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

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ABSTRACT. Pingualuk Lake fills a deep crater in the Parc National des Pingualuit on the Ungava Peninsula (Nunavik, Canada) and is isolated from nearby surface waters. The main objectives of this study were to determine and compare the concentrations of two atmospherically derived contaminants, mercury and perfluorinated chemicals (PFCs), in the lake water column and fish of Pingualuk Lake and to assess the physical and biological factors influencing contaminant concentrations. Mercury concentrations in arctic char muscle tissue were comparable to those of char in other Arctic lakes, while the total amount of PFCs was below reported levels for remote lakes in the Arctic and elsewhere. Stable isotope and stomach content analyses were made to investigate the feeding ecology of the Pingualuk Lake arctic char population and indicated the possibility of multiple feeding groups. Genetics characteristics (MH and mtDNA) of fish from Pingualuk Lake revealed that this population is likely distinct from that of nearby Laflamme Lake. However, both arctic char populations exhibit differential variation of their allele families. Physical characteristics determined for Lake Pingualuk revealed that the water column was inversely stratified beneath the ice and extremely transparent to visible and ultraviolet radiation. The highest mercury concentrations (3–6 pg mL^{-1} THg) occurred just beneath the ice surface in each lake. Pingualuk Lake, given its near pristine state and exceptional limnological features, may serve as a most valuable reference ecosystem for monitoring environmental stressors, such as contaminants, in the Arctic.

Key words: Pingualuit Crater, *Salvelinus alpinus*, mercury, PFOS, PFOA, polyfluoroalkyl chemicals, atmospheric deposition, cannibalism, genetic isolation, meteor-impact crater

RÉSUMÉ. Le lac Pingualuk, qui remplit un cratère profond situé dans le parc national des Pingualuit, dans la péninsule d'Ungava (Nunavik, Canada), est isolé des eaux de surface avoisinantes. Les principaux objectifs de la présente étude consistaient à déterminer et à comparer les concentrations de deux contaminants dérivés dans l'atmosphère, soit le mercure et les produits chimiques perfluorés, se trouvant dans la colonne d'eau lacustre et les poissons du lac Pingualuk, ainsi qu'à évaluer les facteurs physiques et biologiques influençant les concentrations de contaminants. Les concentrations de mercure décelées dans le tissu musculaire de l'omble chevalier étaient comparables à celles de l'omble d'autres lacs de l'Arctique, tandis que la quantité totale de produits chimiques perfluorés était inférieure aux niveaux répertoriés dans les lacs éloignés de l'Arctique et d'ailleurs. L'analyse des isotopes stables de même que l'analyse du contenu de l'estomac ont permis d'enquêter sur l'écologie alimentaire de la population d'ombles chevaliers du lac Pingualuk, et ont indiqué la possibilité qu'il existe plusieurs groupes d'alimentation. Les caractéristiques génétiques (MH et ADN mt) des poissons du lac Pingualuk ont révélé que cette population est vraisemblablement distincte de celle du lac Laflamme situé tout près. Toutefois, les deux populations d'ombles chevaliers affichent une variation allélique différentielle. Les caractéristiques physiques déterminées dans le cas du lac Pingualuk ont révélé que la colonne d'eau était inversement stratifiée sous la glace et extrêmement transparente au rayonnement visible et au rayonnement ultraviolet. Les concentrations de mercure les plus prononcées (3–6 pg mL^{-1} THg) se trouvaient juste sous la surface de glace de chaque lac. Étant donné l'état quasi originel et les caractéristiques limnologiques exceptionnelles du lac Pingualuk, ce lac pourrait servir d'écosystème de référence des plus précieux pour surveiller les agresseurs environnementaux, tels que les contaminants, dans l'Arctique.

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Mots clés : cratère de Pingualuit, *Salvelinus alpinus*, mercure, PFOS, PFOA, produits chimiques perfluorés, dépôt atmosphérique, cannibalisme, isolement génétique, cratère résultant de l'impact d'un météorite

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INTRODUCTION

Pingualuk Lake fills a 1.4 ± 0.1 million-year-old meteorite crater, known as Pingualuit Crater, on the Ungava Peninsula in northern Quebec, Canada (Bouchard, 1989). It is one of the clearest lakes in the world, which gave rise to its local name, which means the "Crystal Eye of Nuavik" (Bouchard, 1989). Sediment records from Pingualuk Lake, like those of ancient crater lakes elsewhere (e.g., El'gygytgyn Crater Lake, Siberia, (Russia) (Melles et al., 2007), have the potential to provide important paleolimnological and paleoclimatic records spanning multiple glacial-interglacial cycles (details at www.cen.ulaval.ca/pingualuit/index.html) (Guyard et al., 2011). Several scientific surveys have investigated the Pingualuit Crater's origin and geology (Bouchard, 1989); however, only a few expeditions to Pingualuk Lake have focused on its aquatic fauna, limnology, and contaminants (e.g., mercury) (Meen, 1952; Martin, 1955; Delisle et al., 1986; Bouchard, 1989; Ouellet et al., 1989).

As a crater lake, Pingualuk (~2.7 km diameter) represents the rare case of a lake that has an extremely restricted catchment (total catchment area to lake area ratio = 0.4). The lake has steeply sloping sides dropping off to a maximum-recorded depth of 267 m (Martin, 1955). The surrounding rim of the crater rises from 56 to 163 m above the lake, creating a barrier that leaves the lake hydrologically isolated from nearby surface waters. Inputs to the system are therefore mainly through precipitation, as indicated by oxygen stable isotope ratios (Ouellet et al., 1989), while outflow likely occurs through groundwater drainage, as the surface of the lake is located at a higher elevation than the water table of the surrounding area (Pienitz et al., 2008). A water residence time of 330 years has been estimated for Pingualuk Lake (Ouellet et al., 1989).

Mercury (Hg) and perfluorinated chemicals (PFCs), the two contaminants selected for the present study, are dispersed by long-range atmospheric transport (Lindberg et al., 2007; Stock et al., 2007; Schenker et al., 2008). Hg is a natural element with a long history of anthropogenic atmospheric emissions, particularly from combustion of fossil fuels (Lindberg et al., 2007; AMAP/UNEP, 2008; Durnford et al., 2010), while PFCs are a group of entirely human-made substances that have a comparatively short history of use and emission (i.e., within the last 60 years) (Schenker et al., 2008). Biomagnification in aquatic food webs has been demonstrated both for methyl mercury (MeHg) and for PFCs with 8 to 12 perfluorinated carbon chains (Gantner et al., 2010a; Houde et al., 2011).

Arctic char (*Salvelinus alpinus* (L.)) is the only fish species reported from the lake (Martin, 1955), although the genetic makeup of the population is not known. The elevated rim of the crater completely encircles the lake, preventing migration between Pingualuk Lake and nearby lakes. Therefore, it is likely that arctic char in Pingualuk Lake became isolated from other char populations about 6000 years ago, following the last deglaciation during the Holocene (Bouchard, 1989). Little is known about the behavior and ecology of this population except that cannibalism was previously observed among larger individuals (Martin, 1955; Bouchard, 1989). Contaminant accumulation can vary among species (Evans et al., 2005; Lockhart et al., 2005; Swanson et al., 2011), making genetic identification highly relevant. Arctic char have been used as a species for monitoring contaminants undergoing long-range atmospheric transport because of their circumpolar distribution (Evans et al., 2005; Muir et al., 2006; Evenset et al., 2007; Gantner et al., 2010b; Swanson et al., 2011). However, char are highly variable in terms of life history patterns (growth and age at maturity), habitat use (freshwater or marine), the role they play in those ecosystems (predator or prey), and their food sources (benthic or pelagic) (Klemetsen et al., 2003; Power et al., 2008). Therefore, it is critical to understand the ecology of a particular population when assessing contaminant bioaccumulation because these differences might influence bioaccumulation. The main objective of the present study was to compare the concentrations of Hg with concentrations of PFCs (among fish and water) of both Pingualuk Lake and nearby Laflamme Lake, in order to broaden our understanding of contaminant dynamics in freshwater ecosystems dominated by atmospheric inputs. To aid our interpretation of the contaminant concentrations, we also conducted a basic survey of the unique physical properties of the lake water column (underwater solar radiation, temperature, water chemistry) and selected biological factors (lake productivity, char feeding ecology, genetics) that might influence Hg and PFC concentrations. We assessed previously unknown genetic characteristics of the arctic char population of Pingualuk Lake using two end points: major histocompatibility complex (MH) and mitochondrial deoxyribonucleic acid (mtDNA). Examination of stomach contents and analysis of carbon (^{13}C) and nitrogen (^{15}N) stable isotopes were also used to better understand the feeding ecology of this fish population. The $\delta^{13}C$ and $\delta^{15}N$ signatures were used to determine habitat use and the trophic position of individual arctic char (Vander Zanden and Rasmussen, 2001; Post, 2002). Comparative measurements were made in Laflamme Lake, which is thought to host a genetically related arctic char population (Bouchard, 1989).

MATERIALS AND METHODS

Study Sites

Pingualuk Lake (61˚17′ N, 73˚40′ W) and Laflamme Lake (61˚20′ N, 73˚43′ W) are located on the Ungava Peninsula, with Laflamme Lake located approximately 4 km to the northwest of Pingualuk Lake (Fig. 1). The two lakes differ in their catchment characteristics. Laflamme reflects a more "normal" lake, with a catchment area approximately four times the size of the lake area, while the catchment area of the crater lake (Pingualuk) is minimal, with less than half the area of the lake itself (CA/LA ~0.4) (Gantner et al., 2010b). Basic limnological information was first established for Pingualuk Lake in 1988, when a Secchi depth of ~37 m and a euphotic zone (delimited by 1% of surface irradiance level) down to a depth of 89 m were recorded (Bouchard, 1989; Ouellet et al., 1989). Hydrologic separation of both lakes is thought to have occurred following the uplifting of the land (isostatic rebound) during the retreat of the Laurentide ice sheet.

Fish collection, water sampling, and water column profiling were conducted during the two-week Pingualuk Crater Lake Project sampling expedition in early spring (4–19 May) 2007. A single horizontal tow for zooplankton under the ice resulted in a single bulk zooplankton sample (> 64 µm). At that time, the 6.68 km^2 surface area of Pingualuk Lake was covered by 1.55 m of ice and 10–15 cm of hard-packed snow (R. Pienitz, U. Laval, pers. comm. 2007). Additional water samples for PFC analysis were obtained during the ice-free season (25 August 2008).

Char Sampling

Char sampling was conducted using hook and line through the ice by local personnel from Park National de Pingualuit (Yaaka Yaaka and Peter Kiatainaq) of Kangiqsujuaq (formerly Wakeham Bay). A total of 20 arctic char were captured by angling near the western shore of Pingualuk Lake, while a combination of arctic char (n = 7) and lake trout, *S. namaycush* (W.), (n = 2) were caught near the south shore of Laflamme Lake. The maximum catch per lake was 20 specimens as per the license permit issued by the Ministère des Ressources Naturelles et de la Faune du Québec. All fish were frozen immediately after collection and shipped to the Canadian Centre for Inland Waters (CCIW) in Burlington, Ontario (Canada) for further processing. After a brief thawing period, individual weight, fork length, and samples of dorsal muscle and liver tissue were obtained, and sex was determined by gonadal inspection of each fish. Muscle and liver tissue samples were frozen to preserve them for stable isotope, Hg, and PFC analyses, while additional muscle and adipose fin tissue were placed in ethanol or dimethyl sulfoxide (DMSO), respectively, for genetic analyses. During dissections, eight additional arctic char were obtained from the stomach contents of larger arctic char from Pingualuk Lake. Muscle

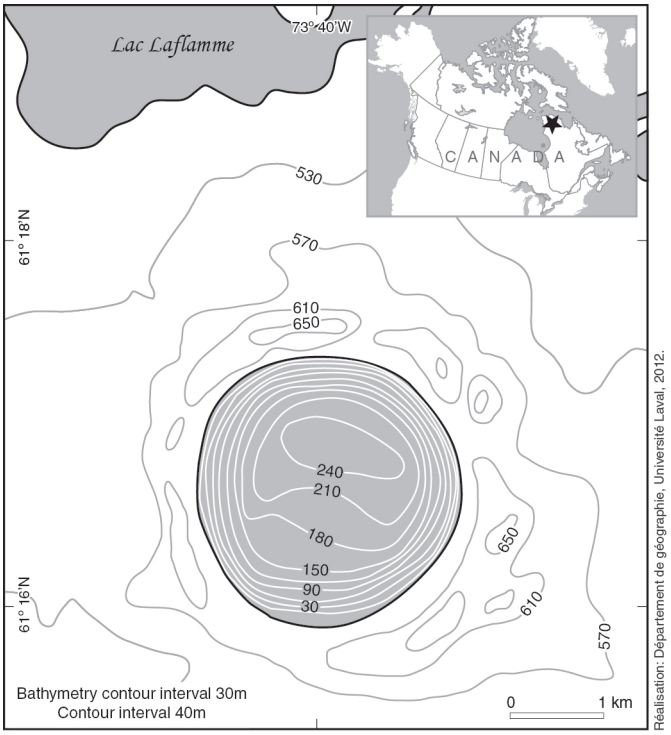

FIG. 1. Map showing the geographic location and bathymetry of Pingualuk Lake, as well as the topographic contours of Pingualuit Crater and its surrounding area (from Pienitz et al., 2008).

tissue samples were taken from these eight specimens for stable isotope analysis; however, accurate weight and fork length determination were possible for only three of these fish, as the rest were partially digested. Ageing was performed on extracted otoliths at the Freshwater Institute, Fisheries and Oceans Canada (Winnipeg, MB, Canada) using the break-and-burn method (Chilton and Beamish, 1982). Stomach contents were assessed qualitatively by recording the presence or absence of fish and chironomid larvae or pupae in the foregut of each fish. Longer-term diet information, reflected in the values of dorsal muscle tissue $\delta^{13}C$ and $\delta^{15}N$ signatures, was collected for the fish originally obtained from Pingualuk Lake (n = 20) and Laflamme Lake (n = 9), as well as for eight char removed from the stomach contents of larger individuals from Pingualuk Lake. Samples were processed according to the methods described in Guiguer et al. (2002), and all stable isotope ratios are reported in conventional δ notation, where $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \cdot 1000$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Char Hg and PFC Analyses

Concentrations of total Hg (THg) in dorsal muscle tissue were determined for fish collected from Pingualuk Lake (n = 22) and Laflamme Lake (n = 9) using the methods outlined in Gantner et al. (2010a). Briefly, 0.1–0.2 g of homogenized tissue was analyzed with U.S. EPA method 7473 using a Direct Mercury Analyzer (DMA 80, Milestone Instruments). All THg concentrations are presented

here on a wet weight basis. MeHg was not measured separately, as we have shown that more than 95% of THg in char muscle tissue is present in the methylated form (Gantner et al., 2010a, b). Geometric means of Hg concentrations are reported here, and results were $\log_{10}$-transformed prior to regression against length or age to satisfy criteria of normality.

PFCs were determined in 21 arctic char muscle samples from Pingualuk Lake and nine muscle samples from Laflamme Lake. These samples were analyzed for perfluorosulfonates (PFSAs; PFBS, PFHxS, PFHpS, PFOS, PFDS), perfluorocarboxylates (PFCAs; C4 – C14, C16, C18), perfluorooctane sulfonamide (PFOSA) and unsaturated fluorotelomer acids (6:2, 8:2, 10:2 PFUA) (see [online] Appendix 1: Table 1 for definitions of abbreviations) using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (API 4000, Applied Biosystems Inc.). An internal standard (^{13}C-mass-labelled PFCAs C8-12, PFOS, 6:2, 8:2, 10:2 PFUA; Wellington Laboratories, Guelph, Ontario) was added to ~0.2 g of homogenized muscle samples in a 15 mL polypropylene centrifuge tube. Three subsequent methanol extractions were made, and the resulting supernatants were evaporated under high-purity nitrogen gas (Tomy et al., 2006). These concentrates were filtered through 0.2 µm disposable syringes into polypropylene vials. Laboratory blanks were made of PFC-free methanol. A five-point calibration curve was used to quantify the PFCs (range 3.6 – 322.0 pg mL^{-1}). Information on detection limits for PFCs in fish is provided in [online] Appendix 1.

Genetic Analyses

Major Histocompatibility Class II β allele analysis (hereafter referred to as MH) and mitochondrial DNA (mtDNA) analysis were used in the present study. Subunits of the MH molecule are highly polymorphic and can be used to characterize wild fish populations (Dorschner et al., 2000; Landry and Bernatchez, 2001; Miller et al., 2001), while mtDNA is a standard method to distinguish char lineages (Brunner et al., 2001). Because lake trout, a closely related salmonid species, was present in nearby Laflamme Lake, we aimed to confirm that the fish in Pingualuk Lake are arctic char and not lake trout. All detailed methods pertaining to the two genetic analyses (MH and mtDNA) are presented in [online] Appendix 2.

Water Hg and PFCs Analyses

Depth profiles of THg in the water column from Pingualuk and Laflamme Lakes were obtained through the ice at the site of (but prior to) sediment coring. Water samples were taken at 2, 5, 10, 15, 30, and 60 m depth at Pingualuk Lake and at 2 and 5 m at Laflamme Lake, using a precleaned Teflon® Kemmerer sampler. Two additional surface water samples for analysis of PFCs were collected by hand, using 500 mL polypropylene bottles. All samples were stored at 4°C after collection prior to shipment to CCIW. Details of water analysis for Hg using atomic fluorescence spectroscopy (AFS) are from Gantner et al. (2010a). To minimize contamination, water for PFC analysis was processed in a clean room (HEPA, carbon filtered air) along with other Arctic samples using the method described in Veillette et al. (in press), as modified by Yamashita et al. (2004). Method detection limits are provided in [online] Appendix 1.

Water Chemistry, Temperature, and Light Conditions

Surface water samples (n = 1 at each lake) for standard water chemistry parameters were obtained through the ice and analyzed at CCIW. Water chemistry, temperature, and underwater solar radiation profiles were taken at Pingualuk Lake with an XR-420 CTD (conductivity-temperature-depth profiler; RBR Ltd.) and a PUV-500 light profiler (Biospherical Instruments Inc.). Both were lowered through a drilled hole. The diffuse attenuation coefficient (K_d) for 308 (UVB), 320 (UVB), 340 (UVA), and 380 (UVA) nm radiation, as well as photosynthetically available radiation (PAR, 400 – 700 nm, visible light) were calculated over the log-linear portion of the irradiance vs. depth plot, from well below the ice (7.5 m) to a maximum depth of 26.4 m. The euphotic zone, the depth at which 1% of visible light remains, was estimated from the K_{dPAR} value.

RESULTS AND DISCUSSION

Char Ecology

Arctic char collected from Pingualuk Lake (n = 20 + 3 from stomach contents; total of 23 fish) ranged in size from 97 mm to 576 mm in fork length, and from 10 to 1721 g in weight. The oldest individual collected was 32+ years old. Of the 23 char, five were females and ten were males; gender of the remaining eight fish could not be determined (Table 1).

The majority (13 of 23) of Pingualuk char had empty stomachs, while arctic char remains were found in eight char stomachs (i.e., cannibalism was evident; Fig. 2). Stable isotope data suggest that arctic char in this population may undergo an ontogenic shift at 200 mm in length (Fig. 3A), as there is a significant difference in δ^{15}N values between individuals smaller than 200 mm (mean δ^{15}N ± 95% CI = 9.26 ± 0.55‰) and those greater than 200 mm (12.38 ± 0.21‰) (ANOVA: $F_{(1,26)} = 206.08$; $p < 0.05$) (Fig. 3B). Stomach contents and δ^{15}N signatures indicate that arctic char larger than 225 mm fed at a trophic level consistent with piscivory (Fig. 3B). Among those arctic char with fork lengths less than 200 mm, there appeared to be some differentiation with respect to δ^{13}C signatures as well. A group of seven individuals formed one group (mean ± 95% CI: δ^{13}C = 30.33 ± 1.06‰) with δ^{13}C signatures that were significantly more negative than the group of three individuals with

TABLE 1. Biological data and Hg concentrations (muscle) of arctic char and lake trout.

Lake	Fish (#)	Species	Fork length (mm)	Weight (g)	Sex	Age (yr)	$\delta^{15}N‰$	[THg][1] (μg g^{-1})
Laflamme	1	Arctic char	488	1343	U	118.90	0.11	
	2		507	1530	F	119.15	0.14	
	3		550	1732	F	179.07	0.24	
	4		350	483	F	78.75	0.10	
	5		621	2605	M	1610.03	0.13	
	6		456	1220	F	139.35	0.14	
	9		639	2770	M	199.79	0.15	
	7	Lake trout	265	203	M	79.35	0.07	
	8		585	2178	M	2211.42	0.30	
Pingualuk	1	Arctic char	501	971	M	3212.33	0.46	
(present study)	2		409	632U	20	12.52	0.20	
	3		498	1097	M	2412.95	0.29	
	4		334	301U	17	12.55	0.15	
	5		387	454	M	1612.59	0.11	
	6		383	460	F	1512.30	0.19	
	7		338	343	M	1612.65	0.15	
	8		566	1708	M	2112.37	0.20	
	9		354	401	M	1812.17	0.12	
	10		576	1721	M	2411.61	0.23	
	11		520	1132	U	2812.00	0.40	
	12		374	356F	22	13.06	0.38	
	13		407	645	F	1712.30	0.14	
	14		414	603	U	2012.65	0.22	
	15		423	605	U	2112.03	0.31	
	16		229	207	M	1412.58	0.12	
	17		244	107U	12	12.07	0.16	
	18		149	36F	9	9.93	0.04	
	19		301	250	M	1713.02	0.14	
	20		242	127	M	1911.38	0.12	
	2a[2]		195	59F	12	8.42	0.05	
	8a[2]		97	10U	6	8.22	N/A	
	19a[2]		104	10U	7	9.48	N/A	
Pingualuk	1	Arctic char	425	N/A	N/A	N/A	N/A0.13	
(from Delisle et al., 1986)	2		458			0.21		
	3		478			0.15		
	4		526			0.20		
	5		479			0.18		
	6		601			0.36		
	7		681		16	0.22		
	8		655		14	0.25		
	9		672		13	0.23		
	10		607		12	0.30		
	11		676		18	0.35		

[1] Results of dorsal muscle tissue in wet weight.

[2] Samples were obtained from mouth or stomach of adult char.

more positive $\delta^{13}C$ values ($\delta^{13}C$ = -23.17 ± 2.52) (ANOVA: $F_{(1,8)} = 82.84$; $p < 0.05$). Variation among the $\delta^{13}C$ signatures of smaller char suggests that differentiation between littoral and pelagic feeding strategies may exist among individual Arctic char. However, we do not have any information regarding the difference in $\delta^{13}C$ signatures between the littoral and pelagic food webs with which to test this hypothesis. Additional biological information on all char collected is presented in Table 1.

Char Hg and PFC Concentrations

The mean Hg concentration in Pingualuk char was 0.18 ± 0.11 μg g^{-1} (all wet weight; geometric mean ± 1 SD) and 0.14 ± 0.07 μg g^{-1} in Laflamme char (Table 1; mean concentrations of Thg [Thg] from Gantner et al., 2010a). The mean [THg] of Pingualuk char analyzed in the present study was similar ($p > 0.05$, t-test) to the mean [THg] reported by Delisle et al. (1986) (0.23 ± 0.07 μg g^{-1}) for 11 slightly larger Pingualuk char captured in 1983 (Table 1). Statistical adjustment of char Hg concentrations using analysis of covariance (ANCOVA) was performed as part of a comparative study of 27 populations (Gantner et al., 2010b), revealing that length-adjusted Hg concentrations were not significantly different (ANOVA $p > 0.05$) between Pingualuk and Laflamme char. Concentrations of Hg in char muscle were positively correlated ($p < 0.05$ for all linear regressions below) with fork length and age in Pingualuk Lake ($r^2 = 0.57$ and $r^2 = 0.71$, respectively) and Laflamme Lake ($r^2 = 0.47$ and $r^2 = 0.72$, respectively). The strong correlation of Hg with age indicates slow growth of arctic char in the ultra-oligotrophic water of Pingualuk, which is supported by the water column characteristics described below. Concentrations of Hg in Pingualuk and Laflamme

FIG. 2. Arctic char from Pingualuk Lake (Nunavik, Canada) exhibit cannibalism, as demonstrated here. The char on the bottom was retrieved from the mouth and stomach of the individual above. Photo © N. Gantner.

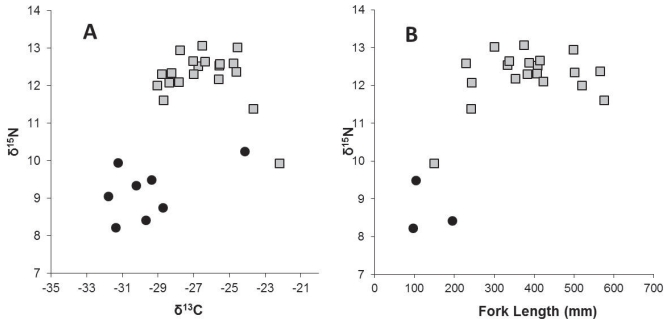

FIG. 3. Trophic signatures of Pingualuk char muscle tissues. A) Stable isotope plots for Pingualuk arctic char muscle tissue. Squares denote individuals captured by angling, while circles denote individuals sampled from the gut contents of larger char. B) Individual fork lengths plotted against $\delta^{15}N$, as an indication of relative trophic position. Note: The lengths of five char removed from stomach contents could not be accurately determined because the caudal fin was partially digested, so they are not included in this figure.

char were not correlated with $\delta^{15}N$ (linear regression $p > 0.05$), possibly owing to the presence of two feeding types (Fig. 4). Although there is some evidence that higher [THg] in arctic char occurs in lakes with higher catchment to lake area (CA/LA) ratios (Gantner et al., 2010b), Pingualuk char have high [THg] compared to fish in the lakes in that study, despite the very low CA/LA of Pingualuk Lake. This fact could be explained by the high number of piscivorous individuals collected from Pingualuk Lake compared to the other lakes. Analysis of food web structure in several Arctic lakes has suggested that benthic coupling may be the dominant energy pathway in Arctic lakes (Sierszen et al., 2003; Chételat et al., 2008; Gantner et al., 2010a, b). However, the complete food web of Pingualuk Lake has yet to be characterized in order to elucidate to what extent Pingualuk char may use the lake bottom, limited littoral zone (chironomids), or pelagic habitat (zooplankton) during feeding. No isotope data for primary producers (baseline of the food web) or chironomids are currently available. The lone isotopic information on food web items available remains a zooplankton sample ($\delta^{13}C$ = -25.3 ‰) taken in 2007.

A suite of PFC compounds was detected in char from both lakes (Fig. 5). PFOS, PFUnA, PFDA, and PFDoA were consistently above method detection limits (MDLs) and were the most abundant PFCs in char muscle in both

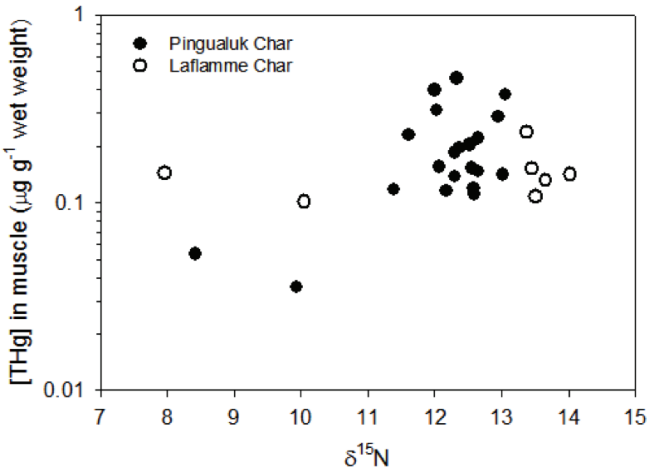

FIG. 4. Concentrations of Hg versus $\delta^{15}N$ values in Pingualuk and Laflamme fish muscle.

lakes. Mean total PFCs detected in Pingualuk (28 pg g^{-1} wet weight) and Laflamme fish (64 pg g^{-1} wet weight) were not statistically different (Mann-Whitney rank sum test, $p = 0.161$). There was no significant relationship between the concentrations of PFCs and $\delta^{15}N$ or char fork length for each lake (linear regression, $p > 0.05$). To our knowledge, the PFC concentrations detected in char from both lakes are the lowest ever reported for fish (Houde et al., 2006, 2011). As expected, the PFCs with less than eight carbons were not prominent contaminants in the fish although they predominated in water (Table 3 in [online] Appendix 1). This pattern reflects their rapid elimination by the fish, which reduces their potential for bioaccumulation (Houde et al., 2006).

Comparing our result with those of other studies, total PFC concentrations of 5–2149 pg g^{-1} wet weight were reported for arctic char (muscle) from Lake A and Lake C2 on Ellesmere Island (Nunavut, Canada) (Veillette et al., in press). Mean total PFCs were in the range of 400–900 pg g^{-1} in char muscle from Amituk Lake and Char Lake on Cornwallis Island (Nunavut, Canada), while they averaged 350 pg g^{-1} wet wt in char from Lake Hazen (Quttinirpaaq National Park, Ellesmere Island) (Muir et al., 2009b). Concentrations of PFCs found for lake trout (whole fish homogenates) in the Great Lakes (13–152 ng g^{-1}) (Furdui et al., 2007) were more than 40 times as high as those in Pingualuk and Laflamme Lakes.

Char Genetics

All fish samples from Pingualuk Lake were identified as arctic char using mitochondrial DNA (mtDNA). All Pingualuk char mtDNA displayed haplotype ARC19 (Genebank accession EU310899), which is the most widely observed haplotype of arctic char in the Canadian Arctic, with a distribution from the Mackenzie River to Newfoundland and Labrador (J. Reist et al., Fisheries and Oceans Canada, unpubl. data). The absence of variation suggests that

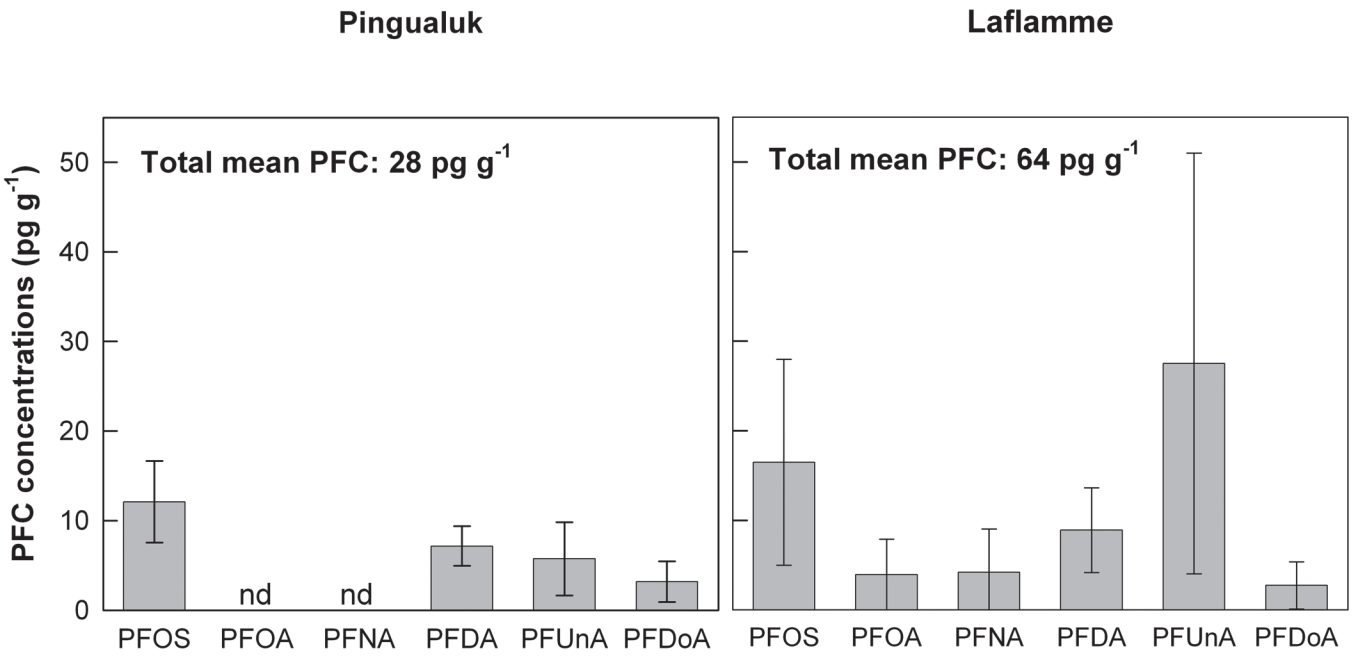

FIG. 5. Mean PFC concentrations of dorsal muscle tissue (all wet weight) of arctic char from Pingualuk Lake (n = 21) and arctic char and lake trout from Laflamme Lake (n = 9). The analytes included are those detected in at least three fish from both lakes. They were above instrument detection limits but below method detection limits. Error bars indicate the 95% confidence interval and "nd" indicates that the analyte was detected in only one fish or no fish.

Pingualuk char likely originated from a small founding population that had been genetically bottlenecked during glacial times, or from recolonization of recently deglaciated locations, or both. This is typical for char from glaciated regions of northern Canada (Wilson et al., 1996). At Laflamme Lake, seven fish exhibited the haplotype ARC19, and two fish (#7 and 8) had the lake trout haplotype eLT01. As in the Pingualuk Lake char, we observed no variation in Laflamme Lake char mtDNA.

Analyses of the MH alleles indicated that arctic char from Pingualuk and Laflamme Lakes do not interbreed (Dixon et al., 1996; Miller et al., 2001). The clusters in the phylogenetic tree (Fig. 6) contain many sequences that suggest allele families have expanded in each lake since the two char populations were separated. The branch length of the clusters and low bootstrap values suggest that shared allele families diverged before the populations invaded the respective lakes. Because of the low number of samples and shared alleles (n = 42), it was not possible to determine whether the char belong to previously identified Arctic or Atlantic char lineages (Brunner et al., 2001) using MH. The two populations seem to be distinct from each other, although the time of separation could not be determined here. Further information on the mtDNA and MH results is available in [online] Appendix 2.

Mercury and PFCs in Lake Water

Concentrations of THg in water were greatest directly under the ice (6 pg mL^{-1} in Pingualuk Lake, 3.5 pg mL^{-1} in Laflamme Lake), and declined rapidly in the first 15 m (Fig. 7A). These concentrations are higher than those found in several other Arctic lakes, where values ranged from 0.3 to 0.8 pg mL^{-1} (Gantner et al., 2010b), but the under-ice profile was similar to that of Char Lake, Nunavut (D. Muir and G. Lawson, Environment Canada, unpubl. data). The extent to which these higher THg under-ice concentrations ultimately influence concentrations in the food web is unclear. Further study, including measurements of bioaccumulative MeHg, is needed to investigate this pathway.

Surface water from Pingualuk Lake had a total PFC concentration of 231 pg L^{-1}, and the main PFCs detected were PFOA, PFOS, PFHpA, PFNA, PFUnA, and PFDA (Fig. 7B). The PFC concentrations were similar to those reported for Lake A, Ellesmere Island (~ 500 pg L^{-1}) (Veillette et al., in press), and much lower than those found in Amituk and Char Lakes on Cornwallis Island (2–5 ng L^{-1}) (Stock et al., 2007) and the North American Great Lakes (~15 ng L^{-1}) (Furdui et al., 2008). However, PFOA and PFOS were the predominant PFCs detected in the water, a result which is similar to those from other remote Arctic lakes (Stock et al., 2007; Veillette et al., in press). The very low concentrations of PFCs detected in Pingualuk Lake (fish and surface water) and in Laflamme Lake (fish) indicate that the amount of PFCs entering both lakes is similar and that the catchment effect is negligible for this chemical. A short duration of open water (4–6 weeks) might further limit the input of PFCs in these lakes. Local sources of PFC contamination can be important for some systems (Moody et al., 2002; Stock et al., 2007); however, these are likely to be negligible for Pingualuk Lake given that very strict legislation regulates activities in the Parc National des Pingualuit. Furthermore, the remote location of Pingualuk and Laflamme Lakes, far from PFC source regions of North

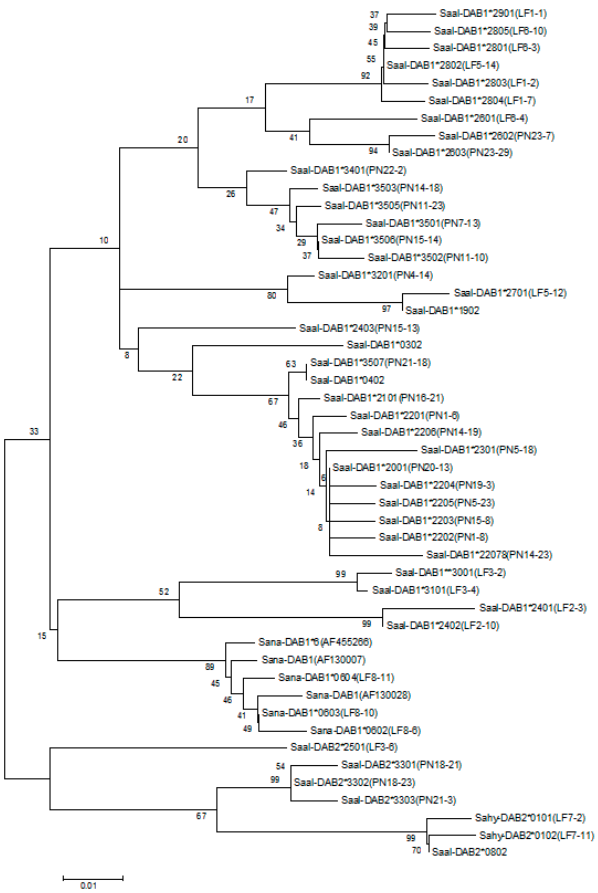

FIG. 6. Phylogenetic tree of MH class II beta alleles isolated from the Pingualuk Lake and Laflamme Lake arctic char populations. Allele names are given, with representative clone names in brackets to help identify the population the alleles were found in (PN = Pingualuk Lake, LF = Laflamme Lake). If alleles were shared with other populations, the location of those other populations is indicated. The bar at the bottom represents genetic distance, and the numbers at branch points indicate percentage bootstrap values after 5000 rounds of replication. Brackets and numbers have been inserted to facilitate discussion of the clustering pattern.

America, might explain the extremely low concentrations. Both lakes are located approximately 90 km from the nearest coastline, making any oceanic contribution from marine aerosols very unlikely.

The apparent difference in the concentration of these contaminants (i.e., extremely low PFCs, but unexpectedly high Hg) could be due to the duration of deposition of each chemical into Pingualuk Lake. While the volatile precursors of PFCs have been emitted over only the past 60 years, and the majority since 1970, anthropogenic Hg deposition has been occurring in northern Québec since the beginning of the 20th century, doubling natural background concentrations of Hg in lake sediments in the region (Lucotte et al., 1995; Muir et al., 2009a). A potential source of additional local mercury inputs could be the newly mobilized rock dusts transported across the tundra landscape from mining operations of a nearby X-Strata Nickel Mine (formerly the Raglan Mine) located about 70 km to the north of Pingualuk Lake.

Water Chemistry, Light and Temperature Conditions

Water chemistry, solar radiation and temperature indicate that both lakes Pingualuk and Laflamme are ultra-oligotrophic, with Pingualuk Lake surface water typically lower in most parameters (Table 2). Further, these ultra-oligotrophic conditions only allow very limited growth of char, which could contribute to the Hg concentrations in char muscle. The concentration of chlorophyll *a* (Chl *a*) in Pingualuk and Laflamme surface water was extremely low (< 0.1 and 0.3 µg L^{-1} Chl *a*, respectively), indicating very low phytoplankton stocks and productivity during ice cover conditions. Pingualuk Lake was inversely stratified (Fig. 8A), with temperatures increasing with depth through the entire water column. Conductivity measures were below the detection limits (of 20 µS cm^{-1}) of the instruments used, indicating the extremely dilute major ion concentrations of Pingualuk Lake.

The light measurements emphasized the exceptional clarity of the waters of Pingualuk Lake, placing it among the clearest lakes in the world. The diffuse attenuation coefficient for PAR (K_{dPAR}) in the deeper waters of Pingualuk Lake (in the log-linear portion of the curve; i.e., below 7.5 m) was 0.040 m^{-1} (Fig. 8B), which is near or below values for ultra-oligotrophic lakes renowned for their transparency. For instance, K_{dPAR} was 0.034 m^{-1} in the upper water column of Lake Vanda in Antarctica (Vincent et al., 1998) and 0.066 m^{-1} in subalpine Lake Tahoe (USA) (Rose et al., 2009). The clearest natural waters sampled to date, the hyperoligotrophic South Pacific gyre near Easter Island, have K_{dPAR} values at 420 nm around 0.02 m^{-1} (Morel et al., 2007). The Pingualuk Lake K_{dPAR} value would translate into a 1% irradiance level (under ice-free conditions) at 115 m, indicating a remarkably deep euphotic zone. The zone of net primary production would be substantially smaller during ice-covered conditions because of the attenuating effects of the snow and ice and the greater attenuation of PAR in the upper meters of the water column under the ice. The irradiance curves in all wavebands showed higher attenuation values in the upper 7.5 m of the water column, which is consistent with stratified conditions and suggests the development of a phytoplankton community immediately under the ice. Pingualuk Lake is also highly transparent to UVA and UVB radiation (Fig. 8B), although its overlying snow and ice cover would reduce the exposure of the aquatic food web to these damaging wavebands throughout much of the year (Belzile et al., 2001).

Summary and Future Research at Pingualuk Lake

This study provided information on the present THg and PFC concentrations in Pingualuk Lake arctic char and water column during ice-cover conditions. Mercury in char was found in concentrations comparable to the only previous measurement in 1983 and was similar to that of other Arctic lakes, despite the minimal catchment influence. As expected, [THg] was greater in larger and older piscivorous

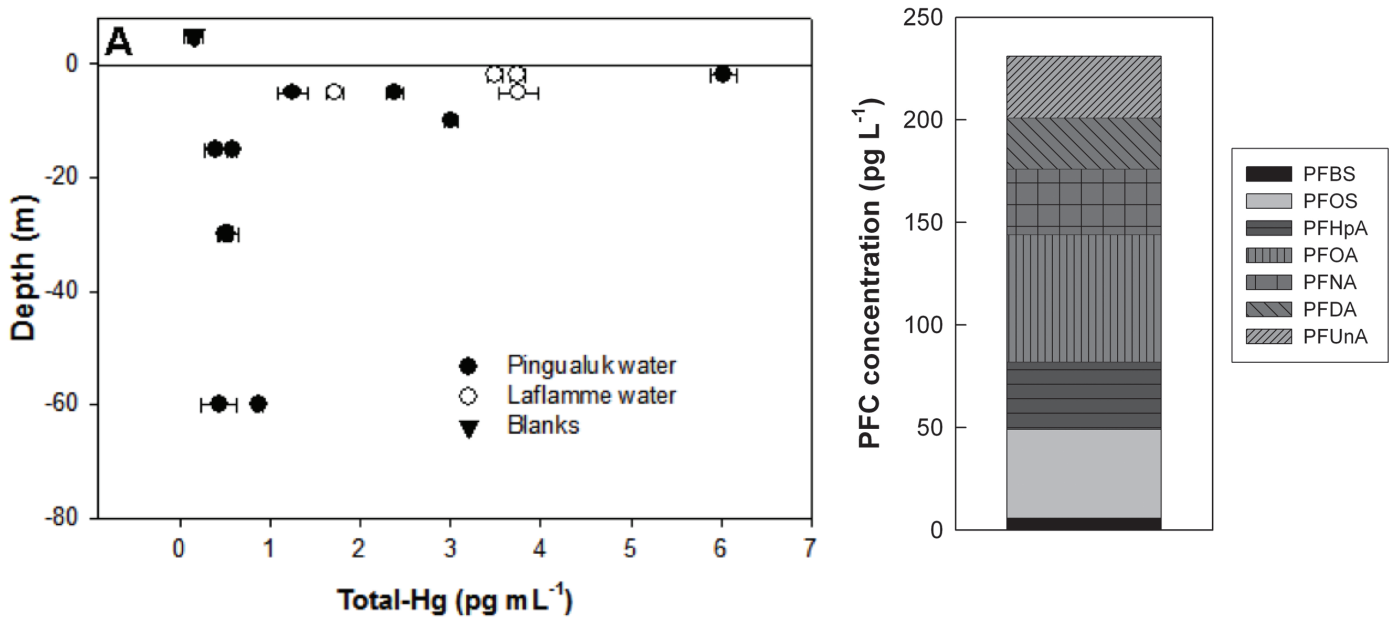

FIG. 7. Water contaminant concentrations detected in Pingualuk and Laflamme Lakes. A) Depth profile of [THg] in water sampled at various depths in Pingualuk and Laflamme Lakes; B) Surface water PFC concentration in Pingualuk Lake. Total PFC = 231 pg L^{-1}. Error bars in (A) represent ± 1 SD of three analytical replicates.

individuals. Piscivory and thus greater biomagnification could explain the greater [THg], while slow growth in the ultra-oligotrophic conditions is likely a contributing factor. The reported PFC concentrations were amongst the lowest known from the literature.

The analyses presented in this study provide important baseline data for future environmental change studies. The diet and trophic positions of Pingualuk char revealed evidence for cannibalism, as well as the potential for multiple feeding strategies within this lake. Using genetic measurements, all fish collected in Pingualuk were identified as *S. alpinus* (all haplotype ARC19) of a common eastern North American complex. The parent population of the present-day Pingualuk char population could not be identified, nor could the timing of separation from that parent population be determined by this study. For the first time, an under-ice profile of [THg] in the water column was presented for Arctic lakes and indicated elevated concentrations under the ice surface. Water-column profiling data confirmed that Pingualuk Lake is among the most transparent lakes of the world. Pingualuk Lake, with its minimal drainage basin, its hydrologic isolation, and its remote location, is a highly valuable ecosystem to monitor contaminant inputs from the atmosphere (e.g., PFCs) and climate warming effects.

Many questions remain about the ecology of Pingualuit Crater Lake. The unique catchment characteristics make this system particularly attractive for biogeochemical and optical studies, with an opportunity to analyze atmospheric inputs, including additional contaminants, dissolved organic carbon, dust, and microbiota (Mladenov et al., 2009). The seasonal dynamics of phytoplankton and zooplankton communities require attention, as do the benthos, in future sampling efforts. To determine parent population and timing of separation, a genetic comparison of arctic char of the same ecotype from multiple lakes in the vicinity of the crater, as well as Pingualuk Lake, is necessary. In addition, future sampling should be directed by the degree of anthropogenic inputs of relevant contaminants, from highly impacted (e.g., local mining), to less impacted (lakes nearby), to least impacted (Pingualuk Lake) lakes for further studies of atmospheric contaminants. A subset of all biological material collected should be preserved and archived for future investigations. Any future collection efforts at Pingualuk Lake should continue to be conducted with great care to avoid disruption of the crater itself and the biota within. In the longer term, the ongoing observation of Pingualuk Lake limnology and char would provide a valuable monitoring system for contaminant inputs and climate warming effects (Wrona et al., 2006), especially those induced by changes in ice cover (Prowse et al., 2006).

TABLE 2. Selected water chemistry parameters in Pingualuk and Laflamme Lakes, Nunavik. Chlorophyll *a* is measured in µg L^{-1} and the other parameters are measured in mg L^{-1}.

Parameter	Pingualuk	Laflamme
Chl *a*	< 0.1	0.3
DOC	0.7	1.7
DIC	1.1	1.1
POC	0.128	0.365
PON	0.018	0.046
SiO_2	0.28	0.31
SO_4	0.77	0.62
Cl	1.10	0.91
Ca	0.56	0.49
Mg	0.16	0.19
K	0.29	0.26
Na	0.75	0.70

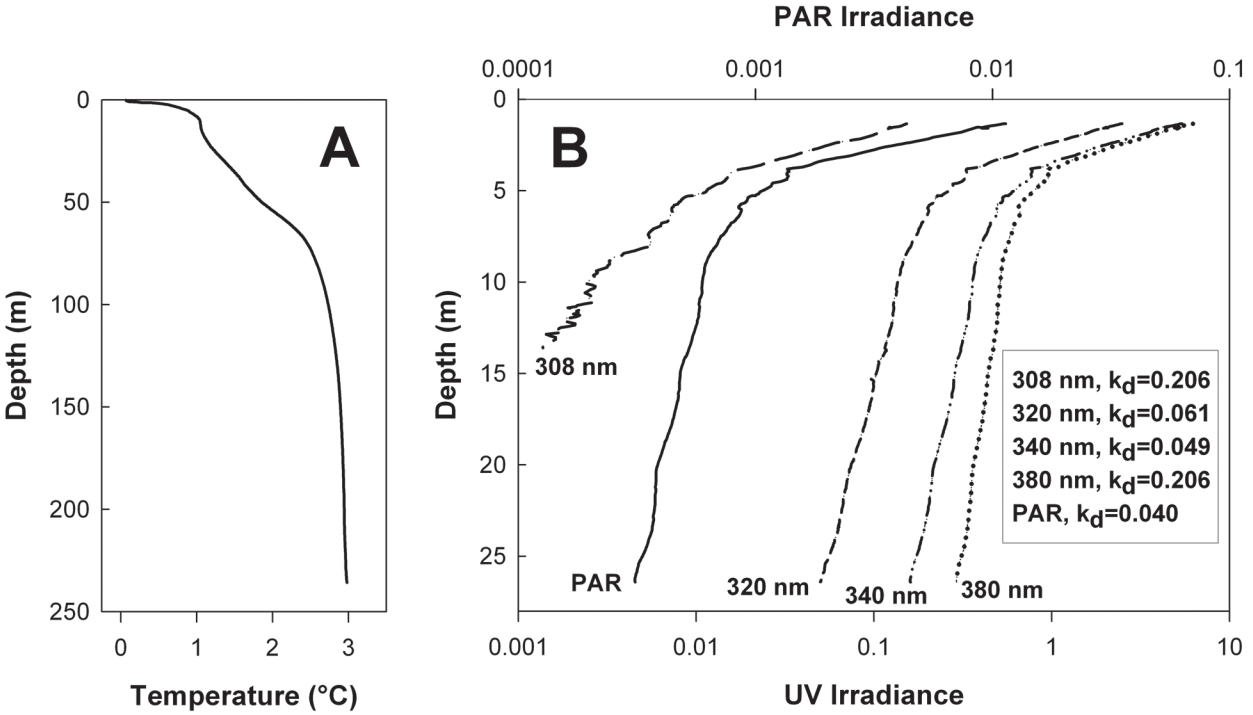

FIG. 8. Water column profiles of A) temperature and B) downwelling PAR and UV irradiance in the top 25 m of the water column for Pingualuk Lake. Note the different log scales for irradiance: PAR values are in µmol photons cm^{-2} s^{-1} and UV values are in µW cm^{-2} nm^{-1}.

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APPENDICES

Appendix 1: Contaminants and Appendix 2: Arctic Char Genetics are available in the supplementary file attached to the online version of this article at http://arctic.synergiesprairies.ca/arctic/index.php/arctic.

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