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Addressing the impact of mercury estuarine contamination in the European eel (*Anguilla anguilla* L., 1758) – An early diagnosis in glass eel stage based on erythrocytic nuclear morphology

D. Castro[a], C.L. Mieiro[b,*], J.P. Coelho[a,c], S. Guilherme[b], A. Marques[b], M.A. Santos[b], A.C. Duarte[a], E. Pereira[a], M. Pacheco[b]

[a] Department of Chemistry & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[b] Department of Biology & CESAM, University of Aveiro, 3810-193 Aveiro, Portugal
[c] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), University of Porto, Rua das Bragas, 289, 4050-123 Porto, Portugal

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ABSTRACT

The decline of the European eel (*Anguilla anguilla* L., 1758) population throughout Europe has been partially attributed to pollution. As glass eel estuarine migration may represent a considerable threat, the impact of mercury (Hg) contamination at this stage was evaluated through an *in situ* experiment (7 days). Total Hg (tHg) bioaccumulation was evaluated concomitantly with erythrocytic nuclear morphology alterations: erythrocytic nuclear abnormalities assay (ENA), frequency of immature erythrocytes (IE) and the erythrocytic maturity index (EMI). The ENA results suggested a genotoxic pressure at the most contaminated sites, in line with the tHg increase. The EMI data, together with IE frequency, showed that fish exposed to high levels of Hg exhibited alterations of haematological dynamics, translated into an erythropoiesis increment. Despite the presence of these compensatory mechanisms, the present findings suggest a harmful impact of Hg on genome integrity at this early development stage, potentially affecting eels' condition and ultimately the population sustainability.

1. Introduction

Estuaries are coastal transition areas with high productivity, considered as important nursery areas for several fish species (Vasconcelos et al., 2007; Martinho et al., 2013; Neves, 2014). However, due to their location and high value of goods and services provided, estuaries have been subjected to high anthropogenic pressure, receiving hazardous chemical waste discharges, suffering from margin interventions, flow regulation, overfishing and even illegal fishing (Neves, 2014; Pan et al., 2014). Thus, estuaries have become the most endangered and modified aquatic ecosystems over the years, which contributed to the degradation of their ecological functions (Blaber et al., 2000; Vasconcelos et al., 2007).

Several diadromous migratory fish rely on estuaries' high productivity, which offer favourable conditions for their ontogenic migrations between marine and riverine environments. This is the case of the European eel (*Anguilla anguilla* L., 1758), which is widely distributed across almost all the European territory. In the last decades, the European eel population has demonstrated a steep decline all over Europe, which has been attributed to several factors such as overfishing, anthropogenic barriers to their migration and aquatic contamination (Gravato et al., 2010; ICES ADVICE, 2013; Blanchet-Letrouvé et al., 2014). Estuarine contamination is particularly hazardous to fish juvenile stages, such as glass eel, due to its higher vulnerability comparing to adults, which relies on specific behavioural, morphological, physiological and biochemical characteristics (Mohammed, 2013). Specifying, the apparent susceptibility of early life stages is due to factors such as the greater uptake of toxicants and lower capacity to sequester them in fat tissue or specific proteins (preventing them from reaching target organs), underdeveloped homeostatic mechanism to deal with the toxicants and poorly developed organs (*e.g.* liver and kidney) critical in detoxification/elimination processes (Mohammed, 2013). Therefore, exposure of fish to estuarine contamination during early life stages may compromise survival rates, growth, recruitment, reproductive success and, ultimately, affect the population's dynamics.

The degradation of the estuarine environmental quality status has been associated mainly to the presence of polycyclic aromatic hydrocarbons, organometallic and metallic compounds (Pacheco et al., 2005; Kerambrun et al., 2012; Carreira et al., 2013), being the latter group of

* Corresponding author.
E-mail address: cmieiro@ua.pt (C.L. Mieiro).

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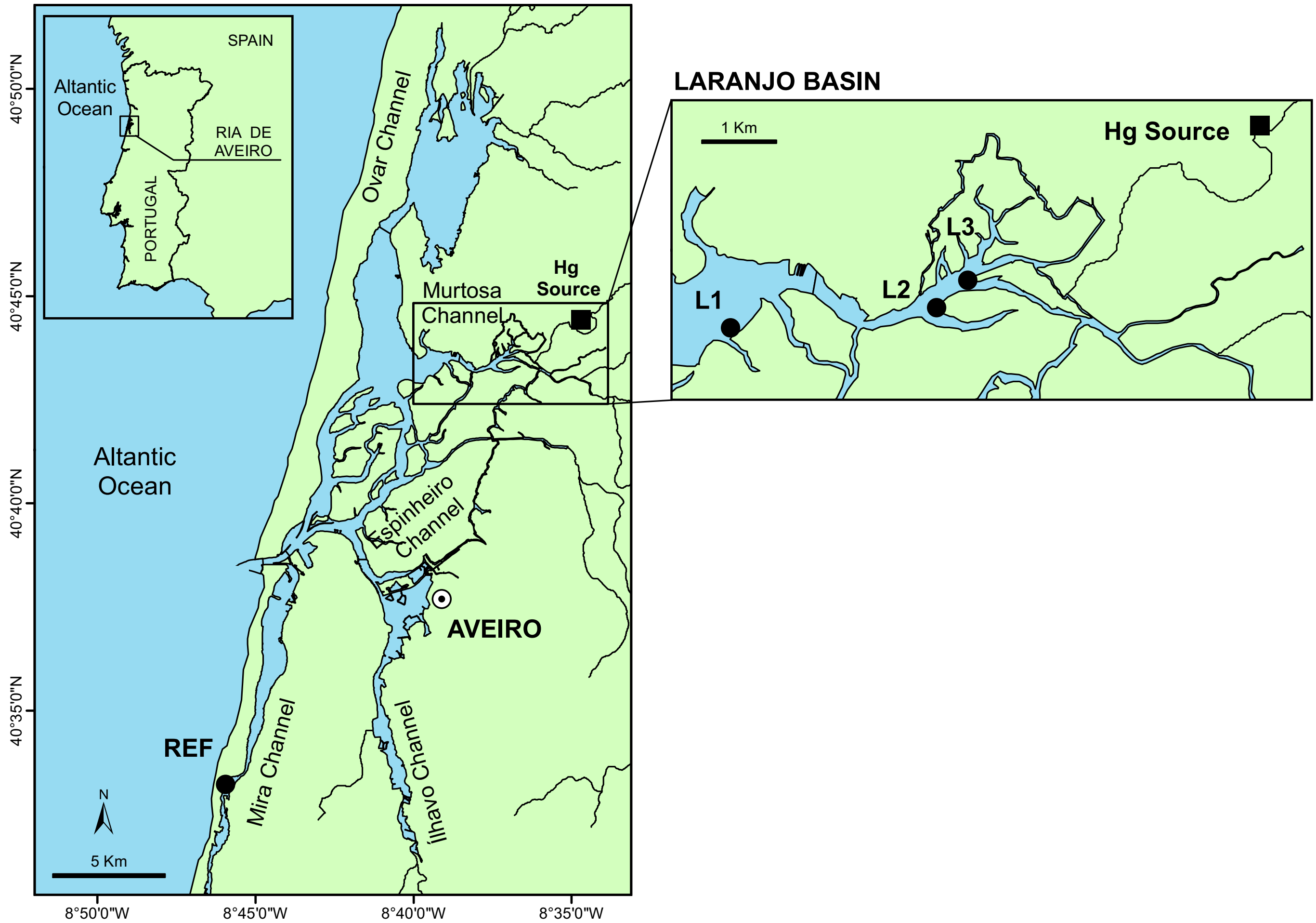

Fig. 1. Map of the Ria de Aveiro (Portugal) with caging locations of glass eels (black circles): Areão as reference (REF – 40°31′53.2″N, 8°46′18.9″W), Bico (L1–40°43′33.5″N, 8°36′42.5″W), Entrada do Esteiro (L2–40°43′49″N, 8°36′53″W) and Esteiro (L3–40°43′24.7″N, 8°36′21.6″W). Mercury source is indicated with a black square.

contaminants reported as highly toxic (Serafim et al., 2013). Among metallic contaminants, mercury (Hg) has been highlighted for exceeding the EU environmental quality standard (EQS) levels in fish from several estuaries across Europe (Nguetseng et al., 2015). Mercury is persistent and highly toxic, being recognized to adversely affect fish physiology, growth, health and behaviour (Scheuhammer et al., 2007; Vieira et al., 2009; Mieiro et al., 2015). The underlying molecular mechanisms of Hg toxicity are predominantly related with oxidative stress, which in turn may induce lipid and DNA damage (Mohmood et al., 2012; Pereira et al., 2016).

Several fish studies addressed DNA damage associated to Hg exposure (Guilherme et al., 2008b; Costa et al., 2011; Mohmood et al., 2012), highlighting genotoxicity as a critical diagnostic endpoint to evaluate the impact of Hg contamination. Moreover, genotoxicity biomarkers have recurrently been used to assess contamination impact on different fish species (Costa et al., 2011; Ameur et al., 2012; Marques et al., 2014; Vignardi et al., 2015), reinforcing their importance towards the assessment of the health status (van der Oost et al., 2003). In the last decade, the erythrocytic nuclear abnormalities (ENA) assay, performed in peripheral blood, emerged as one of the most applied methodology to assess genotoxicity in wildlife, including fish (Maceda-Veiga et al., 2015). The formation of erythrocyte nuclei deformities is associated to chromosome breakage (clastogenicity) or total loss of chromosome, as well as to mitotic spindle apparatus dysfunction leading to chromosome missegregation (aneugenicity) (Fenech, 2000). This assay efficiently signals unrepairable nuclear lesions, but is unable to point out alterations on cell cycle progression and subsequent implications on turnover of circulating erythrocytes (balance erythropoiesis/cell removal). This information has not only an intrinsic interest towards a physiologic evaluation but also as a factor affecting genotoxicity expression (as ENA frequency, for instance) (Pacheco and Santos, 2002). In this way, the frequency of immature erythrocytes (IE) and indices of erythrocyte maturity, highly sensitive to environmental pollution (Maceda-Veiga et al., 2010), can provide critical and complementary information on fish haematological dynamics. These endpoints rely on the analysis of nucleus size and the ratio between nuclear and cytoplasmic volumes (karyoplasmic ratio), important features for cell function (Webster et al., 2009). Disturbance of nuclear size and karyoplasmic ratio has been associated with certain types of cancer in humans (Zink et al., 2004). Moreover, this type of haematological parameters has been recognized as a suitable tool to detect early warning signs of physiological stress in fish, showing to be particularly responsive to metal contamination (Maceda-Veiga et al., 2010; Sadeghi et al., 2015; Seriani et al., 2014).

Hence, the present research was designed to improve knowledge on the effects of estuarine contamination, namely Hg, in European eel by using an active biomonitoring approach applied to the glass eel stage. The active biomonitoring consisted in caging homogeneous specimens in different sites throughout the contaminated area of the Ria de Aveiro, Portugal. The Ria de Aveiro is considered a hotspot in terms of Hg contamination, yet with Hg circumscribed to an inner region, originating an environmental gradient suitable for assessing Hg threat to wildlife. To accomplish this general goal, the following specific objectives were pursued: i) to evaluate Hg bioaccumulation along a