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Head shape disparity impacts pollutant accumulation in European eel☆

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ABSTRACT

Several aspects of the life cycle of the critically endangered European eel (*Anguilla anguilla*) remain poorly understood. One such aspect is the broad-versus narrow-head dimorphism, and how this impacts their overall performance at different stages of their life cycle. At the yellow eel stage, the phenotypes show a trophic divergence. We investigated whether pollutant accumulation is affected by this disparity. We show that broad-headed eels contained higher concentrations of mercury and several lipophilic organic pollutants, compared to narrow-headed ones, irrespective of their fat content. The hereby confirmed link between the phenotypic disparity, its associated feeding ecology and its impact on pollutant accumulation thus raises further concerns about their migratory and reproductive success. Considering that pollution is an important contributor to the European eel's decline, our results demonstrate that broad-headed eels are more vulnerable to detrimental pollutant accumulation. This compromises their successful contribution to their population's reproduction and its restoration.

1. Introduction

While European eels (*Anguilla anguilla*) have been studied extensively, many questions regarding their unique life cycle still remain unanswered. Dependent on oceanic current dynamics, this catadromous fish arrives as an un-pigmented glass eel at the continental waters of Europe (Baltazar-Soares et al, 2014). They continue their journey up the rivers, where they feed and grow into yellow eels. For several years, they accumulate a fat reserve of at least 12% of their body weight, which is required for their migration back across the ocean and for gonadal development, to become the mature silver eels that will eventually spawn in the Sargasso Sea (Tesch, 2003, Van den Thillart et al., 2007). Worryingly, the analysis of the current glass eel recruitment indices at European monitoring stations have revealed a decrease to only 1%, compared to the late 1970's, in the North Sea region and to less than 5% in the rest of Europe (ICES, 2016). Consequently, the European eel is considered critically endangered on the International Union for Conservation of Nature (IUCN) red list. Following the EU Eel Regulation put in place in 2007 to restore the European eel stock (Council Regulation (EC) No. 1100/2007), EU Member States have developed Eel Management Plans (EMPs) with the objective to obtain a silver eel biomass escapement of 40%, compared to a situation without anthropogenic disturbances. Alarmingly, more than 50% of the current EMP progress reports show a failing to meet this target (ICES, 2013), indicating that much more effort is needed to be put wards the European eel's recovery. A thorough understanding of its ecology is thereby crucial to develop proper management measures. Among other factors, such as habitat loss (Kettle et al., 2011), migration barriers (Durif et al., 2002), parasites (Palstra et al., 2007), overfishing (Dekker, 2003), climate change and changes in oceanic conditions (Friedland et al., 2007, Bonhommeau et al, 2008), the accumulation of pollutants could be one of the possible synergistic

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causes that are contributing to the decline of the eel population (Belpaire et al., 2016).

Eels bio-accumulate many lipophilic pollutants in their fat tissue throughout their life. When eels start migrating and turn into silver eels, they stop feeding and thrive on their fat reserves. The continuous fat burning, in combination with endocrine-induced morphological and physiological changes during migration, induces a remobilization of pollutants into the bloodstream and leads to a subsequent increase in tissue concentrations of target organs, such as the developing gonads (Lewander et al., 1974, Geeraerts and Belpaire, 2010, Freese et al, 2017). Bio-accumulation of toxic compounds can lead to physiological disturbances, lowered resistance, disturbed reproduction and possibly even death, before eels reach the spawning area (Geeraerts and Belpaire, 2010, Robinet and Feunteun, 2002). Even when eels successfully complete the migration, evidence has shown that 17–52% of the original fat reserves, together with its pollutants, are incorporated in the oocytes. This maternal transfer of contaminants to eggs is expected to increase the mortality of larvae, especially when the eels come from highly polluted environments (Foekema et al., 2016). Thus, pollution impacting physiological processes during the European eel's life cycle is thought to be a crucial factor contributing to their decline.

While eels can absorb pollutants through their gills and skin, the major uptake of Persistent Organic Pollutants (POPs) and trace metals, such as mercury, occurs through the ingestion of contaminated food. European yellow eels demonstrate trophic divergence which is associated with a head shape dimorphism: broad-headed eels consume more fish and crustaceans, whereas narrow-headed eels mainly feed on benthic invertebrates (Törlitz, 1922, Thurow, 1958, Lammens and Visser, 1989, Proman and Reynolds, 2000). Also anatomically, the dimorphism involves variation in the musculoskeletal components of the feeding apparatus, which increases biting performance in broader heads (De Schepper, 2007). Here, we investigated whether this disparity in trophic ecology could also affect the accumulation of pollutants, which could alter reproductive ability. We hypothesize that broad-headed eels, which feed higher in the food chain (Cucherousset et al, 2011), are more vulnerable to pollutant accumulation and are thus more susceptible to the detrimental pollutant effects, due to the consumption of more contaminated prey. Gaining insight into the interaction of the eel's morphology with its feeding ecology and pollutant accumulation would not only be a key element for improving recovery efforts, but it would also shed some much needed light onto how feeding-associated morphological variation can cause differential pollutant accumulation in eels.

2. Material and methods

2.1. Experimental design

2.1.1. Sample collection

European eels (N = 377) were captured by fyke nets and electric fishing in Lake Weerde (Belgium) in October 2015. Lake Weerde is a small (14 ha) lake in the Scheldt catchment, which is located in a recreational and agricultural area, with no important industrial activity. There is no open connection to a river system and all eels originated from glass eel restocking. It was initially a lake with a depth of approximately 8 m, resulting from sand excavation between 1968 and 1973. In order to create a more shallow lake with different shallow water zones to increase biodiversity, 250,000 m^3 of inert demolition material was used to partially fill the lake during the 1990s. However, apparently, also toxic material had been dumped, resulting in Lake Weerde being a highly contaminated lake for polychlorinated biphenyls (PCBs) and other contaminants (considering the Flanders Eel Pollution Monitoring Network). Because of these conditions, this lake was chosen for the present study. By annual monitoring, it was shown that PCB body burden of eels were decreasing from 1998 to 2005 (Belpaire, 2008). However, a steep increase of PCB contamination was observed in 2006, which was suspected to be due to a local discharge or spilling of toxic waste containing PCBs (Belpaire et al., 2011).

All eels were anaesthetized with MS222 (Tricaine methanesulfonate). Subsequently, total length (TotL) and total weight (W) were measured. Additionally, pictures of the head were taken in dorsal view and head width (HW) was measured between the jaw hinges at the nearest 0.1 mm, using a Mauser digital caliper. HW/TotL was calculated in the field. Eye size was used to determine whether an eel was in the yellow or silver eel stage, as eye size increases extensively during silvering (Pankhurst, 1982). Out of the 377 eels, 75 yellow eels were selected over a wide range of HW/TotL for further analyses. All the selected eels were larger than 46 cm ($TotL_{min-max}$: 46.5–67 cm), and thus were also all female (Durif et al., 2009), hence a sex effect could be excluded. The other eels were released again into the wild once they had recovered from anesthesia. The condition of the 75 eels was determined by calculating the Le Cren's condition factor K (Le Cren, 1951) as follows: K = W/W′ where W is the observed weight and W′ the calculated weight based on the length-weight relationship ($W = a \times TotL^b$, where a is the intercept of the slope and b the slope of the relationship). Subsequently, the 75 eels were anaesthetized by MS222 and euthanized by an MS222 overdose, in accordance with the Belgian legislation. The eels were decapitated and the heads were fixed in 10% formalin and preserved in 70% ethanol. The body was skinned, intestines were removed and after decapitation, the body was cut into four equal parts. The muscle tissue of the first three parts was used for further analyses, while the final part acted as a reserve (Fig. S3). The muscle tissue of the first part was used for stable isotope analysis, while the muscle tissue of the second part was used for analysis of Persistent Organic Pollutants and the third part for the analysis of trace metals. This protocol has been applied according to the methods developed by INBO during the Eel Pollution Monitoring Network in Flanders (Maes et al., 2008) and described in ICES (ICES (2015). It has been further used during other international eel assessments (CORDIS, 2013; Pujolar et al., 2012).

2.1.2. Head shape determination

We used a mixture analysis in PAST to visualize a bimodal distribution, based on the HW/TL of the selected eels. We selected the point where the two unimodal distributions of the frequency histogram overlapped as the separation value between broad- and narrow-heads. The separation value was found at a HW/TL of 0.030. Based on this value, we considered eels with a HW/TL lower than 0.0275 as narrow-heads, eels with a HW/TL higher than 0.0325 as broad-heads and eels with a HW/TL between these values as intermediates. Using these cut-off values, our dataset consisted of 26 narrow-heads (NH), 25 intermediates (INT) and 24 broad-heads (BH).

2.2. Analyses

2.2.1. Age determination

The left and right sagittal otoliths were removed from the head, to determine the age of the eels by the burning and cracking method (Hu and Todd, 1981; Moriarty, 1973). This method is recommended for eel ageing, especially for large eels (ICES, 2011). In short, the otoliths were first cut into two equal pieces and were subsequently burned in a flame, revealing the annuli on the broken face. The otoliths were then mounted cut face up onto a glass slide in silicone. Pictures of the otoliths were taken using an SZX9