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Biotransformation, antioxidant and histopathological biomarker responses to contaminants in European and American yellow eels from the Gironde and St. Lawrence estuaries

Géraldine Patey [a, b], Catherine M. Couillard [b], Fabien Pierron [c], Magalie Baudrimont [d], Patrice Couture [a, *]

[a] Institut National De La Recherche Scientifique (INRS), Centre Eau Terre Environnement, 490 De La Couronne, Québec, Québec, G1K 9A9, Canada
[b] Fisheries and Oceans Canada, Maurice Lamontagne Institute, 850 Route de la Mer, Mont-Joli (Québec), G5H 3Z4, Québec, Canada
[c] CNRS, UMR EPOC CNRS 5805, F-33400, Talence, France
[d] Univ. Bordeaux, UMR EPOC CNRS 5805, F-33400, Talence, France

HIGHLIGHTS

- Patterns of contaminants and biological characteristics differed among sites and species.
- In American eels, biomarker responses did not differ clearly among sites and years.
- In European eels, biomarker responses were higher in contaminated sites.
- High spleen hemosiderin deposition was observed in eels from the most brackish sites.
- European eels may be more affected by contaminants than American eels.

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ABSTRACT

Since the early 1980s, populations of American (*Anguilla rostrata*) and European eels (*Anguilla anguilla*) have suffered a sharp decline. The causes of their decline are likely multifactorial and include chemical pollution. A field study was conducted in eight sites varying in organic and metal contamination along the St. Lawrence (Eastern Canada) and Gironde (France) systems to investigate the relationships among contaminants, biological characteristics and biotransformation, antioxidant and histopathological biomarkers in eels from both species. For *A. rostrata*, no major influences of persistent organic contaminants on biomarkers were identified. For *A. anguilla*, eels from the most contaminated site expressed higher surface of MelanoMacrophage Centers (MMCs) and eels from another contaminated site expressed higher amount of spleen lipofuscin pigment. These two histopathological biomarkers were also associated with aging. Compared to eels from the cleanest French site, higher hepatic catalase activity and density of MMC in eels from contaminated sites was related to higher concentration of organic (DDT and metabolites, sum of PCBs, sum of PBDEs) and inorganic (Hg and Cd) contaminants. In both species, a higher deposition of spleen hemosiderin pigment was measured in eels from the most brackish sites compared to eels living in freshwater environments. Our results suggest an association between higher hemosiderin pigment and metal contamination (As for *A. anguilla* and Pb for *A. rostrata*). Parasitism by *A. crassus* was observed in European eels from freshwater sites but not in eels from brackish habitats. Overall, contamination may pose a greater risk for the health of European compared to American eels.

1. Introduction

In the Northern hemisphere, the abundance of European eel (*Anguilla anguilla*) and American eel (*Anguilla rostrata*), here called “Atlantic eels”, has severely declined since the early 1980's (FAO/ICES, 2009; Cosewic, 2012). The causes of their decline are likely

* Corresponding author.
E-mail addresses: geraldine.patey@ete.inrs.ca (G. Patey), catherine.couillard@dfo-mpo.gc.ca (C.M. Couillard), f.pierron@epoc.u-bordeaux1.fr (F. Pierron), magalie.baudrimont@u-bordeaux.fr (M. Baudrimont), patrice.couture@ete.inrs.ca (P. Couture).

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multifactorial, including variables such as oceanographic and climate changes, overfishing, barriers to migration, habitat degradation, parasites and chemical pollution (Castonguay et al., 1994; Geeraerts and Belpaire, 2010). Although management and restoration programs have been conducted in several hydrosystems in Europe and North America, eel stocks have not recovered to levels of the past (ICES, 2016). An increasing number of recent reports support that pollutants might be among the possible synergistic causes contributing to the collapse of eel stocks (Belpaire et al., 2016). Due to their unique ecological and physiological traits, eels are particularly prone to bioaccumulation of contaminants through gills, skin and contaminated food during their feeding and growth stage (yellow eel). Eels are facultative catadromous fish that can be in contact with contaminated sediments for extended periods, leading to the accumulation of high concentrations of organic contaminants due to their high lipid content (Robinet and Feunteun, 2002; Daverat and Tomás, 2006; Thibault et al., 2007). Finally, eels are considered good bio-indicators of contaminated habitats and are expected to reflect the impacts of local pollutants (Belpaire and Goemans, 2007).

Recently, decreasing muscle concentrations of organic contaminants such as PolyChlorinated Byphenyls (PCBs) and OrganoChlorine Pesticides (OCPs) have been reported in eels from some historically contaminated habitats since their ban 40 years ago (Belpaire et al., 2016). From 1988 to 2008, a decreasing trend in PCB and OCP concentrations has been reported in the muscle of *A. rostrata* from the historically contaminated Lake Ontario (Byer et al., 2013). This trend was confirmed by a significant decrease in the embryotoxic potential of organic mixtures extracted from Lake Ontario eels captured in 1988, 1998 and 2008 for developing *Fundulus heteroclitus* (Rigaud et al., 2016). However, these contaminants are persistent in sediments and levels of PCBs, OCPs and PolyBrominated Diphenyl-Ethers (PBDEs) still remain high and preoccupying in yellow eels, for example in eels from the Gironde system (Tapie et al., 2011; Guhl et al., 2014). In addition to organic contaminants, metals such as mercury (Hg) and cadmium (Cd) show no trend of decrease and persist in contaminated areas (Maes et al., 2008). Consequently, eels inhabiting contaminated areas could be still affected by a combination of contaminants acting synergistically.

Several field and laboratory studies have reported effects of contaminants in eels at several levels of biological organization from the cellular, molecular and tissue levels to the level of whole organisms using various biological biomarkers to investigate their health status. They have shown that contaminants can delay growth, reduce lipid storage efficiency, cause oxidative stress, induce DNA damage and histopathological lesions, which can ultimately negatively affect migratory and reproductive capacities (Couillard et al., 1997; Robinet and Feunteun, 2002; Palstra et al., 2006; Pierron et al., 2007; Geeraerts and Belpaire, 2010; Gravato et al., 2006). Others effects such as disturbances of the immune and endocrine systems and a reduction of migratory and reproductive capacities have been also reported (Belpaire et al., 2009). In addition, ecological gradients such as latitudinal cline, longer distances to the spawning site and differences in salinity directly or indirectly affect growth rate, body condition, size at metamorphosis and lipid storage, which in turn could affect contaminant uptake (Vøllestad, 1992; Edeline et al., 2007; Thibault et al., 2007; Belpaire and Goemans, 2007; Jessop, 2010; Daverat et al., 2012). For *A. anguilla*, studies have suggested that the nematode *Anguillicola crassus* in the swimbladder of yellow eels could affect their swimming performance, reduce their resistance to other stressors such as contamination and increase spleen size, macrophage and lymphocyte production to combat parasites (Kirk et al., 2000; Lefebvre et al., 2004).

In a series of related studies performed by our team along the St. Lawrence and Gironde hydrosystems (Eastern Canada and Southwest France, respectively), we have identified that certain metals and organic contaminants could disturb physiological functions in yellow Atlantic eels (Baillon et al., 2015a, 2015b, 2016; Caron et al., 2016; Pannetier et al., 2016). In Baillon et al. (2015a, 2015b, 2016), we conducted a large scale and without *a priori* transcriptomic based approach. The global hepatic transcriptome of animals was determined by RNA-Seq. Genes that most likely responded to single factors were identified. We detected transcriptomic responses typical of PCB-170 and Cd exposure in wild *A. anguilla* collected in the most contaminated site in the Gironde Estuary, associated with changes in the transcription levels of genes involved in hepatic energy metabolism. In another paper (Caron et al., 2016), we focused on biomarkers involved in glycolytic, aerobic and anaerobic capacities and lipid metabolism. We reported an association between silver (Ag), lead (Pb) and arsenic (As) contamination in wild *A. rostrata* from the St. Lawrence River system and inhibition of the enzyme G6PDH, involved in lipid metabolism in liver and which also plays a role in oxidative stress response. This study has also shown an association between zinc (Zn), copper (Cu) and Pb contamination and altered glycolytic and anaerobic capacities in wild *A. anguilla* from the Gironde system. More recently, we have demonstrated a significant difference in early size-at-age (a proxy of early growth rate) between yellow eels captured upstream compared to downstream sites of the St. Lawrence and the Gironde systems (Patey et al., in press). These differences were present as early as 1 year old and could influence contaminant accumulation.

In the present study, we investigated the relationships among tissue concentrations of inorganic and organic contaminants, biological characteristics such as size, age, condition factor and muscle lipids and a set of complementary biomarkers such as hepatic biotransformation enzymes, antioxidant enzymes and splenic histopathological measurements in Atlantic eels. The selected biomarkers have been successfully used, alone or in combination, in previous biomonitoring studies on eels (Couillard and Hodson, 1996; Pacheco and Santos, 2002; Schwindt et al., 2008; Van der Oost et al., 2003; Buet et al., 2006) and are among the list of recommended biomarkers in the Report of the Workshop of the Working Group on Eels and the Working Group on Biological Effects of Contaminants (ICES, 2016). First, we examined the induction of Cytochrome P450-dependent monooxygenase, measured as ethoxyresorufin *O*-deethylase (EROD) in eel livers. This biotransformation marker is involved in a well-documented biochemical reaction of fish to organic planar contaminants such as PCBs, polycyclic aromatic hydrocarbons (PAHs), dioxins and furans and may be related to other effects such as oxidative stress (Van der Oost et al., 2003; Whyte et al., 2008). Secondly, we measured hepatic activities of two antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD), commonly used as biomarkers of antioxidant capacities in fish and induced by several contaminants such as PCBs, PAHs, organochlorine pesticides (OCPs) and metals (Van der Oost et al., 1996; Livingstone, 2001). In addition, we examined a suite of histopathological biomarkers induced by chronic exposure to contaminants, including density and surface of melano-macrophage centers (MMCs) and deposition of lipofuscin and hemosiderin pigments in spleen (Wolke, 1992; Couillard et al., 1997; Fournie et al., 2001; Au, 2004). In general, biomolecular biomarkers such as EROD, CAT and SOD are more sensitive and specific short-term responses (days to weeks), whereas histopathological biomarkers are responses to chronic exposure to contaminants and reveal more persistent damage.

In this study, yellow eels were sampled in four sites varying in anthropogenic pressure and contamination level in each of the Saint Lawrence System (SLS, Quebec, Canada) and the Gironde