation of long fatty acids. It is interesting to  
530 note that *oxysterol-binding protein 2 (osbp2)*, involved in lipid transport and cholesterol  
531 metabolism, was also up-regulated. Thus, our results tend to show that chronic exposure to  $\gamma$ -  
532 HCH triggers an increased lipolysis and energy production in eels. Such a result is consistent  
533 with a previous study carried out on *Daphnia magna* which showed that exposure to sub-  
534 lethal concentrations of  $\gamma$ -HCH triggers an increase in the Krebs's cycle activity (De Coen et  
535 al. 2001). These up-regulations of transcriptional activity and energy metabolism were further  
536 associated with changes in the transcription level of genes that are known to be involved in  
537 tumorigenesis. First, the gene *serine protease hepsin (hpn)* was found to be up-regulated.  
538 This gene encodes for a protein that plays an essential role in cell growth (Nakamura et al.  
539 2008). This gene is known to have prominent expression in human liver, prostate and ovarian  
540 tumors (Herter et al. 2005). Such a potential role of  $\gamma$ -HCH in tumorigenesis in eel liver is  
541 further supported by the significant decrease in *zinc-finger protein 148 (znf148)* gene  
542 transcription level in  $\gamma$ -HCH-contaminated fish. This gene presents anti-tumor properties  
543 when over-expressed in hepatocellular carcinoma by increasing apoptotic processes (Zhang et  
544 al. 2012). Furthermore, it is noteworthy that a gene encoding a protein involved in hemophilic  
545 cell-cell adhesion, the *myelin protein zero-like protein 2* gene (*mpzl2*) was also down-  
546 regulated. A loss of hemophilic cell-cell adhesion molecules induces signals promoting cell  
547 proliferation and migration and thus, tumor progression (Craig and Brady-Kalnay 2011). In  
548 contrast, a gene described to have a tumor suppressor role in colorectal cancers and in

549 malignant thyroid tumors (Mlakar et al. 2009; Arnaldi et al. 2005), i.e. the decorin gene (*dcn*),  
550 was up-regulated in our findings ( $\beta = 0.12$ ). We must note however that the primary role of  
551 *dcn* is to control extracellular matrix assembly. DCN is known to be expressed during hepatic  
552 fibrosis (Baghy and László 2010). This could be in accordance with a study that reported  
553 hepatic fibrosis in several fish species exposed to accidental discharge of  $\gamma$ -HCH (Ortiz et al.  
554 2003). To conclude, our results show that chronic  $\gamma$ -HCH exposure is associated with an up-  
555 regulation of global transcriptional activity and lipolysis. This up-regulation of cellular  
556 metabolism was further associated with changes in the transcription level of genes involved in  
557 tumorigenesis, suggesting that  $\gamma$ -HCH could lead to cancer development in eels. This tumor  
558 promoter role of  $\gamma$ -HCH had already been described in fish by Kroll et al., 1999 (Kroll et al.  
559 1999). An alternative hypothesis would be that this increase in cellular metabolism and cell  
560 proliferation are signs of liver regeneration (King and Newmark 2012). Indeed,  $\gamma$ -HCH was  
561 reported to trigger hepatocyte necrosis in fish (Ortiz et al. 2003).

562

## 563 **Conclusion**

564

565 Our approach allowed us to establish significant relationships between the hepatic expression  
566 levels of specific transcripts and the concentrations of individual contaminants measured in  
567 fish. The aim was to identify some genes that are more likely related to a given factor. The  
568 genes identified for each anthropogenic or natural factor were involved in numerous and  
569 common biological processes. For example, fish HSI and Cd contamination were associated  
570 with changes in the transcription levels of genes involved in the p53 signaling pathway and in  
571 the lipid and protein metabolism, suggesting an effect of Cd on the hepatic energy  
572 metabolism. In this view, it is interesting to note that some discrepancies appear between our  
573 work and previous field transcriptomic studies. For example, in the studies carried out by  
574 Pujolar et al. (2012 and 2013), during which the transcriptome of eels from a clean site was



575 compared with the transcriptome of eels from polluted sites by means of DNA microarray,  
576 significant changes in the transcription level of genes involved in detoxification, in the fight  
577 against oxidative stress and in the general stress response (e.g. metallothioneins, heat shock  
578 proteins) were observed. Such genes have not been highlighted in our study. This could be  
579 linked to the fact that our statistical approach (i.e. FAMT analysis) aimed to eliminate as  
580 much as possible genes for which transcription levels are correlated with several factors.  
581 However, such genes are known to vary in response to many factors, including both  
582 anthropogenic (including both metals and organic pollutants) and natural (e.g. temperature,  
583 hypoxia, age..; (Boeuf et al., 2001, Podrabsky et al., 2004) factors. Our study thus proposes  
584 an alternative method that could give more specific “signatures” of exposure. Further studies  
585 are however required at the protein level to test whether such variations in gene transcription  
586 levels are associated with adverse effects in eels and to deepen our understanding about the  
587 mechanisms of toxicity of pollutants in wild contaminated fish.

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602 **Figure captions**

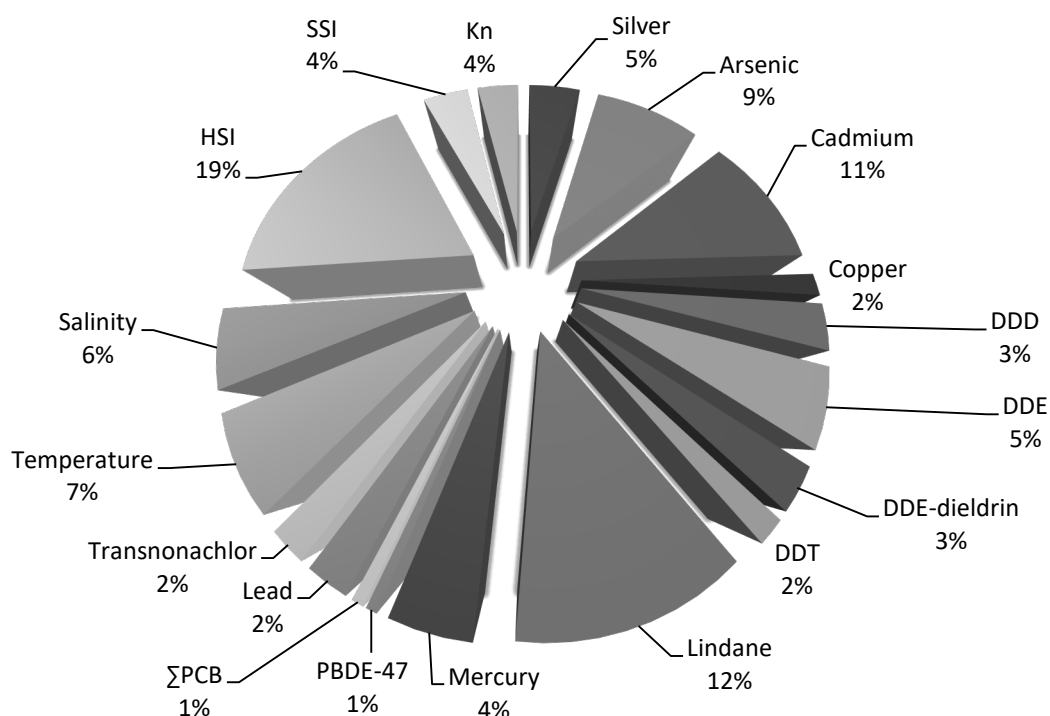
603 **Fig.1** Results of FAMT analyses with low stringency: Percentage representation of genes  
604 correlated with a single factor

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607 **Figures**608 **Figure 1**

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Table 1 Morphometric measures (mean  $\pm$  SE, n= 6) and average concentrations of muscle organic pollutants (expressed as ng.g<sup>-1</sup>, dw) and kidneys metal concentrations (expressed as  $\mu$ g.g<sup>-1</sup>, dw) in Atlantic eels sampled in 8 sites located in Canada and France. For each country, the four sites are stored in a gradient of contamination. All data are expressed as means  $\pm$  SE (n= 6 per site). Means designated with different letters are significantly different (LSD test,  $P < 0.05$ ).

| Groups of variables        | Variables                       | Canadian sites ( <i>Anguilla rostrata</i> ) |                                |                                   |                                  | French sites ( <i>Anguilla anguilla</i> ) |                                   |                                   |                                   |                                   |
|----------------------------|---------------------------------|---|--------------------------------|-----------------------------------|----------------------------------|---|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
|                            |                                 | St Jean                                     | Sud-Ouest                      | St François                       | St Pierre                        | Certes                                    | Dordogne                          | Garonne                           | Gironde                           |                                   |
| Biometry                   | Length                          | 529.26 $\pm$ 22.43                          | 347.53 $\pm$ 18.47             | 827.93 $\pm$ 14.12                | 890.87 $\pm$ 10.53               | 434.53 $\pm$ 19.2                         | 338 $\pm$ 12.78                   | 442.66 $\pm$ 17.73                | 578.66 $\pm$ 15.02                |                                   |
|                            | Weight                          | 290.13 $\pm$ 37.6                           | 77.87 $\pm$ 18.66              | 1222.33 $\pm$ 32.17               | 1434.66 $\pm$ 53.92              | 157.33 $\pm$ 31.25                        | 63 $\pm$ 10.65                    | 156.26 $\pm$ 22.32                | 349.46 $\pm$ 32.0                 |                                   |
|                            | SSI                             | 0.27 $\pm$ 0.07 <sup>a</sup>                | 0.22 $\pm$ 0.05 <sup>a</sup>   | 0.09 $\pm$ 0.01 <sup>b,c</sup>    | 0.09 $\pm$ 0.01 <sup>b</sup>     | 0.11 $\pm$ 0.01 <sup>b,c</sup>            | 0.09 $\pm$ 0.01 <sup>b,c</sup>    | 0.12 $\pm$ 0.01 <sup>c</sup>      | 0.06 $\pm$ 0.004 <sup>d</sup>     |                                   |
|                            | HSI                             | 2.09 $\pm$ 0.13 <sup>a</sup>                | 1.62 $\pm$ 0.06 <sup>b</sup>   | 1.04 $\pm$ 0.05 <sup>c,d,e</sup>  | 1.07 $\pm$ 0.06 <sup>c,d,e</sup> | 1.18 $\pm$ 0.02 <sup>d</sup>              | 0.96 $\pm$ 0.04 <sup>e,f</sup>    | 1.14 $\pm$ 0.04 <sup>d,c</sup>    | 0.88 $\pm$ 0.03 <sup>f</sup>      |                                   |
|                            | Kn                              | 0.4 $\pm$ 0.01 <sup>a,c</sup>               | 0.39 $\pm$ 0.01 <sup>a,c</sup> | 0.48 $\pm$ 0.02 <sup>a</sup>      | 0.38 $\pm$ 0.01 <sup>c</sup>     | 0.51 $\pm$ 0.02 <sup>b</sup>              | 0.48 $\pm$ 0.01 <sup>b</sup>      | 0.48 $\pm$ 0.01 <sup>b</sup>      | 0.46 $\pm$ 0.01 <sup>b</sup>      |                                   |
| Natural factor             | Temperature (°C)                | 17.0  | 19.1                           | 22.0                              | 12.0                             | 21.9                                      | 22.2                              | 21.4                              | 21.3                              |                                   |
|                            | Salinity (‰)                    | 1.9   | 16.5                           | 0.0                               | 0.11                             | 19.9                                      | 0.32                              | 0.13                              | 7.78                              |                                   |
| Contaminants               | Metals                          | Cd  | 2.35 $\pm$ 0.75 <sup>a</sup>   | 2.23 $\pm$ 0.14 <sup>a,c</sup>    | 1.75 $\pm$ 0.19 <sup>a</sup>     | 4.76 $\pm$ 1.29 <sup>c</sup>              | 0.47 $\pm$ 0.17 <sup>d</sup>      | 27.29 $\pm$ 5.26 <sup>b</sup>     | 9.09 $\pm$ 2.51 <sup>c</sup>      | 52.3 $\pm$ 11.35 <sup>b</sup>     |
|                            |                                 | Cu  | 6.01 $\pm$ 0.67 <sup>a</sup>   | 9.3 $\pm$ 1.04 <sup>b</sup>       | 5.94 $\pm$ 0.39 <sup>c</sup>     | 8.78 $\pm$ 1.16 <sup>b</sup>              | 7.18 $\pm$ 0.80 <sup>a,b,c</sup>  | 6.54 $\pm$ 0.35 <sup>a,b,c</sup>  | 6.06 $\pm$ 0.98 <sup>a,c</sup>    | 8.7 $\pm$ 0.65 <sup>b</sup>       |
|                            |                                 | Zn  | 253.2 $\pm$ 32.8 <sup>a</sup>  | 250.51 $\pm$ 22.64 <sup>a</sup>   | 182.7 $\pm$ 40.9 <sup>a</sup>    | 205.1 $\pm$ 17.6 <sup>a</sup>             | 227.0 $\pm$ 17.4 <sup>a</sup>     | 255.7 $\pm$ 7.9 <sup>a</sup>      | 198.6 $\pm$ 16.9 <sup>a</sup>     | 246.7 $\pm$ 31.9 <sup>a</sup>     |
|                            |                                 | Ag  | 0.08 $\pm$ 0.01 <sup>a</sup>   | 0.39 $\pm$ 0.14 <sup>a</sup>      | 0.09 $\pm$ 0.02 <sup>a</sup>     | 0.63 $\pm$ 0.41 <sup>a</sup>              | 0.01 $\pm$ 0.001 <sup>a</sup>     | 0.09 $\pm$ 0.03 <sup>a</sup>      | 0.13 $\pm$ 0.05 <sup>a</sup>      | 3.38 $\pm$ 1.48 <sup>b</sup>      |
|                            |                                 | As  | 2.58 $\pm$ 0.36 <sup>a,c</sup> | 1.59 $\pm$ 0.24 <sup>c,d</sup>    | 0.97 $\pm$ 0.10 <sup>d</sup>     | 2.36 $\pm$ 0.14 <sup>d</sup>              | 4.25 $\pm$ 0.83 <sup>a,b</sup>    | 2.98 $\pm$ 0.76 <sup>a,c</sup>    | 1.08 $\pm$ 0.16 <sup>d</sup>      | 6.33 $\pm$ 1.48 <sup>b</sup>      |
|                            |                                 | Pb  | 0.19 $\pm$ 0.06 <sup>a,c</sup> | 0.12 $\pm$ 0.02 <sup>a,c</sup>    | 0.09 $\pm$ 0.02 <sup>c</sup>     | 0.36 $\pm$ 0.16 <sup>a</sup>              | 0.25 $\pm$ 0.03 <sup>a</sup>      | 0.73 $\pm$ 0.16 <sup>d</sup>      | 2.7 $\pm$ 1.54 <sup>b,d</sup>     | 2.68 $\pm$ 0.62 <sup>b</sup>      |
|                            |                                 | Cr  | 0.62 $\pm$ 0.06 <sup>a</sup>   | 0.65 $\pm$ 0.04 <sup>a</sup>      | 0.85 $\pm$ 0.07 <sup>a</sup>     | 0.69 $\pm$ 0.05 <sup>a</sup>              | 0.93 $\pm$ 0.13 <sup>a</sup>      | 1.4 $\pm$ 0.45 <sup>a</sup>       | 0.7 $\pm$ 0.09 <sup>a</sup>       | 0.91 $\pm$ 0.25 <sup>a</sup>      |
|                            |                                 | Ni  | 0.66 $\pm$ 0.08 <sup>a,b</sup> | 0.55 $\pm$ 0.07 <sup>a,b</sup>    | 0.26 $\pm$ 0.03 <sup>c</sup>     | 0.47 $\pm$ 0.15 <sup>b,c</sup>            | 0.97 $\pm$ 0.33 <sup>a,d</sup>    | 0.56 $\pm$ 0.17 <sup>b,d</sup>    | 0.46 $\pm$ 0.08 <sup>b,c</sup>    | 0.88 $\pm$ 0.17 <sup>a,d</sup>    |
|                            | Hg                              | 0.08 $\pm$ 0.01 <sup>a</sup>                | 0.30 $\pm$ 0.07 <sup>c</sup>   | 0.66 $\pm$ 0.06 <sup>b</sup>      | 1.07 $\pm$ 0.21 <sup>b</sup>     | 0.13 $\pm$ 0.05 <sup>a,d</sup>            | 0.27 $\pm$ 0.05 <sup>c</sup>      | 0.09 $\pm$ 0.01 <sup>d</sup>      | 1.19 $\pm$ 0.21 <sup>b</sup>      |                                   |
|                            | POPs                            | PBDE_47                                     | 1.98 $\pm$ 0.4 <sup>a</sup>    | 1.61 $\pm$ 0.4 <sup>a</sup>       | 7.56 $\pm$ 0.94 <sup>c,e</sup>   | 24.33 $\pm$ 9.77 <sup>b,c</sup>           | 1.19 $\pm$ 0.79 <sup>d</sup>      | 6.37 $\pm$ 1.25 <sup>e</sup>      | 22.63 $\pm$ 8.53 <sup>b,c</sup>   | 19.71 $\pm$ 4.25 <sup>b</sup>     |
|                            |                                 | Sum of all PCBs                             | 33.44 $\pm$ 3.86 <sup>a</sup>  | 28.41 $\pm$ 3.42 <sup>a</sup>     | 142.11 $\pm$ 15.79 <sup>c</sup>  | 164.25 $\pm$ 33.19 <sup>c</sup>           | 77.48 $\pm$ 12.54 <sup>d</sup>    | 325.65 $\pm$ 56.25 <sup>c</sup>   | 1753.72 $\pm$ 750.92 <sup>b</sup> | 1477.69 $\pm$ 309.64 <sup>b</sup> |
|                            |                                 | HCB   | 4.85 $\pm$ 1.22 <sup>a,d</sup> | 1.52 $\pm$ 0.17 <sup>b</sup>      | 2.73 $\pm$ 0.36 <sup>a,c</sup>   | 4.01 $\pm$ 0.42 <sup>a,d</sup>            | 2.24 $\pm$ 0.45 <sup>c</sup>      | 3.89 $\pm$ 0.65 <sup>c,d</sup>    | 4.4 $\pm$ 0.95 <sup>a</sup>       | 4.67 $\pm$ 0.99 <sup>a</sup>      |
|                            |                                 | $\gamma$ HCH                                | 0.19 $\pm$ 0.02 <sup>a</sup>   | 0.13 $\pm$ 0.02 <sup>a</sup>      | 0.15 $\pm$ 0.01 <sup>a</sup>     | 0.12 $\pm$ 0.01 <sup>a</sup>              | 0.84 $\pm$ 0.17 <sup>b</sup>      | 5.57 $\pm$ 1.39 <sup>c</sup>      | 4.01 $\pm$ 1.07 <sup>c</sup>      | 0.98 $\pm$ 0.17 <sup>b</sup>      |
|                            |                                 | Trans-nonachlor                             | 9.08 $\pm$ 1.73 <sup>a,c</sup> | 4.33 $\pm$ 0.56 <sup>b</sup>      | 7.01 $\pm$ 0.42 <sup>c</sup>     | 11.25 $\pm$ 1.71 <sup>a,c</sup>           | 1.55 $\pm$ 0.95 <sup>d</sup>      | 3.33 $\pm$ 0.67 <sup>b</sup>      | 10.23 $\pm$ 1.99 <sup>a,c</sup>   | 11.32 $\pm$ 2.26 <sup>a</sup>     |
|                            |                                 | 2,4'DDE                                     | 2.30 $\pm$ 0.34 <sup>a,e</sup> | 1.90 $\pm$ 0.28 <sup>c,e</sup>    | 3.49 $\pm$ 0.37 <sup>a,c,d</sup> | 4.44 $\pm$ 0.71 <sup>a,d</sup>            | 1.33 $\pm$ 0.56 <sup>e</sup>      | 2.35 $\pm$ 0.59 <sup>e</sup>      | 10.88 $\pm$ 3.27 <sup>b</sup>     | 4.54 $\pm$ 0.95 <sup>b,d</sup>    |
| 4,4'DDE+dieldrin           |                                 | 19.36 $\pm$ 5.1 <sup>a,c</sup>              | 14.08 $\pm$ 2.7 <sup>c</sup>   | 34.63 $\pm$ 2.71 <sup>a,b</sup>   | 96.13 $\pm$ 24.59 <sup>b,e</sup> | 7.11 $\pm$ 4.68 <sup>f</sup>              | 52.08 $\pm$ 9.79 <sup>b</sup>     | 136.58 $\pm$ 30.03 <sup>d,e</sup> | 164.19 $\pm$ 33.43 <sup>d</sup>   |                                   |
| 4,4'DDD                    | 2.48 $\pm$ 0.57 <sup>a</sup>    | 1.32 $\pm$ 0.12 <sup>a</sup>                | 6.48 $\pm$ 0.43 <sup>b</sup>   | 14.23 $\pm$ 5.26 <sup>b</sup>     | 1.96 $\pm$ 0.64 <sup>a</sup>     | 10.09 $\pm$ 1.8 <sup>b</sup>              | 43.46 $\pm$ 10.27 <sup>c</sup>    | 50.87 $\pm$ 10.82 <sup>c</sup>    |                                   |                                   |
| 2,4'DDT                    | 6.83 $\pm$ 1.72 <sup>a</sup>    | 1.82 $\pm$ 0.39 <sup>c</sup>                | 12.07 $\pm$ 0.18 <sup>b</sup>  | 15.90 $\pm$ 2.79 <sup>b</sup>     | 2.26 $\pm$ 1.77 <sup>e</sup>     | 4.31 $\pm$ 0.91 <sup>a</sup>              | 23.02 $\pm$ 4.81 <sup>b,d</sup>   | 25.79 $\pm$ 5.35 <sup>d</sup>     |                                   |                                   |
| Sum of DDT and metabolites | 7.74 $\pm$ 1.92 <sup>a</sup>    | 4.79 $\pm$ 1.29 <sup>a</sup>                | 14.38 $\pm$ 2.68 <sup>d</sup>  | 32.68 $\pm$ 9.70 <sup>b,d</sup>   | 1.46 $\pm$ 0.26 <sup>e</sup>     | 17.26 $\pm$ 4.83 <sup>d</sup>             | 51.31 $\pm$ 11.57 <sup>b,c</sup>  | 72.83 $\pm$ 15.8 <sup>c</sup>     |                                   |                                   |
| Sum of all OCPs            | 44.91 $\pm$ 9.79 <sup>a,c</sup> | 25.00 $\pm$ 3.29 <sup>c</sup>               | 66.42 $\pm$ 3.2 <sup>a,e</sup> | 145.97 $\pm$ 31.54 <sup>b,e</sup> | 16.45 $\pm$ 8.99 <sup>f</sup>    | 76.07 $\pm$ 13.57 <sup>a,e</sup>          | 228.60 $\pm$ 47.15 <sup>b,d</sup> | 261.40 $\pm$ 52.46 <sup>d</sup>   |                                   |                                   |

618 Table 2: Results of FAMT analysis for the most significant correlations ( $\delta_{i,1} = 0.005$  and  $\delta_{i,2} = 0.1$ ) established between gene transcription levels and concentrations in eels in As or Cd or  
619 lindane or with fish HSI.

620

| Factor  | P        | $\beta$                   | Gene name  | Biological process  |
|---------|----------|---------------------------|--|---|
| Arsenic | 0.002    | -0.12                     | Zinc finger protein 36, C3H1 type-like 1   | (1) mRNA metabolic process (2) Vasculogenesis   |
|         | 0.004    | 0.06                      | E3 ubiquitin-protein ligase RNF115   | Ubiquitin conjugation pathway   |
|         | 0.004    | -0.05                     | Transient receptor potential cation channel subfamily M member 5                     | Ion transport   |
|         | 0.004    | -0.05                     | Metalloprotease TIKI2  | Wnt signalling pathway  |
|         | 0.002    | -0.09                     | Trehalase  | Trehalose catabolic process   |
|         | 0.004    | -0.005                    | Heterogeneous nuclear ribonucleoprotein A0   | (1) 3'-UTR-mediated mRNA stabilization (2) inflammatory response                            |
|         | 0.001    | 0.1                       | 15 kDa selenoprotein   | Protein folding   |
|         | 0.002    | -0.21                     | Zinc transporter ZIP11   | (1) Ion transport (2) zinc influx   |
|         | 0.002    | -0.21                     | 25-hydroxyvitamin D-1 alpha hydroxylase, Mitochondrial                               | Vitamin D metabolic process   |
| Cadmium | 1.27e-05 | -0.007                    | Pericentriolar material 1 protein  | Centrosome organization   |
|         | 0.0002   | -0.003                    | Utrophin   | Positive regulation of cell-matrix adhesion   |
|         | 0.0002   | -0.004                    | E3 ubiquitin-protein ligase UBR5   | Protein polyubiquitination  |
|         | 0.0003   | 0.03                      | Activin receptor type-2A   | Positive regulation of activin receptor signaling pathway                                   |
|         | 0.0005   | 0.02                      | Transcription factor E2-alpha  | Cell differentiation  |
|         | 0.0005   | -0.007                    | Protein 4.1  | Cytoskeleton organization   |
|         | 0.001    | 0.003                     | Band 4.1-like protein 3  | Apoptosis   |
|         | 0.001    | 0.01                      | Ubiquitin carboxyl-terminal hydrolase 20   | Ubiquitin-dependent protein catabolic process   |
|         | 0.001    | 0.01                      | C-terminal-binding protein 2   | (1) Transcription corepressor activity  |
|         | 0.001    | -0.008                    | Acetylcholinesterase collagenic tail peptide   | Neurotransmitter degradation  |
| 0.004   | 0.002    | Sortilin-related receptor | (1) Cholesterol metabolism (2) Lipid metabolism and transport (3) Steroid metabolism |   |
| Lindane | 0.0001   | -0.13                     | Zinc finger protein 148  | (1) Regulation of apoptotic process (2) Regulation of cellular amino acid metabolic process |
|         | 0.0003   | 0.04                      | Oxysterol-binding protein 2  | (1) Lipid transport   |
|         | 5.53e-06 | 0.1                       | Mitochondrial coenzyme A transporter SLC25A42  | ADP transmembrane transporter activity  |
|         | 0.0005   | 0.14                      | Carnitine O-palmitoyltransferase 2, mitochondrial                                    | Fatty acid metabolism   |
|         | 0.002    | 0.06                      | Mediator of RNA polymerase II transcription subunit 13                               | Mediator complex  |
|         | 5.31e-06 | -0.03                     | Myelin protein zero-like protein 2   | Cell adhesion   |
|         | 3.15e-05 | 0.06                      | Peroxisomal membrane protein PEX13   | Fatty acid alpha-oxidation  |
|         | 0.0004   | 0.09                      | Serine protease hepsin   | (1) Serine-type endopeptidase activity (2) Positive regulation of hepatocyte proliferation  |
|         | 0.002    | 0.12                      | Decorin  | Organ morphogenesis   |
|         | 0.002    | 0.06                      | RNA-binding protein 4B   | mRNA processing   |
| HSI     | 0.004    | -2.10                     | Glioma tumor suppressor candidate region gene 2 protein                              | /   |
|         | 0.003    | -2.82                     | 60S ribosomal protein L5   | Translation   |
|         | 0.003    | 0.51                      | 40S ribosomal protein S29  | Translation   |
|         | 1.29e-06 | 1.93                      | Putative ferric-chelate reductase 1  | Electron transport chain  |
|         | 0.005    | 0.86                      | Cathepsin B  | Proteolysis   |
|         | 0.004    | 0.98                      | 60S ribosomal protein L23  | Translation   |
|         | 0.002    | -0.93                     | Ubiquitin-60S ribosomal protein L40  | (1) Translation (2) DNA repair (3) G1/S transition of mitotic cell cycle                    |
|         | 0.004    | -0.96                     | Ethanolaminephosphotransferase 1   | Phospholipid biosynthesis   |
|         | 0.002    | 0.63                      | Hephaestin-like protein 1  | Iron homeostasis  |
|         | 0.0009   | 0.13                      | Transmembrane channel-like protein 7   | Ion transport   |
|         | 0.003    | 1.01                      | UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminophosphotransferase    | Protein glycosylation   |
|         | 0.001    | 1.54                      | Thioredoxin  | (1) Cell redox homeostasis (2) Cell proliferation   |
|         | 0.0005   | 1.08                      | Proteasome activator complex subunit 2   | Proteasome activator complex  |
| 0.004   | -0.10    | Acyl-CoA-binding protein  | Triglyceride metabolic process   |   |

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630

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