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Thyroid endocrine status of wild European eels (*Anguilla anguilla*) in the Loire (France). Relationships with organic contaminant body burdens

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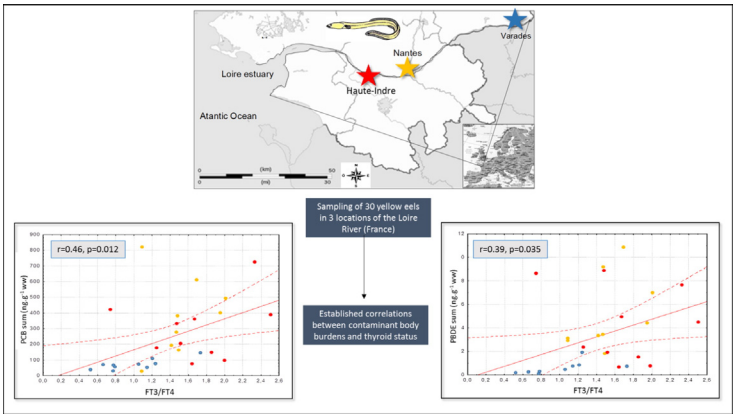
HIGHLIGHTS

- The thyroid status of yellow and silver eels from the Loire River was investigated.
- The variations observed were analyzed according to the organic contaminants loads.
- Correlations were found between free T4 and some PCBs and PBDEs in the yellow eels.
- On the whole, the silver eels exhibited lower thyroid activity.

GRAPHICAL ABSTRACT



Variations of free T3/T4 ratios in the yellow European eels of the Loire River, according to their PCB and PBDE body burdens and their sampling site.

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ABSTRACT

In teleost fish, thyroid function is involved in various critical physiological processes. Given the complexity of the hypothalamo–pituitary–thyroid (HPT) axis, a large number of genes and proteins can be the potential target of endocrine-disrupting compounds (EDCs). The aim of this study was to evaluate, in yellow and silver European eels (*Anguilla anguilla*), potential effects of EDCs on thyroid status by analyzing the associations between EDC body burdens and thyroid hormones (THs). In yellow individuals, greater free T3/T4 ratios (FT3/FT4) and lower plasma FT4 levels were associated with greater concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), as highlighted by significant correlations with many congeners. Few positive relationships with alkylphenols were noticed. In contrast, silver eels usually exhibited less significant correlations between THs and contaminant loads. Expression of a series of genes involved in the HPT axis was also investigated in the silver individuals. Concerning mRNA expression in silver females, some main correlations were noticed: thyroid-stimulating hormone (TSHβ) gene expression was significantly correlated to numerous PCBs, and hepatic mRNA levels of deiodinase 2 (Deio 2) were negatively correlated to 2-hydroxyfluorene (2-OHF) and 2-naphtol (2-OHNa). Thyroid receptor (TRα and TRβ) mRNA levels exhibited weak negative correlations with some PBDEs in silver females and males. Hepatic vitellogenin (Vtg) mRNA levels were detected in

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all silver males but at lower levels than in silver females. In males, Vtg mRNA levels were positively associated to FT4/TT4. In silver females, strong positive correlations were found between congeners of PCBs, PBDEs and PFAS suggesting potential estrogenic effects. Overall, the observed results indicate that several organic contaminants, mainly dl-, ndl-PCBs and PBDEs, could be associated with changes in thyroid homeostasis in these fish, via direct or indirect interactions with peripheral deiodination, metabolism of T4 and mechanisms involved in TSHβ, Deio 2 and Vtg gene transcription.

1. Introduction

During recent decades, growing human activities have led to an increase of pollutant emissions into the environment leading to a widespread contamination of many ecosystems, notably the estuaries which represent the ultimate receptacle of pollutants before their discharge into the sea. The effects of these contaminant mixtures on fish and other wildlife are not fully understood. Among wild fish species living in European estuaries, the European eel (*Anguilla anguilla*) was listed as "critically endangered" in the International Union for Conservation of Nature (IUCN) Red List as its population has been declining since 1950 (Dekker, 2003; Robinet and Feunteun, 2002). Several causes were suggested to explain this decline, such as overfishing, climate change, habitat reduction, numerous hydraulic barriers to migrations, diseases, and parasitic infection (Dekker, 2003; Elie and Girard, 2009). Additionally, this fatty species is prone to bioaccumulation of organic lipophilic pollutants such as Endocrine Disrupting Compounds (EDCs), which may threaten their population (Geeraerts and Belpaire, 2010). As suggested by laboratory studies (Carr and Patiño, 2011), a number of chemicals were known to be potential disruptors of endocrine systems in fish, including the hypothalamo–pituitary–thyroid (HPT) axis. Thyroid function is involved in various critical physiological processes in teleost fish, such as growth, metabolism, osmoregulation, and reproduction (Cyr and Eales, 1996). Therefore, its disruption raises special concern.

The thyroid system is highly complex and sensitive to several biotic and abiotic factors (Jarque and Pina, 2014). It is mainly regulated by thyroid hormone receptors (TRs) that bind to thyroid hormones (THs). The pro-hormone thyroxine, or T4, is synthesized in the thyroid gland under the control of the hypothalamus and pituitary axis. Its active form, the triiodothyronine, also known as T3, is produced in peripheral tissues by deiodinases (Kohrle, 2000). In fish, 2 deiodinase enzymes (Deio1 and Deio2) convert T4 to T3 by removing iodine from the outer ring of T4 (Orozco and Valverde, 2005). Another group of deiodinase enzymes (Deio 3) convert T4 and T3 to inactive forms by inner ring deiodination. In non-mammalian vertebrates, the corticotropin-releasing hormone (CRH) appears to stimulate the thyroid-stimulating hormone (TSH) secretion (De Groef et al., 2006) and therefore functions as a common regulator of the thyroidal axis. Given this complexity, a number of genes and proteins can be the potential target of endocrine-disrupting compounds (EDCs).

Few studies evaluating the effects of environmental contaminant mixtures on the thyroid endocrine system in wild resident fish are available to date (Brar et al., 2010; Jarque et al., 2014; Jenkins et al., 2014; Simmons et al., 2014). Brar et al. (2010) characterized the thyroid endocrine-related effects and their association to accumulated contaminants in two fish species, the Pacific staghorn sculpin and the shiner surfperch (*Leptocottus armatus* and *Cymatogaster aggregata*, respectively) sampled from different locations in San Francisco Bay. In individuals of both species sampled around industrialized areas of the bay, plasma concentrations of T4 were significantly reduced relative to fish from less contaminated sites. Simmons et al. (2014) and Gilroy et al. (2012) found histological evidence of thyroid de-activation in male brown bullhead with high dl-PCB body loads and modifications in T4 and T3 deiodinase activities. Another study demonstrated some positive correlations between hepatic Deio 2 mRNA levels and highly-brominated PBDE body burdens in trout (*Salmo trutta*) (Jarque et al., 2014).

In European eels, thyroid endocrine status was studied regarding the silvering process but nothing is known about its variation according to contaminant burdens (Aroua et al., 2005; Van Ginneken et al., 2007). The global aim of our study was to assess the potential consequences of accumulated contaminants on eel thyroid profiles. For this purpose, we integrated the whole dataset of contaminants and biometric data related to the physiological status of female (Couderc et al., 2015) and male eels at different life stages and sampled in different sites of the Loire River. For this fish, the Loire River (France) represents one of the most important continental migration paths of glass eels. The Loire River is also the last wild, large, and most preserved river in Europe. Its estuary constitutes an interesting study area since it is characterized by important human pressure. Recently, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), perfluorinated and polyfluorinated alkyl substances (PFAS), alkylphenols (APs), bisphenol A (BPA) and polycyclic aromatic hydrocarbon metabolites (mPAHs) were investigated in yellow and silver female eels sampled in several locations of the Loire River with different contamination pressures (Couderc et al., 2015). Our specific objective was to analyze the eventual associations between organic contaminant body burdens and THs according to each developmental and sexual stage of individuals. Moreover, as silver stage represents a critical stage for the reproductive success of the species, mRNA expression of a series of genes involved in the HPT axis [thyroid hormone synthesis (Deio 2), binding (TRα and TRβ) and regulation (TSHβ)] and the reproductive function (Vtg) were investigated in silver eels as well as their possible associations with biometric parameters, thyroid status and contaminants.

2. Material and methods

2.1. Fish sampling locations and sample collection

Within the Loire River, European eels (*A. anguilla*) were collected according to the fishing authorizations, by local fishermen, at two life stages (silver: S and yellow: Y) in November 2011 and June 2012, respectively. Yellow eels were collected with fyke and silver eels with stow nets. A total of 59 individuals were randomly collected: 30 yellow females [in three sampling areas: 10 sampled at Varades (YV), 10 at Bellevue (YB) and 10 at Haute Indre (YHI)], as well as 15 migrant silver female eels (SVf) and 14 migrant silver male eels (SVm) sampled during downstream migration at Varades site. The three sampling locations of yellow individuals were detailed in Couderc et al. (2015) and were expected to present different signatures of environmental chemicals. Varades (47°21′58″N–1°1′31″W) is a small city (about 3550 locals), located upstream of the estuary (after the limit of the dynamic tide); it presents few industrial activities and is particularly under agricultural pressure. The two other sampling locations are located in the estuary, and upstream and downstream of an important city, Nantes (about 600,000 locals), near two peripheral localities, Bellevue (47°14′15″N–1°28′2″W) (B) and Haute Indre (47°11′39″N–1°40′4″W) (HI) respectively. Nantes city, located at approximately 50 km from the mouth, is characterized by an industrial harbor and an urban zone including two incineration factories. These two areas are under a strong influence of the North Atlantic Ocean. Animal manipulations were performed