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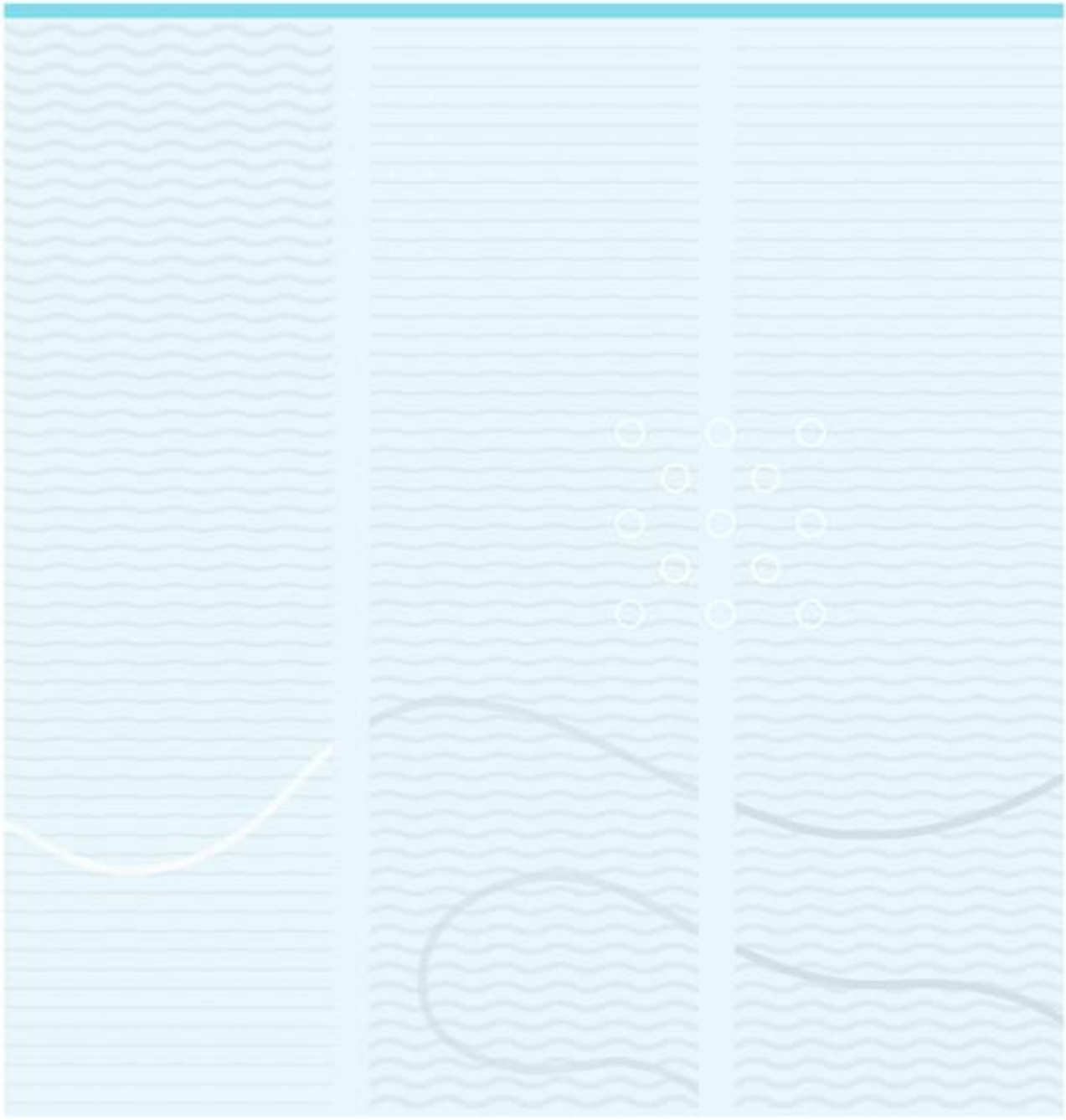

Effects of nutrients and temperature on mobilization of mercury from sediment of the industrial contaminated Gunneklevfjorden, southern Norway

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Abstract

Mobilization of mercury (Hg) and Hg methylation rates in sediment and water from the contaminated fjord Gunneklevfjorden, Telemark, Norway, were investigated in a laboratory experiment with addition of nutrients (as glucose ($C_6H_{12}O_6$) and ammonium (NH_4^+)) to the water in 56 different treatments under two different temperature regimes (4ºC and 20ºC). After storage for four months, the concentration of total Hg (TotHg) and methylmercury, CH_3Hg^+ (MeHg), in water above the contaminated sediment were measured in the different treatments. Correlations were assessed between TotHg/MeHg and nutrient consumption, redox potential (Eh), sulfate (SO_4^{2-})- and sulfide (S^{2-}) concentrations, as well as other possible influencing variables such as pH, nitrate (NO_3^--N) and total phosphorous (Tot-P). The amount of nutrients added and nutrient consumption were strongly correlated ($p = < 2.2 \times 10^{-16}$ for both glucose and NH_4^+), indicating a stimulation of bacterial activity with increasing nutrient availability. The Eh 1 cm above the sediment surface ($Eh_{(1)}$) was significantly negatively correlated with nutrient consumption ($\alpha = 6.9 \times 10^{-9}$ and $\alpha = 0.0023$ for glucose and NH_4^+, respectively) and significantly lower at storage temperature 20°C ($\alpha = 0.0152$), indicating that enhanced bacterial activity reduced the amount of oxygen above the sediment, and thereby lowered $Eh_{(1)}$. A significant negative correlation between consumed glucose and SO_4^{2-} concentrations in the water ($\alpha = 3.3 \times 10^{-9}$) indicated presence of sulfate-reducing bacteria (SRB), further demonstrated by a significant negative correlation between S^{2-} 1 cm below the sediment surface ($S^{2-}_{(-1)}$) and SO_4^{2-} in the water ($p = 0.0088$). TotHg concentrations in the water after storage showed a large variation, ranging from 1.9 - 74.8 ng L^{-1}. Storage temperature appeared to be the strongest explanatory variable for TotHg, with a significant difference between TotHg at 4ºC (34.2 ± 22.9 ng L^{-1}) and 20ºC (9.1 ± 3.8 ng L^{-1}) ($p = 5.9 \times 10^{-6}$). MeHg concentrations in the water after storage ranged from below detection limit (DL: 0.02 ng L^{-1}) to 8.60 ng L^{-1}. In a multiple regression model fitted for MeHg, $Eh_{(1)}$ and storage temperature explained 50 % of the variations in MeHg (interpreted by $R^2 = 0.50$). There was no significant correlation between NH_4^+ consumed and MeHg ($p = 0.2563$). Thus it was assumed that NH_4^+ did not directly affect the bacterial MeHg formation.

1. Introduction

Mercury (Hg) is a highly toxic element in ecosystems all over the world (Wang and Liu, 2008, Ullrich et al., 2001, DeLaune et al., 2004). Earlier and present industrial use (Clarkson and Magos, 2006, KLIF, 2010) has resulted in contamination of surface waters (e.g., (Malm et al., 1990, Nakamura et al., 1988, Wershaw, 1970), sediments (Lamborg et al., 2002), and floodplain soils (Rinklebe et al., 2010). Elemental Hg (Hg^0) has an atmospheric residence time up to one or two years, and is uniformly distributed throughout the troposphere (Lindqvist, 1985). The standard reduction potential for the Hg^0/Hg^{2+} redox pair is within the redox (Eh) interval commonly found in natural environments (defined by Eh). Accordingly, oxidation and reduction of Hg continuously occur in atmospheric, aquatic and terrestrial environments (Lehnherr, 2014). Hg forms stable bonds with soft bases such as sulfides, thiols (-SH) and other reduced sulfur (S) containing ligands, besides entering into many complexes with dissolved organic matter (DOM) (Lehnherr, 2014). Depending on the prevailing physical, chemical and biological conditions, Hg compounds in the aquatic system can be released from sediment to water phase, taken up by aquatic biota, be lost to the atmosphere, or be transported with sediment particulate matter to new, previously uncontaminated locations (Ullrich et al., 2001). The cycling and distribution of Hg between the sediment and water phase can be physically, biologically or chemically mediated, and subsequently affected by variations in pH, temperature, Eh, nutrient status (nitrogen (N), phosphorous (P), S, carbon (C)) and complexing agents (Ullrich et al., 2001). Sediments are the largest storage of heavy metals (Peng et al., 2009), and once associated with sediments, metals undergo various biogeochemical transformations (Lee et al., 2000). Sediments are thought to be the main locator for bacterial methylation of Hg and possible release of methylmercury, CH_3Hg^+ (MeHg), to sediment pore water, the water column, and consequently food webs (Mason et al., 2006). Hg can be methylated both by biotic and abiotic processes (Bjerregaard, 2005), but the abiotic formation is assessed not being environmentally relevant in most aquatic ecosystems (Lehnherr, 2014). MeHg is considerably more toxic than inorganic forms (Berntssen et al., 2004) and bioaccumulates in organisms and biomagnify in food chains (Lehnherr, 2014). MeHg is a neurotoxin (Barkay and Wagner-Döbler, 2005), and has been linked to several human diseases, including numbness, loss of balance, blindness, loss of muscle control, tremors and cancer (Zahir et al., 2005, Barkay and Wagner-Döbler, 2005, Mergler et al., 2007, IARC, 1993).

As Hg methylation is controlled by microbial activity and Hg^{2+} availability, temperature, Eh, organic carbon (OC) and sulfate (SO_4^{2-}) concentrations are essential environmental factors for this methylation (Lehnherr, 2014). Sulfate-reducing bacteria (SRB) are often the main producers of MeHg in water (Branfireun et al., 1999, Ekstrom et al., 2003), but iron-reducing bacteria and methanogenic bacteria are also Hg methylators (Fleming et al., 2006, Parks et al., 2013). However, SRB tend to outcompete methanogens because of their more efficient electron sink, which allow them to generate more energy from the same organic substrate (Compeau and Bartha, 1987). Methylation is, according to Bjerregaard (2005), highest in the transition between aerobic and anaerobic zones, typically in the upper parts of the sediment. Sulfide (S^{2-}) is reported to both increase and decrease MeHg production, dependent on the amount present (Craig and Moreton, 1983). S^{2-} affects the bioavailability of Hg by controlling Hg speciation, and by this how much Hg which is available for methylation (Benoit et al., 1999). This mechanism is also pH dependent (Isa et al., 1986).

Several studies have investigated how Hg methylation can be manipulated by changing environmental parameters, including both physical and chemical factors. Additions of nitrate (NO_3^-) to the hypolimnion have been suggested to suppress accumulation of MeHg in Hg-contaminated lakes (Matthews et al., 2013, Todorova et al., 2009), while additions of glucose ($C_6H_{12}O_6$) to the water has

been reported to increase the Hg methylation rate (Shukla and Pandey, 1993, Larsson, 2005). Other studies have documented that the availability of OC is a controlling factor for Hg methylation (Choi and Bartha, 1994, Lambertsson and Nilsson, 2006), as increased levels of OC enhance Hg methylation (Olson and Cooper, 1976, Fjeld and Rognerud, 1993, Callister and Winfrey, 1986, Gilmour et al., 1992). Others have claimed that additions of glucose and OC to freshwater have no significant effect on MeHg formation (Mitchell et al., 2008, Callister and Winfrey, 1986), while a combination of glucose and SO_4^{2-} additions were shown to increase the Hg methylation (Mitchell et al., 2008). Accordingly, in a study of Hg speciation in Subarctic and Boreal lakes, Braaten et al. (2014a) conclude that the relationship between methylation and nutrient status are poorly understood, and deserves more attention. Temperature is believed to affect Hg cycling, transformation and kinetics in bottom sediments (Boszke et al., 2003, Callister and Winfrey, 1986, Rothenberg et al., 2008), but little information is available on the direct effect of temperature on mobilization of Hg from sediments to the water column.

The aim of this study was to evaluate the effects of nutrient additions to water (as glucose and ammonium (NH_4^+)) and temperature on mobilization of Hg and Hg methylation rates in water and contaminated sediments from the fjord Gunneklevfjorden, situated in southern Norway. Temperature was hypothesized to affect partitioning and solubility of Hg, and thereby the amount of Hg available for methylation. Temperature will also impact the bacterial activity, likely with direct impact on Hg methylation rates. Glucose was added because it is an easily accessible carbon source for the bacteria (Shukla and Pandey, 1993), while N (as NH_4^+) was added because N often is the most limiting nutrient in seawater (Howarth, 1988). Other essential nutrients as P and S (as SO_4^{2-}) were evaluated to be present at sufficient levels, both in the sediment and the overlying brackish water. Since SO_4^{2-} reduction and Eh was expected to affect the methylation rate, these measurements were also incorporated in the study. The main hypothesis of our study was that addition of easily bioavailable C and N will stimulate the Hg methylation rates, but that the rates are temperature dependent.

2. Material and methods

2.1. Study area

Sediment and water were collected in October 2014 from one site in the Hg-contaminated Gunneklevfjorden (Fig.1), a brackish fjord located in Telemark County, Norway. The fjord size and depth are presented in Table 1.

Table 1
Geometry of the Gunneklevfjorden.

Parameter	Unit	Value
Area	km^2	0.8
Length	km	1.8
Width	km	0.5
Max depth	m	≈ 10
Overall depth	m	3 - 6

The fjord receives freshwater from the River Skienselva through a narrow canal in the north end, and salt water from the Frierfjord in the south end. Because of shallow sills in both ends, the supply of salt water to the Gunneklevfjorden is limited (Ottesen et al., 2001). The surface waters in the fjord has therefore a low salinity (0.5 – 6.0 PSU), but during stagnation periods salinity levels up to 10-20 PSU has been measured in deeper waters (Molvær, 1989). Thus, both salt tolerant freshwater organisms and

some saltwater organisms live in the fjord (Olsen et al., 2015). Former industrial discharges from magnesium- and chloralchali plants are the reason for high levels of chlorinated organic compounds (approximately 2.5 tons) and heavy metals (approximately 25 tons Hg) in the sediment.

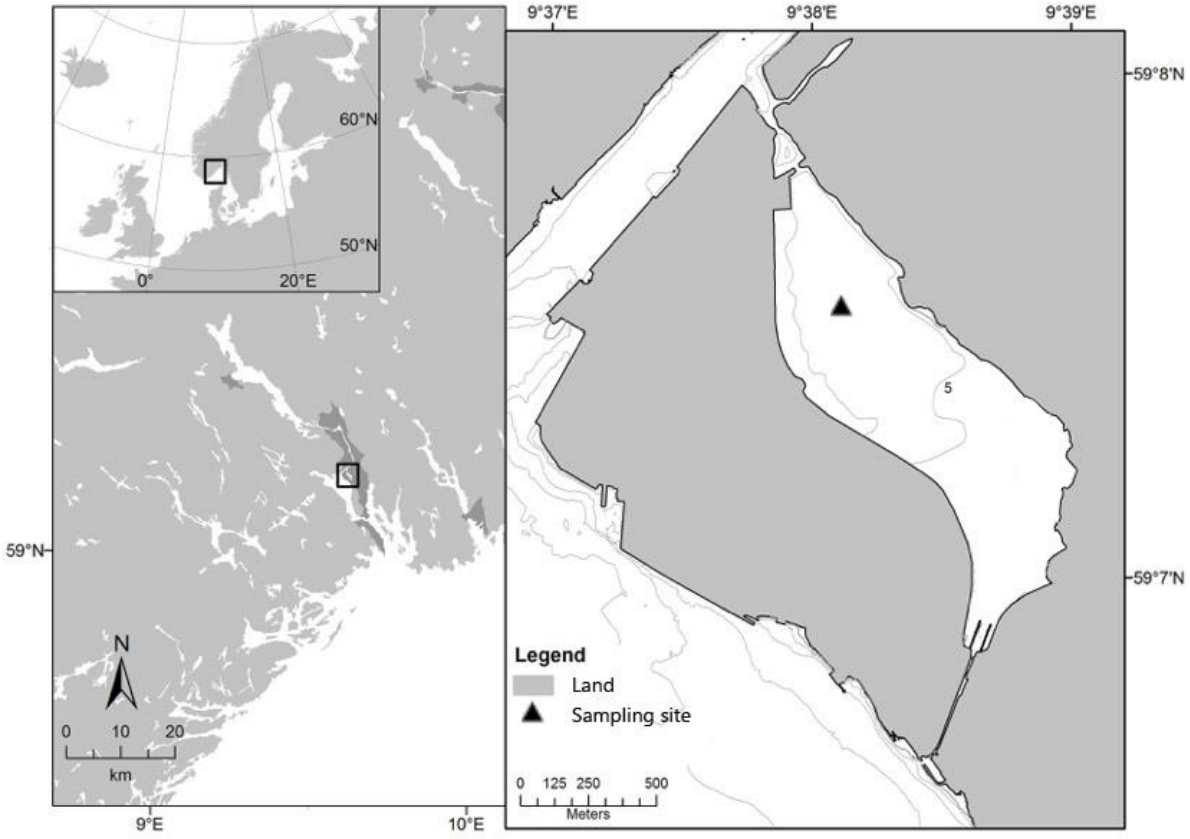

Fig. 1. Map of the Gunneklevfjorden and the sampling site.

2.2. Sample collection

The sediment was collected at 6.5 m deep in the Gunneklevfjorden using an Ekman bottom grab, resulting in samples of the uppermost sediment (approximately 0-15 cm). Several samples were taken at the same site to obtain sufficient material. Water samples were collected with a Ramberg sampler, a 2 m long PVC-tube (inner diameter: 42 mm), resulting in integrated 0-2 m samples. Sediment and water was collected in separate plastic containers, and stored in a dark cooling room (4°C) until initiation of the experiment four months later.

2.3. Experimental setup

The whole sediment bulk sampled from the Gunneklevfjorden was homogenized by a strong electric hand mixer, after overlaying water had been carefully removed. Three subsamples were withdrawn for reference analysis of total Hg (TotHg), while another 56 subsamples of about 250 mL of sediment each were transferred into 2 L polyethylene bottles (diameter: 120 mm, height: 247 mm). To secure homogenized subsamples the bulk sediment was continuously mixed by the hand mixer during the subsampling process. Thereafter, the bottles were filled up with the brackish water sampled from the Gunneklevfjorden. Subsamples of water were collected for reference analysis of TotHg, MeHg, salinity, pH, conductivity, total phosphorous (Tot-P), total nitrogen (Tot-N), total organic carbon

(TOC), nitrate as nitrogen (NO_3^--N), ammonium as nitrogen (NH_4^+-N) and SO_4^{2-}. All 2 L bottles were weighed first empty, then after sediment addition, and finally after water was added (Appendix 1), to be able to normalize the chemical measures to equal sediment-water ratio if necessary.

The bottles with sediment and water were treated with different concentrations of glucose ($C_6H_{12}O_6$ (H_2O)) and ammonium chloride (NH_4Cl) and combinations of these chemicals according to a preassessed dosage scheme (Table 2). The chemicals were added to the overlying water of the sediments. For all combinations of added nutrients, triplicates of each treatment were made (except for ID 25-26, which were a duplicate) to increase the statistical confidence of the results. Most combinations of nutrients were stored at 4ºC (n = 35), while the rest were stored at 20ºC (n = 21). The bottles were stored dark and undisturbed in upright position for four months, before chemical analysis.

Table 2
Dosage scheme of NH_4^+ and glucose to the overlaying water of the sediments.
µmolar of NH_4^+-N and mmolar of $C_6H_{12}O_6$ were calculated from the molar mass of N and C, respectively.

Sample	Temp	NH_4^+-N		$C_6H_{12}O_6$		molar
ID	°C	mg L^{-1}	µmolar	mg L^{-1}	mmolar	C/N
1	**4**	0.00	0.00	0.00	0.00	N.A
2	**4**	0.00	0.00	0.00	0.00	N.A
3	**4**	0.00	0.00	0.00	0.00	N.A
4	20	0.00	0.00	0.00	0.00	N.A
5	20	0.00	0.00	0.00	0.00	N.A
6	20	0.00	0.00	0.00	0.00	N.A
7	**4**	0.25	17.86	0.00	0.00	N.A
8	**4**	0.25	17.86	0.00	0.00	N.A
9	**4**	0.25	17.86	0.00	0.00	N.A
10	**4**	1.25	89.29	0.00	0.00	N.A
11	**4**	1.25	89.29	0.00	0.00	N.A
12	**4**	1.25	89.29	0.00	0.00	N.A
13	**4**	2.50	178.57	0.00	0.00	N.A
14	**4**	2.50	178.57	0.00	0.00	N.A
15	**4**	2.50	178.57	0.00	0.00	N.A
16	**4**	0.00	0.00	2.50	0.21	N.A
17	**4**	0.00	0.00	2.50	0.21	N.A
18	**4**	0.00	0.00	2.50	0.21	N.A
19	**4**	0.00	0.00	12.50	1.04	N.A
20	**4**	0.00	0.00	12.50	1.04	N.A
21	**4**	0.00	0.00	12.50	1.04	N.A
22	**4**	0.00	0.00	25.00	2.08	N.A
23	**4**	0.00	0.00	25.00	2.08	N.A
24	**4**	0.00	0.00	25.00	2.08	N.A
25	**4**	0.00	0.00	50.00	4.17	N.A
26	**4**	0.00	0.00	50.00	4.17	N.A
27	**4**	0.25	17.86	2.50	0.21	11.67
28	**4**	0.25	17.86	2.50	0.21	11.67

Sample	Temp	NH_4^+-N		$C_6H_{12}O_6$		molar
ID	°C	mg L^{-1}	µmolar	mg L^{-1}	mmolar	C/N
29	**4**	0.25	17.86	2.50	0.21	11.67
30	**4**	1.25	89.29	12.50	1.04	11.67
31	**4**	1.25	89.29	12.50	1.04	11.67
32	**4**	1.25	89.29	12.50	1.04	11.67
33	**4**	2.50	178.57	25.00	2.08	11.67
34	**4**	2.50	178.57	25.00	2.08	11.67
35	**4**	2.50	178.57	25.00	2.08	11.67
36	**4**	5.00	357.14	50.00	4.17	11.67
37	**4**	5.00	357.14	50.00	4.17	11.67
38	**4**	5.00	357.14	50.00	4.17	11.67
39	20	0.25	17.86	0.00	0.00	N.A
40	20	0.25	17.86	0.00	0.00	N.A
41	20	0.25	17.86	0.00	0.00	N.A
42	20	2.50	178.57	0.00	0.00	N.A
43	20	2.50	178.57	0.00	0.00	N.A
44	20	2.50	178.57	0.00	0.00	N.A
45	20	0.00	0.00	2.50	0.21	N.A
46	20	0.00	0.00	2.50	0.21	N.A
47	20	0.00	0.00	2.50	0.21	N.A
48	20	0.00	0.00	25.00	2.08	N.A
49	20	0.00	0.00	25.00	2.08	N.A
50	20	0.00	0.00	25.00	2.08	N.A
51	20	0.25	17.86	2.50	0.21	11.67
52	20	0.25	17.86	2.50	0.21	11.67
53	20	0.25	17.86	2.50	0.21	11.67
54	20	2.50	178.57	25.00	2.08	11.67
55	20	2.50	178.57	25.00	2.08	11.67
56	20	2.50	178.57	25.00	2.08	11.67

After the storage period, Eh, S^{2-} and pH were measured in water and sediment in all bottles (Eh and S^{2-}: 12 cm above the sediment surface, 1 cm above the sediment surface and 1 cm below the sediment surface, pH: 12 cm above the sediment surface). For chemical analysis, the uppermost water was carefully sampled by a 50 mL plastic syringe, to avoid sediment disturbance. The syringe was rinsed with 5M hydrochloric acid (HCl) between each sampling, to avoid sample contamination. Water for

TotHg and MeHg analysis were transferred into two separate 250 mL fluoropolymer pre-tested bottles (quality tested by Brooks Rand Labs: mean TotHg concentration = 0.02 ng L^{-1}). To avoid loss of MeHg during preservation, MeHg samples were preserved with 1 mL 37 % HCl analytical grade solution (Parker and Bloom, 2005, Braaten et al., 2014b). Samples for Tot-P and Tot-N were collected in 100 mL glass bottles, and preserved by adding 1 mL 4M sulfuric acid (H_2SO_4). Samples for main water chemistry were collected in 1 L polyethylene bottles. Water samples for TotHg and MeHg were stored frozen (> -18ºC) until analyzed, while samples for main water chemistry were stored in a dark cooling room (4ºC). Sediment from each bottle was collected in preweighed small plastic boxes, and frozen (> -18ºC) for later determination of TotHg.

2.4. Water and sediment analysis

Water

In addition to Eh-, S^{2-}- and pH measurements in each bottle, the uppermost water were analyzed for conductivity, Tot-P, Tot-N, SO_4^{2-}, NO_3^--N, NH_4^+-N, TOC, TotHg and MeHg. All results are presented in Appendix 2. pH was measured using a Hamilton Polilyte Bridge Lab pH electrode with a Radiometer Analytical pH meter (Model PHM210), which was calibrated against standardized pH 4 and 7 buffers before use. S^{2-} and Eh were measured with a S^{2-} electrode (ISE25S) and a combined platinum electrode (MC3051Pt-9), respectively, both with a mercury chloride (Hg_2Cl_2) reference electrode (REF401) and a pH meter (Model PHM210) from Radiometer Analytical. The S^{2-} electrode was calibrated following manufacturer instructions. pH-, S^{2-}- and Eh measurements (in water and sediment) were registered when stable values were achieved, normally after 3-4 min. Conductivity and salinity were measured with a WTW meter (LF320), precalibrated with a stock solution of 0.00100M potassium chloride (KCl). All electrodes were rinsed with distilled water and dried between each sampling, to avoid sample contamination.

Water chemistry were analyzed according to Norwegian Standards (NS) and European Standards (EN-ISO). All water samples were analyzed unfiltered due to low particulate matter content, unless otherwise stated. Tot-P and Tot-N were measured by spectrophotometry according to NS-EN 1189 and NS 4743, respectively, while TOC was measured by infrared spectrophotometry (ISO 8245). SO_4^{2-} and NO_3^--N were measured by liquid chromatography (NS-EN-ISO 10304-1). Due to high concentrations of sodium (Na^{2+}), NH_4^+-N was measured by spectrophotometry (NS 4746), after being filtered through 0.45 µm cellulose nitrate membrane filters (47 mm). Detailed description of analytical methods for TotHg and MeHg is available in Braaten et al. (2014a). In short, every TotHg sample was oxidized with bromine monochloride (BrCl) before analysis, in order to oxidize all Hg species to Hg^{2+}. Sampling and analytical method for TotHg were based on USEPA Method 1669 (USEPA, 1996) and 1631 (USEPA, 2002), respectively. Prior to the MeHg analysis, the water samples were thawed 24 hours before distillation. Sampling and analysis of MeHg were based on USEPA Method 1630 (USEPA, 1998). Both Hg species were analyzed by Brooks Rand Labs MERX automated systems with Model III Atomic Fluorescence Detector. The detection limit (DL) for TotHg and MeHg was 0.1 ng L^{-1} and 0.02 ng L^{1-}, respectively. TotHg and MeHg were analyzed at the Norwegian Institute for Water Research (NIVA), while all other analysis were performed at University College of Southeast Norway (USN). Tot-N measurements were later discarded due to large instrument problems and subsequent unreliable data. Also, four bottles tipped over during storage (ID 37 and 54-56 in Table 2), and were excluded in the statistical analysis.

Sediment

Eight sediment samples from the various N and C treatments (ID: 2, 5, 15, 25, 30, 38, 45 and 53 in Table 2) and one reference sample were weighed before and after the samples were dried at 105°C, to estimate water content and dry weight. TotHg was measured for dried sediments by a Lumex RA-915M instrument, with a PYRO 915-unit. The TotHg analysis is based on differential Zeeman atomic absorption spectrometry using high frequency modulation of light polarization, by gradually heating the sample at 500-580°C (thermal desorption). The atomized Hg is swept by carrier gas into the absorption cell at 254 nm. Interferences is eliminated by Zeeman background correction. The samples were analyzed in triplicates, and the mean value reported.

2.5. Calculation and statistical analysis

Consumption of glucose and NH_4^+ (mg L^{-1}) were calculated from the concentration of TOC and NH_4^+-N (mg L^{-1}) in the water before and after treatment and storage:

$$Glucose_{consumed} = [TOC_{ref} + glucose_{added}]_{before} - [TOC]_{after}$$

$$NH_4^+{}_{consumed} = [NH_4^+\text{-}N_{ref} + NH_4^+{}_{added}]_{before} - [NH_4^+\text{-}N]_{after}$$

As this estimate should reflect assimilation of added nutrients during the experimental period, we assumed this parameter being a proxy for the variation in bacterial activity between the different bottles. In addition, pre- and post treatment and storage concentrations of NO_3^--N (mg L^{-1}) were compared to reveal potential nitrification or denitrification processes during the storing period: Net NO_3^--N = $[NO_3^-\text{-}N]_{after} - [NO_3^-\text{-}N_{ref}]_{before}$. These three calculations are presented in Appendix 2.

The statistical program R (R Core Team, 2014) was used for all the statistical analysis, with significance level $\alpha = 0.05$ (unless otherwise stated). The significance of variables included in multiple regression models are presented as α, while significance of all other statistical tests are presented as *p*. Statistical tests were chosen with respect to the type of variables of interest (all statistical tests and results are presented in Appendix 3). Parametric tests were used when the data was normally distributed, and met the assumptions of parametric tests. For data that violated the assumptions of parametric tests, also after attempt of logarithmic transformation, non-parametric tests were used. For multiple regression models, the plots of the residuals vs. fitted values and the normal quantile plots (Q-Q plots) were assessed after the respective plot requirements described in Whitlock and Schluter (2015), and the models were only included if they fitted the data satisfyingly. Categorical variables which were put first in multiple regression models were specified as factors, to avoid that they were wrongly treated as continuous variables (Liaw and Wiener, 2002). To be able to distinguish differences between groups, storing temperature and nutrient additions were defined as categorical variables. The magnitude of multicollinearity among variables included in a multiple regression model was tested with the variance inflation factor (VIF) in R package "car", version 2.0-25 (Fox and Weisberg, 2011). VIF were assessed against VIF levels accepted in other Hg-related studies (Donald et al., 2015, Burns et al., 2012), and accordingly accepted if the value was < 4. Plots were created using R package "ggplot2", version 2.1.0 (Wickham and Chang, 2016). MeHg samples which were under DL were included in the study with a conservative concentration of 0.01 ng L^{-1} (half the DL). All average concentrations are presented as (mean ± standard deviation).

3. Results

3.1. Initial water and sediment characteristics

Initial physico-chemical analysis of the bulk-water and bulk-sediment used in the experiment are presented in Table 3. The salinity of the water (0.4 PSU) classified the fjord as oligohaline (European Commission, 2003), similar to the conclusion drawn in a previous study from the same fjord (Mjelde, 2014). The pH was 7.2 and the concentration of NO_3^--N was higher than NH_4^+-N, consistent with the fact that NO_3^- normally is the predominate inorganic N ion in saltwater (Tait, 1972). The nutrients NO_3^-, NH_4^+ and phosphate (PO_4^{3-}) in the fjord primarily derive from enriched surface water from the River Skienselva (Skarbøvik et al., 2015) and municipal waste water discharge (Gulbrandsen and Sørensen, 1990, NGI, 2013). Also, ground water runoff from a local landfill may contribute with N to the fjord (SFT, 2009), in addition to local wet and dry deposition (Valiela, 1991). The majority of SO_4^{2-} likely derives from seawater input from the Frierfjord, as SO_4^{2-} is one of the major anions in seawater (Tait, 1972). In addition, SO_4^{2-} derives from the River Skienselva due to impacts of acid rain, and likely also from the local landfill (Direktoratsgruppa, 2013), containing waste from the former magnesium production at Herøya (SFT, 2009). Annual contribution of nutrients, metals and contaminants to the Grenland Fjords (including the Gunneklevfjorden) can be found in Olsen (2012).

Table 3
Initial physico-chemical characterization of bulk water and bulk sediment.

Parameter	Unit	Value
Temperature	°C	10.2
pH	-log[H^+]	7.2
Salinity	PSU	0.4
Conductivity	mS cm^{-1}	1.3
Tot-P	µg L^{-1}	14.5
TOC	mg L^{-1}	2.9
NO_3^--N	mg L^{-1}	0.5
SO_4^{2-}	mg L^{-1}	144.3
NH_4^+-N	mg L^{-1}	0.01
TotHg	ng L^{-1}	3.4
MeHg	ng L^{-1}	0.01 (< DL)
TotHg, sediment	mg kg^{-1} dw	52.6

The TotHg concentration in the fjord of 3.4 ng L^{-1} was within the range of 2 to 15 ng L^{-1} reported for coastal estuarine waters (Schroeder, 1989), and almost equal to other brackish Norwegian fjords (Pakhomova et al., 2014). The MeHg concentration was 0.01 ng L^{-1} (< DL), and lower than the concentrations normally found in Norwegian surface waters (0.16 ± 0.13 ng L^{-1}) (Braaten et al., 2014a). The sediment TotHg concentration before treatment was 52.6 mg kg^{-1} dry weight (dw) (Table 3). The TotHg concentrations in the eight sediment samples analyzed after storage varied from 55.1 – 58.8 mg kg^{-1} dw, with an average of 56.7 ± 1.4 mg kg^{-1} dw (Appendix 4). This indicated highly Hg polluted sediments with minor variations within sediment subsamples used in our experiment. Accordingly, variations in sediment TotHg concentrations were not included as an explanatory variable in our study. The sediment TotHg concentration *per se* should neither be a limiting factor for MeHg formation in our setup, as high levels of Hg has been reported not to limit MeHg production (Han et al., 2007, Zhao, 2009).

3.2. Water chemical conditions after treatment

Chemical analysis of the water above the sediments were carried out four months after addition of glucose and NH_4^+. This time of storage is in accordance to the duration of the summer-stagnation period in many dimictic surface waters at our latitudes. In samples with both nutrients added, the molar ratio between C and N (C:N ratio) was identical in all additions, i.e. 11.76 (Table 2). This ratio is within normal C:N ratios of marine (C:N = 4-10) (Meyers, 1994) and lacustrine organic carbon (C:N = 11-18) (Meyers and Ishiwatari, 1993, Ishiwatari et al., 1977).

Conductivity and pH

Both conductivity and pH in the overlaying water showed minor variations between stored untreated reference samples and stored treated samples, at both 4°C and 20°C. Average conductivity in all samples was 2.3 ± 0.1 mS cm^{-1}, while median pH was 9.5, varying from 8.3 – 9.8. The pH levels in the stored samples were higher than prior to storage (7.2, Table 3). Minor pH variation between treatments indicated that neither variations in storage temperature nor variations in nutrient addition directly caused this pH increase.

Eh

The Eh measurements revealed a redox gradient within the overlaying water and the upper parts of the sediment, i.e. 130.1 ± 132.7 mV 12 cm above the sediment surface ($Eh_{(12)}$), -20.3 ± 67.9 mV 1 cm above the sediment surface ($Eh_{(1)}$), and -309.2 ± 66.1 mV 1 cm below the sediment surface ($Eh_{(-1)}$). As NH_4^+ oxidation normally occurs at Eh values > 400 mV, while ferric iron (Fe^{3+}) and SO_4^{2-} reduction normally occur at Eh ≈ 250 mV and Eh ≈100 mV, respectively in oxygen depleted/anoxic aquatic environments (Wetzel, 1975), the measured Eh in our samples indicated favorable conditions for formation of hydrogen sulfide (H_2S), ferrous iron (Fe^{2+}) and NH_4^+. Due to the high pH (pH > 9), theoretically S would primarily be present as hydrosulfide ions (HS^-), iron (Fe) as Fe^{2+}/iron carbonate ($FeCO_3$), and NH_4^+ as ammonia (NH_3)/ammonium hydroxide (NH_4OH).

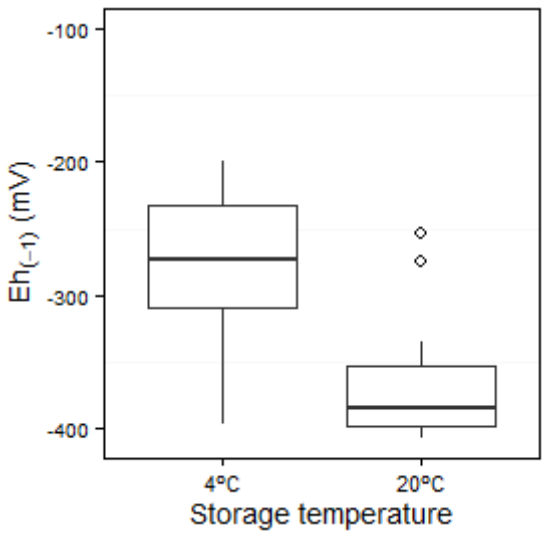

Fig. 2. Measured Eh 1 cm below the sediment surface ($Eh_{(-1)}$) at storage temperatures 4°C and 20°C.

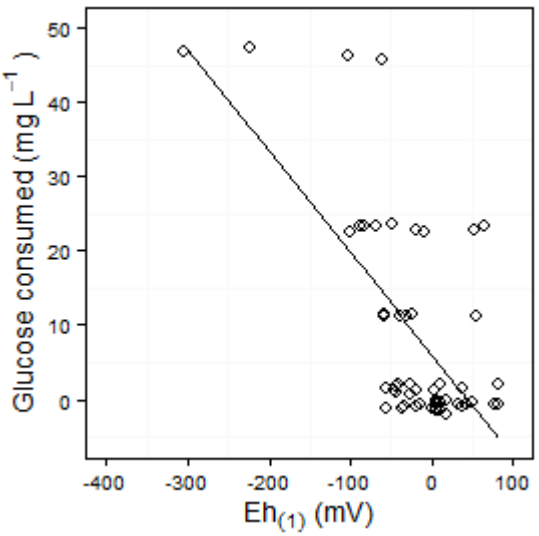

Fig. 3. Relationship between Eh 1 cm above the sediment surface ($Eh_{(1)}$) and glucose consumed.

Fitting a multiple regression model for $Eh_{(-1)}$ with glucose consumption, NH_4^+ consumption and storage temperature as explanatory variables explained 64 % of the variation in $Eh_{(-1)}$ ($R^2 = 0.64$). $Eh_{(-1)}$ was significantly lower at storage temperature 20ºC ($\alpha = 1.8 \times 10^{-11}$) (Fig.2) and was significantly negatively correlated with consumption of glucose ($\alpha = 5.3 \times 10^{-6}$), while the correlation with NH_4^+ consumption was not significant ($\alpha = 0.6540$). Accordingly, a multiple regression model fitted for $Eh_{(1)}$ showed that $Eh_{(1)}$ also was significantly lower at storage temperature 20ºC ($\alpha = 0.0152$) and significantly negatively correlated with consumption of glucose ($\alpha = 6.9 \times 10^{-9}$) (Fig.3), in addition to being significantly negatively correlated with consumption of NH_4^+ ($\alpha = 0.0023$) (model $R^2 = 0.57$). The regression models fitted for prediction of Eh with storage temperature and nutrient consumption as predictors can be understood as increased bacterial activity with subsequently higher oxygen consumption, resulting in a decrease in Eh and thereby suitable conditions for SRB and Hg methylation (Compeau and Bartha, 1984).

TOC

The pre-treatment concentration of TOC in the overlaying water was 2.9 mg L^{-1} (Table 3), while the average post concentration was 4.3 ± 1.0 mg L^{-1}. The addition of glucose should theoretically imply an increase in TOC by 2.50 mg L^{-1} at lowest additions, to 50.00 mg L^{-1} at highest additions (Table 2). The nominally calculated consumption of glucose showed a strong positive significant correlation with the amount of glucose added ($p = < 2.2 \times 10^{-16}$), and as the consumption of glucose ranged from almost 0.0 to 47.3 mg L^{-1} (Fig.4), almost all the glucose had presumably been catabolized/consumed during the four month storing period.

Fig. 4. Relationship between glucose added and glucose consumed.

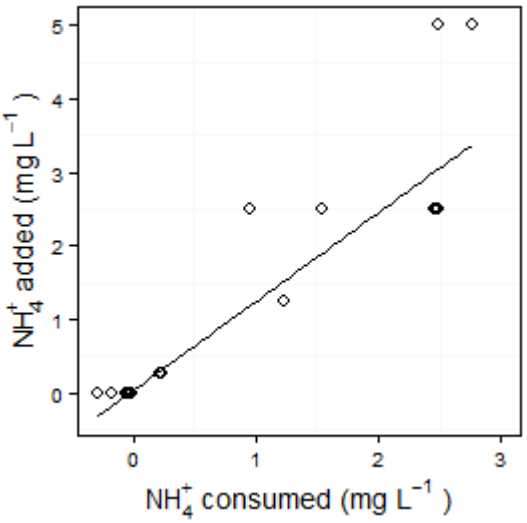

Fig. 5. Relationship between NH_4^+ added and NH_4^+ consumed.

NH_4^+-N

The pre-treatment concentration of NH_4^+-N was 0.01 mg L^{-1} (Table 3), while the average post concentration was 0.2 ± 0.5 mg L^{-1}. The addition of NH_4^+-N should theoretically imply an increase in NH_4^+ by 0.25 mg L^{-1} at lowest additions, to 5.00 mg L^{-1} at highest additions (Table 2). The maximum nominally calculated consumption of NH_4^+ was 2.8 mg L^{-1} (Fig.5), and the highest increases in consumption occurred in the samples with the highest additions. NH_4^+ consumed had a strong positive significant correlation with the amount of NH_4^+ added ($p = < 2.2 \times 10^{-16}$). The consumption of both glucose and NH_4^+ was assumed to reflect bacterial activity (anabolic and catabolic processes).

NO_3^--N and Tot-P

The pre-treatment concentration of NO_3^--N was 0.5 mg L^{-1} (Table 3), while the average post concentration was 0.4 ± 0.3 mg L^{-1}. The calculated net NO_3^--N concentration ranged from -0.5 to 0.7 mg L^{-1} (0.0 ± 0.3 mg L^{-1}). There was no significant correlation between NH_4^+ consumed and net NO_3^--N (p = 0.2364). However, in some samples the increase in NO_3^--N during storage were relatively high, and these samples also had some of the highest amounts of NH_4^+ consumed. This could indicate that formation of NO_3^- took place in these samples, and implies that nitrifying bacteria was present. The average post concentration of Tot-P in the overlying water was 60.6 ± 19.6 µg L^{-1}, while the pre-treatment concentration was 14.5 µg L^{-1} (Table 3). The much higher Tot-P concentrations in the stored samples indicated a release of P from the sediment during storage.

SO_4^{2-} and S^{2-}

While the pre-treatment concentration of SO_4^{2-} in the overlaying water was 144.3 mg L^{-1} (Table 3), the average post concentration was 110.8 ± 19.1 mg L^{-1}. Fitting a multiple regression model for SO_4^{2-} in the water after storage with glucose consumption, NH_4^+ consumption and storage temperature as explanatory variables explained 57 % of the variation in SO_4^{2-} (R^2 = 0.57). SO_4^{2-} showed a significant negative correlation with the amount of glucose consumed ($\alpha = 3.3 \times 10^{-9}$) (Fig.6) but not with the amount of NH_4^+ consumed (α = 0.0957), and was not significantly lower at storage temperature 20°C (α = 0.8108).

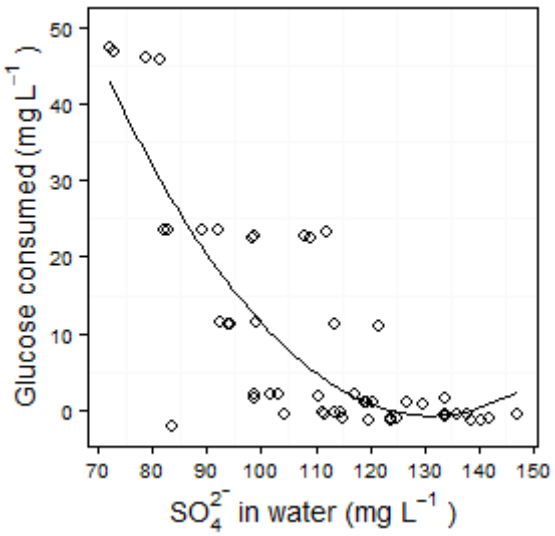

Fig. 6. Relationship between glucose consumed and SO_4^{2-} concentrations in the water.

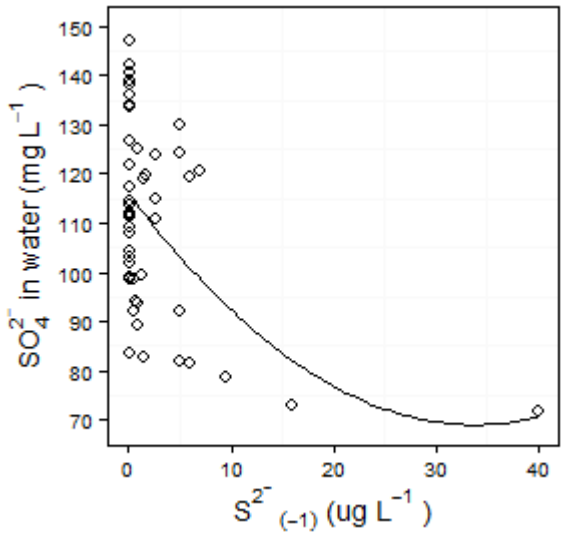

Fig. 7. Relationship between SO_4^{2-} in water and S^{2-} 1 cm below the sediment surface ($S^{2-}_{(-1)}$).

As the reduction in SO_4^{2-} was largely dependent on the amount of glucose consumed, an increased amount of reduced S ($H_2S/HS^-/S^{2-}$) should be expected. The average measured S^{2-} concentrations in our samples was relatively low, i.e. 2.0 ± 8.1 µg L^{-1} 12 cm above the sediment surface ($S^{2-}_{(12)}$), 0.4 ± 1.2 µg L^{-1} 1 cm above the sediment surface ($S^{2-}_{(1)}$), and 2.5 ± 6.1 µg L^{-1} 1 cm below the sediment surface ($S^{2-}_{(-1)}$). A significant negative correlation was observed between reduced S ($S^{2-}_{(-1)}$) and oxidized S (SO_4^{2-}) in our samples (p = 0.0088) (Fig.7). The highest S^{2-} concentrations were measured in the sediment ($S^{2-}_{(-1)}$), significantly and positively correlated with $S^{2-}_{(1)}$ ($p = 1.1 \times 10^{-6}$). Thus, S^{2-} production in the sediment likely affected the S^{2-} concentration in the overlaying water by diffusion across the sediment-water interface. There was also a significant negative correlation between $S^{2-}_{(-1)}$

and $Eh_{(-1)}$ ($p = 2.5 \times 10^{-8}$), indicating that S^{2-} formation was favored under reducing conditions, and that SRB was present.

3.3. TotHg and MeHg in water after treatment

TotHg

While the TotHg concentration in the overlaying water prior to storage was 3.4 ng L^{-1} (Table 3), the TotHg concentrations ranged from 1.9 – 74.8 ng L^{-1} in the stored samples (n = 52). The average TotHg concentration in the samples stored at 4ºC (n = 34) (34.2 ± 22.9 ng L^{-1}) was higher compared with samples stored at 20ºC (n = 18) (9.1 ± 3.8 ng L^{-1}) (Fig.8). In the reference samples (n = 3 at both temperatures), TotHg was 47.9 ± 15.2 ng L^{-1} at 4°C and 10.7 ± 0.9 ng L^{-1} at 20°C. Accordingly, it was a significant difference in TotHg between 4°C and 20°C in all samples ($p = 5.9 \times 10^{-6}$). TotHg did not correlate significantly with the amount of glucose or NH_4^+ consumed during storage ($p = 0.4606$ and $p = 0.3709$, respectively), indicating that the consumption of nutrients did not affect TotHg to a large degree. TotHg was significantly positively correlated with $Eh_{(-1)}$ ($p = 0.0002$), indicating that $Eh_{(-1)}$ affected the partitioning coefficient of Hg in the sediment, and the mobilization of Hg from the sediment to the overlaying water. TotHg was also significantly positively correlated with Tot-P ($p = 0.0059$), hence a correlation between Tot-P and the amount of glucose added was tested, which appeared to be positively significant ($p = 0.0117$). TotHg also showed a significant positive correlation with pH ($p = 0.0024$), indicating that pH affected release of TotHg from the sediment to the overlying water. It was no significant correlation neither between TotHg and SO_4^{2-} in water ($p = 0.8865$), nor between TotHg and $S^{2-}_{(-1)}$ ($p = 0.1792$). Thus it did not seem that Hg was significantly removed to the sediment by formation of mercury sulfide (HgS).

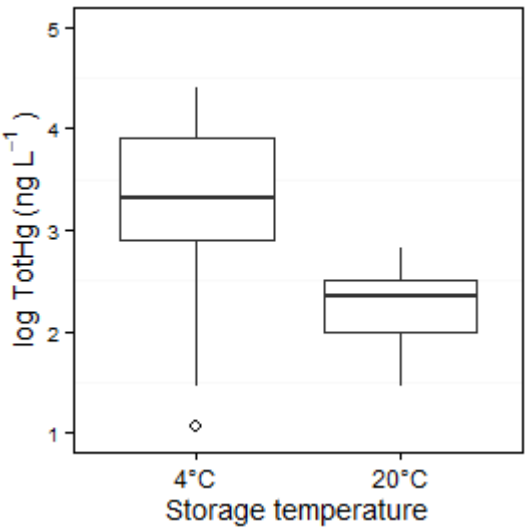

Fig. 8. TotHg concentrations in the water at 4°C and 20°C.

Because storage temperature, $Eh_{(-1)}$, pH and Tot-P were the variables best correlated with TotHg, these variables were chosen as explanatory variables in a multiple regression model fitted for TotHg. However, as $Eh_{(-1)}$ and pH were not found to be significant predictors, they were excluded in the final model. The two remaining variables explained 46 % of the variation in TotHg (interpreted by $R^2 = 0.46$), where TotHg was significantly lower at 20°C ($\alpha = 1.9 \times 10^{-6}$), and significantly positively correlated with Tot-P ($\alpha = 0.0016$).

MeHg

The pre-treatment concentration of MeHg in the overlying water was < DL, i.e. 0.01 ng L^{-1} (Table 3). The average post concentration of MeHg in the stored, untreated reference samples (n = 3 at both temperatures) were approximately equal in samples stored at 4ºC (0.02 ± 0.01 ng L^{-1}) and at 20ºC (0.01 ± 0.01 ng L^{-1}, < DL). This indicated that minor Hg methylation occurred in the reference samples, at both temperatures. In the treated samples (n = 46), MeHg concentrations were also generally low with a few exceptions, and ranged from 0.01 ng L^{-1} (< DL) to 8.60 ng L^{-1} (0.28 ± 1.22 ng L^{-1}).

MeHg was not found to have a significant correlation with TotHg (p = 0.9205), indicating that the amount of TotHg in water did not affect the formation of MeHg to a large degree. There was no significant difference in MeHg between 4°C and 20°C (p = 0.3400), i.e. temperature did not seem to directly affect MeHg formation in water and/or release of MeHg from sediment to water. MeHg had a significant positive correlation with the amount of glucose consumed (p = 0.0001), but not with the amount of NH_4^+ consumed (p = 0.2563). The highest additions of glucose (50.00 mg L^{-1}, Table 2) with or without NH_4^+ addition were the only glucose additions which significantly caused an increase in MeHg ($\alpha = 2.4 \times 10^{-7}$) (Fig.9). A significant negative correlation was revealed between MeHg and SO_4^{2-} concentrations in water (p = 0.0019), together with a strong positive significant correlation between MeHg and $S^{2-}_{(-1)}$ ($p = 2.8 \times 10^{-5}$). There was no significant correlation between MeHg and $S^{2-}_{(1)}$ (p = 0.0978), indicating that Hg methylation primarily took place in the sediment. There were significant negative correlations between MeHg and both $Eh_{(-1)}$ and $Eh_{(1)}$ (Fig.10) (p = 0.0119 and p = 0.0006, respectively), indicating that MeHg formation was favored under low Eh conditions. Net NO_3^--N did not correlate significantly with MeHg (p = 0.9941), indicating that variation in NO_3^--N did not affect MeHg formation considerably. Neither pH nor Tot-P correlated significantly with MeHg (p = 0.6962 and p = 0.4453, respectively), thus it was assumed that variations in these variables did not affect MeHg to a large degree.

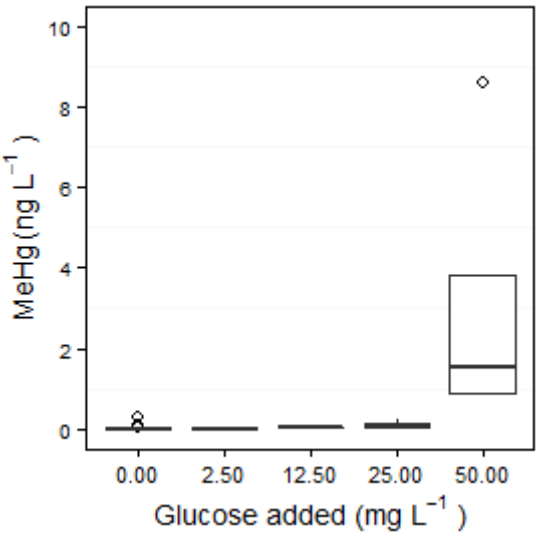

Fig. 9. Response in MeHg at different glucose additions.

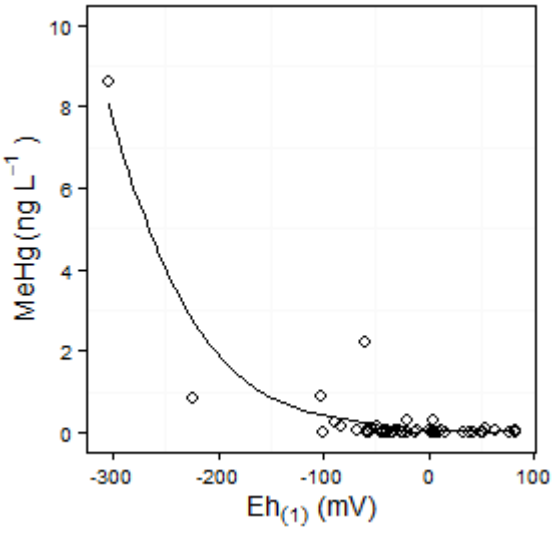

Fig. 10. Relationship between MeHg and Eh 1 cm above the sediment surface ($Eh_{(1)}$).

Fitting a multiple regression model for MeHg showed that 50 % of the variation in MeHg could be explained by $Eh_{(1)}$ and an interaction term between $Eh_{(1)}$ and storage temperature (R^2 = 0.50). MeHg was significantly negatively correlated with $Eh_{(1)}$ ($\alpha = 1.5 \times 10^{-8}$), while an increase in storage temperature to 20°C did not affect MeHg significantly (α = 0.7691). The interaction between $Eh_{(1)}$ and

storage temperature 20°C appeared however to be significant ($\alpha = 0.0407$), indicating that temperature indirectly affected MeHg by influencing $Eh_{(1)}$. An alternative multiple regression model for MeHg prediction was also tested, with glucose consumption and an interaction term between glucose consumption and storage temperature as explanatory variables. Here, MeHg was significantly positively correlated with glucose consumption ($\alpha = 1.8 \times 10^{-5}$), while an increase in storage temperature to 20°C did not affect MeHg significantly ($\alpha = 0.3841$). The interaction between glucose consumed and storage temperature was close to significant ($\alpha = 0.0656$), indicating that temperature may indirectly have affected MeHg through influencing the amount of glucose consumed. This model explained 33 % of the variation in MeHg (interpreted from $R^2 = 0.33$). Thus, the first model with $Eh_{(1)}$ and storage temperature best explained the variations in MeHg concentrations.

4. Discussion

Increase in pH and Tot-P during storage

As neither storing temperature nor variations in nutrient addition seemed to directly explain the pH increase observed during storage, secondary effects of these variables presumably caused this increase. At pH levels as those measured in our experiment (< 9), the dominant dissolved carbonate species is bicarbonate (HCO_3^-) (Zeebe, 1999). Proton consumption during reduction processes can cause pH to rise (Yu et al., 2007), and Grybos et al. (2009) reported that a decline in Eh was accompanied by an increase in pH from 5.5 – 7.4. Also, Strawn et al. (2015) found that prolonged reduced conditions could increase the pH in soils, because of volatilization of carbon dioxide (CO_2) and conversion of organic acids to methane (CH_4). As methanogenic bacteria use molecular hydrogen (H_2) and CO_2 to produce CH_4 (Daniels et al., 1987, Økland and Økland, 2006), the pH of the system can rise due to removal of CO_2. Thus, reducing conditions at the sediment-water interface (shown by $Eh_{(-1)}$ and $Eh_{(1)}$) likely caused the pH increase during storage. As release of P also is significantly influenced by Eh of the upper sediment layers (Miao et al., 2006), the much higher Tot-P concentrations in the stored samples compared to the prestored, original water could possibly have been an effect of increased mobilization of P under low Eh conditions (Nowlin et al., 2005). The Eh measured in our experiment indicated favorable conditions for Fe^{3+} reduction (Wetzel, 1975), and as production of Fe^{2+} can release PO_4^{3-} anions chemically associated to Fe^{3+} (Baldwin and Mitchell, 2000), the concentration of dissolved P can increase. Our Eh and SO_4^{2-} concentrations also suggested favorable conditions for formation of H_2S, which in turn can remove Fe by formation of iron sulfide (FeS) and cause a higher release of P to the water (Økland and Økland, 2006).

Nutrient consumption and bacterial activity

Because glucose is an easy accessible carbon source for the bacteria (Shukla and Pandey, 1993) it was assumed that the increasing glucose consumption was a result of increased bacterial activity, by supplying the bacteria with both energy and protons. While the glucose added to our samples probably had been utilized for bacterial metabolism and growth during the storage period, the fate of the NH_4^+ added was more complicated, as many more chemical and biological processes might be involved. N can be transformed by denitrification, nitrification and ammonification (Kuenen and Robertson, 1988). Thus, the relatively high net NO_3^--N concentration in some of our samples could indicate the presence of nitrifying bacteria, which oxidized NH_4^+ to NO_3^-. Some of the NH_4^+ added could also have been used as an N source by SRB. The NH_4^+ ion is the primary source of nitrogen for SRB (Hao et al.,

1996), and has been found to be closely related to breeding of SRB (Wang et al., 2013). Some strains of the bacteria *Desulfovibrio* can reduce NO_3^- to NH_4^+, and some SRB can use NO_3^- and nitrite (NO_2^-) instead of SO_4^{2-} as a terminal electron acceptor in the support of growth (Barton and Tomei, 1995). As NO_3^- respiration gives more energy than SO_4^{2-} respiration, NO_3^- is the preferred electron acceptor in the absence of oxygen (O_2) (DeBusk et al., 2001).

Consumption of both nutrients and a higher storage temperature showed to significantly lower $Eh_{(1)}$, presumably by increasing the bacterial activity and thereby the oxygen consumption. Only glucose consumption and storage temperature significantly lowered $Eh_{(-1)}$. This may indicate that the strongly reducing conditions in the sediment not were suitable for nitrifying bacteria, consistent with nitrification taking place under oxic conditions (Nishio et al., 1983). Both $Eh_{(-1)}$ and $Eh_{(1)}$ were within Eh limits defined for SRB (+115 mV – -450 mV) in Becking et al. (1960). The availability of SO_4^{2-} (electron acceptor) and highly bioavailable OC (electron donor) are two major factors for SRB population growth (Lambertsson and Nilsson, 2006). Thus, the significant reduction in SO_4^{2-} by increasing consumption of glucose in our study could be an effect of increased SRB activity. Jong and Parry (2003) stated that an increased activity of SRB will cause a higher SO_4^{2-} reduction rate resulting in gradually increasing S^{2-} concentrations, which is consistent with our significant increase in $S^{2-}_{(-1)}$ with decreasing SO_4^{2-} concentrations in the water. However, the relatively low increase in S^{2-} concentrations might also be an effect of H_2S and HS^- formation due to the high pH in our samples (< 9), or because SRB preferred NO_3^- as a terminal electron acceptor over SO_4^{2-}.

TotHg in water after storage

The significantly higher TotHg concentrations in the overlying water in samples stored at 4°C compared to 20°C indicated that storage temperature had a significant effect on mobilization of Hg from the sediment. Temperature has earlier been reported to influence the kinetics of Hg in bottom sediments (Boszke et al., 2003), by affecting other TotHg explanatory variables such as Eh (Scarlatos, 1996). Hg is a redox sensitive metal (Weiner, 2008), and Mason et al. (2006) and Jonge et al. (2012) suggested that Hg was removed to the sediment by co-precipitation or adsorption as the oxygen concentration decreases, and subsequently remobilized under more oxic conditions. Accordingly, we found significantly lower $Eh_{(-1)}$ at 20°C compared to 4°C, and a significant increase in TotHg with increasing $Eh_{(-1)}$. This indicated that the generally higher Eh conditions observed in our samples at 4°C might be the reason for the higher TotHg mobilization from sediment to the water, in the more oxic environments present at 4°C compared to 20°C.

Tot-P concentrations have earlier been found to be the strongest explanatory variable for TotHg (Braaten et al., 2014a), because of the strong inter-correlation between Tot-P and TOC with a subsequent strong link between Hg and DOM. The significant increase in TotHg caused by Tot-P in our experiment might support this theory as Tot-P significantly increased by increased addition of glucose, despite glucose being far more bioavailable than natural organic matter in natural waters (Figueroa et al., 2016, Lane et al., 2013), which primarily consists of well decomposed allochthonous matter (Meili, 1992). Also, an increased Tot-P concentration *per se* is known to increase the bacterial activity (Roos-Barraclough et al., 2002, Aldén et al., 2001), and bacterial uptake of Hg is an acknowledged process (Mason et al., 1996, Benoit et al., 1999, Benoit et al., 2003, Golding et al., 2002, Kelly et al., 2003). As our TotHg analysis also included bacteria-assimilated Hg, increasing Tot-P concentrations with subsequent increase in bacterial activity might explain a part of the increase in TotHg concentrations.

Area of the sediment-water interface and sediment resuspension seem to be important for mobilization of Hg from the sediment to the overlying water. In our study, four bottles were unintendedly stored horizontally during the four month storing period. Consequently, the TotHg concentrations in the water column were higher than in the vertically stored bottles. Sample ID 37 had a TotHg concentration that was 61.2 % higher than the mean concentration of the two other bottles in the same triplicate, and sample ID 54-56 had a mean TotHg concentration that was 485.2 % higher than the mean concentration in rest of the samples stored at the same temperature. This is not unexpected, as we have a defined water volume in the bottles and an area-dependent mobilization rate of Hg through the sediment surface. Another factor for this increase might be increased resuspension when these bottles tipped over. Latimer et al. (1999), Calvo et al. (1991) and Petersen et al. (1997) stated that resuspension could play a major role in the mobility and bioavailability of trace metals and other contaminants. Particulate associated metals as Hg reside primarily in the particulate phase in anoxic sediments, and are largely associated with organic matter and metal sulfides. A resuspension of the sediment can release metals from the particulate phase, and increase the metal bioavailability due to oxidation processes (Cantwell et al., 2002).

MeHg in water after storage

The results presented in our study are based upon alterations of strictly physico-chemical properties as storage temperature, nutrient consumption, Eh, S^{2-}- and SO_4^{2-} concentrations, and their effects on TotHg and MeHg. Despite no qualitative or quantitative bacteriological measurements, we assume that our responses in MeHg concentrations are bacteriologically motivated, as MeHg is primarily a by-product of a metabolic pathway within SRB (Choi et al., 1994).

As in our experiment, increased methylation rates with decreasing Eh have been found in many studies (e.g., (DeLaune et al., 2004, Compeau and Bartha, 1984)). Mason et al. (2006) suggested that Eh of the sediment surface was the most important factor for Hg methylation in the sediment and subsequent transport of MeHg to the water column, which is consistent with the significant negative correlation between MeHg and $Eh_{(1)}$ in our study. Based on the significant correlations between MeHg and both $Eh_{(-1)}$ and $Eh_{(1)}$, we assume suitable conditions between sediment surface and overlaying water for MeHg formation (Bjerregaard, 2005). Although no direct significant relationship was found between MeHg and storage temperature, we suggest that temperature indirectly affected formation of MeHg, as temperature had a significant effect on both $Eh_{(-1)}$ and $Eh_{(1)}$. Processes of methylation are reported to be temperature dependent, and mainly related to increased activity of micro-organisms by increasing temperature (King et al., 1999). This is consistent with our results, where nutrient consumption and storage temperature (20°C) proved to significantly lower Eh, probably due to increased bacterial activity. Korthals and Winfrey (1987) found that temperature only accounted for about 30 % of the variation in MeHg formation, and said that other factors like nutrient loading and oxygen conditions also influenced Hg methylation rates. This is in harmony with our study showing that Eh, $S^{2-}{}_{(-1)}$ and glucose consumption better explained the variations in MeHg than storage temperature.

Enhanced bacterial MeHg formation due to glucose additions have earlier been documented in many studies (e.g., (Larsson, 2005, Hines et al., 2012)). Supply of organic compounds stimulates Hg methylation (Kim et al., 2006, Wright and Hamilton, 1982), through increased metabolism of heterotrophic organisms (Gilmour et al., 1992). As in our study, Wright and Hamilton (1982) found that MeHg production and release from sediments increased with increasing nutrient levels, and

Shukla and Pandey (1993) found significantly enhanced MeHg formation in the presence of carbohydrates (glucose and starch). In our study, only the highest additions of glucose (50.00 mg L^{-1}) resulted in considerable MeHg formation. This indicated that the other glucose additions were too low to significantly stimulate bacterial MeHg formation. In contrast to the above mentioned glucose studies, Mitchell et al. (2008) and Callister and Winfrey (1986) reported no effect of glucose on MeHg formation. These studies were however conducted in freshwater, and by adding SO_4^{2-} in combination with glucose, Mitchell et al. (2008) found considerably higher MeHg production. As MeHg did not correlate significantly with NH_4^+ consumption in our study, we assume that increased availability of N did not directly stimulated MeHg formation. The consumption of NH_4^+ may still have had an indirect effect, by affecting $Eh_{(1)}$ and thereby improving conditions for SRB and Hg methylation. If the concentration of nitrate (NO_3^-) or other electron acceptors (such as oxidized manganese (Mn^{4+}) and Fe^{3+}) are elevated, the activity and Hg methylation rates in SRB can be reduced, because these compounds are favored over SO_4^{2-} during microbial respiration of organic matter (Todorova et al., 2009). This can explain that many samples had low MeHg concentrations, if SRB used NO_3^- instead of SO_4^{2-} as a terminal electron acceptor. Samples with elevated MeHg concentrations should in this case have a low or negative value of net NO_3^--N, since it is suggested that SO_4^{2-} reduction not takes place if the system is well supplied with NO_3^- (McGill, 2007). It was however no significant correlation between MeHg and net NO_3^--N, and although the samples with the highest MeHg concentrations had negative net NO_3^--N concentrations, this was also the case for several samples which not exhibited elevated MeHg concentrations.

The strong, significant negative correlation between MeHg and SO_4^{2-} found in our experiment indicated that SRB were the main methylators. This is consistent with Watras et al. (1995), who reported maximum methylation rates in areas with maximum SO_4^{2-} reduction. However, Gilmour and Henry (1991) proposed an optimal SO_4^{2-} concentration range of 0.2 to 0.5 mM (19.2 – 48.0 mg L^{-1}) for Hg methylation by SRB in sediments, which is lower than SO_4^{2-} concentrations in our experiment. Above this concentration methylation may be limited, due to inhibition of SRB by pore water S^{2-} (Gilmour et al., 1992), or decreased availability of Hg to SRB in S^{2-} rich pore water (Benoit et al., 1999). As many of our samples exhibited low MeHg concentrations, SO_4^{2-} inhibition was considered as a possible explanation. This was however not likely the main reason, since all S^{2-} concentrations were lower than what is believed to be limiting for MeHg production (Craig and Moreton, 1986, Compeau and Bartha, 1987), and because the non-significant correlations between TotHg and SO_4^{2-} in water/ $S^{2-}_{(-1)}$ suggested that Hg was not significantly removed to the sediment by formation of HgS. The strong positive significant correlation between MeHg and $S^{2-}_{(-1)}$ in our study substantiated the assumption of SRB being responsible for Hg methylation, as S^{2-} is a product of SO_4^{2-} reduction by SRB (Elliott et al., 1998, Ullrich et al., 2001). The non-significant correlation between MeHg and $S^{2-}_{(1)}$ indicated that methylation mainly took place in the sediment, and that elevated MeHg concentrations in some samples was a result of MeHg diffusion from the sediment to the overlying water.

5. Conclusion

The results showed that release of Hg from sediment to water and transformation of Hg into MeHg are complex processes, involving several physico-chemical and biological parameters and important interactions. Storage temperature and Eh 1 cm below the sediment surface ($Eh_{(-1)}$) appeared to be important explanatory variables for mobilization of TotHg from the sediment to the overlaying water. Still, the direct effect of temperature on mobilization of Hg from sediments to the water column are poorly understood, and deserves more attention. Eh can be significantly lowered by increased inputs of

glucose and NH_4^+, to Eh levels favorable for Hg methylation by SRB. Accordingly, Eh 1 cm above the sediment surface ($Eh_{(1)}$) explained much of the variations in MeHg in water. The concentration of TotHg seem to be less important for the formation of MeHg, as no significant correlation was found between TotHg and MeHg in water. Although physico-chemical conditions suitable for MeHg formation by SRB was present in many of our samples, most of them exhibited low MeHg concentrations. SO_4^{2-} inhibition at high SO_4^{2-} concentrations and the use of NO_3^- as a terminal electron acceptor by SRB has been discussed, but lack of MeHg response in many of the samples might also simply be due to cultivation problems with SRB under our "semi-natural" conditions. In samples where cultivation of SRB was successful, we documented significant physico-chemical effects with subsequent importance for bacterial MeHg formation. Thus, as MeHg formation depends on several factors and interactions between them, more Hg studies should be performed under more controlled "semi-natural" conditions, where it is possible. This in order to simplify the very complex systems involved in Hg methylation in natural aquatic systems, and thereby reveal some more information about the sever toxicant MeHg.

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Appendix

Appendix 1: Weight of bottles, water and sediment

Appendix 2: Measurements in each bottle after storage

Appendix 3: Statistical tests and results

Appendix 4: Dried sediment samples analyzed for TotHg

Appendix 1

Weight of bottles, water and sediment

SAMPLE ID	Weight of empty bottle with cap (g)	Weight of added sediment (g)	Weight of bottle + sediment (g)	Weight of added water (g)	Total weight (bottle + sediment + water) (g)
1	185	269	454	1567	2021
2	184	286	470	1590	2060
3	183	264	447	1559	2006
4	185	291	476	1552	2028
5	185	276	461	1582	2043
6	186	283	469	1538	2007
7	185	283	468	1618	2086
8	184	282	466	1531	1997
9	185	282	467	1540	2007
10	185	293	478	1511	1989
11	185	291	476	1577	2053
12	184	296	480	1490	1970
13	184	282	466	1606	2072
14	184	284	468	1485	1953
15	185	280	465	1536	2001
16	183	269	452	1561	2013
17	184	283	467	1545	2012
18	183	280	463	1574	2037
19	185	278	463	1578	2041
20	184	276	460	1505	1965
21	183	263	446	1529	1975
22	184	256	440	1566	2006
23	184	287	471	1527	1998
24	184	276	460	1555	2015
25	184	291	475	1556	2031
26	185	276	461	1588	2049
27	183	274	457	1602	2059
28	184	290	474	1573	2047
29	184	280	464	1615	2079
30	185	274	459	1572	2031
31	184	281	465	1613	2078
32	185	268	453	1586	2039
33	183	266	449	1556	2005
34	185	260	445	1763	2208
35	185	269	454	1771	2225
36	184	282	466	1754	2220
37	183	252	435	1786	2221
38	183	265	448	1779	2227
39	183	255	438	1785	2223
40	184	265	449	1784	2233
41	184	261	445	1775	2220
42	184	279	463	1759	2222
43	185	281	466	1752	2218
44	185	278	463	1763	2226
45	185	254	439	1812	2251
46	184	277	461	1766	2227
47	185	237	422	1797	2219
48	185	280	465	1757	2222
49	185	275	460	1740	2200
50	185	275	460	1747	2207
51	185	284	469	1750	2219
52	186	272	458	1739	2197
53	185	271	456	1777	2233
54	183	275	458	1744	2202
55	186	282	468	1759	2227
56	184	284	468	1755	2223

Appendix 2

Measurements in each bottle after storage: basis for statistical analysis

SAMPLE ID	TotHg (ng L^{-1})	MeHg (ng L^{-1})	pH (-log[H^+])	Conductivity (mS cm^{-1})	Tot-P (µg L^{-1})	Tot-N* (µg L^{-1})	TOC (mg L^{-1})	SO_4^{2-} (mg L^{-1})
1	38.3	0.02	9.5	2.2	59.3	452.0	3.8	142.0
2	65.5	0.02	9.6	2.3	63.2	421.7	3.4	147.2
3	40.0	0.02	9.5	2.3	52.8	239.4	3.4	136.2
4	9.6	0.01 (<DL)	9.3	2.4	64.2	342.0	4.8	83.7
5	11.0	0.01 (<DL)	9.4	2.4	59.6	254.4	4.1	138.7
6	11.4	0.01 (<DL)	9.4	2.4	59.7	291.2	4.1	140.7
7	52.6	0.01 (<DL)	9.6	2.3	66.2	356.6	3.5	134.0
8	34.0	0.01 (<DL)	9.6	2.3	58.5	353.9	3.4	137.9
9	74.8	0.01 (<DL)	9.6	2.3	50.5	305.4	3.3	133.7
10	35.8	0.01 (<DL)	9.6	2.3	61.3	962.2	3.1	113.7
11	64.6	0.01 (<DL)	9.6	2.3	55.4	706.6	3.2	111.7
12	80.2	0.01 (<DL)	9.6	2.3	56.1	640.4	3.2	111.8
13	24.8	0.01 (<DL)	9.3	2.3	58.0	1677.8	3.4	104.3
14	39.6	0.01 (<DL)	9.5	2.3	63.4	1385.0	3.0	114.6
15	40.1	0.01 (<DL)	9.5	2.3	63.2	1450.6	3.0	111.3
16	16.0	0.03	9.6	2.3	58.3	452.2	3.3	117.3
17	1.9	0.02	9.4	2.3	40.5	439.5	3.8	98.7
18	7.3	0.03	9.5	2.3	45.4	635.5	3.3	101.7
19	15.2	0.07	9.3	2.3	25.3	380.1	4.0	93.8
20	23.3	0.04	9.7	2.4	74.5	360.3	4.2	121.8
21	6.6	0.05	9.3	2.2	27.5	545.3	4.0	94.2
22	18.0	0.02	9.8	2.3	95.4	289.6	5.1	108.1
23	35.2	0.03	9.8	2.4	107.1	215.7	4.5	112.1
24	22.0	0.03	9.8	2.4	104.4	801.3	5.3	109.1
25	63.7	2.20	9.8	2.4	117.7	719.9	7.0	81.3
26	68.5	0.88	9.8	2.4	94.2	565.7	6.7	78.7
27	18.2	0.01 (<DL)	9.7	2.3	77.3	249.9	3.8	133.7
28	12.3	0.01 (<DL)	9.4	2.3	49.3	484.4	3.3	103.3
29	3.3	0.01 (<DL)	9.4	2.2	43.8	430.0	3.1	98.8
30	25.1	0.03	9.6	2.3	69.2	404.9	4.1	113.6
31	27.0	0.02	9.3	2.3	51.7	1166.1	3.8	99.3
32	8.6	0.02	9.0	2.2	27.7	1089.0	3.9	92.3
33	17.1	0.12	8.3	2.3	43.7	1939.9	4.2	89.2
34	21.2	0.23	9.3	2.2	28.8	1585.9	4.4	81.9
35	25.6	0.15	9.5	2.2	41.5	809.0	4.4	82.9
36	68.7	0.84	9.0	2.2	61.6	1625.6	5.5	71.9
37**	110.3	2.15	9.8	2.2	106.8	2283.1	6.5	74.0
38	67.5	8.60	9.4	2.2	91.6	1635.6	6.2	73.0
39	15.1	0.27	9.5	2.2	70.5	101.5	4.1	119.7
40	10.0	0.04	9.5	2.2	73.6	45.6	4.0	123.8
41	11.2	0.31	9.5	2.2	64.2	118.6	3.9	114.8
42	3.8	0.07	9.5	2.3	70.5	61.2	4.0	125.0
43	14.7	0.02	9.4	2.3	70.8	113.7	3.8	123.9
44	6.0	0.01 (<DL)	9.4	2.3	72.9	263.2	4.0	124.0
45	9.2	0.01 (<DL)	9.5	2.2	67.6	104.9	4.1	119.3
46	7.3	0.03	9.5	2.3	53.2	118.0	4.3	120.5
47	3.6	0.04	9.4	2.2	61.7	124.6	4.6	129.7
48	3.3	0.02	9.5	2.2	45.0	384.5	5.1	98.7
49	5.1	0.02	9.5	2.2	50.0	351.5	5.3	98.4
50	8.0	0.03	9.5	2.3	44.3	580.1	4.4	92.0
51	9.8	0.02	9.4	2.3	48.2	133.8	4.1	126.7
52	9.2	0.01 (<DL)	9.5	2.2	44.0	146.4	3.4	110.7
53	15.9	0.01 (<DL)	9.5	2.3	47.9	114.0	4.2	119.1
54**	49.5	0.04	9.5	2.3	42.3	285.9	5.5	116.2
55**	60.0	0.04	9.6	2.3	36.5	103.3	7.1	101.0
56**	45.1	0.05	9.5	2.3	37.5	237.2	5.7	101.1

* All measurements discarded

** Tipped over during storage, excluded in statistical analysis

SAMPLE ID	$S^{2-}_{(12)}$ ($\mu g\ L^{-1}$)	$S^{2-}_{(1)}$ ($\mu g\ L^{-1}$)	$S^{2-}_{(-1)}$ ($\mu g\ L^{-1}$)	$Eh_{(12)}$ (mV)	$Eh_{(1)}$ (mV)	$Eh_{(-1)}$ (mV)
1	0.1	0.1	0.1	294	38	-201
2	0.1	0.1	0.1	282	-13	-228
3	0.1	0.1	0.1	262	43	-200
4	0.1	0.1	0.1	116	17	-274
5	0.1	0.1	0.1	133	11	-351
6	0.1	0.1	0.1	142	7	-254
7	0.1	0.1	0.1	246	83	-220
8	0.1	0.1	0.1	234	7	-207
9	0.1	0.1	0.1	230	33	-250
10	0.1	0.1	0.1	226	5	-226
11	0.1	0.1	0.1	221	10	-258
12	0.1	0.1	0.1	216	51	-311
13	0.1	0.1	0.1	216	78	-229
14	0.1	0.1	0.1	211	17	-240
15	0.1	0.1	0.1	200	7	-231
16	0.1	0.1	0.1	196	83	-248
17	0.1	0.1	0.1	194	38	-397
18	0.1	0.1	0.1	191	12	-320
19	0.1	0.1	0.9	183	54	-320
20	0.1	0.1	0.1	177	-58	-278
21	0.1	0.1	0.7	178	-39	-321
22	0.1	0.1	0.1	185	-20	-247
23	0.1	0.1	0.1	179	64	-304
24	0.1	0.1	0.1	170	-10	-290
25	11.0	0.1	6.0	-151	-60	-337
26	43.0	0.1	9.5	-269	-103	-391
27	2.2	0.1	0.1	-60	-57	-268
28	0.8	0.1	0.1	37	-25	-248
29	0.1	0.1	0.2	83	-40	-229
30	0.1	0.1	0.1	87	-31	-255
31	0.1	0.1	1.2	89	-24	-292
32	0.1	0.1	0.4	96	-58	-289
33	0.1	0.1	0.9	110	-48	-294
34	0.1	0.1	5.0	101	-88	-281
35	0.1	0.2	1.5	91	-83	-306
36	3.0	3.0	40.0	-312	-224	-346
37**	4.3	7.0	16.0	-369	-173	-352
38	40.0	8.0	16.0	-347	-305	-381
39	0.1	0.1	1.7	153	5	-384
40	0.1	0.1	2.7	148	1	-395
41	0.1	0.1	2.7	151	-20	-335
42	0.1	0.9	0.9	154	-55	-394
43	0.1	0.2	2.7	153	-33	-374
44	0.1	0.2	5.0	156	-36	-383
45	0.1	0.3	5.9	158	3	-402
46	0.1	0.3	7.0	165	-43	-391
47	0.1	0.5	4.9	166	-27	-338
48	0.1	0.1	0.5	163	52	-399
49	0.1	0.1	0.1	156	-100	-361
50	0.1	0.1	5.0	158	-68	-406
51	0.1	0.2	0.2	156	-45	-386
52	0.1	0.1	2.7	155	-43	-407
53	0.1	1.4	1.5	156	-20	-401
54**	0.1	0.2	6.5	152	-70	-396
55**	0.1	0.4	12.0	146	-133	-405
56**	0.1	1.3	10.0	143	-116	-406

** Tipped over during storage, excluded in statistical analysis

SAMPLE ID	NO_3^--N (µg L^{-1})	NH_4^+-N (µg L^{-1})	Glucose consumed (mg L^{-1})	NH_4^+ consumed (mg L^{-1})	Net NO_3^--N (mg L^{-1})
1	314.1	48.0	-1.0	0.0	-0.2
2	327.8	45.0	-0.6	0.0	-0.1
3	24.2	28.0	-0.6	0.0	-0.4
4	535.4	35.5	-2.0	0.0	0.1
5	273.2	27.0	-1.3	0.0	-0.2
6	860.4	37.0	-1.3	0.0	0.4
7	868.6	27.5	-0.7	0.2	0.4
8	25.9	25.0	-0.6	0.2	-0.4
9	93.4	23.0	-0.5	0.2	-0.4
10	453.2	27.5	-0.3	1.2	0.0
11	262.2	32.0	-0.4	1.2	-0.2
12	447.4	26.5	-0.4	1.2	0.0
13	952.0	32.0	-0.5	2.5	0.5
14	1208.5	26.5	-0.2	2.5	0.7
15	1141.2	42.0	-0.2	2.5	0.7
16	99.0	42.5	2.0	0.0	-0.4
17	403.1	25.0	1.5	0.0	-0.1
18	202.4	38.0	2.0	0.0	-0.3
19	170.7	43.5	11.3	0.0	-0.3
20	65.8	64.0	11.1	-0.1	-0.4
21	199.2	63.0	11.3	-0.1	-0.3
22	851.7	55.0	22.8	0.0	0.4
23	897.7	33.5	23.3	0.0	0.4
24	346.4	74.5	22.5	-0.1	-0.1
25	885.7	183.5	45.8	-0.2	0.4
26	867.3	290.5	46.1	-0.3	0.4
27	78.6	29.0	1.5	0.2	-0.4
28	362.8	39.5	2.0	0.2	-0.1
29	71.2	31.0	2.2	0.2	-0.4
30	147.0	29.5	11.2	1.2	-0.3
31	232.4	31.0	11.5	1.2	-0.2
32	530.4	22.5	11.4	1.2	0.1
33	15.9	51.5	23.6	2.5	-0.5
34	230.1	1550.0	23.4	1.0	-0.2
35	346.1	975.0	23.4	1.5	-0.1
36	925.0	2525.0	47.3	2.5	0.5
37**	896.3	922.5	46.3	4.1	0.4
38	913.3	2247.5	46.6	2.8	0.6
39	306.3	30.0	-1.3	0.2	-0.2
40	316.7	35.0	-1.2	0.2	-0.2
41	334.5	19.0	-1.0	0.2	-0.1
42	881.1	16.0	-1.2	2.5	0.4
43	349.7	52.5	-1.0	2.5	-0.1
44	328.0	14.0	-1.2	2.5	-0.1
45	312.9	13.5	1.2	0.0	-0.2
46	331.3	25.5	1.0	0.0	-0.1
47	830.5	20.5	0.7	0.0	0.4
48	337.9	12.5	22.7	0.0	-0.1
49	1092.6	23.0	22.5	0.0	0.6
50	18.3	20.0	23.4	0.0	-0.5
51	9.2	16.0	1.2	0.2	-0.5
52	312.7	23.5	1.9	0.2	-0.2
53	325.0	29.0	1.1	0.2	-0.1
54**	854.5	21.5	22.3	2.5	0.4
55**	929.7	10.0	20.7	2.5	0.5
56**	967.2	10.0	22.1	2.5	0.5

** Tipped over during storage, excluded in statistical analysis

Appendix 3

Statistical tests and results

Datasets	Statistical test	p-value	Multiple R^2	Pearson's r/ Spearman's rho
Nutrient consumption, Eh, SO_4^{2-} and S^{2-}				
$Eh_{(-1)}$ vs. storage temperature (categorical) + glucose consumed + NH_4^+ consumed	Multiple regression	1.8×10^{-10}	0.6378	
Significance of each variable (α) and VIF-levels:	Storage temperature 20°C ($\alpha = 1.8 \times 10^{-11}$, VIF = 1.1), glucose consumed ($\alpha = 5.3 \times 10^{-6}$, VIF = 1.1), NH_4^+ consumed ($\alpha = 0.6540$, VIF = 1.0)			
$Eh_{(1)}$ vs. storage temperature (categorical) + glucose consumed + NH_4^+ consumed	Multiple regression	8.8×10^{-9}	0.5655	
Significance of each variable (α) and VIF-levels:	Storage temperature 20°C ($\alpha = 0.0152$, VIF = 1.1), glucose consumed ($\alpha = 6.9 \times 10^{-9}$, VIF = 1.1), NH_4^+ consumed ($\alpha =$ 0.0023, VIF = 1.0)			
Glucose consumed vs. additions of glucose	Spearman correlation	$< 2.2 \times 10^{-16}$		0.9566
NH_4^+ consumed vs. additions of NH_4^+	Spearman correlation	$< 2.2 \times 10^{-16}$		0.9409
NH_4^+ consumed vs. net NO_3^--N	Spearman correlation	0.2364		0.1671
SO_4^{2-} vs. glucose consumed + NH_4^+ consumed + storage temperature (categorical)	Multiple regression	7.3×10^{-9}	0.5688	
Significance of each variable (α) and VIF-levels:	Storage temperature 20°C ($\alpha = 0.8108$, VIF = 1.1), glucose consumed ($\alpha = 3.3 \times 10^{-9}$, VIF = 1.1), NH_4^+ consumed ($\alpha =$ 0.0957, VIF = 1.0)			
$S^{2-}_{(-1)}$ vs. SO_4^{2-} in water	Spearman correlation	0.0088		-0.3598
$S^{2-}_{(-1)}$ vs. $S^{2-}_{(1)}$	Spearman correlation	1.1×10^{-6}		0.6178
$S^{2-}_{(-1)}$ vs. $Eh_{(-1)}$	Spearman correlation	2.5×10^{-8}		-0.6823

Datasets	Statistical test	p-value	Multiple R^2	Pearson's r/ Spearman's rho
TotHg				
logTotHg vs. storage temperature (categorical)	Pairwise t-test	5.9×10^{-6}		
logTotHg vs. glucose consumed	Spearman correlation	0.4606		0.1045
logTotHg vs. NH_4^+ consumed	Spearman correlation	0.3709		0.1267
logTotHg vs. $Eh_{(-1)}$	Spearman correlation	0.0002		0.4936
logTotHg vs. Tot-P	Pearson correlation	0.0059		0.3763
logTotHg vs. pH	Spearman correlation	0.0024		0.4124
Tot-P vs. glucose added	Pearson correlation	0.0117		0.3473
logTotHg vs. SO_4^{2-} in water	Spearman correlation	0.8865		0.0203
logTotHg vs. $S^{2-}_{(-1)}$	Spearman correlation	0.1792		-0.1892
logTotHg vs. storage temperature (categorical) + Tot-P	Multiple regression	2.5×10^{-7}	0.4622	
Significance of each variable (α) and VIF-levels:	Storage temperature 20°C ($\alpha = 1.9 \times 10^{-6}$, VIF = 1.0), Tot-P ($\alpha$ = 0.0016, VIF = 1.0)			
MeHg				
MeHg vs. logTotHg	Spearman correlation	0.9205		0.0142
MeHg vs. storage temperature (categorical)	Pairwise t-test	0.3400		
MeHg vs. glucose consumed	Spearman correlation	0.0001		0.5023
MeHg vs. NH_4^+ consumed	Spearman correlation	0.2563		-0.1603
MeHg vs. additions of glucose (categorical)	Multiple regression	6.0×10^{-6}	0.4604	
Significance of each variable (α):	Glucose 2.50 mg L^{-1} (α = 0.9450) Glucose 12.50 mg L^{-1} (α = 0.9920) Glucose 25.00 mg L^{-1} (α = 0.9380) Glucose 50.00 mg L^{-1} ($\alpha = 2.4 \times 10^{-7}$)			

Datasets	Statistical test	p-value	Multiple R^2	Pearson's r/ Spearman's rho
MeHg vs. SO_4^{2-}	Spearman correlation	0.0019		-0.4200
MeHg vs. $S^{2-}_{(-1)}$	Spearman correlation	2.8×10^{-5}		0.5460
MeHg vs. $S^{2-}_{(1)}$	Spearman correlation	0.0978		0.2321
MeHg vs. $Eh_{(-1)}$	Spearman correlation	0.0119		-0.3463
MeHg vs. $Eh_{(1)}$	Spearman correlation	0.0006		-0.4581
MeHg vs. net NO_3^--N	Spearman correlation	0.9941		0.0011
MeHg vs. pH	Spearman correlation	0.6962		-0.0554
MeHg vs. Tot-P	Spearman correlation	0.4453		0.1082
MeHg vs. glucose consumed + glucose consumed*storage temperature (categorical)	Multiple regression	0.0002	0.3343	
Significance of each variable (α) and VIF-levels:	Glucose consumed ($\alpha = 1.8 \times 10^{-5}$, VIF = 1.3), storage temperature 20°C (α = 0.3841, VIF = 1.3), glucose consumed*storage temperature 20°C (α = 0.0656, VIF = 1.3)			
MeHg vs. $Eh_{(1)}$ + $Eh_{(1)}$*storage temperature (categorical)	Multiple regression	2.4×10^{-7}	0.4998	
Significance of each variable (α) and VIF-levels:	$Eh_{(1)}$ ($\alpha = 1.5 \times 10^{-8}$, VIF = 1.1), storage temperature 20°C (α = 0.7691, VIF = 1.3), $Eh_{(1)}$*storage temperature 20°C (α = 0.0407, VIF = 1.4)			

* = interaction term between variables

Appendix 4

Dried sediment samples analyzed for TotHg

SAMPLE ID	Weight of dried sediment analyzed (mg)	TotHg (mg kg^{-1} dw)	Average TotHg (mg kg^{-1} dw)
Ref. (before storage)	19.5	52.9	52.6
	20.0	53.2	
	20.5	51.7	
2	19.7	61.5	58.8
	20.5	55.9	
	20.6	59.0	
5	20.5	63.2	56.3
	19.5	49.5	
	19.8	56.1	
15	21.2	54.2	58.2
	20.8	57.8	
	21.0	62.5	
25	20.2	53.1	55.1
	19.7	56.3	
	20.0	55.9	
30	20.9	65.1	56.9
	20.7	62.8	
	21.8	63.6	
38	20.0	58.2	56.9
	20.0	56.9	
	19.6	55.7	
45	19.5	49.8	55.6
	20.3	58.2	
	20.4	59.0	
53	21.6	50.1	55.7
	21.1	60.1	
	19.9	57.0	