

Are dioxin-like contaminants responsible for the eel (*Anguilla anguilla*) drama?

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Abstract

Eel populations world-wide are dangerously close to collapse. Our study is the first to show that current levels of dioxin-like contaminants are strong candidates, because of their devastating effects on development and survival of eel embryos. Female and male silver eels were artificially stimulated to maturation and reproduction by treatment with carp pituitary extracts and hCG respectively. During maturation of female European silver eels about 60 g fat per kg eel is incorporated in the oocytes. Together with the fat however, persistent organic pollutants such as dioxin-like polychlorinated biphenyls (PCBs) are incorporated too. The total dioxin-like toxic potency of the individual gonad batches was determined as TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxine) – equivalents (TEQs), using an in vitro reporter gene assay. The observed differences in development and survival showed a significant negative correlation with the TEQ levels in the gonads, already at levels far below the maximal allowable level for fish consumption i.e. 4 ng TEQ/kg fish. The clear inverse relationship between the TEQ-level and the survival period of the fertilised eggs strongly suggest that the current levels of dioxin-like compounds seriously impair the reproduction of the European eel. The peak of the environmental levels of dioxin-like PCBs and the decline of eel coincide world-wide, further suggesting that, in addition to other threats, these contaminants contributed significantly to the current collapse of eel populations.

Keywords: Sargasso, fish, decline, migration, maturation, reproduction, spawning, contamination, teratogenicity, polychlorinated biphenyls PCBs, fertility, embryology

Introduction

Recruitment of the European eel (glass eel arrivals) has collapsed since the early 80s. The influx of glass eels has even declined with 99% (Anonymous, 2003). Two groups of potential causes of decline have been suggested: 1) quantitative causes such as climate changes, fisheries, habitat reduction and restocking, and migration barriers, and 2) qualitative causes such as insufficient fat reserves, virus infection (EVEX), parasite infection (*Anguillicola crassus*), and contamination (PCBs).

Migrating silver eels do not feed 'en route' and totally depend on their fat stores to fuel migration and oocyte development. The fate of mobilised fat reserves of the semelparous eels is as follows: 39% is used as an energy source (van Ginneken & van den Thillart, 2000; van den Thillart et al., 2004; van Ginneken et al., 2005; Palstra et al., unpublished data), and 28% is incorporated into the maturing oocytes (Palstra et al., submitted and unpublished data) during the eels' 5,500-km migration to the Sargasso Sea. With fat consumption, however, internal concentrations of lipophilic pollutants rise, thus increasing the risk for toxic effects. Especially eels are vulnerable to dioxin-like contamination since they often reside in contaminated sediments and accumulate high levels of especially PCBs. These have been shown to have adverse effects on fertility in fish, amphibians and mammals.

The quest for successful artificial reproduction has a long history, but is still open. If successful, aquaculture could be provided with artificially bred stock while the natural populations have a chance to recover without fishing pressure on the yearly arriving glass eel stock. Recently, we have been able to fertilise eggs and follow embryonic development (Palstra et al., 2005). Large differences were observed with respect to development in fertilised egg batches. In a recently published study (Palstra et al., 2006) we have investigated whether these developmental differences were caused by maternal dioxin-like contaminants deposited in the egg yolk.

Concepts & results

Twenty-five female silver eels (500-1,700 g) and 50 male silver eels (100-150 g) were caught during their seaward migration in a relatively PCB-clean habitat (van Leeuwen et al., 2002). Males were hormonally stimulated to mature by weekly injections with human chorionic gonadotropin and females by weekly injections of carp pituitary extract. Ovulation was induced by a single injection of dihydroxyprogesterone. Eels were hand-stripped and eggs were fertilised and reared (procedure after Palstra et al., 2005). After stripping, eels were sampled for: 1) otoliths for age estimation (after Daverat et al., 2005), and 2) muscle and gonad samples for fat & TEQ measurements by DR-CALUX-assay (after Aarts et al., 1995 and Murk et al., 1998).

DR-CALUX is a dioxin responsive chemical activated luciferase gene expression – bioassay. It measures TEQ which are dioxin equivalents. These are dioxins (TCDDs) and dioxin-likes (PCDDs, PCDFs, PCBs). In eel, 86% of the TEQ are PCBs. Some PCBs are metabolizable, others partly and some are not. These are all PHAHs: polyhalogenated aromatic hydrocarbon pollutants. They bind to the AhR (aryl hydrocarbon receptor) which leads to expression at specific genes controlled by dioxin responsive elements. DR-CALUX measures by stably transfecting rat hepatoma cells (H4IIE) with a plasmid carrying the luciferase gene of fireflies as a reporter gene. These cells produce luciferase in presence of PHAHs. Luciferase reacts with luciferine under the production of light that is measured in a luminometer.

The TEQ levels in hormone treated eels tended to be higher. All hormone-treated females fully matured reaching GSIs (relative gonad mass) of 27-51% after 12-25 weekly injections (0.8-1.7 million eggs). Absolute fat in gonads of hormone-treated females was 60 g and 12 times higher than in control females (Fig. 1). The amount of fat in gonads positively correlated with age.

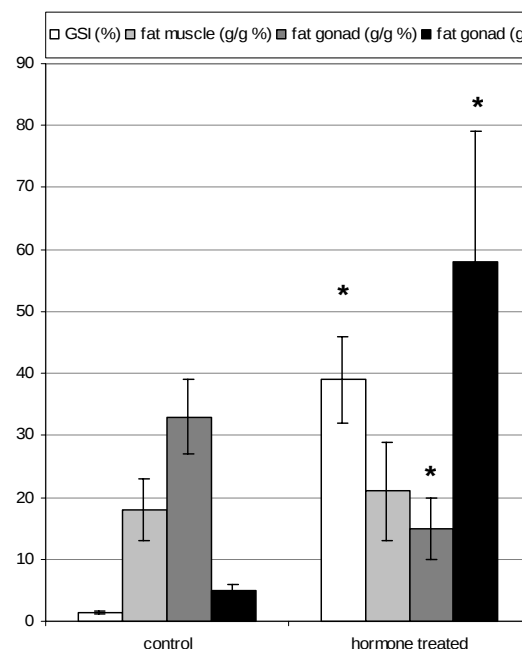


Figure 1 GSI (relative gonad weight), relative fat content in muscle and gonad (g fat/g tissue *100%) and total fat in gonad (g/kg eel) in control and hormone-treated eels. Highly significant differences ($P < 0.001$) between groups are indicated with an asterisk. (taken from Palstra et al., 2006)

The eggs from 8 out of 13 hormone-treated females showed cleavage. Seven of these 8 batches showed development up to 15 hours post fertilisation (hpf) but died thereafter. One of these 7 batches resulted in 1,500 embryos that showed serious oedema of the yolksac, a deformed head region and absence of heartbeat (Fig. 2). A negative correlation between TEQ values and embryo survival time was found (Fig. 2).

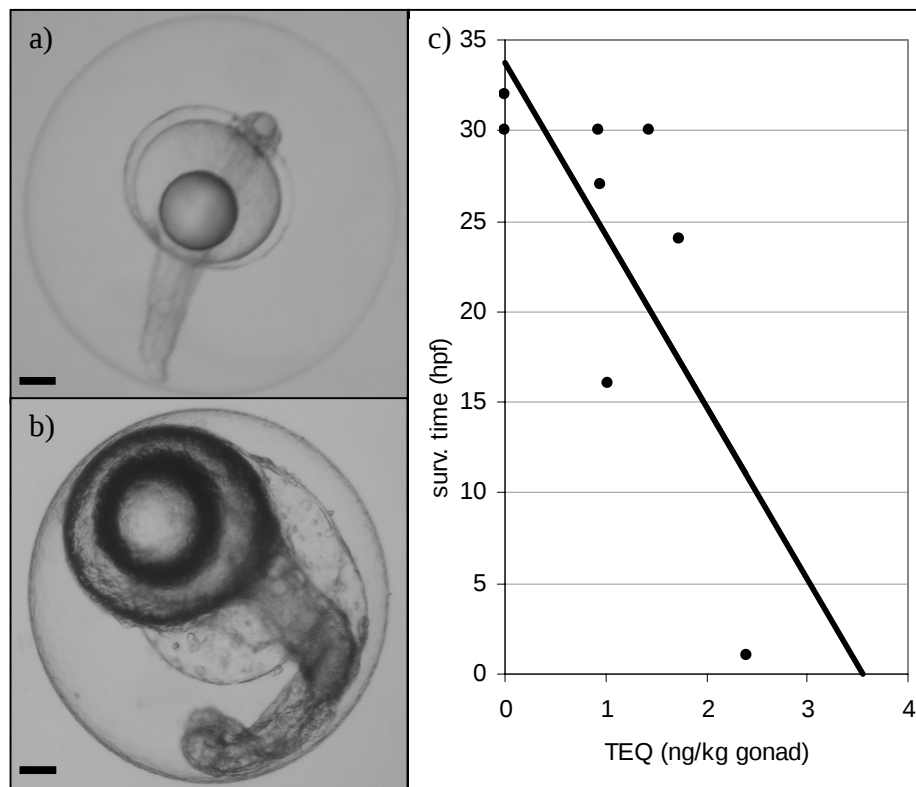


Figure 2 a) Healthy European eel embryo at 30-35 hpf with heartbeat and yolk sac with large fat droplet, b) Larger embryo of an unhealthy batch at identical time of development displaying yolk sac oedema, deformed head region and absence of heartbeat. Scale bars represent 100 µm. c) Negative correlation between total TEQ values (ng/kg gonad) and embryo survival time (hours post fertilisation) of fertilised eggs of 8 hormone induced, stripped females. (taken from Palstra et al., 2006)

Conclusions

Eels accumulate dioxin-like compounds since they are bottom fish, predators, have a long generation time, and have high fat levels. During their long distance migration they will further increase the levels due to fat consumption. Thus eels can be used for biomonitoring of the chemical status of surface waters with respect to hazardous substances (ICES/EIFAC work group on eel, 2006).

The observed embryonic malformations are typical for PCB-exposed eggs such as observed in pike *Esox lucius*, carp *Cyprinus carpio*, lake trout *Salvelinus namaycush* and rainbow trout *Oncorhynchus mykiss* (Walker and Peterson 1991; Helder 1980; Stouthart et al. 1998; Walker et al. 1994). Similar symptoms are described for fish-eating birds and are known as GLEMEDS or 'Great Lakes embryo mortality, edema and deformities syndrome' (Gilbertson et al. 1991). From this, we can conclude that the observed correlation between embryo survival time and TEQ levels in the gonads implies TEQ-induced teratogenic effects. A dose-effect relation study is needed to provide conclusive evidence.

Results of this study suggest that current gonad levels of dioxin-like contaminants in eels from most European locations impair normal embryonic development (Fig. 3). Emission of PCBs in the environment (Fig. 4) coincides with decline of eel. Therefore, we consider it likely that dioxin-like contaminants contributed to the current collapse.

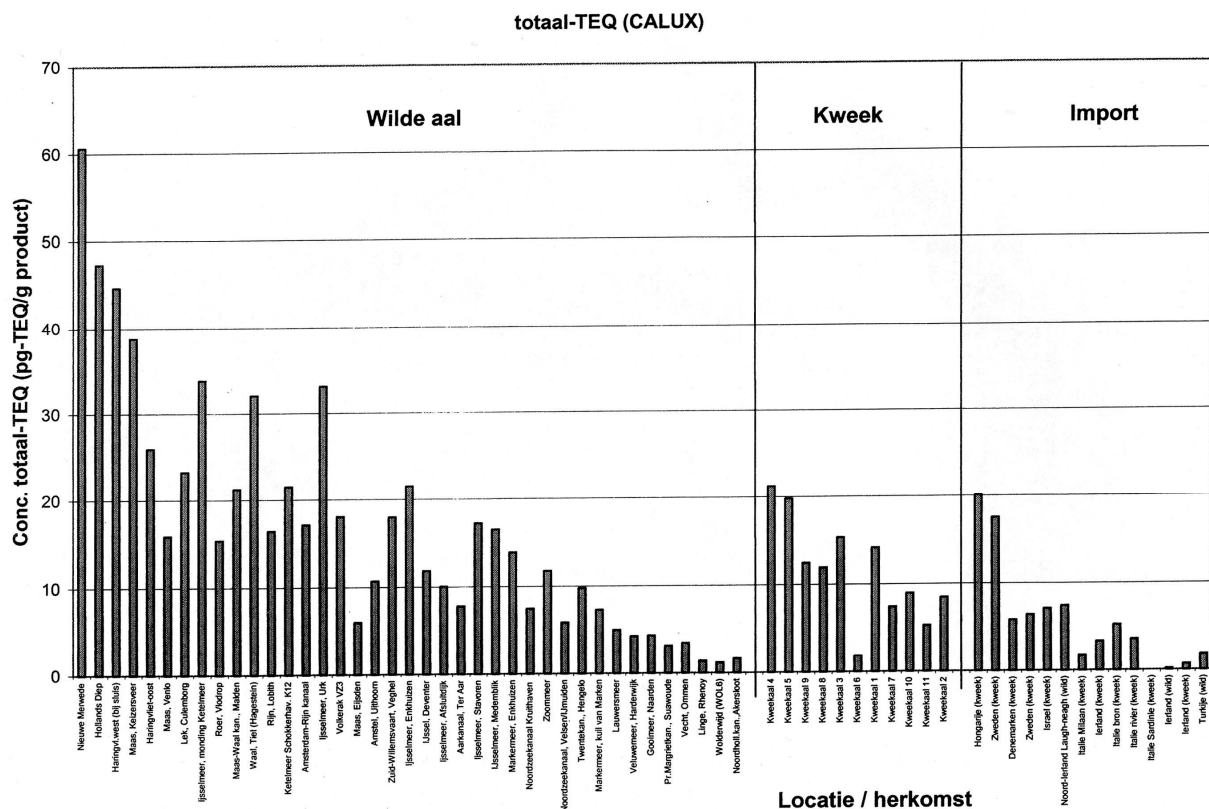


Figure 3 Total TEQ values (pg-TEQ/g product) measured by CALUX of eels from 39 locations in Holland (wilde aal), from 11 cultured eels (kweek) and from 14 imported eels either wild or cultured (import). (taken from van Leeuwen et al., 2002). This indicates that dioxin-like contaminant levels in eels from most locations impair normal embryonic development.

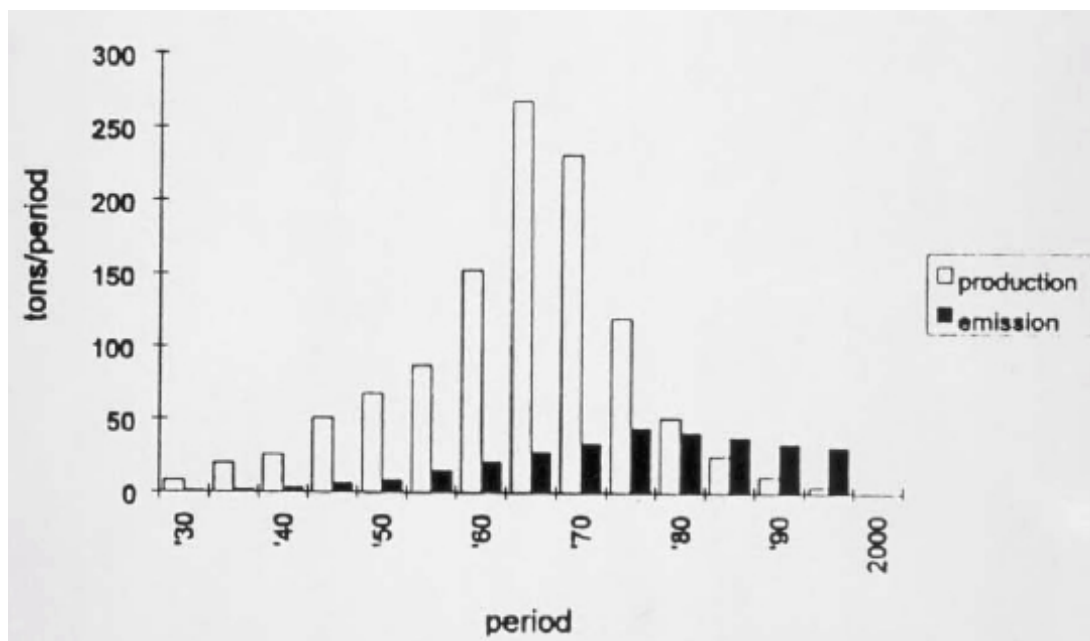


Figure 4 Estimated production and emissions of PCBs in OECD countries. (taken from van Leeuwen & Hermens, 1995)

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