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Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels

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

Keywords Transcriptome · Multi-pollutant · Wild fish populations · Atlantic eels

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Introduction

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

In this context, particular emphasis was placed in recent years on the potential use of "omics" technologies in ecotoxicology. By allowing a global approach, "omics" technologies have indeed the potential to discover new mechanisms of toxicity and to classify the

toxicity of contaminants. In molecular medicine, where these technologies (i.e. genomic, transcriptomic, epigenomic, proteomic or metabolomic) are already widely used, successful prospective and diagnostic cases studies were conducted with such approaches (Vlaanderen et al. 2010). In 2008, the fish toxicogenomics consortium thus proposed to use "omic" technologies in the aquatic risk assessment process (Van Aggelen et al. 2010). Among the "omics" technologies, the recent development of next generation sequencing (NGS) technologies and bioinformatic tools to analyze these large datasets offers the opportunity for ecotoxicologists to investigate the effects of contaminants on genome-wide evolution or on transcriptome-wide response in non-model but environmentally relevant species (Webster and Bury 2013; Regier et al. 2013). Gene expression profiling using RNA-seq (also called whole transcriptome shotgun sequencing) could play a key role to identify new biomarkers of exposure and adverse effects and to discover new toxicity pathways.

In the present study, we thus used RNA-seq technology on two threatened species, the European eel (*Anguilla anguilla*) and the American eel (*Anguilla rostrata*), to test whether changes in transcriptome profiles in wild fish may be used to detect and unravel the toxicity of pollutants in a context of in situ exposure. Atlantic eels are catadromous fish species with a complex life cycle including marine (spawning, larval phase and sexual maturation) and continental (feeding and somatic growth) environments. Historically abundant and widespread in Europe and North America, populations of Atlantic eels have suffered a sharp decline. European eel's recruitment is only one tenth of what it was in the early 1980s (Stone 2003; ICES report 2009). In Canada, and more precisely in one of the most important rivers of the North American continent, the St. Lawrence River, the situation appears to be equally worrying. Available data show a decrease in recruitment of around 40 to 80 % depending on the sites and years, some sites having registered a decrease of 98 % since early 1980s (de Lafontaine et al. 2010). In view of these data, the European species is currently considered as critically endangered (IUCN red list) and the American eel was recently designated as threatened species by the Committee on the Status of Endangered Wildlife in Canada. As the unusual life cycle of Atlantic eels makes them particularly vulnerable to pollution, Atlantic eels are considered as sentinel species in ecotoxicology (Belpaire and Goemans 2007; Geeraerts and Belpaire 2010).

Thus, wild immature yellow eels (i.e. growth somatic phase) were sampled in 8 sites (4 Canadian and 4 French sites) presenting a contamination gradient and also a wide range of physicochemical conditions notably in term of salinity and temperature. In France, European eels were sampled along a historically cadmium-contaminated hydrosystem, the Garonne–Gironde continuum (Durrieu et al. 2005). In addition, recent investigations have reported high concentrations of PCBs in the muscle of eels inhabiting the Gironde estuary (up to 3,399 ng g^{-1} dw, Tapie et al. 2011). In Canada, fish were sampled along the St. Lawrence River. Past studies carried out on the muscle of American eels inhabiting this hydrosystem (Hodson et al. 1994) have reported high concentrations in PCBs (up to 0.83 µg g^{-1} wet w), organochlorinated pesticides (up to 0.70 µg g^{-1} wet w) and in mercury (up to 0.26 µg g^{-1} wet w). The individual hepatic transcriptome of 6 replicate specimens of eel per sampling site was determined by high throughput RNA sequencing using Illumina Hiseq 2000 technology. In parallel, the contamination levels of fish in 25 organic pollutants and 8 metals were determined. Then, we carried out correlation analyses between the transcriptional level of a given gene and contaminant concentrations in each individual fish. Rather than perform comparisons among sampling sites, we combined fish from all sites before carrying out the statistical analyses. In addition, data from both European and American eels were combined before carrying our statistical analyses in order to (i) identify common genes and thus potential biomarkers of exposure that could be extrapolated across fish species and (ii) to work with the widest range of physic-chemical conditions and thus to remove as much as possible genes that are more likely to be affected by natural factors. Finally, condition indices such as the relative condition index (Kn), the hepatosomatic index (HSI) and the spleen somatic index (SSI) were added in the model in order to identify genes for which transcription levels could be associated to the general health status of animals.

Materials and methods

Wild eel sample collection

Eight sampling sites were selected in Quebec (Canada) and in France on the basis of their known gradient of contamination by metallic and organic pollutants (Lee et al. 1999; Durrieu et al. 2005). In Quebec, yellow eels (sexually undetermined fish) were collected in four sites (see Table 1) located near or in the St Lawrence Estuary (St Jean Lake: 48°51′40″N 64°28′47″O, Sud-Ouest River: 48°22′27″N 68°43′02″O, St Pierre Lake: 46°09′18″N 74°23′04″WO, St François Lake: 46°19′50″N, 74°23′04″WO). In France, immature yellow eels were collected in four sites along the Gironde aquatic system (Dordogne: 44°54′30.30″N 0°15′00.61″O, Garonne: 45°12′06.62″N 0°43′34.72″O and

Table 1 Morphometric measures (mean ± SE, $n = 6$) and average concentrations of muscle organic pollutants (expressed as ng g^{-1}, dw) and kidneys metal concentrations (expressed as μg g^{-1}, dw) in Atlantic eels sampled in 8 sites located in Canada and France

Groups of variables	Variables	Canadian sites (*Anguilla rostrata*)				French sites (*Anguilla anguilla*)			
		St Jean	Sud-Ouest	St François	St Pierre	Certes	Dordogne	Garonne	Gironde
Biometry	Length	529.26 ± 22.43	347.53 ± 18.47	827.93 ± 14.12	890.87 ± 10.53	434.53 ± 19.2	338 ± 12.78	442.66 ± 17.73	578.66 ± 15.02
	Weight	290.13 ± 37.6	77.87 ± 18.66	1,222.33 ± 32.17	1,434.66 ± 53.92	157.33 ± 31.25	63 ± 10.65	156.26 ± 22.32	349.46 ± 32.0
	SSI	0.27 ± 0.07^{a}	0.22 ± 0.05^{a}	0.09 ± 0.01^{b,c}	0.09 ± 0.01^{b}	0.11 ± 0.01^{b,c}	0.09 ± 0.01^{b,c}	0.12 ± 0.01^{c}	0.06 ± 0.004^{d}
	HSI	2.09 ± 0.13^{a}	1.62 ± 0.06^{b}	1.04 ± 0.05^{c,d,e}	1.07 ± 0.06^{c,d,e}	1.18 ± 0.02^{d}	0.96 ± 0.04^{e,f}	1.14 ± 0.04^{d,c}	0.88 ± 0.03^{f}
	Kn	0.4 ± 0.01^{a,c}	0.39 ± 0.01^{a,c}	0.48 ± 0.02^{a}	0.38 ± 0.01^{c}	0.51 ± 0.02^{b}	0.48 ± 0.01^{b}	0.48 ± 0.01^{b}	0.46 ± 0.01^{b}
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 ± 0.75^{a}	2.23 ± 0.14^{a,c}	1.75 ± 0.19^{a}	4.76 ± 1.29^{c}	0.47 ± 0.17^{d}	27.29 ± 5.26^{b}	9.09 ± 2.51^{c}	52.3 ± 11.35^{b}
	Cu	6.01 ± 0.67^{a}	9.3 ± 1.04^{b}	5.94 ± 0.39^{c}	8.78 ± 1.16^{b}	7.18 ± 0.80^{a,b,c}	6.54 ± 035^{a,b,c}	6.06 ± 0.98^{a,c}	8.7 ± 0.65^{b}
	Zn	253.2 ± 32.8^{a}	250.51 ± 22.64^{a}	182.7 ± 40.9^{a}	205.1 ± 17.6^{a}	227.0 ± 17.4^{a}	255.7 ± 7.9^{a}	198.6 ± 16.9^{a}	246.7 ± 31.9^{a}
	Ag	0.08 ± 0.01^{a}	0.39 ± 0.14^{a}	0.09 ± 0.02^{a}	0.63 ± 0.41^{a}	0.01 ± 0.001^{a}	0.09 ± 0.03^{a}	0.13 ± 0.05^{a}	3.38 ± 1.48^{b}
	As	2.58 ± 0.36^{a,c}	1.59 ± 0.24^{c,d}	0.97 ± 0.10^{d}	2.36 ± 0.14^{d}	4.25 ± 0.83^{a,b}	2.98 ± 0.76^{a,c}	1.08 ± 0.16^{d}	6.33 ± 1.48^{b}
	Pb	0.19 ± 0.06^{a,c}	0.12 ± 0.02^{a,c}	0.09 ± 0.02^{c}	0.36 ± 0.16^{a}	0.25 ± 0.03^{a}	0.73 ± 0.16^{d}	2.7 ± 1.54^{b,d}	2.68 ± 0.62^{b}
	Cr	0.62 ± 0.06^{a}	0.65 ± 0.04^{a}	0.85 ± 0.07^{a}	0.69 ± 0.05^{a}	0.93 ± 0.13^{a}	1.4 ± 0.45^{a}	0.7 ± 0.09^{a}	0.91 ± 0.25^{a}
	Ni	0.66 ± 0.08^{a,b}	0.55 ± 0.07^{a,b}	0.26 ± 0.03^{c}	0.47 ± 0.15^{b,c}	0.97 ± 0.33^{a,d}	0.56 ± 0.17^{b,d}	0.46 ± 0.08^{b,c}	0.88 ± 0.17^{a,d}
	Hg	0.08 ± 0.01^{a}	0.30 ± 0.07^{c}	0.66 ± 0.06^{b}	1.07 ± 0.21^{b}	0.13 ± 0.05$^{a\ d}$	0.27 ± 0.05^{c}	0.09 ± 0.01^{d}	1.19 ± 0.21^{b}
POPs	PBDE_47	1.98 ± 0.4^{a}	1.61 ± 0.4^{a}	7.56 ± 0.94^{c,e}	24.33 ± 9.77^{b,c}	1.19 ± 0.79^{d}	6.37 ± 1.25^{e}	22.63 ± 8.53$^{b\ c}$	19.71 ± 4.25^{b}
	Sum of all PCBs	33.44 ± 3.86^{a}	28.41 ± 3.42^{a}	142.11 ± 15.79^{c}	164.25 ± 33.19 c	77.48 ± 12.54^{d}	325.65 ± 56.25^{c}	1753.72 ± 750.92^{b}	1477.69 ± 309.64^{b}
	HCB	4.85 ± 1.22^{a,d}	1.52 ± 0.17^{b}	2.73 ± 0.36^{a,c}	4.01 ± 0.42^{a,d}	2.24 ± 0.45^{c}	3.89 ± 0.65^{c,d}	4.4 ± 0.95^{a}	4.67 ± 0.99^{a}
	γHCH	0.19 ± 0.02^{a}	0.13 ± 0.02^{a}	0.15 ± 0.01^{a}	0.12 ± 0.01^{a}	0.84 ± 0.17^{b}	5.57 ± 1.39^{c}	4.01 ± 1.07^{c}	0.98 ± 0.17^{b}
	Trans-nonachlor	9.08 ± 1.73^{a,c}	4.33 ± 0.56^{b}	7.01 ± 0.42^{c}	11.25 ± 1.71^{a,c}	1.55 ± 0.95^{d}	3.33 ± 0.67^{b}	10.23 ± 1.99^{a,c}	11.32 ± 2.26^{a}
	2.4′DDE	2.30 ± 0.34^{a,e}	1.90 ± 0.28^{c,e}	3.49 ± 0.37^{a,c,d}	4.44 ± 0.71^{a,d}	1.33 ± 0.56^{e}	2.35 ± 0.59^{e}	10.88 ± 3.27^{b}	4.54 ± 0.95^{b,d}
	4.4′DDE + dieldrin	19.36 ± 5.1^{a,c}	14.08 ± 2.7^{c}	34.63 ± 2.71^{a,b}	96.13 ± 24.59^{b,e}	7.11 ± 4.68^{f}	52.08 ± 9.79^{b}	136.58 ± 30.03^{d,e}	164.19 ± 33.43^{d}
	4.4′DDD	2.48 ± 0.57^{a}	1.32 ± 0.12^{a}	6.48 ± 0.43^{b}	14.23 ± 5.26^{b}	1.96 ± 0.64^{a}	10.09 ± 1.8^{b}	43.46 ± 10.27^{c}	50.87 ± 10.82^{c}
	2.4′DDT	6.83 ± 1.72^{a}	1.82 ± 0.39^{c}	12.07 ± 0.18^{b}	15.90 ± 2.79^{b}	2.26 ± 1.77^{e}	4.31 ± 0.91^{a}	23.02 ± 4.81^{b,d}	25.79 ± 5.35^{d}
	Sum of DDT and metabolites	7.74 ± 1.92^{a}	4.79 ± 1.29^{a}	14.38 2.68^{d}	32.68 ± 9.70^{b,d}	1.46 ± 0.26^{e}	17.26 ± 4.83^{d}	51.31 ± 11.57^{b,c}	72.83 ± 15.8^{c}
	Sum of all OCPs	44.91 ± 9.79^{a,c}	25.00 ± 3.29^{c}	66.42 ± 3.2^{a,e}	145.97 ± 31.54^{b,e}	16.45 ± 8.99^{f}	76.07 ± 13.57^{a,e}	228.60 ± 47.15^{b,d}	261.40 ± 52.46^{d}

For each country, the four sites are stored in a gradient of contamination. All data are expressed as mean ± SE ($n = 6$ per site). Means designated with different letters are significantly different (LSD test, $p < 0.05$)

Gironde estuary: 45°12′06.62″N 0°43′34.72″O), and in the Arcachon Bay, considered as a pristine environment (Certes: 44°41′18″N 1°1′39″W). For all sites, temperature, salinity and dissolved oxygen concentrations were measured.

All fish were collected between May 24 and June 24 of 2011, using a trawl, a fyke net or by electrical fishing. All yellow eels were size selected to minimize potential allometric bias. The total length of American and European eels (672.8 ± 38.55 and 482.04 ± 31.01 mm, respectively; mean ± SE, $n = 24$) and weight (811.42 ± 118.41 and 221.33 ± 34.06 g, respectively; mean ± SE, $n = 24$) were recorded for each fish in order to estimate the relative condition factor [Kn: (total weight (g)/(0. 0004 × (total length (cm))3.3828) × 100], the Hepato-Somatic Index [HSI: (weight liver/total weight) × 100] and spleen condition index [SSI: (spleen weight/total weight) × 100]. For each sampling region, a total of 6 fish were collected and used for subsequent analyses. Fish were dissected as soon as possible and organs were divided into several samples. Samples for gene transcription analyses were stored in RNA later at −20 °C until needed. For both organic and metal analyses, samples were stored at −80 °C.

Metal analyses

Metal analyses were determined from the kidneys, i.e. a main organ of metal bioaccumulation (Barbier et al. 2005). Kidney samples were freeze-dried in PFA vials (Savillex) and kept in these vials during the whole digestion process. A 50 mg subsample was taken after the freeze-drying step to perform the mercury analysis. These sub-samples were processed in a mercury analyser (Milestone DMA-80) and MESS-3 certified reference material (National Research Council Canada) was used, with a recovery rate of 98.8 ± 2.5 %. The rests of the samples were digested overnight at room temperature in pure trace metal grade nitric acid (HNO_3). The following day, sample digestion was completed by autoclave (All American 25X-1) for 2 h at 15 PSI. Samples were then diluted in Milli-Q water to obtain a final concentration of 10 % HNO_3. Metal concentrations for Ag, As, Cd, Cr, Cu, Ni, Pb and Zn were measured by inductively coupled plasma–mass spectrometry (ICP–MS; Thermo Scientific XSeries 2), inductively coupled plasma–atomic emission spectrometry (ICP–AES; Varian Vista AX) or both methods. Blanks and standards (TORT-2 and DOLT-4, National Research Council Canada) were submitted to the same digestion protocol as kidney samples to monitor the efficiency of the procedure. Recovery rates (expressed as % of certified values) were: Ag = 80.2 ± 1.2 %; As = 106.5 ± 3.8 %; Cd = 102.9 ± 1.2 %; Cr = 90.8 ± 5.2 %; Cu = 88.8 ± 1.9 %; Ni = 86.1 ± 2.7 %; Pb = 82.9 ± 26.0 %; Zn = 100.4 ± 1.6 %.

Organic pollutants analyses

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

As described by Labadie et al. 2010, POPs levels were blank corrected and the method detection limit (LoD) was derived from the blank value variability, For PCBs LoQs were comprised between 0.2 and 2 ng/g dw; for OCPs they were comprised between 0.1 and 0.4 ng/g dw; for PBDEs they were comprised between 0.1 and 0.2 ng/g dw.

Preparation of cDNA libraries and contig assembly

For the transcriptomic sequencing, the liver was selected due to its wide range of functions, including detoxification and energy metabolism. For each sampling site (Quebec and France), six biological replicates were used ($n = 48$ eels). Briefly, total RNAs were individually extracted from fish liver using the PureLink™ RNA mini kit (Invitrogen). During this step, samples were submitted to DNAseI treatment, according to the manufacturer's instructions. Then, preparation of cDNA libraries for Illumina HiSeq 2000 sequencing was done using the Truseq™ Stranded mRNA sample prep kit, following the manufacturer's instructions. The 48 individually tagged libraries were randomly pooled in equal amounts and sequenced on 4 lanes at the Genome Quebec innovation Center (McGill University, Montreal, QC, Canada) using Illumina HiSeq 2,000 technology (100 bp paired-ends reads).

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

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

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

Eight genes that showed strong variations in their transcription levels among sampling sites were quantified by RT-qPCR on the same liver samples used for RNA-seq. For each gene, specific primer pairs were determined (see supplementary material Table S1) using the lightcycler probe design software (Roche). RNAs were extracted from 30 mg of tissue using the SV total RNA isolation system (Promega) according to the manufacturer's instruction. One µg of total RNAs was used for cDNA synthesis using Goscript reverse transcriptase (Promega), oligodT primer and random primers according to manufacturer's instructions. Resulting cDNAs were diluted 2-fold for PCR reactions. RT-qPCR reactions of 20 µl were performed in a Stratagene *Mx 3,000p* system using Brilliant III Fast SYBR Green QPCR Master Mix (Stratagene-Agilent) and 1 µl of diluted cDNAs. The reaction specificity was determined for each reaction from the dissociation curve of the PCR product and electrophoresis. Data analysis was performed using the *β-actin* gene as reference. Hence, during our experiment, total RNAs were quantified and 1 µg was used to be reverse-transcribed. During the subsequent qPCR amplifications, the output cycle corresponding to the *β-actin* was examined. This output cycle did not show significant differences among fish, demonstrating the relevance of the *β-actin* as reference gene in our conditions.

Statistical analyses

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

Due to the wide distribution area of eels and since factors unique to each site but unrelated to metal or organic contamination (e.g. temperature, salinity,…), can also affect the gene transcription level, the FAMT approach was adopted to increase statistical power between a variation in the transcription level of an individual gene and the contamination level of fish for a given contaminant. Thus, in addition to contaminants, natural factors that showed significant variations among sites (mainly temperature and salinity) as well as some morphometric measurements (length and weight) were added to the model in order to remove, at least in part, genes for which transcription levels could be more likely related to these factors rather than with contaminants. Then, the variables such as pollutant concentrations, condition indices (i.e. the relative condition index (Kn), the hepato-somatic index (HSI) and the spleen somatic index (SSI)) or physicochemical conditions of sampling sites (i.e. temperature and salinity) were gathered in a matrix made of independent observations from a standard normal distribution. This model has been studied in Friguet et al. (2009) and modified for our transcriptomic data (for more information see Coudret 2013). By using this test, we also controlled the False Discovery Rate (FDR) over the set of contigs for a given variable. The R package FAMT (Causeur 2011) allowed us to produce a *p value* between a factor and contigs. For every studied variables, we then introduced two thresholds $\delta_{i,1}$ and $\delta_{i,2}$ and we selected a contig according to the two thresholds. In this way, the *pvalue* calculated between a given variable and one contig had to satisfy the thresholds $\delta_{i,1}$ and *p value* calculated between another variable for the same contig had to satisfy the thresholds $\delta_{i,2}$. For each contig and each variable, the β represents the correlation length (see supplementary material for more details).

Results and discussion

Condition and contamination levels of eels

Morphometric measurements, conditions indices and contamination levels of all eels and their difference among sampling sites are presented in Table 1. Generally, the highest levels of contamination in both metallic and organic contaminants were observed in eels from France. Significant increases in pollutant concentrations were observed along the French gradient from Certes → Dordogne → Garonne → Gironde. To a lesser extent, we observed a contamination gradient from St. Jean → Sud-Ouest → St. François → St. Pierre for Quebec. Concerning metals, the highest variations were observed for cadmium (Cd) and silver (Ag). For Cd, animals inhabiting the Gironde estuary were 112-fold more contaminated than eels from Certes, i.e. the cleanest site in France, and 11-fold superior to Lake St. Pierre, i.e. the

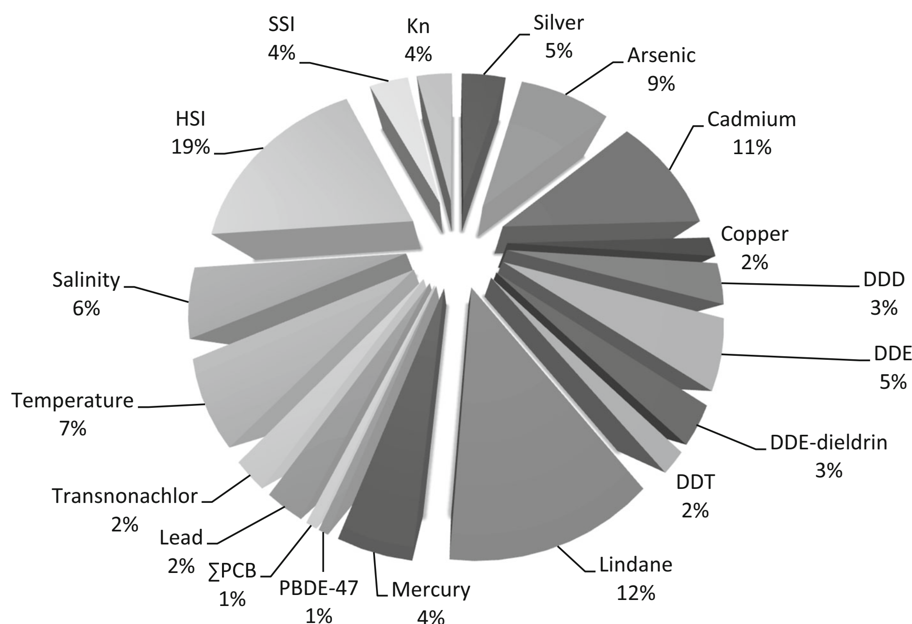

Fig. 1 Results of FAMT analyses with low stringency: Percentage representation of genes correlated with a single factor

most contaminated site in Quebec. For Ag, eels from Gironde were 338-fold superior to eels from Certes with low concentrations of 3.4 ± 1.5 and 0.01 ± 0.001 ng g^{-1} respectively. Concerning organic pollutants, the highest variations were observed for metabolites of DDT and PCBs. For Gironde, eels were 56 and 2.3-fold more contaminated than eels from Certes and St Pierre Lake, respectively. For PCBs, eels from Gironde were 24-fold more contaminated than eels from Certes and 10.6-fold more contaminated than eels from St Pierre Lake. These high contamination pressures in Gironde were associated with a poor health status of eels. Eels from Gironde presented the lowest values for Kn, HSI and SSI in comparison to eels from the other French sites.

RNA-seq data

RNA-seq generated 2.7 billion fragments averaging 75 bases in length. The assembly of these reads generated a total of 54,140 contigs, 95 % of the contigs were longer than 200 bp and 11.7 % were longer than 1,000 bp. A total of 18,115 contigs showed high homology with known sequences (BLASTX, Evalue $\leq 10^{-10}$). A total of 11,547 unique genes of known function were identified (i.e. multiple contigs that had the same annotation were removed).

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

Quantitative influence of factors on eel's transcriptome

In order to get an overview of the effects of natural and anthropogenic factors on the transcriptome profiles of eels, we first used the FAMT method with low stringency thresholds: $\delta i,1 = 0.009$ and $\delta i,2 = 0$. From the 11,547 unique genes, the transcription level of 1,523 single genes of known function was significantly correlated with pollutants or natural factors. We then counted how many contigs were linearly linked with each of the $n = 19$ variables (Fig. 1 and Table S3). For biometric variables, 284 genes were identified for HSI, 60 for SSI, and 55 for Kn. This corresponds to 19, 4, and 4 % of the total number of genes, respectively. The dominant percentage of genes associated to HSI appears to be consistent with the fact that transcriptome analyses were carried out on the liver tissue.

Among metals, the number of genes reached 140 (9 %) for As, 164 (11 %) for Cd and 4 % for Hg and 5 % for Ag. Despite that the highest contamination gradient was observed for Ag, Cd and As seemed to have more influence on the transcriptome of eels. For POPs, γ-HCH (lindane) was associated with the greatest number of genes ($n = 182$, 12 %), followed by DDT and its metabolites 4,4′DDD, 2,4′DDE, 4,4′DDE + dieldrin represented around 2 and 5 % of total genes (Fig. 1). Interestingly, despite the fact that we observed very high concentrations of PCBs in eel muscle, few genes were associated with this

contaminant. In contrary, numerous genes were associated with lindane whereas it was found weakly accumulated in eels. Temperature and salinity were respectively linked with 6 and 5 % of genes. Since they are essential factors controlling the food intake and growth rates in fish, it is quite surprisingly that these two factors were less represented than pollutants. This could suggest that contaminants have more influence on the liver transcriptome of eels than temperature, which could be related to the fact that the liver is a major target of pollutant metabolism, accumulation and toxicity.

Genes associated with several factors

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

Genes associated with arsenic, cadmium, lindane and HSI

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

Hepato-somatic index

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

The transcription level of 14 genes (Table 2) showed significant correlations with the HSI of fish. Among these genes, 4 genes encode for ribosomal proteins, i.e. *60S ribosomal protein L5* (*rpl5*), *40S ribosomal protein S29* (*rps29*), *60S ribosomal protein L23* (*rpl23*) and *ubiquitin-60S ribosomal protein L40* (*uba52*). Interestingly, the transcription level of these genes was found to increase or decrease with increasing fish HSI. This could be linked, at least in part, to the fact that these genes are not only involved in protein translation but also in cell cycle by regulating the accumulation of the p53 protein in cell (Horn and Vousden 2008; Lee et al. 2012). The p53 protein is involved in cell cycle arrest and apoptosis. In this view, it is interesting to note that increasing fish HSI were significantly correlated with a drastic and similar decrease in *rpl5* ($\beta = -2.82$) and *glioma tumor suppressor candidate region gene 2 protein* (*gltscr2*, $\beta = -2.10$) transcription levels. Both *gltscr2* and *rpl5* genes are involved in the ribosomal protein-Mdm2-p53 pathway. Its activation is known to induce p53 stabilization. Moreover, recent discoveries tend to show that this pathway could play a pivotal role in cellular energy homeostasis, the ribosomal protein-Mdm2-p53 pathway serving as a molecular metabolizing switch to direct nutrient utilization in an effort to promote cell survival (Deisenroth and Zhang 2011). Our results could suggest an important role of ribosomal proteins as well as the ribosomal protein-Mdm2-p53 pathway in liver development and hepatic accumulation of energy reserves. Another potential marker of cell division and hepatic growth is the gene encoding for *thioredoxin* (*txn*). The

Table 2 Results of FAMT analysis for the most significant correlations (δi,1 = 0.005 and δi,2 = 0.1) established between gene transcription levels and concentrations in eels in As or Cd or lindane or with fish HSI

Factor	*p*	β	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

Table 2 continued

Factor	*p*	β	Gene name	Biological process
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*-acetylglucosaminephosphotransferase	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

transcription level of *txn* was positively associated with fish HSI (β = 1.54). The TXN protein is known to play an important role in cell viability by inducing cell proliferation and increasing cell lifetime (Yoshida 2005). The transcription levels of 2 other genes involved in protein metabolism were found to be positively correlated with fish HSI. These genes, i.e. *proteasome activator complex subunit 2* (*psme2*) and *cathepsin B* (*ctsb*), encode proteins that are responsible for proteolysis (Min et al. 2013; Mohamed and Sloane 2006). Cathepsin B is known to play a key role in protein turnover (Mohamed and Sloane 2006).

In addition to genes involved in protein metabolism and cell division, significant correlations were observed with genes that encode proteins involved in lipid and iron metabolism. The two genes involved in lipid metabolism, i.e. *Acyl-CoA-binding protein* (*acbp*) and *Ethanolamine-phosphotransferase 1* (*ept1*), were negatively correlated with fish HSI (β = −0.10 and β = −0.96, respectively). The EPT1 protein catalyzes the biosynthesis of phosphatidylethanolamine (PE), i.e. a major phospholipid (Gibellini and Smith 2010). It is somewhat surprising that an increase in fish HSI was associated with a decrease in *ept1* transcription level. This could be linked to the fact that PE is the precursor of many biologically active molecules such as pro-apoptotic substances (Gibellini and Smith 2010). The ACBP protein has been shown to act as intracellular acyl-CoA pool former, transporter and regulator of gene transcription (Oikari et al. 2008). In a recent study, overexpression of *acbp* in transgenic rats was found to trigger a down-regulation of peroxisome proliferator-activated receptor γ and sterol regulatory element-binding protein-1 gene transcription levels, i.e. metabolic regulators involved in lipogenesis and lipid synthesis (Oikari et al. 2008). Thus, a decrease in *acbp* transcription level could indicate lipid storage. Finally, an increase in fish HSI was associated with an up-regulation of genes involved in both iron secretion and influx, i.e. *hephaestin-like protein 1* (*hephl1*) and *putative ferric-chelate reductase 1*(*frrs1*), respectively. Such results appear consistent with the fact that iron plays a key role in cellular respiration and energy metabolism. This is also consistent with the fact that fish liver plays a central role in iron metabolism and hemoglobin turn-over (Graham and Chua 2007).

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

Arsenic

The FAMT method with highly restrictive thresholds allowed us to identify 9 genes for which transcription levels were significantly correlated with the As content of eels. The majority of these genes, i.e. 7 genes (Table 2), were found to be down-regulated and 2 genes were up-

regulated. These 2 up-regulated genes, i.e. *E3 ubiquitin-protein ligase RNF115* (*Rnf115*) and *15 kDA selenoprotein* (*sep15*), are involved in protein metabolism. RNF115 is an ubiquitin-protein involved in the ubiquitination pathway and proteolysis (Burger et al. 2006). SEP15 is involved in the quality control of protein folding and in the endoplasmic reticulum stress response (Labunskyy et al. 2007). Such a pattern could be linked to the fact that As is well known to interfere with protein folding, leading to accumulation of protein aggregates in cell (Martín-Pardillos et al. 2013). The up-regulation of these genes could aim to limit the accumulation of unfolded proteins in cell in response to As exposure. Interestingly, among the down-regulated genes, numerous genes are involved in biological functions that are known to be involved in the vasculotoxicity of As in mammals. The various mechanisms that have been observed during As vasculotoxicity include endoplasmic reticulum stress (as described above), apoptosis, oxidative stress, inflammatory mediators and vasular smooth muscle cells (VSMC) calcification (Martín-Pardillos et al. 2013). Thus, a down-regulation of the *heterogeneous nuclear ribonucleoprotein A0* (*HNRPA0*) gene that encodes a protein involved in the inflammatory response by controlling cytokine synthesis was observed in As-contaminated fish. The *zinc finger protein 36* (*zfp36l1*) that is known to be involved in vasculogenesis (Bell et al. 2006) was also found to be down-regulated. The *metalloprotease TIKI2* (*trabd2*) gene was down-regulated in eels exposed to As. *Trabd2* encodes for a protein that antagonizes the Wnt signaling pathway (Clevers and Nusse 2012). Recent works have shown that Wnt signaling pathway activation can tigger calcification of VSMCs (Martínez-Moreno et al. 2012). We also observed a decrease of *threhalase* gene transcription level. This gene encodes for an enzyme that hydrolyses trehalose. Trehalose is a disaccharide known to protect membranes in case of extreme environmental conditions (Higashiyama 2002). Moreover, in osteoporosis mice model, trehalose has been shown to decrease the development of osteoporosis (Higashiyama 2002). In the same way, it is interesting to note that we observed a strong effect of As on the transcription of *25-hydroxyvitamin D-1 alpha hydroxylase* gene (*cyp27b1*). This gene encodes an enzyme that synthetizes the active form of vitamin D_3 (Rowling et al. 2007). Vitamin D_3 under its active form acts as a hormone that controls growth and bone remodeling. In this context, we must note that VSMCs calcification during As exposure in rats was found to be accelerated by intraperitoneal injection of vitamin D_3 (Martín-Pardillos et al. 2013). We could thus hypothesize that the down-regulation of *cyp27b1* could aim to protect the hepatic vasculatory system against calcification. Two last genes involved in ion transport were found down-regulated in response to As exposure, the *zinc transporter* ZIP11 (*slc39a11*) and *transient receptor potential cation channel subfamily M member 5* (*trpm5*), suggesting an effect of As on cellular homeostasis. To conclude, it is interesting to pinpoint that many mechanisms that have been reported in mammals during As vasculotoxicity are affected in fish chronically exposed to As, thus suggesting that the hepatic vascular system could be a main target of As toxicity in wild fish.

Cadmium

Among genes that showed significant correlations with Cd concentrations in fish, 5 genes were found to be down-regulated and 6 were found to be up-regulated.

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

Interestingly, such biomarkers of tissue damage were associated with changes in the transcription level of genes involved in cell cycle regulation and apoptosis. First, the *activin receptor type-2A* (*acvr2a*) gene that encodes a receptor complex for Activin A protein was found to be up-regulated in Cd-contaminated fish ($\beta = 0.03$). Activin A protein is a member of the transforming growth factor-β super family (Letterio and Roberts 1998).These factors are known to be important mediators of apoptosis in liver (Rodgarkia-Dara et al. 2006). Such a result is consistent with the up-regulation ($\beta = 0.003$) of the *Band 4.1-like protein 3* (*epb41l3*) gene. This gene encodes a protein that inhibits cell proliferation and induces apoptosis (Li et al. 2011). In addition, the *pericentriolar material 1 protein* gene (*pcm1*) was found to be down-regulated in Cd-contaminated fish. PCM1 is known to be responsible for the correct localization of several centrosomal proteins that are required for regulation of centrosome structure at the G2/M transition and thus, for cell division (Hames and Crookes 2005). Cd contamination seems to affect genes involved in cell division and particularly mechanisms that are responsible to cell cycle arrest. Our results could indicate that chronic Cd exposure represses hepatocyte division. This

hypothesis is further supported by the fact that Cd contamination was also associated with an up-regulation of the *C-terminal binding protein 2* gene (*ctbp2*). CtBPs are transcriptional co-repressors acting via epigenetic mechanisms and more precisely by recruiting histone deacetylase and histone methyltransferase (Kim et al. 2005). It has been shown that these proteins serve as energy sensing-repressor. Their activation by low energy or NADH levels leads to repression of gene transcription and particularly to the down-regulation of genes involved in cell division. In this view, it is interesting to note that our results also showed that Cd exposure affects the transcription of genes involved in lipid transport and protein metabolism. Cd contamination was associated with an increase of *sortilin-related receptor (sorl1)* gene transcription level, a multifunctional endocytic receptor that is involved in the uptake of lipoproteins and proteases from plasma. This enzyme notably presents a high affinity for apoE-rich lipoproteins and β-VLDL (Taira et al. 2011). Such an increase could appear in accordance with previous results obtained by Pierron et al. 2007 who reported a significant increase in lipolysis and energy needs in the liver of eels experimentally exposed to dissolved Cd at 5 $\mu g\ L^{-1}$ during 30 days. Finally, two genes encoding enzymes involved in ubiquitin conjugation pathway were also affected by Cd contamination. The gene *E3 ubiquitin-protein ligase UBR5* (*ubr5*) which encodes a protein responsible for protein ubiquitination and thus to their degradation by the proteasome (Ernst et al. 2011), was found to be down-regulated in response to metal contamination. In contrast, the *ubiquitin carboxyl terminal hydrolase 20* gene (*usp20*) that encodes an enzyme that leads to protein deubiquitination was found to be up-regulated. This could potentially limit protein turnover and thus limit energy expenditure. It is interesting to note that a similar pattern was reported in wild yellow perch (*Perca flavescens*) chronically exposed to Cd pollution (Pierron et al. 2011). Using RNA-seq, authors hypothesized that chronic Cd exposure leads to energy restriction and to the activation of epigenetic mechanisms responsible for gene silencing, notably on genes involved in lipid and protein metabolism.

Lindane

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

This potential increase in transcriptional activity was coupled with an up-regulation of several genes involved in lipid catabolism. Indeed, our results showed an up-regulation of the *carnitine-O-palmitoyltransferase* 2 (*cpt2*) and *mitochondrial coenzyme A transporter SLC 25A42* (*slc25a42*) genes. These genes encode for enzymes involved in mitochondrial fatty acid β-oxidation (Kang et al. 2007). Moreover, these up-regulations were associated to an up-regulation of the *peroxisomal membrane protein PEX13* gene (*pex13*; $\beta = 0.06$). This gene encodes for a protein that is involved in the peroxisomal degradation of long chain fatty acids (Mannaerts 2000). Because mitochondrial β-oxidization can only use short and median fatty acids to generate energy (Mannaerts 2000), the up-regulation of *pex13* could contribute to provide to mitochondria short fatty acids via degradation of long fatty acids. It is interesting to note that *oxysterol-binding protein 2* (*osbp2*), involved in lipid transport and cholesterol metabolism, was also up-regulated. Thus, our results tend to show that chronic exposure to γ-HCH triggers an increased lipolysis and energy production in eels. Such a result is consistent with a previous study carried out on *Daphnia magna* which showed that exposure to sub-lethal concentrations of γ-HCH triggers an increase in the Kreb's cycle activity (De Coen et al. 2001). These up-regulations of transcriptional activity and energy metabolism were further associated with changes in the transcription level of genes that are known to be involved in tumorigenesis. First, the gene *serine protease hepsin* (*hpn*) was found to be up-regulated. This gene encodes for a protein that plays an essential role in cell growth (Nakamura et al. 2008). This gene is known to have prominent expression in human liver, prostate and ovarian tumors (Herter et al. 2005). Such a potential role of γ-HCH in tumorigenesis in eel liver is further supported by the significant decrease in *zinc-finger protein 148* (*znf148*) gene transcription level in γ-HCH-contaminated fish. This gene presents anti-tumor properties when over-expressed in hepatocellular carcinoma by increasing apoptotic processes (Zhang et al. 2012). Furthermore, it is noteworthy that a gene encoding a protein involved in hemophilic cell–cell adhesion, the *myelin protein zero-like protein 2* gene (*mpzl2*) was also down-regulated. A loss of hemophilic cell–cell adhesion molecules induces signals promoting cell proliferation and migration and thus, tumor progression (Craig and Brady-Kalnay 2011). In contrast, a gene described to have a tumor suppressor role in colorectal cancers and in malignant thyroid tumors (Mlakar et al. 2009; Arnaldi et al. 2005), i.e. the decorin gene (*dcn*), was up-regulated in our findings ($\beta = 0.12$). We must note however that the primary role of *dcn* is to control extracellular matrix assembly. DCN is

known to be expressed during hepatic fibrosis (Baghy and László 2010). This could be in accordance with a study that reported hepatic fibrosis in several fish species exposed to accidental discharge of γ-HCH (Ortiz et al. 2003). To conclude, our results show that chronic γ-HCH exposure is associated with an up-regulation of global transcriptional activity and lipolysis. This up-regulation of cellular metabolism was further associated with changes in the transcription level of genes involved in tumorogenesis, suggesting that γ-HCH could lead to cancer development in eels. This tumor promoter role of γ-HCH had already been described in fish by Kroll et al. (1999). An alternative hypothesis would be that this increase in cellular metabolism and cell proliferation are signs of liver regeneration (King and Newmark 2012). Indeed, γ-HCH was reported to trigger hepatocyte necrosis in fish (Ortiz et al. 2003).

Conclusion

Our approach allowed us to establish significant relationships between the hepatic expression levels of specific transcripts and the concentrations of individual contaminants measured in fish. The aim was to identify some genes that are more likely related to a given factor. The genes identified for each anthropogenic or natural factor were involved in numerous and common biological processes. For example, fish HSI and Cd contamination were associated with changes in the transcription levels of genes involved in the p53 signaling pathway and in the lipid and protein metabolism, suggesting an effect of Cd on the hepatic energy metabolism. In this view, it is interesting to note that some discrepancies appear between our work and previous field transcriptomic studies. For example, in the studies carried out by (Pujolar et al. 2012, 2013), during which the transcriptome of eels from a clean site was compared with the transcriptome of eels from polluted sites by means of DNA microarray, significant changes in the transcription level of genes involved in detoxification, in the fight against oxidative stress and in the general stress response (e.g. metallothioneins, heat shock proteins) were observed. Such genes have not been highlighted in our study. This could be linked to the fact that our statistical approach (i.e. FAMT analysis) aimed to eliminate as much as possible genes for which transcription levels are correlated with several factors. However, such genes are known to vary in response to many factors, including both anthropogenic (including both metals and organic pollutants) and natural (e.g. temperature, hypoxia, age..) (Bœuf and Payan 2001; Podrabsky and Somero 2004) factors. Our study thus proposes an alternative method that could give more specific "signatures" of exposure. Further studies are however required at the protein level to test whether such variations in gene transcription levels are associated with adverse effects in eels and to deepen our understanding about the mechanisms of toxicity of pollutants in wild contaminated fish.

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Conflict of interest The authors declare that they have no conflict of interest.

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