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Gonadal transcriptome analysis of wild contaminated female European eels during artificial gonad maturation

Lucie Baillon [a,b], Jennifer Oses [a,b], Fabien Pierron [a,b,*], Sarah Bureau du Colombier [c], Antoine Caron [d], Eric Normandeau [e], Patrick Lambert [c], Patrice Couture [d], Pierre Labadie [a,b], Hélène Budzinski [a,b], Sylvie Dufour [e], Louis Bernatchez [f], Magalie Baudrimont [a,b]

[a] Univ. Bordeaux, UMR EPOC CNRS 5805, F-33400 Talence, France
[b] CNRS, EPOC, UMR 5805, F-33400 Talence, France
[c] Irtsea, UR EABX, 50 avenue de Verdun-Gazinet, 33612 Cestas, France
[d] Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, 490 de la Couronne, Québec, Québec G1K 9A9, Canada
[e] Muséum National d'Histoire Naturelle, UMR BOREA UPMC, CNRS 7208, IRD 207, UCBN, 7 rue Cuvier CP 32, F-75231 Paris, France
[f] Département de biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, Québec G1V 0A6, Canada

HIGHLIGHTS

- Strong differences were observed on the contamination levels of eels from the two sites.
- Eels from the reference site showed a higher gonad growth compared to contaminated fish.
- Genes associated to gonad growth were involved in cell division and gametogenesis.
- Genes associated to contaminants were involved in FOXO1 regulation and steroidogenesis.

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ABSTRACT

Since the early 1980s, the population of European eels (*Anguilla anguilla*) has dramatically declined. Nowadays, the European eel is listed on the red list of threatened species (IUCN Red List) and is considered as critically endangered of extinction. Pollution is one of the putative causes for the collapse of this species. Among their possible effects, contaminants gradually accumulated in eels during their somatic growth phase (yellow eel stage) would be remobilized during their reproductive migration leading to potential toxic events in gonads. The aim of this study was to investigate the effects of organic and inorganic contaminants on the gonad development of wild female silver eels. Female silver eels from two sites with differing contamination levels were artificially matured. Transcriptomic analyses by means of a 1000 candidate gene cDNA microarray were performed on gonads after 11 weeks of maturation to get insight into the mechanisms of toxicity of contaminants. The transcription levels of several genes, that were associated to the gonadosomatic index (GSI), were involved in mitotic cell division but also in gametogenesis. Genes associated to contaminants were mainly involved in the mechanisms of protection against oxidative stress, in DNA repair, in the purinergic signaling pathway and in steroidogenesis, suggesting an impairment of gonad development in eels from the polluted site. This was in agreement with the fact that eels from the reference site showed a higher gonad growth in comparison to contaminated fish.

1. Introduction

Historically abundant and widespread in Europe, European eels (*Anguilla anguilla*) have suffered a sharp decline and the species' recruitment now represents only one tenth of what it was in the early 1980s (Stone, 2003; ICES report 2013). It is currently considered as critically endangered of extinction by the International Union for Conservation of Nature (IUCN). European eel is a catadromous fish species with a complex life cycle including marine (larval phase, sexual maturation and spawning) and continental (feeding and somatic growth) environments. The unusual life cycle

* Corresponding author at: CNRS, EPOC, UMR 5805, F-33400 Talence, France.
E-mail address: f.pierron@epoc.u-bordeaux1.fr (F. Pierron).

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of European eels makes them particularly vulnerable to pollution. Reproduction of the species takes place in the Sargasso Sea. Eel larvae drift back to the European coasts following oceanic currents. After metamorphosis of the larvae into glass eels, the organisms reach the juvenile growth phase stage (yellow eel) in continental habitats. During this stage, for up to 20 years, eels feed in order to grow and accumulate fat reserves. Then, yellow eels undergo a second metamorphosis called silvering which prepares the future genitors (silver eels) for their transoceanic reproductive migration (Tesch, 2003; Van Ginneken and Maes, 2005). However, when silver eels leave the European coasts, their gonads are still immature and maturation is blocked at a prepubertal stage (Vidal et al., 2004). This implies that gonad development must occur during the 5500 km transoceanic migration. During the migration, silver eels no longer feed and use their lipid reserves accumulated during the growth phase (yellow eel stage) for swimming and gonad maturation. Finally, this life cycle ends with the death of genitors after reproduction. During the growth phase, yellow eels will accumulate lipids but also metal and organic contaminants present in continental environments (Durrieu et al., 2005; Tapie et al., 2011). The lipid reserves will be remobilized but contaminants could be also remobilized toward gonads during their reproductive migration (Robinet and Feunteun, 2002; Palstra et al., 2006; Pierron et al., 2008). Consequently, a particular emphasis was placed in recent years on the hypothesis that spawner quality might play an essential role in the decline of the European eel. However, despite the increasing awareness that pollution might impair the reproductive success of silver eels, the potential effects of pollution on the eels' gonad development remain poorly known. Investigating the effects of contaminants on developing gonads in future spawners is highly relevant since effects observed at the individual level have implications at the population level.

The aim of this study was to investigate the possible impacts of contaminants on gonad growth as well as the toxic mechanisms of contaminants on gonads during artificial maturation of European female silver eels by means of a transcriptomic approach using a DNA microarray comprising 1000 candidate gene targets (Baillon et al., 2015).

2. Materials and methods

2.1. Experimental design

All procedures used in this experiment were approved by the Aquitaine fish-birds ethics committee. Migrating female silver eels (*Anguillla anguilla*) were captured during their continental downstream migration in winter 2012–2013, in the Arcachon Bay in the Domaine de Certes salt marshes (southwest of France, 44°41′18″N 1°1′39″W) a site considered as "clean", and in the Gironde estuary (45°12′06.62″N 0°43′34.72″O), considered as highly contaminated (Baillon et al., 2015). The animals were transferred to the laboratory (Irstea experimental station), individually marked with pit-tags and randomly mixed in two separate swim tanks (salinity 30‰, natural seawater) thermostated at 15 °C. After at least one month of acclimation period, 5 eels from Gironde and 8 from Certes were removed and dissected.

Remaining eels were then submitted to water current to force them to swim at a speed around 16 cm s^{-1} (Davidsen et al., 2011). To induce gonad maturation, eels received one perivisceral injection per week of CPE without anesthesia at a dose equivalent to 20 mg of pituitary powder/kg body weight (Durif et al., 2006). Sixteen animals, 8 fish per sampling site, were removed for analysis after 11 CPE injections.

At each sampling time, the total length and weight were recorded for each fish in order to estimate the Fulton condition factor (K): (weight (g) $\times 10^5$)/(length (mm)^3). Fish ovaries were weighed to calculate the gonadosomatic index (GSI expressed as a percentage: (gonad weight/total body weight) $\times$ 100). Samples for genetic analyses were stored in RNA later at −20 °C until needed. For both organic and metal analyses, samples were stored at −80 °C.

During the experiment, fish were not fed as eels undergo a natural period of fasting at the silver stage.

2.2. Metal and organic contaminant analyses

Metal analyses and organic contaminants analyses were carried out as previously described in Baillon et al. (2015). Metal concentrations were measured by inductively coupled plasma–mass spectrometry (Thermo Scientific XSeries 2), inductively coupled plasma–atomic emission spectrometry (Varian Vista AX) or both methods. Extraction 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) was performed using microwave assisted extraction and analyses were carried out on an HP 5890 series II gas chromatograph coupled to a ^{63}Ni electron capture detector. For PCBs, LoQs were comprised between 0.2 ng/g dw and 2 ng/g dw; for OCPs they were comprised between 0.1 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 dw.

2.3. RNA extraction, labeling, and cDNA hybridization

Total RNA was extracted from the gonads of 8 individuals per condition. RNA was extracted from 20 to 25 mg of tissue using the SV total RNA isolation system (Promega) with minor modifications. Briefly, for the first step of reverse-transcription (RT), 15 μg of total RNA were used. After RT reaction, cDNA purification was made with Qiagen PCR purification kit following the manufacturer's protocol. Purified cDNA were then labeled with CyDye™ Post-Labeling Reactive Dye Pack (Cyanine 3 for sample and Cyanine 5 for reference). In order to normalize microarray data, we used a common reference design. The reference was composed by pooling total RNA from liver of 30 wild eels from the clean site; i.e. Certes (15 fish collected in year 2011 and 15 in year 2012). This reference was combined in equal amounts with each sample before to be hybridized on the microarray slide (15 h at 55 °C). A total of 16 microarrays were used, 8 microarrays were performed for each sampling site (i.e. Certes and Gironde). Each sample has been hybridized once on DNA microarray. Data acquisition was carried out by means of the Innoscan 710 microarray scanner (Innopsys) using Mapix software. More details on sample preparation and microarray processing are available in NCBI/Gene Expression Omnibus (GEO) under the accession number GPL19017.

2.4. Statistical analysis

For the contaminant analyses, comparisons among fish groups were performed by analysis of variance (ANOVA), after checking assumptions of normality and homoscedasticity of the error terms. When the assumptions were not met as deduced graphically and from ad-hoc tests, we used box-cox data transformations or the non-parametric Kruskal–Wallis test. If significant effects were detected, the Least Square Deviation (LSD) or U-Mann Whitney tests were used to determine whether means between pairs of samples were significantly different from one another. Computations were performed using STATISTICA version 6.1 software (StatSoft, USA).