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The Arctic has long been considered to be a pristine environment, far from population centers and pollution sources. The detection of synthetic organochlorine compounds in various elements of the Arctic food web has confirmed the global dispersion of pollutants, particularly of persistent compounds such as organochlorines and heavy metals. Levels of heavy metals, although elevated, appear to be naturally so throughout much of the Arctic. Arctic pollution is both a humanitarian and ecological concern. Many arctic coastal communities depend heavily on marine mammal fat for sustenance, and are therefore potentially exposed to high levels of organochlorines and some metals. From an ecological perspective, the structure of arctic food webs, the importance of lipid mobilization for winter survival, and the adaptive physiologies of arctic organisms may result in an enhanced response to contaminant exposure. This thesis assesses the exposure and effect of organochlorine and heavy metal exposure in inland freshwater ecosystems of Arctic Alaska, and evaluates the physiological response of arctic grayling to experimental polychlorinated biphenyl (PCB) exposure.

Assessment of Exposure and Response to Atmospherically-Derived Contaminants in U.S. Arctic Freshwater Fish

by

Susan M. Allen-Gil

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Susan M. Allen-Gil

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CONTRIBUTION OF AUTHORS

The manuscripts included in this dissertation represent integrated and interdisciplinary efforts. Manuscript co-authors have contributed within the following areas of expertise: Lawrence Curtis - principal investigator and thesis advisor, Dixon Landers - director of Arctic Contaminants Research Program, Chad Gubala - sediment research, Rose Wilson - complementary organochlorine analysis in fish and snails, Brenda Lasorsa and Eric Crecelius - inorganic analysis, Terry Wade - organochlorine analysis, Vincent Palace - vitamin analysis for grayling study, Robert Evans - histology for grayling study, Don Metner - liver enyzme analysis for grayling study, and Scott Brown - retinol analysis for grayling study, and Lyle Lockhart - coordinator of laboratory study on grayling.

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INTRODUCTION

The Arctic has long been considered to be a pristine environment, far from population centers and pollution sources. The detection of synthetic organochlorine compounds in various elements of the Arctic food web, including the breast milk of Inuit women (Dewailley *et al.*, 1989, 1992), has confirmed the global dispersion of pollutants, particularly of persistent compounds such as organochlorine and metallic compounds. There are two predominant contributing factors explaining the presence of elevated levels of certain pollutants in the North American Arctic: 1) the semivolatility of many organochlorine compounds may result in recurrent volatilization and deposition in response to latitudinal temperature gradients, a phenomena described as global distillation (Wania and Mackay, 1993), and 2) the high percentage of body mass present as fat compared to organisms living in more temperate climates, and the importance of these lipid stores as a dietary component at all stages of the food chain. This second factor is particularly important in the marine food web structure.

Arctic contamination is both a humanitarian and ecological concern. Some arctic coastal communities depend heavily on marine mammal fat for sustenance, and are therefore potentially exposed to high levels of organochlorines and other lipophilic pollutants. Levels of polychlorinated biphenyls (PCBs) in breast milk of Inuit women

in Hudson Bay, Canada, are among the highest reported in any population, five times higher than women from southern Quebec (Dewailley *et al.*, 1989, 1992). It is suspected that neurological development of the children of these Inuit women may be compromised as a result of PCB exposure (Dewailley *et al.*, 1992). From an ecological perspective, the structure of arctic food webs, the importance of lipid mobilization for winter survival, and the adaptive physiologies of arctic organisms may result in an exagerrated response to organochlorine exposure.

In addition to the concerns regarding organochlorine contamination, heavy metal concentrations appear to be naturally high throughout much of the Arctic. The largest copper/nickel and lead/zinc mines in the world are both located in the Arctic. Smelting operations potentially lead to the local atmospheric dispersal of these metals throughout the northern region. The high sensitivity of some species inhabiting the Arctic may render them more vulnerable to contaminant exposure Alaskan arctic grayling (*Thymallus arcticus*), for example, are more sensitive to exposure to metals than other salmonids, including arctic grayling from Montana (Buhl and Hamilton, 1990, 1991). Lake trout (*Salvelinus namaycush*), also distributed widely through the Arctic, are extremely sensitive to organochlorine toxicity, as elucidated by laboratory experiments with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Walker *et al.*, 1991).

This thesis assesses the exposure and effect of heavy metal and organochlorine exposure in inland freshwater ecosystems of Arctic Alaska, and evaluates the physiological response of arctic grayling to experimental PCB exposure. Presented in manuscript format, the first chapter addresses the presence of organochlorines in several components of U.S. arctic freshwater ecosystems, the second chapter evalutes the sensitivity of a large suite of biomarkers to PCB exposure in arctic grayling, and the third chapter discusses the presence and effects of heavy metals in lake trout and arctic grayling. In addition to the contents herein, I have conducted

similar research on the arctic ground squirrel (*Spermophilus parryi*) in the U.S. Arctic, and three fish species (*Salvelinus alpinus*, *Lota lota* and *Coregonus sp.*) and collared lemming (*Dicronstonyx torquatus*) in the Taimyr region of Russia as part of my graduate studies that will be published in separate manuscripts. Raw data from all aspects of the field research projects in the U.S. and Russian Arctic are compiled in the appendix for archival purposes.

Chapter 1

ORGANOCHLORINE PESTICIDES AND POLYCHORINATED BIPHENYLS (PCBS) IN U.S. ARCTIC AQUATIC ECOSYSTEMS

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Abstract

Organochlorine (OC) concentrations in surface sediment, snails (*Lymnea sp.*), and two freshwater fish species (grayling-*Thymallus arcticus*; and lake trout-*Salvelinus namaycush*) from four freshwater lakes in the U.S. Arctic were determined. In surface sediment, chlorinated benzenes, (CBZs), hexachlorobenzene (HCB), and p,p'-DDT were detected in the highest concentrations (maximum of 0.7 ng/g dry wt.), while individual polychlorinated (PCB) congeners were always below 0.1 ng/g dry wt. The largest number of compounds and the highest concentrations were found in lake trout, the top predatory fish species in U.S. Arctic lakes. The range of concentrations for hexachlorocyclohexanes (HCHs), chlordane compounds (CHLORs), DDTs, and PCBs were similar to those reported for other arctic freshwater fish (1-100 ng/g wet wt.), but 1-2 orders of magnitude lower than Great Lakes salmonids. Nitrogen isotope analysis confirmed that differences in OC concentrations between grayling and lake trout are explained in part by their different food web positions. OC concentrations in fish muscle are typically greater than 50% lower than those of marine mammals, indicating that freshwater fish are probably not a major source of these contaminants to northern communities in the U.S. Arctic.

Introduction

Contamination of remote regions of the world, such as the Arctic, has been recognized for several decades. In the 1970s, detection of DDT in fur seals from the Pribiloff Islands (Anas and Wilson, 1970) and in peregrine falcons on the Alaskan tundra (Cade *et al.*, 1971) served as important indicators of the extent of DDT's global dispersion (Buckley, 1986). More recently, numerous other OC compounds (such as toxaphene, CHLORs, HCHs, PCBs and dioxins), have been detected in North American and European Arctic fauna (e.g., Bowes and Jonkel, 1975; Muir *et al.*, 1988, 1990; Norstrom *et al.*, 1988; Oehme *et al.*, 1988; Koistinen *et al.*, 1989; Larsson *et al.*, 1990; Hargrave *et al.*, 1992; Lockhart *et al.*, 1992; Norheim *et al.*, 1992).

The lack of local sources of OCs, combined with the detection of various classes of pollutants in arctic air and snow samples (Maenhaut *et al.*, 1989; Ottar, 1989; Bidleman *et al.*, 1990; Gregor, 1990; Barrie *et al.*, 1992), has led researchers to conclude that atmospheric transport is a primary pathway for organic pollutants to the arctic (Gregor and Gummer, 1989). Certain classes of organic compounds are believed to be prone to long-range atmospheric transport to the arctic, based on their vapor pressure, water solubility, and octanol:water partition coefficients (Wania and Mackay, 1993). Also, arctic conditions seem to favor long-term accumulation of semi-volatile OC compounds. At low temperatures, greater partitioning to aerosols leads to more rapid deposition (Wania and Mackay, 1993). Slower reaction times, lower annual primary productivity, and fat-based trophic energy transfer enhance the susceptibility to contaminant exposure and its adverse effects in the Arctic. The slow growth rates and longevity of arctic freshwater fish have also been suggested to increase the likelihood of higher contaminant burdens than similar species in temperate climates (Lockhart *et al.*, 1992; Schindler *et al.*, 1995).

To date, Arctic contaminant research has predominantly focused on determining residue concentrations with little, if any, research conducted to evaluate potential ecological impact. In addition, there is a paucity of information available for the U.S. Arctic (see Sci. Total Environ. 160/161, 1995). The purpose of this study, therefore, was to document presence of OCs in surface sediment, snails and freshwater fish from arctic Alaska. Heavy metal accumulation and its relationship to reproductive status in freshwater fish are being published separately.

Methods

Sediment, snails (*Lymnea sp.*) and fish (arctic grayling; *T. arcticus*, and lake trout; *S. namaycush*) were collected from Elusive, Schrader, Feniak and Desperation lakes, located in the foothills of the Brooks Range (Fig 1.1). Limnological and drainage basin characteristics of the four lakes are provided in Table 1.1.

Sample collection

Sediment cores were retrieved and processed during the late winters of 1991-1993, using the methods described previously by Gubala *et al.* (1995). Sediment samples were stored cold (approx. 4°C) until the time of analysis. Sediment intervals were analyzed sequentially for total water and carbon content, radionuclides, and metals. Radiometric analyses of ^{210}Pb, ^{137}Cs and ^{7}Be were utilized in a constant rate of supply model (Robbins, 1978) to assign approximate dates of deposition to individual sediment intervals.

Fish were caught by hook and line. Blood samples (~5 ml) were taken from the caudal vein. Whole livers were removed, weighed, and divided for organic and inorganic analysis and transferred to pre-cleaned I-Chem™ jars and acid-cleaned borosilicate vials with teflon screw-tops respectively. Epaxial muscle samples were excised and processed in the same manner as liver samples. Snails were collected from submerged rocks in the littoral zone of the lakes, and placed directly in pre-cleaned I-Chem™ jars. All samples were frozen (or nearly frozen) on site and shipped by air to the laboratories. Fish gonads were excised and weighed. Sex, weight, length and stomach contents were recorded. Scale samples of grayling were taken for age determination, which was performed following the method described by Nielsen and Johnson (1983). Characteristics of sampled fish populations for each lake are provided in Table 1.2. Liver and gonadal somatic indices (LSI, GSI) were

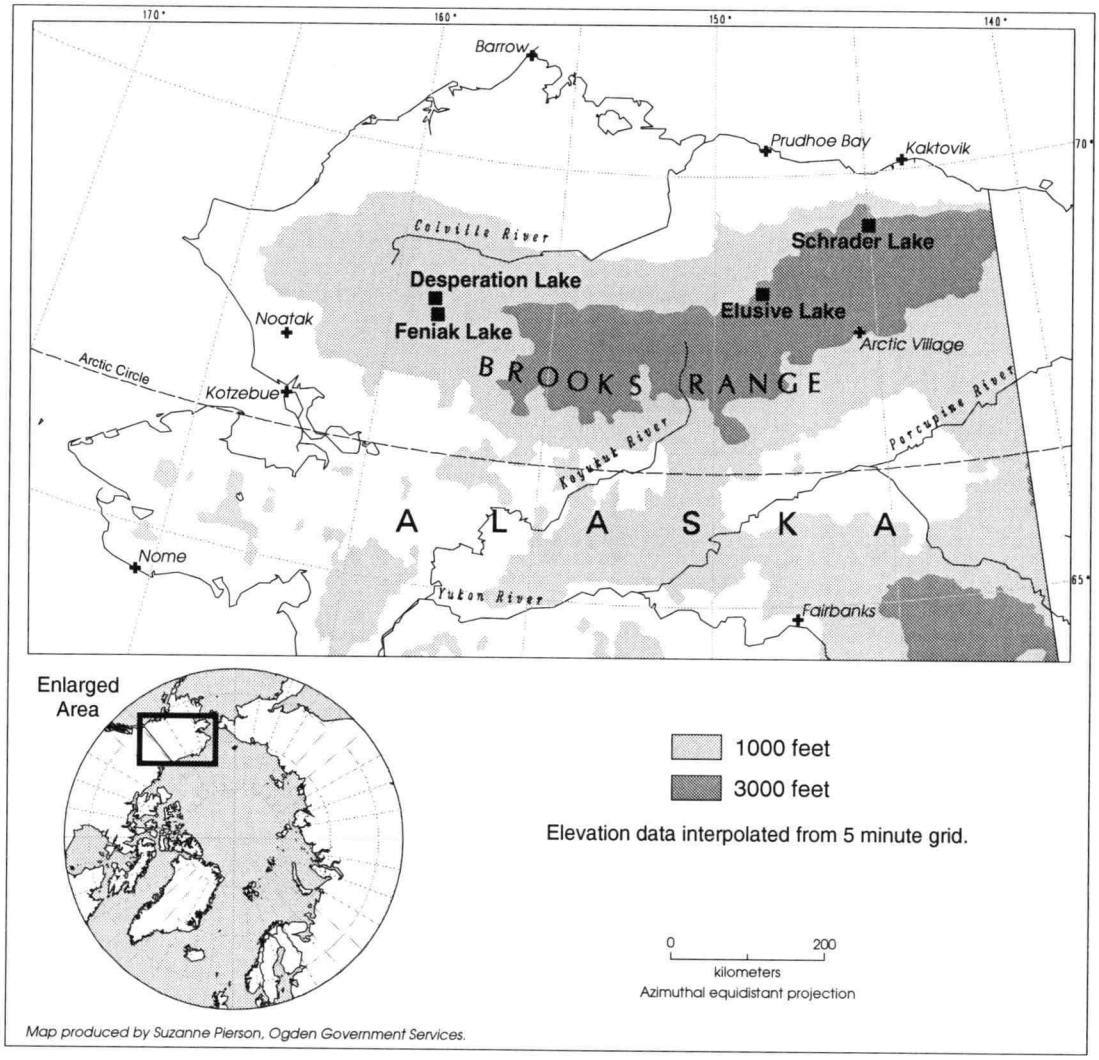

Figure 1.1 Location of sampling sites in the U.S. Arctic

Table 1.1 Limnological characteristics of study lakes

		Elusive	Schrader/ Peters	Feniak	Desperation
Latitude		68° 50' N	69° 22' N	68° 16' N	68° 19' N
Longitude		148° 30' W	144° 60' W	158° 18' W	158° 45' W
Drainage Basin Area	*(km2)*	15	469	213	296
Surface area	*(km2)*	3.3	20.4	17.6	5.9
Drainage basin area/lake surface area		5	24	13	51
Max. Depth	*(m)*	11.4	57	35.7	24.3
Mean Depth	*(m)*	6.8	33	10.2	6.4
pH		na	na	7.92	7.67
Acid neutralizing capacity	*(μeq/L)*	na	na	464	263
Dissolved organic carbon	*(mg/L)*	na	na	1.41	3.09
Chloropyll a (trichromatic)	*(mg/L)*	na	na	<0.001	0.002
Phosphorous (total)	*(μg/L)*	na	na	1	3
Nitrogen (total)	*(μg/L)*	na	na	898	252
Phosphorous/nitrogen		na	na	0.001	0.012

na=not available
Drainage basin area does not include lake surface
Drainage basin and surface area calculated from ARC/INFO by Suzanne Pierson, Computer Services Corporation.
Schrader Lake mean and max. depth from Hobbie (1961).

Table 1.2 Characteristics of fish sampled from U.S. Arctic lakes

		Elusive	Schrader/Peters	Feniak	Desperation
Years sampled		1991 1993	1991 1992	1992 1993	1992
Lake trout					
N		21	9	12	0
Males		12	3	8	
Females		9	6	4	
Standard Length	*(cm)*	46 ± 6	48 ± 10	52 ± 4	
Weight	*(g)*	1147 ± 630	1355 ± 494	1388 ± 274	
Percent lipid	*liver*	1.93 ± 1.06	11.18 ± 11.61**	1.7 ± 0.73	
	muscle	0.72 ± .72	1.87 ± 1.52	0.60 ± .071	
Grayling					
N		21*	25	16	10
Males		13	12	11	9
Females		6	13	5	1
Standard Length	*(cm)*	32 ± 4	31 ± 3	38 ± 2	34 ± 4
Weight	*(g)*	347 ± 71	327 ±63	539 ± 91	398 ± 127
Age	*(yrs)*	6 ± 1	8 ± 2	7 ± 1	7 ± 1
Percent lipid	*liver*	3.84 ± 5.44	1.75 ± 1.06	1.48 ± 0.58	2.02 ± 1.34
	muscle	2.70 ± 3.12	2.70 ± 2.59	2.45 ± 2.41	5.75 ± 6.4***

Values are presented as arithmetic mean ± standard deviation.
* Sex not determined on two individuals.
** One fish had 38% lipid in liver.
*** One fish had 11% lipid in muscle.

calculated as (liver wt/(total wt-liver wt))*100 and (gonad wt/(total wt-gonad wt))*100, respectively. Fulton-type condition factor was calculated as (weight/(fork length)^3*100) (Jearld, 1983).

Extraction and separation

OC analysis of fish and sediment was performed by the Geochemical and Environmental Research Group at Texas A&M University. The analytical procedure for sediment and fish was adapted from those employed for the U.S. National Oceanic and Atmospheric Administration's Status and Trends Program and the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (Wade *et al.*, 1988, 1993; Sericano *et al.*, 1990). This procedure was modified to provide the lower method detection limits required for samples from the Arctic.

After freeze-drying or drying with anhydrous Na_2SO_4, 10-30 g of sediment was Soxhlet extracted for 4 h with 300 ml CH_2Cl_2. A homogenized tissue sample, 0.5-15 g (wet wt.), was weighed into a 200 ml centrifuge tube. Prior to extraction, internal standards 4:4, dibromooctafluoro-biphenyl (DBOFB), PCB 103 and PCB 198 were added to all tissue and sediment samples, blanks, and quality control samples. For tissue samples, 50 g of anhydrous Na_2SO_4 and 100 ml CH_2Cl_2 were added to the centrifuge tube and the mixture macerated with a Tekmar™ Tissumizer for 3 minutes. The CH_2Cl_2 was decanted into a 500 ml flat bottom flask after centrifugation at about 2000 rpm for 5 min, when necessary. The mixture in the centrifuge tube was extracted two more times with 100 ml of CH_2Cl_2 each time. The CH_2Cl_2 extracts were combined and a 20 ml aliquot removed for gravimetric lipid determination. The remaining tissue sample extract, and sediment extracts following Soxhlet extraction, were concentrated to approximately 10-20 ml by attaching a 3-ball Snyder column to the 500 ml flat bottom flask, adding a Teflon boiling chip and heating the extract in a

water bath at 60-70°C. The extract and hexane rinses of the flat bottom flask were transferred to a 25 ml concentration tube and evaporated in a water bath to 1 ml.

The tissue and sediment extracts were fractionated by alumina:silica open column chromatography. The silica gel was activated at 170°C for 12 hr and partially deactivated with 5% distilled water (v/w). Twenty g of silica gel were slurry-packed in CH_2Cl_2 over 10 g of alumina. The alumina was activated at 400°C for 4 hr and partially deactivated with 1% distilled water (v/w). The CH_2Cl_2 was replaced with pentane by elution. The extract in hexane was then applied to the top of the column. The extract was sequentially eluted from the column with 50 ml of pentane (aliphatic fraction) and 200 ml of 1:1 pentane: CH_2Cl_2 (pesticide/PCB fraction). The pesticide/PCB fraction was further purified by HPLC (Spectro Physics Model 8100, Waters 440 uv detector) to remove the lipids. Samples were injected in 1 ml CH_2Cl_2 with a Gilsen 231/401 auto sampler. The lipids were removed by size exclusion using CH_2Cl_2 as an isocratic mobile phase (7 ml/min) and two 22.5 x 250 mm Phenogel 100 columns (Krahn *et al.*, 1988). The purified pesticide/PCB fraction was collected in a LKB Bromma 2211 fraction collector from 1-5 min prior to the elution of perylene. The retention times of the two marker peaks were checked prior to the beginning and at the end of each set of 10 samples. The purified pesticide/PCB fraction was concentrated to 1 ml of hexane using a Kuderna-Danish tube heated in a water bath at 60°C.

Gas chromatography

Chlorinated pesticides and PCB congeners were analyzed by fused-silica capillary column gas chromatography with an electron capture detector (GC-ECD) using a Hewlett-Packard 5880A GC in the splitless mode. The primary column was a DB-5 capillary column, 30 m x 0.25 mm i.d. (0.25 μm film thickness). The secondary column was a DB-17HT, 30 m x 0.25 i.d. (0.15 μm film thickness). Both columns

were temperature-programmed from 100 to 140°C at 5°C/min, from 140 to 250°C at 1.5°C/min., and from 250 to 300°C at 10°C/min. with 1 minute hold time at the beginning of the program and before each program rate change. The final temperature was held for 5 minutes. Injector and detector temperatures were set at 275°C and 325°C, respectively. The analytes were quantitated against a set of standards that were injected at four different concentrations to calibrate the instrument and to compensate for the non-linear response of the detector.

Analyte concentrations were determined on both columns, and only reported when detected on both columns. The lower of the two column concentrations was reported. The presence of analytes in 10% of samples was confirmed by gas chromatography electron-impact mass spectrometry in the selected ion mode.

Quality assurance

Quality assurance for each set of ten samples included a procedural blank, matrix spike, duplicate, and standard reference material (tissues: NIST-SRM 1974 or 1974a, sediment: NIST-SRM 1941 or 1941a) which were carried through the entire analytical scheme. Internal standards (surrogates) were added to samples prior to extraction and were used for quantitation. To monitor the recovery of the surrogates, a chromatography internal standard, tetrachlorometaxylene (TCMX), was added prior to gas chromatographic analysis. For all data reported, SRM values were within 80-120% of the concensus value (unless the relative standard deviation of the concensus value was greater than 50%), spike recoveries were between 80-120%, and analytes were not detected in the blanks above the minimum detection level (MDL). Duplicate samples were included in each batch, but were not used to reject data because of the difficulty of achieving precision at extremely low concentrations. Relative percent difference of duplicates was typically <30%.

Snails (shells removed) were analyzed at the EPA Research Laboratory in Corvallis, OR following the methods previously described in Wilson *et al.* (1995).

Nitrogen isotope analysis

Ten lake trout and ten grayling samples were analyzed for stable nitrogen isotopes by the National Hydrological Research Laboratory, Saskatoon, Canada. For $\delta^{15}N$, 10-15 mg samples of freeze-dried muscle tissue were prepared using the CaO combustion technique described by Kendall and Grim (1990). The purified dinitrogen gas was analyzed for $^{15}N/^{14}N$ on a VG Optima isotope mass spectrometer. All stable isotope data are reported relative to the air standard in the delta (*o/oo*) notation, $\delta^{15}N$ (Fritz and Fontes, 1980).

Statistical analysis

All OC data was log(10) transformed as preliminary data exploration revealed proportional relationships between means and standard deviations for some analytes. In addition, standard length, LSI, GSI and condition factor were log(10) transformed prior to correlation/regression analysis. Site and sex differences were examined by ANOVA, followed by Neuman-Keuls post-hoc multiple comparison test. Spearman rank-order correlations (r_s) and regression analysis were used to identify relationships between contaminant concentrations in tissues and physiological parameters. For steroid analysis, outliers from replicate analyses were identified using Dixon's test for outliers, $p=0.01$ (as described in Sokal and Rohlf, 1981). In all other cases, a significance level of $p<0.05$ was applied. Distributions of OC concentrations are presented in figures using the analytical estimate for all observations in which a peak was observed even if below the MDL.

Results

Surface sediment from Alaskan Arctic lakes typically contained detectable concentrations of HCB, p,p'-DDT and chlorinated benzenes (Table 1.3). Concentrations of all analytes were less than 1 ng/g dry wt. The highest concentrations of many OC pesticides were observed in Feniak Lake. Concentrations of PCB congeners in sediment from all lakes were less than 0.1 ng/g dry wt.

A more extensive suite of analytes was detected in biological samples. For example, dieldrin and PCBs 170 and 180 were present in snail composite samples from lakes where these analytes were not detectable in surface sediment (Table 1.4). With the exception of o,p'-DDE and PCB 29, all analytes were detected in some grayling liver samples (Table 1.5). All analytes were detected in some lake trout liver samples (Table 1.6). In liver and muscle of both species, a-HCH and p,p'-DDE comprised greater than 75% ∑HCHs and ∑DDTs respectively. Concentrations of analytes were typically higher in lake trout than grayling and snails (Fig. 1.2). Liver burdens of OCs were usually higher than muscle burdens for both fish species. Liver samples also showed a wider spectrum of PCBs, including less-chlorinated congeners that were typically absent in muscle (Fig. 1.3). PCBs 138 and 153 (hexachlorobiphenyls) comprised approximately 40% of total PCBs in muscle compared with 20% in liver.

Overall, ∑CHLORs, ∑HCHs, ∑DDTs, and ∑PCBs concentrations were generally low in fish tissue. In grayling, maximum concentrations were less than 50 ng/g wet wt. for all classes of compounds. In lake trout, ∑CHLORs, ∑HCHs, ∑DDTs concentrations were less than 100 ng/g wet wt., while ∑PCBs concentrations reached 200 ng/g wet wt.

Table 1.3 Organochlorine concentrations in sediment (ng/g dry weight)

	Elusive Lake	Desperation Lake	Feniak Lake	Schrader Lake*
Minimum detection limit	0.04	0.05	0.07	0.06
Analyte				
HCB	0.21	0.08	0.27	0.11
a-HCH	nd	0.03	0.09	0.01
b-HCH	nd	nd	nd	0.09
g-HCH	0.07	0.01	0.02	0.09
d-HCH	nd	nd	nd	nd
heptachlor epoxide	nd	nd	nd	nd
oxychlordane	0.01	nd	nd	nd
g-chlordane	nd	nd	nd	nd
a-chlordane	0.01	nd	0.05	nd
trans-nonachlor	0.01	0.005	nd	nd
cis-nonachlor	nd	0.01	nd	nd
dieldrin	0.003	nd	nd	nd
endrin	nd	nd	nd	nd
mirex	nd	nd	nd	nd
endosulfan-2	nd	nd	nd	nd
TCB1234	0.19	0.00	0.40	0.01
TCB1245	nd	0.06	0.70	0.02
PCA	0.01	0.002	0.01	0.07
pentachlorobenzene	0.07	0.03	0.26	0.02
o,p'-DDE	nd	nd	nd	nd
p,p'-DDE	0.03	nd	0.06	0.02
o,p'-DDD	nd	nd	nd	nd
p,p'-DDD	0.06	0.05	nd	nd
p,p'-DDT	nd	0.07	0.25	0.03
Total DDTs	0.09	0.12	0.31	0.09
PCB 8	nd	nd	nd	0.07
PCB 18	nd	0.01	nd	nd
PCB 28	0.02	0.02	nd	0.01
PCB 29	nd	nd	nd	nd
PCB 44	0.05	0.02	nd	nd
PCB 50	0.02	0.02	nd	nd
PCB 52	nd	nd	nd	nd
PCB 66	0.02	nd	nd	nd
PCB 87	nd	0.01	nd	nd
PCB 101	nd	0.01	nd	nd
PCB 105	nd	nd	nd	nd
PCB 118/108/149	nd	nd	nd	nd
PCB 128	nd	nd	nd	nd
PCB 138	0.07	nd	0.04	0.03
PCB 153	nd	nd	nd	nd
PCB 170	nd	nd	nd	nd
PCB 180	nd	nd	nd	nd
PCB 187	nd	0.02	nd	nd
PCB 195	nd	nd	nd	nd
PCB 200	nd	nd	nd	nd
PCB 206	nd	0.02	nd	nd
PCB 209	nd	nd	nd	nd
Total PCBs*	0.18	0.25	0.04	0.11

nd: not detected
Total PCBs*: sum of all congeners analyzed
Schrader Lake*: data from Gubala et al. 1995

Table 1.4 Organochlorine concentrations in snails (ng/g lipid)

	Elusive Lake	Feniak Lake
	n=5	n=2
Analyte		
a+g-HCH	50.6 ± 31.3	
HCB	7.1 ± 4.4	8.8 ± 2.0
heptachlor epoxide		nd
a-chlordane	17.6 ± 8.7	6.4 ± 1.9
t-nonachlor	9.0 ± 7.1	6.4 ± 1.9
dieldrin	41.4 ± 27.3	15.5 ± 7.2
p,p'-DDE	45.7 ± 15.9	32.7 ± 4.9
PCB 101	199.08 ± 64.3	95.8 ± 4.2
PCB 118	105.0 ± 29.2	56.4 ± 1.9
PCB 153	68.4 ± 16.2	39.0 ± 2.7
PCB 105	27.9 ± 14.6	19.5 ± 1.3
PCB 138	93.7 ± 18.8	49.8 ± 4.4
PCB 187	10.3 ± 2.7	6.4 ± 1.9
PCB 128	18.8 ± 8.7	10.8 ± 1.7
PCB 180	9.6 ± 3.7	10.6 ± 6.1
PCB 170	4.4 ± 5.4	2.3
PCB 195	1.5 ± 1.9	nd
PCB 206	nd	nd
PCB 209	nd	nd
Total PCBs	538.8 ± 136.7	290.75

Values presented as mean ± 1 standard deviation.
nd: not detected

Table 1.5 Organchlorine concentrations in grayling (ng/g lipid)

	Liver												Muscle											
	Elusive Lake			Schrader Lake			Fenisk Lake			Desperation Lake			Elusive Lake			Schrader Lake			Fenisk Lake			Desperation Lake		
Analyte	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev.	N	Mean	St. Dev
HCB	14	19.7 ± 13.6		16	59.2 ± 37.4		16	37.7 ± 30.9		9	27.9 ± 20.4		6	16.6 ± 13.7		6	14.1 ± 11.5		8	34.9 ± 22.2		8	29.5 ± 18.8	
a-HCH	3	10.7 ± 7.9		1	27.7			nd			nd		1	2.9		12	38.2 ± 28.9		9	9.7 ± 8.8		4	13.5 ± 8.2	
g-HCH	2	40.3 ± 8.1		1	454.1		3	46.1 ± 14.9			nd			nd			nd			nd		1	1.1	
b-HCH	2	14.5 ± 6.1		4	304.8 ± 182.1		1	27.1			nd			nd		1	6.1			nd			nd	
d-HCH	2	18.0 ± 8.4		1	12.5			nd			nd			nd			nd		1	1.5			nd	
Heptachlor	3	10.5 ± 6.3		2	5.9 ± 1.4		1	9.2			nd			nd			nd			nd			nd	
g-chlordane	2	18.0 ± 3.5		1	126.3		1	16.4			nd			nd		1	0.8		2	10.0 ± 4.7			nd	
heptachlor epoxide	2	5.1 ± 2.5			nd			nd			nd			nd		6	6.2 ± 5.9		3	0.5 ± 0.2		5	4.9 ± 2.9	
oxychlordane	6	17.9 ± 11.4		2	20.0 ± 18.2		3	55.5 ± 33.0			nd		2	3.0 ± 2.4		8	9.9 ± 5.2		11	9.0 ± 13.0		1	10.8	
a-chlordane	4	46.8 ± 76.7		1	28.1		2	14.2 ± 4.7			nd		3	1.9 ± 1.5		8	8.6 ± 7.0		8	11.4 ± 15.1		1	0.1	
trans-nonachlor	6	18.8 ± 15.3		7	58.4 ± 86.6		5	61.3 ± 52.9		1	24.7		6	8.3 ± 3.6		17	40.4 ± 31.3		13	31.7 ± 39.1		8	15.9 ± 12.0	
dieldrin		nd			nd			nd			nd			nd			nd			nd			nd	
cis-dieldrin	3	4.8 ± 1.7		2	27.3 ± 4.0		5	36.6 ± 13.5		1	23.9			nd		1	3.5		4	1.2 ± 0.2		1	1.5	
endrin	3	39.9 ± 13.8			nd			nd			nd			nd		1	4.9		1	0.6 ±			nd	
mirex	3	1.3 ± 0.9			nd		2	9.0 ± 1.3			nd			nd		8	7.4 ± 5.7		4	4.9 ± 7.3			nd	
endosulfan-2		nd		2	13.1 ± 3.5			nd			nd			nd		8	13.8 ± 18.3			nd		2	11.3 ± 10.7	
TCB1234	5	21.9 ± 9.7		7	86.6 ± 68.2		4	39.2 ± 43.7		1	322.3		2	55.5 ± 55.3			±			nd		1	0.2	
TCB1245	3	333.9 ± 43.1		5	222.2 ± 81.0		3	24.5 ± 12.3			nd		1	133.7			±		3	26.8 ± 28.4		2	9.1 ± 5.8	
PCA	9	8.1 ± 5.8		4	295.4 ± 351.9		4	15.5 ± 5.5		4	70.9 ± 40.9		7	5.5 ± 9.6		7	1.8 ± 1.5		13	2.7 ± 2.3		5	1.8 ± 1.2	
pentachlorobenzene		nd		1	8.8			nd		1	249.4		5	10.9 ± 19.4		7	4.0 ± 3.4		3	2.9 ± 1.6		2	0.4 ± 0.2	
o,p-DDE		nd			nd			nd			nd			nd			nd		1	4.8			nd	
o,p-DDD		nd		1	6.1		1	11.3			nd		1	0.5		14	2.1 ± 2.8		4	2.1 ± 3.0		3	2.1 ± 0.8	
p,p'-DDE	11	41.1 ± 35.6		13	135.3 ± 176.8		11	145.9 ± 151.4		3	17.1 ± 7.1		10	10.0 ± 9.3		21	24.6 ± 28.2		8	46.7 ± 74.8		3	1.3 ± 0.9	
p,p'-DDD	1	57.3			nd		4	74.0 ± 53.2			nd		1	2.0		4	8.7 ± 8.9		4	3.4 ± 1.9			nd	
p,p'-DDT	3	3.6 ± 0.4			nd		1	97.4			nd		3	5.4 ± 1.4		5	4.2 ± 4.0		5	5.2 ± 4.0		2	8.4 ± 8.1	
Total HCHs	4	19.8 ± 10.4		6	249.9 ± 230.9		3	55.2 ± 3.5			nd			±		8	48.6 ± 29.7			nd		2	21.3 ± 2.6	
a+g-HCH	3	10.7 ± 7.9		2	230.9 ± 303.2		2	61.3 ± 6.1		1	24.7		1	2.9		12	38.2 ± 28.9			nd		8	20.3 ± 15.0	
Total chlordanes		na		5	83.1 ± 94.3		2	61.3 ± 6.1		1	24.7		1	2.2		17	51.1 ± 38.6			nd		8	19.0 ± 12.9	
Total DDTs	5	55.6 ± 8.4		13	135.3 ± 176.8		12	165.7 ± 176.5		3	17.1 ± 7.1		9	9.4 ± 9.5		12	8.1 ± 5.9		8	51.0 ± 79.8		3	1.4 ± 0.9	
Total p,p'-DDTs	7	51.0 ± 39.1		13	135.3 ± 176.8		12	165.7 ± 176.5		3	17.1 ± 7.1		10	11.8 ± 11.6		20	24.2 ± 29.2		8	51.0 ± 79.8		3	1.4 ± 0.9	
PCB congeners																								
8	5	421.6 ± 583.5		10	636.2 ± 553.1		8	147.7 ± 184.4		2	92.3 ± 65.5			nd			nd			nd			nd	
18	7	36.3 ± 36.5			nd			nd			nd			nd			nd			nd			nd	
28	4	15.7 ± 11.0		3	372.9 ± 151.4		1	30.5			nd			nd			nd			nd			nd	
29		nd			nd			nd			nd		1	0.1			nd		3	4.1 ± 4.6			nd	
44	2	6.6 ± 1.9			nd			nd			nd			nd			nd			nd			nd	
52	12	37.2 ± 27.3		4	107.0 ± 25.7		6	18.1 ± 5.2		1	77.0		1	2.3			nd		2	2.4 ± 1.9			nd	
66	5	24.2 ± 27.7		2	98.0 ± 50.7			nd			nd			nd		1	1.4		6	3.1 ± 3.5		1	0.4	
87	4	1.5 ± 1.3		3	15.8 ± 10.7		1	6.6		1	52.9			nd		1	5.7		2	0.3 ± 0.2			nd	
101	8	20.2 ± 13.0		2	14.3 ± 7.8		4	23.0 ± 11.2			nd		11	1.8 ± 1.0		20	4.8 ± 3.8		10	4.9 ± 3.0		3	4.6 ± 5.9	
105		nd			nd		1	12.9			nd		14	5.5 ± 3.7		8	10.5 ± 7.2		12	13.8 ± 14.6			nd	
118/108/149	1	3.5		1	2.4			nd			nd		1	6.8		1	6.9 ±		2	7.9 ± 6.2			nd	
128		nd		1	56.2		1	13.8			nd		6	3.5 ± 3.4		5	16.4 ± 19.9		10	11.9 ± 15.4		3	4.5 ± 1.9	
138	13	23.3 ± 12.0		17	134.7 ± 136.6		10	79.1 ± 67.2		9	66.2 ± 41.4		1	1.5			nd		4	6.5 ± 3.0			nd	
153	2	9.5 ± 4.3		4	69.6 ± 63.3		2	75.7 ± 12.7			nd		16	11.3 ± 9.7		16	38.2 ± 35.8		16	23.7 ± 19.7		9	10.6 ± 9.8	
170	3	41.3 ± 18.5		6	90.3 ± 77.3		5	46.7 ± 22.5			nd		14	10.1 ± 8.8		8	41.5 ± 49.0		9	47.4 ± 41.8		6	28.1 ± 16.8	
180	3	18.8 ± 9.1		2	22.7 ± 13.5		4	22.3 ± 7.8			nd		14	6.2 ± 3.8		1	0.6		5	11.5 ± 9.7			nd	
187	5	4.0 ± 2.2			nd		2	19.3 ± 2.8			nd		5	5.9 ± 2.8		10	14.5 ± 21.0		10	8.2 ± 4.6		4	13.0 ± 5.6	
195		nd			nd		1	12.1			nd		7	4.9 ± 3.6		7	16.8 ± 17.7		14	8.0 ± 11.5		2	8.4 ± 8.1	
200		nd		1	1.8			nd			nd			nd			nd			nd			nd	
206		nd			nd		1	16.2			nd			nd		2	296.3 ± 285.7		2	1.6 ± 1.2			nd	
209		nd			nd		1	9.4			nd			nd			nd			nd			nd	
Total PCBs*		na		10	902.3 ± 501.9		9	177.1 ± 123.4		7	109.2 ± 53.6			na		1	23.0			nd			nd	
Total PCBs **	10	250.6 ± 493.9		12	706.0 ± 514.5		10	144.1 ± 98.3		7	109.2 ± 53.6			na			na			na			na	
													3	196.6 ± 109.5		5	82.0 ± 50.3		6	56.5 ± 33.4				

Values presented as arithmetic means ± 1 standard deviation.
N= Number of samples in which analyte was detected.
nd=not detected
na=data not available
Total PCBs*: sum of 23 congeners analyzed
Total PCBS**: includes only PCBs 8, 52, 87, 101, 105, 138, 153, and 180

Table 1.6 Organchlorine concentrations in lake trout (ng/g lipid)

	Liver												Muscle											
	Ekalute Lake				Schrader Lake				Fenish Lake				Ekalute Lake				Schrader Lake				Fenish Lake			
Analyte	N	Mean		St. Dev.	N	Mean		St. Dev.	N	Mean		St. Dev.	N	Mean		St. Dev.	N	Mean		St. Dev.	N	Mean		St. Dev.
HCB	19	39.5	±	26.5	3	59.43	±	24.51	11	110.87	±	69.37	14	30.38		16.65	11	57.50		29.1	9	39.4	±	10.9
a-HCH	4	16.4	±	10.5	6	28.1	±	23.4				nd	3	19.7	±	7.8	7	39.57	±	27.0	1	19.9		
g-HCH	4	113.1	±	102.4	5	48.6	±	51.1	1	674.0						nd	5	13.17	±	5.9				nd
b-HCH	9	77.7	±	72.8	1	16.2						nd				nd				nd				nd
d-HCH	7	137.9	±	101.3				nd	1	38.6	±					nd				nd				nd
heptachlor	5	8.7	±	10.5				nd	2	30.9	±	19.3				nd				nd				nd
g-chlordane	2	2.8	±	0.3				nd	1	51.6	±					nd				nd				nd
heptachlor epoxide				nd	3	3.2	±	2.4				nd				nd	1	6.94		0.0	4	2.8	±	0.6
oxychlordane	5	14.5	±	8.9	6	12.0	±	11.8	5	94.3	±	50.1				nd	8	15.99	±	7.3	2	10.2	±	1.5
a-chlordane	6	158.9	±	117.0	5	14.6	±	10.0	5	137.7	±	102.2	10	10.5	±	5.5	7	28.8	±	13.4	8	26.1	±	29.6
trans-nonachlor	16	31.9	±	35.5	8	39.9	±	28.0	8	409.4	±	340.8	4	3.0	±	3.6	10	29.0	±	21.8	9	30.4	±	29.7
aldrin	2	26.5	±	26.5	1	1.2						nd	21	44.5	±	33.2	11	127.7	±	118.0	12	259.0	±	252.8
dieldrin	4	27.2	±	11.5	5	11.3	±	7.5	4	27.1	±	19.4				nd				nd				nd
endrin	4	48.1	±	36.8				nd				nd				nd	2	16.2	±	2.5	1	8.5		
mirex				nd	3	4.5	±	2.9	6	124.1	±	106.5				nd				nd				nd
endosulfan-2				nd	5	18.9	±	26.1	2	291.0	±	91.2				nd				nd	4	43.3	±	31.6
TCB1234	8	23.2	±	17.4	4	38.1	±	53.4	3	115.0	±	46.0	4	17.0	±	27.4	3	9.8	±	11.5	4	77.3	±	65.2
TCB1245	3	167.6	±	194.8	5	78.9	±	63.3	3	658.9	±	255.2	3	171.7	±	113.4				nd				nd
PCA	6	11.2	±	13.2	3	10.3	±	7.6	3	14.8	±	2.5				nd				nd				nd
pentachlorobenzene	6	31.8	±	39.2	4	20.2	±	4.8	2	16.3	±	0.5	11	4.4	±	3.8	6	2.2	±	1.0	4	19.9	±	17.4
o,p-DDE	2	1.4	±	1.2				nd				nd	7	579.4	±	1243.7	4	2.7	±	1.8	1	12.9		
o,p-DDD	3	3.7	±	2.9	5	6.3	±	6.7	3	31.6	±	17.3				nd				nd	3	2.3	±	0.3
p,p'-DDE	18	185.5	±	113.9	7	91.6	±	84.2	9	497.8	±	493.5	1	10.5			3	0.9	±	0.6	6	8.6	±	3.6
p,p'-DDD	5	56.5	±	45.0	5	17.8	±	14.5	1	133.4			17	103.1	±	67.6	11	281.1	±	359.6	4	326.3	±	318.9
p,p'-DDT	4	8.9	±	7.3	3	2.2	±	2.5	3	51.4	±	29.7	4	32.0	±	35.7	3	24.3	±	21.7	1	23.0		
Total HCHs	3	20.3	±	9.3	5	56.9	±	40.8	1	674.0			14	14.1	±	7.9	3	42.4	±	48.3	5	16.8	±	11.1
a+g-HCH	6	86.3	±	91.5	8	39.3	±	36.5	1	674.0						nd	6	48.3	±	31.6				
Total chlordanes				na	6	83.5	±	30.5	2	683.0	±	238.4	1	9.3			9	38.1	±	29.7				nd
Total DDTs	7	222.9	±	156.0	7	104.8	±	99.6	9	546.7	±	524.9	7	56.3	±	49.7	5	121.6	±	100.6	4	204.4	±	119.8
Total p,p'-DDTs	18	200.2	±	122.2	7	100.4	±	94.0	9	532.0	±	500.6	7	71.6	±	56.4	5	335.8	±	435.1	4	376.4	±	360.3
													17	114.8	±	89.7	11	299.3	±	383.5	4	335.6	±	312.1
PCB congeners																								
8	1	1067.8			1	78.6			9	2001.2	±	2333.1				nd				nd				nd
18	3	189.1	±	137.3	4	39.3	±	47.2				nd	1	3.0						nd				nd
28	10	141.4	±	160.1				nd	8	272.1	±	263.3				nd				nd				nd
29	1	74.0	±					nd	1	65.5						nd				nd				nd
44				nd	1	1.9	±		1	148.2						nd				nd				nd
52	9	154.2	±	85.8				nd	5	67.7	±	61.9	3	7.1	±	2.9	2	8.8	±	3.7	1	30.4		
66	8	159.1	±	107.7	2	146.4	±	145.1	1	227.6						nd	1	13.8						nd
87	11	19.4	±	17.0	4	8.2	±	7.0				nd	9	8.1	±	9.5	5	7.0	±	4.9	11	23.3	±	32.9
101	2	6.8	±	1.9	3	25.3	±	22.5	7	174.2	±	165.7	10	19.5	±	11.8	10	48.5	±	47.1	12	123.3	±	144.9
105	2	8.3	±	6.2	1	8.4						nd	1	14.3			4	11.0	±	11.7				nd
118/108/149	5	10.6	±	9.8	3	7.9	±	5.0	2	188.6	±	77.6	14	13.2	±	13.0	7	23.1	±	29.9	9	78.8	±	82.5
128	2	10.8	±	8.7	2	11.1	±	3.1	3	74.4	±	31.7	8	6.6	±	5.3	5	9.4	±	11.7	9	39.8	±	42.1
138	17	70.6	±	53.3	9	80.2	±	64.1	5	389.7	±	276.1	20	48.4	±	39.2	11	84.1	±	90.8	12	272.5	±	363.7
153	12	61.5	±	73.6	7	49.8	±	39.1	8	683.2	±	766.6	7	84.1	±	59.4	8	119.5	±	122.9	7	641.0	±	756.3
170	7	77.1	±	38.4	4	24.5	±	26.9	4	76.4	±	46.9	14	28.9	±	15.2	6	17.9	±	11.0	3	23.4	±	26.5
180	3	28.9	±	15.1	7	18.7	±	19.1	8	205.7	±	232.8	8	32.4	±	24.6	9	27.1	±	32.9	10	152.2	±	186.3
187	4	14.8	±	8.3	4	10.1	±	8.9	5	324.8	±	354.6	16	14.2	±	8.9	5	39.2	±	46.6	9	184.7	±	297.9
195	1	4.0			1	3.2			2	20.7	±	6.6				nd				nd	1	30.3		
200	1	8.5			1	0.6						nd				nd				nd	6	10.5	±	11.8
206				nd	1	2.6			4	22.2	±	2.9				nd				nd				nd
209				nd	1	6.5			2	12.0	±	0.6				nd				nd				nd
Total PCBs*				na	4	250.2	±	170.1	6	2140.3	±	2417.3				na	9	352.4	±	365.3	4	685.2	±	782.8
Total PCBs**	3	44.2	±	53.3	6	194.1	±	118.6	7	1609.1	±	1993.9	3	110.8	±	63.4	9	295.1	±	295.2	7	1429.9	±	1570.7

Values presented as arithmetic means ± 1 standard deviation.
N= Number of samples in which analyte was detected.
nd=not detected
na=data not available
Total PCBs*: sum of 23 congeners analyzed
Total PCBS**: includes only PCBs 8, 52, 87, 101, 105, 138, 153, and 180

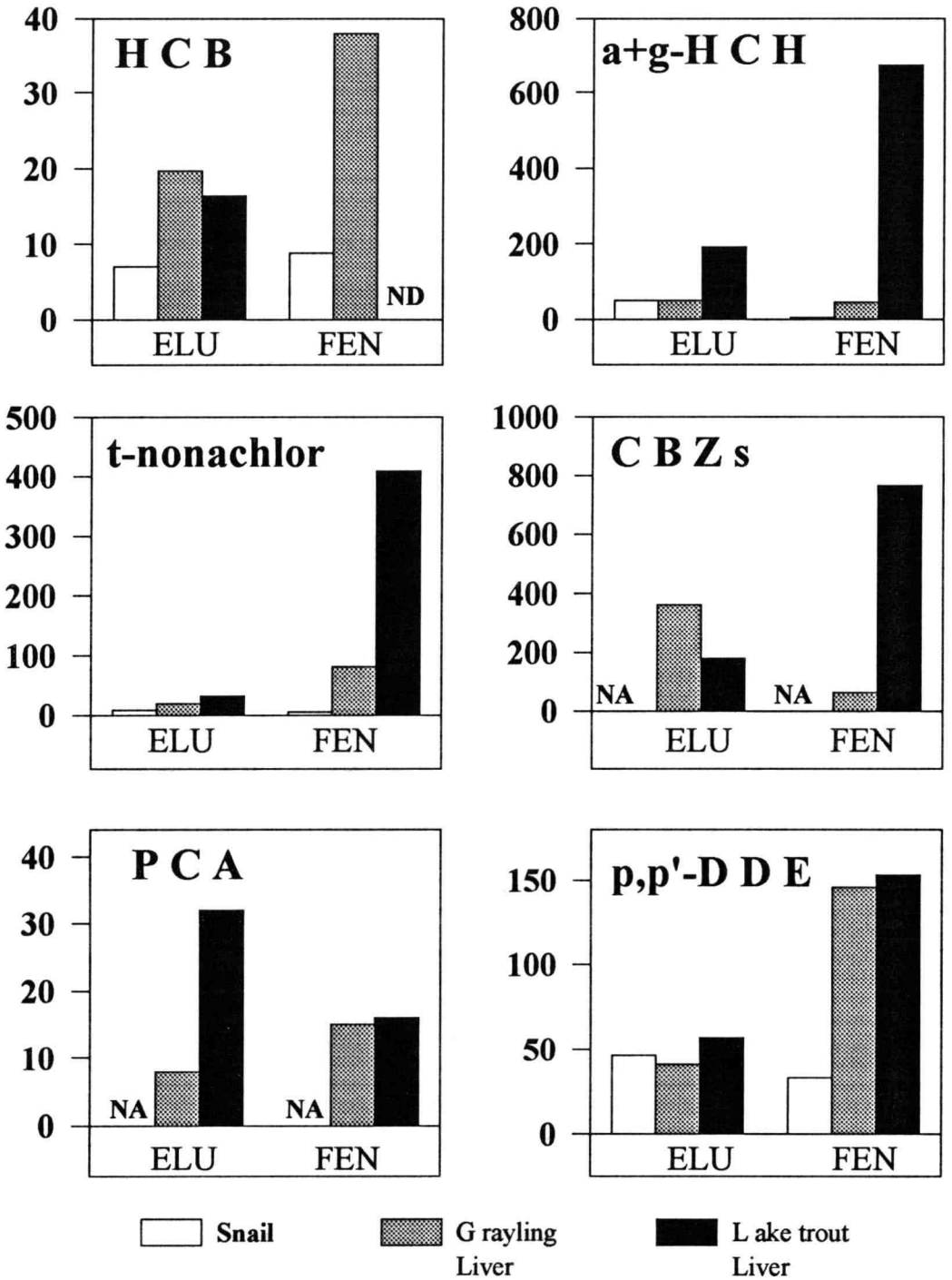

Figure 1.2 Mean concentrations of select organochlorines in aquatic organisms in U.S. Arctic lakes (ng/g lipid). ELU=Elusive Lake, FEN=Feniak Lake, NA=not analyzed in snails.

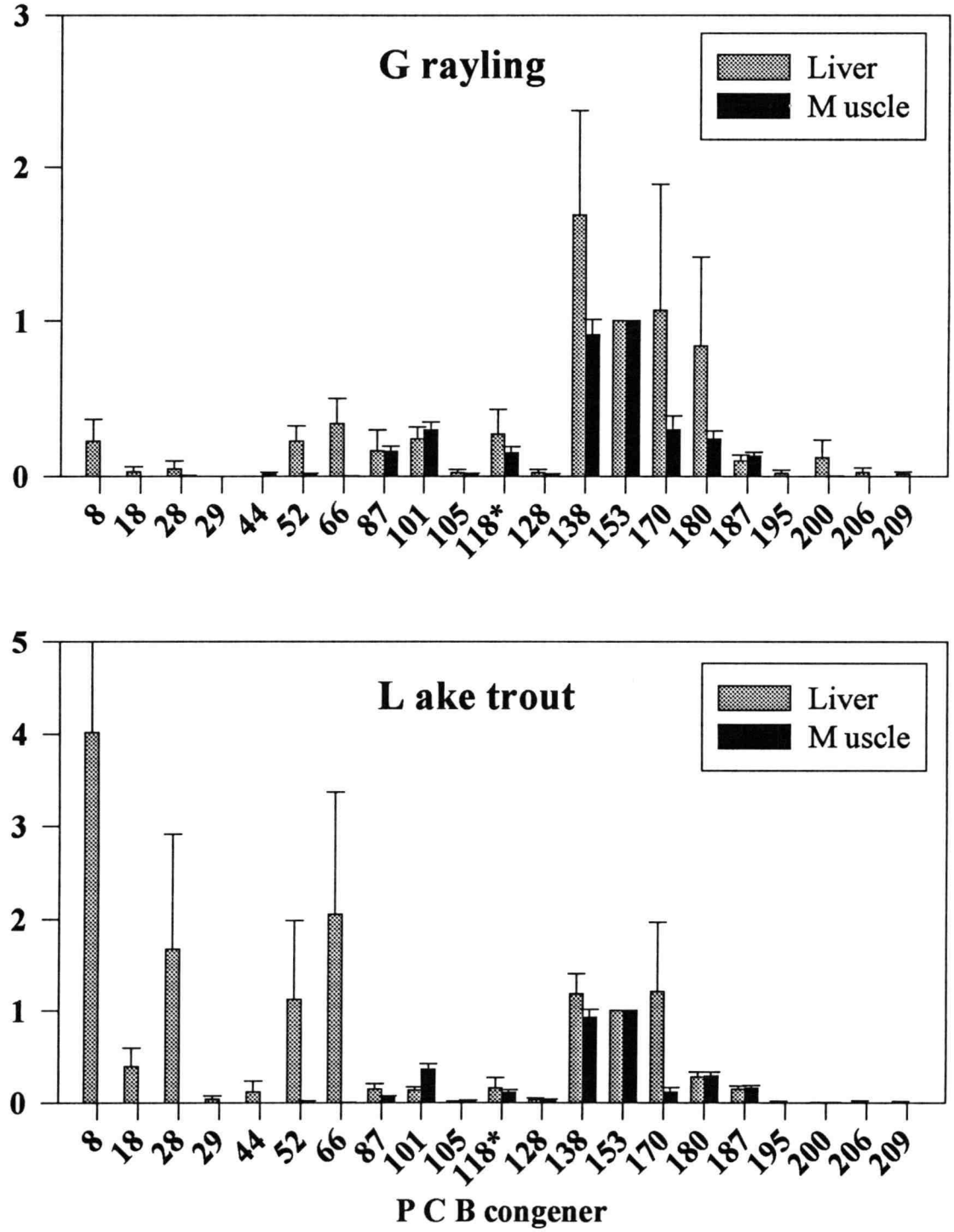

Figure 1.3 PCB composition relative to PCB 153

* PCB 118 coeluted with PCB 108 and 149

Lake trout from Feniak Lake had significantly higher liver concentrations of p,p'-DDE and PCBs 138 and 153. No site differences were observed for grayling.

Results from ^{15}N analysis on a subset of lake trout and grayling samples showed a clear relationship between tissue burdens of select OCs and trophic positioning between lake trout and grayling (Fig. 1.4). However, trophic position alone did not account for observed variability in lake trout from the same lake, since individuals with similar ^{15}N concentrations did not have comparable OC burdens.

Considerable variability in OC concentrations was observed in fish sampled from the same lake, even following lipid normalization. In some instances, a portion of the variability may be explained by age/length factors (age and length were positively correlated in grayling, r_s=0.39). For example, p,p'-DDE in grayling liver was positively correlated with both age and length (r_s=0.45 and 0.39 respectively). In lake trout liver, however, p,p'-DDE was not related to length. *Trans*-nonachlor in grayling and lake trout muscle was positively correlated with length. HCH isomers in muscle (*g*-HCH in lake trout and *a*-HCH in grayling) were negatively correlated with standard length. We did not observe any sex-related differences in tissue burdens, nor any consistent relationships between contaminant burdens and LSI, GSI or condition factor in either lake trout or grayling.

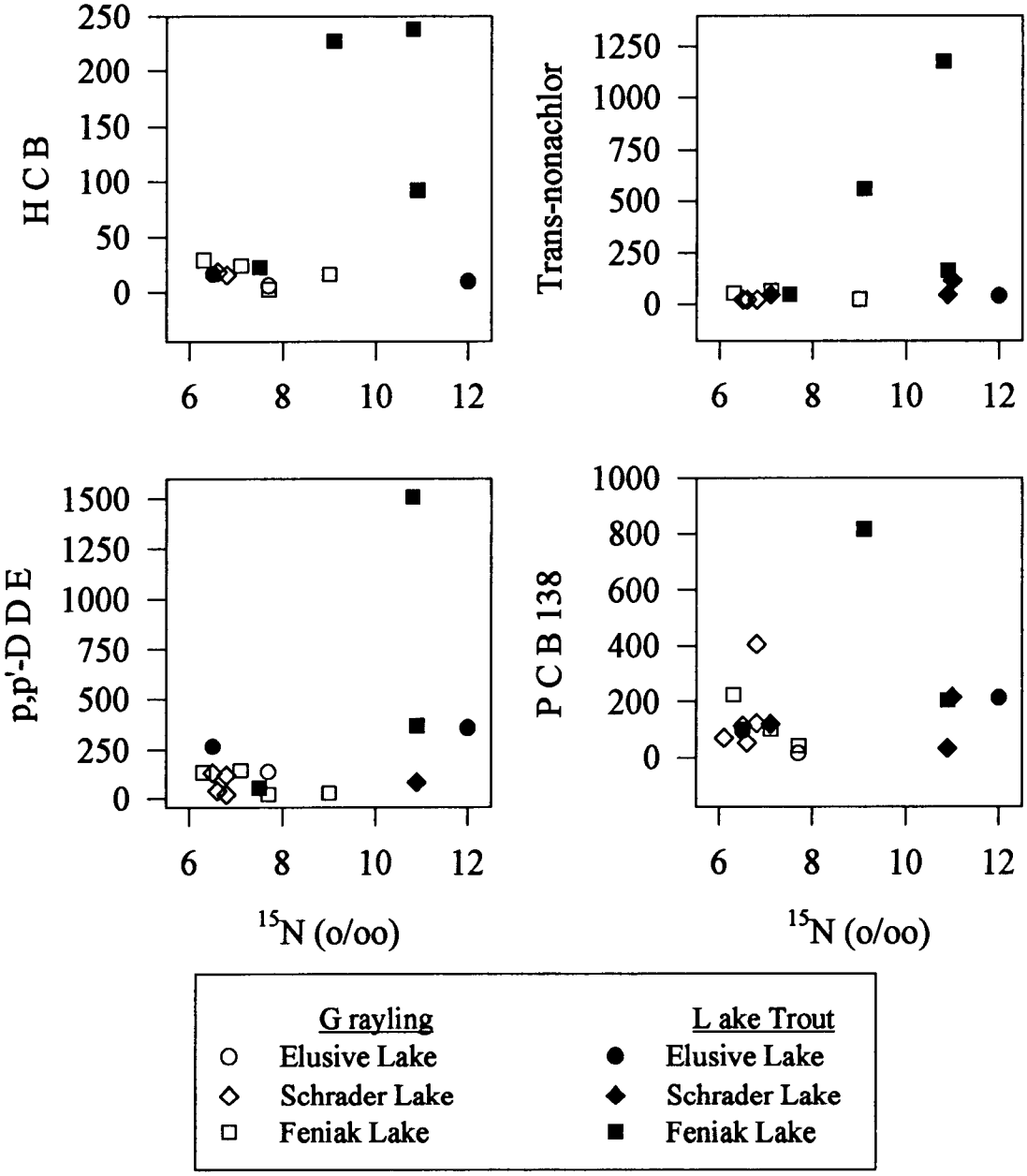

Figure 1.4 δ^{15}Nitrogen ratios (*o/oo*) versus organochlorine concentrations (ng/g lipid) for lake trout and grayling from U.S. Arctic lakes

Discussion

The results of this study support those of Gubala *et al.* (1995) and Wilson *et al.* (1995) for Schrader Lake: surface sediment OC concentrations from Feniak Lake are typically higher than those reported for Schrader Lake (Gubala *et. al.*, 1995), and this corresponds to the higher burdens of p,p'-DDE and PCBs 138 in lake trout from Feniak Lake. Our results for OC concentrations in lake trout and grayling from Schrader Lake also generally agree well with those reported previously for Schrader Lake (Wilson *et. al.*, 1995) (Table 1.7). Wilson *et al.* (1995) used comparable techniques to analyze complementary fish collections in 1992. Taken together, these results allow for an interlaboratory comparisons and contribute to a more robust assessment of OC contamination in Schrader Lake fish.

Circumpolar perspective

Detection of OC contaminants in remote freshwater systems of the U.S. Arctic confirms the distribution of these pollutants throughout much of the circumarctic region. Previously, OC contamination has been reported in arctic freshwater systems in Canada (Muir *et al.*, 1990, 1995; Lockhart *et al.*, 1992; Murdoch *et al.*, 1992; Bright *et al.*, 1995; Kidd *et al.*, 1995), and Scandinavia (Pyysalo *et al.*, 1983). In all arctic regions, background concentrations of HCHs, DDTs, CHLORs, and PCBs are typically within an order of magnitude, and maximum concentrations of individual OC compounds in sediment and fish are in the range of 5-10 ng/g dry wt. and 50-100 ng/g wet wt. respectively.

Although comparisons between $\sum$DDTs and $\sum$PCBs among studies should be treated cautiously as different analytes may be included in these figures, there is no alternative in cases where individual congener concentrations are not reported. Based on this technique, $\sum$DDTs and $\sum$PCBs concentrations in the Arctic are considerably

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lower than values reported for more temperate locations, such as the Great Lakes. For example, maximum concentrations of ∑DDTs and ∑PCBs in surface sediment were 0.31 and 0.25 ng/g dry wt. and in lake trout muscle in this study were 30 and 13 ng/g wet wt. respectively. By contrast, Oliver and Niimi (1988) reported ∑PCBs in Lake Ontario sediment and salmonids of 1100 ± 520 ng/g dry wt. and 4300 ± 640 ng/g wet wt. respectively. Concentrations of ∑DDTs and ∑PCBs in lake trout muscle samples collected from Lake Michigan in 1990 were 1830 ± 670 and 2440 ± 470 ng/g wet wt. (Miller *et. al.*, 1993).

We believe that OC burdens in lake trout and grayling are insufficient to result in sublethal toxicity. Maximum liver ∑PCBs burdens (lake trout=166 ng/g wet wt, grayling=44 ng/g wet wt.) were similar to concentrations of coplanar PCB congeners causing induction of P450 enzyme activity (Gooch *et al.*, 1989; Lindstrom-Seppa *et al.*, 1994). Therefore, if the entire PCB burden was present as the more potent coplanar congeners, one might observe physiological changes. Furthermore, when we explored correlations between OC burdens and plasma sex steroid data, no convincing relationships surfaced.

Distribution of organochlorines in freshwater systems

This study provides an integrated assessment of OC distribution with arctic freshwater systems by combining results from sediment, snails, and freshwater fish. The largest suite of contaminants and the highest concentrations were found in lake trout, the top predatory fish species. Accumulation rates based solely on sediment concentrations are therefore likely to underestimate the flux of OCs into Arctic aquatic systems.

Our demonstration of a strong relationship between OC concentration in tissues and trophic positioning in cases where lake trout and grayling occupied

different trophic positions agrees well with the results of other studies using food web positioning or nitrogen isotope analysis to explain differences in observed OC concentrations (Rasmussen *et al.*, 1990; Hesslein *et al.*, 1991; Broman *et al.*, 1992; Rolff *et al.*, 1993; Madenjian *et al.*, 1994; Kidd *et al.*, 1995). The fact that lake trout from the same lake showed considerable variability in OC concentrations that could not be explained by food web position suggests that either our data set was too limited to observe a strong relationship or that other factors are involved. Other researchers have suggested the importance of growth dilution, sex composition, and number of reproductive cycles as important determinants (Vodicnik and Peterson, 1985; Swackhamer and Hites, 1988; Sjim *et al.*, 1992; Larsson *et al.*, 1993). Although ANOVA analysis did not reveal any sex-related differences in OC concentrations, the lake trout showing the highest concentrations of select OC compounds (Fig. 1.4) were all male fish. Female fish reduce their OC burden during spawning to a greqater extent than males (Vodicnik and Peterson, 1985; Sjim *et al.*, 1992). Observed variability in lake trout from one lake may, therefore, be attributable to a combination of sex-related differences and trophic status.

Relevance for human exposure

Concentrations of OCs in fish tissues reported in this study are similar to those reported in other arctic studies and are typically 1-2 orders of magnitude lower than those reported for marine mammals (Table 1.7). In studies of human OC exposure in communities in the Canadian Arctic, Cameron and Weis (1993), Kinloch *et al.* (1992), and Dewailly *et al.* (1993) concluded that the majority of OC intake is from consumption of tissues of marine mammals, such as ringed seal, beluga, narwhal and walrus. In the first two studies, fish consumption was reported to account for approximately 10% of OC intake. Assuming that the proportional dietary intake of fish in Northern Alaska is similar to Northern Canada, we would predict that fish would not account for a significant percentage of OC exposure in humans.

Conclusion

In this first integrated assessment of OC contamination of freshwater ecosystems in the U.S. Arctic, we have documented the presence of HCHs, chlordanes, chlorinated benzenes, DDTs and PCBs in sediment, snails and two fish species. OC concentrations in fish are in the range of those reported for other arctic regions, but are considerably lower than burdens observed in similar species from more temperate regions of North America. Many contaminants that were not detectable in sediment were found in biological samples, suggesting the sediment monitoring alone would have underestimated the scope of contamination. A combination of site, sex, and bioaccumulation appear to affect OC concentrations in fish. We predict that if the proportional human dietary intake of fish is similar to northern communities in Canada, fish would account for less than 10% of OC intake by humans.

Chapter 2

UPTAKE, TISSUE DISTRIBUTION AND BIOMARKER RESPONSES IN ARCTIC GRAYLING (*THYMALLUS ARCTICUS*) EXPOSED TO 3,3',4,4'-TETRACHLOROBIPHENYL (TCB)

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Abstract

Arctic grayling (*Thymallus arcticus*) were given a single oral dose (10, 100 and 1000 ng/g nominal) of [^{14}C]-3,3',4,4'-tetrachlorobiphenyl (TCB; IUPAC No. #77) and sacrificed after 7, 30 and 90 days. A suite of 25 biomarkers (including plasma electrolytes, sex steroids, and thyroid hormones; hepatic cytochrome P-450 enzyme induction; hepatic, renal and plasma vitamins; and histopathology) was evaluated. After 7 days, hepatic ethoxyresorufin-*O*-deethylase (EROD) was the most sensitive biomarker, which increased at 10 ng/g; hepatic reintol and retinyl palmitate were elevated in the 1000 ng/g group. After 30 days, EROD induction in the 100 and 1000 ng/g groups remained higher than in control fish. The considerable variability in the biomarker response and percent of initial dose recovered within groups suggested that correlation analysis may be more appropriate: liver burdens of TCB were most highly correlated with EROD (Spearman correlation (r_s) of 0.93), followed by weak correlations with plasma osmolarity (r_s=0.36) and plasma Na (r_s=0.34). Less than 50% of the administered dose was recoverable in nine fish for which mass balance sampling and calculations were conducted, suggesting either a low assimilation efficiency, rapid elimination, or a combination of the two. Most of the dose resided in muscle, followed by intestine and gonads.

Introduction

Polychlorinated biphenyls (PCBs) account for the most of the toxic equivalents (TEQs) in many investigations of organochlorine contamination in feral fish (Niimi and Oliver, 1989; Tarhanen *et al.*, 1989; Janz and Metcalfe, 1991; Petreas *et al.*, 1992). Among PCBs, the non-*ortho* coplanar congeners (IUPAC #77, #126, and #169) are the most toxic, based on the ability to activate the A*h* receptor (Safe, 1990). In Aroclor and Kanechlor mixtures, the 3,3',4,4'-tetrachlorobiphenyl (PCB 77; TCB) is present at higher concentrations than PCBs 126 and 169 (Kannan *et al.*, 1987). Fish are unable to metabolize TCB to the same extent as mammals, and therefore usually have higher burdens of TCB relative to more highly chlorinated coplanar congeners (Kannan *et al.*, 1989; Koistinen *et al.*, 1989; Murk *et al.*, 1994a).

PCBs are associated with a number of toxicological responses in fish, including disruption of metabolic processes, reproduction and immune systems (Von Westernhagen *et al.*, 1981; Folmar *et al.*, 1982; Johnson *et al.*, 1988; Casillas *et al.*, 1991; Goksoyr *et al.*, 1991; Hontela *et al.*, 1992). TCB, in particular, impairs thymic, hepatic, reproductive, and immune function in fish in laboratory studies (Folmar *et al.*, 1982; Gooch *et al.*, 1989; Van Der Oost *et al.*, 1991; Harrad *et al.*, 1992; Lindstrom-Seppa *et al.*, 1994). The toxicity of TCB, based on the ability to induce cytochrome P-450 enzyme activities in rat hepatoma H-4-II E cells, is an order of magnitude greater than mono-*ortho* and three orders of magnitude greater than di-*ortho* substituted coplanar PCB congeners respectively (Safe, 1990). The relative sensitivity of toxicological endpoints to TCB exposure has not yet been established in fish.

The objective of this research was to investigate the relative sensitivity of several biomarkers to TCB exposure in Arctic grayling (*Thymallus arcticus*).

Conducted as an international collaborative effort between U.S. and Canadian scientists investigating arctic contamination, the intent of the study was to examine the potential toxicity of one of the most potent low-weight PCB congeners in an arctic freshwater fish species sensitive to contaminant exposure (Buhl and Hamilton, 1990, 1991). Our selection of doses was designed to produce environmentally-relevant tissue burdens, based on reported TCB concentrations in feral fish (Melancon and Lech, 1976; Koistinen *et al.*, 1989; Muir *et al.*, 1993) The suite of biomarkers examined is shown in Table 2.1.

Table 2.1. Study design: biomarkers evaluated

Hepatic Enzyme Induction:	•	ethoxyresorufin O-deethylase (EROD)
Plasma sex steroids:	•	testosterone
	•	17ß-estradiol
Plasma thyroid hormones:	•	T3: triiodothyronine
	•	T4: thyroxine
Vitamins:	•	tocopherol (vitamin E)
liver, kidney and plasma	•	retinol (vitamin A)
	•	didehydroretinol
	•	retinyl palmitate (liver and kidney only)
Plasma chemistry:	•	osmolarity
	•	glucose
	•	electrolytes: Na, Cl, K
Histology:	•	IRND: interrenal nuclear diameter
	•	IRCA: interrenal cell area
	•	TECH: thyroid gland epithelial cell height
	•	THSAP: thyroid follicule shape factor
	•	P1: cell height in kidney first proximal tubule
	•	P2: cell height in kidney second proximal tubule
	•	LND: nuclear diameter of liver cells
	•	LCA: liver cell area

Methods

Experimental conditions

Fish maintenance. Arctic graylingwere wet-lab reared at the Freshwater Institute. Fish were reared in 500-L fiberglass tanks with flowing, aerated, dechlorinated Winnipeg city water.

Chemicals. Uniformly ring-labelled [^{14}C]3,3',4,4'-tetrachlorobiphenyl (PCB 77) was obtained from Sigma Chemical Co (St. Louis, MO). Unlabelled PCB 77 was purchased from UltraScientific (N. Kingstown, RI). Gavage solutions were prepared by dissolving PCB 77 in ethanol and warmed gelatin (Sjim *et al.*, 1990).

Dosing of fish. Fiberglass tanks (168 L) were used to hold exposed fish in groups of 12 per tank. All tanks had translucent white plexiglass tops and each tank received at least 2 L of aerated water per gram of fish each day. Temperature ranged from 10.1 to 10.5°C and the photoperiod was adjusted to 12 h light and 12 h darkness. Fish were fed Martin Feeds trout food at a ration of 1% wet body weight every second day. All fish were starved for 2 d prior to sampling. Prior to dosing, fish were lightly anaesthetized in water containing tricaine methanesulphonate (TMS, 0.38 mmol/L) solution neutralized to tank pH with ammonium hydroxide and NaCl (150 mmol/L) approximately iso-osmotic with fish plasma. Fish were fitted with visual implant tags (Northwest Marine Technology, Seattle, WA) and allowed 2 wks recovery and acclimation prior to dosing.

After acclimation, fish were again lightly anaesthetized in pH-neutral TMS. When the fish lost equilibrium, they were weighed and either given a single oral dose

of gelatin/ethanol containing concentrations of 0 (control), 10, 108 or 998 ng [^{14}C] TCB/kg fish weight (0.03, 0.37, and 3.42 µmol/kg respecively). Fish recovered from the procedure in anaesthetic-free water within 3 min.

Blood and tissue sampling. After 7, 30, or 90 days, fish were anaesthetized by adding pH-neutralized TMS (0.76 mmol/L) solution. Immobilization was complete within 30 s. Blood was removed from the caudal vessels in 2-3 min using preheparinized 3- or 5- mL syringes and 18 gauge needles. Plasma was immediately separated by centrifugation and stored at -90°C in polyethylene vials. To minimize potential diurnal effects, samples were taken between 0930 and 1100 h each day. Immediately after blood sampling, tissues were quickly dissected out, weighed and processed as required.

Disposition

Subsamples (0.01-0.5 g) of bile, blood, head kidney, gill, gonad, intestine, kidney, liver, fat and muscle and stomach were weighed and oxidized using a sample oxidizer (Model 306, Packard Instrument Company, Downer Grove, IL). Radioactivity was collected using 4-5 mL Carbosorb and 12-13 mL PCS:xylene. Oxidation burn times ranged from 15-45 secs depending on the amount and moisture content of the tissue. Calibration was performed using a 5000 dpm standard (Amersham International plc, Amersham, UK). Blanks and standards were run at a rate of 1:50 samples. Radioactivity was measured in a liquid scintillation counter (Model LS 7500, Beckman). Initial mass balance calculations suggested that recovery of the administered dose was lower than expected. To test recovery of the radioactive ^{14}C in wet tissues, subsamples of select tissues (gonad, liver, intestine, muscle, and carcass) were subsequently freeze-dried, homogenized, and extracted with toluene. The toluene extracts were then analyzed in the same methods as described above. Values from extracted samples were typically lower (28-77%) than

non-extracted samples in tissues other than muscle, in which extracted samples were higher than unextracted samples (38%). Data from extracted samples (rather than direct oxidation) are reported.

Blanks were consistently below detection and calibration standards fell within the acceptable range in all cases. Intestine, gonad and muscle samples were analyzed in replicate (13-22% of samples); average coefficient of variation was 19%, 11%, and 13% respectively by tissue.

Percent of total dose recovered was based on summing the recovered dose in each tissue analyzed, assuming that muscle comprises 70% of the body weight (Fok *et al.*, 1990), and that 1 ml blood=1.1 g and that blood comprises 3% of the body weight (Gingerich and Pityer, 1989). As the total weight of visceral fat was not determined, this could not be included in the calculation of total dose recovered. Stomach subsamples were only analyzed in the fish exposed for 7 days.

EROD assays

Microsomes were prepared immediately from the fresh livers, following the methods of James *et al.* (1979) and Stegeman *et al.* (1981). EROD analysis was performed using methods modified from Pohl and Fouts (1980), Prough *et al.* (1978), and Klotz *et al.* (1984).

Reproductive steroid analysis

Plasma 17ß-estradiol and testosterone were determined by radioimmunoassay (RIA) following the methods of Fitzpatrick *et al.* (1986). 300 µl plasma was extracted with diethyl ether and reconstituted in 1 ml phosphate-buffered saline with gelatin. 100 µl aliquots of extract were assayed for both 17ß-estradiol and testosterone, using 1-1250 pg standards. All samples were analyzed in replicate.

Intra-assay variability was <10% for testosterone and <4% for 17ß-estradiol. Inter-assay variability was 5.6% for 17ß-estradiol and 29% for testosterone.

Plasma chemistry

Plasma electrolytes, glucose and osmolarity were determined using the methods outlined in Brown *et al.* (1986).

Vitamins

Tocopherol (vitamin E), retinol (vitamin A), didehyrdoretinol, retinyl palmitate in plasma, liver and kidney were analyzed by HPLC, following the methods described in Palace and Brown (1994).

Thyroid hormones

L-Thyroxine (T4) and L-triiodothyronine (T3) were determined in plasma using the method of Omeljaniuk *et al.* (1984).

Histology

Following plasma sampling, the subpharyngeal tissue containing the thyroid follicles was dissected out. Also, pieces of liver, anterior and posterior kidney (removed from the mid-region) were sampled. All tissues were preserved in Bouin's solution for 24 h. Subsequently, tissues were washed for several days in 70% ethanol, dehydrated in n-butanol and embedded in paraffin. Tissues were sectioned at 8 µm and microscopic slides were stained with Harris' hematoxylin and eosin (Edwards, 1967). The microslides were magnified and projected onto a Summagraphics data tablet connected to a microcomputer. Measurements were performed using SigmaScan software.

Thyroid activity was assessed by measuring the height of the follicular epithelial cells (TECH) and follicular shape factor (deviation from perfect sphere = 1.0). Four cell heights were measured from each of the ten follicles (40) per fish. Shape factor (THSAP) was calculated from the same follicles based on the formula: $THSAP=(4\pi \times area/perimeter^2)$.

To determine interrenal nuclear size (IRND), 50 round nuclear diameters for each fish were randomly selected and measured. Because salmonid interrenal tissue is diffusely distributed cords or clusters of cells, measurements were made from three different areas of the anterior or head kidney. The relative size of the interrenal cells, as mean interrenal cell cross-sectional area (IRCA), was estimated by the number of nuclei per unit area (approx. 15,000 μm^2).

The diameters of 50 round hepatocyte nuclei (LND) were similarly determined as an indicator of liver cell activity. Relative hepatocyte size (LCA) was also determined as for interrenal cells.

In order to determine effects on kidney cells and tubules, measurements were made of the first (P1) and second (P2) proximal tubule cells for each fish. Two cell heights were measured from opposite sides of the first 12 symmetrical cross sections from both first and second proximal tubules.

Statistical analysis

For each time after exposure, dose-related differences in biomarker response were examined using t-tests. Spearman correlation and regression analyses identified statistically significant relationships between tissue burdens and biomarker responses. A significance level of $p<0.05$ was applied in all cases.

All results are presented based on extractable ^{14}C concentrations, and therefore may represent parent compound or metabolites. Although TCB is not easily metabolized by fish (Niimi and Oliver, 1983; Kulkarni and Karara, 1990), one might expect some conversion to hydroxy-metabolites (Melancon and Lech, 1976).

Results

Tissue distribution

The relative tissue distribution of the recovered dose did not differ by treatment, and did not significantly change with treatment time, with the exception of the gut, for which the recovered dose at 7 days was greater than at 90 days (Table 2.2). In addition, there was a tendency toward a greater percentage of the recovered dose to reside in the muscle with time. A greater percentage of the recovered dose was recovered in the gonads of females than males, reflecting the higher gonadal somatic index (GSI) for females, and the high lipid content of ovary tissue (Larsson *et al.*, 1993). At 7 and 30 days, the percent of dose recovered in the gonads of female fish was strongly correlated with GSI (r_s=0.94-1.0), while there was no relationship between these factors in males at any sampling time. Less than 50% of the dose was recovered in fish (representing all doses and treatment times) for which mass balance sampling and analysis were conducted (n=9, data not shown). Total recovered dose was typically highest at 30 days, followed by a gradual elimination by 90 days.

On a concentration basis, bile, gut and fat generally contained the highest levels of TCB. Extractable concentrations of TCB in gonad, liver, and muscle were typically an 5-10x lower than the administered oral dose (Table 2.3).

Biomarker response

Biomarker response by treatment and sampling time are shown in Table 2.4. EROD was the most sensitive biomarker, showing a pronounced induction in the 10, 100 and 1000 ng/g treatment groups over controls after 7 days (Fig. 2.1). Hepatic retinol, retinyl palmitate and plasma glucose were elevated in the 1000 ng/g group at 7 days. After 30 days, the 100 and 1000 ng/g treatment groups continued to show EROD induction, while retinyl palmitate and plasma glucose concentrations were

Table 2.2 Percent of recovered [^{14}C]TCB dose in select tissues

	Males			Females		
Days post-exposure	**7**	**30**	**90**	**7**	**30**	**90**
Tissue	**10 ng/g**			**10 ng/g**		
	n=2	n=4-5	n=1	n=1	n=1	n=3-4
Gonad	2.3 ± 1.2	8.4 ± 13.3	0.7	56.9	23.2	41.9 ± 34.2
Gut	20.7 ± 0.4	4.7 ± 3.5	7.6	22.6	27.0	10.6 ± 3.3
Liver	1.4 ± 0.8	3.9 ± 4.0	0.6	3.1	1.2	1.4 ± 1.0
Muscle	69.7 ± 4.3	83.6 ± 4.2	87.9	16.9	43.3	59.6 ± 4.5
	100 ng/g			**100 ng/g**		
	n=1-2	n=2-3	n=3-4	n=1	n=3	n=1
Gonad	19.4 ± 22.6	2.9 ± 1.4	4.0 ± 6.8	11.1	49.6 ± 17.8	26.9
Gut	19.2 ± 5.0	19.8 ± 13.9	3.9 ± 2.3	15.3	13.9 ± 8.9	6.5
Liver	9.5 ± 11.6	6.8 ± 4.5	7.6 ± 13.9	3.0	7.3 ± 3.2	3.4
Muscle	74.9	74.1 ± 11.0	93.1 ± 3.7	58.5	59.7 ± 11.4	54.2
	1000 ng/g			**1000 ng/g**		
	n=2	n=3-4	n=1-2	n=1	n=2	n=3-4
Gonad	2.1 ± 0.1	3.1 ± 1.8	7.2 ± 7.5	3.4	18.1 ± 10.6	30.0 ± 13.2
Gut	17.5 ± 10.1	21.0 ± 27.5	4.9	50.4	4.7 ± 2.1	7.4 ± 4.6
Liver	2.1 ± 0.4	4.8 ± 3.0	11.4 ± 12.3	5.8	5.9 ± 2.3	4.1 ± 2.5
Muscle	70.9 ± 10.5	79.4 ± 7.1	79.9	40.4	62.5 ± 8.8	55.6 ± 18.3

Values presented as mean ± 1 standard deviation.

Table 2.3 Concentration of [^{14}C]TCB in select tissues (ng/g w.w.)

Days post-exposure		7		30		90	
Tissue		10 ng/g					
Bile		36.1 ±	41.7	48.3 ±	57.0	3.5 ±	0.3
Blood		0.7 ±	0.3	1.1 ±	0.7	0.4 ±	0.4
Fat		63.9 ±	35.6	166.7 ±	95.8	34.2 ±	3.2
Gill		1.2 ±	1.3	60.5 ±	38.3	0.3 ±	0.1
Gonad		2.3 ±	2.2	3.5 ±	2.6	1.2 ±	0.5
	males	2.9 ±	2.8	2.9 ±	2.2	0.5	
	females	1.2		6.7		1.4 ±	0.4
Gut		19.4 ±	6.0	3.4 ±	5.5	NA	
Kidney		2.5 ±	2.0	NA		0.8 ±	0.4
Liver		2.2 ±	2.3	3.8 ±	4.4	0.4 ±	0.1
Muscle		1.3 ±	2.0	1.6 ±	0.4	0.7 ±	0.2
		100 ng/g					
Bile		50.8 ±	29.8	153.9 ±	69.1	105.4 ±	108.6
Blood		6.4 ±	7.0	2.0 ±	0.7	3.0 ±	3.6
Fat		172.3 ±	55.8	400.0 ±	97.8	319.2 ±	184.3
Gill		5.7 ±	6.0	2.4 ±	1.4	4.9 ±	4.6
Gonad		18.1 ±	26.0	6.8 ±	4.9	9.5 ±	6.4
	males	27.1 ±	29.6	5.3 ±	2.9	7.3 ±	5.0
	females	0.6		8.2		18.1	
Gut		60.1 ±	57.0	8.5 ±	5.5	NA	
Kidney		9.6 ±	12.4	NA		9.1 ±	5.6
Liver		30.3 ±	44.9	15.8 ±	13.3	14.2 ±	13.1
Muscle		8.2 ±	8.9	5.1 ±	2.4	10.8 ±	7.0
		1000 ng/g					
Bile		5548.0 ±	4639.6	2413.7 ±	1230.5	2637.1 ±	2047.8
Blood		33.6 ±	25.3	44.4 ±	22.8	39.1 ±	24.8
Fat		2922.9 ±	2968.5	3490.0 ±	2870.7	3661.4 ±	2555.3
Gill		64.6 ±	93.7	60.4 ±	38.3	31.2 ±	15.7
Gonad		77.1 ±	87.6	118.3 ±	42.3	128.5 ±	98.7
	males	111.5 ±	90.2	129.4 ±	35.6	152.4 ±	3.2
	females	7.7		96.4 ±	60.7	116.5 ±	125.3
Gut		371.3 ±	465.4	170.5 ±	137.8	NA	
Kidney		81.9 ±	95.6	NA		53.1 ±	56.1
Liver		211.1 ±	217.7	274.7 ±	114.7	179.3 ±	126.4
Muscle		342.3 ±	276.8	81.8 ±	54.9	47.0 ±	44.1

Sample sizes: n=3 for all treatment groups 7 days post-exposure, and n=6 for all treatment groups 30 and 90 days post-exposure
Values presented as mean ± 1 standard deviation.
NA=not available, not determined

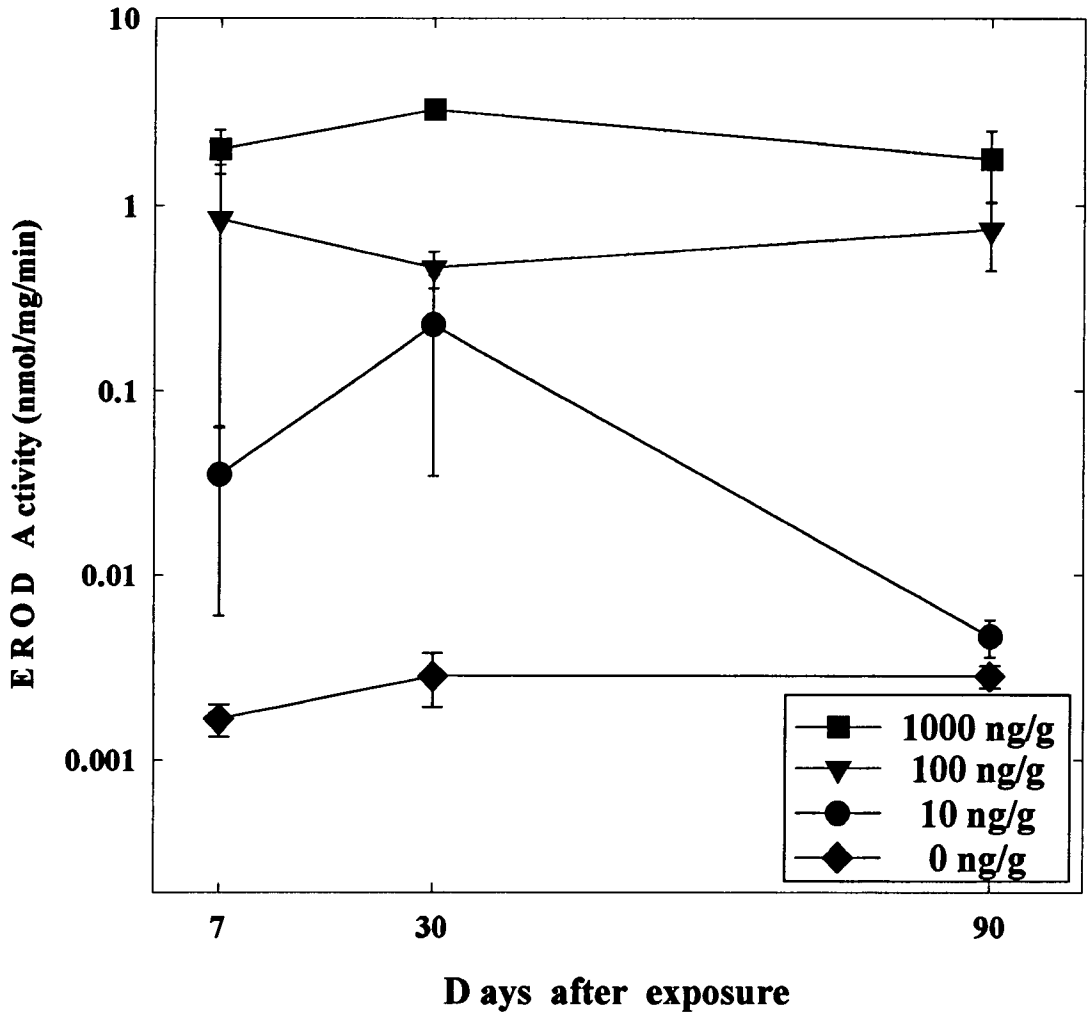

Figure 2.1 TCB-induced EROD induction in arctic grayling

Table 2.4 Biomarker response by dose and treatment time

	Days post-exposure		7 days			
	Dose (ng/g)		0	10	100	1000
			n=3	n=3	n=3	n=3
General Health Parameters		*units*				
	Initial Wt	*g*	285.0 ± 58.8	291.3 ± 1.2	307.0 ± 98.3	329.7 ± 34.9
	Post-experimental Wt	*g*	304.7 ± 21.2	289.7 ± 11.6	322.3 ± 95.2	314.3 ± 33.2
	Condition Factor		1.0 ± 0.2	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.1
	Specific Growth		0.008 ± 0.018	-0.001 ± 0.020	-0.002 ± 0.007	-0.002 ± 0.005
	LSI		1.3 ± 0.2 a	1.1 ± 0.6 ab	0.9 ± 0.1 b	1.0 ± 0.4 ab
	GSI					
	males			1.3 ± 0.1 a	2.4 ± 0.1 b	2.9 ± 1.4 b
	females		8.6 ± 6.2	19.5	14.7	4.5
Hepatic enzyme induction						
	EROD	*nmol/mg/min*	0.002 ± 0.001 a	0.035 ± 0.050 b	0.846 ± 1.356 c	1.984 ± 0.905 c
Vitamins						
Hepatic	Retinol	*µmol/kg*	9.0 ± 0.9 a	10.9 ± 3.9 ab	9.5 ± 7.8 ab	13.0 ± 1.9 b
	Didehyrdroretinol	*µmol/kg*	40.9 ± 7.5	52.4 ± 14.1	50.0 ± 31.8	54.0 ± 6.4
	Didehyrdroretinol/retinol		4.6 ± 0.8	5.0 ± 1.4	6.2 ± 2.3	4.2 ± 0.6
	Tocopherol	*µmol/kg*	108.2 ± 37.3	213.7 ± 103.1	203.1 ± 118.7	221.0 ± 74.3
	Retinyl palmitate	*µmol/kg*	854.3 ± 132.9 a	1622.2 ± 1495.0 ab	877.3 ± 755.3 ab	1964.9 ± 201.7 b
Renal	Retinol	*µmol/kg*	11.9 ± 7.5	11.3 ± 1.0	9.1 ± 2.1	15.2 ± 3.8
	Didehyrdroretinol	*µmol/kg*	69.3	74.2 ± 10.9	65.8 ± 35.6	56.8 ± 11.4
	Didehyrdroretinol/retinol		3.4	6.7 ± 1.6 a	7.3 ± 1.8 a	3.8 ± 0.5 b
	Tocopherol	*µmol/kg*	283.7 ± 35.1	264.7 ± 102.6	264.5 ± 44.1	247.9 ± 44.5
	Retinyl palmitate	*µmol/kg*	296.5 ± 473.2	350.1 ± 175.4	201.0 ± 63.2	987.0 ± 416.8
Plasma	Retinol	*µmol/L*	0.7 ± 0.1	0.8 ± 0.4	0.8 ± 0.2	0.9 ± 0.2
	Didehyrdroretinol	*µmol/L*	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
	Didehyrdroretinol/retinol		0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.0	0.4 ± 0.2
	Tocopherol	*µmol/L*	85.5 ± 17.8	96.9 ± 54.2	119.6 ± 41.4	86.8 ± 25.5
Plasma sex steroids						
	17ß-estradiol					
	males	*ng/ml*		0.3	0.5 ± 0.0	0.6 ± 0.4
	females	*ng/ml*	1.2 ± 0.5	0.7		1.1
	Testosterone					
	males	*ng/ml*		1.9 ± 0.3	2.2 ± 0.8	2.5
	females	*ng/ml*	2.5 ± 0.8	1.2		1.4
Plasma chemistry						
	Osmolarity	*mosm/L*	297.0 ± 6.0 a	297.0 ± 3.5 a	305.7 ± 3.5 b	301.7 ± 3.5 ab
	Glucose	*mmol/L*	4.3 ± 0.3 a	4.1 ± 0.2 ab	5.0 ± 0.7 ab	4.8 ± 0.5 b
	Cl	*mmol/L*	123.3 ± 2.9	128.0 ± 5.2	129.3 ± 6.4	132.7 ± 3.1
	K	*mmol/L*	2.7 ± 0.2	2.8 ± 0.2	2.3 ± 0.6	2.6 ± 0.4
	Na	*mmol/L*	144.2 ± 2.5	143.1 ± 0.8	148.8 ± 3.6	148.6 ± 4.2
Plasma thyroid hormones						
	T3	*nmol/L*	7.9 ± 2.7	5.1 ± 1.5	5.3 ± 1.4	6.7 ± 1.7
	T4	*nmol/L*	3.7 ± 0.7	2.6 ± 0.8	2.9 ± 0.4	3.9 ± 1.6
	T3/T4		2.1 ± 0.4	2.2 ± 1.2	1.9 ± 0.6	1.8 ± 0.4
Histology						
	Interrenal Cell area	*sq/µm*	124.2 ± 18.4	120.8 ± 19.2	115.0 ± 9.7	117.2 ± 4.9
	Interrenal Nuclear Diam.	*µm*	6.1 ± 0.2	5.9 ± 0.2	5.8 ± 0.1	5.4 ± 0.4
	TECH	*µm*	4.6 ± 0.9	4.9 ± 0.9	4.3 ± 0.6	3.6 ± 0.5
	THSAP		0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0	0.9 ± 0.1
	Kidney P1	*µm*	19.6 ± 1.1	18.9 ± 2.2	17.7 ± 0.1	18.1 ± 0.5
	Kidney P2	*µm*	16.9 ± 0.8 a	17.4 ± 0.5 ab	16.3 ± 0.5 ac	16.9 ± 0.2 a
	Liver Nuclear Diam.	*µm*	5.3 ± 0.1 a	5.4 ± 0.4 ab	5.0 ± 0.1 bc	5.4 ± 0.0 ab
	Liver Cell Area	*sq/µm*	155.1 ± 16.1	147.7 ± 27.0	156.8 ± 15.6	159.1 ± 15.1

Table 2.4 Biomarker response by dose and treatment time (continued)

	Days post-exposure		30 days			
	Dose (ng/g)		0	10	100	1000
			n=-6	n=6	n=6	n=6
General Health Parameters		*units*				
	Initial Wt	*g*	259.3 ± 13.7	288.2 ± 61.0	310.7 ± 53.1	268.0 ± 66.9
	Post-experimental Wt	*g*	265.0 ± 16.3	286.2 ± 69.3	332.2 ± 60.2	271.0 ± 70.1
	Condition Factor		0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
	Specific Growth		-0.007 ± 0.001	-0.000 ± 0.001	<0.01 ± 0.004	-0.001 ± 0.001
	LSI		1.3 ± 0.5	0.9 ± 0.3	1.3 0.5	1.1 ± 0.3
	GSI					
	males		1.7 ± 0.7	1.7 ± 1.0	1.4 ± 0.1	1.5 ± 0.3
	females		12.0 ± 6.9 a	9.2	17.5 ± 3.4 ab	7.0 ± 0.1 ac
Hepatic enzyme induction						
	EROD	*nmol/mg/min*	0.003 ± 0.002 a	0.227 ± 0.473 ab	0.462 ± 0.261 b	3.236 ± 0.917 c
Vitamins						
Hepatic	Retinol	*µmol/kg*	13.3 ± 3.5	13.4 ± 5.5	10.6 ± 2.9	11.0 ± 2.2
	Didehyrdroretinol	*µmol/kg*	51.6 ± 11.9	57.0 ± 21.3	44.0 ± 13.3	47.4 ± 10.3
	Didehyrdroretinol/retinol		3.9 ± 0.4	4.3 ± 0.3	4.1 ± 0.5	4.3 ± 0.5
	Tocopherol	*µmol/kg*	242.8 ± 97.3	303.8 ± 121.8	239.0 ± 95.1	269.6 ± 96.7
	Retinyl palmitate	*µmol/kg*	1106.0 ± 660.0	1470.0 ± 882.0	1180.0 790.0	924.0 ± 491.0
Renal	Retinol	*µmol/kg*	10.7 ± 4.2	10.4 ± 5.1	10.6 ± 7.4	14.4 ± 10.5
	Didehyrdroretinol	*µmol/kg*	68.8 ± 14.4 a	88.5 ± 15.5 ab	61.8 ± 6.8 ac	74.8 ± 19.8 abc
	Didehyrdroretinol/retinol		7.2 ± 3.5	6.9 ± 2.2	6.9 ± 4.0	4.9 ± 1.9
	Toocopherol	*µmol/kg*	242.2 ± 56.4 a	280.9 ± 116.5 a	171.2 ± 56.2 ab	259.3 ± 77.7 ac
	Retinyl palmitate	*µmol/kg*	893.7 ± 465.9	718.5 ± 519.3	590.2 ± 392.5	851.5 ± 847.7
Plasma	Retinol	*µmol/L*	0.9 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.2
	Didehyrdroretinol	*µmol/L*	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
	Didehyrdroretinol/retinol		0.4 ± 0.2 a	0.5 ± 0.1 ab	0.4 ± 0.1 ac	0.4 ± 0.1 ac
	Tocopherol	*µmol/L*	98.9 ± 26.8	108.3 ± 41.2	73.0 ± 25.5	115.1 ± 71.4
Plasma sex steroids						
	17ß-estradiol					
	males	*ng/ml*	0.3	0.4 ± 0.2	0.2	0.3 ± 0.1
	females	*ng/ml*	0.9 ± 0.3 a	0.5	0.8 ± 0.1 ab	0.5 ± 0.1 ac
	Tesoterone					
	males	*ng/ml*	3.5 ± 0.0	3.5 ± 1.5	3.7 ± 1.8	3.3
	females	*ng/ml*	4.3 ± 4.2	2.6	3.1 ± 0.2	1.6 ± 0.0
Plasma chemistry						
	Osmolarity	*mosm/L*	301.3 ± 3.7 a	299.3 ± 4.3 a	309.2 ± 5.4 b	308.5 ± 3.7 b
	Glucose	*mmol/L*	5.2 ± 0.3 a	4.8 ± 0.3 ab	4.7 ± 0.8 abc	5.3 ± 0.4 ac
	Cl	*mmol/L*	128.3 ± 4.0 a	129.8 ± 4.8 a	133.0 ± 6.1 ab	126.2 ± 3.7 ac
	K	*mmol/L*	2.5 ± 0.4	2.3 ± 0.8	2.6 ± 1.6	2.5 ± 1.0
	Na	*mmol/L*	144.9 ± 2.1	145.4 ± 3.9	146.9 ± 2.9	146.3 ± 1.6
Plasma thyroid hormones						
	T3	*nmol/L*	5.0 ± 1.2	5.5 ± 2.0	5.1 ± 1.7	4.3 ± 1.3
	T4	*nmol/L*	3.8 ± 1.3	3.2 ± 0.9	3.0 ± 0.8	2.7 ± 0.7
	T3/T4		1.4 ± 0.5	1.8 ± 0.4	1.8 ± 0.5	1.7 ± 0.7
Histology						
	Interrenal Cell Area	*sq/µm*	111.9 ± 2.4	108.6 ± 15.1	110.5 ± 6.9	119.8 ± 13.3
	Interrenal Nuclear Diam.	*µm*	5.5 ± 0.3	5.8 ± 0.2	5.5 ± 0.2	5.7 ± 0.1
	TECH	*µm*	4.3 ± 0.8	4.2 ± 0.4	4.4 ± 0.8	4.4 ± 0.7
	THSAP		0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
	Kidney P1	*µm*	19.4 ± 1.6	18.6 ± 1.9	19.2 ± 1.5	19.4 ± 0.9
	Kidney P2	*µm*	17.1 ± 0.8	16.9 ± 0.9	17.4 ± 0.7	17.2 ± 0.5
	Liver Nuclear Diameter	*µm*	5.3 ± 0.1	5.3 ± 0.3	5.6 ± 0.3	5.4 ± 0.1
	Liver Cell Area	*sq/µm*	149.6 ± 30.1	156.8 ± 25.7	167.9 ± 15.8	172.2 ± 13.3

Table 2.4 Biomarker response by dose and treatment time (continued)

	Days post-exposure		90 days			
	Dose (ng/g)		0	10	100	1000
			n=6	n=5	n=5	n=6
General Health Parameters		*units*				
	Initial Wt	*g*	290.7 ± 50.6	278.6 ± 57.3	322.4 ± 119.6	292.0 ± 70.5
	Post-experimental Wt	*g*	301.2 ± 47.4	299.2 ± 59.0	331.6 ± 115.1	294.3 ± 73.0
	Condition Factor		1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.0
	Specific Growth		<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01	<0.01 ± <0.01
	LSI		1.1 ± 0.6 a	1.6 ± 0.4 ab	1.0 ± 0.2 ac	1.3 ± 0.5 abc
	GSI					
	males		1.4 ± 0.4	1.1	1.3 ± 0.2	1.1 ± 0.0
	females		14.7 ± 9.3	17.1 ± 7.1	7.0	19.7 ± 12.3
Hepatic enzyme induction						
	EROD	*nmol/mg/min*	0.003 ± 0.001	0.005 ± 0.002	0.743 ± 0.664	1.760 ± 1.778
Vitamins						
Hepatic	Retinol	*µmol/kg*	6.2 ± 1.4 a	4.8 ± 1.4 ab	9.0 ± 3.7 a	6.8 ± 1.5 ac
	Didehyrdroretinol	*µmol/kg*	17.2 ± 8.8	11.6 ± 5.5	22.2 ± 10.6	13.1 ± 4.6
	Didehyrdroretinol/retinol		2.6 ± 1.0	2.4 ± 0.6	2.3 ± 0.6	1.9 ± 0.5
	Tocopherol	*µmol/kg*	150.9 ± 135.7	72.7 ± 52.3	163.6 ± 99.2	65.1 ± 41.9
	Retinyl palmitate	*µmol/kg*	1330.0 ± 931.0	1138.0 ± 844.0	1879.0 ± 1394.0	669.0 ± 293.0
Renal	Retinol	*µmol/kg*	8.0 ± 6.3	7.8 ± 4.3	12.1 ± 12.5	8.8 ± 6.9
	Didehyrdroretinol	*µmol/kg*	11.3 ± 13.8	4.5 ± 2.6	5.9 ± 4.4	4.4 ± 3.3
	Didehyrdroretinol/retinol		1.2 ± 1.3	0.5 ± 0.2	0.7 ± 0.4	0.4 ± 0.2
	Tocopherol	*µmol/kg*	175.3 ± 76.9	151.7 ± 92.1	176.0 ± 35.7	149.0 ± 43.2
	Retinyl palmitate	*µmol/kg*	595.0 ± 470.0	611.0 ± 420.0	738.0 ± 660.0	463.0 ± 334.0
Plasma	Retinol	*µmol/L*	0.8 ± 0.3	0.8 ± 0.3	1.1 ± 0.3	1.0 ± 0.3
	Didehyrdroretinol	*µmol/L*	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
	Didehyrdroretinol/retinol		0.4 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.2
	Tocopherol	*µmol/L*	109.4 ± 35.2	143.1 ± 86.7	147.4 ± 66.3	91.6 ± 28.8
Plasma sex steroids						
	17ß-estradiol					
	males	*ng/ml*	0.3 ± 0.0	0.3	0.3	2.7 ± 3.4
	females	*ng/ml*	1.1 ± 0.9	1.0 ± 0.5		1.1 ± 0.5
	Testosterone					
	males	*ng/ml*	3.4 ± 0.6	2.4	2.5 ± 1.3	4.6 ± 1.9
	females	*ng/ml*	3.3 ± 1.8	2.9 ± 1.9	1.5	2.9 ± 0.9
Plasma chemistry						
	Osmolarity	*mosm/L*	290.8 ± 5.4 a	295.2 ± 3.7 ab	296.6 ± 3.0 ab	297.5 ± 4.6 b
	Glucose	*mmol/L*	5.0 ± 0.7 a	4.6 ± 0.4 ab	5.4 ± 0.7 abc	5.6 ± 0.3 ac
	Cl	*mmol/L*	124.3 ± 8.4	122.6 ± 4.6	123.2 ± 2.6	124.5 ± 1.8
	K	*mmol/L*	2.1 ± 0.4	2.6 ± 0.5	2.0 ± 0.4	2.4 ± 0.6
	Na	*mmol/L*	144.9 ± 2.0	145.7 ± 4.7	145.4 ± 3.4	147.3 ± 3.1
Plasma thyroid hormones						
	T3	*nmol/L*	5.7 ± 1.1	4.5 ± 1.4	6.6 ± 2.6	5.4 ± 2.8
	T4	*nmol/L*	2.0 ± 0.9	2.5 ± 1.5	2.2 ± 0.5	2.9 ± 1.1
	T3/T4		4.2 ± 4.1	2.1 ± 1.0	3.0 ± 1.4	1.9 ± 0.7
Histology						
	Interrenal Cell Area	*sq/µm*	111.3 ± 13.0	102.9 ± 8.2	97.9 ± 8.6	100.8 ± 13.4
	Interrenal Nuclear Diam.	*µm*	5.8 ± 0.1	5.8 ± 0.2	5.8 ± 0.2	5.6 ± 0.2
	TECH	*µm*	4.2 ± 0.3	4.1 ± 0.4	4.5 ± 0.7	4.2 ± 0.4
	THSAP		0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0
	Kidney P1	*µm*	18.4 ± 1.5	20.0 ± 1.2	18.2 ± 1.2	19.8 ± 1.9
	Kidney P2	*µm*	17.9 ± 0.9	17.8 ± 1.0	17.6 ± 1.2	17.5 ± 0.6
	Liver Nuclear Diameter	*µm*	5.6 ± 0.2	5.6 ± 0.2	5.5 ± 0.2	5.6 ± 0.3
	Liver Cell Area	*sq/µm*	187.7 ± 32.8	173.8 ± 33.8	156.3 ± 41.5	185.6 ± 26.3

Values presented as means ± 1 standard deviation.
Values with different postscripts are statistically different from one another, based on unpaired t-testing with unequal variances when appropriate.

similar to controls in all treatment groups. After 30 days, the 100 and 1000 ng/g treatment groups were showed plasma hyperosmolarity relative to control fish, and after 90 days, plasma osmolarity remained elevated in the highest dose group. After 90 days, EROD activities were not statistically different, probably because of high variability within treatment groups. There was an increase in plasma osmolarity in the high-dose fish relative to controls after 90 days. There were no significant changes in biomarker response for plasma sex steroids, thyroid hormones, or histological parameters.

To adjust for the variability in tissue burdens within dose groups, biomarker responses were also examined by correlation analysis (Table 2.5). EROD and liver [^{14}C]TCB were highly correlated (r_s=0.93). On a log-log scale, EROD activity displayed a sigmoidal relationship to liver [^{14}C]TCB burdens, with induction threshold below 10 pmol/g (2.9 ng/g w.w.) and approached saturation at approximately 1 nmol/g (290 ng/g w.w.) (Fig. 2.2). Significant, but weak, positive correlations were also observed between liver [^{14}C]TCB and plasma osmolarity and Na.

Table 2.5. Correlations (r_s) between biomarkers and target organ [^{14}C]TCB

		[14-C] TCB in tissue		
		Liver	Gonad	Kidney
Tissue burdens				
	Liver (extracted + oxidized)	**1.00** *	**0.93** *	**0.90** *
	Gonad (extracted + oxidized)	**0.93** *	**1.00** *	**0.89** *
	Kidney (oxidized)	**0.90** *	**0.89** *	**1.00** *
General indicators				
	Specific Growth	0.22	-0.10	-0.13
	Condition Factor	-0.28	-0.18	-0.34
	Liver somatic index	-0.26	-0.10	-0.38
	Gonadal somatic index			
	males	0.18	0.11	0.14
	females	-0.26	-0.24	0.08
Hepatic enzyme induction				
	EROD	**0.93** *	**0.87** *	**0.84** *
Plasma sex steroids				
	17ß-estradiol			
	males	0.04	0.12	0.35
	females	-0.06	0.03	-0.41
	testosterone			
	males	0.20	0.15	0.56
	females	-0.02	0.01	-0.05
Plasma thyroid hormones				
	T3	0.07	0.10	0.21
	T4	0.07	0.12	0.31
	T3/T4	0.09	0.08	-0.04
Vitamins				
Hepatic	Retinol	0.22	0.14	0.23
	Didehydroretinol	0.20	0.06	0.18
	Didehydroretinol/retinol	0.09	-0.08	-0.09
	Retinyl palmitate	-0.03	-0.08	0.09
Renal	Retinol	0.14	0.15	0.16
	Didehydroretinol	0.01	-0.13	-0.02
	Didehydroretinol/retinol	-0.10	-0.28	-0.21
	Retinyl palmitate	0.13	0.18	0.23
	Tocopherol	-0.06	-0.16	-0.06
Plasma	Retinol	-0.16	-0.19	-0.15
	Didehydroretinol	0.01	0.03	-0.01
	Didehydroretinol/retinol	0.24	**-0.32** *	-0.13
	Tocopherol	-0.24	-0.19	-0.14
Plasma chemistry				
	Osmolarity	**0.36** *	0.29	0.15
	Glucose	0.50	0.50	0.28
	Cl	0.14	0.04	0.22
	Na	**0.34** *	**0.39** *	0.29
	K	-0.11	-0.08	-0.09
Histology				
	Interrenal nuclear diam.	-0.29	-0.22	-0.22
	Interrenal cell area	0.13	-0.00	-0.31
	TECH	-0.12	-0.12	-0.45
	THSAP	0.08	0.08	0.11
	P1	-0.16	0.01	-0.26
	P2	-0.10	-0.04	-0.07
	Liver cell area	0.07	0.12	0.23
	Liver cell nuclear diam.	-0.00	0.08	0.19

* denotes significant Spearman correlation coefficient ($p<0.05$)

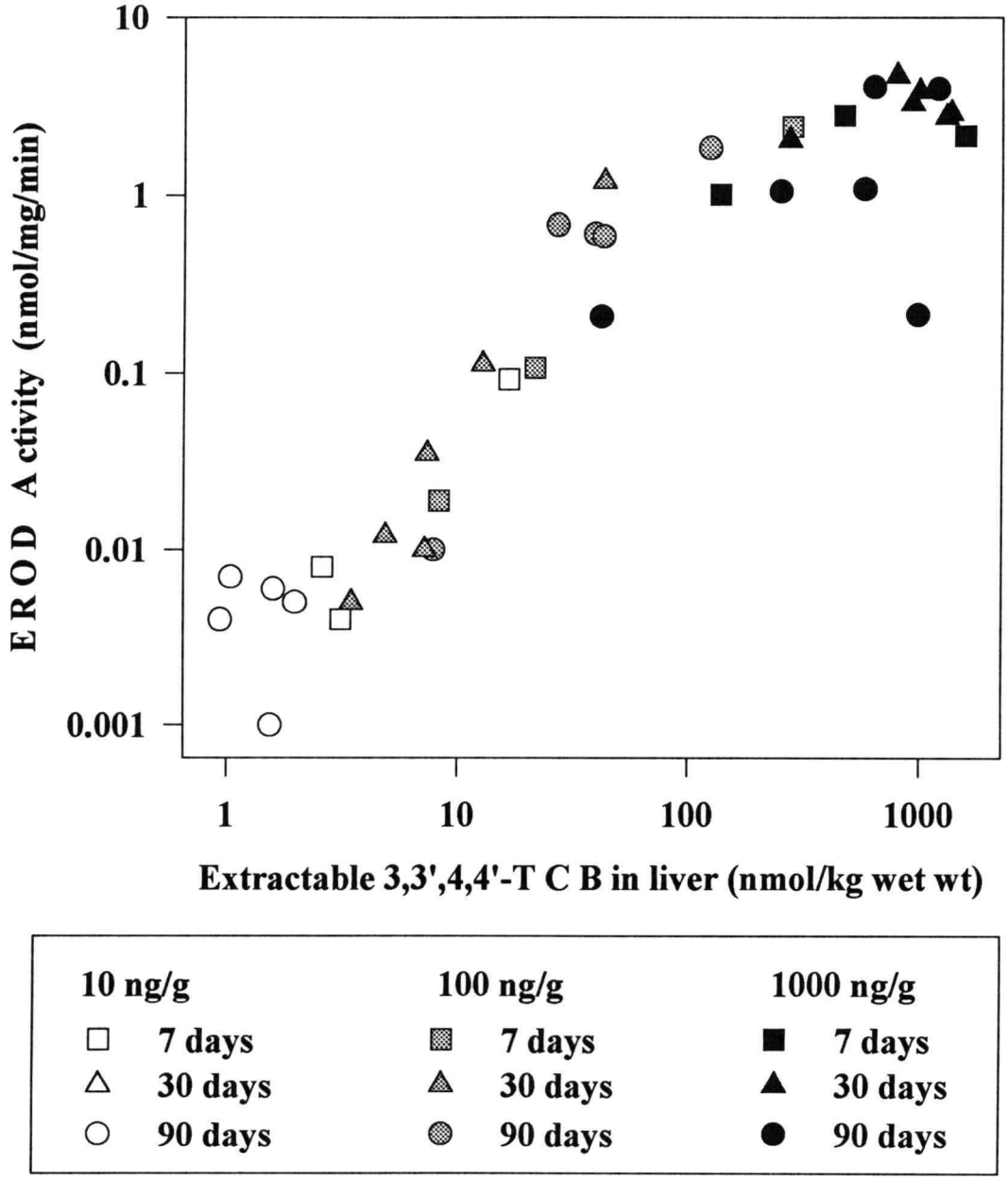

Figure 2.2 TCB-induced EROD induction relative to extractable TCB in liver

Discussion

Tissue distribution

The disposition of [^{14}C]TCB in grayling was similar to PCBs in other salmonids. For example, redistribution from gut to liver, muscle and fat over time was reported for other PCB congeners in fish (Guiney *et al.*, 1977; Gobas *et al.*, 1989; Kulkarni and Karara, 1990). The large variation in tissue distribution among individual fish within a treatment that we experienced also occurred in studies on rainbow trout (Niimi and Oliver, 1983) and birds (Murk *et al.*, 1994b). The higher percent recovered dose in the gonads of female fish reflected both a higher GSI and greater lipid content reported by Larsson *et al.* (1993).

The low recovery (<50%) of the administered dose corresponded well with previous research on the disposition of PCB congeners in fish. Niimi and Oliver (1983) reported that 68% of a single oral TCB dose was absorbed by rainbow trout, which was lower than that for many mono- and di-*ortho* substituted congeners. The same study calculated the half-life of TCB in whole fish as 44 days, an order of magnitude lower than other tetrachlorobiphenyls. Therefore, after 44 days, recovery only 34% of the administered dose would not be unreasonable. Similarly, in a study of TCB in rats, 64% of the oral dose was presumably never absorbed in the gastrointestinal tract (Yoshimura and Yamamoto, 1973). These findings suggested that low assimilation in combination with some elimination, accounts for the low recovery in our experiment. Additional processes that could result in lowered PCB concentrations in fish include growth dilution and maternal transfer to eggs (Sjim *et al.*, 1992).

Biomarker response

EROD was clearly the most sensitive and consistent biomarker response. This was consistent with a similar study by Murk *et al.* (1994b) in eider ducklings. EROD activity, thyroid and retinol status were evaluated 10 days after ducklings were injected with TCB (5-50 mg/kg). Although correlations were observed between liver TCB and thyroid hormones and retinol concentrations in ducklings, only EROD displayed dose-related differences. The results of these two studies reinforced the possibility that EROD induction alone may not be indicative of more ecologically-relevant processes (Lockhart, 1995).

EROD. EROD induction was an anticipated outcome of TCB exposure, as this congener induced P450 1A1 and 1A2 (Clarke, 1986; De Vito *et al.*, 1993), and AHH and EROD in fish (Melancon and Lech, 1983; Janz and Metcalfe, 1991; Monosson and Stegeman, 1991; Tyle *et al.*, 1991; Murk *et al.*, 1994b). We showed increased EROD activity at liver TCB concentrations below 1 μg/g w.w. and decreased activity at liver TCB of 3-6 μg/g, which agreed very well with other studies (Gooch *et al.*, 1989; Monosson and Stegeman, 1991; Lindstrom-Seppa *et al.*, 1994). For example, Lindstrom-Seppa *et al.* (1994) reported increased EROD activity over controls at liver TCB concentrations less than 1 μg/g w.w., followed by decreased activity at TCB concentrations greater than 1.8 μg/g in fathead minnows. Gooch *et al.* (1989) found EROD induction at low doses and strong EROD inhibition at 2.0 μg/g liver TCB in scup. Although there is evidence that TCB may competitively inhibit EROD binding to P-450 enzymes at high microsomal concentrations (Gooch *et al.*, 1989), other researchers have questioned whether other mechanisms may be active (Monosson and Stegeman, 1991).

Sex steroids. Absence of significant differences in plasma sex steroid concentrations after TCB treatment in this experiment perhaps involved the low

sample number in the treatment groups. As sex in grayling is indistinguishable based on external characteristics, the number of fish of each sex in the treatment groups was not known until fish were killed. Unfortunately, there were several dose/duration groups in which the sex ratio was 5:1 or 1:5. Despite the fact that male and female salmonids may have similar levels of androgens and estrogens (Schreck *et al.*, 1973), they may not respond in the same manner to PCB exposure. Although laboratory studies documented decreased plasma sex steroids and other reproductive dysfunction after exposure to Aroclor mixtures (Freeman and Idler, 1975; Sivarajah *et al.*, 1978; Thomas, 1988, 1989; Casillas *et al.*, 1991), target organ dose in this experiment was possibly below the threshold for this toxicological response.

Thyroid hormones. Lack of response of circulating thyroid levels in this study may indicate life-stage or species differences in sensitivity, or again, insufficient dose. In another study, adult rainbow trout fed 500 µg/g Aroclor 1254 for 25-50 days showed no changes in thyroid physiology or serum thyroid hormone levels (Leatherland and Sonstegard, 1979). By contrast, yearling coho salmon fed the same concentration of PCBs experienced depressed serum T3 (Leatherland and Sonstegard, 1978). Aroclor 1254 delayed plasma T3 elevation during smoltification in yearling cohgo salmon (Folmar *et al.*, 1982). Depressed serum T3 and T4 after TCB treatment was also reported for mammals (van den Berg *et al.*, 1988).

Hepatic vitamins. Increased hepatic retinyl palmitate after 7 days following administration of 1000 ng TCB/g was not substantiated by a similar positive correlation between tissue burdens and retinyl palmitate concentrations. And, this increase was opposite of that reported after PCB 126 exposure in lake trout (Palace and Brown, 1994). Oral exposure of lake trout to a similar concentration PCB 126 (1, 40 ng/g fish wt.) resulted in depressed retinol, and didehydroretinol concentrations in liver, and increased renal concentrations of retinyl palmitate (Palace and Brown,

1994). The differences in response between our study and that of Palace and Brown (1994) perhaps reflected the higher potency of PCB 126 to elicit A*h*-receptor mediated effects (Safe, 1984, 1990; Brunström, 1991) as muscle burdens were similar.

We observed a transcient increase in liver retinol concentrations, which is the opposite of observations in mammals (Mercier *et al.*, 1990), birds (Murk *et al.*, 1994b) and fish (Palace and Brown, 1994) after coplanar PCB exposure.

Histology. The lack of sensitivity of histological parameters observed in grayling was similar to that found in other studies. For example, Gooch *et al.* (1989) observed no histological changes in liver after TCB exposure. Leatherland and Sonstegard (1979) reported normal thyroid histology in rainbow trout fed Aroclor 1254.

Plasma chemistry. Our finding of a weak positive correlation between plasma Na and liver TCB burdens was not documented in other studies of PCB-exposed fish. However, increased plasma Na was observed after exposure to endrin and bleached kraft mill effluent (Eisler and Edmunds, 1966; Lindstrom-Seppa and Oikara, 1990).

Relative to environmental exposures. EROD induction was observed in fish with liver and muscle burdens in the range of 2-5 ng/g. w.w. Similar burdens were reported in fish from the Great Lakes, North Atlantic, and Baltic Sea (Niimi and Oliver, 1989; Tarhanen *et al.*, 1989; Monosson and Stegeman, 1991). In North Atlantic flounder, fish from contaminated sites had higher TCB burdens and EROD activities compared to offshore fish. Fish from the Arctic typically have much lower TCB burdens (0.02 - 1 ng/g) (Koistinen *et al.*, 1989; Muir *et al.*, 1993). Therefore,

we would not predict that EROD induction, hypothyroidism, vitamin A deficiency, reproductive impairment or histological changes would be observed at current TCB exposure levels in feral arctic fish.

Conclusion

Tissue distribution of TCB in grayling, in which the most of the recovered dose resided in muscle, gut and gonad, was similar to that of other organochlorines in fish. TCB concentrations in visceral fat and bile were typically an order of magnitude higher than in other tissues. Our finding of a low recovery of TCB supported the findings of other researchers that the assimilation efficiency and half-life of TCB was lower than that of many mono- and di-*ortho* substituted PCBs. Among the biomarkers examined, EROD was clearly the most sensitive and robust response to TCB exposure. However, this also suggested that EROD induction alone was not indicative of other physiologically-relevant responses. Applying our findings to levels of TCB reported in arctic fish, indicated current exposure levels would not result in TCB-related changes in cytochrome P450 activity, vitamin A status, thyroid, or reproductive performance.

Chapter 3

SEX STEROIDS AS A BIOMARKER FOR HEAVY METAL ACCUMULATION IN FRESHWATER FISH IN U.S. ARCTIC LAKES

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Abstract

Metal concentrations in sediment and two species of freshwater fish (lake trout-*Salvelinus namaycush*, grayling-*Thymallus arcticus*) were examined in four arctic lakes in Alaska. Concentrations of mercury (Hg) are naturally high in the sediment relative to uncontaminated lakes in other arctic regions and more temperate locations. If any anthropogenic enrichment has occurred, it is not distinguishable from background variability. The rank-order of metal concentrations in sediment and freshwater lakes is similar for some metals (Hg, Ni) while relative concentrations of other metals (Cd, Pb) in fish tissue among lakes does not directly follow those of sediment, suggesting that calcium (Ca) may mediate metal bioavailability. Concentrations of Cu and Zn in fish do not parallel those in sediment, and are not subject to Ca modulation, indicative of physiological regulation of uptake by fish. At these naturally-occurring levels of metal concentrations, physiological processes may be affected by their occurrence, as evidenced by the negative correlation between plasma hormone levels and lead (Pb) concentrations in *T. arcticus*. Concentrations of metals observed in fish muscle are generally lower than those of marine mammals, indicating that freshwater fish are probably not a major source of these contaminants to Inuit populations.

Introduction

The circumpolar arctic is generally considered to have a mineral-rich geology, as evidenced by the number of mineral deposits located within the Arctic (Crane and Pfirman, 1993). Ecosystem loading of metals locally and regionally can be exacerbated through mining of these deposits. Long-range transport of anthropogenically-derived metals, which has been documented in arctic systems (Maenhaut *et al.*, 1989; Barrie *et al.*, 1992), may also contribute the metal burdens in the arctic environment. There are only three investigations of the contribution of atmospherically-derived metals relative to local geologic material in Arctic ecosystems (Hermanson, 1991, Gubala *et al.*, 1995, Landers *et al.*, in press). The rate of atmospheric deposition of mercury in the mid-continental U.S. has increased 3-4x since pre-industrial levels (Swain *et al.*, 1992). For lakes in Ontario, Canada, Johnson (1987) found that present Pb loadings were 17x higher than historical background, and As, Cd, Hg and Zn loadings were 1-3 x higher.

Heavy metal inputs from natural sources, mining practices and long-range atmospheric transport, are likely to enter inland aquatic systems in the Arctic. In these systems, metals may accumulate in fish and may result in physiological perturbations. In addition, consumption of fish with heavy metal burdens represents a potential source of toxic metals to subsistence communities. There have been several studies documenting tissue burdens of heavy metals in arctic fauna, but sources have not been identified (Smith and Armstrong, 1975; Bohn and Fallis, 1978; Goldblatt and Anthony, 1983; Ronald *et al.*, 1984; Norstrom *et al.*, 1986; Wagemann, 1989; Norheim *et al.*, 1992; Snyder-Conn and Lubinski, 1993). These studies have primarily been monitoring efforts, with little attention on probable sources or effects from heavy metal exposure. There is no published information using lake sediment stratigraphies in conjunction with fish tissue concentrations to evaluate probable

sources in Arctic fauna. Furthermore, until the recent work of Wagemann *et al.* (1993), and McLeay *et al.*(1987) and others, arctic contaminant research has not addressed potential ecological or physiological consequences from heavy metal exposure.

Exposure to heavy metals has numerous sublethal toxicological manifestations in fish, including changes in morphology, histology, physiology, biochemistry, behavior and reproduction. Effects are most often caused by metals binding to sulfhydryl groups of enzymes, by displacing essential trace elements (Ca, Cu, Zn) in physiologically active molecules, or by altering structural and/or functional integrity through oxidation-reduction reactions (Connell and Miller, 1984). Reproductive and developmental systems in particular are very sensitive to many toxicants, including metals. Lead, cadmium, copper, mercury and zinc impair fish reproduction (e.g., Holcombe *et al.*, 1976; McFarlane and Franzin, 1978; Katti and Sathyanesan, 1983, 1984; Tulasi *et al.*, 1992; Pereira *et al.*, 1993). Fish exposed to some metals in the part per billion range in water may exhibit reproductive dysfunction (Connell and Miller, 1984).

The U.S. Environmental Protection Agency (USEPA) Arctic Contaminant Research Project investigated the distribution and effects of metals in arctic lakes by comparison of lake sediments and key aquatic species (Landers *et al.*, 1992). Analysis of sediment cores from four arctic Alaskan lakes provided the basis of comparison of accumulation in fish collected from the same systems. Concentrations of metals and calcium found in the surface sediments of the lakes served as a proxy for the between-lake differences in bioavailable materials. Comparison of the surface sediments with those buried prior to the onset of the significant industrial activity (pre-1900) quantified the additional burden of metals resulting from human activity.

The concentration of metals found in lake trout and grayling muscle and liver were compared to those determined in surface sediments at each site. This comparison, tempered by the relative availability of Ca, described the bioavailability of the metals in the lakes of the U.S. Arctic. Further analysis of specific growth and reproductive factors within each species established the likely significance of the cumulative uptake of these metals.

Methods

Sample collection

Sediment and fish were collected from Elusive, Schrader, Feniak and Desperation lakes, located in the foothills of the Brooks Range (Fig. 3.1). At Elusive Lake, snails were also collected. Limnological and drainage basin characteristics of the four lakes are provided in Table 3.1.

Sediment cores were retrieved and processed during the late winters of 1991 - 1993, using the methods described previously by Gubala *et al.*(1995). Sediment samples were stored cold (approx. 4°C) until the time of analysis. Sediment intervals were analyzed sequentially for total water and carbon content, radionuclides, and metals. Radiometric analyses of ^{210}Pb, ^{137}Cs and ^{7}Be were utilized in a constant rate of supply model (Robbins, 1978) to assign approximate dates of deposition to individual sediment intervals.

Fish were sampled by hook and line during the summers of 3 years (1991-1993). Blood samples (~5 ml) were taken from the caudal vein. Whole livers were removed, weighed, and divided for organic and inorganic analysis and transferred to pre-cleaned I-Chem™ jars and acid-cleaned borosilicate vials with teflon screw-tops respectively. Epaxial muscle samples were excised and processed in the same manner as liver samples. Snails were collected from submerged rocks in the littoral zone of one lake, (Elusive Lake), and placed directly in pre-cleaned I-Chem jars. All samples were frozen (or nearly frozen) on site and shipped by air to the laboratories. Fish gonads were excised and weighed. Sex, weight, length and stomach contents were recorded. Scale samples of grayling were taken for age determination, which was

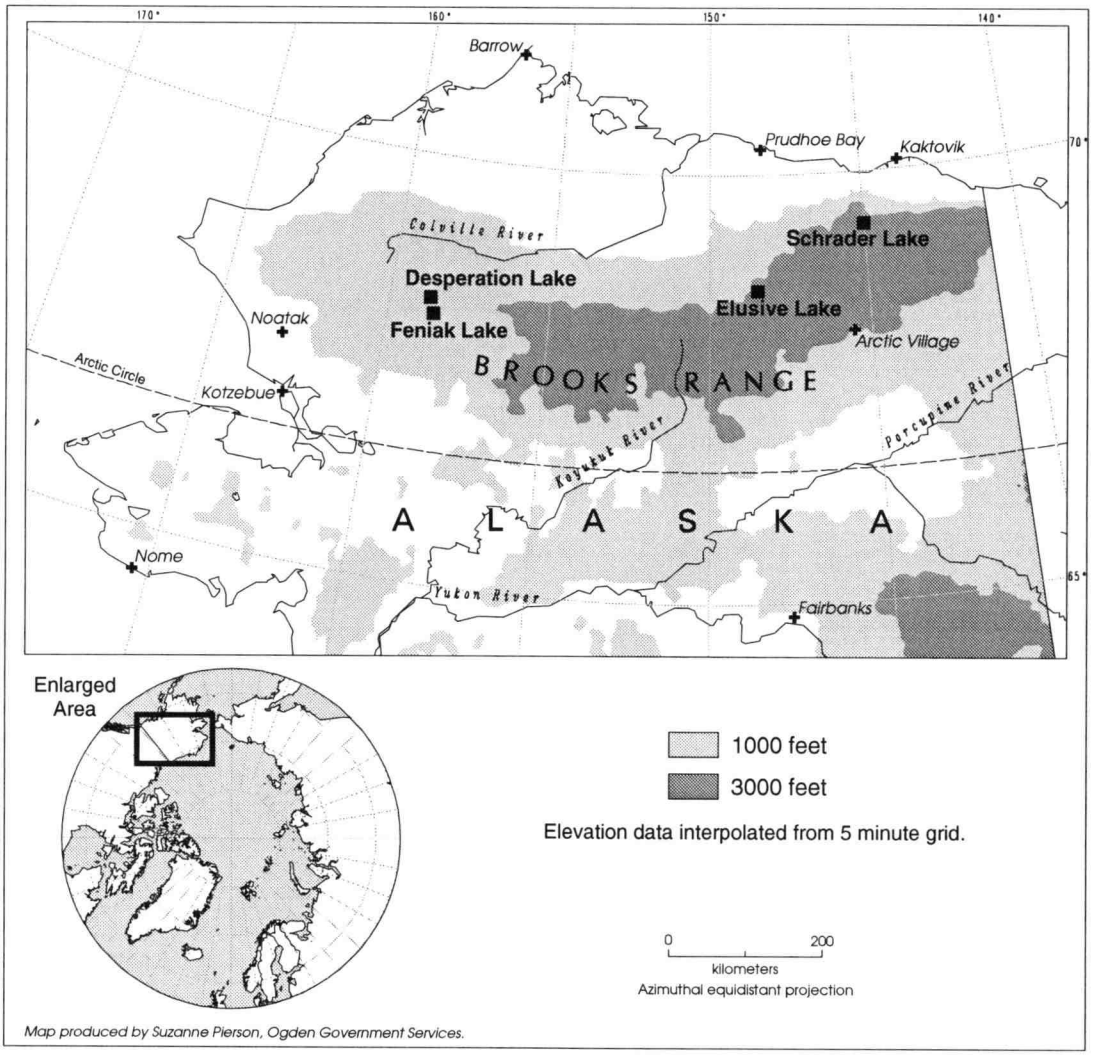

Figure 3.1. Location of sampling sites in the U.S. Arctic

Table 3.1 Limnological characteristics of study lakes

		Elusive	Schrader/ Peters	Feniak	Desperation
Latitude		68° 50' N	69° 22' N	68° 16' N	68° 19' N
Longitude		148° 30' W	144° 60' W	158° 18' W	158° 45' W
Drainage Basin Area	*(km2)*	15	469	213	296
Surface area	*(km2)*	3.3	20.4	17.6	5.9
Drainage basin area/lake surface area		5	24	13	51
Max. Depth	*(m)*	11.4	57	35.7	24.3
Mean Depth	*(m)*	6.8	33	10.2	6.4
pH		na	na	7.92	7.67
Acid neutralizing capacity	*(μeq/L)*	na	na	464	263
Dissolved organic carbon	*(mg/L)*	na	na	1.41	3.09
Chloropyll a (trichromatic)	*(mg/L)*	na	na	<0.001	0.002
Phosphorous (total)	*(μg/L)*	na	na	1	3
Nitrogen (total)	*(μg/L)*	na	na	898	252
Phosphorous/nitrogen		na	na	0.001	0.012

na=not available
Drainage basin area does not include lake surface
Drainage basin and surface area calculated from ARC/INFO by Suzanne Pierson, Computer Services Corporation.
Schrader Lake mean and max. depth from Hobbie (1961).

performed following the method described by Nielsen and Johnson (1983). Liver and gonadal somatic indices (LSI, GSI) were calculated as (liver wt/(total wt-liver wt))*100 and (gonad wt/(total wt-gonad wt))*100, respectively. Fulton-type condition factor was calculated as (weight/(fork length)^3*100) (Jearld, 1983). Characteristics of sampled fish populations for each lake are provided in Table 3.2.

Analysis of sediment

Quantification of metals in the extract was performed using a combination of ICP-MS, graphite furnace and flame atomic absorption, energy dispersive x-ray fluorescence (XRF), and (CVAA) following the methods of Crecelius *et al.* (1991) and Bloom and Crecelius (1983).

Analysis of tissues

All metal analyses (except methylmercury) was performed by Battelle Marine Sciences Laboratory (MSL) on a 500 mg subsample of freeze-dried tissue using the method recommended by the National Oceanic and Atmospheric Administration (1993). With the exception of Hg and Ni, all metals were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Hg was analyzed by stannous chloride reduction, gold amalgamation, and cold vapor atomic absorption (CVAA). Ni was either analyzed by ICP-MS or graphite furnace atomic absorption. Methylmercury analysis was performed on a 50-250 mg subsample of tissue, following the method of Bloom (1989).

Table 3.2 Characteristics of fish sampled from U.S. Arctic lakes

		Elusive	Schrader/Peters	Feniak	Desperation
Years sampled		1991 1993	1991 1992	1992 1993	1992
Lake trout					
N		21	9	12	0
Males		12	3	8	
Females		9	6	4	
Standard Length	*(cm)*	46 ± 6	48 ± 10	52 ± 4	
Weight	*(g)*	1147 ± 630	1355 ± 494	1388 ± 274	
Percent lipid	*liver*	1.93 ± 1.06	11.18 ± 11.61**	1.7 ± 0.73	
	muscle	0.72 ± .72	1.87 ± 1.52	0.60 ± .071	
Grayling					
N		21*	25	16	10
Males		13	12	11	9
Females		6	13	5	1
Standard Length	*(cm)*	32 ± 4	31 ± 3	38 ± 2	34 ± 4
Weight	*(g)*	347 ± 71	327 ±63	539 ± 91	398 ± 127
Age	*(yrs)*	6 ± 1	8 ± 2	7 ± 1	7 ± 1
Percent lipid	*liver*	3.84 ± 5.44	1.75 ± 1.06	1.48 ± 0.58	2.02 ± 1.34
	muscle	2.70 ± 3.12	2.70 ± 2.59	2.45 ± 2.41	5.75 ± 6.4***

Values are presented as arithmetic mean ± standard deviation.
* Sex not determined on two individuals.
** One fish had 38% lipid in liver.
*** One fish had 11% lipid in muscle.

Quality assurance

All tissue analytical batches were accompanied by standard reference materials (SRMs): (sediment: SRM 1646, NIST; fish: DORM-1, DOLT-1 and DOLT-2, National Research Council of Canada, Ottawa, Ontario), matrix spikes, blank spikes, and blanks at a minimum rate of 1:20 samples. Replicate analyses were conducted on 10% of the samples to assess precision of analytical techniques. Following digestion, calibration and minimum detection limit (MDL) check standards were added to the analytical stream at a minimum rate of 1:20 samples. Acceptance criteria by batch were: 1) for SRMs: not more than 20% of the recoveries were outside the certified range, and more than one value was outside the certified range by more than 20%, 2) for spikes: not more than 20% of the recoveries were outside the acceptable 80-120% range, and 3) for duplicates: not more than one set of replicates having a relative percent difference greater than 20%. Duplicate values of the concentrations of analytes reported in sediment in this manuscript varied by less than 5%.

Reproductive steroid analysis

Plasma 17ß-estradiol and testosterone were determined by radioimmunoassay (RIA) following the methods of Fitzpatrick *et al.*(1986). Between 200 - 500 µl plasma was extracted with diethyl ether and reconstituted in 1 ml phosphate-buffered saline with gelatin. 100-200 µl aliquots of extract were assayed for both 17ß-estradiol and testosterone, using 1-1250 pg standards. Concentrations of steroids were determined by the relative percent of ^{3}H-steroid bound to the antibody against known unlabelled standards, as quantified by liquid scintillation counting. All sample analyses were replicated. Extraction efficiency for all species averaged 79 ± 5% for testosterone and 74 ± 6% for 17ß-estradiol. For all three years, intra-assay variability was 10.5 ± 0.6% for testosterone and 10.2 ± 5.5% for 17ß-estradiol. Inter-assay variability was 14.8 ± 4.8% for 17ß-estradiol and 20 ± 3.0% for testosterone.

Statistical analysis

All tissue heavy metal data was log(10) transformed as preliminary data exploration revealed proportional relationships between means and standard deviations for some metals. In addition, standard length, 17ß-estradiol, testosterone, LSI, GSI and condition factor were log(10) transformed prior to correlation/regression and ANOVA analyses. Data for each species were analyzed by GLM ANOVA to determine if length (and age for grayling) differed by site. Since length and weight were highly correlated ($r_s > 0.92$) and because fish weights were determined prior to evisceration and therefore influenced by the mass of the gut contents (which were estimated to range from 0-300 g), correlations were performed only on length measurements. Standard length (tip of upper jaw to the end of the caudal peduncle) was selected for statistical analysis instead of total or fork lengths to avoid the influence of caudal fin abnormalities (Anderson and Gutreuter, 1983). Data were then examined to identify correlations (Spearman, r_s) between tissue heavy metal concentrations and length or age. Site effects for heavy metal concentrations were determined using analysis of covariance (accounting for site and metal covariation with length and age). Species differences in tissue concentrations were examined by ANOVA/ANCOVA by site (adding length as a covariate for those metals exhibiting covariance with length). If the ANCOVA suggested that length was a significant covariant, regression slopes between the two species were tested for equality. The effects of sex and year of sampling were examined by ANOVA.

Individual comparisons among sites, sex, species and year were performed using Neuman-Keuls post-hoc multiple comparison test on the results from the appropriate model. In this manner, if tissue metal concentrations were correlated with fish length, means were adjusted prior to individual comparisons.

Spearman correlations (r_s) and regression analysis were used to identify relationships between heavy metal concentrations in tissues and physiological parameters. For steroid analysis, outliers from replicate analyses were identified using Dixon's test for outliers, $p=0.01$ (as described in Sokal and Rohlf, 1981). In all other cases, a significance level of $p<0.05$ was applied.

Distributions of heavy metal concentrations are presented in figures substituting the MDL for all observations less than MDL. In instances in which it was desirable to calculate summary statistics for datasets with values <MDL, adjusted arithmetic means and standard deviations by site were calculated using Helsel's robust method of the UNCENSOR program, as recommended by Newman *et al.*(1989) and Helsel (1990). These statistics were then compared by ANOVA, followed by Neuman-Keuls multiple comparison testing. This technique was applied only to Ni and Pb in liver.

Results

Heavy metals in sediment

Trace metals in the top 2 cm of sediment are assumed to reside within an "active" layer, capable of interacting with the lake biota, based upon visual observations of insect activity within sediment cores collected from each site. Further evidence from other Alaskan lakes (Gubala *et al.*, 1995), indicates that the top two centimeters of sediment in each lake were deposited during approximately the last 30-40 years. Metals entering the system during this time period may have interacted with the current active biota. The average concentration of metals within the top 2 cm from each lake are then considered to fairly reflect the difference in potentially bioavailable metals between systems.

Cu, Ni and Zn account for the highest concentrations of heavy metals in each system (Fig. 3.2: d,e,h). Ni is particularly high in the sediment of Feniak Lake, reaching nearly 300 μg/g dry weight (dw). Feniak and Desperation Lakes, which reside in close proximity to each other and within a particularly mineral-rich region of the Brooks Range (U.S. Geological Survey., 1991), are higher in Ni and Cu than Elusive and Schrader lakes (Fig. 3.2:d,e). Differences between lakes in Zn are minimal (Fig. 3.2h). Of the other three metals, Cd is highest in Elusive Lake, Hg in Feniak Lake and Pb in Schrader Lake (Fig. 3.2:c,g,f). These pronounced differences between sites allow for a clear comparison with metal accumulation in fish.

Lastly, note that the surface concentrations of Ca in sediments varies markedly between sites (Fig. 3.2a). Elusive Lake is enriched in Ca nearly 10-fold over each of

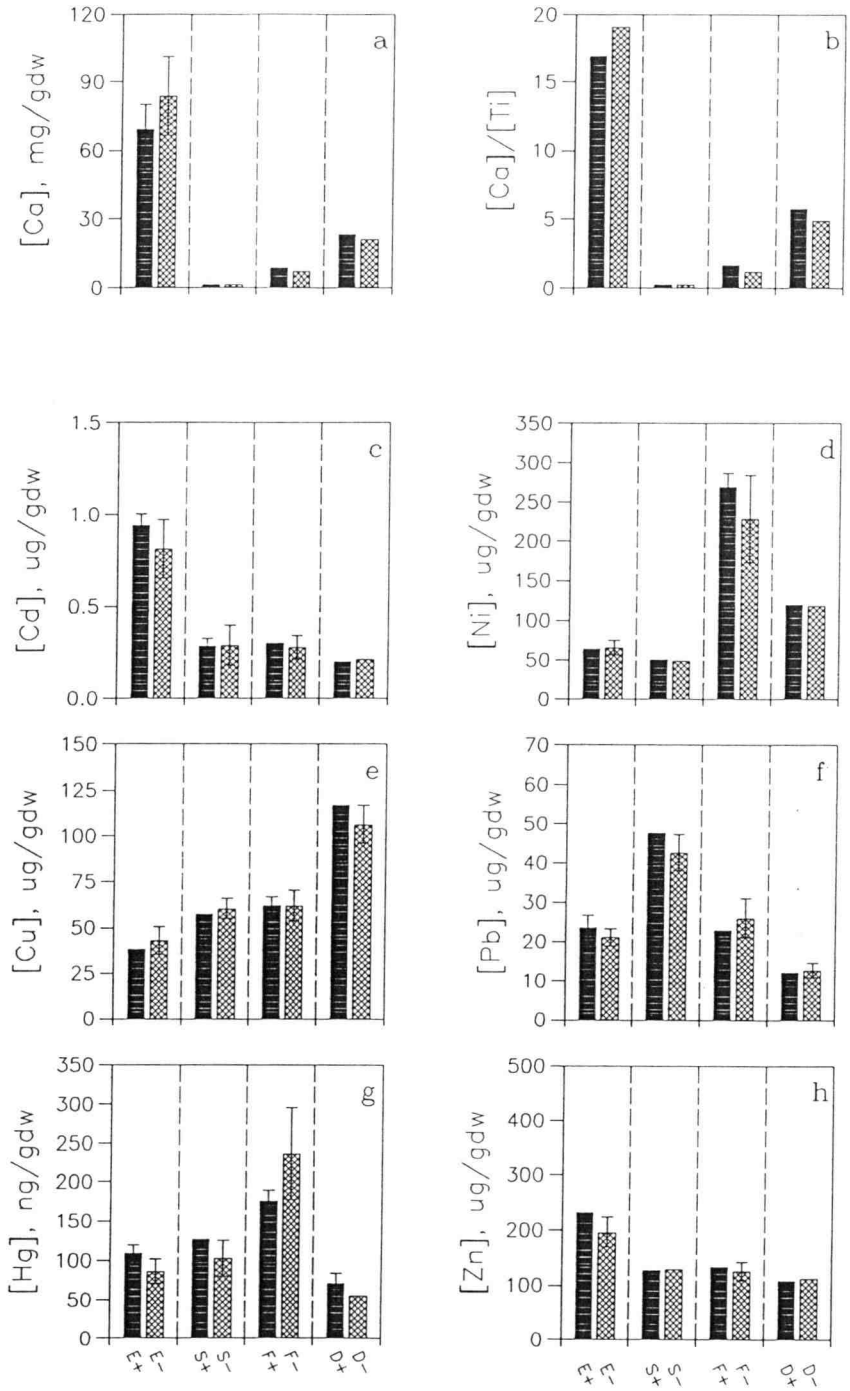

Figure 3.2 Lake sediment characteristics from four U.S. Arctic lakes E=Elusive Lake, S=Schrader Lake, F=Feniak Lake, and D=Desperation Lake. +/- denotes surface sediment (+) and down-core sediment (-).

the other three lakes. Normalizing the Ca concentrations by the surface concentrations of Ti (Fig. 3.2b), a resistant crustal element, suggests that the Ca found in Elusive Lake probably entered the system in a bioavailable form. Hence, the uptake of metals in Elusive Lake may be diminished relative to the other three lakes, due to the markedly higher concentrations of calcium found in that system.

In almost all instances, the concentrations of metals in top two centimeters of sediments are quite similar to those found in the lower, pre-1900 regions of the sediment cores. This suggests that there is no significant enrichment of the metals due to recent (ca 40 years) anthropogenic activity. The only exception to this pattern is the slight (4%), albeit statistically significant ($p<0.05$), increase of Hg in the top 2 cm of Schrader Lake sediment relative to down-core values (Fig. 2g; Landers *et al.*, in press).

Heavy metal concentrations in fish tissue

At each site, there was considerable variation in heavy metal concentrations in liver among individuals for both species (Fig. 3.3). In general, lake trout from Feniak Lake had higher metal burdens that fish from the other two sites, while grayling showed little variation among sites (except for Ni and Pb). Maximum concentrations of all metals (except Cu and Zn) in liver and muscle were 2.5 μg/g in lake trout and 1 μg/g in grayling (all values in dry weight unless otherwise specified).

Lake trout standard lengths were significantly lower at Elusive Lake than Schrader or Feniak lakes ($p=0.003$). Liver [Hg] and [Pb], and muscle [Cu] and [Hg], were positively correlated with fish length ($p<0.035$), although analysis of covariance

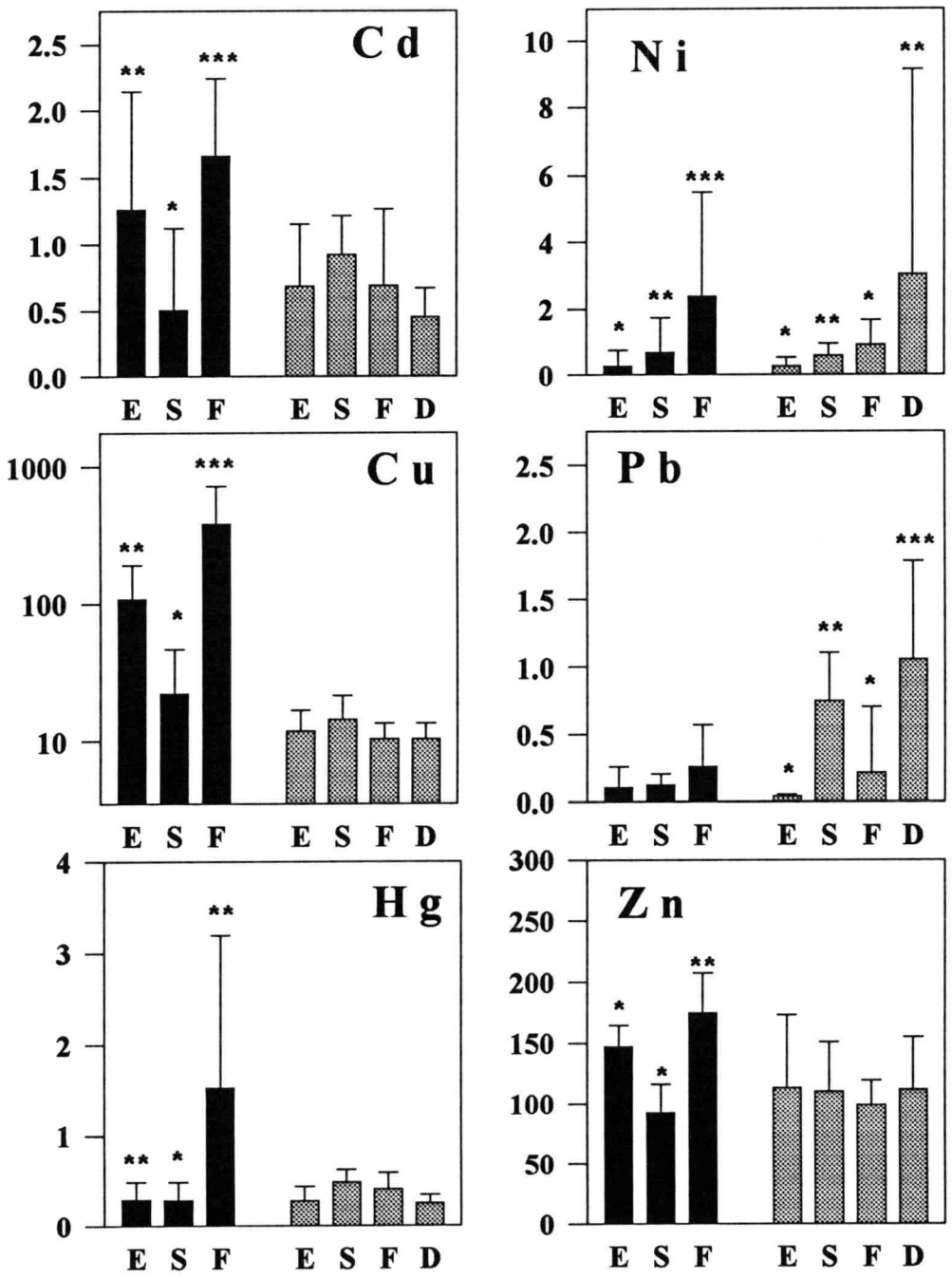

Figure 3.3 Heavy metal concentrations in lake trout and grayling liver from four U.S. Arctic lakes (µg/g dw). Lake trout are in black, grayling are in gray.

E=Elusive Lake, S=Schrader Lake, F=Feniak Lake, and D=Desperation Lake.

Error bars represent ± 1 st. dev. Hg site differences based on length-adjusted means.

*,**,*** indicate statistically different at p=0.05.

demonstrated that standard length was a significant covariant only for [Hg] in liver and muscle. Regression slopes (liver, muscle [Hg] vs. standard length) did not differ among sites. Length-adjusted means for total Hg in liver and muscle were significantly higher in lake trout from Feniak Lake than those from Schrader Lake.

Metal concentrations in liver or muscle of lake trout or grayling did not differ significantly by sex and were generally consistent between the two sampling years for any given lake. The only significant year effects were observed for muscle [Cd] and [Pb] for which the minimum detection limit varied considerably between 1991-92 samples and 1993 samples as a result of analytical instrumentation.

Comparing species at each site using ANCOVA with standard length as a covariant (when significant), lake trout generally had higher metal burdens than grayling (Fig. 3.3). In all cases for which standard length was a significant covariant, regression slopes between the two species were not significantly different.

Heavy metal concentrations in lake trout were compared directly to their primary food source (snails), at Elusive Lake (Fig.3.4). Concentrations of Ni and Pb were higher in snails than lake trout, whereas Cd, Cu, Hg, and Zn were higher in lake trout than snails.

Metal concentrations were lower in muscle than liver (except Hg) for both species and did not differ by site for either species. Median liver [Cd] and [Cu] were 50-100x higher than muscle levels, while Ni, Pb, and Zn were 2-15 fold higher in liver than muscle. In lake trout, muscle [Hg] was 1.5 times higher than liver [Hg], while

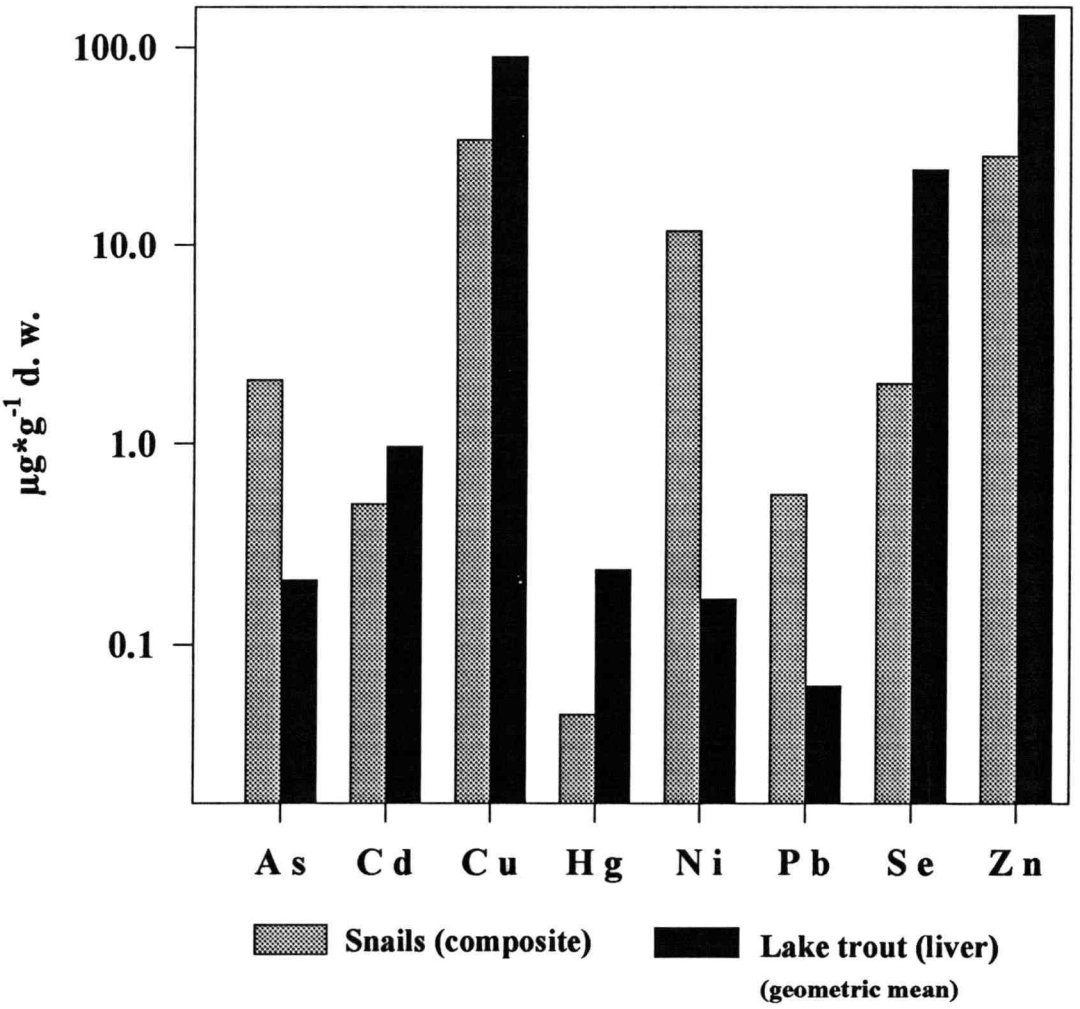

Figure 3.4 Comparison of heavy metal concentrations (µg/g dw) in lake trout and their primary dietary item (snails) from Elusive Lake, U.S. Arctic

liver [Hg] exceeded muscle [Hg] (1.7x) for grayling. The pattern for [MeHg] was the same, but less pronounced, for both species. This shift was not related to the relative liver and muscle lipid content in the two species. The proportion of Hg as methyl-Hg was higher in muscle than liver (90% vs. 60%) for both species.

In grayling, age and standard length were positively correlated (r_s=0.37). Liver [Ni] and muscle [Hg] were positively correlated with fish length (r_s=0.41, 0.28), while muscle [Cu] was positively correlated with age (r_s=0.48).

In both lake trout and grayling, muscle [Hg] was positively correlated with fish length. In addition, muscle [Cu] was positively correlated with fish length for lake trout and with age for grayling. This combination indicates an accumulation of both metals with fish age/size. Although age was not determined in lake trout, given the difficulty of accurate age determination from lake trout scales and the lack of validated aging methods for lake trout residing in Arctic regions (McCart *et al.*, 1972), it is assumed that length increases with age (Johnson, 1976), and therefore both correlations indicate increased accumulation over the lifetime of fish.

Distribution coefficients between fish and sediment

Comparing the ratio of heavy metal burdens in fish liver to surficial sediment among lakes suggests that a lower proportion of metals is bioavailable to fish in Elusive Lake (Fig. 3.5). This relationship holds for all metals except Cu.

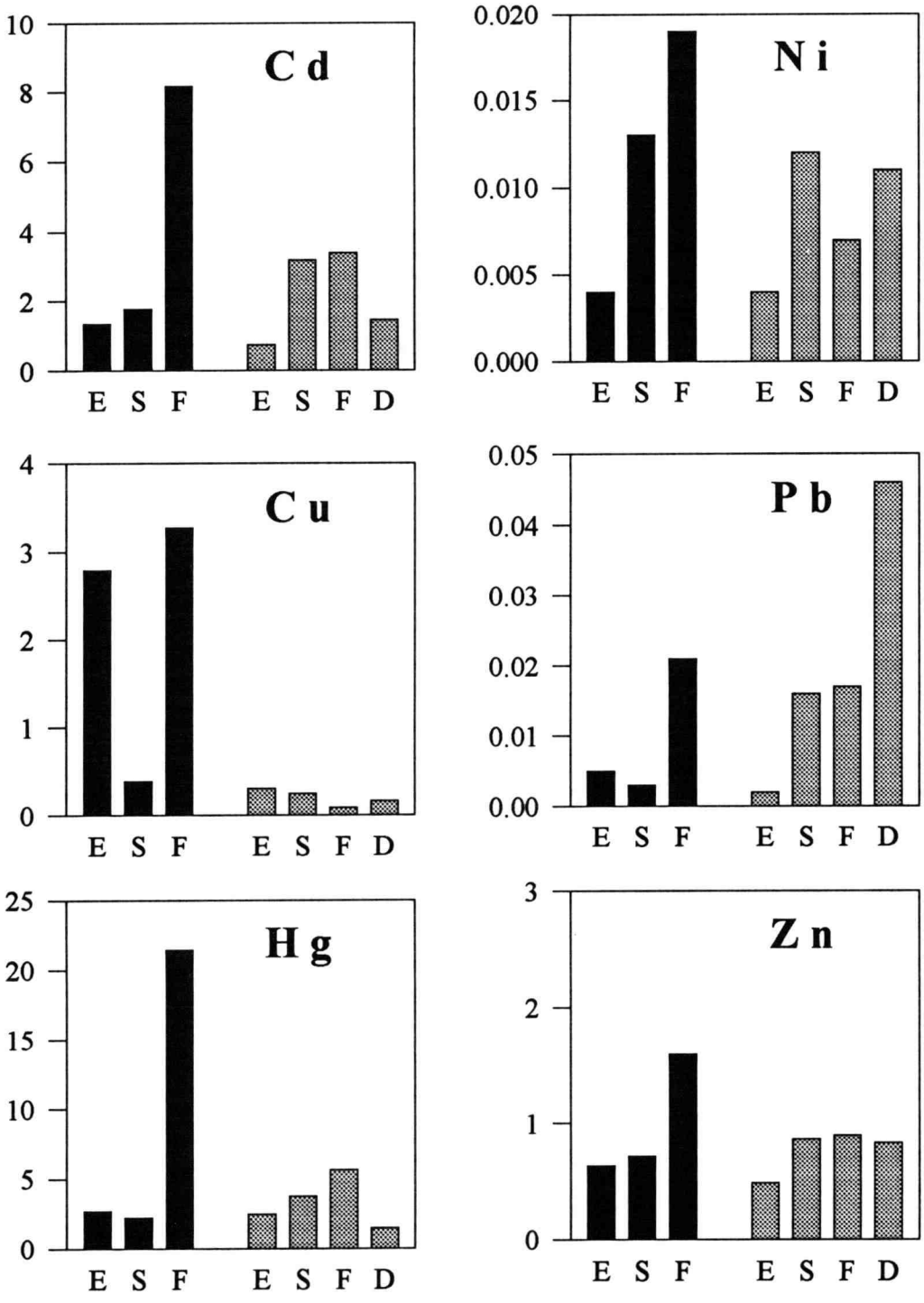

Figure 3.5 Distribution coefficients between fish liver and sediment for four U.S. Arctic lakes. E=Elusive Lake, S=Schrader Lake, F=Feniak Lake, and D=Desperation Lake. Based on mean values (fish liver/top 2 cm of sediment).

Effect on plasma sex steroids

In lake trout, muscle [Pb] was negatively correlated with plasma testosterone. As 25% of the observations for muscle [Pb] were less than the detection limit, the correlation was performed twice; once with all the data (substituting the MDL for values <MDL) (r_s=-0.47), and once with only those observations above the detection limit (r_s=-0.10). When values <MDL were omitted, the correlation with plasma testosterone was not significant. Muscle [Zn] were also negatively correlated with plasma testosterone (r_s=-0.52), but the association was weaker than that for [Pb]. Stepwise regressions revealed that the best model included only muscle [Pb] (R^2=0.23). There were no significant correlations between heavy metal levels in liver or muscle and plasma 17ß-estradiol.

In grayling, negative correlations were observed between Pb in both liver and muscle and plasma 17ß-estradiol and testosterone (Fig. 3.6). In addition, muscle [Cu] and [Zn] were negatively correlated with both sex steroids. Stepwise regressions revealed that the best model to explain plasma 17ß-estradiol levels contained only liver [Pb] (R^2=0.36). The model providing the best fit included liver [Pb] in liver and muscle [Cd] and [Pb] (R^2=0.72); however, since both muscle [Cd] and [Pb] in muscle are below the detection limit in nearly 50% of the observations, it appears that lead levels in liver best explains variation in plasma testosterone values (R^2=0.70). When liver [Pb] values <MDL were omitted from the analysis, the relationship with plasma testosterone was still significant, although the R^2 dropped to 0.56.

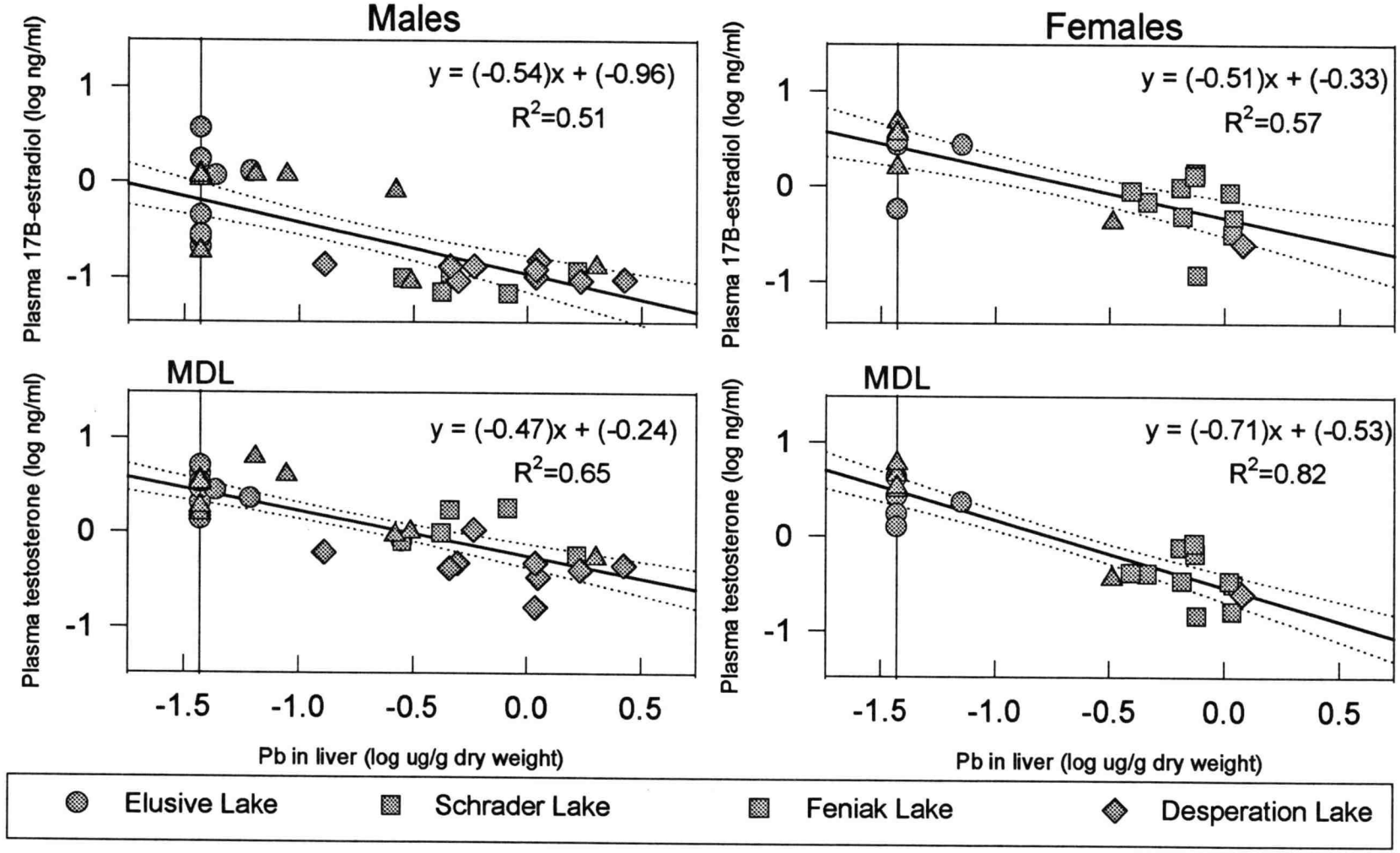

Figure 3.6 Relationship between lead [Pb] and plasma sex steroids in grayling

Discussion

Heavy metals in sediment

Our results document a fairly high level of metals occurring naturally in lakes in the U.S. Arctic with no definite indication of recent anthropogenic enrichment. This agrees well with the findings of Ford *et al.*(1995), who reported no evidence of long-range atmospheric transport of Pb, Cd, or Zn to the U.S. Arctic based on vegetation sampling. It has also been shown that Pb, Cu and Zn are not transported long distances in the atmosphere from smelters in the Arctic (Kelley *et al.*, 1995). The lack of anthropogenic enrichment of metals in the these systems is somewhat surprising given the conjecture surrounding the concept of arctic haze (Ottar, 1989; Pacyna, 1995). However, the metals found in these lakes are present at concentrations that are naturally quite high. Therefore, a small enrichment from regional or distant sources may be lost in the variability of the natural signal. The bioaccumulation of metals in fish in these lakes can then be considered as a largely "natural" phenomenon.

Although there are very few published results of metal concentrations in arctic lake sediments with which to compare our findings, heavy metal levels in the lakes we sampled are equal or higher than those documented as background levels in northern Sweden (Johansson *et al.*, 1995).

Among our lakes, there are significant differences in the metal concentrations in both sediments and fish, although the rank order among the lakes differs between metals and fish. While we are not certain of the metal speciation in sediment and therefore the bioavailability, the differences in total metal concentrations among sites combined with the mitigating influence of Ca, seem to explain most of the variability of fish accumulation among sites. We believe that the presence of large quantities of

Ca in the Elusive Lake system may mitigate metal bioavailability through its ability to both decrease the availability of other metals through calcium carbonate-metal complexation and to competitively inhibit uptake of divalent cations (Prosi, 1979; Connell and Miller, 1984). Other researchers have found fish and sediment [Hg] and [Cd] to be correlated, but not [Cu], [Pb] or [Zn] (Johnson, 1987).

Heavy metals in fish

Differences in uptake between two fish species suggest that insectivorous species, such as grayling, accumulate lower levels of metals than species feeding higher on the food chain. Pb is the only metal showing higher levels in grayling than lake trout. The heavy reliance of lake trout on snails in Feniak and Elusive lakes may explain the high levels observed in this species and the generally higher levels in fish from these lakes compared to fish from Schrader Lake. The higher proportion of Hg as methyl-Hg in muscle may be explained by enhanced retention of inorganic Hg in liver through binding to metallothionein (Angelow and McEwen Nicholls, 1991; Konovalov, 1994). Metallothionein is concentrated in liver and kidney, and negligible in muscle (Klaassen, 1986) and is induced only by inorganic mercury (Goyer, 1986). The fact that muscle [Cu] was correlated with age, but not standard length, could be attributed to variability in the age-length relationship.

The inconsistency of correlations between metals and growth parameters among studies in fish may relate to the range of ages included in particular studies. Mercury, for which bioaccumulation in fish has been demonstrated in numerous studies, can remain stable among certain age/size classes in which there are no prey shifts (Driscoll *et al.*, 1994). Within one study area, correlations between fish muscle [Hg] and fish weight were significant in only 23 of 40 lakes (Johnson, 1987). Correlations with length and age observed in this study are not as strong as those observed in lake trout living in temperate regions (Gutenmann *et al.*, 1992). This is

most likely due to the slower growth rates of fish in arctic environments (McCart *et al.*, 1972; Scott and Crossman, 1973).

Circumarctic perspective

Maximum concentrations of most metals were equal to or higher in fish collected in this study than those collected from remote arctic lakes and rivers in Canada, Finland and Russia. For Cd and Ni, ranges and maximum concentrations were similar in fresh and saltwater fish across the arctic (Snyder-Conn and Lubinski, 1993; Allen-Gil and Martynov, 1995). [Hg] in lake trout from Feniak Lake was higher than those of other arctic freshwater and saltwater fish, except arctic charr from Amituk Lake Canada, which were comparable (Muir and Lockhart, 1993). Maximum [Pb] in grayling and lake trout liver from Schrader and Desperation lakes were approximately 5x higher than arctic charr from Kuhulu Lake, Canada (Bohn and Fallis, 1978) and saltwater fish from Arctic National Wildlife Refuge lagoons (Snyder-Conn and Lubinski, 1993).

Effect on plasma sex steroids

Our results indicate that Pb may have an effect on circulating steroid levels in lake trout and grayling in the U.S. Arctic. Although our observation is a correlation, and does not necessarily imply causation, there is evidence from laboratory studies that: 1) grayling are a relatively sensitive species that heavy metals including Pb (McLeay *et al.*, 1987; Buhl and Hamilton, 1990), 2) that Pb causes decreases in plasma 17ß-estradiol and testosterone in fish (Kumar and Pant, 1984; Thomas, 1988) and 3) plasma sex steroids are a sensitive biomarker for lead exposure (Winder, 1993).

Grayling mortality occurs at [Pb] in water that are half that required to elicit toxic effects in other fish species (20 vs 47 µg/L) (Tewari *et al.*, 1987; Buhl and

Hamilton, 1990). Lead decreases cholesterol levels in blood, liver, ovary and testes, suggesting either the disruption of plasma membranes and/or altered steroidogenesis (Tewari *et al.*, 1987), as cholesterol is the precursor molecule for steroid synthesis. The fact that relationships occur between both plasma testosterone and 17ß-estradiol and liver [Pb] reflects the common steroidogenic and degradation pathways for both hormones (Kupfer, 1975; Hanukoglu, 1992). Pb has been shown to target several regulatory steps of the steroidogenic pathway; it inhibits adenyl cyclase (a critical enzyme for the activation of mitochondrial P450scc for conversion of cholesterol to pregnenolone), activates phosphodiesterase (responsible for terminating the cAMP signal which activates P450scc), and activates protein kinase C (which phosphorylates P450scc) (Nathanson and Bloom, 1975; Wiebe *et al.*, 1983; Goldstein, 1993).

Our results also indicate that reproductive hormone levels may be a sensitive biomarker for Pb exposure in fish. Decreased plasma 17ß-estradiol and testosterone occurred at lead liver levels of 2-5 ug/g d.w., whereas 80% delta-aminolevulinic acid dehydrase (delta-ALAD) inhibition was observed at Pb liver levels above 7 ug/g d.w. in feral whitefish (Haux *et al.*, 1986). In laboratory rats and mice, Pb decreased steroid synthesis at an order of magnitude lower blood [Pb] than other male reproductive disorders (Winder, 1993). Although clearly species differences are an important consideration in evaluating biomarker sensitivity, our results indicate the desirability of including reproductive hormones in the suite of biomarkers examined in laboratory and field studies of Pb exposure in fish.

Without the link between decreased levels of basal circulating steroid hormones and reproductive success, it is not possible to state whether grayling populations could be threatened by heavy metal exposure in arctic Alaskan lakes. However, laboratory studies by Weber (1993) indicated that sublethal Pb exposure to fathead minnows decreased spermatocyte production, secondary sex characteristic

development and display of mating behaviors.

Relevance for human exposure

The importance of freshwater fish as a source of heavy metals for humans depends on the concentration of heavy metals in dietary components, the relative percentage of the select food items, and the extent to which various metals are absorbed and excreted by humans. As humans either have low absorption, effective inactivation or rapid excretion of Cu, Ni, and Zn, these metals are not thought to pose human health hazards from fish consumption (Biddinger and Gloss, 1984).

Relative to other items in Inuit diet, concentrations of heavy metals in the two species of freshwater fish we sampled are generally lower than those of mammals. Maximum [Cd] were approximately 5x higher in harp seal (Ronald *et al.*, 1984). [Cd] in caribou liver from Svalbard (Norheim and Nilssen, 1990) were approximately equal or slightly higher than observed in fish liver. Although maximum [Hg] in lake trout from Feniak Lake exceeded the U.S. FDA tolerance limit for human consumption of commercially-caught fish (1.0 μg/g w.w.), these excessive levels occurred in only a small percentage of lake trout from Feniak Lake and were considerable lower than found in other Inuit dietary items. For example, maximum [Hg] in polar bear liver was 2x higher than fish liver (Norstrom *et al.*, 1986; Norheim *et al.*, 1992), and seals from the arctic were 20-200x higher than fish (Smith and Armstrong, 1975; Ronald *et al.*, 1984). Maximum [Pb] in polar bear, harp seal liver were 3-4x higher than freshwater fish (Ronald *et al.*, 1984; Norheim *et al.*, 1992), while saltwater fish (Snyder-Conn and Lubinski, 1993) had maximum tissue [Ni] 3-4x higher than freshwater fish. Thus, if freshwater fish are not a major dietary item, then its contribution to total heavy metal intake can be expected to be rather low, as is the case for most coastal-dwelling communities. However, in inland Arctic communities where freshwater fish from systems naturally high in metals are the dominant food

item, (consumed at 3 times the rate of other subsistence foods) (Nobmann *et al.*, 1992), fish could provide a significant amount of dietary intake for select heavy metals, particularly for Hg and Pb.

Conclusion

Heavy metal burdens in sediments and fish from four lakes in the U.S. Arctic show high background levels, which may be typical of lakes throughout much of the mineral-rich arctic environment. If any anthropogenic enrichment has occurred, it is not distinguishable from background variability in these lakes. Among lakes, heavy metals burdens in fish are related to sediment concentrations, except in calcareous systems, such as Elusive Lake. It also appears that naturally-occurring levels of metals in arctic freshwater systems may be sufficiently high to affect physiological process, as evidenced by the negative correlation between Pb burdens and plasma hormone levels in arctic grayling. Relative to other items in Inuit diets, it does not appear that lake trout and grayling area major source of heavy metal exposure to northern communities.

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