Gene transcription reflects poor health status of resident European eel chronically exposed to environmental pollutants

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ABSTRACT

Understanding the effects of chronic exposure to pollutants on the genome and transcriptome of diadromous fish populations is crucial for their resilience under combined anthropogenic and environmental selective pressures. The catadromous European eel (Anguilla anguilla L.) has suffered a dramatic decline in recruitment for three decades, necessitating a thorough assessment of the transcriptional effects of environmental pollutants on resident and migrating eels in natural systems. We investigated the relationship between muscular bioaccumulation levels of metals (Hg, Cd, Pb, Cu, Zn, Ni, Cr, As and Se), PCBs and organochlorine pesticides (DDTs), the health status (condition factor and lipid reserves) and the associated transcriptional response in liver and gill tissues for genes involved in metal detoxification (metallothionein, MT) and oxidative metabolism (cytochrome P4501A, CYP1A) of xenobiotic compounds. In total 84 resident eels originating from three Belgian river basins (Scheldt, Meuse and Yzer) were analyzed along with five unpolluted aquaculture samples as control group. There was a large spatial variation in individual contaminant intensity and profile, while tissue pollution levels were strongly and negatively associated with condition indices, suggesting an important impact of pollution on the health of sub-adult resident eels. Gene transcription patterns revealed a complex response mechanism to a cocktail of pollutants, with a high variation at low pollution levels, but strongly down-regulated hepatic and gill gene transcription in highly polluted eels. Resident eels clearly experience a high pollution burden and seem to show a dysfunctional gene transcription regulation of detoxification genes at higher pollutant levels, correlated with low energy reserves and condition. To fully understand the evolutionary implications of pollutants on eel reproductive fitness, analyses of mature migrating eels and the characterization of their transcriptome-wide gene transcription response would be appropriate to unveil the complex responses associated with multiple interacting stressors and the long-term consequences at the entire species level. In the meanwhile, jointly monitoring environmental and tissue pollution levels at a European scale should be initiated, while preserving high quality habitats to increase the recovery chance of European eel in the future.

1. Introduction

Human pressure on aquatic habitats has led to a tremendous decrease in aquatic biodiversity in the last century, through the synergetic negative impact of global environmental changes (e.g. climate), harvesting pressure, pollution and habitat degradation (Allan and Flecker, 1993; Lynch and Lande, 1993; Vitousek et al., 1997; Dietz et al., 2007). Unveiling the causes and consequences of biodiversity loss in aquatic habitats due to pollution pressure remains poorly documented, both at the species diversity and at the intraspecific genetic level (Lynch and Lande, 1993; Dietz et al., 2007; Perrings et al., 2010). The study of diadromous species completing their life-cycle through ontogenic shifts between marine and freshwater habitats (such as sturgeons, salmonids and anguillids) is particularly important in that aspect, as they represent natural models to understand the impact of continental human-induced pressures combined with marine environmental fluctuations. Diadromous species seem particularly vulnerable to extinction, due to their costly physiological and osmoregulatory plasticity, potentially leading to an increased sensitivity to multiple anthropogenic stressors at both local and global scales. Very little is known about the

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long-term functional responses and evolutionary resilience to human pressures in species with a catadromous life-strategy, such as anguillids (*Anguilla* spp.), spending their pre-reproductive phase in human-impacted continental waters, while reproducing in the open ocean (Maes et al., 2005; Maes and Volckaert, 2007).

Understanding the effects of chronic pollutant exposure on the genome and transcriptome is crucial to safeguard the adaptive potential of populations under heavy anthropogenic pressure (Cheung and Spielman, 2002; Hoffmann and Willi, 2008; Oleksiak, 2008). Although experimental controlled populations are ideal to assess the level of transcriptional responses under specific environmental stressors (Cossins and Crawford, 2005), studying the toxic effects and transcriptional responses in natural populations is essential to understand the synergetic effects of multiple environmental stressors under field conditions. The detrimental combination of pollutants with concomitant stressors (such as fishing, migration barriers, habitat degradation, parasites and thermal or osmotic stress) may indeed bring a species to the brink of extinction (Brown et al., 2009). Intra-generational differential gene transcription directly caused by local environmental conditions is best studied within a widely distributed species with a broad physiological tolerance to stressors and with a homogeneous neutral genetic background (population structure), to avoid signals of neutral evolution in transcriptional variation between diverging populations (Khaitovich et al., 2005; Oleksiak, 2008). In addition to well established enzymatic analyses, quantifying gene transcription levels during exposure to pollutants and parasites, enable the early detection of decreased fitness and survival for improved conservation measures for several fish species, such as rainbow trout (Hook et al., 2006), striped sea bream (Auslander et al., 2008), gudgeon (Knapen et al., 2007), barbel (Quiros et al., 2007), (mangrove) killifishes (Oleksiak, 2008; Rhee et al., 2009; Kessabi et al., 2010), zebrafish (Holth et al., 2008) and three-spined stickleback (Sanchez et al., 2010).

The catadromous European eel *Anguilla anguilla* has a fascinating life-history. After their transoceanic migration from the spawning site at the Sargasso Sea, eel larvae, called leptocephali, metamorphose into glass eels ascending rivers. Here, in continental waters, they grow as resident eels (now called yellow eels) for 3–20 years, to finally metamorphose into mature eels (called silver eels) migrating all the way back towards their spawning site (van Ginneken and Maes, 2005). A growing concern for the poor stock status (more than 90% drop in recruitment) has tagged the CITES listed European eel as another species in danger of extinction (Dekker et al., 2003; ICES, 2009). A number of stress factors make eel recovery particularly challenging, such as pollution of coastal zones and rivers, habitat loss, infections by invasive parasites and over-exploitation, all potentially affecting the reproductive success of eel. Moreover, the potential synergistic vs. additive effects of global climate change may weaken the species to a point of no return (Bonhommeau et al., 2008). As pollutants have been reported to substantially affect the eel's physiological homeostasis and ability to successfully swim back to the Sargasso Sea, there is now compelling evidence that silver eels from specific areas are unlikely to produce any offspring (Palstra et al., 2006; Belpaire et al., 2009; van Ginneken et al., 2009). Various biometric and molecular indicators have been used to assess the reproductive potential of local eel stocks, such as lipid content (Pierron et al., 2007a; Belpaire et al., 2009), infection level with the swimbladder parasite *Anguillicoloides crassus* (Palstra et al., 2007; Fazio et al., 2009) and the bioaccumulation of pollutants (Gorbi et al., 2005; Buet et al., 2006; Storelli et al., 2007; Pierron et al., 2008a; Van Campenhout et al., 2008; Gravato et al., 2010). As eel is a longlived semelparous species, being amongst the fattiest fish in freshwater, they continuously accumulate lipophilic toxic compounds, until

their detrimental lipolysis during sexual maturation and reproductive migration (Palstra et al., 2006). Eel can thus be considered as an important sentinel species in an *in situ* ecotoxicological context. They record past/current environmental pollutant concentrations at a local scale (Belpaire and Goemans, 2007a) and provide a view on the local extreme transcriptional responses in a high gene-flow species (Maes et al., 2005; Maes and Volckaert, 2007). Past ecotoxicological studies on eels have documented biometric, enzymatic and transcriptional responses to pollutants under either field or experimental conditions (Gorbi et al., 2005; Hoff et al., 2005; Aubry et al., 2007; Pierron et al., 2007b, 2008a, 2009a; Marohn et al., 2008; Van Campenhout et al., 2008; Belpaire et al., 2009; Gravato et al., 2010; Pujolar et al., 2012). All of them showed clear genetic responses to pollutants together with impairments of lipid content, oocyte development and hypoxic responses. However, no quantitative knowledge exists about pollution induced molecular responses at specific detoxification genes under natural conditions, combined with a thorough assessment of individual health and viability potential. The only evolutionary ecotoxicology study in eel to date documented a very poor health status due to metal contaminants and found an intra-generational differential genotype distribution resulting from local selective pressure (Maes et al., 2005). Maes and Volckaert (2007) suggested that investigating the functional part of genomes might yield additional insights to quantify the degree of phenotypic plasticity to pollutants in eel and its consequences at the population/species level.

Based on previous knowledge, the study of the transcriptional responses of main candidate genes for detoxification, such as Metallothionein and Cytochrome P4501A genes, would be of high priority in natural eel populations. Metallothioneins (*MTs*) are low-molecular-mass cysteine-rich metal-binding proteins with a high affinity for heavy metal ions and commonly used as a biomarker of metal exposure (Bourdineaud et al., 2006; Van Campenhout et al., 2008). Although the biological functions of *MTs* have not been fully elucidated, the response to pollutants is not always predictable and several isoforms may have different functions, they are thought to play an important role in detoxification of toxic metals such as cadmium (Cd) and mercury (Hg) (Gorbi et al., 2005). They might also display an antioxidant function and are involved in the homeostasis of the essential metals zinc (Zn) and copper (Cu). Cytochrome P450 (*CYP1A*) represents a super-family of enzymes playing a major role in the oxidative metabolism and biotransformation of chlorinated and aromatic hydrocarbons.

The objective of this study was to better understand the biological and transcriptional phenotypic response of European eels chronically exposed to a cocktail of pollutants (PCBs, organochlorine pesticides (OCPs) and metals) in their natural environment. We were interested to know whether the level of multiple interacting pollutants and the health status of resident eels played a role on the individual pollutant sequestration potential, measured by gene transcription variation. Our specific objectives were threefold: (1) we quantified the relationship between heavy metal, PCB and DTT bioaccumulation and several fitness measures (e.g. condition indices and lipid content) in yellow eels originating from three Flemish river basins, along with five unpolluted aquaculture samples as control group; (2) we investigated the relation between individual bioaccumulation, fitness and tissue-specific overall gene transcription patterns for genes involved in metal detoxification (metallothionein 1, *MT*) and the oxidative metabolism (cytochrome P4501A, *CYP1A*) of xenobiotic compounds, to unveil potential transcriptional and physiological impairment during the growth phase; (3) we assessed the usefulness of both genes as biomarkers for monitoring and to assess the eel's resilience to a cocktail of interacting toxicants in the context of European eel recovery.