



Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox



PCBs and the energy cost of migration in the European eel (*Anguilla anguilla* L.)

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ARTICLE INFO

Article history:

Received 26 August 2008

Received in revised form 15 January 2009

Accepted 17 January 2009

Keywords:

Eel decline

PCBs

Migration

Metabolic rate

Cortisol

Immune function

ABSTRACT

The effect of polychlorinated biphenyls (PCBs) on the energy consumption of fasting silver European eel (*Anguilla anguilla* L.) was studied over a 27-day period during which the animals were at rest or were swimming 800 km in Blazka swim tunnels. Three-year-old female hatchery eels (silver stage) between 73 and 80 cm long weighing around 1 kg were dosed intraperitoneally with PCBs at a nominal dosage of 10× the consumption standard as a mixture representative for planar (7 µg PCB126/kg eel), non-planar (5 mg PCB153/kg eel) and metabolizable PCBs (50 µg PCB77/kg eel) found in wild eel, or only with the vehicle (corn oil, 10 ml/kg eel). Four major observations were made: (1) PCB-exposed animals lose less weight compared to their unexposed controls; (2) PCB-concentrations on a lipid basis are 2.8–14 times higher in swimming compared to resting animals; (3) the standard metabolic rate is significantly lower in the PCB-exposed animals than in unexposed controls. In addition, PCB-exposure significantly reduces oxygen consumption during swimming, and starting at 400 km (18 days) this effect increases with time; (4) the relative spleen and liver weight significantly increased in the PCB-swim animals but not in the PCB-rest animals. The swimming animals lost about 75% more weight compared to resting animals and had about 50% lower plasma fat content. Hematocrit, haemoglobin, plasma pH, ion levels (sodium and potassium), and plasma lactate were not affected by PCB-exposure or swimming. Apparently, the current levels of PCBs and other dioxin-like compounds may seriously impair the reproduction of the European eel.

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1. Introduction

Since 1970s the population of European eel (*Anguilla anguilla* L.) is dwindling. A dramatic decrease has been observed in the number of glass eel that enters the Netherlands and other European countries (Dekker, 2004). Similar steep declines in glass eel biomass of 90–99% were reported for other eel species such as the Japanese eel (*Anguilla japonica*), and American eel (*Anguilla rostrata*) (Castonguay et al., 1994; Stone, 2003). The eel is a catadromic fish species with spawning grounds thousands of miles away, the Sargasso sea for the European eel. As a result sufficient energy reserves and an efficient metabolism may be more critical for this species to successfully spawn compared to other fish species. As a result the eel may be very vulnerable to persistent toxic con-

taminants that are released from fat stores during migration and which may interfere with energy metabolism and reproduction. Among the causes advanced for the decline of eel populations are oceanographic changes, which may interfere with larval transport (Knights, 2003), over-fishing, viruses (Van Ginneken et al., 2004, 2005b) or swim bladder parasites (Haenen et al., 1994; Palstra et al., 2007). Eels that resided for significant times in inland waters may contain high levels of especially polychlorinated biphenyls (PCBs) in the ranges up to 1.5–10 mg/g (De Boer and Hagel, 1994), values that exceed the Dutch standard for human eel consumption of 0.5 mg/kg for PCB 153.

Polyhalogenated aromatic hydrocarbon (PHAH) pollutants, including PCBs and polychlorinated dibenzo-dioxins/-furans (PCDD/Fs, further referred to as 'dioxins'), are lipophilic, persistent and widely spread in the environment and known to accumulate in the food chain. It is estimated that over 30% of the one million tons of PCBs produced are still present in aquatic and terrestrial ecosystems (Voltura and French, 2000). Exposure to PHAHs, especially 'dioxins' and dioxin-like PCBs, leads to adverse effects in many species, including disturbance of retinoid and thyroid hormone endocrinology, adverse effects on male and female reproduction,

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teratogenicity, developmental toxicity, hepatotoxicity, immunotoxicity, progressive weight loss ('wasting syndrome') and tumor promotion (Goldstein and Safe, 1989; Mukerjee, 1998; DeVito and Birnbaum, 1994; Palstra et al., 2006; Foekema et al., 2008). As it has not yet been possible to study physiological changes and toxicological effects in eel during migration, little is known about effects of PCB-exposure on spawning migration of eels. During this period the starving eel relies completely on its energy stores in adipose tissue. PCBs stored in fat will be concentrated when fat is being used, increasing the internal concentrations to possibly toxic levels. We therefore hypothesise that release of PCBs from fat may interfere with eel physiology and energy metabolism and impairs the capacity to reach the spawning grounds.

In this study we exposed eels to a PCB-mixture consisting of a di-ortho-, a planar and a metabolizable PCB in a relative and absolute amount which is environmentally relevant and $10\times$ the "2001" PCB-limit for human consumption. This standard was 0.5 mg Σ 7 PCBs/kg eel and 70 ng dioxin equivalents (TEQ)/kg eel. Because of possible specific metabolite effects and because eels are known to metabolize PCBs well (De Boer and Hagel, 1994, and own unpublished results) PCB-77 was chosen in an amount based on the average environmental ratio to persistent PCB-126. Morphological parameters, energy metabolism, PCB-levels and blood parameters were assessed in eels that experienced a simulated partial migration of 800 km in Blazka swim tunnels compared to resting eel exposed to the same levels of PCBs.

2. Materials and methods

2.1. Animal treatment and husbandry conditions

Three-year-old female hatchery eels (silver stage) between 73 and 80 cm long weighing around 1 kg were obtained from an eel farm in The Netherlands (Royaal BV, Helmond, The Netherlands). The eels were transported to Wageningen–Imares IJmuiden, The Netherlands, where they were gradually adapted to natural seawater over a period of 4 weeks. After the adaptation period of 4 weeks, 44 eels were injected intraperitoneally (ip) with the PCB-mixture exposure consisting of PCB-153 (5 mg/kg bw), PCB-126 (7 μ g/kg bw), and PCB-77 (50 μ g/kg bw) dissolved in corn oil or with corn oil (controls, 10 ml/kg). The most common, di-ortho, PCB-153 was chosen to represent $10\times$ the standard of 0.5 mg Σ 7 PCBs/kg eel. The most relevant dioxin-like PCB, the planar PCB-126, has a relative dioxine-like potency (TCDD equivalency factor or TEF) of 0.1 therefore 7 μ g/kg bw represents 70 ng TCDD-equivalents (TEQ)/kg eel. The metabolizable PCB-77 was dosed at 50 μ g PCB-77/kg eel based on the average environmental ratio to the 10-fold higher concentration of PCB-126). The eels were kept for 2 weeks in a recirculation system with seawater to allow the PCBs to distribute over the body. Next, the animals were transported to Leiden University and, immediately upon arrival in Leiden, anaesthetised with 100 mg/l benzocaine, weighed and body length measured, given a tag (Trovan ID 100 implantable transponder microchip in a bio-compatible glass capsule in the dorsal muscle 10 cm behind the head of every eel) and treated with Flumequine (50 mg/l) for 3 h before transfer to the experimental units, to avoid any bacterial source of skin infections as occurred in two earlier experiments with more than 2 weeks swimming (unpublished results). The animals were divided into four experimental groups of 11 animals each, PCB-exposed or control animals were individually assigned to one of the 22 swim tunnels of 127 or 22 50 l flow-through containers (PCB-swim or Control-swim). Eels in swim tunnels were stimulated to swim for 750–800 km over a period of approximately 27 days, or kept resting in one of 22 50 l flow-through boxes for the same period (PCB-rest or Control-rest). Tunnels and containers were all

connected to the same water recirculation system of 6000 l of artificial seawater (Instant Ocean, Smulders B.V., Maastricht). The NH_3 and NO_2^- value of the water was checked daily. At values above 0.1-ppm NH_3 and/or 0.1-ppm NO_2^- the water was refreshed from a separate 3000-l stock tank. The temperature of the water was 19.0 ± 0.3 °C. Experiments were performed in the dark (containers) or infra-red light 670 nm light (bandwidth 20 nm) (tunnels) which is invisible for eel (Pankhurst and Lythgoe, 1983).

2.2. Blazka swim tunnel

The Blazka swim tunnel has a length of 200 cm, a diameter of the outer swim tunnel tube of 28.8 cm and a diameter of the inner swim tunnel tube of 19.0 cm. The volume was 127.14 ± 0.90 l ($n = 5$). Flow patterns were measured with a Laser Doppler technique at the Delft Hydraulics Laboratory, Technical University Delft. The experimental set-up is described elsewhere (Van den Thillart et al., 2004) with the exception that salt water was used. The water flow in the swim tunnels was set for each animal at 0.5 body length per second (ca. 0.4 m/s).

2.3. Measuring of oxygen consumption and standard metabolic rate

In each tunnel the oxygen level was monitored continuously with an oxygen electrode (Mettler, Toledo). The oxygen consumption rate was calculated from the oxygen decline after automatic closure of the water-inlet by a magnetic valve. The oxygen levels varied between 85% and 75% air saturation. The valve was normally open allowing a refreshment rate of 5–7 l min^{-1} and automatically closed between 14.00 and 17.00 h to measure oxygen consumption. The oxygen value was not allowed to fall below 75%, at that value the valve would automatically reopen to avoid hypoxia (Van den Thillart and van Waarde, 1985). From the decrease in O_2 -concentration, the rate of oxygen consumption ($\dot{M}\text{O}_2$) was calculated following the formula:

$$\dot{M}\text{O}_2 = 127 \cdot \Delta[\text{O}_2] \cdot \Delta t^{-1} (\text{mg O}_2 \cdot \text{h}^{-1} \cdot \text{kg}^{-1}),$$

where $\Delta[\text{O}_2] \cdot \Delta t^{-1}$ is the decrease of the oxygen content per hour (Van den Thillart et al., 2004). Oxygen consumption data were normalised for body mass assuming a linear decline in mass of the animals between start and end of the experiment.

At the end of the experiment, after swimming 800 km, PCB-swim and Control-swim animals were allowed to rest while their oxygen consumption was measured for a period of 4 h to determine the standard metabolic rate (SMR) (Fry, 1971). Hereafter the animals were anaesthetised and blood was collected (see below). Eels were weighed and their length and horizontal eye diameter was measured. Next the animals were sacrificed by spinal transection. Tissues (liver, spleen, gonads, and muscle) were collected, weighed and snap-frozen in liquid nitrogen. Subsequently, the PCB-rest and Control-rest animals were placed in the tunnels and after 2 days of acclimation to the experimental set-up, their oxygen consumption in rest was determined for a period of 4 h. Then these eels were sacrificed and sampled as described for the swimming group.

2.4. Analysis of blood parameters

Blood was collected by puncture of the caudal vessels with a heparinised (Leo Pharmaceuticals products, Ltd.) tuberculin syringe fitted with a 25 Gauge needle.

Haemoglobin content in 20 μ l blood was detected after 3 min using the cyan-methaemoglobin method (Boehringer Mannheim, F.R.G.). Plasma was obtained by centrifugation in an Eppendorf centrifuge for 5 min at $13,000 \times g$; the supernatant plasma was divided

over several cups as required for further analyses and snap-frozen in liquid nitrogen. Hematocrit value was measured directly in 9 μ l whole blood sample using a hematocrit micro-centrifuge (Bayer, FRG). Plasma potassium concentration was determined by flame photometry (Radiometer Copenhagen FLM3). Plasma was further analysed with a Stat Profile pHox Plus analyser with automated two-point calibration; this analyser provides electrode readings for Na^+ and Ca^{2+} concentration, and pH, and is equipped with enzymatic electrodes (Nova Biomedical, Waltham, MA, USA) for measurement of glucose and lactate concentrations. Plasma cortisol level was determined by radioimmunoassay as described by Arends et al. (1998).

2.5. Bio-analysis of the internal dose in a reporter gene assay

The internal dosage of the given PCBs was determined in the extractable lipids from muscle and blood plasma by applying an *in vitro* reporter gene assay for dioxin-like toxic potency according to the method developed by Murk et al. (1998). This assay is also known as the DR-CALUX (Dioxin Receptor-mediated Chemical Activated Luciferase eXpression) assay and is based on activation of the Aryl hydrocarbon (Ah)-receptor by dioxin-like chemicals in H4IIE rat hepatoma cells, which results in transcription and translation of the newly introduced gene for luciferase activity. Of the three PCB-congeners dosed in this experiment the DR-H4IIE.Luc mainly quantifies PCB-126 (which is not metabolized by the eel) and the small amount of PCB-77 that could be left after 6 weeks. PCB-153 is not active in the DR-CALUX.

Muscle samples for bio-analysis of the TEQ-values were homogenized by scraping and minced with a sharp razor. To a portion of 1 g of this thick muscle homogenate 2 ml water was added and for 1 min further homogenized with a high speed emulsifier (Ultra Thurrax T25, Janke & Kunkel, Germany) after which 2 ml of isopropyl alcohol was added. This mixture was sonicated for 10 min and a liquid/liquid extraction was performed with 3 ml of hexane/di-ethylether (97:3, v/v). To approximately 1 g plasma samples 1 ml nanopure water was added and mixed, after which 2 ml of isopropyl alcohol was added. These mixtures were sonicated for 10 min and a liquid/liquid extraction was performed with 3 ml of hexane/di-ethylether (97:3, v/v).

The extractions were repeated twice after the addition of two drops of concentrated hydrochloric acid to enhance the extraction. The organic layers were pooled and evaporated under a gentle stream of N_2 at 30 °C. The amount of fat extracted was determined gravimetrically. The clean up of the extracts was performed using a multi-layer sulphuric acid silica column consisting of 1 g Na_2SO_4 on top of 2 g of dried silica with 10% hexane pre-washed H_2SO_4 , 4 g of dried silica with 20% H_2SO_4 and 4 g of dried silica with 33% H_2SO_4 . The column was eluted with 40 ml of hexane:diethyl ether (97:3, v/v) as described for sediment samples by Murk et al. (1998). The eluate was dried with N_2 at 30 °C and 15 μ l of DMSO was added just before complete evaporation of the organic solvent.

Exposure of the DR-H4IIE.Luc cells was performed in white 96-well view plates (Packard) according to the method described before (Murk et al., 1998). Cells were exposed in triplicate to eel muscle extracts in DMSO (0.4% maximum). Luciferase activity was quantified in a luminometer after addition of the substrate luciferin. For the quantification of induced responses on each microtiter plate, a concentration series of the reference compound 2,3,7,8-TCDD was included and 1-site-ligand curve fitted was performed using Slidewrite 6.0. The limit of quantification (LOQ) was set at the DMSO-response plus three times the standard deviation. In these cases 50% of the LOQ was used. After correction for the background signal of the DMSO solvent control, luciferase activities of sample dilutions interpolated on this curve and expressed as TCDD-

Equivalents (TEQ)-values per gram lipid when the response was between 1 and 10 pM TCDD.

2.6. Statistics and calculations

Mean values \pm standard deviation (S.D.) are presented of 6–7 animals that survived the experimental period and were able to swim continuously (for swimmers), unless otherwise indicated. The following indices were calculated according to the formulae below: Eye Index (EI); indicating the degree of maturation (Pankhurst, 1982), GonadoSomatic Index (GSI), HepatoSomatic Index (HSI), Spleen Somatic Index (SSI).

1. Eye Index (EI) = $100 \times (((\text{EDh} + \text{EDv}) \times 0.25)^2 \pi \times (10 \times \text{BL})^{-1})$; EDh: eye diameter horizontal (mm), EDv: eye diameter vertical (mm) (Pankhurst, 1982; in our study we calculated $2 \times \text{EDh}$ instead of $\text{EDh} + \text{EDv}$).
2. GonadoSomatic Index (GSI) = $(\text{GW} \text{BW}^{-1}) \times 100\%$.
3. HepatoSomatic Index (HSI) = $(\text{LW} \text{BW}^{-1}) \times 100\%$; LW: liver weight (g).
4. Spleen Somatic Index (SSI) = $(\text{SW} \text{BW}^{-1}) \times 100\%$; SW: spleen weight (g).

Statistical analysis of the results was performed with SPSS 10.0 software. A one-way ANOVA was performed and mean squares of the ANOVA were compared using *F*-tests. $P \leq 0.05$ was taken as fiducial limit. Normality of data distribution was assessed and homogeneity of variances was checked by Kolmogorov–Smirnov and F_{max} tests, respectively. Because the cortisol values were not normally distributed and showed a large variance, these data were compared by non-parametric analysis (Mann–Whitney *U*-test). Differences between experimental groups in liver, gonad and spleen weight were tested by covariance analysis (ANCOVA) with body weight as co-factor. Deduced index parameters were compared non-parametrically like cortisol values.

3. Results

3.1. General condition and performance

We included only (female) silver eels weighing between 900 and 1100 g in our studies. The resting animals survived the whole experimental period without any signs of adverse effects. Several swimming fish however, unexpectedly developed non-PCB-related skin problems after about 2 weeks such as a bloody inflammatory red lateral line and four PCB-swim and five Control-swim eels died during the 27 days of experimentation, in a not PCB-related manner. Initially, the swimming groups were supplemented with formerly resting animals until 6–7 animals remained per group. The recorded swimming distances at 27 days, when the experiment was terminated, were not significantly different for the PCB-swim (767 ± 112 km, $n=7$) and the Control-swim (781 ± 124 km, $n=6$) animals (Table 1). The swimming animals lost 74% ($P=0.03$) and 78% ($P=0.12$) more weight compared to the resting groups for the PCB-exposed and C-animals, respectively (Table 1). The PCB-exposed animals only lost 70% (in rest) and 72% (during swimming) of the weight of the Control animals. As can be seen from Table 1 the animals also lost weight in the 4 weeks period during which they were not fed any more and had to adapt to salt water. The eye indices confirmed that the eels were silver after the adaptation period (Table 1).

3.2. Internal PCB–TEQ-levels

PCB-levels in muscle from the PCB-exposed animals were 12–62 times higher compared to their respective swimming and rest-

Table 1
Averages (\pm S.D.) for several parameters for the four experimental groups: PCB-exposed or Control eel that either have been resting or swimming during 27 days. The standard metabolic rate (SMR) is determined during 4 h, 2 days after 27 days swimming or resting.

	PCB-exposed		Control	
	Rest	Swim	Rest	Swim
N	6 ^a	7	7	6
Swim distance (km)	56 \pm 68 ^a	767 \pm 112	0	781 \pm 124
Total eel length (cm)	74.5 \pm 2.1	77.4 \pm 2.5	74.8 \pm 3.7	73.9 \pm 4.1
Eye Index end swim period	10.73 \pm 1.73	10.57 \pm 2.20	10.03 \pm 0.90	10.26 \pm 3.00
Bodyweight before exposure (g)	941 \pm 46	1068 \pm 136	991 \pm 60	1036 \pm 150
Bodyweight start swim period (g)	893 \pm 88	998 \pm 90	895 \pm 65	936 \pm 127
Bodyweight end swim period (g)	857 \pm 99	936 \pm 99	845 \pm 87	847 \pm 103
Weight loss (g) over 27 days	36.1 \pm 10.4	62.8 \pm 30.9 [*]	50.1 \pm 28.2	89.2 \pm 58.4
PCB in muscle (pmol EQ/g fat)	138 \pm 150 [*]	381 \pm 44 ^{***} &	2.2 \pm 3.7 ^{b,***} &	31 \pm 29 [*]
PCB in plasma (pmol TEQ/g fat)	1570 \pm 1367 [*] &	1526 \pm 913 [*] &	63 \pm 103 ^c	64 \pm 75
% fat muscle	25 \pm 6.1	27 \pm 3.5	34 \pm 9.6	27 \pm 7.7
% fat plasma	2.4 \pm 0.9	1.3 \pm 0.7	3.4 \pm 2.0	1.5 \pm 1.1
SMR (mg kg ⁻¹ h ⁻¹) (at the end)	43.4 \pm 6.4 [*] &	54.2 \pm 12.4	58.2 \pm 16.7	58.4 \pm 12.4
Liver weight (g)	7.15 \pm 1.21	11.38 \pm 4.10 [*]	8.45 \pm 2.54	9.60 \pm 2.34
HepatoSomatic Index (HSI)	0.80 \pm 0.09	1.19 \pm 0.39 [*]	0.93 \pm 0.32	1.09 \pm 0.14
Ovary weight (g)	11.45 \pm 1.69	11.08 \pm 2.09	11.45 \pm 1.54	7.72 \pm 1.23 ^{**} &
GonadoSomatic Index (GSI)	1.29 \pm 0.17	1.13 \pm 0.15	1.25 \pm 0.16 ^{***}	0.89 \pm 0.18 ^{**} &
Spleen Somatic Index (SSI)	0.056 \pm 0.0093 [*]	0.129 \pm 0.063 [*] &	0.07 \pm 0.023	0.056 \pm 0.019
pH-plasma	7.65 \pm 0.07	7.68 \pm 0.07	7.65 \pm 0.05	7.64 \pm 0.06
Sodium (meq/l)	163.3 \pm 1.9	161.8 \pm 5.5	176.5 \pm 22.0	173.3 \pm 14.5
Potassium (meq/l)	2.91 \pm 0.49	2.62 \pm 0.37	2.57 \pm 0.76	2.97 \pm 0.89
Calcium (mmol/l)	1.43 \pm 0.08	1.66 \pm 0.24	1.63 \pm 0.31	1.54 \pm 0.54
Cortisol (ng/ml)	11.20 \pm 9.14	7.81 \pm 5.99	26.1 \pm 28.5	25.9 \pm 21.9
Glucose (mM)	5.44 \pm 2.01	5.38 \pm 1.03	7.13 \pm 2.75	8.44 \pm 5.52
Lactic acid (mM)	1.18 \pm 0.53	1.13 \pm 0.58	1.02 \pm 0.35	1.95 \pm 1.34
Haemoglobin (mM)	0.33 \pm 0.10	0.35 \pm 0.07	0.35 \pm 0.03	0.37 \pm 0.09
Hematocrit (%)	33.0 \pm 3.74	37.6 \pm 4.96	37.7 \pm 3.51	33.8 \pm 4.27

^a Three animals were placed in the resting group after they stopped swimming.

^b 6 of the 7 levels were below limit of quantification.

^c 4 of the 7 levels were below limit of quantification.

^{*} Significant difference between rest and swim group at $P \leq 0.05$ and in combination with the & added between PCB and control group (either swimming or resting).

^{**} Significant difference between rest and swim group at $P \leq 0.01$ and in combination with the & added between PCB and control group (either swimming or resting).

^{***} Significant difference between rest and swim group at $P \leq 0.001$ and in combination with the & added between PCB and control group (either swimming or resting).

ing C-animals (Table 1). This difference was highly significant ($P \leq 0.0001$). Levels in the PCB-swim animals were 2.8 times higher compared to the PCB-rest group (Table 1). This difference was highly significant ($P = 0.0017$). This difference was even greater (14 \times) in the unexposed animals, where 6 out of 7 TEQ-levels were below the limit of quantification (LOQ). Plasma TEQ-levels of the PCB-exposed eels were 24 times higher than the background levels in the plasma of the C-eel. In contrast to levels in muscle no significant differences were observed in plasma levels between swimming and non-swimming animals (Table 1).

3.3. Organs and blood parameters

The liver weight was higher in the PCB-swim compared to the PCB-rest group ($P \leq 0.05$). This effect was reflected in the HSI. Covariance analysis indicated there was a swimming effect which was significant between the PCB-groups. Also in the Control-swim group the mean liver weight tended to be higher than in the Control-rest group, but this difference was just not significant.

The C-swim group had lowest ovary weights of all groups (Table 1), and ovary weights of both swimming groups were lower than that of their respective resting groups. These effects were reflected in the GSIs.

The Spleen Somatic Index (SSI) was 2 \times higher in the PCB-swim group in comparison to all other experimental groups, and these differences were all significant ($P \leq 0.05$) (Table 1). Covariance analysis indicated this was a significant effect of PCB-exposure in swimmers ($P \leq 0.01$). There was no significant difference in SSI between the PCB-rest and Control-rest groups.

None of the measured blood parameters differed significantly among groups, although a tendency for lower glucose levels in PCB-exposed animals was seen (64% and 76% compared to, respectively, Control-swim and Control-rest groups; Table 1). Average cortisol levels in the PCB-swim and the PCB-rest groups were 30% and 43% higher compared to their control groups (Table 1), but due to a large variation this difference was not statistically significant (swim: $P = 0.083$ and rest: $P = 0.33$). Plasma pH, ion levels (sodium and potassium), plasma lactate acid, haemoglobin and hematocrit did not show any difference that could be related to PCB-exposure.

3.4. Oxygen consumption

Oxygen consumption in the PCB-swim group was significantly lower than in the Control-swim group ($P < 0.05$) as of 18 days of swimming, i.e. after about 400 km of swimming. Subsequently the difference further increased (Fig. 1 and Table 2). The SMR value of the PCB-rest group was 25% lower compared to the C-groups, a significant difference (Table 1). The SMR values of the PCB-rest group were not different from those of the PCB-swim group. The SMR of the PCB-rest group not statistically significantly ($P = 0.07$) compared to the PCB-swim group (Table 1). The SMR of the Control-swim and Control-rest groups did not differ.

The cost of transport (COT, Table 3), i.e. the total amount of oxygen consumed in 1 kg fish to swim 1 km, is already significantly lower ($P \leq 0.05$) in the PCB group after 100 km and continues to decrease up to 800 km ($P \leq 0.0001$).

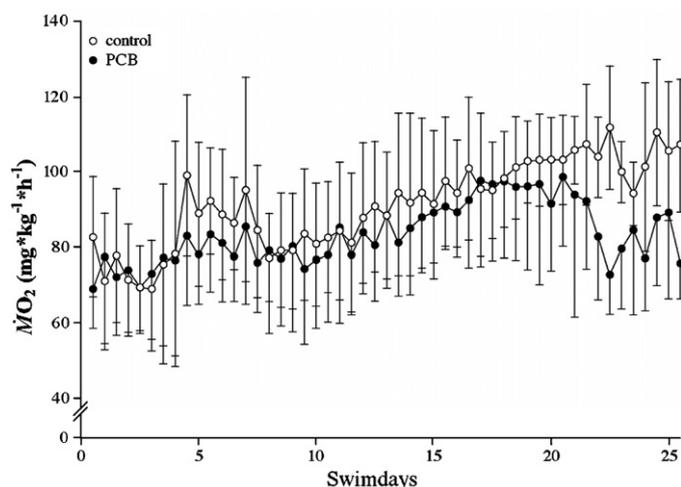


Fig. 1. Average oxygen consumption (\pm S.D.) profiles of control and PCB-exposed eels swimming at 0.5 BL/s for 27 days (800 km). The oxygen consumption of the PCB-exposed eels is significantly depressed compared to the controls from day 18 onwards. The PCB-group was dosed intraperitoneally with the environmentally relevant mix of 5 mg PCB-153/kg eel, 7 μ g PCB-126/kg and 50 μ g PCB-77/kg eel PCB-77.

Table 2

Mean oxygen consumption in ($\text{mg kg}^{-1} \text{ h}^{-1}$) in the Control-swim and PCB-swim groups related to distance. The oxygen consumption is significantly less in the PCB-swim group than the Control-swim group from 400 km onwards, and this effect increases towards 800 km.

km	Control (mean \pm S.D.) ($\text{mg kg}^{-1} \text{ h}^{-1}$)	PCB (mean \pm S.D.) ($\text{mg kg}^{-1} \text{ h}^{-1}$)	P-value
100	73.8 \pm 15.9	71.2 \pm 15.2	0.42
200	79.5 \pm 19.9	75.6 \pm 17.6	0.17
300	80.8 \pm 19.4	77.0 \pm 17.5	0.09
400	81.4 \pm 18.7	77.5 \pm 17.7	0.04*
500	83.6 \pm 19.2	79.2 \pm 17.3	0.009**
600	85.9 \pm 19.3	81.7 \pm 18.2	0.009**
700	87.9 \pm 19.1	83.3 \pm 19.1	0.003**
800	88.9 \pm 19.2	83.3 \pm 18.9	0.0001***

* Significant difference with $P \leq 0.05$.

** Significant difference with $P \leq 0.01$.

*** Significant difference with $P \leq 0.001$.

4. Discussion

4.1. General condition

All animals from the resting groups survived in good health. Most animals swam during the 27-day experimental period, but several of the swimming eels exhibited PCB-unrelated skin problems, some fishes even died. This health problem occurred in two earlier experiments, but only with eel swimming in seawater

(unpublished results) and was not prevented by antibiotic (flumequine) pre-treatment and application of UV-irradiation to the water. No infectious diseases were found by fish specialists from the Practical Research Institute for Animal Husbandry (CDI) in Lelystad, The Netherlands. Considering the fact that the resting groups did not show the same problem, it is likely that the combination of seawater and forced swimming has induced this disease. A similar phenomenon was observed in 2000 with swimming silver eels in contrast to resting silver eels. In that experiment the swimming eels (also in seawater) developed a disease that was caused by the EVEX virus (Van Ginneken et al., 2005b). The resting eels carried the same virus, but did not develop the disease. In another study with eels performing several swimming tests during 1 week, it was shown that eels consume 20% more energy for swimming in seawater than in fresh water, which relates to the higher osmotic gradient between blood and seawater (Palstra et al., 2008). In this study the experiments were terminated when still 6–7 eels were present in each swimming group, allowing studying the effects of PCBs on energy consumption.

4.2. Internal PCB-levels

The nominal PCB-dose given was 10 times the consumption standard, but within environmentally relevant concentrations (De Boer and Hagel, 1994). The background TEQ-concentration in the muscle of the Control-rest animals was 2.2 pmol TEQ/g fat. After swimming 800 km this level rose 14 times to 31 pmol TEQ/g lipid in the Control-swim group. The same effect occurred in the muscle tissue of the PCB-exposed animals, where the absolute increase is higher (from 138 to 381 pmol TEQ/g lipid), but the relative increase comparable (2.8 times, Table 1). Due to the exercise, fat is mobilised and consumed as can be deduced from the greater weight loss in the swimming groups compared to their respective resting groups. This is corroborated by the observation that the total fat content of the plasma in the swimming groups was much lower than in the resting groups, which also indicate high lipid oxidation rates. As the dioxin-like PCB-126 is hardly metabolised, the TEQ-levels in the muscle tissue increase (Table 1). The higher concentration of PCBs suggests further concentration of PCBs when fat is being used for metabolic activity in the fat cells in the muscle tissue. The PCB-levels in the plasma did not differ between swimming and resting animals in either of the groups, but the PCB-levels in the plasma of the PCB-exposed animals was much higher than in the muscle on a lipid basis. This may be due to the difference in mode of mobilisation between fatty acids (regulated by hormone sensitive lipases, energy driven) and the passive process of release of PCBs. Also the difference in fat-composition between plasma lipids and muscle lipids may influence the relative PCB-concentrations. The percentage total fat was about two times higher in plasma from resting fish compared to the swimming fish. In an earlier experiment we observed

Table 3

Cost of transport (COT) in [$\text{mg O}_2 \text{ kg}^{-1} \text{ eel km}^{-1}$] in the Control-swim group and PCB-swim group related to distance. The COT already is significantly reduced from 100 km onwards.

km	COT of a control group (mean \pm S.D.) ($\text{mg O}_2 \text{ kg}^{-1} \text{ eel km}^{-1}$)	COT of a PCB group (mean \pm S.D.) ($\text{mg O}_2 \text{ kg}^{-1} \text{ eel km}^{-1}$)	P-value
100	56.9 \pm 11.3	51.9 \pm 10.7	0.03*
200	61.3 \pm 14.6	55.1 \pm 12.5	0.002***
300	62.3 \pm 14.4	56.0 \pm 12.3	0.0001***
400	62.8 \pm 13.6	56.4 \pm 12.5	0.0001***
500	64.6 \pm 14.3	57.7 \pm 12.4	0.0001***
600	66.4 \pm 14.5	59.6 \pm 13.3	0.0001***
700	68.1 \pm 14.5	60.7 \pm 14.2	0.0001***
800	69.0 \pm 14.7	60.7 \pm 14.0	0.0001***

* Significant difference at $P \leq 0.05$.

*** Significant difference at $P \leq 0.001$.

strongly reduced triglyceride levels in swimming eel (unpublished results).

4.3. Effects on organ weights

The absolute and relative liver weights (HSI) are 49% higher in the PCB-swim group compared to the PCB-rest group. Liver enlargement is a well-known effect for animals exposed to dioxin-like PCBs, which is in accordance with the observed highest hepatic PCB-level in the PCB-swim group in which the PCBs have become more concentrated (Table 1).

The GSI was higher in the resting compared to the swimming groups, and higher in the PCB-swim group in comparison with the Control-swim group. ANCOVA showed that this PCB-effect was significant, but as the GSI of the Control-swim group was lower than that in all other groups it cannot be excluded that this is a coincidence. There was no difference in GSI between PCB-rest and Control-rest groups (Table 1).

The Spleen Somatic Index (SSI) significantly increased in the PCB-swim eels. This could indicate immune activation, although it cannot be excluded that the erythrocyte concentration could also influence the SSI. As the Control-swim group did not have an increased SSI, this induced extra spleen activity could perhaps be related to the combination of swimming in seawater plus PCB-exposure. Dioxin-like compounds like PCB-126 have been related directly to immuno-suppression and reduced resistance to diseases, viruses and parasites. In field studies, increased disease incidence in benthic fish from various coastal areas has been related to PCB-contamination (Vethaak and Reinhalt, 1992). Several field studies reported evidence of disrupted immune function in fish sampled from inshore areas known to be contaminated with PCBs and other xenobiotics (Warriner et al., 1988; Weeks et al., 1990; Arkoosh et al., 1991, 1994). In fish the most important organs in the immune function are the head kidney, thymus and spleen. In European flounder (*Platichthys flesus*) exposed to 50 mg PCB-126/kg (Grinwis et al., 2001) thymus atrophy was reported suggesting an impact on the specific resistance against infectious diseases (viral, bacterial, and parasitical) in the field situation. The reported effects of PCBs on the immunologically important spleen (Taysse et al., 1998) can be quite different, as in some studies a reduction of the spleen weight is observed (Nakata et al., 2002) while in other studies a spleen hypertrophy was reported (Greichus et al., 1975). Such differences can be related to the exposure period and concentration of the xenobiotic and require further study. Although the spleen mainly produces leucocytes (Iwama et al., 1996) it has been shown before for carp that this organ (as well as the immunologically important head-kidney) contributes to biotransformation of xenobiotics (Taysse et al., 1998). Therefore it cannot be excluded that the hypertrophy of the spleen observed in our study is due to its increased biotransformation activity in the PCB-swim group which also has the highest internal PCB-levels.

4.4. Energy use

In our experiment swimming animals lost about 75% more body weight during the 27-day experimental period than their respective resting controls. This could be predicted, as energy consumption during swimming is higher than during resting. Recently we could show that during a 5500-km simulated migration of yellow eel in swim tunnels (over 173 days in fresh water) the metabolic rate of swimming animals (at 0.5 BL/s) was two times higher compared to resting control animals (Van Ginneken et al., 2005a). This is still low because the active metabolic rate in fish species can be 10–20 times the standard metabolic rate (Fry, 1971). The initial oxygen consumption levels in this study were similar to values measured in previous studies. Swimming eels from the control group, for

instance, consumed 74 ± 16 mg O₂/(kg h) at a swimming speed of 0.5 BL/s, corresponding to approximately 0.4 m/s (Table 2). During the swimming period the oxygen consumption per kilogram body weight increased. This could be due to the decreased body weight in this period. Eels from the same farm and similar size, swimming at a speed of 0.5 m/s in seawater in the same swim tunnel set-up consumed in a study by Palstra et al. (2008) 89 ± 15 mg O₂/(kg h). Consequently, the costs of transport (COT) values were slightly higher for eels in this study, respectively 57 ± 11 mg O₂/(kg km) (Table 3) vs. 49 ± 8 mg O₂/(kg km) in the study of Palstra et al. (2008). Recently we found that optimal swimming speeds are 0.61–0.68 m/s (0.74–1.02 BL/s), much higher than the swimming speed applied in this study. This indicates that the eels were not stressed by a too high swimming speed.

The loss in body weight in the PCB-dosed animals was 29% lower than in the respective controls (Table 1). This is surprising because a well-known effect of dioxin-like PCBs in many species is the 'wasting syndrome' which results in enhanced weight loss in spite of a normal appetite. This 'wasting syndrome' has also been reported in fish (Kleeman et al., 1988). As our eels were not fed during the experiments, the significantly reduced weight loss in the PCB-exposed groups does not relate to altered food intake. We speculate therefore that the PCB-treatment affected intermediary metabolism and metabolic processes and made the animals somehow economize on energy expenditure.

Thyroid hormone is very important for both energy balance and seawater migration in eels (Leloup and de Luze, 1985) and PCBs and their metabolites have been shown to interfere via multiple pathways with the thyroid hormone system. Eels have been shown to metabolize PCBs in the liver (De Boer and Hagel, 1994). PCB 77 was shown to be converted to hydroxylated metabolites (Murk et al., 1994) that can disrupt thyroid hormone transport actions and metabolism (Brouwer et al., 1998; Marchesini et al., 2008) and with thyroid hormone receptor function (Schriks et al., 2006; Freitas et al., submitted) part of the PCB-induced changes in energy metabolism could be related to their thyroid hormone disruption.

The low glucose and cortisol levels in the PCB-exposed animals, point to a lowered catabolism of structural protein, a diminished conversion of amino acids in gluconeogenesis and an altered carbohydrate metabolism. The reduced energy consumption and cost of transport in the PCB-swim group (Fig. 1, Tables 2 and 3), the lower SMR in the PCB-rest group and the reduced weight loss in the PCB-exposed groups compared to the control groups, could therefore perhaps be explained by a reduced protein synthesis.

4.5. Possible consequences of PCB-contamination for eel reproduction

This, and earlier studies show that eel migration and reproduction could be seriously affected by currently occurring PCB-levels. During the 6000 km migration to the Sargasso Sea much fat will be used for energy consumption causing the PCB-levels to increase even further in the body fat of the eel. This could mean further interference with the energy metabolism of the eel and possibly with the thyroid status reducing the chance of the animals to reach their spawning grounds.

When eels reach their spawning grounds, elevated PCB-levels may also interfere with steroid hormone function and therefore with reproduction and hatching success of the larvae. It has been shown that PCBs and other persistent organic pollutants can be passed on from the mother to their eggs and impair larval survival and development (Gutleb et al., 1999, 2007). In sole (*Solea solea*), the LD₅₀ for larval survival is 1 ng TEQ/g lipid (Foekema et al., 2008), equivalent to 3 pmol TEQ/g lipid. PCB-effects on impaired larval development are not only mediated by thyroid hormone dis-

ruption, but also via disruption of retinoid homeostasis (Brouwer, 1991; Zile, 1992). In an earlier study we found a 30-fold decrease of retinylpalmitate in eel liver with TEQ-levels increasing from 2 to 20 pg TEQ/g fat. So females with higher internal PCB-levels will not only transfer the PCBs to their eggs, but will also have a reduced amount of retinoids, and possibly thyroid hormones, to pass on to the eggs, which is required for the early development of the larvae. These effects are in accordance with the inverse relationship found between the TEQ-level in both muscle and gonads of female eel and the survival period of eel embryos (Palstra et al., 2006). This further adds to the suggestion that the current levels of PCBs and other dioxin-like compounds in eels may seriously impair the survival and reproduction of the European eel. It is to be expected that the toxicological risk from other persistent organic pollutants found in eel, such as brominated flame retardants and perfluorinated compounds, will further add to the multi-causal problem of world-wide decline of eel populations.

Acknowledgments

The eel migration project at the University Leiden is supported by a grant of the Technology Foundation (STW), which is subsidized by the Netherlands Organization for Scientific Research (NWO), STW-project no. LBI66.4199 and was supported also by the EU EEL-REP project no. Q5RS-2001-01836, and by EURO CHLOR.

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