Science of the Total Environment 571 (2016) 92–102

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Gene transcription profiling in wild and laboratory-exposed eels: Effect of captivity and in situ chronic exposure to pollution

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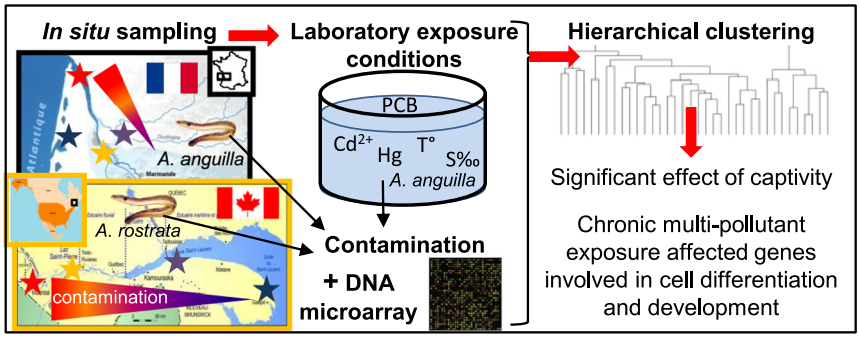
HIGHLIGHTS

- Need of improved method to predict and discriminate in situ effects of pollutants
- Fish were caught in the wild or laboratory-exposed to different abiotic factors.
- Hepatic transcriptome profiles of wild and laboratory-exposed fish were compared.
- Captivity markedly altered the profiles, especially genes involved in histone marks.
- Genes affected by pollution were involved in cell differentiation and development.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form 13 July 2016
Accepted 18 July 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Transcriptomics
Multi-stress
Atlantic eels
Ecotoxicology
In situ
Experimental

ABSTRACT

Aquatic ecosystems are subjected to a variety of man-induced stressors but also vary spatially and temporally due to variation in natural factors. In such complex environments, it remains difficult to detect, dissociate and evaluate the effects of contaminants in wild organisms. In this context, the aim of this study was to test whether the hepatic transcriptome profile of fish may be used to detect in situ exposure to a particular contaminant. Transcriptomic profiles from laboratory-exposed and wild eels sampled along a contamination gradient were compared. During laboratory experiments, fish were exposed during 45 days to different pollutants (Hg, PCBs, OCPs or Cd) or natural factors (temperature, salinity or low food supply) at levels close to those found in the sampling sites. A strong difference was observed between the transcriptomic profiles obtained from wild and laboratory-exposed animals (whatever the sites or experimental conditions), suggesting a general stress induced by captivity in the laboratory. Among the biological functions that were up-regulated in laboratory eels in comparison to wild eels, histone modification was the most represented. This finding suggests that laboratory conditions could affect the epigenome of fish and thus modulate the transcriptional responses developed by fish in response to pollutant exposure. Among experimental conditions, only the transcription profiles of laboratory animals exposed to cold temperature were correlated with those obtained from wild fish, and more significantly with fish from contaminated sites. Common regulated genes were mainly involved in cell differentiation and liver

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http://dx.doi.org/10.1016/j.scitotenv.2016.07.131

development, suggesting that stem/progenitor liver cells could be involved in the adaptive response developed by fish chronically exposed to pollutant mixtures.

1. Introduction

Human activities have dramatically increased the concentration of metallic and organic pollutants in aquatic environments (Thrush et al., 2009). The goal of ecotoxicology is to assess and predict the impact of these contaminants on organisms, populations and ecosystems. Towards this aim, experimental approaches were developed with aquatic model animals to understand and assess the impacts of contaminants in natural ecosystems. However, it remains difficult to extrapolate the results obtained in the laboratory to a realistic effect in natural environments due to the presence of other natural factors (temperature, salinity, dissolved oxygen concentration, parasitism…) as well as variations of these factors from one sampling site to another. Indeed, aquatic ecosystems are complex and dynamic entities in structure, composition and functioning change on a daily and seasonal basis in response to variations in natural factors. A huge challenge for ecotoxicologists is thus to distinguish the effects triggered by contaminants from those triggered by natural factors. It is even more difficult to detect and assess the contribution of individual contaminants in a multi-pollutant field context (Denslow et al., 2007).

In this context, gene transcription profiling has received increasing attention in recent years. By allowing the simultaneous measurement of the transcription level of a large number of genes belonging to various metabolic pathways, gene expression profiling analysis has been used to identify and provide mechanistic insights into pollutant toxicity as well as to provide chemical signatures of toxicity (Denslow et al., 2007; Pierron et al. 2011; Bougas et al. 2013; Baillon et al. 2015a, 2015b). Such signatures are constituted by a set of genes that respond to a particular factor and may be used to discern the effects of chemicals in pollutant mixtures (Poynton et al., 2008a, b). The emergence of analytical tools allowing high-throughput sequencing of mRNA molecules has allowed ecotoxicologists to develop such approaches in wild and non-model organisms. In a previous work, we took advantage of RNA-Seq to discover without any a priori method statistically confident "candidate genes" for which transcription levels were more likely related to contaminant exposure than to natural stressors in wild Atlantic eels, i.e. in European (*Anguilla Anguilla*) and American (*Anguilla rostrata*) eels (Baillon et al. 2015a). A total of 1000 candidate genes were retained and used to construct a DNA microarray (Baillon et al. 2015b). Historically abundant and widespread in Europe and North America, populations of Atlantic eels have suffered a sharp decline since the 1980′s. The European species (*Anguilla anguilla*) is currently considered as critically endangered of extinction by the International Union for Conservation of Nature. The Committee on the status of Endangered Wildlife in Canada (COSEWIC) has revised the status of the American eel from "special concern" in 2006, to "Threatened" in 2012. Among hypotheses advanced to explain these declines, the possible contribution of pollution has received considerable attention in recent years. The unusual life cycle of Atlantic eels makes them particularly vulnerable to pollution (Belpaire and Goemans 2007; Geeraerts and Belpaire 2010). Spawning takes place in the Sargasso Sea and leptocephali larvae are transported by ocean currents until they reach the European or American coasts. After metamorphosis of the larvae into glass eels, the organisms reach the juvenile growth phase stage (yellow eel) in continental habitats. During this stage, eels adopt a more sedentary lifestyle and accumulate substantial energy reserves in the form of lipids. This stage can last from several years to >20 years, depending on the hydrosystem, and ends with a second metamorphosis called silvering which prepares the future genitors (silver eels) for their transoceanic reproductive migration without feeding (Tesch 2003; van Ginneken and Maes 2005).

Previous transcriptomic studies carried out on silver European eels chronically exposed to pollutants in their natural environment have reported an altered pattern of transcription of genes involved in detoxification and a down-regulation of genes involved in oxidative phosphorylation in eels inhabiting a highly polluted site (contaminated notably by polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants) in comparison to eels inhabiting clean sites, suggesting that pollutants may have a significant effect on energy metabolism in these fish (Pujolar et al. 2012, 2013). However, many biotic and abiotic factors can influence the transcription of fish in nature, leading to potential ambiguities in their interpretation. Here, we first established specific transcriptomic profiles for different anthropogenic (mercury (Hg), cadmium (Cd), PCBs, or OCPs) and natural (temperature, salinity or low food supply) factors under controlled laboratory conditions. The aim was to obtain a "gene transcription signature" elicited by each stressor (Ramaswamy et al. 2001; Denslow et al., 2007) by means of a DNA microarray. The second objective was to test whether these gene transcription signatures could help to detect and separate the effects of each stressor in wild fish chronically exposed to multiple stressors.

2. Material and methods

2.1. Wild eel sample collection

Seven sampling sites were selected in Québec (Canada; St. Jean, Sud-Ouest, St. François, St. Pierre) and in France (Dordogne, Garonne, Gironde) on the basis of their known gradients of contamination by metallic and organic pollutants as previously described in Baillon et al. (2015a). European eels (*Anguilla anguilla*) and American eels (*Anguilla rostrata*) were collected between May 24 and June 24 of 2012, using a trawl, a fyke net or by electrical fishing. For each sampling site, a total of five immature and sexually undifferentiated eels (mean ocular index 3.32 ± 0.1) were used for subsequent analyses. Fish were dissected as soon as possible at the proximity of the sampling sites and organs (liver and muscle) 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.

2.2. Experimental design

For laboratory experiments, European yellow eels were exposed to only one factor at a time. While environmental exposures rarely contain a single stressor, these single physicochemical studies are important because they set the stage against which responses to more complex exposures can be compared (Denslow et al., 2007). During laboratory experiments, fish were exposed to different pollutants or natural factors at levels close to those found in the sampling sites Table 1 and Table 2A and B). Pollutants selected for laboratory exposures were those found in high concentration levels in eels sampled in St. Lawrence and Gironde hydrosystems such as PCBs, Cd, Hg and OCPs (Baillon et al. 2015a). Finally, immature eels were exposed to natural stressors that showed the strongest variations among the natural sites, i.e. salinity and temperature. Eels were also submitted to food restriction, a factor known to influence fish metabolism (Dave et al. 1975). Experimental conditions are summarized in Table 1.