Supporting Information for:

Ratio of Methylmercury to Dissolved Organic Carbon in Water Explains Methylmercury Bioaccumulation Across a Latitudinal Gradient from North-Temperate to Arctic Lakes

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Table S1. Location, sampling year, and size of the 35 study lakes.

Study Area	Lake	Year	Latitude	Longitude	Lake Area (km^2)	Catchment Area (km^2)	Mean Depth (m)
Gatineau Park (mixed forest)	Black	2011-12	45°29'30"	75°51'51"	0.02	0.10	5.8
	Brown	2011-12	45°36'33"	75°55'56"	0.27	6.24	2.9
	Clair	2011-12	45°35'46"	76°03'29"	0.11	0.5	13.7
	Kidder	2011-12	45°36'17"	76°05'19"	0.06	1.78	9.9
	La Pêche	2011-12	45°37'23"	76°11'07"	7.06	49.4	5.7
	Meech	2011-12	45°32'07"	75°53'24"	2.89	60.2	13.3
	Phillipe	2011-12	45°36'15"	76°00'05"	1.75	18.1	9.1
	Pink	2011-12	45°28'05"	75°48'28"	0.11	0.92	11.7
	Ramsay	2011-12	45°35'54"	76°05'58"	0.11	2.71	7.1
	Taylor	2011-12	45°36'17"	76°03'04"	0.30	3.82	8
Kuujjjuaraapik-Whapmagoustui (sub-Arctic taiga)	Site 1	2012	55°19'11"	77°42'41"	0.08	2.70	0.7
	Site 2	2012	55°22'10"	77°37'04"	0.11	3.35	1.0
	Site 3	2012	55°18'16"	77°42'56"	0.01	0.62	0.3
	Kachishayoot	2012	55°20'03"	77°37'31"	0.29	1.78	1.9
	Site 5	2012	55°17'26"	77°43'08"	0.05	1.00	1.3
	Site 6	2012	55°19'12"	77°38'32"	0.08	0.39	0.6
	Site 7	2012	55°20'01"	77°35'48"	0.39	2.31	0.9
	Site 8	2012	55°20'14"	77°36'42"	0.10	0.19	1.2
Iqaluit (tundra)	Site 1	2013	63°47'49"	68°32'46"	0.03	0.21	1.8
	Site 2	2013	63°45'29"	68°26'38"	0.08	2.00	6
	Site 3	2013	63°39'07"	68°17'49"	0.28	4.03	12
	Site 4	2013	63°47'53"	68°32'17"	0.09	0.37	5
	Site 5	2013	63°44'56"	68°23'53"	0.36	1.87	6
	Site 6	2013	63°54'58"	68°34'23"	0.42	12.5	3
	Iqalugaajuruluit	2013	63°41'06"	68°22'34"	0.54	3.74	8
	Site 8	2013	63°49'30"	68°36'14"	0.56	14.2	12
	Tasirluk (Crazy)	2014	63°52'29"	68°28'40"	4.5	41.1	7.5
Resolute Bay (polar desert)	Ruins	2014	74°40'48"	94°54'52"	0.13	20.2	1.8
	Meretta	2014	74°41'24"	94°59'24"	0.27	5.18	3.1
	RZ2	2014	74°43'15"	94°51'42"	0.03	1.51	0.8
	Teardrop	2014	74°41'03"	94°59'22"	0.04	0.42	4.3
	Small	2014	74°45'33"	95°03'37"	0.15	1.56	2.7
	North	2014	74°46'37"	95°05'47"	0.63	83.7	6. 7
	Resolute	2014	74°41'15"	94°56'33"	1.21	19.8	9
	RZ-P3	2014	74°44'38"	94°57'18"	0.04	1.40	0.8

Water Sampling Methods:

At each lake, *in situ* water temperature, specific conductivity, pH, and dissolved oxygen were measured with a YSI multi-parameter sonde (YSI Inc., Yellow Springs, Ohio, USA). In Gatineau Park lakes, *in situ* chlorophyll (Chl) was measured with a YSI fluorescence probe, validated with Chl analyses by ethanol extraction of filtered seston in the laboratory for a subset of six lakes (Supplemental Figure S1). Water for mercury analysis was collected using clean protocols for trace metals (e.g., clean hands/dirty hands method, acid washing of sampling equipment). Surface water was collected as sub-surface grabs in Nalgene® PETG bottles for mercury analysis and in HDPE bottles for analysis of dissolved organic carbon (DOC), total nitrogen (TN), and Chl. Lakes in the Arctic study areas were not stratified or only weakly stratified, and surface grab samples were representative of water column concentrations. Five lakes at Resolute Bay were partially ice covered during sampling in July 2014. For those lakes, water was collected as surface grabs from the ice moat (open water area) and under the ice pan by drilling a hole through the ice to collect water at 4 m depth with a peristaltic pump and acid-washed teflon tubing. Water THg and MMHg concentrations reported for those partially ice-covered lakes are the means of the surface grab and under ice measurements, which were similar in concentration. In thermally stratified lakes at Gatineau Park, deep hypolimnetic waters (1 m above the sediments) were sampled for water chemistry by peristaltic pump and acid-washed teflon tubing or by acid-washed teflon Kemmerer bottle. Total and filtered (0.45 or 0.7 µm) water samples were collected for mercury analysis, preserved with ultra-pure HCl (0.4% by volume) and refrigerated.

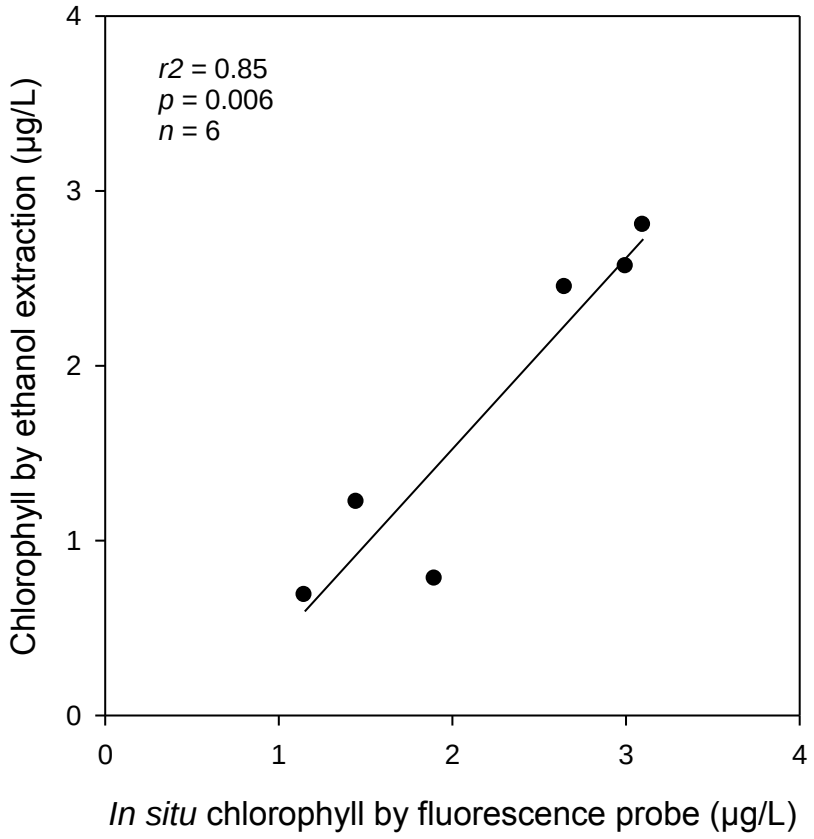

Figure S1. Comparison of two methods for measurement of chlorophyll concentration in surface water using either an *in situ* chlorophyll probe or ethanol chlorophyll extraction from filtered seston of six lakes in Gatineau Park. Data points are lake-mean estimates of surface water chlorophyll concentration that were calculated with 2 to 10 measurements in each lake.

Derivation of Lake and Watershed Morphometrics

Lake and watershed morphometrics including lake area, lake depth, catchment area to lake area ratio (CA:LA) and water residence time (WRT) were determined using GIS-based terrain analysis methods. The bathymetry of each water body was measured in a boat using a GPS-linked echosounder. Lake areas were obtained from 1:50,000 scale CANVEC National Vector hydrography dataset and the catchment area (or gross drainage area) for each water body was extracted from a hydrologically pre-processed, 1:50,000 Canadian Digital Elevation Data (CDED) Digital Elevation Model using well-established methods within the System for Automated Geoscientific Analysis (SAGA) GIS software.[1] The water residence time of a water body is determined by the ratio of annual discharge volume (q, m^3 yr^-1) entering or exiting the lake to the total volume (V, m^3) of the water body itself.[2] Since data on annual discharge were not available for the study lakes, we estimated it using mean mean annual runoff (MAR) from the landscape for each of the four study regions combined with the catchment area of each lake. MAR was estimated using Water Survey of Canada discharge records for nearby rivers at each site and normalizing to the associated catchment area. Combining this estimate with watershed area (A_ws), lake area (A_lk) and average lake depth (D_lk) for each waterbody (all in units of m or m^2), WRT in days was calculated as follows):

WRT (days) = (V/q) = ((A_lk*D_lk)/((A_ws+A_lk)*(MAR)/1000))*365

LV:CA is a proxy for WRT that assumes constant MAR. Within any one of the four study regions, the information content of LV:CA is equivalent to WRT since MAR is a constant. Across the four study sites, the estimation of MAR is incorporated to improve the estimate of WRT. Finally, CA:LA is a simpler, and more commonly used morphometric that can be calculated in the absence of both MAR and lake depth.

Analytical Methods for Water Mercury: Water samples for MMHg analysis were predistilled with additions of KCl and H_2SO_4 to remove matrix interferences. MMHg extract was derivatized by aqueous ethylation using $NaBEt_4$, trapped with Tenax and measured with a Tekran 2700 (Tekran® Instruments Corporation, Toronto, Canada) cold vapor atomic fluorescence spectrometer (CVAFS) with a detection limit of 0.01 ng L^{-1}. Relative standard deviations (RSDs) for field and analytical duplicates were 5.5 ± 5.8% (*n* = 64) and 7.5 ± 5.8% (*n* = 13), respectively. A MMHg standard spiked in Milli-Q water was analyzed after every 12 samples during water MMHg analyses with a recovery of 97.6 ± 11.6 % (n=37). Tort-2 was used as a standard (added to Milli-Q water) to verify the distillation method and potential matrix interferences associated with the presence of organic matter in the water. Recoveries of the distilled Tort-2 additions in water were 103 ± 7.8 % (n = 43).

Water total mercury (THg) was determined on 50 mL samples by BrCl oxidation, $SnCl_2$ reduction, two-stage gold amalgamation and gas-phase detection with a Tekran 2600 CVAFS with a detection limit of 0.05 ng L^{-1}. RSDs of field and analytical duplicates were 2.12 ± 3.2% (*n* = 64) and 1.9 ± 5.0% (*n* = 14), respectively. The average recovery of a standard spike for total mercury was 100% ± 4.2% (n = 43). In addition, internal reference waters of known concentration (inter-laboratory calibration solutions) were analyzed for total mercury after every 12 water samples with a recovery of 105% ± 10.7% (n = 34).

Analytical Methods for Solid-Phase MMHg: Freeze-dried and homogenized samples of chironomid larvae (*n* = 84) and biofilm organic matter (*n* = 70) from Arctic lakes were analyzed for MMHg at the Laboratory for the Analysis of Natural and Commercial Environmental Toxins (LANCET, University of Ottawa, Ottawa, Canada). Sample masses of 1–10 mg (chironomids) or 30–100 mg (biofilm organic matter) were pretreated with an alkaline digestion in KOH followed by acidic digestion in KBr and CuSO4. Bromide derivative of MMHg was extracted in dichloromethane, isolated with sodium thiosulfate and back extracted in dichloromethane for determination by capillary gas chromatography coupled with atomic fluorescence spectrometry. Concentrations of MMHg in chironomid larvae from Gatineau Park (*n* = 38) and all seston (*n* = 48) and zooplankton (*n* = 74) samples were determined at the Laboratoire de biogéochimie environnementale (Université de Montréal, Montreal, Canada). For those samples, MMHg was extracted from sample masses of 1–10 mg (chironomids), 0.5–2 mg (seston) or 3-30 mg (zooplankton) by digestion in 4 M HNO_3 at 55°C for 16 h, derivatized by aqueous ethylation using $NaBEt_4$, trapped with Tenax and measured with a Tekran 2700 CVAFS.

Table S2. Recoveries for THg or MMHg from certified reference materials, precision of duplicate samples (relative standard deviation, RSD), and method detection limits for solid-phase Hg analysis of various environmental matrices.

Sample Type	Hg Analysis Method	CRM Analyzed	% Recovery of Hg	Sample Duplicate RSD	Method Detection Limit
Sediment	Direct mercury analyzer	MESS-3	THg: 97 ± 7% (n = 12)	2 ± 2% (n = 8)	0.2 ng of Hg
Chironomid (Arctic), rock biofilm	Alkaline digestion in KOH followed by acidic digestion in KBr and CuSO4, extraction with dichloromethane, detection by GC-AFS	TORT-2 DORM-4	MMHg: 100 ± 5% (n = 17) MMHg: 94 ± 7% (n = 17)	5 ± 4% (n = 19)	3 ng/g (for 5 mg invertebrate sample)
Seston	Nitric acid digestion, aqueous ethylation, detection by CVAFS	TORT-2	MMHg: 100 ± 13% (n = 12)	Insufficient sample to do analytical duplicates	0.4 ng/g (for 1 mg of seston sample)
Zooplankton, chironomid (north-temperate)	Nitric acid digestion, aqueous ethylation, detection by CVAFS	TORT-2	MMHg: 113± 7% (n = 22)	13 ± 10% (n = 22)	0.09 ng/g (for 5 mg invertebrate sample)

Latitudinal Trend of Surface Sediment THg

Surface sediment THg concentrations were normalized for organic matter content using percent nitrogen (%N), which declined with latitude. Means (± 1 standard deviation) of %N in surface sediment from each study area were: 1.9 ± 0.5 % (Gatineau Park), 1.8 ± 0.3 % (Kuujjuaraapik), 0.7 ± 0.4 % (Iqaluit), and 0.6 ± 0.5 % (Resolute Bay). After normalizing for organic matter content (%N), lake-mean concentrations of sediment THg were positively correlated with lake depth (Figure S2).

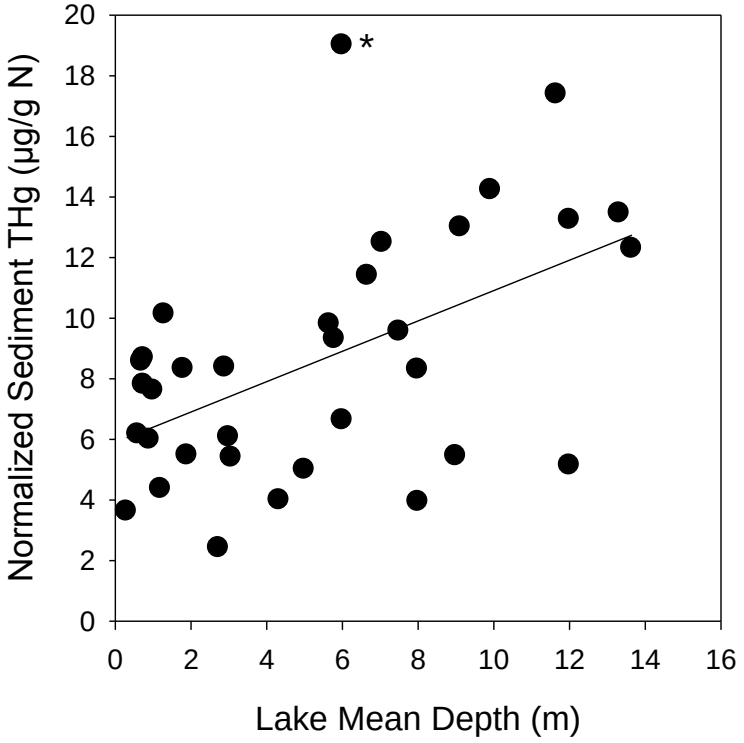

Figure S2. Relationship between lake mean concentration of sediment THg (normalized for nitroge content) and lake depth (regression model: THg/N = 5.900 + 0.501 * Mean Depth; r^2_{adj} = 0.31, $p < 0.001$, n = 33 lakes). Note that 1 outlier (identified by the asterix) was removed for the regression model.

After controlling for both organic matter content (%N) and lake mean depth, surface sediment THg concentrations declined with latitude (Figure S3), similar to the trend for uncorrected THg concentrations presented in Figure 1. A multiple regression model including both lake mean depth and latitude was highly significant (regression model: THg/N = 13.902 + (0.391 * Mean Depth) - (0.132 * Latitude); r^2_{adj} = 0.44, p < 0.001, n = 33 lakes).

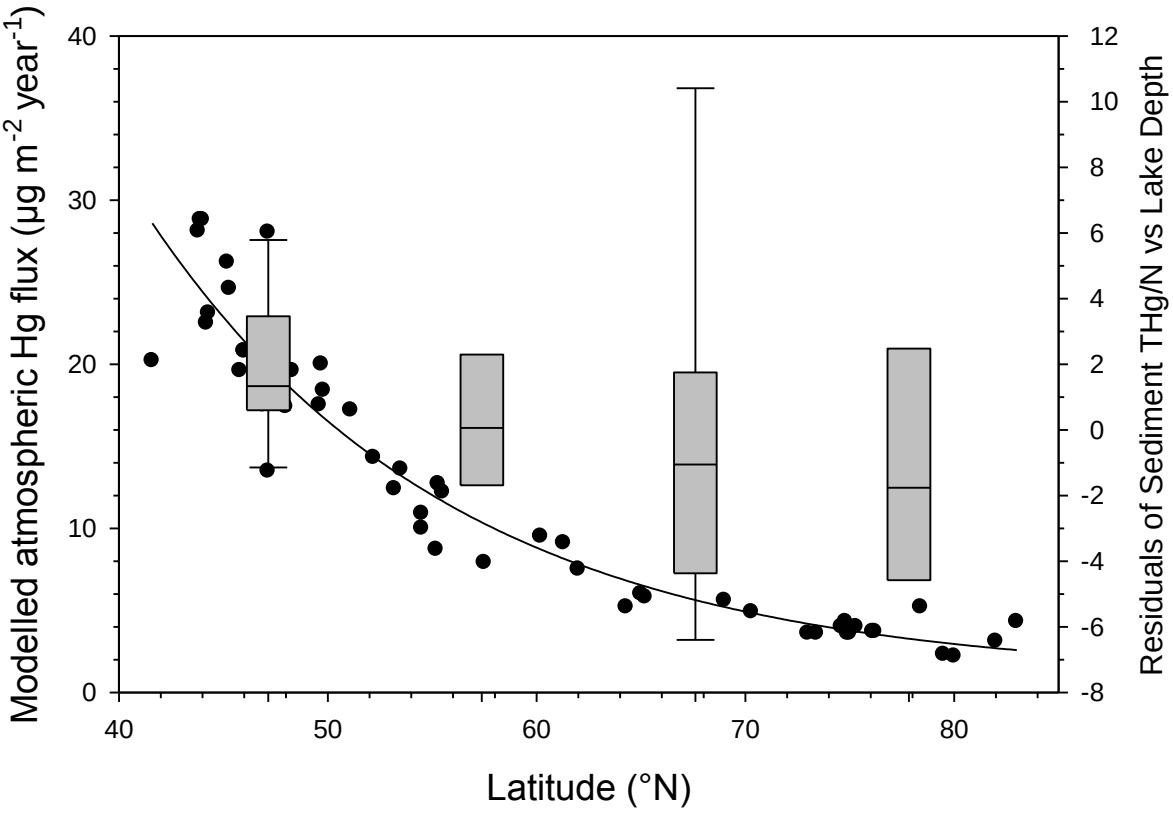

Figure S3. Latitudinal trends in modelled atmospheric mercury deposition (solid circles; from Muir et al.[3]) and normalized concentrations of THg in surface sediment from lakes in the four study regions.

Table S3. Means and ranges of chemical variables of surface waters, and mercury concentrations of surface sediment and aquatic invertebrates in lakes in the four study regions.

		Surface Water							Sediment	Biotic MMHg	
Region	Descriptor	DOC (mg/L)	TP (µg/L)	TN (mg/L)	Chl (µg/L)	THg (ng/L)	MMHg (ng/L)	% MMHg	THg (µg/g)	Chironomids (ng/g)	Zooplankton (ng/g)
Gatineau Park (46°N)	Mean	4.9	---	0.21	1.8	0.83	0.03	4	0.22	2	24
(*n* = 10 lakes)	Range	3.2–6.7		0.14–0.29	1.2–3.0	0.38–1.37	0.01–0.07	2–9	0.10–0.27	0.3–7	8–53
Kuujjuaraapik (55°N)	Mean	5.0	6.3	0.27	1.9	2.30	0.11	5	0.11	60	93
(*n* = 8 lakes)	Range	3.9–7.6	3.1–8.7	0.21–0.34	0.8–2.9	1.23–3.09	0.06–0.18	3–6	0.05–0.14	23–160	64–155
Iqaluit (64°N)	Mean	1.9	4.7	0.08	0.5	0.95	0.02	2	0.06	19	49
(*n* = 9 lakes)	Range	1.0–3.0	1.2–9.6	0.04–0.17	0.2–1.2	0.19–1.57	<0.01–0.04	1–4	0.01–0.14	4–57	26–81
Resolute Bay (75°N)	Mean	1.5	4.5	0.17	0.3	0.62	0.03	6	0.03	69	65
(*n* = 8 lakes)	Range	0.8–2.5	2.8–5.6	0.07–0.26	0.1–0.6	0.28–0.95	0.02–0.05	4–10	0.01–0.09	8–120	10–154

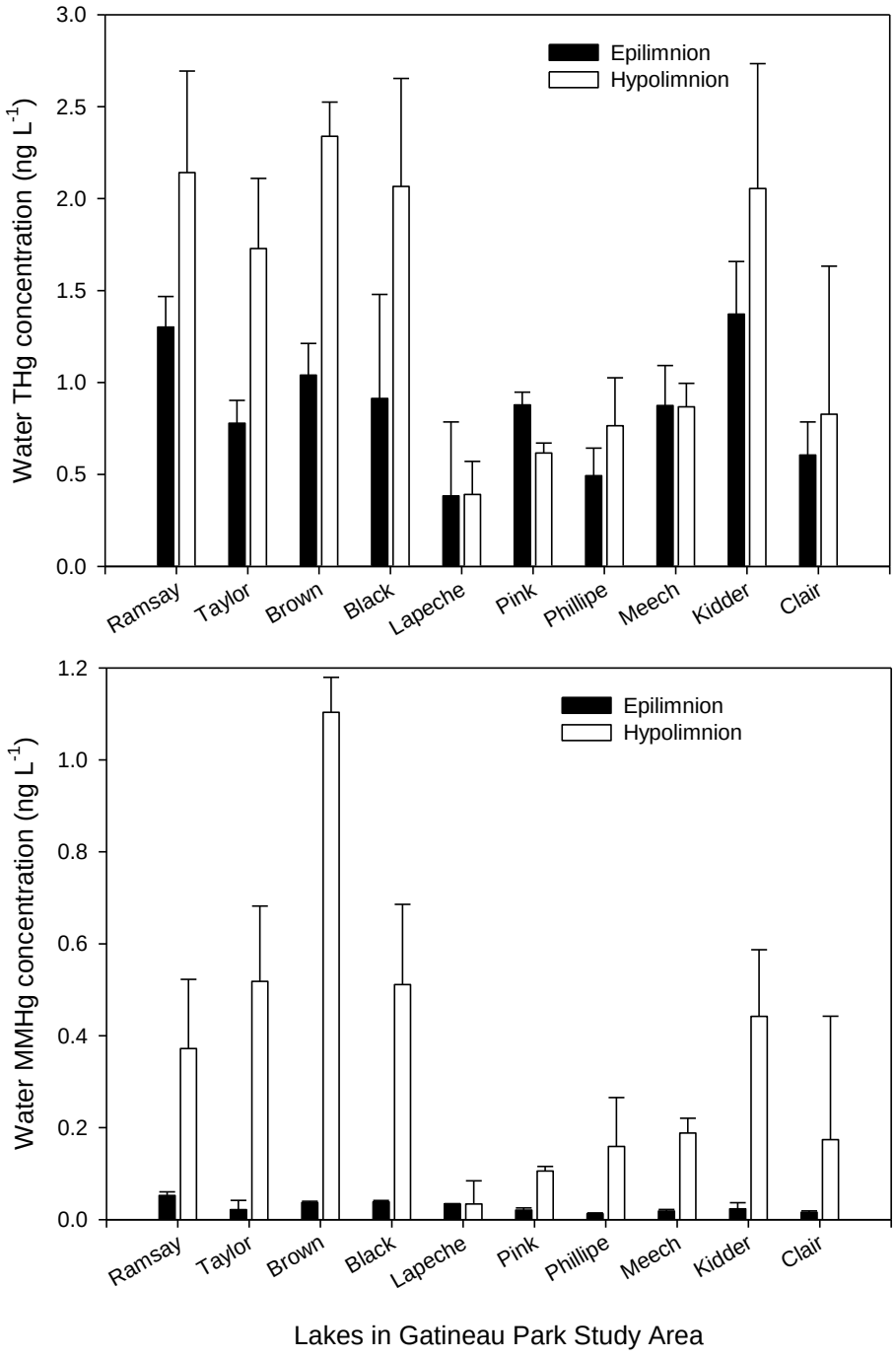

Figure S4. Comparison of surface water (epilimnion) and bottom water (hypolimnion) concentrations of THg (top panel) and MMHg (bottom panel) in stratified Gatineau Park lakes. Values are means (± 1 standard error) of early summer and fall measurements in 2011 and 2012 (3-4 sampling dates).

Table S4. Multiple regression models explaining MMHg concentrations of aquatic invertebrates in relation to surface water concentrations of MMHg, DOC, Chl and TN.

Response Variable	Model Equation	Model r^2_{adj}	Model p
Log Chironomid MMHg (n = 35)			
	Model 1 = 1.408 + 13.113 $MMHg_{water}$*** – 2.049 log DOC***	0.55	<0.001
	Model 2 = 0.459 + 10.995 $MMHg_{water}$*** – 1.121 log Chl***	0.44	<0.001
	Model 3 = -0.374 + 10.877 $MMHg_{water}$*** – 1.211 log TN*	0.22	0.007
Zooplankton MMHg (n = 34)			
	Model 4 = 45.676 + 723.733 $MMHg_{water}$*** – 54.798 log DOC*	0.45	<0.001
	Model 5 = -19.543 + 720.888 $MMHg_{water}$*** – 51.391 log TN*	0.41	<0.001
	Model 6 = 21.699 + 649.684 $MMHg_{water}$*** – 26.005 log Chl	0.39	<0.001

log = log-transformed

Significance of individual variables: * $p < 0.05$, *** $p < 0.001$

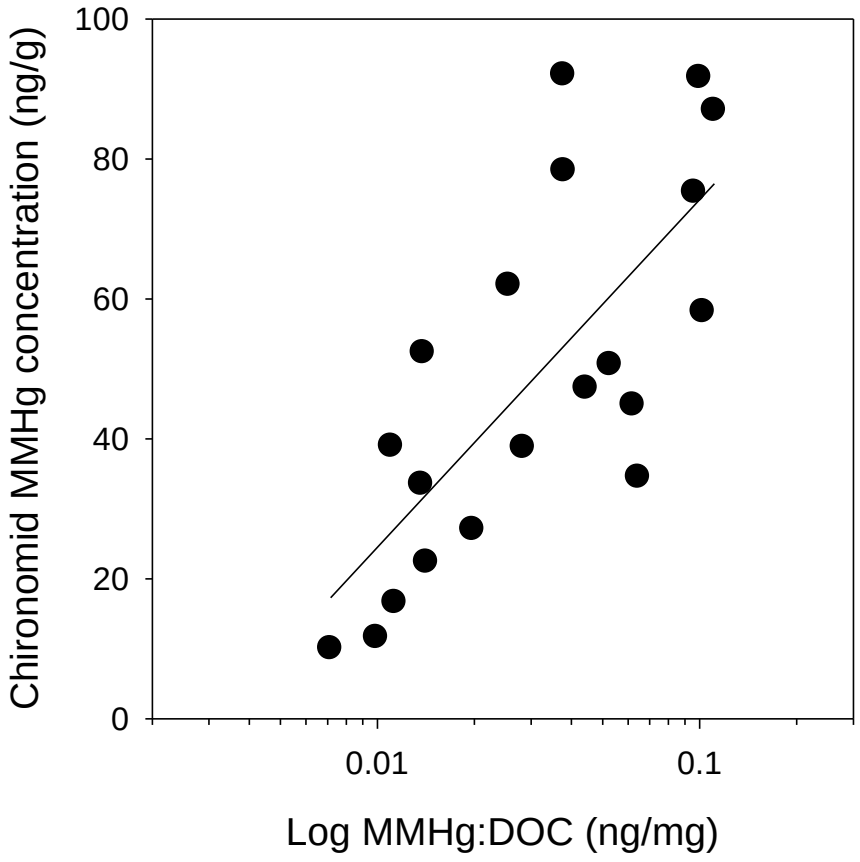

Figure S5. Relationship between MMHg concentration in chironomid larvae and the surface water MMHg:DOC ratio (r^2_{adj} = 0.52, p < 0.001, n = 20 lakes), following re-analysis of a previously published dataset for the Canadian high Arctic. Data points are lake-mean concentrations measured on 1 or 2 occasions in 2005 and/or 2006. An outlier lake with only 1 chironomid MMHg measurement was excluded from the regression. Ranges of surface water concentrations of DOC (< 0.6 – 7.4 mg/L) and MMHg (< 0.02 – 1.5 ng/L) were observed among high Arctic study lakes because more productive polar oasis sites on Devon Island were sampled in addition to polar desert sites on Cornwallis and Somerset Islands. See Chetelat et al.[4] for more detail on methods used to generate the dataset.

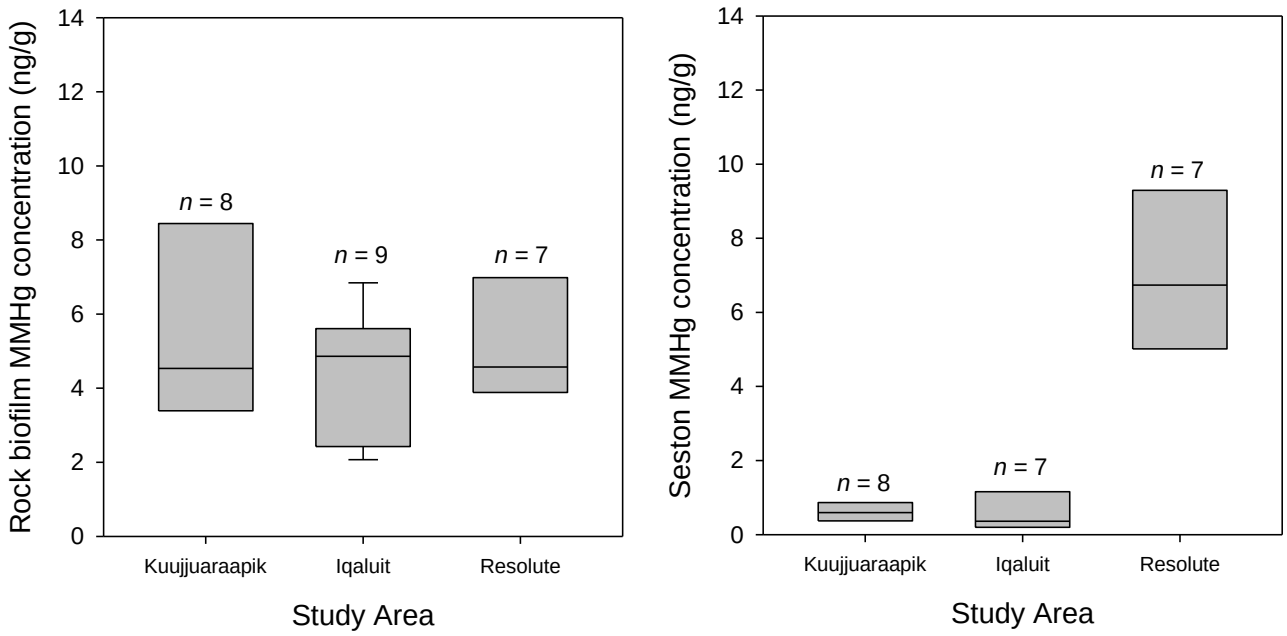

Figure S6. Boxplots of MMHg concentrations in rock biofilms and water seston from lakes in the three Arctic study areas.

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