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The silvering process of European eel (*Anguilla anguilla*) influences PAH metabolite concentrations in bile fluid: Consequences for monitoring

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ABSTRACT

The stock of the catadromous European eel (*Anguilla anguilla* L.) continues to decline and there is growing evidence that poor health status due to contaminants might be a key element in this decrease. Organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) belong to the major threats to yellow eel in their growth habitat and their metabolites are detectable in the bile. Starting the silvering process eels undergo physiological and morphological changes including cessation of feeding and downstream migration back to their spawning grounds. Reduced feed intake results in a diminishment of bile production and induces accumulation of e.g. PAH-metabolites in bile. Therefore, the aim of the present study was to demonstrate the impact of silvering on biliary PAH metabolite concentrations and to utilize normalization procedures to overcome silvering related accumulation effects of PAH-metabolites in eel bile. We investigated the hydroxyl-metabolites of pyrene (1-OH Pyr) and phenantrene (1-OH Phen) in the bile of different maturation stages of eels (silvering index I–V) from nine German rivers. We detected increasing absolute PAH metabolite levels in bile during the silvering process. The highest rise could be observed at the transition from pre migration stage III to the migrating stage IV, suggesting the onset of cessation of feeding at this stage. A cessation bias in PAH metabolite measurement could be diminished by normalization of absolute values against bile pigments (A_{380}, biliverdin). In conclusion, we demonstrated the impact of silvering on PAH metabolite concentrations in eel bile and present suitable normalization procedures to overcome silvering related accumulation effects. Thus, for a future eel monitoring we recommend (1) to regularly monitor PAH metabolites in bile, (2) to determine silvering index of eel and (3) to normalize PAH metabolite values in bile based on maturation/silvering stages. The knowledge of the silvering stage is mandatory for an unbiased evaluation of PAH contamination of European eel towards an international harmonized eel monitoring program.

1. Introduction

Since the early 1980s, the panmictic stock of European eel (*Anguilla anguilla* L.) has undergone a general decline with decreased recruitment of young eels to 1–5% of its mean level in the late 1970s (Dekker, 2003). In the last 10 years the stock continues to decline dramatically with an all time-low of recruited glass eels in 2009 (ICES, 2010). Therefore, the stock is still considered outside safe biological limits (ICES, 2010). The recent stock decline has been related to natural as well as to anthropogenic factors. Changes in climate and oceanic conditions may have influenced reproductive success, larval development and recruitment (Friedland et al., 2007; Kettle et al., 2008; Durif et al., 2010). Exploitation, habitat alterations, including migration barriers and deterioration in water quality (parasites, diseases and contaminants) contribute to the anthropogenic stresses on eels (ICES, 2010). Impacts of pollution on fitness and fecundity have been suggested to be a key factor in the decline of the eel stock (Palstra et al., 2007; Geeraerts and Belpaire, 2010; ICES, 2010). Potential restrictive factors for eel migration and successful reproduction are insufficient fitness (Svedäng and Wickström, 1997) and bioaccumulation of organic pollutants (Larsson et al., 1990; Robinet and Feunteun, 2002; Palstra et al., 2006; Belpaire et al., 2008).

Major lipophilic contaminants in freshwater sediments are polycyclic aromatic hydrocarbons (PAHs) (Ruddock et al., 2002; Nagel et al., 2011), which are a group of ubiquitous, aquatic pollutants derived from pyrogenic and petrogenic sources (Van der Oost et al., 2003; Vuorinen et al., 2006). After uptake from the environment PAHs are metabolized primarily in the fish liver and the metabolites are secreted into the bile and temporarily stored in the gall bladder (Meador et al., 1995). PAHs and intermediate metabolic compounds have been demonstrated to induce delayed

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growth, reduced survival and increased prevalence of developmental malformation at early life history stages (Heintz et al., 2000; Incardona et al., 2004, reviewed in Beyer et al., 2010). These findings are linked to DNA adducts (van Schooten et al., 1995) and neoplasia-related aberrations or tumors in fish liver (Stein et al., 1990; Aas et al., 2000; Myers et al., 2003). PAH metabolites are used as markers for monitoring PAH exposure of fish, because the parent compounds are quickly metabolized. However, several factors may impede the use of biliary PAH metabolites as a biomarker for e.g. the feeding status (Vuorinen et al., 2006). While bile volume increases during feeding, the gall bladder is emptied into the intestine shortly after feeding (Varanasi and Stein, 1991; Au et al., 1999). Brumley et al. (1998) investigated the influence of fish feeding status on biliary 2-chlorosyringaldehyde metabolite levels in sand flathead (Platycephalus bassensis) suggesting that bile volume and biliary metabolite concentrations are both related to the dietary status of fish.

Among fish, the European eel is the predestinated species to examine the influence of feeding status on biliary PAH metabolite levels for two reasons: Firstly, as PAHs tend to accumulate in sediments (Notar et al., 2001) the benthic and largely benthivorous, fat-rich eel is directly exposed to PAHs and thus feasible for the screening of toxic organic substances compared to other freshwater and diadromous fish species (Belpaire et al., 2011). Therefore, eel has been proposed as an indicator species for the chemical status of fresh water habitats (Belpaire and Goemans, 2007). Secondly, the eel has a peculiar life cycle with an oceanic and a continental phase with distinct changes in feeding status. At the end of their continental phase adult eels undergo a metamorphosis called silvering corresponding to morphological and physiological changes that prepare the eel for the migration back to the spawning grounds in the Sargasso Sea (Schmidt, 1922). The dynamics of silvering are linked to environmental factors like temperature, flood events and lunar phase (Haro, 2003; Tesch and Rohlf, 2003) triggering final events e.g. cessation of feeding for the whole migratory phase back to the spawning grounds, whereas any prediction of the precise onset is still challenging. A first step to classify silvering of eel has been done by Durif et al. (2005), who developed stages to characterize yellow resident and silver migrating eels. Regarding the known link between PAH metabolites in bile and the feeding status of fish on one hand and the fact that the European eel stops feeding during the silvering process on the other hand it could be assumed that silvering influences PAH metabolite concentration in the bile. However, no information on PAH metabolite levels in relation to different life stages of freshwater eels has yet been published. Moreover, this problem was completely ignored in several studies on PAH metabolites in eel (Pointet and Milliet, 2000; Ruddock et al., 2002; Ruddock et al., 2003; Ribeiro et al., 2005), despite PAH metabolites have been suggested to be part of a future harmonized eel monitoring strategy over Europe in view of eel management plans and the identification of suitable eel habitats (Belpaire et al., 2011; Nagel et al., 2011). A prerequisite for any such monitoring action is the unbiased assessment of PAH contamination in individual fish.

The primary scope of this study was therefore to demonstrate the impact of silvering on biliary PAH metabolite concentrations and the evaluation of suitable normalization procedures. In a second step we provide an advice for future use of PAH metabolite data towards a pan-European eel monitoring program.

2. Materials and methods

Thirty eels of different maturation stages were caught at one location each of the rivers Rhine, Ems, Weser, Elbe, Schlei, Eider, Warnow, Peene and Uecker between May and November in 2009

Table 1
Number, mean length, and mean weight of eel in relation to the silvering index (SI I to V).

River		Silvering index				
		I	II	III	IV	V
Rhine	*n*	1	7	7	8	6
	Mean length (cm)	43.5	53.3 ± 5.4	64.6 ± 4.9	80.0 ± 4.3	73.0 ± 6.8
	Mean weight (g)	137	245 ± 67	473 ± 99	1004 ± 96	727 ± 164
Ems	*n*	2	9	4	1	9
	Mean length (cm)	43.0 ± 2.8	48.8 ± 3.1	68.6 ± 6.1	86.5	73.1 ± 6.2
	Mean weight (g)	148 ± 32	212 ± 36	671 ± 157	1180	750 ± 146
Weser	*n*	2	10	7	8	3
	Mean length (cm)	41.8 ± 6	59.1 ± 8.9	69.3 ± 5.7	78.6 ± 8.3	67.5 ± 3
	Mean weight (g)	117 ± 37	378 ± 196	665 ± 237	1182 ± 437	640 ± 170
Elbe	*n*	1	9	14	2	3
	Mean length (cm)	40.5	52.4 ± 3.6	65.5 ± 8.0	81.8 ± 7.4	65.5 ± 5.8
	Mean weight (g)	99	232 ± 55	504 ± 215	1141 ± 239	520 ± 209
Schlei	*n*	6	5	6	11	2
	Mean length (cm)	39.6 ± 2.7	59.0 ± 9.9	74.8 ± 4.5	86.2 ± 5.5	69.8 ± 0.4
	Mean weight (g)	100 ± 22	407 ± 274	825 ± 257	1455 ± 212	696 ± 44
Eider	*n*	2	10	4	0	3
	Mean length (cm)	42.5 ± 2.1	47.8 ± 3.3[a]	61 ± 7.0		66 ± 1.0
	Mean weight (g)	104 ± 9	149 ± 31	386 ± 123		571 ± 78
Warnow	*n*	1	3	13	4	8
	Mean length (cm)	41.5	55.3 ± 6.0	64.8 ± 6.1	75 ± 3.4	65.5 ± 5.6
	Mean weight (g)	139	350 ± 133	518 ± 159	919 ± 79	496 ± 139
Peene	*n*	0	12	8	0	11
	Mean length (cm)		55.2 ± 2.4	62.6 ± 3.6		64.4 ± 5.4
	Mean weight (g)		276 ± 39	459 ± 78		532 ± 120
Uecker	*n*	0	2	15	5	9
	Mean length (cm)		68.8 ± 0.4[a]	68 ± 6.6	82.4 ± 7.6	62.7 ± 6.4
	Mean weight (g)		506 ± 74	549 ± 172	1233 ± 329	481 ± 147
Σ	*n*	15	67	78	39	54
	Mean length (cm)	41.8 ± 1.3	55.5 ± 6.0	66.6 ± 3.9	81.5 ± 3.8	67.5 ± 3.5
	Mean weight (g)	120.6 ± 19.0	306.1 ± 105.9	561.1 ± 127.6	1159.1 ± 158.6	601.4 ± 97.9

All values are expressed as means ± SDs. Comparing mean length and mean weight for each silvering index letters in bold type indicate values that are significantly greater (ANOVA, Scheffe's post hoc test, $p < 0.05$) than values with the same letters in regular type.