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Subcellular partitioning of non-essential trace metals (Ag, As, Cd, Ni, Pb, and Tl) in livers of American (*Anguilla rostrata)* and European (*Anguilla anguilla*) yellow eels

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ABSTRACT

We determined the intracellular compartmentalization of the trace metals Ag, As, Cd, Ni, Pb, and Tl in the livers of yellow eels collected from the Saint Lawrence River system in Canada (*Anguilla rostrata*) and in the area of the Gironde estuary in France (*Anguilla anguilla*). Differential centrifugation, NaOH digestion and thermal shock were used to separate eel livers into putative "sensitive" fractions (heat-denatured proteins, mitochondria and microsomes + lysosomes) and detoxified metal fractions (heat-stable peptides/proteins and granules). The cytosolic heat-stable fraction (HSP) was consistently involved in the detoxification of all trace metals. In addition, granule-like structures played a complementary role in the detoxification of Ni, Pb, and Tl in both eel species. However, these detoxification mechanisms were not completely effective because increasing trace metal concentrations in whole livers were accompanied by significant increases in the concentrations of most trace metals in "sensitive" subcellular fractions, that is, mitochondria, heat-denatured cytosolic proteins and microsomes + lysosomes. Among these "sensitive" fractions, mitochondria were the major binding sites for As, Cd, Pb, and Tl. This accumulation of non-essential metals in "sensitive" fractions likely represents a health risk for eels inhabiting the Saint Lawrence and Gironde environments.

1. Introduction

North Atlantic eel populations (*Anguilla* spp.) have declined drastically over the last 30 years (Haro et al., 2000; ICES, 2011). For *A. anguilla,* which is commercially exploited in European fresh, estuarine, and coastal waters, a substantial reduction in abundance has been reported (Dekker, 2003; ICES, 2011). Recent data for *A. anguilla* have shown that the recruitment of glass eels has dropped to about 4000 t/year, which represents less than 10% of the recruitment reported in the 1980s (ICES, 2011). Thus, *A. anguilla* is considered to be critically endangered by the International Union for the Conservation of Nature (Dekker, 2003; ICES, 2011). The European Community has implemented a regional eel management plan, involving fishing regulations and the assessment of anthropogenic impacts and eel stocks in most regions (ICES, 2011), including the Gironde estuary.

North American eels have experienced a similar decline in their stocks in recent decades (COSEWIC, 2006; Haro et al., 2000). For example, juvenile eel recruitment in Lake Ontario, Lake Champlain, and the upper Saint Lawrence River has dramatically decreased by as much as 80–90% since the 1980s (Haro et al., 2000). In addition, the number of *A. rostrata* eels migrating up the Saint Lawrence River to Lake Ontario decreased 1000-fold between the early 1980s and the mid-1990s (Castonguay et al., 1994). A growing concern for the poor stock status of American eels led to their designation as a "species of special concern" in Canada in 2006 and as an "endangered species" in the province of Ontario (COSEWIC, 2006).

A variety of factors, including habitat loss, migration barriers, overfishing, introduced parasites, and changes in climate and oceanic sea currents, have been suggested as possible causes of

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Table 1
Range (minimum–maximum) and mean (±standard deviation, SD) trace metal concentrations (nmol g^{-1} dw) in the liver of yellow eels collected in Europe (*Anguilla anguilla*) and North America (*Anguilla rostrata*). Also given are ranges in eel age, length and weight.[a]

Variable	Trace metals					
	Ag	As	Cd	Ni	Pb	Tl
Anguilla anguilla (France)						
Number of samples (*n*)[b]	8	22	25	11	25	24
[M] range (nmol g^{-1} dw)	0.9–24	5.2–320	0.2–40	1.1–24	0.6–13	0.018–0.44
$[M]_{max}/[M]_{min}$	27	63	200	22	22	24
Age range (years)	5.0–15	3.0–15	3.0–18	3.0–15	3.0–18	3.0–18
Length range (mm)	390–500	390–600	350–690	390–500	350–690	350–690
Weight range (g)	78–230	73–400	73–480	97–240	78–480	74–480
Anguilla rostrata (Canada)						
Number of samples (*n*)	20	25	28	28	27	26
[M] range (nmol g^{-1} dw)	1.7–16	4.4–44	0.8–11	7.4–73	0.4–3.2	0.024–0.44
$[M]_{max}/[M]_{min}$	9.4	10	103	9.8	8.0	18
Age range (years)	6.0–16	6.0–16	6.0–16	6.0–16	6.0–16	6.0–16
Length range (mm)	310–830	310–830	310–910	310–910	310–910	310–910
Weight range (g)	80–1000	50–1100	50–1400	50–1400	50–1400	50–1400

[a] Note that in comparisons among metals for a given eel species, the number of livers varied for different metals, but the ranges of total length, body weight and age were the same for all metals. Maximum total length and body weight were however higher for the *A. rostrata* specimens than for those of *A. anguilla*.
[b] *n* represents the number of individual fish for which the mass balance recovery was between 60% and 150% for each metal studied.

the decline in recruitment of both eel species (Castonguay et al., 1994; COSEWIC, 2006; Dekker, 2003). Contaminants have also been included in the list of potential factors leading to these downward trends (Geeraerts and Belpaire, 2010; Maes et al., 2005) since eels are reported to have high concentrations of lipophilic organic contaminants and non-essential trace metals (Bordajandi et al., 2003; Durrieu et al., 2005; Pérez Cid et al., 2001; Pointet and Milliet, 2000; Usero et al., 2004). For example, indigenous *A. rostrata* are reported to be the fish with the highest concentrations of polychlorinated biphenyls and mercury (Hg) in certain parts of the Saint Lawrence River (Abdelouahab et al., 2008; Hodson et al., 1994). In the Gironde ecosystem, European eels were ranked as the fish species having the second highest concentration of cadmium (Cd) in gills, muscles, and kidneys among 7 other fish species (e.g., *Alosa fallax*, *Dicentrarchus labrax*, *Argyrosomus regius*, and *Solea vulgaris*) (Durrieu et al., 2005). Since non-essential trace metals can affect physiological functions (Mason and Jenkins, 1995), the accumulation of such contaminants in *Anguilla* spp. might well contribute to population declines (Pierron et al., 2008a).

Despite reports of contamination of both eel species by trace metals (Geeraerts and Belpaire, 2010; Hodson et al., 1994), the role that these contaminants play in the observed population declines has been little studied. In this context, the determination of the subcellular partitioning of these metals could in principle provide useful diagnostic information about their potential metabolic effects (Campbell and Hare, 2009; Wallace et al., 2003). Such approaches allow one to distinguish between metal accumulation in detoxified metal fractions (e.g., heat-stable proteins and granule-like structures), which do not represent a toxicological risk, from metal binding to physiologically sensitive target molecules (e.g., cytosolic enzymes) and organelles (e.g., mitochondria), where the inappropriate binding of non-essential metals can induce deleterious effects that could compromise the fitness and performance of both eel species.

With this in mind, we determined the subcellular partitioning of silver (Ag), arsenic (As), cadmium (Cd), nickel (Ni), lead (Pb), and thallium (Tl) in the livers of American and European yellow eels collected from the Saint Lawrence and Gironde environments, respectively. A subcellular partitioning procedure using differential centrifugation, NaOH digestion, and thermal shock steps was applied to separate the liver samples into putative metal-sensitive fractions (heat-denatured proteins, mitochondria and microsomes + lysosomes) and detoxified-metal fractions (heat-stable proteins and NaOH-resistant granules). Trace metals were then measured in each subcellular fraction and changes in the partitioning between sensitive and detoxified compartment along the metal bioaccumulation gradient were used to assess the extent to which American and European eels are able to detoxify metals effectively.

2. Material and methods

2.1. Site sampling and fish collection

A. anguilla and *A. rostrata* immature yellow eels were captured in late May and early June 2012 from the Saint Lawrence and Gironde region (Table 1). American eels were collected in the Saint Jean River (48°51′40″N; 64°28′47″W), the Sud-Ouest River (48°22′27″N; 68°43′02″W), Lake Saint Pierre (45°09′18″N; 74°23′04″W) and Lake Saint François (46°19′50″N; 72°32′06″W), whereas the European eels were collected from three sites in southwest France along the Gironde system (Dordogne: 44°54′30″N, 0°15′01″W; Garonne: 44°43′51″N, 0°43′35″W; Gironde estuary: 45°12′07″N, 0°43′35″W) and from Arcachon Bay which is considered to be a pristine environment (Certes salt marshes: 44°41′18″N; 1°1′39″W). The sampling sites were selected on the basis of information on metal concentrations in water, sediments, and biota previously reported for the Saint Lawrence River system (Carignan et al., 1994; Desrosiers et al., 2008; Saulnier and Gagnon, 2006) and the Gironde region (Durrieu et al., 2005; Pierron et al., 2008b). Some of the collection sites are highly metal-contaminated (e.g., Lake Saint Pierre, Lake Saint François, and Garonne) compared to others that we consider to be reference sites (e.g., Saint Jean, Sud-Ouest, and Certes).

Yellow eels from each sampling site were collected either by electrofishing or with fyke nets. After collection, fish were kept alive in well-oxygenated freshwater. Efforts were made to minimize stress on the eels during their capture and handling. Once the total length (±1 mm) and weight (±0.1 g) had been determined, the eels were decapitated. The liver was chosen as the target organ because of its importance in the detoxification of toxic substances, and because in previous eel studies the liver was shown to have high concentrations of trace metals (Durrieu et al., 2005; Pierron et al., 2008b). Livers were removed and held in 2-mL cryo-vials (Corning, Sigma–Aldrich, Oakville, ON, Canada), frozen in liquid nitrogen in the field and then kept at −80 °C in a laboratory freezer until analysis. The American eel capture and sampling protocols were approved by the INRS animal-care committee.