ARCTIC
VOL. 65, NO. 2 (JUNE 2012) P. S1 – S6

Physical and Biological Factors Affecting Mercury and Perfluorinated Contaminants in Arctic Char (*Salvelinus alpinus*) of Pingualuit Crater Lake (Nunavik, Canada)

NIKOLAUS GANTNER,[1,2] JULIE VEILLETTE,[3] WENDY K. MICHAUD,[4] ROBERT BAJNO,[5] DEREK MUIR,[2] WARWICK F. VINCENT,[3] MICHAEL POWER,[4] BRIAN DIXON,[4] JAMES D. REIST,[5] SONJA HAUSMANN[6,7] and REINHARD PIENITZ[7]

(*Received 3 December 2010; accepted in revised form 15 November 2011*)

APPENDIX 1: CONTAMINANTS

INTRODUCTION

Contaminants: Mercury and PFCs

In the Arctic, concentrations of the neurotoxic form of mercury (Hg), methylmercury, in aquatic, marine, and terrestrial apex predators often exceed consumption guidelines for humans (WHO or Health Canada), posing risk to local populations that harvest these traditional food items (AMAP, 2009). The majority of anthropogenic mercury in the Arctic environment is considered to originate from emissions in industrialized areas to the south, particularly from Eurasia (Pacyna et al., 2006). Atmospheric transfer is facilitated by the long atmospheric residence time of mercury (up to 1.5 years) (Lindberg et al., 2007). Arctic lakes receive Hg through wet and dry deposition, as well as runoff from the surrounding catchments (Muir et al., 2009; Gantner et al., 2010). Anthropogenic emissions have doubled the background concentrations of Hg in northern Quebec lakes (Muir et al., 2009). Once in lakes, Hg is methylated by microbial communities in sediments and anoxic lake bottom waters, and it enters the food web, where it is biomagnified along trophic levels. Catchments can also influence Hg concentrations in char; large catchments surrounding small lakes yield greater Hg concentrations in char (Gantner et al., 2010). Lake Pingualuk, widely lacking this catchment influence, represents a unique opportunity to determine anthropogenic Hg concentrations in arctic char, which (theoretically) must originate solely from atmospheric deposition to the lake itself.

Perfluorinated chemicals have been detected worldwide in humans, in wildlife, and in the global environment, including remote locations such as the High Arctic (Martin et al., 2004; Houde et al., 2006; Stock et al., 2007; Young et al., 2007). These persistent, human-made substances have been used over the last 50 years in an array of industrial and commercial products such as cosmetics, and water and grease repellent coatings for fabrics and food packaging (e.g., 3M: "Scotch Gard", DuPont: "Zonyl") (Kissa, 2001). However, unlike legacy POPs that accumulate in lipid-rich tissues, PFCs bind to blood proteins and are found mainly in the liver, kidneys, and bile secretions. The two most widely studied PFCs globally have an eight-carbon backbone: perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Both PFOS and PFOA are the final degradation products of volatile precursors that are subject to long-range atmospheric transport to the Arctic (Table 1; Stock et al., 2007; Young et al., 2007). An alternative pathway that would explain their presence in remote regions is via oceanic transport, as suggested by several groups (Yamashita et al., 2005, 2008; Prevedouros et al., 2006). PFCs with eight or more carbons have a tendency to bioaccumulate and biomagnify through food webs (Tomy et al., 2004; Houde et al., 2006; Conder et al., 2008), and fish consumption has been identified as a contributing source of PFCs to human populations (Falandysz et al., 2006; Gulkowska et al., 2006). Toxicological studies have demonstrated that these anthropogenic contaminants could have an impact on human and wildlife health (Lau et al., 2007).

MATERIALS AND METHODS

Detection Limits for PFCs

Instrument detection limits (IDLs) were defined as three times the signal-to-noise ratio for the lowest standard on the calibration curve divided by the volume or the mass of the sample analyzed. IDLs of the different compounds ranged from 1 to 11 pg g^{-1} for fish and from 0.1 to 0.9 pg L^{-1} for

[1] Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada; present address: Department of Geography, University of Victoria, Victoria, British Columbia V8W 3R4, Canada; gantnern@uvic.ca
[2] Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, Ontario L7R 4A6, Canada
[3] Département de Biologie and Centre d'Études Nordiques, Université Laval, Québec, Québec G1V 0A6, Canada
[4] Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada
[5] Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba R3T 2N6, Canada
[6] Department of Geosciences, University of Arkansas, Fayetteville, Arkansas 72701, USA
[7] Département de Géographie and Centre d'Études Nordiques (CEN), Université Laval, Québec, Québec G1V 0A6, Canada

TABLE 1. Nomenclature of PFCs.

Compound	Acronym	Number of carbons
Perfluorocarboxylates (PFCAs):		
perfluorohexanoate	PFHxA	C6
perfluoroheptanoate	PFHpA	C7
perfluorooctanoate	PFOA	C8
perfluorononanoate	PFNA	C9
perfluorodecanoate	PFDA	C10
perfluoroundecanoate	PFUnA	C11
perfluorododecanoate	PFDoA	C12
perfluorotridecanoate	PFTrA	C13
perfluorotetradecanoate	PFTA	C14
Perfluorosulfonates (PFSAs):		
perfluorobutane sulfonate	PFBS	C4
perfluorohexane sulfonate	PFHxS	C6
perfluoroheptane sulfonate	PFHpS	C7
perfluorooctane sulfonate	PFOS	C8
perfluorodecane sulfonate	PFDS	C10
perfluorooctane sulfonamide	PFOSA	C8
Unsaturated Fluorotelomer Acids:		
2H-hexadecafluoro-2-octenoicacid	6:2 PFUA	
2H-hexadecafluoro-2-decenoicacid	8:2 PFUA	
2H-octadecafluoro-2-dodecenoicacid	10:2 PFUA	

the water (Tables 2 and 3). Method detection limits (MDLs) were defined as three times the standard deviation of the mean blank divided by the volume or the mass of the sample analyzed (Table 2). Because the PFC levels in the lab blanks were low, it was possible to calculate MDLs for all compounds in the water sample (MDLs: 0.3 – 16.3 pg L^{-1}). Lab blanks for fish analysis typically had non-detectable analyte concentrations, and therefore IDLs were used as the MDLs. Values less than IDLs were reported as non-detectable (nd). All reported concentrations were blank-subtracted with the mean lab blank values. For the calculation of the means, nd values were treated as IDLs.

TABLE 2. Mean recoveries (SD) of ^{13}C-labelled internal standards from fish and water. Results are from Veillette et al. (in press) because fish and water samples were analyzed at the same time as those from Pingualuk and LaFlamme Lakes.

Analyte	Fish (n = 56)	Water (n = 29)
PFHxS	na[1]	180 (23)
PFOS	84 (29)	123 (19)
PFOA (C8)	122 (35)	102 (16)
PFNA (C9)	92 (29)	54 (9)
PFDA (C10)	71 (25)	88 (13)
PFUnA (C11)	71 (25)	72 (19)
PFDoA (C12)	48 (26)	55 (13)
6:2 PFUA	na	56 (22)
8:2 PFUA	na	63 (31)
10:2 PFUA	na	43 (29)

[1] na = not analyzed.

TABLE 3. Range of PFC concentrations and percent of samples with detectable PFCs, along with instrument detection limit (IDL) and method detection limit (MDL) for fish and water samples.

	Fish (pg g^{-1})		Pingualuk char (n = 20)		Laflamme char (n = 9)		Water (pg L^{-1})		
	IDL	MDL	Range	% detected	Range	% detected	IDL	MDL	Pingualuk (n = 1)
PFBS	11.0	11.0	< 11	0	< 11	0	0.9	0.8	6.4
PFHxS	2.8	2.8	< 2.8 – 26	19	< 2.8	0	0.2	0.7	3.1
PFHpS	6.0	6.0	< 6.0	0	< 6.0	0	0.5	0.3	0.8
PFOS	6.0	6.0	< 6.0 – 48	86	< 6.0 – 55	56	0.5	14.5	28
PFDS	3.0	3.0	< 3.0	0	< 3.0 – 4.0	33	0.2	1.0	1.8
PFHxA (C6)	2.5	2.5	< 2.5 – 34	5	< 2.5	0	0.2	16.3	30
PFHpA (C7)	2.5	2.5	< 2.5 – 40	90	< 2.5 – 52	56	0.2	16.3	33
PFOA (C8)	1.3	1.3	< 1.3	0	< 1.3 – 19	33	0.1	15.3	62
PFNA (C9)	1.2	1.2	< 1.2 – 6	5	< 1.2 – 23	22	0.1	9.0	32
PFDA (C10)	1.4	1.4	< 1.4 – 24	90	< 1.4 – 19	78	0.1	6.6	25
PFUnA (C11)	1.0	1.0	< 1.0 – 31	24	< 1.0 – 114	67	0.1	3.4	30
PFDoA (C12)	1.2	1.2	< 1.2 – 25	33	< 1.2 – 13	33	0.1	3.6	2.9
PFOSA	1.1	1.1	< 1.1 – 8.0	14	< 1.1 – 14	56	0.1	1.0	3.8
6:2 PFUA	4.2	4.2	< 4.2	0	< 4.2	0	0.3	4.4	4.7
8:2 PFUA	1.0	1.0	< 1.0 – 1.0	10	< 1.0 – 2.0	67	0.1	1.5	5.3
10:2 PFUA	1.0	1.0	< 1.0 – 2.0	29	< 1.0	0	0.1	1.5	0.1
ΣPFCs (mean)			28		64				
ΣPFCs (range)			< 10 – 87		< 10 – 179				Total = 269

APPENDIX 2: ARCTIC CHAR GENETICS

INTRODUCTION

Genetics Endpoints

Major Histocompatibility (MH) receptors present pathogen-derived peptides to T-lymphocytes, which then initiate an immune response (Dixon and Stet, 2001). There are two types of MH receptors present in most jawed vertebrates, MH Class I and MH Class II. The latter are composed of two subunits: Class II α and Class II β. The second exon of the genes encoding these molecules encodes the α 1 and β 1 domains, respectively. These form the groove in the receptor that binds to the pathogenic peptides, the peptide binding region, and are highly variable in sequence (Goldsby et al., 2000).

MH receptors are very polymorphic at the population level because of the large number of potential pathogens that may be encountered (Grimholt et al., 2003). Thus, populations that confront similar pathogens carry common patterns in their MH alleles, while populations that encounter different pathogens accumulate different allele compositions (Bonneaud et al., 2006), and a large number of different alleles can be maintained within a given population (Messaoudi et al., 2002). Therefore, the set of MH alleles within distinct populations arises from genetic background of the founding population as a result of selective pressure by pathogens in their environment (de Eyto et al., 2007). This selection can happen rapidly, making MH allele studies useful in cases of recent population divergences, like that of Pacific salmonids (Miller et al., 2001) or, as in this study, arctic char.

MATERIALS AND METHODS

Major Histocompatibility Complex

Major Histocompatibility (MH) allele analyses were used to infer genetic relationships of the arctic char populations of Pingualuk and Laflamme Lakes, as it has been proven to be useful in cases of recent population divergences, like Pacific salmonids (Miller et al., 2001). For amplification of MH class II β exon 2, the following primers were used for cloning the initial PCR products: P1 (5–GATACTCCTCAAAGGACCTG–3′) and P3 (5′-TCATACTGTAATGKCACTAC-3′), which should amplify an approximate product of 300 bp, including all of exon 2 and part of intron 2. The PCR amplicons were separated by agarose electrophoresis in a 1% gel to verify size and then extracted directly from the gel using GenEluteAgarose Spin Columns (Sigma-Aldrich, St Louis, Missouri). The fragments were inserted in pGEM-T vectors as per manufacturer instructions (Promega Corporation, Madison, Wisconsin). XL-blue strains of *E. coli* bacteria were made competent by washing them twice and then re-suspending them with thawing buffer (10 mM PIPES, 15 mM CaCl-$2H_2O$, 250 mM KCl, 55 mM MnCl2-$4H_2O$, pH 6.7). The bacteria were transformed by the Inoue procedure (Sambrook et al., 2001) and grown in LB plates with ampicillin (100 µg/ml) plus 1 µM IPTG and 100 µg/ml X-gal. Eight to twelve white colonies were selected and grown in LB media.

For amplification of the products to be analyzed by denaturing gradient gel electrophoresis (DGGE), the primers used were P, as used above, and P2 (5′-CGGGCGGGGGCGGCGGGCCGGGCGCGGGGCGCGGCGGGCGCCAGTACGGCGCTGTAG), which amplifies 191 bp of exon 2. Amplifications were performed in a PJ 200, in 30 µl PCR reaction mixture containing 150 ng of DNA template, 30 pmol of each of the primers, 1× PCR reaction buffer, 30 µm dNTP and 0.21 units Taq polymerase (Fisher Scientific). PCR conditions were as follows: denaturation for 2 min at 95˚C, followed by 35 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 57˚C and primer extension for 1 min at 72˚C. The final extension was for 5 min at 72˚C. PCR products resulting from amplification with P1 and P2 were run on 8% 19:1 acrylamide:bisacrylamide parallel DGGE gels containing 1× TAE buffer and a 20–45% gradient of urea and formamide at 60 V for 15.5 h (Bio-Rad Laboratories, Richmond, California).

Only the alleles that differed between samples were selected for DNA sequencing in order to avoid redundancies. Those plasmids selected for sequencing were extracted from the grown bacteria using Gen Elute Plasmid Miniprep Kit (Sigma-Aldrich, St Louis, Missouri) according to manufacturer instructions, and sequenced using the T7 and Sp6 primers with a big dye terminator v3.1 (Applied Biosystems, Foster City, California) in a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California). Alleles were named according to the convention for naming MH alleles (Klein et al., 1990): the first two letters of the genus and species are combined (*Saal* for char and *Sana* for lake trout), followed by DAB, indicating that they are alleles of beta chain (B) of the first gene (A) MH class II (D), followed by a four digit number, in which the first two digits designate the allele family, while the second two indicate the allele number within the family (e.g., *0101, first allele in the first family).

Bioedit (Hall, 1999) was used to define the open reading frames and to translate DNA sequences into protein sequences. Phylogenetic trees were constructed using the neighbor-joining method (Nei and Gojorobi, 1986) with MEGA 4 software. Arlequin 3.01 software was used to calculate the pairwise F-statistics (FST).

mtDNA

A total of 30 fish tissue samples, 21 from Pingualuk Lake and 9 from Laflamme Lake, were analyzed for species identification and mitochondrial DNA sequence variation. Total DNA from the tissue samples was extracted using the DNeasy® Tissue Kit (Qiagen Inc.) according

to manufacturer's instruction. The mitochondrial control region was PCR amplified and sequenced following the methods described in Alekseyev et al. (2009). For the 30 fish samples from Pingualuk and Laflamme Lakes, along with additional reference sequences, a 507 base pair sequence was analyzed for variability with the program Seqscape® Software version 2.5 (Applied Biosystems Foster City, California). Mitochondrial DNA sequences (Table 1) were compared to sequences from other studies of species in various geographic locations (Table 2). Included in the comparison were representative samples from the major phylogeographic groups reported in Brunner et al. (2001). Lake trout identification was verified by sequence comparison to lake trout reference samples from previous studies (J. Reist and R. Bajno, Freshwater Institute, Winnipeg, Manitoba, unpubl. data), and brook trout sequences (not shown). A UPGMA tree was constructed using maximum composite likelihood model bootstrapped using 1000 replications with MEGA4 (Tamura et al., 2007) to summarize the relationship between observed haplotypes.

TABLE 1. Sequence results for Pingualuk and Laflamme samples. See Figure 2 for sequence data.

Sample ID#	Haplotype	Species
PIN07-01	ARC19	*S. alpinus*
PIN07-02	ARC19	*S. alpinus*
PIN07-03	ARC19	*S. alpinus*
PIN07-04	ARC19	*S. alpinus*
PIN07-05	ARC19	*S. alpinus*
PIN07-06	ARC19	*S. alpinus*
PIN07-07	ARC19	*S. alpinus*
PIN07-08	ARC19	*S. alpinus*
PIN07-09	no data	unknown
PIN07-10	ARC19	*S. alpinus*
PIN07-11	ARC19	*S. alpinus*
PIN07-12	ARC19	*S. alpinus*
PIN07-13	ARC19	*S. alpinus*
PIN07-14	ARC19	*S. alpinus*
PIN07-15	ARC19	*S. alpinus*
PIN07-16	ARC19	*S. alpinus*
PIN07-17	ARC19	*S. alpinus*
PIN07-18	ARC19	*S. alpinus*
PIN07-19	ARC19	*S. alpinus*
PIN07-20	ARC19	*S. alpinus*
PIN07-20a	ARC19	*S. alpinus*
LAF07-01	ARC19	*S. alpinus*
LAF07-02	ARC19	*S. alpinus*
LAF07-03	ARC19	*S. alpinus*
LAF07-04	ARC19	*S. alpinus*
LAF07-05	ARC19	*S. alpinus*
LAF07-06	ARC19	*S. alpinus*
LAF07-07	eLT01	*S. namaycush*
LAF07-08	eLT01	*S. namaycush*
LAF07-09	ARC19	*S. alpinus*

TABLE 2. Reference samples, locations, and haplotypes identified in this study. Haplotype sequence information is reported in Figure 2. Genbank accession numbers are given for samples reported in Brunner et al. (2001) and for haplotype sequences previously described in Alekseyev et al. (2009).

Species	Location	Haplotype	Genbank
S. alpinus	Nalusiaq Lake, Baffin Island	ARC19	EU310899
S. alpinus	Lake Aigneau, Quebec	ARC19	EU310899
S. alpinus	Beverly Inlet, Melville Island	ARC19	EU310899
S. alpinus	Parker River, Banks Island	ARC19	EU310899
S. alpinus	Lake Hazen, Ellesmere Island	ARC19	EU310899
S. alpinus	Kasegalik Lake, Belcher Island	ARC19	EU310899
S. alpinus	Cambridge Bay, Victoria Island	ARC19	EU310899
S. alpinus	Gander Lake, Newfoundland	e13A	
S. alpinus	Floods Pond, Maine	ACD09	EU310898
S. alpinus	Loch Rannoch, Scotland	e39A	
S. malma	Rat River, Yukon	BER12	EU310902
S. malma	Nome River, Alaska	e31A	
S. alpinus	Thun Lake, Switzerland	ATL01	AF297991
S. alpinus	Goltsovoe Lake, Baikal region	SIB04	AF298012
S. alpinus	Lac Rond, Quebec	ACD04	AF298048
S. alpinus	Hall Lake, Melville Peninsula	ARC06	AF298032
S. alpinus	Charr Lake, Cornwallis Isl.	ARC10	AF298036
S. alpinus	River near Isorto Fiord, Greenland	ARC15	AF298041
S. malma	Auke Creek, Alaska	BER9	AF298026

RESULTS AND DISCUSSION

MH

A total of 41 alleles of arctic char MH Class II β were found in the 30 individuals examined from Laflamme and Pingualuk Lakes. Among them, the population from Laflamme Lake had 16 unique Class II β alleles and the population from Pingualuk had 24 unique alleles. A single allele, *Saal*-DAB*0402, was the only one shared between the two populations. F-statistic analysis of population allele compositions revealed a significant difference between the two populations (FST = 0.18879, $p < 0.001$). A phylogenetic analysis of the two populations showed that alleles from each lake clustered together with high bootstrap values (> 75%) in seven of nine cases (Fig. 1). Where clusters contain sequences from both lakes (i.e., *Saal*-DAB*2601 and cluster II) the bootstrap value is low and the branch lengths are long, suggesting that the relationship between the alleles is ancient and probably predates the split of the two populations. These distinct clusters support the FST analysis. Two lake trouts were collected from Laflamme Lake. Only three of the 41 char alleles found in Laflamme and Pingualuk Lakes were shared with alleles found in other char populations, and two of those alleles were found in the nearby Lake Aigneau population (Ungava peninsula, Quebec) (P. Conejeros, University of Waterloo and Catholic University of Valparaiso, Chile, unpubl. data). It is interesting to note that one of the alleles shared with other populations was *Saal*-DAB*0402, the allele that both populations shared, which was by far the most frequently found sequence (21 of 86 sequences obtained).

The two populations share only a single allele (*Saal*-DAB*0402), which was the most common, found in 2 of 7 Lake Laflamme individuals and 15 of 23 individuals from Pingualuk. This allele, shared only with the char population in Aigneau Lake, is one of three alleles shared with other char populations. The second shared allele was found in the populations of the de la Trinité River in southern Quebec, while the third was found in the Aigneau population and the distant population in Resolute, Cornwallis Island,

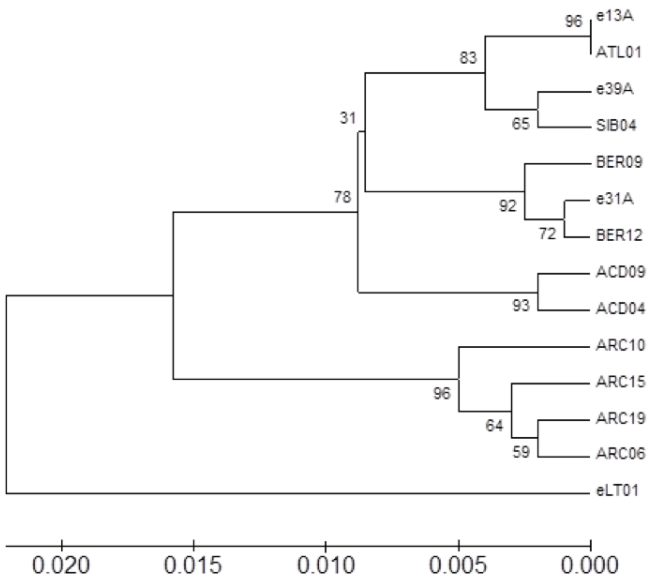

FIG. 1. UPGMA tree summarizing relationships of observed haplotypes. Bootstrap values calculated by 1000 replications using a maximum composite likelihood model of nucleotide substitution. See Table 2 for accession numbers of additional Genbank sequences.

Nunavut (P. Conejeros, University of Waterloo and Catholic University of Valparaiso, Chile, unpubl. data). Since only 3 of 41 alleles are shared, the data are insufficient for a definitive analysis.

mtDNA

The results of the mitochondrial sequence data for Pingualuk and Laflamme samples are reported in Table 1 and Figure 2. All fish except one (PIN07-09) were successfully analyzed for mitochondrial DNA sequence data. Sequences were edited, aligned and compared to the reference haplotype (ARC19, Table 2). This haplotype appears to be fairly common throughout the Canadian range of arctic char. The genetic relationship among haplotypes is shown in Figure 2. The sequences for LAF07-07 and LAF07-08 were identical to haplotype sequences found in lake trout samples from George Lake, MB, and Lake Quamutissiat, QC (data not shown). The position of ARC19 within the UPGMA tree was expected and is consistent with the Arctic phylogeographic group reported by Brunner et al. (2001). The lake trout haplotype eLT01 was expectedly outside of the char clusters.

REFERENCES

Alekseyev, S.S., Bajno, R., Gordeeva, N.V., Reist, J.D., Power, M., Kirillov, A.F., Samusenok, V.P., and Matveev, A.N. 2009. Phylogeography and sympatric differentiation of the arctic charr *Salvelinus alpinus* (L.) complex in Siberia as revealed by mtDNA sequence analysis. Journal of Fish Biology 75(2):368–392.

AMAP (Arctic Monitoring and Assessment Programme). 2009. AMAP Assessment 2009: Human health in the Arctic. Oslo, Norway: AMAP. 256 p.

Bonneaud, C., Chastel, O., Federici, P., Westerdahl, H., and Sorci, G. 2006. Complex Mhc-based mate choice in a wild passerine. Proceedings of the Royal Society B 273:1111–1116.

Brunner, P.C., Douglas, M.R., Osinov, A., Wilson, C.C., and Bernatchez, L. 2001. Holarctic phylogeography of arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. Evolution 55(3):573–586.

Conder, J.M., Hoke, R.A., de Wolf, W., Russell, M.H., and Buck, R.C. 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. Environmental Science & Technology 42(4):995–1003.

	1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5
	3 7 7 9 0 1 3 3 4 6 5 5 5 6 6 6 7 7 8 1 2 2 9 9 9 9 9 0 0 2 3 4 5 6 7 7 7 8 8 9 0 0 0
	1 2 4 3 5 3 2 9 0 8 8 4 3 8 9 0 8 9 3 9 5 5 4 5 1 4 5 8 9 4 5 3 0 9 4 8 1 7 8 5 9 9 3 4 5
ARC19	A A T C C C G T T C A A G T G T A T G A C - T G T T A G G G T T C A G G A T A A T T C G T
e09A	. . . . . A . C G . . . C . . . C . . . . G A . . C . . . G . . .
BER12	. . . . T . A . C G . . G C A C . C A . . . C . . . . T G A . . C . . . G . . .
e31A	. . . . . A . C G . . . C . . . C . . . . G A A . C . . . G . . .
ACD09	. . . . . A . C G . . G C A . G C A . . . C C . . . A . . . C . G A . . C . . . G . . .
e39A	. . . . T . A . C G . . G . A . . C A . T . C . . . . T G A . . C . . . G . . .
ATL1	. . . . T . A . C G . . A C A C . C A . . . C . . . . T G A . . C . . . G . . .
SIB4	. . . . T . A . C G . . A . A . . C A . T . C . . . . A . . C . . . G . . .
BER9	. . A . . . A . C G . . . C . . . C . . . . C . G A . . C . . . G . . .
ACD4	. . A T . . A . C G . . A C A . G C A . . . C C . . . A . . . C . G A . . C . . . G . . .
ARC6	. . . T . . . . C . . . .
ARC10	. . A T . . . . . G G . . . . T . . . .
ARC15	. . A . . . . G . . . .
eLT01	G G . . T T A C G G . . A . A . . C . G . . . . C C C . A - - . T . A . . C G G . G - . C

FIG. 2. Mitochondrial DNA sequence variation. Variable sites are numbered according to their position in the reference sequence ARC19. A period (.) denotes similarity to the reference sequence ARC19, and a dash (–) indicates a deletion. Haplotype eLT01 is representative of the two lake trout samples caught in Laflamme Lake.

de Eyto, E., McGinnity, P., Consuegra, S., Coughlan, J., Tufto, J., Farrell, K., Megens, H.-J., Jordan, W., Cross, T., and Stet, R.J.M. 2007. Natural selection acts on Atlantic salmon major histocompatibility (MH) variability in the wild. Proceedings of the Royal Society B 274(1611):861–869.

Dixon, B., and Stet, R.J.M. 2001. The relationship between major histocompatibility receptors and innate immunity in teleost fish. Developmental & Comparative Immunology 25 (8-9):683–699.

Falandysz, J., Taniyasu, S., Gulkowska, A., Yamashita, N., and Schulte-Oehlmann, U. 2006. Is fish a major source of fluorinated surfactants and repellents in humans living on the Baltic coast? Environmental Science & Technology 40(3):748–751.

Gantner, N., Muir, D.C., Power, M., Iqaluk, D., Reist, J.D., Meili, M., Babaluk, J.A., et al. 2010. Mercury concentrations in landlocked arctic char (*Salvelinus alpinus*) from the Canadian Arctic. Part II: Influence of lake biotic and abiotic characteristics on geographic trends in 27 populations. Environmental Toxicology and Chemistry 29(3):633–643.

Goldsby, R.A., Kindt, T.J., Osborne, B.A., and Kuby, J.I. 2000. Kuby immunology, 4th ed. New York: W.H. Freeman.

Grimholt, U., Larsen, S., Nordmo, R., Midtlyng, P., Kjoeglum, S., Storset, A., Saebo, S., and Stet, R.J.M. 2003. MHC polymorphism and disease resistance in Atlantic salmon (*Salmo salar*); facing pathogens with single expressed major histocompatibility class I and class II loci. Immunogenetics 55(4):210–219.

Gulkowska, A., Jiang, Q., Ka So, M., Taniyasu, S., Lam, P.K.S., and Yamashita, N. 2006. Persistent perfluorinated acids in seafood collected from two cities of China. Environmental Science & Technology 40(12):3736–3741.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Houde, M., Bujas, T.A.D., Small, J., Wells, R.S., Fair, P.A., Bossart, G.D., Solomon, K.R., and Muir, D.C.G. 2006. Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food web. Environmental Science & Technology 40(13):4138–4144.

Kissa, E. 2001. Fluorinated surfactants and repellents, 2nd ed. New York: Marcel Dekker, Inc.

Klein, J., Bontrop, R.E., Dawkins, R.L., Erlich, H.A., Gyllensten, U.B., Heise, E.R., Jones, P.P., Parham, P., Wakeland, E.K., and Watkins, D.I. 1990. Nomenclature for the major histocompatibility complexes of different species: A proposal. Immunogenetics 31(4):217–219.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., and Seed, J. 2007. Perfluoroalkyl acids: A review of monitoring and toxicological findings. Toxicology Science 99(2):366–394.

Lindberg, S., Bullock, R., Ebinghaus, R., Engstrom, D., Feng, X., Fitzgerald, W., Pirrone, N., Prestbo, E., and Seigneur, C. 2007. A synthesis of progress and uncertainties in attributing the sources of mercury in deposition. Ambio 36(1):19–32.

Martin, J.W., Smithwick, M.M., Braune, B.M., Hoekstra, P.F., Muir, D.C.G., and Mabury, S.A. 2004. Identification of long-chain prefluorinated acids in biota from the Canadian Arctic. Environmental Science & Technology 38(2):373–380.

Messaoudi, I., Guevara Patiño, J.A., Dyall, R., LeMaoult, J., and Nikolich-Zugich, J. 2002. Direct link between *mhc* polymorphism, T cell avidity, and diversity in immune defense. Science 298:1797–1800.

Miller, K.M., Kaukinen, K.H., Beacham, T.D., and Withler, R.E. 2001. Geographic heterogeneity in natural selection on an MHC locus in sockeye salmon. Genetica 111(1-3):237–257.

Muir, D.C.G., Wang, X., Yang, F., Nguyen, N., Jackson, T.A., Evans, M.S., Douglas, M., et al. 2009a. Spatial trends and historical deposition of mercury in eastern and northern Canada inferred from lake sediment cores. Environmental Science & Technology 43:4802–4809.

Nei, M., and Gojorobi, T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Molecular Biology and Evolution 3(5):418–426.

Pacyna, E.G., Pacyna, J.M., Steenhuisen, F., and Wilson, S. 2006. Global anthropogenic mercury emission inventory for 2000. Atmospheric Environment 40(22):4048–4063.

Prevedouros, K., Cousins, I.T., Buck, R.C., and Korzeniowski, S.H. 2006. Sources, fate and transport of perfluorocarboxylates. Environmental Science & Technology 40(1):32–44, doi:10.1021/es0512475.

Sambrook, J., and Russell, D.W. 2001. Molecular cloning: A laboratory manual, 3rd ed. New York: Cold Spring Harbor Laboratory Press.

Stock, N.L., Furdui, V.I., Muir, D.C.G., and Mabury, S.A. 2007. Perfluoroalkyl contaminants in the Canadian Arctic: Evidence of atmospheric transport and local contamination. Environmental Science & Technology 41(10):3529–3536.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24(8):1596–1599, doi:10.1093/molbev/msm092.

Tomy, G.T., Budakowski, W., Halldorson, T., Helm, P.A., Stern, G.A., Friesen, K., Pepper, K., Tittlemier, S.A., and Fisk, A.T. 2004. Fluorinated organic compounds in an eastern Arctic marine food web. Environmental Science & Technology 38(24):6475–6481.

Veillette, J., Muir, D.C.G., Antoniades, D., Small, J.M., Spencer, C., Loewen, T.N., Babaluk, J.A., Reist, J.D., and Vincent, W.F. In press. Perfluorinated chemicals in meromictic lakes on the northern coast of Ellesmere Island, High Arctic Canada. Arctic 65(3).

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Petrick, G., and Gamo, T. 2005. A global survey of perfluorinated acids in oceans. Marine Pollution Bulletin 51:658–668.

Yamashita, N., Taniyasu, S., Petrick, G., Wei, S., Gamo, T., Lam, P.K.S., and Kannan, K. 2008. Perfluorinated acids as novel chemical tracers of global circulation of ocean waters. Chemosphere 70(7):1247–1255.

Young, C.J., Furdui, V.I., Franklin, J., Koerner, R.M., Muir, D.C.G., and Mabury, S.A. 2007. Perfluorinated acids in Arctic snow: New evidence for atmospheric formation. Environmental Science & Technology 41(10):3455–3461.