

1 High Methylmercury in Arctic and Subarctic Ponds
2 is Related to Nutrient Levels in the Warming Eastern
3 Canadian Arctic

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12 **ABSTRACT**

13 Permafrost thaw ponds are ubiquitous in the eastern Canadian Arctic, yet little
14 information exists on their potential as sources of methylmercury (MeHg) to freshwaters. They
15 are microbially-active and conducive to methylation of inorganic mercury, and are also affected
16 by Arctic warming. This multi-year study investigates thaw ponds in a discontinuous permafrost
17 region in the Subarctic taiga (Kuujjuarapik-Whapmagoostui, QC) and a continuous permafrost
18 region in the Arctic tundra (Bylot Island, NU). MeHg concentrations in thaw ponds were well
19 above levels measured in most freshwater ecosystems in the Canadian Arctic ($> 0.1 \text{ ng L}^{-1}$). On
20 Bylot, ice-wedge trough ponds showed significantly higher MeHg ($0.3 - 2.2 \text{ ng L}^{-1}$) than
21 polygonal ponds ($0.1 - 0.3 \text{ ng L}^{-1}$) or lakes ($< 0.1 \text{ ng L}^{-1}$). High MeHg were measured in the
22 bottom waters of Subarctic thaw ponds near Kuujjuarapik ($0.1 - 3.1 \text{ ngL}^{-1}$). High water MeHg
23 concentrations in thaw ponds were strongly correlated with variables associated with high inputs
24 of organic matter (DOC, a_{320} , Fe), nutrients (TP, TN), and microbial activity (dissolved CO_2 and
25 CH_4). Thawing permafrost due to Arctic warming will continue to release nutrients and organic
26 carbon into these systems and increase ponding in some regions, likely stimulating higher water
27 concentrations of MeHg. Greater hydrological connectivity from permafrost thawing may
28 potentially increase transport of MeHg from thaw ponds to neighbouring aquatic ecosystems.

29 **KEYWORDS**

30 Mercury, Methylmercury, Thaw Pond, Arctic, Subarctic, Lake

31 **INTRODUCTION**

32 The Minamata Convention on Mercury, a global legally-binding treaty designed to
33 reduce the emission of mercury to the environment, has recently been adopted by 198 countries.¹
34 Nearly a half-century after the discovery of Minamata disease, mercury (Hg) remains a high-

35 priority global contaminant, especially in the form of methylmercury (MeHg), which
36 bioaccumulates and biomagnifies to high levels in aquatic food webs. Exposure to MeHg can
37 affect the nervous, reproductive, and immune systems of vertebrates, including fish, birds, and
38 humans.² Arctic ecosystems are especially vulnerable to Hg pollution due to atmospheric
39 deposition and higher rates of biomagnification in the cold and unproductive food webs of the
40 Arctic.³⁻⁵

41 Mercury reaches the Arctic through long-range atmospheric transport in the form of
42 elemental mercury, or Hg(0), where it is deposited into the environment after oxidation into
43 Hg(II).⁶ Once deposited, inorganic Hg(II) can be microbially methylated *in situ* to the toxic and
44 biomagnifying form, organic MeHg. A key area of current Arctic research is to establish where
45 Hg(II) methylation occurs in Arctic systems.⁷ For inland fresh waters, MeHg is produced in
46 anaerobic sediments and hypolimnia of lakes and ponds or in wetlands.^{8,9} Spring snowmelt may
47 also be an important source of MeHg to freshwater ecosystems.^{10,11}

48 In the High Arctic, small ponds have been identified as important sites of microbial
49 Hg(II) methylation.^{8,9,12} However, only a few studies have examined the mercury cycle in
50 permafrost thaw lakes and ponds. Although often overlooked, these systems are now considered
51 the most abundant type of aquatic ecosystem at circumpolar Arctic and Subarctic latitudes.¹³
52 They are formed in depressions created by permafrost thawing and may persist from days to
53 hundreds of years, depending on local geomorphology and hydrology.^{13,14} Most are small and
54 shallow systems receiving nutrients and organic matter from thawing permafrost and are often
55 colonized by biofilms.¹⁵

56 One recent study in the western Canadian Arctic found that lakes affected by the
57 development of retrogressive thaw slumps had lower Hg levels in surface sediments when

58 compared to reference lakes.¹⁶ In this case, slumping of permafrost soils resulted in high
59 inorganic sedimentation rates and the dilution of Hg in the sediments. However, a study of a peat
60 *palsa mire* in Norway showed that long-term changes in climate can cause the release of Hg into
61 lake surface waters through increased permafrost thaw depth and thermokarst erosion¹⁷. Warm
62 and microbially-active thaw ponds receiving inputs from adjacent slumping permafrost soils may
63 in fact be sources of MeHg in the Arctic environment.

64 Unlike other types of shallow ponds, thaw ponds often show stable thermal stratification
65 in summer with hypoxic to anoxic hypolimnia, some keeping unfrozen bottom waters during the
66 winter months potentially allowing for ongoing microbial activity.¹⁸ In stratified thaw ponds
67 having anoxic bottom waters or sediments, reducing conditions promote microbial Hg(II)
68 methylation. Although many ponds are physically isolated in permafrost landscapes, shifts in the
69 hydrological regime may allow for MeHg to reach surrounding lakes, rivers and marine coastal
70 waters.^{17,19–22}

71 Climate warming and rising permafrost temperatures are increasing the impacts of
72 thermokarst processes on Arctic aquatic ecosystems.^{15,23,24} Thawing permafrost may also affect
73 the mercury cycle by modifying hydrological regimes and the transport of mercury from soils
74 and peatlands to nearby aquatic ecosystems.^{4,20–22,25,26} The release of nutrients and organic
75 carbon^{27,28} and the accelerated microbial transformations of contaminants^{29,30} associated with
76 these changes will also likely affect the accumulation or *in situ* production of MeHg in
77 thermokarst aquatic systems.

78 The main objectives of this study were to assess Arctic and Subarctic thaw ponds as a
79 potential source of MeHg by 1) characterizing MeHg levels encountered in different types of
80 thaw ponds and comparing them to other nearby water bodies, and 2) determining the

81 importance of environmental variables, including nutrients and organic carbon, in explaining
82 among-site differences in MeHg levels in thaw ponds. Two geographic areas were investigated
83 in the eastern Canadian Arctic, one located on a discontinuous permafrost landscape in the
84 Subarctic taiga near Kuujjuarapik-Whapmagoostui, Nunavik (Northern Quebec), and the other in
85 an area of continuous permafrost in the High Arctic tundra on Bylot Island, Nunavut.

86

87 **MATERIALS AND METHODS**

88 **Study Sites**

89 Sampling was conducted in the Qarlikturvik Valley on Bylot Island in Nunavut
90 (73°09'23"N, 79°58'19"W) and in the area surrounding the Kuujjuarapik-Whapmagoostui
91 community in Nunavik (55°16'30"N, 77°45'30"W) (Fig. S1). Sites on Bylot Island were sampled
92 in July and August 2008, 2009, 2010 and 2011, whereas sites near Kuujjuarapik-Whapmagoostui
93 were sampled in July and August 2006, 2009, 2012 and 2013. Bylot Island thaw ponds can be
94 classified into two types: 1) polygonal ponds created by the rise of peat polygon ridges and 2)
95 trough ponds that form over melted ice wedges between the polygon mounds. Trough ponds
96 (elsewhere called runnel ponds^{31,32}) are elongated aquatic systems featuring peat erosion and
97 higher turbidity than polygonal ponds, therefore classified as thermokarstic.³³ Both types of
98 ponds examined were no more than a few meters in diameter and generally less than 1.5 m in
99 depth. Seven larger aquatic systems on Bylot Island were categorized as “lakes” for this study,
100 given their much larger surface area and depth. Subarctic sample sites included thermokarst and
101 taiga/rock basin ponds sampled near Kuujjuarapik-Whapmagoostui. Here, thermokarst thaw
102 ponds develop in depressions left after the ice has melted below mineral or besides organic
103 permafrost mounds.^{15,34} They are 10 to 30 m in diameter and have a maximum depth of 3.5

104 m.^{15,33} Taiga/rock basin ponds (pooled into one group) are formed on granite or carbonate-
105 derived bedrock respectively and are 10-20 m in diameter with a maximum depth of 1 m. For
106 more information on the formation of study sites see SI.

107 **Physico-Chemical Sampling**

108 Study lakes and ponds were sampled for water chemistry, including dissolved organic
109 carbon (DOC), total nitrogen (TN), total phosphorus (TP), chlorophyll *a* (Chla), anions, cations,
110 major metals, carbon dioxide (CO₂), methane (CH₄) and aqueous total Hg and MeHg
111 concentrations. Additionally, the physico-chemical properties of the water column, including
112 temperature, pH, conductivity, and dissolved oxygen, were measured at the water surface using a
113 YSI 600QS meter (YSI Incorporated). Vertical profiles were also conducted in 3 Subarctic
114 thermokarst ponds. For details on methods, see SI.

115 **Aqueous Total Mercury (THg) and MeHg Concentrations**

116 Water samples were collected for Hg at the surface and 30 cm above the bottom
117 sediments, either from shore or from a raft. Samples were collected using the clean hands, dirty
118 hands sampling protocol for trace metals.³⁵ Both unfiltered and filtered water samples were taken
119 at each site to determine the proportion of dissolved and particle-bound total mercury (THg or
120 Hg(II) + MeHg) and methylmercury (MeHg). Water samples for total THg and MeHg analyses
121 were pumped through acid-washed Teflon tubing with a peristaltic pump, after the apparatus was
122 flushed with site water for 5 minutes. Samples were stored in acid-cleaned amber glass bottles.
123 Water samples for dissolved Hg and MeHg concentrations were filtered (pore size 0.45 µm)
124 using a peristaltic pump, acid-cleaned Teflon tubing and a GWV High Capacity In-Line
125 Groundwater Sampling Capsule (Pall Corporation) or filtered with pre-ashed glass-fibre filters
126 (0.7 µm pore size, Whatman GF-F) on a clean Teflon filtration tower (HCl 10%). All Hg samples

127 were preserved with ultrapure hydrochloric acid to 0.4% final concentration until laboratory
128 analysis.

129 Aqueous THg concentration was determined following U.S. EPA method 1631, by
130 bromine monochloride (BrCl) oxidation, tin (II) chloride (SnCl_2) reduction, two-stage gold
131 amalgamation and gas-phase detection with a Tekran 2600 Cold-Vapour Atomic Fluorescence
132 Spectrometer (CVAFS) (Tekran Instruments Corporation). The analytical detection limit was
133 0.04 ng L^{-1} , calculated as three times the standard deviation (SD) of ten blanks. New standards
134 (0.5 ng L^{-1}) were run after each set of 12 samples to test for analytical stability (mean recovery
135 $102.5 \pm 9.0\%$, $n = 79$). All water samples were run in duplicate or triplicate with a Relative
136 Standard Deviation (RSD) of usually $<10\%$ for THg.

137 Aqueous MeHg concentration was determined following U.S. EPA method 1630, by
138 acid-distillation to remove matrix interferences, derivatization by aqueous-phase ethylation,
139 purging on Tenax (Tenax Corporation) and separation by gas chromatography, before detection
140 with either a Tekran 2500 or Tekran 2700 CVAFS (Tekran Instruments Corporation). The
141 analytical detection limit was 0.02 ng L^{-1} and 0.01 ng L^{-1} respectively for the Tekran 2500 and
142 Tekran 2700, calculated as three times the SD of ten blanks. New standards (0.5 ng/L) were run
143 after each set of 10-12 samples to test for analytical stability (mean recovery $104.2 \pm 17.3\%$, $n =$
144 53). Analyses were accepted when recovery of certified trace metal reference materials was in
145 the certified range ($152 \pm 13 \text{ ng/g}$ for TORT-2 lobster hepatopancreas, National Research
146 Council of Canada) and the mean (\pm SD) recovery was $99.5 \pm 8.4\%$ ($n = 97$). All water samples
147 were run in duplicate with a Relative Standard Deviation (RSD) of $<12\%$ for MeHg. Hg analyses
148 met the criteria of a Canadian Association for Laboratory Accreditation (CALA) inter-calibration

149 exercise and an Interlaboratory Quality Assurance Program administered by the Northern
150 Contaminants Program (Government of Canada) (see SI).

151 **Statistical Analysis**

152 For all statistical analyses, among-year averages were calculated for each site (from
153 2008-2011 for Bylot and 2006-2013 for Kuujjuarapik) although not all variables were measured
154 at each site for each year. All of the variables were normalized in order to reduce skewness and
155 the effects of outliers using either log transformations (Temp, pH, Cond, DOC, Chla, Cl, Fe, Mg,
156 Mn, Na, SO_4^{2-} , TN, TP, THg, MeHg, %MeHg), square root transformations (Ca, K) or power
157 transformations (square) (DO). Inorganic Hg(II) concentrations were estimated by the difference
158 between THg and MeHg concentrations at each site. Normalized data were used to perform all
159 analyses with the R statistical package (R Development Core Team; <http://cran.r-project.org>).
160 For comparisons of limnological properties and mercury concentrations, the geometric mean
161 (GM) was calculated to better measure the central tendency, calculated as the antilog of the mean
162 of the logarithmic values of the data set. Sites with missing data for multiple variables were not
163 included in the regression analysis, and replacement values were calculated for four sites (each
164 with one missing variable) by imputing the overall variable mean for the type of sample site.

165 Comparisons of limnological properties and mean mercury concentrations were
166 conducted with one-way ANOVAs followed by post-hoc pairwise comparisons using the Tukey
167 HSD correction ($\alpha < 0.05$). Sensitivity analysis with a non-parametric approach (Kruskal-Wallis
168 chi-squared, χ^2 rank sum tests) was conducted to test the assumptions of the analysis of variance
169 model and the non-parametric tests gave the same conclusions as the analyses of variance.³⁶
170 Gradients in environmental characteristics were examined by principal component analysis
171 (PCA) using the vegan package in R on centered and scaled data (n=40). A non-parametric

172 multivariate analysis of variance (MANOVA) was also run to determine whether samples sites
173 differed significantly in terms of measured environmental variables (Adonis test, Vegan package
174 in R).³⁷ Due to high collinearity between many of the variables, multiple regression models
175 were difficult to interpret and therefore only simple linear regression models are presented for
176 the most highly correlated environmental variables.

177

178 **RESULTS AND DISCUSSION**

179 **Thermal Stratification**

180 The ponds sampled on Bylot Island (trough and polygonal ponds) and near Kuujjuarapik
181 (taiga/rock and thermokarst ponds) varied in their vertical thermal structure. Strong seasonal
182 thermal stratification was not observed in polygonal ponds on Bylot Island. While polygonal
183 ponds had well-mixed water columns, trough ponds showed stratified conditions during a large
184 fraction of the summer due to the surrounding microtopography, their small fetch, and high
185 humic contents (data not presented here). Hence bottom waters of Bylot trough ponds were
186 mainly hypoxic (often $< 2 \text{ mgL}^{-1}$) with only occasional mixing of the upper water column.

187 Taiga/rock ponds sampled near Kuujjuarapik were very shallow ($< 1 \text{ m}$) and did not show
188 thermal stratification. However, 9 of the 12 thermokarst ponds (1-3 m in depth) sampled near
189 Kuujjuarapik were strongly thermally stratified. Stratification was sufficiently stable over time to
190 cause low oxygen values in bottom waters, ranging from $0.13 - 3.7 \text{ mg L}^{-1}$ or less than 2%
191 saturation at most sites (Table 1, Fig. S2). On average, temperature in the hypolimnion (bottom
192 waters) was around 10°C cooler than at the surface and mean dissolved oxygen was only 5% of
193 the surface concentrations for the Kuujjuarapik thermokarst ponds (Table 1). Oxygen depletion
194 in bottom waters is caused by very limited mixing of the water column, including in spring, and

195 large microbial respiration.^{15,18,33} Thermokarst ponds in this region are often formed as a result of
196 lithalsa degradation and are therefore prone to stable thermal stratification and long water
197 residence time due to high turbidity and low percolation in silty clay soils.²²

198 **Limnological Properties and Dissolved Gases**

199 Geometric means (\pm standard deviation, GM \pm SD) of limnological properties were
200 compared among sites. Trough and polygonal thaw ponds on Bylot Island showed higher
201 nutrient and DOC levels compared to the (ultra)oligotrophic sites more commonly studied in
202 polar regions (Table 1). Nutrient and DOC concentrations for High Arctic lakes and ponds are
203 typically low, with reported means of 148 – 289 $\mu\text{g L}^{-1}$ for TN, 1.3 – 12.0 $\mu\text{g L}^{-1}$ for TP and 1.5
204 to 2.2 mg L^{-1} for DOC.³⁸⁻⁴¹ Compared to mean concentrations from 204 lakes across the
205 Canadian Arctic Archipelago, mean nutrient (TN, TP) levels were roughly 2 – 4 times higher for
206 Bylot polygonal and trough ponds respectively, whereas DOC levels were 2 – 3 times higher.⁴⁰

207 Bylot thaw ponds also had significantly higher concentrations of solutes than sampled
208 lakes, with higher geometric means for conductivity ($\mu\text{S cm}^{-1}$), chlorine (Cl), and iron (Fe)
209 (ANOVA, Table 1). Trough ponds, in particular, had higher DOC concentrations and
210 significantly darker water colour (a_{320}) than either polygonal ponds or lakes ($p < 0.05$). This
211 supports our field observations of more active peat slumping in trough ponds. Bottom water was
212 not collected in Bylot ponds, but profiles indicated higher specific conductivity and lower
213 oxygen and pH in trough pond bottom waters. Higher levels of lateral erosion in trough ponds
214 result from ice-wedge melting and soil subsidence on the edge of peat polygons leading to higher
215 inputs of organic material. Indeed, a slightly larger fraction of old carbon available for microbial
216 degradation was observed in trough ponds.³¹ Polygonal ponds typically show fewer signs of
217 erosion and had lower DOC concentrations.^{20,31,42}

218 Concentrations of dissolved gases (CH_4 , CO_2) were also significantly higher in trough
219 ponds when compared to polygonal ponds (respectively 3 and 5 times higher, Table 1). High
220 levels of CO_2 and CH_4 in trough ponds compared with polygonal ponds likely reflect anoxic
221 conditions promoting fermentation and methanogenesis,³¹ which may occur in biofilms,
222 sediments or surrounding anaerobic soils.⁴³ High levels of dissolved gases also indicate strongly
223 reducing conditions in sediments at these sites, leading to the remineralisation and remobilization
224 of ions (such as Mn) from anoxic sediments.

225 Subarctic thermokarst ponds similarly displayed higher nutrient and DOC concentrations
226 than neighbouring lakes. Lakes sampled near Kuujjuarapik had mean TN of $267 \pm 50.5 \mu\text{g L}^{-1}$,
227 mean TP of 6.83 ± 3.33 (TP) $\mu\text{g L}^{-1}$ and mean DOC of $4.98 \pm 1.38 \text{ mgL}^{-1}$ (GM \pm SD, G.
228 MacMillan, n = 7, unpublished data 2012). The average concentrations were therefore 1.5 times
229 (TN), 1.7 times (DOC) and 7 times (TP) higher in the surface waters of Kuujjuarapik
230 thermokarst ponds (Table 1). The bottom waters of these thaw ponds also showed distinct water
231 chemistry, with much higher mean specific conductivity (3 \times), TP (4 \times), Chla (8 \times), CO_2 (6 \times) and
232 CH_4 (95 \times) than at the surface (ANOVA, Table 1). The low oxygen measured in the bottom
233 waters of these sites may have caused the remobilization of ions from anoxic sediments and
234 therefore led to higher conductivity. Higher concentrations of dissolved CO_2 and CH_4 in
235 Kuujjuarapik bottom waters also indicates anoxic conditions suitable for microbial gas
236 formation, similar to Bylot trough ponds.

237 Taiga/rock ponds had the highest average concentrations of Na^+ , Cl^- , and sulfate (SO_4^{2-})
238 but had lower TP and Chla than Kuujjuarapik thermokarst ponds. Concentrations of DOC and
239 nutrients (TN, TP) were roughly twice as high as in neighboring lakes, yet TP was significantly

240 lower than in thermokarst ponds. Taiga/rock ponds also showed higher conductivity likely
241 related to their coastal locations and marine aerosol influence from Hudson Bay.

242 **Total Mercury and Methylmercury Levels**

243 On Bylot Island, trough ponds had the highest mean (and median) water concentrations
244 of both THg and MeHg (Table 1, Fig. 1). Geometric mean concentrations of THg from trough
245 ponds were 1.5 times the average found in polygonal ponds and 2.6 times the average in lakes
246 (although the difference was not significant for polygonal ponds). Mean MeHg concentrations
247 were highest in trough ponds, being approximately 3.5 times the average in polygonal ponds and
248 24 times the average found in larger water bodies. Statistical tests showed differences in MeHg
249 concentrations between trough ponds, polygonal ponds and lakes (Table 1, $p < 0.05$). The
250 percentage of THg in the form of MeHg (or %MeHg) was also significantly higher in both types
251 of thaw pond ($26.0 \pm 9.0\%$ in trough ponds and $12.0 \pm 5.1\%$ in polygonal ponds) when compared
252 to the lakes at $1.5 \pm 9.5\%$ (GM \pm SD). It should be noted that maximum values reported here for
253 Bylot thaw ponds are very high due to the sampling of one extreme site (BYL63) over two
254 consecutive years (reaching 30.2 ng L^{-1} THg and 18.2 ng L^{-1} MeHg in 2009, and respectively
255 13.4 and 2.97 ng L^{-1} in 2010). However, median values of THg and MeHg followed the same
256 trends among pond types as the means (SI: Table S1) and statistical tests still showed differences
257 between all groups without these extreme values ($p < 0.05$).

258 Higher levels of MeHg measured in Bylot thaw ponds (particularly trough ponds) may
259 either originate from a) *in situ* methylation in sediments by microorganisms or b) transport from
260 surrounding peaty soils. Our results suggest that *in situ* methylation may be an important source
261 of MeHg in these systems, as high MeHg concentrations combined with high %MeHg often
262 indicates high net methylation rates.⁴⁴ A strong positive correlation between MeHg and inorganic

263 Hg(II) concentrations at these sites ($R^2_{\text{adj}} = 0.53$, $p < 0.01$) suggests that they are suitable
264 aquatic systems for *in situ* production of MeHg and are limited by the availability of inorganic
265 Hg(II) (Fig. 2).

266 On the other hand, MeHg may accumulate in these systems due to high rates of
267 production and transport from surrounding anaerobic soils. Mercury binds strongly to DOC,
268 enhancing the mobilization and transport of this metal within a watershed.^{45,46} However, recent
269 studies in the High Arctic have found variable and relatively low methylation potentials of
270 wetland soils and low export of MeHg to downstream lakes.^{11,47} Reported methylation rates and
271 %MeHg in Arctic soils are also low compared to the MeHg levels measured in Bylot thaw
272 ponds. Since few data are currently available on methylation rates in Arctic soils (and on the
273 soils surrounding our sample sites in particular), the source of MeHg in these Bylot thaw ponds
274 may therefore be either transport from surrounding soils or *in situ* methylation.

275 For stratified Kuujjuarapik thermokarst ponds, both THg and MeHg concentrations were
276 significantly higher in bottom waters than at the surface (Table 1, $p < 0.05$). Bottom water mean
277 concentrations were about 1.7 times higher for THg and 7 times for MeHg compared to surface
278 water concentrations. Taiga/rock ponds showed the highest mean concentrations of THg ($6.50 \pm$
279 2.47 ng L^{-1}) and relatively high concentrations of MeHg ($0.33 \pm 0.33 \text{ ng L}^{-1}$), although MeHg
280 was much lower than in thermokarst pond bottom waters (Fig. 1). Statistical tests showed
281 differences between all groups for THg and higher MeHg in bottom waters of stratified
282 thermokarst ponds relative to other groups ($p < 0.05$, Table 1). The %MeHg was also
283 significantly higher in the bottom waters of stratified thermokarst ponds ($27.2 \pm 24\%$) when
284 compared to surface waters ($6.7 \pm 22\%$) or to taiga/rock ponds ($5.1 \pm 3.7\%$). It should be noted
285 that one shallow thermokarst pond sampled in 2013 (SAS-1G) showed much higher

286 concentrations of THg ($4.35 \pm 0.24 \text{ ng L}^{-1}$) and MeHg ($3.56 \pm 0.11 \text{ ng L}^{-1}$), as well as higher
287 %MeHg (82%) than neighbouring sites. Statistical tests still showed differences between all
288 groups without these extreme values ($p < 0.05$).

289 Strong thermal stratification in Kuujjuarapik thermokarst ponds results in low oxygen or
290 anoxic conditions, which are highly conducive for microbial Hg(II) methylation.⁴⁸ Other studies
291 have even suggested that year-round stable stratification potentially allows for ongoing microbial
292 activity (hence potentially methylation) in bottom waters during the winter months.¹⁸ Moreover,
293 dark bottom waters in these turbid thermokarst ponds precludes photodemethylation losses at
294 depth.⁴⁹ Bottom waters in these ponds have a combination of high MeHg concentrations and
295 high %MeHg suggesting that, as for the Bylot trough ponds, these sites may have high net
296 methylation rates.⁴⁴ However, the lack of correlation between Hg(II) and MeHg at these sites (p
297 > 0.05) either suggests that 1) MeHg production is not limited by the availability of inorganic
298 Hg(II) or 2) measured Hg(II) concentrations do not reflect the Hg(II) bioavailable to methylating
299 microorganisms (Fig. 2). As for Bylot Island, there are limited data available for MeHg
300 production and transport from Subarctic peatlands. However, high MeHg and %MeHg in
301 strongly stratified bottom waters of Kuujjuarapik thaw ponds suggests *in situ* methylation at
302 these sites.

303 Shallow Kuujjuarapik taiga/rock ponds had high THg concentrations and relatively high
304 MeHg concentrations compared to the surface waters of thermokarst ponds (Fig. 1). Proximity of
305 these ponds to the coast of Hudson's Bay may lead to marine inputs of MeHg.⁵⁰ However, the
306 %MeHg was quite low which suggests that these sites may have lower rates of Hg(II)
307 methylation than thaw ponds. The lack of stratification (and therefore reducing conditions for
308 Hg(II) methylation) combined with low organic matter inputs due to little or no peripheral

309 vegetation may help explain the lower %MeHg found at these sites. Higher rates of bio- or
310 photodemethylation may also explain the low %MeHg found in well-lit taiga/rock ponds despite
311 the large pool of potentially bioavailable inorganic Hg.

312 Unfiltered concentrations of THg and MeHg were measured at all sites over all sampling
313 years. For a subset of sites, dissolved (filtered at 0.45 μm) concentrations were also measured at
314 least once over the extended sampling period (2006 to 2013). Overall, both THg and MeHg were
315 primarily in the dissolved phase, with mean \pm SD values of $79.0 \pm 16.7\%$ for THg and $83.8 \pm$
316 25.6% for MeHg on Bylot (n=11), and of $87.0 \pm 8.6\%$ for THg and $83.9 \pm 10.4\%$ for MeHg in
317 the Kuujjuarapik area (n=16). This suggests that the Hg measured at these sites is mobile, with a
318 higher potential for lateral transport into other aquatic systems than Hg bound to settling
319 particles. Concentrations of MeHg found in the dissolved phase are also more bioavailable for
320 uptake by lower trophic levels (algae).⁵¹

321 **Environmental Drivers of Methylmercury Concentrations in Arctic and Subarctic Ponds**

322 PCA was used to identify the dominant environmental gradients in the dataset (Fig. 3).
323 The PCA biplot accounted for 51.9% of the total variation among sites from both study areas
324 (Axis 1: 28.5% and Axis 2: 23.4%). The remaining unexplained variability (48%) can be
325 attributed to a number of different factor, for example, local and regional climatic variation
326 (precipitation, temperature), sedimentation rates, heterogeneity in microbial communities and
327 analytical variability. On the correlation biplot, environmental variables are represented by black
328 arrows, whereas sites are represented by coloured points. The angles relative to axis 1 and 2
329 show the weight of the variable in determining the construction of the ordination axis, and the
330 angles between the arrows are representative of the degree of correlation between variables.⁵²

331 Based on the PCA scores, the distribution of the sites along axis 1 was most strongly
332 driven by environmental gradients in THg, MeHg, TP, TN, Mn, Fe, and DOC. The dominant
333 gradients detected in axis 2 of the PCA were for pH, DO, Chla, SO_4^{2-} and major ions (Ca, Mg,
334 Na, Cl). The Subarctic sites tended to be more productive (higher planktonic Chla) than Bylot
335 sites (Fig. 3), although polygonal ponds had thick cyanobacterial mats.⁵³ Overall, the PCA
336 analysis shows a clustering of the different types of ponds based on distinct water chemistry
337 conditions and the positive association of mercury (THg, MeHg and %MeHg) with
338 environmental variables indicating inputs of organic matter (DOC, Fe), high nutrients (TN, TP)
339 and reducing conditions in the sediments (Mn, Fe, TP). Strongly reducing conditions in
340 sediments or bottom waters leads to the remobilization of ions (such as Hg and P) bound to Fe
341 and Mn oxides back into the water column.⁵⁴ Differences in environmental variables among
342 sample sites (trough, polygonal, subarctic thermokarst and taiga/rock) were evaluated using a
343 permutational MANOVA (Adonis function, Vegan package in R)³⁷ which showed that physico-
344 chemical characteristics of the water differed between sample sites (np-MANOVA, $F = 20.47$, R^2
345 $= 0.71$, $p < 0.01$, 999 permutations).

346 Simple linear regression models were also calculated for THg and MeHg with the most
347 highly correlated environmental variables (SI: Table S4). Surface water THg was significantly
348 correlated with water colour (a_{320} : $R^2_{\text{adj}} = 0.57$, $p < 0.01$), DOC ($R^2_{\text{adj}} = 0.45$, $p < 0.01$), Fe
349 ($R^2_{\text{adj}} = 0.26$, $p = 0.02$) and TN ($R^2_{\text{adj}} = 0.33$, $p = 0.01$). MeHg was most highly correlated with
350 TN ($R^2_{\text{adj}} = 0.61$, $p < 0.01$), DOC ($R^2_{\text{adj}} = 0.57$, $p < 0.01$), water colour (a_{320} : $R^2_{\text{adj}} = 0.39$, $p <$
351 0.01) and CH_4 ($R^2_{\text{adj}} = 0.37$, $p < 0.01$) (Fig. 4). Some regressions showed high leverage due to
352 outlying sample sites, however these relationships were still found to be highly significant when
353 these sample sites were excluded (SI: Fig. S3). Simple linear regression models were also

354 calculated separately for each region (either Bylot: $n = 34$ or Kuujjuarapik: $n = 24$) and on a
355 subset of data from the bottom waters of stratified ponds ($n = 9$). Overall, concentrations of THg
356 and MeHg were correlated with similar (collinear) environmental variables for these data subsets
357 (for R^2_{adj} and p -values, see SI: Table S4).

358 Many of the explanatory variables were collinear and the relative importance of specific
359 correlations is therefore difficult to interpret. For example, DOC, Fe and water colour ($\log a_{320}$)
360 were correlated with each other, as both Fe and DOC concentrations are known to affect water
361 colour.⁵⁵ DOC concentrations were also strongly correlated to CO_2 , TN, and Mn concentrations
362 in surface waters. Concentrations of Fe and Mn were auto-correlated and were negatively
363 correlated with pH and dissolved oxygen (DO), as these metals are only soluble under anoxic,
364 reducing conditions. This notwithstanding, these correlations indicate that MeHg concentrations
365 are strongly correlated with environmental variables indicating high inputs of organic matter
366 (DOC, a_{320} , Fe), high nutrients (TP, TN), microbial activity (dissolved CO_2 and CH_4 gases) and
367 reducing conditions in the sediments (Mn, Fe, TP) at these sites.

368 This study confirms our hypothesis that permafrost thaw ponds may be sources of MeHg
369 in the Canadian Arctic and Subarctic. High concentrations of MeHg ($ng\ L^{-1}$) and %MeHg were
370 measured in Bylot trough ponds (0.72 ± 2.37 , $26\% \pm 9.0$) and in the bottom waters of
371 Kuujjuarapik thaw ponds (0.99 ± 1.17 , $27\% \pm 24$). These values are well above the levels
372 typically found in freshwater ecosystems in the Arctic where average MeHg concentrations
373 generally remain below $0.1\ ng\ L^{-1}$ ⁵⁶ (and less than 15% of total Hg) with a few exceptions (i.e.
374 Ellesmere Island ponds).^{8,9} Thaw pond MeHg concentrations were also high in comparison with
375 temperate lakes and rivers in northeastern North America⁵⁷ ($n = 277$) where they were found to
376 range from 0.01 to $3.12\ ng\ L^{-1}$ with a mean of $0.30\ ng\ L^{-1}$. Interestingly, Bylot polygonal ponds

377 had lower MeHg ($0.21 \pm 0.08 \text{ ng L}^{-1}$) and %MeHg ($12\% \pm 5.1$) than neighbouring trough ponds.
378 This is may be due to more oxic water columns, lower levels of lateral erosion (and hence inputs
379 of OM and nutrients) and/or lower sediment surface area to water volume ratio in polygonal
380 ponds which leads to greater dilution of MeHg from the sediments. Surface waters from
381 Kuujjuarapik thermokarst ponds also showed much lower levels of MeHg than bottom waters
382 (0.14 ± 3.34 , $6.7\% \pm 2.7$). This indicates that thermal stratification and anaerobic bottom waters
383 create favourable conditions for microbial Hg(II) methylation at these sites. High concentrations
384 of MeHg combined with high %MeHg also support the hypothesis of high net methylation rates
385 in Kuujjuarapik thermokarst pond bottom waters.⁴⁴

386 Furthermore, this study highlights differences in the major environmental variables
387 explaining among-site differences in MeHg levels in small Arctic and Subarctic aquatic systems
388 when compared to more temperate systems. The major variables controlling MeHg production at
389 temperate latitudes are typically temperature, pH, redox conditions, sulfate and DOM.^{58,59}
390 Similarly, MeHg concentrations in the present study were positively correlated with inputs of
391 organic matter (DOC) and low redox (anaerobic) conditions in sediments or bottom waters.
392 Positive correlations between MeHg and DOC in freshwaters may indicate the export of Hg
393 bound to DOM from surrounding soils^{45,60,61} or alternatively that organic matter limits microbial
394 activity in lake sediments, thus *in situ* methylation rates.^{62,63} Anoxic bottom waters or sediments
395 also favour both Hg release from sediments⁶⁴ and increased microbial Hg(II) methylation.⁴⁸ Yet
396 THg and MeHg concentrations were not correlated with water column temperature, pH, or SO_4^{2-}
397 at these sites, variables which control MeHg production at more temperate latitudes (Fig. 4).^{58,59}
398 The production of MeHg typically increases at warmer temperatures due to increased microbial
399 activity⁵⁹ yet we found no association between surface water temperature and MeHg

400 concentration. Elevated MeHg concentrations were found in thaw ponds from both study areas,
401 despite their difference in latitude (~20°N) and climate regimes. Lack of correlation between
402 MeHg and SO_4^{2-} concentrations in surface waters may be due to low sulfate concentrations (<10
403 mg L^{-1} or <100 μM), which limit the activity of sulfate-reducing bacteria. Future studies should
404 focus on relationships between sulfate water concentrations and sulfide pore-water
405 concentrations to identify links between the sulfur and mercury cycles at these sites. Lack of
406 correlation between MeHg and SO_4^{2-} may also indicate that other types of bacteria are
407 responsible for Hg(II) methylation in sediments or bottom waters, such as iron-reducing bacteria
408 or methanogens.⁶⁴⁻⁶⁶

409 Unlike for temperate aquatic systems, strong positive correlations were found in these
410 Arctic and Subarctic ponds between MeHg, and higher nutrients (TN, TP) and dissolved
411 greenhouse gases (CO_2 , CH_4). Only a few previous studies have found positive relationships
412 between MeHg and lake nutrient status^{67,68} and the exact relationship between methylation,
413 nutrient status and N availability remains unclear. A previous study in High Arctic ponds also
414 found positive correlations for MeHg concentrations with nitrogen (ratio of ammonium to
415 nitrate, $\text{NH}_4^+:\text{NO}_3^-$) and dissolved CH_4 concentrations.⁸ These results were explained as
416 indicating the relative importance of anaerobic microbial activity on Hg(II) methylation and
417 higher ratios of methylation to demethylation rates. Other recent studies performed at the same
418 sites near Kuujjuarapik show that thermokarst ponds are methanotroph-rich ecosystems,^{69,70}
419 indicating that methane is a potentially important energy source for microorganisms at these
420 sites. In the present study, the lack of correlation between sulfate and MeHg concentrations and
421 the strong correlations found between dissolved CH_4 and MeHg levels may indicate that
422 methane-producing microorganisms (methanogenic archaea) contribute to the production of

423 MeHg in the sample sites, as has been observed in other aquatic systems.⁶⁵ These novel
424 correlations highlight the importance of investigating the role of organic matter erosion and
425 nutrient inputs on the stimulation of anaerobic microbial activity, and hence potential *in situ*
426 methylation by methanogenic archaea, in these ubiquitous aquatic systems.

427 **Ecological Significance**

428 Our findings contrast with the lack of stimulatory effects observed for retrogressive thaw
429 slumps of clay-rich tills entering lakes in western Canadian Arctic,¹⁶ highlighting that permafrost
430 degradation can affect the mercury cycle differently across the Arctic landscape. Permafrost
431 thaw ponds are now considered the most common freshwater aquatic system at circumpolar
432 latitudes¹³ and the impacts of thermokarst thawing on arctic aquatic ecosystems is increasing
433 rapidly with climate warming.^{15,23,24} Our study strongly suggests that increasing inputs of organic
434 matter and nutrients into arctic surface waters^{27,28,71,72} can have potentially major consequences
435 for the transport and/or *in situ* production of MeHg, particularly in these abundant ponds. Small
436 permafrost thaw ponds may play an important role in controlling the local and regional fluxes of
437 contaminants in the warming Eastern Canadian Arctic.

438 MeHg in thaw ponds may enter aquatic food webs through feeding on zooplankton by
439 migratory bird population or through downstream transport to larger water bodies. On Bylot
440 Island, large-scale thermal erosion has led to drainage of the terrain into a nearby river.^{21,73} In
441 ice-rich permafrost areas, thawing may lead to the coalescence of trough and polygonal ponds
442 into larger lakes, which can then be catastrophically drained into nearby rivers by thermal
443 erosion.^{20,74,75} In the Subarctic, there are also signs that rapidly degrading discontinuous
444 permafrost can increase hydrological connectivity and potentially the transport of MeHg to the
445 hydrological network. In the Sheldrake River catchment north of Kuujjuarapik, the thermokarst

446 pond area has increased by 96% over the past 50 years, whereas stream and channel drainage has
447 increased by 18%.²² Sediment and organic material from the degrading permafrost in this area
448 have been tracked many kilometers distant into Hudson Bay, demonstrating the potential for the
449 export of Hg from ponds to coastal waters. On the other hand, Subarctic thaw ponds can be
450 ephemeral and disappear through ‘terrestrialization’ (encroaching peat cover) over a relatively
451 short time frame,⁷⁶ which may not lead to mercury export. Further studies are required in order to
452 understand the large-scale ecological implications of high MeHg concentrations found in thaw
453 ponds in the eastern Canadian Arctic, especially in the context of a rapidly warming North.

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469 manuscript.

470 **Supporting Information.** Details of the experimental design, raw physicochemical data
471 tables, GPS coordinates, simple linear regressions and supporting figures. This material is
472 available free of charge via the Internet at <http://pubs.acs.org>.

473 **REFERENCES**

- 474 (1) UNEP. *Minamata Convention on Mercury*; 2013.
- 475 (2) Donaldson, S. G.; Van Oostdam, J.; Tikhonov, C.; Feeley, M.; Armstrong, B.; Ayotte, P.;
476 Boucher, O.; Bowers, W.; Chan, L.; Dallaire, F.; et al. Environmental Contaminants and
477 Human Health in the Canadian Arctic. *Sci. Total Environ.* **2010**, *408*, 5165–5234.
- 478 (3) Steffen, A.; Douglas, T.; Amyot, M.; Ariya, P.; Aspmo, K.; Berg, T. A Synthesis of
479 Atmospheric Mercury Depletion Event Chemistry Linking Atmosphere, Snow and Water.
480 *Environ. Res.* **2007**, 10837–10931.
- 481 (4) AMAP. *AMAP Assessment 2011: Mercury in the Arctic*; AMAP: Oslo, Norway, 2011;
482 Vol. 14.
- 483 (5) Lavoie, R. A.; Jardine, T. D.; Chumchal, M. M.; Kidd, K. A.; Campbell, L. M.
484 Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis. *Env.*
485 *Sci Technol* **2013**, *47*, 13385–13394.
- 486 (6) Ariya, P.; Dastoor, A.; Amyot, M.; Schroeder, W. H.; Barrie, L.; Anlauf, K.; Raofie, F.;
487 Ryzhkov, A.; Davignon, D.; Lalonde, J.; et al. The Arctic: A Sink for Mercury. *Tellus B*
488 **2004**, *56*, 397–403.
- 489 (7) Barkay, T.; Poulain, A. J. Mercury (micro)biogeochemistry in Polar Environments. *FEMS*
490 *Microbiol. Ecol.* **2007**, *59*, 232–241.
- 491 (8) Lehnerr, I.; St Louis, V. L.; Kirk, J. L. Methylmercury Cycling in High Arctic Wetland
492 Ponds: Controls on Sedimentary Production. *Environ. Sci. Technol.* **2012**, *46*, 10523–
493 10531.
- 494 (9) Lehnerr, I.; St Louis, V. L.; Emmerton, C. a; Barker, J. D.; Kirk, J. L. Methylmercury
495 Cycling in High Arctic Wetland Ponds: Sources and Sinks. *Environ. Sci. Technol.* **2012**,
496 *46*, 10514–10522.
- 497 (10) Loseto, L. L.; Lean, D. R. S.; Siciliano, S. D. Snowmelt Sources of Methylmercury to
498 High Arctic Ecosystems. *Environ. Sci. Technol.* **2004**, *38*, 3004–3010.
- 499 (11) Oiffer, L.; Siciliano, S. D. Methyl Mercury Production and Loss in Arctic Soil. *Sci. Total*
500 *Environ.* **2009**, *407*, 1691–1700.
- 501 (12) St. Louis, V. L.; Sharp, M. J.; Steffen, A.; May, A.; Barker, J.; Kirk, J. L.; Kelly, D. J. a.;
502 Arnott, S. E.; Keatley, B.; Smol, J. P. Some Sources and Sinks of Monomethyl and
503 Inorganic Mercury on Ellesmere Island in the Canadian High Arctic. *Environ. Sci.*
504 *Technol.* **2005**, *39*, 2686–2701.

- 505 (13) Pienitz, R.; Doran, P. T.; Lamoureux, S. F. *Origin and Geomorphology of Lakes in the*
506 *Polar Regions. In Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic*
507 *Ecosystems.*; Vincent, W. F.; Laybourn-Parry, J., Eds.; Oxford University Press: New
508 York, 2008.
- 509 (14) Boike, J.; Langer, M.; Lantuit, H.; Muster, S.; Roth, K.; Sachs, T.; Overduin, P.;
510 Westermann, S.; McGuire, A. D. Permafrost–physical Aspects, Carbon Cycling, Databases
511 and Uncertainties. In *Recarbonization of the Biosphere*; Lal, R.; Lorenz, K.; Hüttl, R. F.;
512 Schneider, B. U.; von Braun, J., Eds.; Springer Netherlands: Dordrecht, 2012; pp. 159–
513 185.
- 514 (15) Breton, J.; Vallières, C.; Laurion, I. Limnological Properties of Permafrost Thaw Ponds in
515 Northeastern Canada. *Can. J. Fish. Aquat. Sci.* **2009**, *66*, 1635–1648.
- 516 (16) Deison, R.; Smol, J. P.; Kokelj, S. V; Pisaric, M. F. J.; Kimpe, L. E.; Poulain, A. J.; Sanei,
517 H.; Thienpont, J. R.; Blais, J. M. Spatial and Temporal Assessment of Mercury and
518 Organic Matter in Thermokarst Affected Lakes of the Mackenzie Delta Uplands, NT,
519 Canada. *Environ. Sci. Technol.* **2012**, *46*, 8748–8755.
- 520 (17) Rydberg, J.; Klaminder, J.; Rosén, P.; Bindler, R. Climate Driven Release of Carbon and
521 Mercury from Permafrost Mires Increases Mercury Loading to Sub-Arctic Lakes. *Sci.*
522 *Total Environ.* **2010**, *408*, 4778–4783.
- 523 (18) Rautio, M.; Dufresne, F.; Laurion, I.; Bonilla, S.; Vincent, W. F.; Christoffersen, K. S.
524 Shallow Freshwater Ecosystems of the Circumpolar Arctic. *Ecoscience* **2011**, *18*, 204–
525 222.
- 526 (19) Yoshikawa, K.; Hinzman, L. D. Shrinking Thermokarst Ponds and Groundwater
527 Dynamics in Discontinuous Permafrost near Council, Alaska. *Permafr. Periglac. Process.*
528 **2003**, *14*, 151–160.
- 529 (20) Allard, M. Geomorphological Changes and Permafrost Dynamics: Key Factors in
530 Changing Arctic Ecosystems. An Example from Bylot Island, Nunavut, Canada. *Geosci.*
531 *Canada* **1996**, *23*, 205–212.
- 532 (21) Fortier, D.; Allard, M.; Shur, Y. Observation of Rapid Drainage System Development by
533 Thermal Erosion of Ice Wedges on Bylot Island, Canadian Arctic Archipelago. *Permafr.*
534 *Periglac. Process.* **2007**, *18*, 229–243.
- 535 (22) Jolivel, M.; Allard, M. Thermokarst and Export of Sediment and Organic Carbon in the
536 Sheldrake River Watershed, Nunavik, Canada. *J. Geophys. Res. Earth Surf.* **2013**, *118*,
537 1729–1745.
- 538 (23) Schuur, E. A. G.; Bockheim, J.; Canadell, J. G.; Euskirchen, E.; Field, C. B.; Goryachkin,
539 S. V; Hagemann, S.; Kuhry, P.; Peter, M.; Lee, H.; et al. Vulnerability of Permafrost

- 540 Carbon to Climate Change : Implications for the Global Carbon Cycle. *Bioscience* **2014**,
541 58, 701–714.
- 542 (24) Kokelj, S. V.; Jorgenson, M. T. Advances in Thermokarst Research. *Permafr. Periglac.*
543 *Process.* **2013**, 24, 108–119.
- 544 (25) Macdonald, R. W.; Harner, T.; Fyfe, J. Recent Climate Change in the Arctic and Its
545 Impact on Contaminant Pathways and Interpretation of Temporal Trend Data. *Sci. Total*
546 *Environ.* **2005**, 342, 5–86.
- 547 (26) Klaminder, J.; Yoo, K.; Rydberg, J.; Giesler, R. An Explorative Study of Mercury Export
548 from a Thawing Palsa Mire. *J. Geophys. Res.* **2008**, 113, G04034.
- 549 (27) Guo, L.; Ping, C.-L.; Macdonald, R. W. Mobilization Pathways of Organic Carbon from
550 Permafrost to Arctic Rivers in a Changing Climate. *Geophys. Res. Lett.* **2007**, 34, 1–5.
- 551 (28) Stern, G. a; Macdonald, R. W.; Outridge, P. M.; Wilson, S.; Chételat, J.; Cole, A.;
552 Hintelmann, H.; Loseto, L. L.; Steffen, A.; Wang, F.; et al. How Does Climate Change
553 Influence Arctic Mercury? *Sci. Total Environ.* **2012**, 414, 22–42.
- 554 (29) Roehm, C. L.; Giesler, R.; Karlsson, J. Bioavailability of Terrestrial Organic Carbon to
555 Lake Bacteria: The Case of a Degrading Subarctic Permafrost Mire Complex. *J. Geophys.*
556 *Res.* **2009**, 114, 2005–2012.
- 557 (30) Faithfull, C.; Huss, M.; Vrede, T.; Karlsson, J.; Bergström, A. K. Transfer of Bacterial
558 Production Based on Labile Carbon to Higher Trophic Levels in an Oligotrophic Pelagic
559 System. *Can. J. Fish Aquat. Sci.* **2011**, 69, 85–93.
- 560 (31) Negandhi, K.; Laurion, I.; Whiticar, M. J.; Galand, P. E.; Xu, X.; Lovejoy, C. Small Thaw
561 Ponds: An Unaccounted Source of Methane in the Canadian High Arctic. *PLoS One* **2013**,
562 8, e78204.
- 563 (32) Negandhi, K.; Laurion, I.; Lovejoy, C. Bacterial Communities and Greenhouse Gas
564 Emissions of Shallow Ponds in the High Arctic. *Polar Biol.* **2014**, 37, 1669–1683.
- 565 (33) Laurion, I.; Vincent, W. F.; MacIntyre, S.; Retamal, L.; Dupont, C.; Francus, P.; Pienitz,
566 R. Variability in Greenhouse Gas Emissions from Permafrost Thaw Ponds. *Limnol.*
567 *Oceanogr.* **2010**, 55, 115–133.
- 568 (34) Bhiry, N.; Delwaide, A.; Allard, M.; Bégin, Y.; Filion, L.; Lavoie, M.; Nozais, C.;
569 Payette, S.; Pienitz, R.; Saulnier-Talbot, É.; et al. Environmental Change in the Great
570 Whale River Region, Hudson Bay: Five Decades of Multidisciplinary Research by Centre
571 D'études Nordiques (CEN). *Ecoscience* **2011**, 18, 182–203.

- 572 (35) St. Louis, V. L.; Rudd, J. W. M.; Kelly, C. a.; Beaty, K. G.; Bloom, N. S.; Flett, R. J.
573 Importance of Wetlands as Sources of Methyl Mercury to Boreal Forest Ecosystems. *Can.*
574 *J. Fish. Aquat. Sci.* **1994**, *51*, 1065–1076.
- 575 (36) Thabane, L.; Mbuagbaw, L.; Zhang, S.; Samaan, Z.; Marcucci, M.; Ye, C.; Thabane, M.;
576 Giangregorio, L.; Dennis, B.; Kosa, D.; et al. A Tutorial on Sensitivity Analyses in
577 Clinical Trials: The What, Why, When and How. *BMC Med. Res. Methodol.* **2013**, *13*, 92.
- 578 (37) Oksanen, J.; Blanchet, F. G.; Roeland, K.; Legendre, P.; Minchin, R. P.; O’Hara, R. B.;
579 Simpson, G. L.; Solymos, P.; Stevens, M. H. H.; Wagner, H. Vegan: Community Ecology
580 Package. R Package Version 2.2-1. <http://cran.r-project.org>,
581 <https://github.com/vegandevs/vegan>.
- 582 (38) Lim, D. S. S.; Douglas, M. S. V. Limnological Characteristics of 22 Lakes and Ponds in
583 the Houghton Crater Region of Devon Island , Nunavut , Canadian High Arctic. **2003**, *35*,
584 509–519.
- 585 (39) Michelutti, N.; Douglas, M. S. V; Lean, D. R. S.; Smol, J. P. Physical and Chemical
586 Limnology of 34 Ultra-Oligotrophic Lakes and Ponds near Wynniatt Bay, Victoria Island,
587 Arctic Canada. *Hydrobiologia* **2002**, *482*, 1–13.
- 588 (40) Hamilton, P. B.; Gajewski, K.; Atkinson, D. E.; Lean, D. R. S. Physical and Chemical
589 Limnology of 204 Lakes from the Canadian Arctic Archipelago. *Hydrobiologia* **2001**,
590 *457*, 133–148.
- 591 (41) Antoniadou, D.; Douglas, M. S. V.; Smol, J. P. Limnology and Autecology of Freshwater
592 Diatoms from Alert, Northern Ellesmere Island, Nunavut. *Proc. 6th Natl. Student Conf.*
593 *North. Stud.* **2000**, 1–11.
- 594 (42) Fortier, D.; Allard, M. Late Holocene Syngenetic Ice-Wedge Polygons Development,
595 Bylot Island, Canadian Arctic Archipelag. *Can. J. Earth Sci.* **2004**, *41*, 997–1012.
- 596 (43) Paytan, A.; Lecher, A. L.; Dimova, N.; Sparrow, K. J.; Kodovska, F. G.-T.; Murray, J.;
597 Tulaczyk, S.; Kessler, J. D. Methane Transport from the Active Layer to Lakes in the
598 Arctic Using Toolik Lake, Alaska, as a Case Study. *Proc. Natl. Acad. Sci.* **2015**, *112*,
599 E2263.
- 600 (44) Gilmour, C.; Riedel, G.; Ederington, M.; Bell, J.; Benoit, J.; Gill, G. A.; Stordal, M. C.
601 Methylmercury Concentrations and Production Rates across a Trophic Gradient in the
602 Northern Everglades. *Biogeochemistry* **1998**, *40*, 327–345.
- 603 (45) Ravichandran, M. Interactions between Mercury and Dissolved Organic Matter - a
604 Review. *Chemosphere* **2004**, *55*, 319–331.

- 605 (46) Watras, C. J.; Morrison, K. A.; Host, J. S.; Bloom, N. S. Concentration of Mercury
606 Species in Relationship to Other Site-Specific Factors in the Surface Waters of Northern
607 Wisconsin Lakes. *Limnol. Oceanogr.* **1995**, *40*, 556–565.
- 608 (47) Loseto, L. L.; Siciliano, S. D.; Lean, D. R. S. Methylmercury Production in High Arctic
609 Wetlands. *Environ. Toxicol. Chem.* **2004**, *23*, 17–23.
- 610 (48) Eckley, C. S.; Hintelmann, H. Determination of Mercury Methylation Potentials in the
611 Water Column of Lakes across Canada. *Sci. Total Environ.* **2006**, *368*, 111–125.
- 612 (49) Watanabe, S.; Laurion, I.; Chokmani, K.; Pienitz, R.; Vincent, W. F. Optical Diversity of
613 Thaw Ponds in Discontinuous Permafrost: A Model System for Water Color Analysis. *J.*
614 *Geophys. Res.* **2011**, *116*, G02003.
- 615 (50) St. Pierre, K. a.; St. Louis, V. L.; Kirk, J. L.; Lehnherr, I.; Wang, S.; La Farge, C.
616 Importance of Open Marine Waters to the Enrichment of Total Mercury and
617 Monomethylmercury in Lichens in the Canadian High Arctic. *Environ. Sci. Technol.*
618 **2015**, *49*, 5930–5938.
- 619 (51) Le Faucheur, S.; Campbell, P. G. C.; Fortin, C.; Slaveykova, V. I. Interactions between
620 Mercury and Phytoplankton: Speciation, Bioavailability, and Internal Handling. *Environ.*
621 *Toxicol. Chem.* **2014**, *33*, 1211–1224.
- 622 (52) Legendre, P.; Legendre, L. *Numerical Ecology*; Elsevier Science, 2012.
- 623 (53) Vézina, S.; Vincent, W. F. Arctic Cyanobacteria and Limnological Properties of Their
624 Environment: Bylot Island, Northwest Territories, Canada (73°N, 80°W). *Polar Biol.*
625 **1997**, *17*, 523–534.
- 626 (54) Borch, T.; Kretzschmar, R.; Kappler, A.; Cappellen, P. van; Ginder-Vogel, M.; Voegelin,
627 A.; Campbell, K. Biogeochemical Redox Processes and Their Impact on Contaminant
628 Dynamics. *Env. Sci Technol* **2010**, *44*, 15–23.
- 629 (55) Kritzberg, E. S.; Ekström, S. M. Increasing Iron Concentrations in Surface Waters – a
630 Factor behind Brownification? *Biogeosciences* **2012**, *9*, 1465–1478.
- 631 (56) Chetelat, J.; Amyot, M.; Arp, P.; Blais, J. M.; Depew, D.; van der Velden, S.; Craig, E.;
632 Evans, M.; Gamberg, M.; Gantner, N.; et al. *Mercury in Freshwater Ecosystems of the*
633 *Canadian Arctic: Recent Advances on Its Cycling and Fate*; 2014.
- 634 (57) Dennis, I.; Clair, T.; Driscoll, C.; Kamman, N.; Chalmers, A.; Shanley, J.; Norton, S.;
635 Kahl, S. Distribution Patterns of Mercury in Lakes and Rivers of Northeastern North
636 America. *Ecotoxicology* **2005**, *14*, 113–123.

- 637 (58) Mitchell, C. P. J.; Branfireun, B. a; Kolka, R. K. Spatial Characteristics of Net
638 Methylmercury Production Hot Spots in Peatlands. *Environ. Sci. Technol.* **2008**, *42*,
639 1010–1016.
- 640 (59) Lehnherr, I. Methylmercury Biogeochemistry: A Review with Special Reference to Arctic
641 Aquatic Ecosystems. *Environ. Rev.* **2014**, 1–15.
- 642 (60) Dennis, I.; Clair, T.; Driscoll, C.; Kamman, N.; Chalmers, A.; Shanley, J.; Norton, S.;
643 Kahl, S. Distribution Patterns of Mercury in Lakes and Rivers of Northeastern North
644 America. *Ecotoxicology* **2005**, *14*, 113–123.
- 645 (61) Watras, C. J.; Back, R. C.; Halvorsen, S.; Hudson, R. J. M.; Morrison, K. A.; Wentz, S. P.
646 Bioaccumulation of Mercury in Pelagic Freshwater Food Webs. *Sci. Total Environ.* **1998**,
647 *219*, 183–208.
- 648 (62) Wang, W.; Driscoll, C. T. Patterns of Total Mercury Concentrations in Onondaga Lake,
649 New York. *Environ. Sci. Technol.* **1995**, *29*, 2261–2266.
- 650 (63) Winfrey, M. R.; Rudd, J. W. M. Environmental Factors Affecting the Formation of
651 Methylmercury in Low pH Lakes. *Environ. Toxicol. Chem.* **1990**, *9*, 853–869.
- 652 (64) Ullrich, S. M.; Tanton, T. W.; Abdrashitova, S. A.; Svetlana, A. Mercury in the Aquatic
653 Environment: A Review of Factors Affecting Methylation. *Crit. Rev. Environ. Sci.*
654 *Technol.* **2001**, *31*, 241–293.
- 655 (65) Hamelin, S.; Amyot, M.; Barkay, T.; Wang, Y.; Planas, D. Methanogens : Principal
656 Methylators of Mercury in Lake Periphyton. *Environ. Sci. Technol.* **2011**, *45*, 7693–7700.
- 657 (66) Kerin, E. J.; Gilmour, C. C.; Roden, E.; Suzuki, M. T.; Coates, J. D.; Mason, R. P.
658 Mercury Methylation by Dissimilatory Iron-Reducing Bacteria. *Appl. Environ. Microbiol.*
659 **2006**, *72*, 7919–7921.
- 660 (67) Braaten, H. F. V; de Wit, H. a; Fjeld, E.; Rognerud, S.; Lydersen, E.; Larssen, T.
661 Environmental Factors Influencing Mercury Speciation in Subarctic and Boreal Lakes.
662 *Sci. Total Environ.* **2014**, *476-477*, 336–345.
- 663 (68) Tjerngren, I.; Karlsson, T.; Björn, E.; Skjellberg, U. Potential Hg Methylation and MeHg
664 Demethylation Rates Related to the Nutrient Status of Different Boreal Wetlands.
665 *Biogeochemistry* **2011**, *108*, 335–350.
- 666 (69) Crevecoeur, S.; Vincent, W. F.; Comte, J.; Lovejoy, C. Bacterial Community Structure
667 across Environmental Gradients in Permafrost Thaw Ponds: Methanotroph-Rich
668 Ecosystems. *Front. Microbiol.* **2015**, *6*, 1–15.
- 669 (70) Rossi, P.-G.; Laurion, I.; Lovejoy, C. Distribution and Identity of Bacteria in Subarctic
670 Permafrost Thaw Ponds. *Aquat. Microb. Ecol.* **2013**, *69*, 231–245.

- 671 (71) McNamara, J.; Kane, D. .; Hinzman, L. D. An Analysis of an Arctic Channel Network
672 Using a Digital Elevation Model. *Geomorphology* **1999**, *29*, 339–353.
- 673 (72) Bowden, W. B.; Gooseff, M. N.; Balser, a.; Green, a.; Peterson, B. J.; Bradford, J.
674 Sediment and Nutrient Delivery from Thermokarst Features in the Foothills of the North
675 Slope, Alaska: Potential Impacts on Headwater Stream Ecosystems. *J. Geophys. Res.*
676 **2008**, *113*, G02026.
- 677 (73) Godin, E.; Fortier, D.; Coulombe, S. Effects of Thermo-Erosion Gullying on Hydrologic
678 Flow Networks, Discharge and Soil Loss. *Environ. Res. Lett.* **2014**, *9*, 105010.
- 679 (74) Billings, W.; Peterson, K. Vegetational Change and Ice-Wedge Polygons through the
680 Thaw-Lake Cycle in Arctic Alaska. *Arct. Alp. Res.* **1980**, *12*, 413–432.
- 681 (75) Jorgenson, M. T.; Shur, Y. Evolution of Lakes and Basins in Northern Alaska and
682 Discussion of the Thaw Lake Cycle. *J. Geophys. Res.* **2007**, *112*, F02S17.
- 683 (76) Vallée, S.; Payette, S. Collapse of Permafrost Mounds along a Subarctic River over the
684 Last 100 Years (northern Québec). *Geomorphology* **2007**, *90*, 162–170.
- 685

686 **FIGURE CAPTIONS**

687

688 **TABLE 1.** Comparison of limnological properties from sampled sites on Bylot Island and near
689 Kuujjuarapik-Whapmagoostui. Surface water geometric mean values (bold) and ranges (min –
690 max) are shown. One-way ANOVA results and post-hoc pairwise comparisons between the 3
691 groups (Tukey’s HSD) are given ($\alpha < 0.05$). *P*-values were corrected for multiple tests (Holm
692 correction) and non-significant tests are shown by n.s. Several variables were not available for
693 the Bylot lakes and for taiga/rock ponds (na) yet with *p*-values for the entire model. Variables
694 include of water temperature (Temp), pH, conductivity (Cond), dissolved oxygen (DO),
695 absorption coefficient of dissolved organic matter at 320 nm (a_{320}), concentrations of dissolved
696 organic carbon (DOC), carbon dioxide (CO₂), methane (CH₄), major ions, total phosphorus (TP),
697 total nitrogen (TN), chlorophyll a (Chla), total mercury (THg), methylmercury (MeHg) and the
698 percentage of MeHg to THg.

699

700 **FIGURE 1.** Box plots showing concentrations in ng L⁻¹ (median ± SD; dots are outliers) for
701 total mercury on the left panel, and methylmercury on the right panel for trough ponds (n=18),
702 polygonal ponds (n=9) and lakes (n=7) on Bylot Island, and for taiga/rock ponds (n=12),
703 thermokarst surface waters (n=12) and thermokarst bottom waters (n=9) near Kuujjuarapik-
704 Whapmagoostui. One trough pond outlier (BYL63) was not included in this figure due to
705 extreme values (21.82 ngL⁻¹ for THg, 10.58 ngL⁻¹ for MeHg).

706

707 **FIGURE 2.** Principal Component Analysis (PCA) correlation biplot showing 47 sample sites
708 (coloured points) and 21 physicochemical/environmental variables (black arrows) for Bylot and
709 Kuujjuarapik. Site name abbreviations in the legend are BYL for Bylot sample sites and KUUIJ
710 for Kuujjuarapik sites. The PCA accounts for 51.9% of the total variation among sites (Axis 1:
711 28.5% and Axis 2: 23.4%). The PCA identified the dominant environmental gradient related to
712 THg, MeHg, TN, TP Mn, Fe and DOC concentrations (axis 1) and a secondary gradient of
713 variables including pH, DO, Chla and major ion concentrations (axis 2).

714

715

716 **FIGURE 3.** Correlations between inorganic Hg(II) and MeHg concentrations in surface and
717 bottom waters from a) Bylot Island ($R^2_{adj} = 0.53$, $p < 0.001$) and b) Kuujjuarapik-
718 Whapmagoostui ($p > 0.05$).

719

720 **FIGURE 4.** Correlations between MeHg concentrations (ngL⁻¹) for surface waters of all
721 samples sites (n = 58) showing significant positive correlations for a) DOC (mgL⁻¹), and b) TN
722 (μgL^{-1}) and no significant correlations for c) pH and d) SO₄²⁻(mgL⁻¹). All axes are shown on
723 logarithmic scales and regressions were performed on log-transformed data. Bottom waters for
724 stratified Kuujjuarapik thaw ponds were not included to preserve independence of observations.

725

726 **ABSTRACT ART**



727

728 Source: Isabelle Laurion, Bylot Island, Nunavut, Canada.

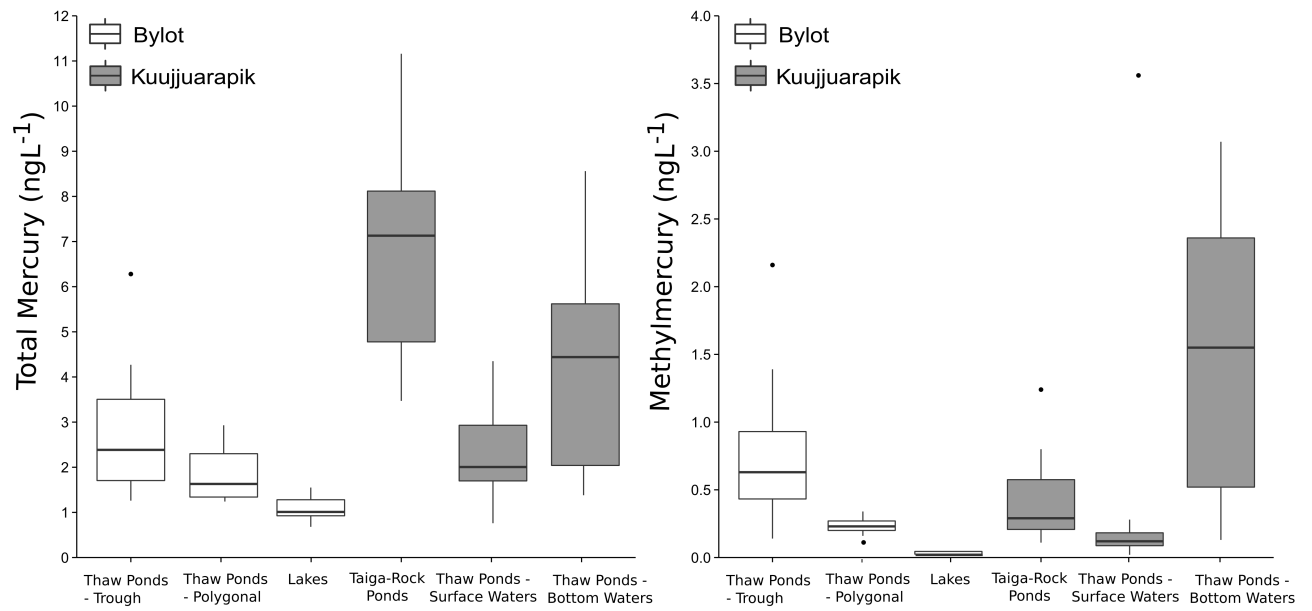
729

TABLE 1

Bylot	a) Trough Ponds (n=18)	b) Polygonal Ponds (n = 9)	c) Lakes (n = 7)	F	P - value	Post-Hoc
Temp (°C)	12.5 (8.0 – 19.2)	13.5 (11.9 – 15.8)	9.4 (6.1 – 16.1)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
pH	6.8 (5.9 – 7.6)	8.0 (6.5 – 8.7)	6.9 (6.6 – 7.6)	14.54	< 0.001	b > a; b > c
Cond (µS cm ⁻¹)	95 (43 – 448)	78 (51 – 119)	18 (9 – 90)	15.87	< 0.001	a > c, b > c
DO (mg L ⁻¹)	8.90 (3.81 – 12.25)	10.46 (6.75 – 12.09)	11.52 (10.43 – 12.85)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
a ₃₂₀ (m ⁻¹)	37.8 (18.4 – 269.4)	16.3 (8.2 – 77.9)	3.3 (1.3 – 6.3)	29.34	< 0.001	a > b > c
DOC (mg L ⁻¹)	12.4 (7.7 – 33.0)	9.0 (6.6 – 15.2)	2.3 (1.0 – 5.3)	36.32	< 0.001	a > c, b > c
CO ₂ (µM)	108.4 (24.3 – 609.1)	20.6 (9.1 – 280.1)	na na	8.206	< 0.01	a > b
CH ₄ (µM)	5.59 (2.09 – 19.90)	1.70 (0.68 – 5.05)	na na	11.58	< 0.001	a > b
SO ₄ ²⁻ (mg L ⁻¹)	1.26 (0.07 – 3.59)	1.49 (0.40 – 6.08)	1.21 (0.66 – 2.47)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Fe (µg L ⁻¹)	90.4 (11.2 – 1637.5)	52.9 (28.68 – 352.4)	19.9 (6.06 – 881.6)	38.87	< 0.001	a > c, b > c
Mn (µg L ⁻¹)	10.5 (2.05 – 556.1)	1.75 (0.58 – 13.94)	na na	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
TP (µg L ⁻¹)	43.5 (14.6 – 359.7)	19.4 (12.9 – 46.8)	4.5 (3.1 – 8.5)	18.68	< 0.001	a > c, b > c
TN (µg L ⁻¹)	743.9 (268.6 – 4366)	418.7 (334.3 – 572.3)	122.8 (94.8 – 194.5)	19.82	< 0.001	a > c, b > c
Chla (µg L ⁻¹)	1.60 (0.40 – 26.60)	0.94 (0.30 – 2.66)	1.13 (0.62 – 1.70)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
THg (ng L ⁻¹)	2.75 (1.26 – 21.82)	1.74 (1.24 – 2.93)	1.05 (0.68 – 1.55)	8.34	< 0.01	a > c
MeHg (ng L ⁻¹)	0.72 (0.14 – 10.58)	0.21 (0.08 – 0.34)	0.03 (0.00 – 0.06)	45.03	< 0.001	a > b > c
MeHg (%)	26.0 (11.1 – 48.5)	12.0 (4.9 – 18.7)	1.5 (0.0 – 5.8)	64.30	< 0.001	a > b > c

Kuujjuarapik	a) Thaw ponds Surface Waters (n = 12)	b) Thaw Ponds Bottom Waters (n = 9)	c) Taiga/Rock Ponds (n=12)	F	P - value	Post-Hoc
Temp (°C)	17.8 (14.4 – 24.2)	7.6 (4.9 – 13.1)	13.5 (11.4 – 16.3)	50.95	< 0.001	a > c > b
pH	6.6 (5.8 – 7.2)	6.2 (5.9 – 6.9)	6.9 (5.7 – 7.8)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Cond (µS cm ⁻¹)	52 (27 – 204)	188 (145 – 265)	77 (26 – 514)	8.77	0.001	b > a, b > c
DO (mg L ⁻¹)	7.76 (2.49 – 9.81)	0.41 (0.41 – 9.31)	10.41 (8.34 – 12.73)	33.73	< 0.001	c > a > b
A ₃₂₀ (m ⁻¹)	31.1 (12.9 – 53.7)	48.3 (19.7 – 106.9)	na na	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
DOC (mg L ⁻¹)	8.71 (4.0 – 28.0)	7.3 (4.2 – 11.9)	12.5 (6.8 – 18.3)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
CO ₂ (µM)	61.0 (33.9 – 141.6)	376.6 (106.9 – 815.5)	na na	39.78	< 0.001	b > a
CH ₄ (µM)	0.44 (0.24 – 1.41)	42.12 (0.48 – 311.9)	na na	28.05	< 0.001	b > a
SO ₄ ²⁻ (mg L ⁻¹)	0.39 (0.05 – 12.52)	0.37 (0.08 – 12.47)	2.31 (0.74 – 12.87)	6.03	< 0.01	c > a, c > b
Fe (µg L ⁻¹)	357.9 (45.9 – 2462.3)	141.4 (31.6 – 512.3)	186.4 (53.6 – 519.7)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Mn (µg L ⁻¹)	6.86 (1.15 – 32.40)	8.09 (0.61 – 30.44)	5.52 (1.51 – 47.82)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
TP (µg L ⁻¹)	53.7 (15.3 – 237.3)	184.1 (48.1 – 431.8)	14.69 (5.2 – 65.3)	30.53	< 0.001	b > a > c
TN (µg L ⁻¹)	409 (228 – 2899)	360 (267 – 496)	530 (208 – 804)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Chla (µg L ⁻¹)	5.91 (1.97 – 14.30)	52.50 (7.4 – 203.4)	1.39 (0.46 – 4.76)	50.30	< 0.001	b > a > c
THg (ng L ⁻¹)	2.12 (0.75 – 4.35)	3.66 (1.38 – 8.56)	6.50 (3.47 – 11.16)	16.23	< 0.001	c > b > a
MeHg (ng L ⁻¹)	0.14 (0.02 – 3.56)	0.99 (0.13 – 3.07)	0.33 (0.11 – 1.24)	9.26	< 0.001	b > a; b > c
MeHg (%)	6.7 (2.7 – 81.9)	27.2 (6.4 – 78.1)	5.1 (1.3 – 12.9)	18.33	< 0.001	b > c, b > a

734

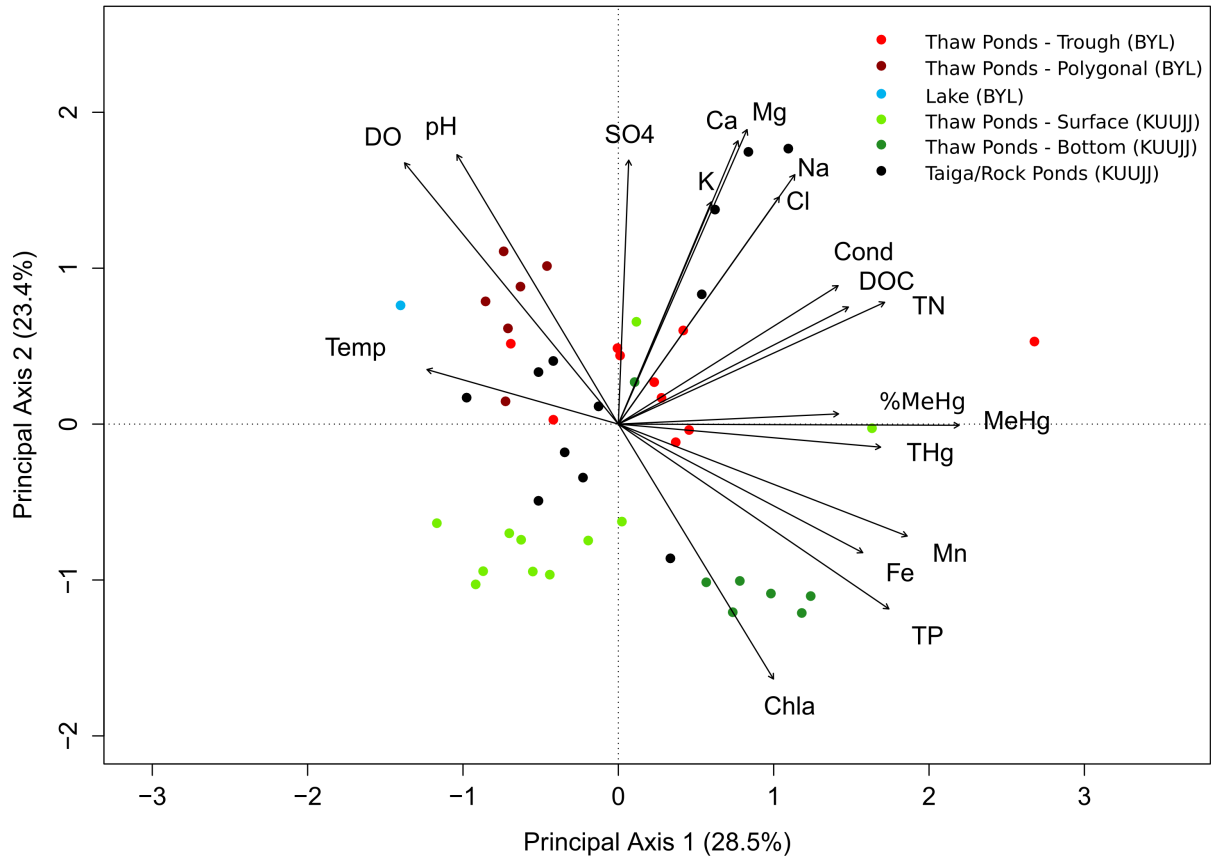


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736 **FIGURE 1**

737

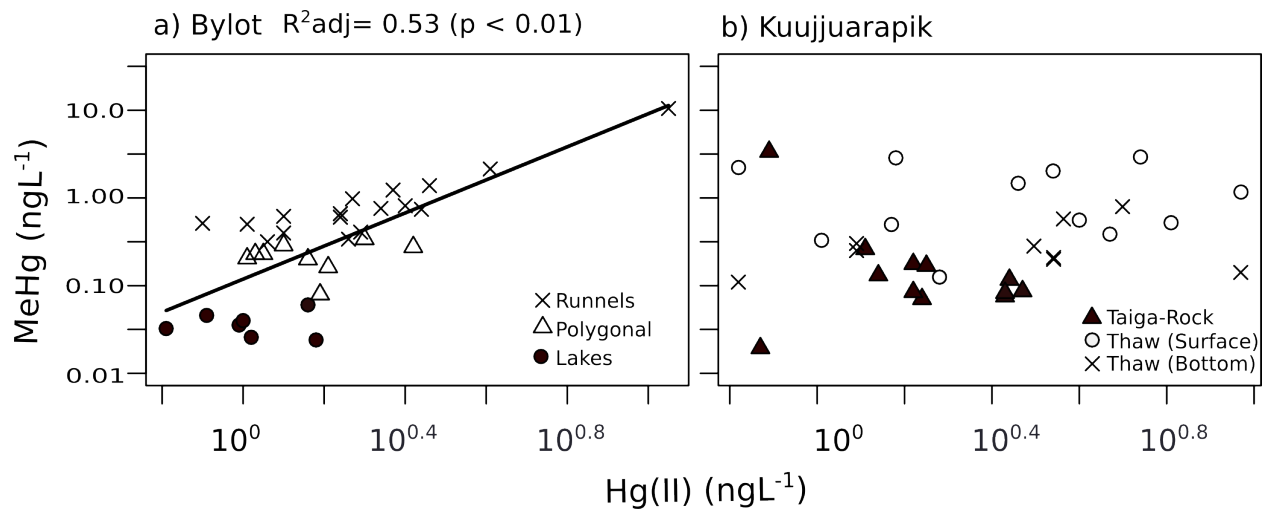
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740 **FIGURE 2**

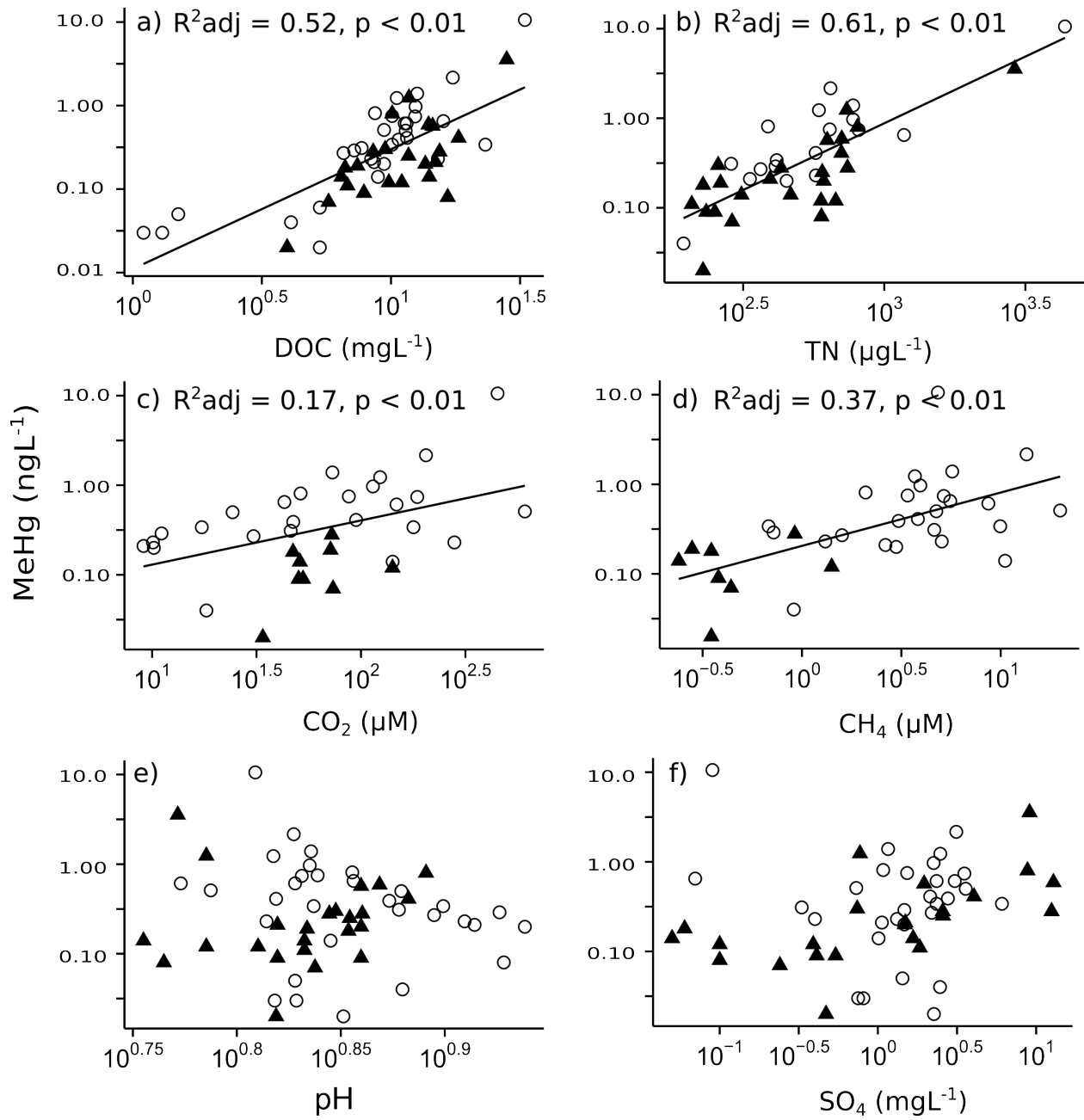
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743 **FIGURE 3**

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745

746 **FIGURE 4**

747