DISPOSITION OF PCB DURING THE WINTER EMACIATION

OF THE ANADROMOUS ARCTIC CHAR

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Introduction

The anadromous Arctic char *Salvelinus alpinus* (L.) undertake annual migrations to the sea in early summer, and usually reside in the sea for 40-50 days before returning to fresh water (Finstad and Heggberget, 1993). During the short seawater residence, individuals may double in body weight (Mathisen and Berg, 1968) and increase body lipid stores several fold (Dutil, 1986; Jørgensen et al., 1997a). In winter the food intake is minimal and stored lipids are mobilised to meet metabolic demands. When new excursions to the sea are to be undertaken, the lipid stores may be depleted (Boivin and Power, 1990; Jørgensen et al., 1997a; Jobling et al., 1998). Such seasonal variations in body lipid stores are characteristic for many high-latitude inhabitants.

Aquatic pollution is an increasing problem, and even in remote Arctic areas high levels of several pollutants have been detected (e.g. Hargrave et al., 1992; Muir et al., 1992). Persistent organic pollutants (POPs) enter the fish primarily via the

food (Sijm et al., 1992). In high-latitude environments, fish are consequently contaminated during the short season in which they eat. Deposition of POPs within the animal appears to be positively correlated with the localisation of non-polar lipids like triacylglycerols (TAG) (Kawai et al., 1988). Hence, in well fed, "fat" fish, a high proportion of the total POP burden is found in tissues containing the majority of the body lipids; i.e. in the muscle, skin and skeleton in the Arctic char (Jørgensen et al., 1997a). During periods of lipid mobilisation, there may be a redistribution of deposited POPs from adipose tissues toward vital organs like the liver, kidney and brain (Boon and Duinker, 1985; Jørgensen et al., 1997a). Periods of POP redistribution would therefore carry a risk of increased pollutant-associated stress.

In order to study these processes, a semi-field model has been developed. In this report we present the results from a pilot study which was conducted in order to reveal the validity of such an experimental approach.

Materials and Methods

The experiment was performed with a 21 immature, anadromous Arctic char (*Salvelinus alpinus*) from Halsvassdraget, located in Finnmark, northern Norway (70°N 23°E). A permanent fish trap located near the river mouth, catches all migrating fish. On the return to freshwater in late July fish were collected in the trap and transferred to a 3 m^3, circular indoor tank supplied with unheated river water.

On August 15, 1996, length and weight of the fish were measured. Two weeks later all fish received an oral dosage of PCB (1.5 µg/gram fish weight) by force feeding of a gelatine capsule containing PCB solved in fish oil. The PCB administered constituted equal amounts of 4 congeners (IUPAC no. 101, 105, 153 and 180), purchased from Promochem AB (Ulricehamn, Sweden).

Until September 15, the fish were held in continuous light, after which the photoperiod was kept at 10 h light, 14 h dark until March 21, and thereafter increased to continuous light. This photoregime was used at the research station in order to give a "normal" parr-smolt development. In order to mimic the non-feeding overwintering in wild, anadromous char, the fish were not fed during this period.

On September 17, February 19 and May 28 seven fish were sacrificed at each date. The fish were weighed and dissected into the following parts: liver, kidney, brain and the remaining carcass (including muscle, gut, skin, head, skeleton and fins). All organs, except the brain, were divided into two parts, one for lipid analysis and one for PCB analysis. Due to the small size, the brain was only analysed for PCB.

Total lipids were analysed from homogenised samples by a mixture of methanol and chloroform, essentially as described by Bligh and Dyer (1959). The proportional amount of triacylglycerols (TAG) was quantified by HPTLC (high-performance thin-layer chromatography), using SiO_2 60 pre-coated plates (Merck, Germany). The developing solvent system consisted of heptane : diethyl ether : acetic acid (80:20:1). After charring with copper acetat the separated lipid classes were quantified by a Camag TLC scanner 3 (Muttenz, Switzerland) at the wavelength 350 nm.

For the PCB analyses, samples were homogenized with acetone and thereafter hexane : acetone (3:1). After clean-up PCB was determined by gas chromatography, using a HPGC 5890 Series II equipped with a splitless injector, electron capture detector (ECD) and a HP-5 capillary column, (50 m x 0.20 μm x 0.11 μm). Nitrogen was used as carrier gas (42 ml/min). The injector and detector temperatures were 280ºC and 320ºC. The temperature program was: 60(1)-15-160(0)-1.5-270(20). Calculations were done on the basis of external (PCB 53) and internal standards with which the chromatograph had been calibrated.

One-way ANOVA (Statistica 5.1, StatSoft Inc., USA) was used to test differences between tissues/organs and sampling dates with regard to PCB concentrations and congener composition. A probability level < 0.05 was considered significant.

Results

Table 1 and 2 shows the weight of the carcass, liver and kidney, and the percentage of total lipids and TAG in these tissues and organs at the different sampling dates.

Table 1 Tissue and organ weights (g) ± s.e.m. (standard error of a mean) in immature anadromous Arctic char sacrificed at different dates throughout the winter.

		Carcass		*Liver*		*Kidney*	
Month	n	Weight	*s.e.m.*	Weight	*s.e.m.*	Weight	*s.e.m.*
September	7	355	*19*	3.9	*0.20*	3.3	*0.29*
February	7	309	*11*	3.3	*0.07*	2.9	*0.13*
May	7	260	*15*	3.1	*0.16*	2.9	*0.22*

All organs and tissues decreased in weight throughout the winter. On the average, the carcass lost 27 % of its weight from September to May, whereas the weight loss of the liver and kidney were slightly less (20 and 12 %, respectively).

Table 2 The percentage of total lipid and TAG (triacylglycerol) in different tissues and organs in anadromous char sacrificed at different dates throughout the winter. Values shown are means (n=7) ± s.e.m. (standard error of a mean).

		Carcass		*Liver*		*Kidney*	
Month		%	*s.e.m.*	%	*s.e.m.*	%	*s.e.m.*
September	Tot.lip	3.4	*0.6*	13.9	*2.4*	3.6	*0.3*
	TAG	2.4	*0.6*	8.1	*1.6*	1.9	*0.3*
February	Tot.lip	1.8	*0.13*	4.1	*0.4*	2.4	*0.07*
	TAG	1.1	*0.11*	1.1	*0.3*	0.6	*0.12*
May	Tot.lip	1.2	*0.08*	2.9	*0.16*	2.0	*0.10*
	TAG	0.5	*0.07*	0.1	*0.03*	0.1	*0.03*

The lipid content decreased in all tissues and organs during the winter. The greatest reduction was seen in the liver, where the amount of lipid on average decreased 83% from September to May. In the carcass, 74% of all the lipids were utilised between September and May. Quantitatively, most lipids were lost form carcass (muscle, skin, bone, fins, and head) during the winter due to the high proportional weight of these tissues (98% of total fish weight). In September more than 50% of the total lipids in all tissues and organs was TAG.

In May, the proportion of TAG had decreased to 4.0, 3.5 and 5% of the total lipids in the carcass, liver and kidney, respectively.

In May, the total body burden of PCB was approximately 80 % of that found in September. No significant differences were found in the proportional amount of the four congeners, neither between organs, nor between sampling dates ($p < 0.05$). Figure 1 shows the concentration of PCB in carcass, liver, kidney and brain in September, February and May.

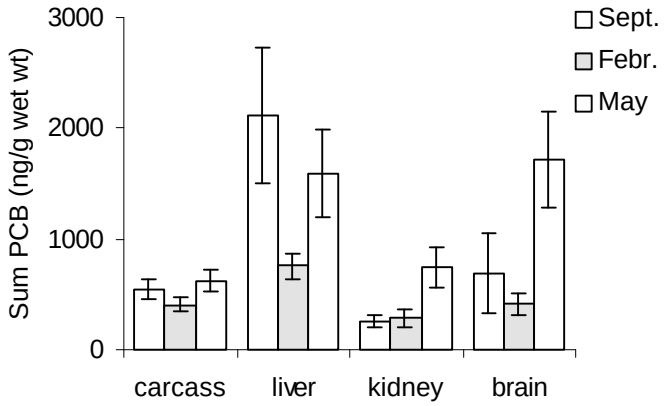

Figure **1** Concentration of PCB (sum of 4 congeners) in different tissues and organs the char sacrificed in September, February and May. Values shown are sample means (n=7) ± s.e.m. (standard error of a mean).

In the carcass, the PCB concentration remained relatively stable during the winter. In the liver a high concentration of PCB was recorded in September, after which there was a significant decrease until February ($p < 0.05$) and a subsequent, significant ($p < 0.05$) increase until May. In the kidney, the PCB concentration did not change from September to February, after which there was an increase (but not significant) until May. In the brain PCB concentration did not differ significantly between September and February, but there was a significant increase (approx. 4 fold) between February and May ($p < 0.05$).

In figure 2 the individual concentration of PCB in the brain is plotted against the percentage of TAG in the carcass. As evident in the figure, there appeared to be

a marked increase in the brain PCB concentration when the percentage of TAG decreased below 1% of the carcass wet weight. A similar increase, although not so dramatic, was seen in liver and kidney.

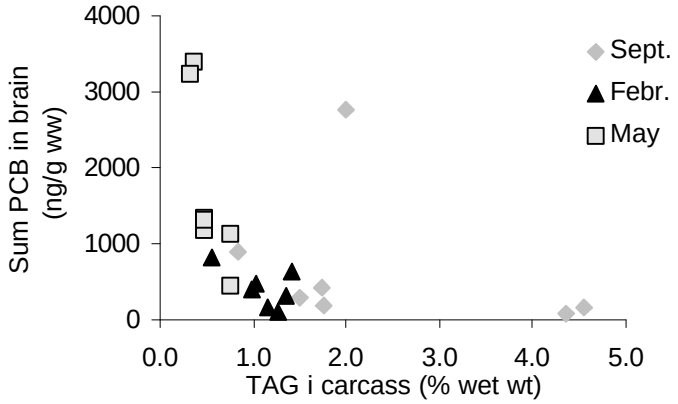

Figure 2 PCB concentrations in brain plotted against the concentrations of triacylglycerol (TAG) in carcass for individual fish, sampled in September (♦), February (▲) and May (■).

Discussion

The changes in body weight, organ weights and lipid status in the char held in captivity throughout the winter were comparable to the changes occurring in their wild counterparts overwintering in the lake (Jørgensen et al., 1997a; Jobling et al., 1998). This indicates that the energy expenditure of wild char overwintering in indoor tanks is comparable to that in the wild. These results confirm the applicability of this model for realistic studies on POP toxicokinetics in relation to the anadromous life strategy of the Arctic char.

The 20 % reduction in the total body burden of PCB between September and May indicated a very long half-life (>600 days) of the PCB mixture used in the present experiment. This is in accordance with the very slow elimination rate of PCB congeners with high chlorine content previously found in fish (e.g. Niimi and Oliver, 1983). All four PCB congeners used in the present experiment were either pure phenobarbital (PB) type inducers (PCB 101, 153 and 180) or mixed (both PB and 3-methylcholanthrene) type inducer (PCB 105) (McFarland and

Clarke, 1989). The metabolisation and excretion rates of these congeners were therefore expected to be relatively similar. This was also the case, in that the temporary changes in the body burden from September to May did not differ between the congeners.

In September the concentrations of PCB were fairly similar in carcass, kidney and brain, as were also the concentrations of TAG in carcass and kidney. At the same time, the liver concentrations of both the PCB and TAG were 3 to 4 times higher than in the other organs. In February PCB and TAG concentrations were comparable for all tissues and organs. In accordance with previous findings in fish (Monod and Keck, 1982; Kawai et al., 1988; Kamman et al., 1990; Jørgensen et al., 1997b), the tissue distribution of PCB in September and February in the present study seemed to be related to the tissue concentration of non-polar lipids (i.e. TAG), at least in the carcass, liver and kidney.

From February to May the PCB concentration increased only slightly in the carcass, whereas a 2 to 4 times increase were seen in the liver/kidney and brain, respectively. In May, the concentration of TAG were substantially lower in liver and kidney (0.1 %) than in carcass (0.5 %), and the change in the tissue distribution of PCB between February and May could therefore not be attributed to corresponding changes in the affinity for PCB between tissues and organs.

Temporary changes in the tissue concentration of PCB in starving fish depend on the relationship between the rates of PCB loss and the reduction of the organ weight. In the present study there was on average 30 % decrease in both the weight and PCB content in carcass from September to May. The fairly stable PCB concentration indicates that there were no apparent changes in the mobilisation of PCB from the carcass during the course of the experiment.

The increased concentration of PCB in liver, kidney and brain from February to May was not accompanied by a corresponding decrease in the weights of these organs. This indicates that there must have been a net input of PCB to these organs from February to May. Hence, we would expect an increased mobilisation of PCB from the carcass in this period, an assumption that is not supported by the data. More research is needed to reveal the mechanisms underlying the disposition of POPs after long-term fasting in fish.

The mean concentration of PCB in the brain increased approximately four-fold from February to May. When the brain PCB concentration of individual fish were plotted against carcass TAG concentration, there seemed to be an

exponential increase in the brain PCB concentration when the TAG concentration in the carcass decreased below 1 %. Apparently, this pattern caused large inter-individual differences in the concentration of PCB in vital organs along with small differences in the degree of emaciation among these individuals. The dramatic increase in the brain PCB concentration, and to a certain extent also in the liver and kidney, at the end of the winter in some individuals must have implications for the toxicological potential of deposited POPs. Since at least some of the toxic effects of halogenated aromatic hydrocarbons such as PCB are mediated through the cytosolic aryl hydrocarbon (Ah) –receptor (Safe, 1990), dose-response relationships depends on the concentration of the toxicant in cells containing the Ah-receptor. In general, Ah-receptors have not been found in skeletal muscle cells (Guengerich, 1993), whereas they are prevalent in both liver, kidney and brain cells in fish (Husøy et al., 1994; Andersson and Goksøyr, 1994). Temporary differences in the tissue distribution of POPs are therefore thought to affect the toxic potential of a certain body burden of POPs. Accordingly, a higher hepatic CYP1A activity was previously found in starved char than in fed charr due to a higher liver concentration of PCB in the former fish (Jørgensen et al., 1999).

In conclusion, the results in the present experiment indicate that the anadromous Arctic char are more vulnerable to negative effects associated to POPs in the late winter/spring and that there are marked differences among individuals in a population in the risk associated with toxicant burdens. The long half-life of POPs with high lipophilicity and temporary emaciation of many Arctic species probably makes them extra sensitive to POPs. In the anadromous Arctic char, winter emaciation coincides with the preparatory changes taking place prior to a new seaward migration and the present result aim for more research on possible effects of POPs on smoltification and seawater performance in this species. The present experimental model appears to be unique in that carefully controlled and ecologically realistic, contamination experiments can be performed with fish displaying natural traits regarding their physiology and body composition. New, comprehensive studies have consequently been undertaken to access 1) PCB disposition in relation to temporary changes in the localisation, content and composition of lipids, 2) dose- and time-dependent biological responses, and 3) effects on the smoltification process and seawater performance in the anadromous Arctic char.

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