Supplementary information

Photodemethylation of methylmercury in Eastern Canadian Arctic thaw pond and lake ecosystems

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Supporting information consists of 16 pages with 4 tables and 7 figures.

TABLE OF CONTENTS

Supplementary methods

Certified standard solutions

MeHg solution used for 5.0 ng L^{-1} amendments was made from a MeHg stock solution (1000 ppm, certified by Alfa Aesar). A 1 ppm MeHg solution was prepared by dilution in methanol (Fisher Scientific, HPLC grade). Intermediate and working solutions (10.0 ug L^{-1}, 600 ng L^{-1} and 10 ng L^{-1}) were prepared in MilliQ water (18.2 MΩ cm, EMD Millipore) and preserved with 0.3% acetic acid (Fisher Scientific, ACS-pur) and 0.2% HCL (EMD, Omni-trace ultra).

Sampling methods

Sampling and experimental solution bottles were rinsed with distilled water, then soaked in a 45% HNO_3, 5% HCl (Fisher Scientific, ACS-pur) bath overnight, before being rinsed 3 times with MilliQ water (18.2 MΩ cm, EMD Millipore).

The peristaltic pump used for sampling was flushed with a 10% HCl solution (Fisher Scientific, ACS-pur) for 10 minutes, then rinsed with site water for 10 minutes.

Vertical profiles in the water column for water conductivity, pH, temperature and dissolved oxygen were measured using a YSI 650 data logger (YSI Incorporated/Xylem Incorporated). Solar radiation data was collected during experiments with Hobo photosynthetic light (PAR) sensors and data loggers (Onset), and light measurements were collected with a Jaz spectrometer (Ocean Optics). Light attenuation coefficients at each study site were calculated with the Beer-Lambert law:

$$I_z = I_0 \, e^{-kz} \qquad \text{(S1)}$$

where z is a given depth in the water column (m), I_z is the intensity of light (W m^{-2}) measured at depth z (Jaz; 290-895 nm), I_o is the intensity of light at the surface (W m^{-2}) and k is the light attenuation coefficient.

Analytical methods

DOC levels were quantified with an Aurora 1030 analyzer (OI Analytical) by persulfate heat oxidation, followed by conductiometric determination of released CO_2. Anions were analyzed via ionic chromatography (Waters), while cations were measured with flame atomic absorption spectroscopy (Varian, Agilent). Total (reduced and oxidized species) thiols (glutathione, cysteine, thioglycolic acid, 3-mercaptopropionic acid, cysteine-glycine) were analyzed by derivatization using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-sulfonate (SBD-F) (Fluka Analytical, Sigma-Aldrich) and high performance liquid chromatography with a fluorescence detector, as described in Moingt et al.[1] Thiol detection limit was defined as three times the standard deviation calculated on 10 low concentration (< 5 nM) standards. These were 1.0 nM for glutathione, 1.0 nM for cysteine, 1.1 nM for thioglycolic, 2.2 nM for 3-mercaptopropionic acid, and 0.4 nM for cysteine-glycine.

Experimental design

Photodemethylation field experiments were conducted in natural sunlight. Water was collected in clear, acid-washed Teflon bottles with no headspace. On Bylot, bottles were incubated in floating rafts approximately 0.5 cm below the surface of the sampled pond. On Cornwallis, due to weather, incubations were conducted in basins on the shore, in water changed periodically to prevent warming. In all cases, bottles were placed horizontally with their caps pointing north to insure uniform irradiation.[2] At every time-point, a triplicate of every treatment series (or duplicate for dark controls) was removed from the incubation set-up and preserved with ultrapure HCl to a 0.4% final concentration. The bottles were then stored in the dark at 4 °C until analysis.

In experiments measuring natural rates conducted in BYL24 and Char Lake, water was collected and separated into three treatment series: one control series kept in the dark, a series exposed to the full spectrum of sunlight and a series exposed to the visible spectrum only using UV filters (transmission of approximately 16% for wavelengths (λ) <400 nm, and 100% for $400 \leq \lambda \leq 700$ nm) (Lee 226, Lee Filters).

Mechanistic experiments aiming to study the effect of chemical actors on photodemethylation were also conducted in the field at BYL22 and Char Lake. All bottles were spiked with 5.0 ng L^{-1} (± 0.4%) of MeHg, and treatments included thiols (10 nM glutathione (*GSH*), 10 nM thioglycolic acid (*TA*)) (formation constants for complexation to MeHg at pH 7.4: $GSH = 11.55$, $TA = 11.47$)[3,4] and chlorides (0.6 M of Cl). Incubations lasted 50 h. This experiment was repeated in the laboratory in a Suntest CPS+ solar simulator (Atlas Material Testing Technology) using BYL22 and MilliQ water. To insure comparability between field experiments and data generated in the laboratory, Hobo photosynthetically active radiation (*PAR*) sensors (Onset) were used to collect light measurements in the field, which were compiled to establish a total irradiance dose received during field experiments. The Suntest CPS+ (Atlas Material Testing Technology) was programmed to deliver constant total irradiance during artificial incubations, with a total dose equal to that received during field incubations but over a shorter period. This required using higher intensity irradiation than what naturally occurred on the field, with a lamp producing a slightly different wavelength spectrum (notably more UV-A radiation than sunlight on the field, and the absence of UV-C radiation) (SI Table S4). No replicates were done in this experiment, due to limited space in the solar simulator.

Finally, to assess the potential impact of photodemethylation on the MeHg budget of an aquatic ecosystem, we sampled BYL22 every 6 h (surface and 30 cm depth) over a 14-day period for total Hg and MeHg and water chemistry parameters. During days 4-8, the pond was covered with a clean opaque plastic tarpaulin anchored into the surrounding permafrost. Pump and sensor tubing were permanently installed at the desired depths to limit disturbances and light penetration associated with sampling. Water was pumped continuously during 5 minutes before each sampling, to thoroughly rinse system.

Supplementary results & discussion

Predicted MeHg losses in covered pond experiment

Theoretical MeHg losses following re-exposure of pond surface to sunlight were calculated using equation 1. This accounts for predicted photodemethylation occurring during Days 9-13, using BYL22 k_{PD} (9.3×10^{-3} m^2 E^{-1}), average daily PAR on Bylot Island (37.76 E d^{-1}) (SI, Table S1) and MeHg concentration on Day 9 (1.35 ng L^{-1}) (Figure 5).

$$\ln([\text{MeHg}]_t) = \ln([\text{MeHg}]_0) - k_{PD}PAR_t \qquad (1)$$

$$\ln([\text{MeHg}]_{\text{Day13}}) = \ln([\text{MeHg}]_{\text{Day9}}) - k_{PD}PAR_t$$

$$\ln([\text{MeHg}]_{\text{Day13}}) = \ln(0.14 \text{ ng L}^{-1}) - (9.3 \times 10^{-3} \text{ m}^2 \text{ E}^{-1}) \times (37.76 \text{ E m}^{-2} \text{ d}^{-1} \times 5 \text{ days})$$

$$\ln([\text{MeHg}]_{\text{Day13}}) = -1.97 - 1.76$$

$$\ln([\text{MeHg}]_{\text{Day13}}) = -3.73$$

$$\text{MeHg}_{\text{Day13}} = 0.02 \text{ ng L}^{-1}$$

$$\text{Predicted \% of MeHg loss} = 1 - \text{MeHg}_{\text{Day13}}/\text{MeHg}_{\text{Day9}} \times 100$$

Predicted % of MeHg loss = $1 - 0.02\ \text{ng L}^{-1}/0.14\ \text{ng L}^{-1} \times 100$

Predicted % of MeHg loss = 85.71%

Note that this loss is only applicable to the surface and should be compared to the surface data in Fig. 5. We only considered the surface here, and assumed that the water was not mixing well after the removal of the cover. This assumption is based on the oxygen data in Fig. 5 that indicated an oxygen stratification from Day 4 to Day 13. We consider the 85% loss as an upper limit.

Supplementary references

(1) Moingt, M.; Bressac, M.; Bélanger, D.; Amyot, M. Role of ultra-violet radiation, mercury and copper on the stability of dissolved glutathione in natural and artificial freshwater and saltwater. *Chemosphere* **2010**, *80*, 1314–1320.

(2) Poulain, A. J.; Lalonde, J. D.; Amyot, M.; Shead, J. A.; Raofie, F.; Ariya, P. A. Redox transformations of mercury in an Arctic snowpack at springtime. *Atmos. Environ.* **2004**, *38*, 6763–6774.

(3) Reid, R. S.; Rabenstein, D. L. Nuclear Magnetic Resonance Studies of the Solution Chemistry of Metal Complexes . 19 . Formation Constants for the Complexation of Methylmercury by Glutathione , Ergothioneine , and Hemoglobin. *J. Am. Chem. Soc.* **1982**, *104*, 6733–6737.

(4) Reid, R. S.; Rabenstein, D. L. Nuclear magnetic resonance studies of the solution chemistry of metal complexes . XVII . Formation constants for the complexation of methylmercury by sulfhydryl- containing amino acids and related molecules. *Can. J. Chem.* **1981**, *59*, 1505–1514.

(5) CEN 2014. *Données environnementales de l'île Bylot au Nunavut, Canada, v 1.4 (1992-2014).*

(6) Weather Canada. Historical Climate Data http://climate.weather.gc.ca/index_e.html#access.

(7) Black, F. J.; Poulin, B. A.; Flegal, A. R. Factors controlling the abiotic photo-degradation

of monomethylmercury in surface waters. *Geochim. Cosmochim. Acta* **2012**, *84*, 492–507.

(8) Fernández-Gómez, C.; Drott, A.; Björn, E.; Diez, S.; Bayona, J. M.; Tesfalidet, S.; Lindfors, A.; Skyllberg, U. Towards universal wavelength specific photodegradation rate constants for methyl mercury in humic waters - exemplified by a boreal lake-wetland gradient. *Environ. Sci. Technol.* **2013**, *47*, 6279–6287.

(9) Lehnherr, I.; St Louis, V. L. Importance of ultraviolet radiation in the photodemethylation of methylmercury in freshwater ecosystems. *Environ. Sci. Technol.* **2009**, *43*, 5692–5698.

(10) Lehnherr, I.; St Louis, V. L.; Emmerton, C. A.; Barker, J. D.; Kirk, J. L. Methylmercury cycling in High Arctic wetland ponds: sources and sinks. *Environ. Sci. Technol.* **2012**, *46*, 10514–10522.

(11) Hammerschmidt, C. R.; Fitzgerald, W. F. Photodecomposition of Methylmercury in an Arctic Alaskan Lake. *Environ. Sci. Technol.* **2006**, *40*, 1212–1216.

(12) Perron, T.; Chételat, J.; Gunn, J.; Beisner, B. E.; Amyot, M. Effects of Experimental Thermocline and Oxycline Deepening on Methylmercury Bioaccumulation in a Canadian Shield Lake. *Environ. Sci. Technol.* **2014**, *48*, 2626–2634.

Supplementary tables

Table S1. Surface water chemistry and Hg values measured at each site in 2010, where *Lat.* is latitude, *Long.* is longitude, *Temp.* is water temperature, *Cond.* Is conductivity, *DO* is dissolved oxygen z_{max} is maximum depth, *k* is light attenuation coefficient, *PAR* is photosynthetically active radiation measured during field experiments, air *temp.* is temperature during field experiments on Bylot[5] and Cornwallis[6] Islands and *DOC* is dissolved organic carbon.

Site		*Lat.* (°N)	*Long.* (°W)	Type	*Temp.* (°C)	*Cond.* (µS cm^{-1})	*DO* (mg L^{-1})	pH	z_{max} (m)	*k*	Average daily *PAR* (E d^{-1})	Average air *temp.* (°C)
Bylot Island	BYL22	73°9’29”	-79°58’44”	Pond	15.37	38	25.90	7.24	1.00	3.42	37.76 ± 16.13	5.71 ± 1.11
	BYL24	73°9’26”	-79°58’42”	Pond	10.5	50	12.09	7.22	0.39	1.37		
Cornwallis Island	Char	74°42’16”	-94°52’56”	Lake	5.59	264	10.60	7.92	27.50	0.84	24.62 ± 13.04	4.93 ± 1.70

(Table S1 continued)

Site		[Hg_{tot}] (ng L^{-1})	[MeHg] (ng L^{-1})	*DOC* (mg L^{-1})	Cl (mg L^{-1})	NO_3 (mg L^{-1})	NO_2 (mg L^{-1})	SO_4 (mg L^{-1})	F (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Na (mg L^{-1})	K (mg L^{-1})
Bylot Island	BYL22	2.38 ± 0.02	0.35 ± 0.04	6.20	2.88	0.12	<0.004	1.81	0.12	-	-	-	-
	BYL24	2.97 ± 0.06	0.70 ± 0.05	9.60	3.99	0.09	<0.004	1.62	0.14	-	-	-	-
Cornwallis Island	Char	0.44 ± 0.11	0.02 ± 0.004	0.94	21.54	<0.62	-	26.35	-	33.59	5.30	10.76	1.07

Table S2. Comparison of photodemethylation first-order rates (k_{PD}) measured in recent studies against photosynthetically active radiation (PAR). Surface measurements in natural freshwater only. *Average over 3 years.

Study	Site	k_{PD} (m^2 E^{-1}) × 10^{-3} from PAR	*DOC* (mg L^{-1})	pH
Present study	Thaw ponds on Bylot Island, Nunavut	6.0 – 9.2	0.94 – 9.60	7.22 – 7.92
Black et al., 2012[7]	Coastal wetlands near San Francisco, California	9.9 ± 2.0 (6.0 – 15.0)	6	6.50
Fernandez-Gomez et al., 2013[8]	Boreal lakes north of Umeå, Sweden	2.3 ± 0.2	17.50 – 81.00	3.80 – 6.60
Lehnherr and St-Louis, 2009[9]	Lake 979 at the Experiment Lakes Area, Ontario	4.4	12.80	N/A
Lehnherr et al., 2012[10]	Wetland ponds near Lake Hazen, Nunavut	3.2 – 3.6	5.73 – 10.87*	8.50 – 8.75
Hammerschmidt and Fitzgerald, 2006[11]	Toolik Lake, Alaska	2.6	4.40	7.60

Table S3. All rates obtained in this study. k_{PDs} (m^2 E^{-1}) are presented with standard error, R^2 adjusted and slope *p*-value. For significant slopes, Bonferroni-corrected ANCOVA results are presented to identify in-experiment differences between slopes. **Figure 1.** Experiment conducted on the field in BYL24, with exposure to full spectrum and *PAR* only. **Figure 2.** Experiment conducted on the field in BYL22. Water samples were amended to 5 ng L^{-1} of MeHg and exposed to full spectrum light. **Figure 3.** Experiment conducted on the field in BYL22 and Char Lake, and solar simulator incubations using BYL22 and MilliQ water. Water samples were amended to 5 ng L^{-1} and treated with *GSH*, *TA* or Cl.

Site	**Treatment**	**$k_{PD} \times 10^{-3}$ m^2 E^{-1}**	**St.err x 10^{-3} m^2 E^{-1}**	**R^2 adjusted**	**k_{PD} *p*-value**	**ANCOVA**
Figure 1 – k_{PDs} at ambient MeHg concentrations in natural waters						
BYL24	Dark control	0.6	0.9	-17.6%	Not significant	-
	Full spectrum	-6.1	0.7	95.5%	< 0.005**	a
	PAR only	0.0	1.0	-33.3%	Not significant	-
Figure 2 – k_{PDs} in spiked natural waters						
A - BYL22	Dark control	-0.9	-	-	-	-
	Full spectrum	-9.3	1.5	82.1%	< 0.0005***	a
Inset (first 12h)	Full spectrum	-3.1	5.3	-14.8%	Not significant	-
B – Char Lake	Dark control	-2.2	-	-	-	-
	Full spectrum	-3.2	1.7	24.6%	Not significant	-
Inset (first 12h)	Full spectrum	-19.3	5.9	66.0%	< 0.05*	a
Figure 3 – k_{PDs} in spiked natural waters according to treatment in the field or in a solar simulator						
A - BYL22	Control	-9.3	1.5	82.1%	< 0.0005***	a
	Cl	-6.7	0.6	93.5%	< 0.00005***	a
	TA	-6.2	1.0	82.1%	< 0.0005***	a
B - Char Lake	Control	-3.2	1.7	24.6%	Not significant	-
	GSH	-0.6	0.7	-4.3%	Not significant	-
	Cl	-3.3	1.6	28.9%	Not significant	-
	TA	0.2	1.0	-13.9%	Not significant	-
Inset (first 12h)	Control	-19.3	5.9	66.0%	< 0.05*	a
	GSH	-19.3	4.0	82.1%	< 0.01**	a
	Cl	-16.3	6.8	48.7%	Not significant	-
	TA	-15.2	7.2	40.8%	Not significant	-
C - BYL22 in solar simulator	Control	-11.3	2.4	84.0%	< 0.05*	a
	GSH	-11.0	0.8	97.7%	< 0.001***	a
D – MilliQ in solar simulator	Dark control	0.8	2.4	-28.4%	Not significant	-
	Control	-6.1	0.5	97.3%	< 0.005**	a
	GSH	-8.0	2.7	65.8%	Not significant	-

Table S4. Comparison on UV spectrum delivered by Suntest CPS+ and sunlight on Bylot Island during experiments.

UV radiation	Irradiance (photons cm^{-2} s^{-1})	
	Suntest CPS+	Bylot Island sunlight
Total UVs (100 – 400 nm)	5.69×10^{15}	2.16×10^{15}
UV-A (320 – 400 nm)	5.61×10^{15}	2.08×10^{15}
UV-B (290 – 320 nm)	9.36×10^{13}	6.44×10^{13}

Supplementary figures

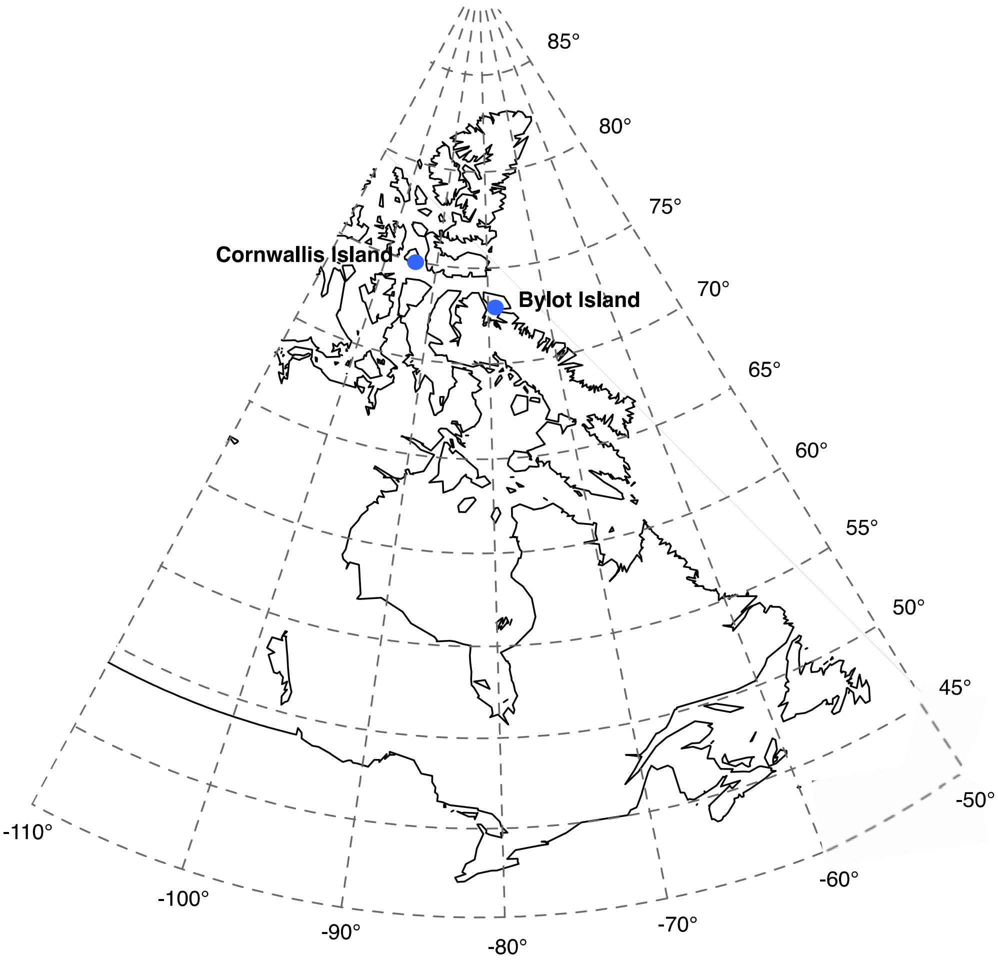

Figure S1. Map of Cornallis and Bylot Islands in the Canadian Arctic Archipelago.

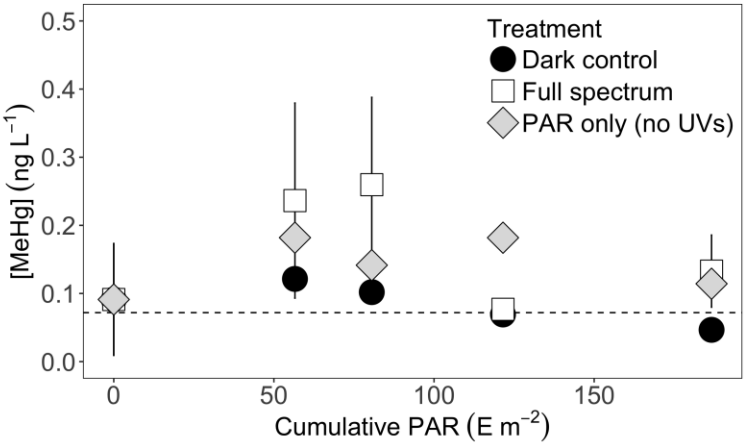

Figure S2. Raw MeHg data for experiment conducted in Char Lake. Some data points were below field detection limit, represented by the dotted line (3x standard deviation of triplicate control = 0.07 ng L^{-1}).

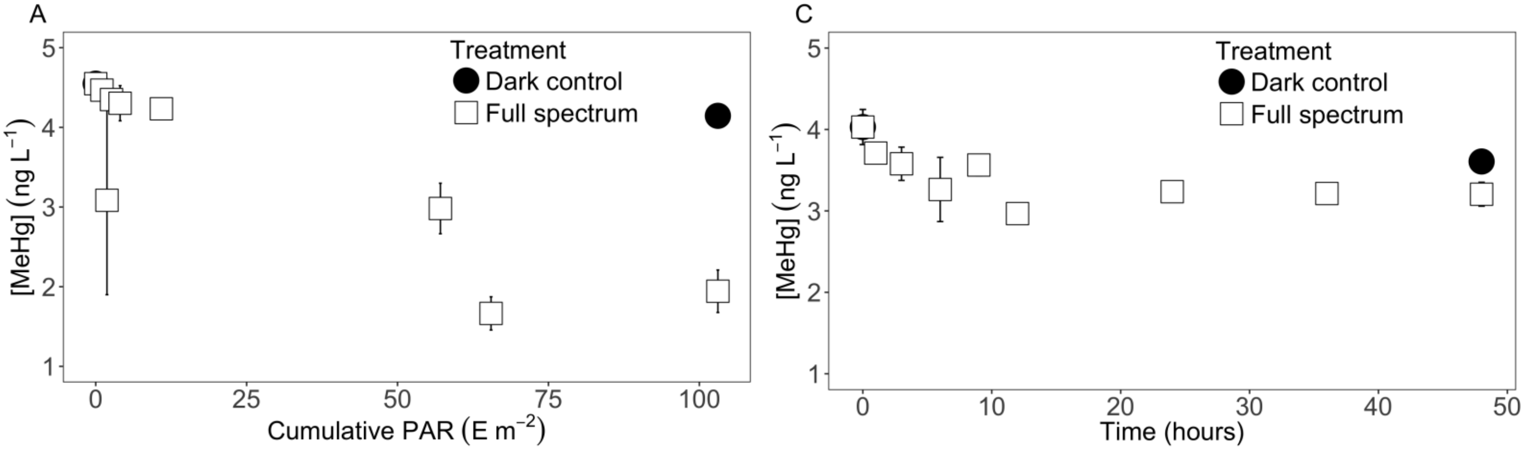

Figure S3. Raw MeHg data for experiment conducted in BYL22 and Char Lake, presented in Figure 2.

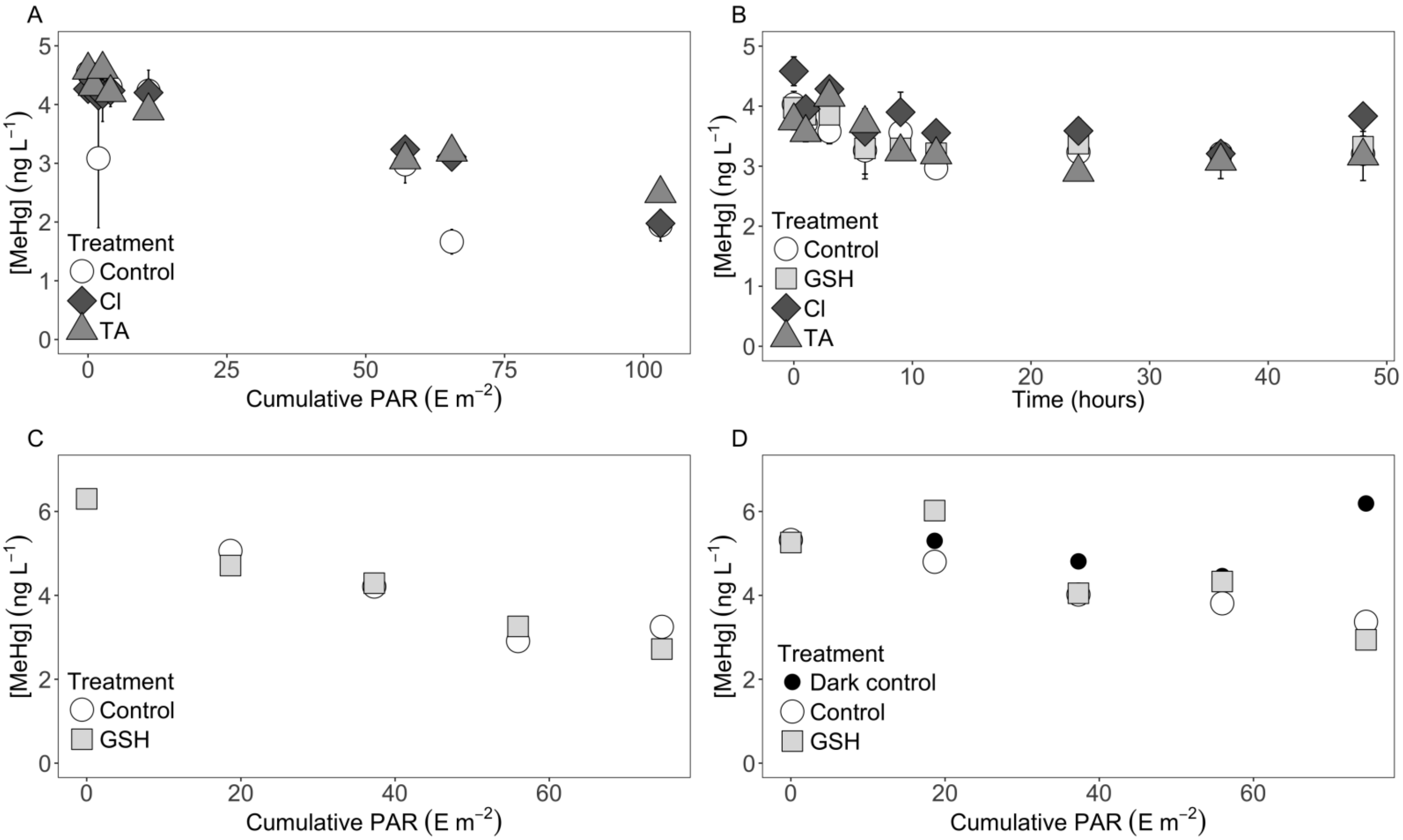

Figure S4. Raw MeHg data for experiment conducted on the field (BYL22 and Char Lake) and in the simulator with (MilliQ and BYL22 water), presented in Figure 3C and 3D.

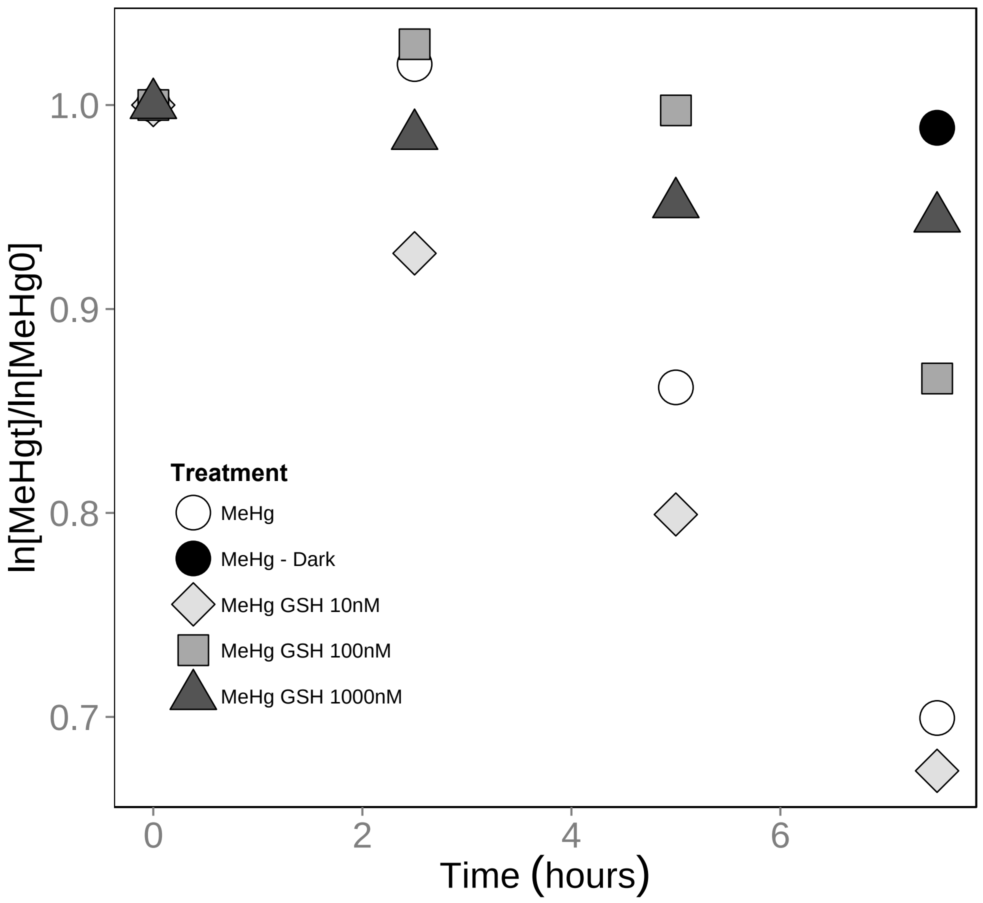

Figure S5. Photodemethylation experiment conducted in a solar simulator using water from a temperate lake (Lake Croche, described in Perron et al.[12]), in the presence of varying concentrations of *GSH* (10-1000 nM) over time (hours).

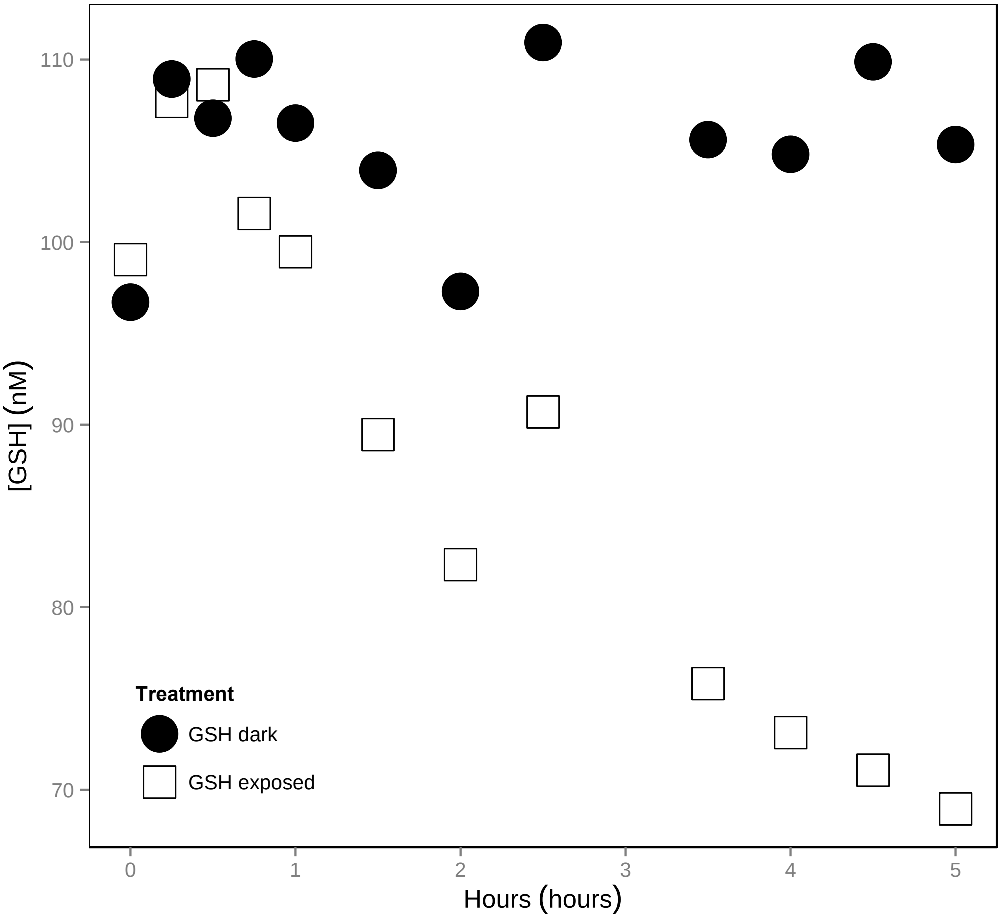

Figure S6. Degradation of *GSH* over time during irradiation in a solar simulator using water from a temperate lake (Lake Croche, described in Perron et al.[12]).

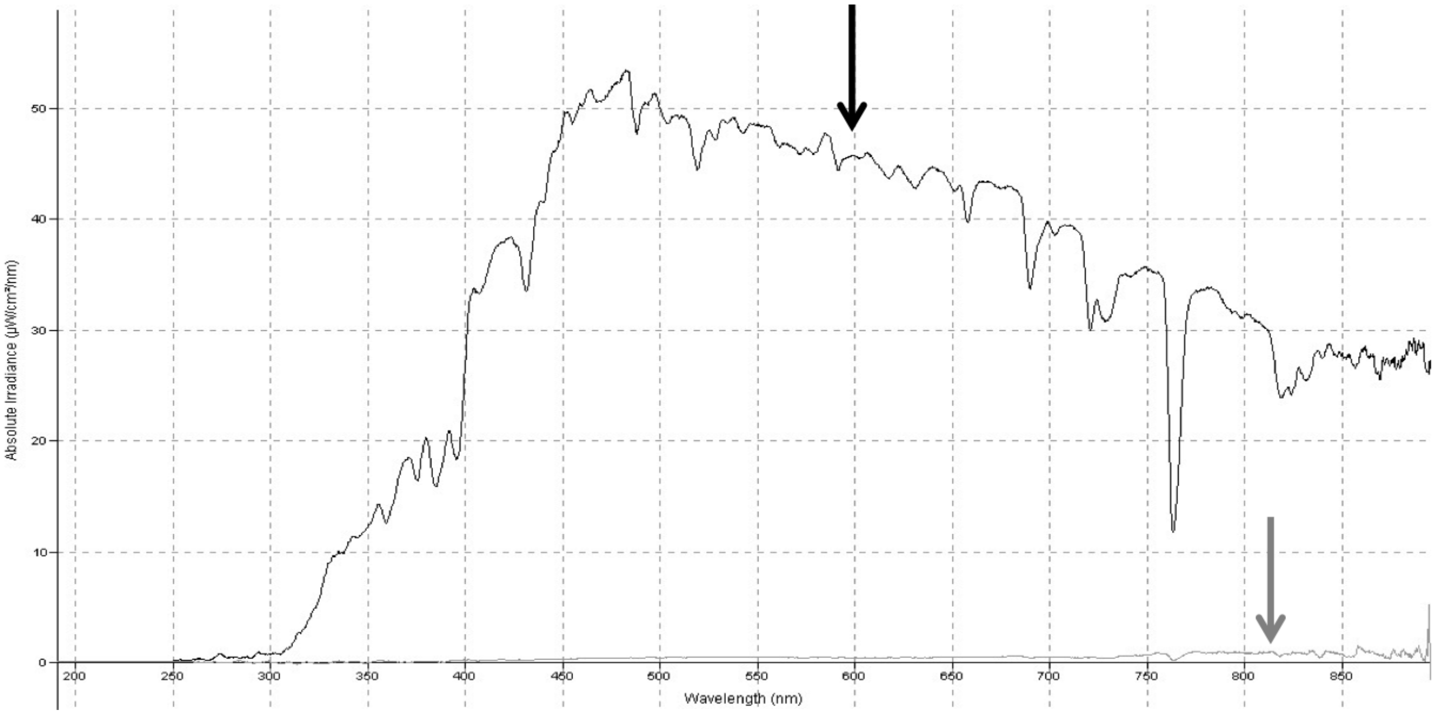

Figure S7. Absolute irradiance (μW cm^{-2} nm^{-1}) spectrum above (in black) and under (in gray) the plastic tarpaulin used in the covered pond experiment, showing radiation obstruction of 98.7% of radiation from 290-895 nm, and 100% of radiation in the UV spectrum (400 nm ≤ λ