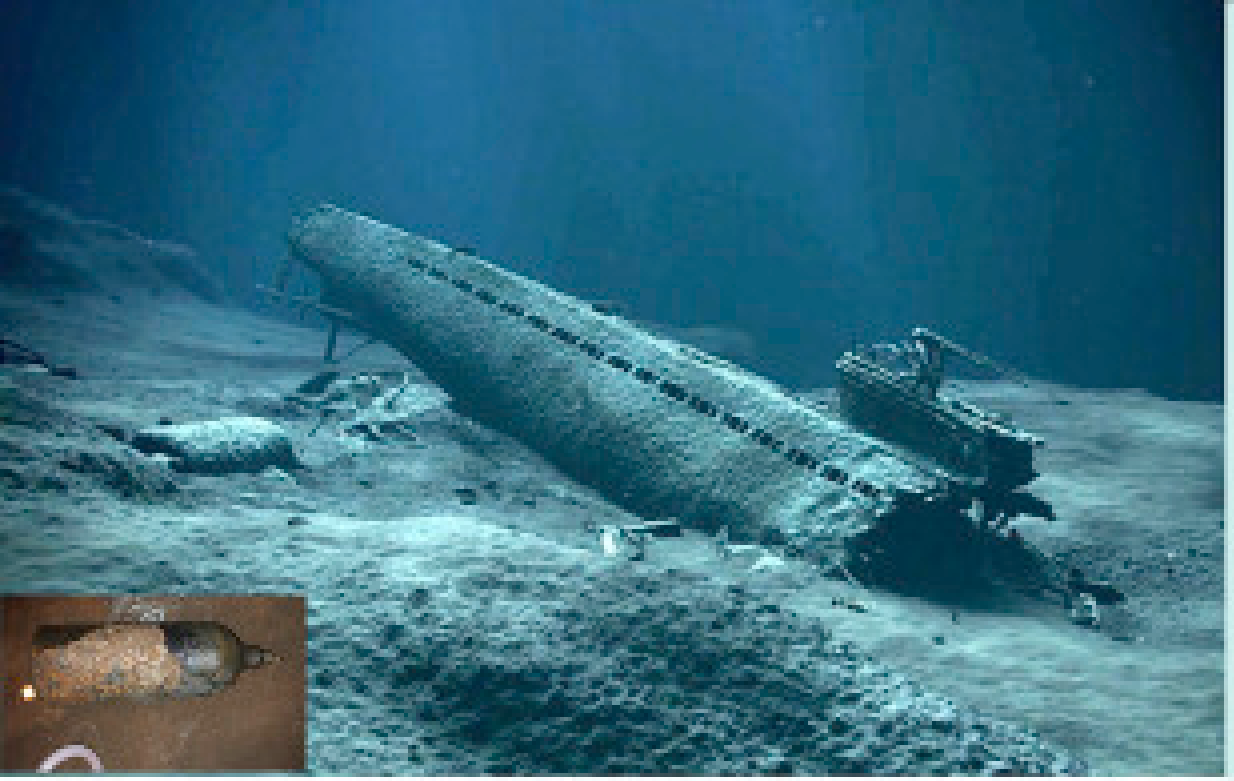

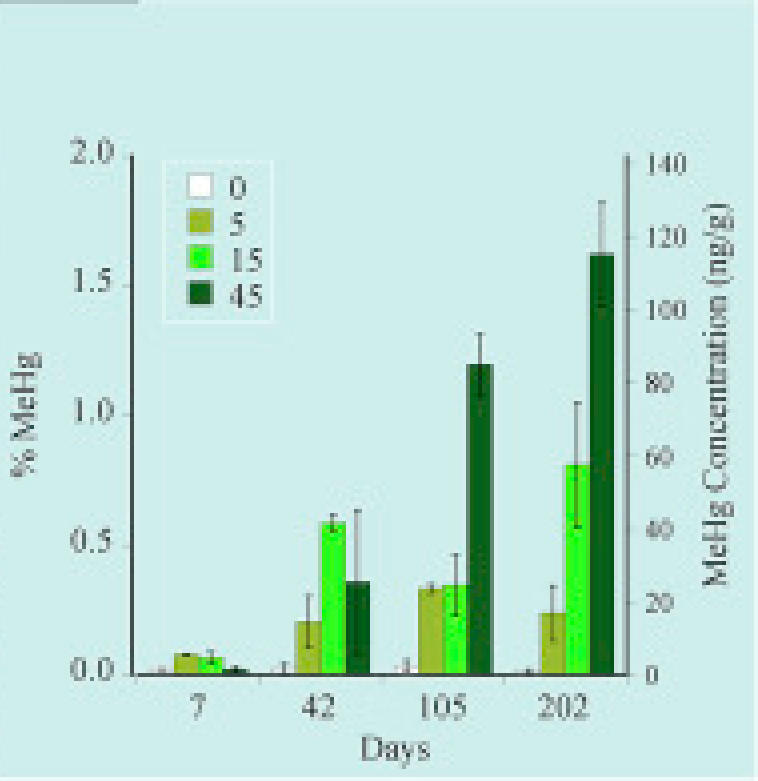
2.0
1.5
1.0
0.5
0.0
% MeHg
0
5
15
45
140
120
100
80
60
40
20
0
MeHg Concentration (ng/g)
7
42
105
202
Days

Effects of organic matter addition on methylmercury formation in capped and uncapped marine sediments

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ABSTRACT: In situ subaqueous capping (ISC) of contaminated marine sediments is frequently proposed as a feasible and effective mitigation option. However, though effective in isolating mercury species migration into overlying water, capping can also alter the location and extent of biogeochemical zones and potentially enhance methylmercury (MeHg) formation in Hg-contaminated marine sediments. We carried out a boxcosm study to investigate whether the addition of organic carbon (OC) to Hg-contaminated marine sediments beneath an in situ cap would initiate and/or enhance MeHg formation of the inorganic Hg present. The study was motivated by ongoing efforts to remediate ca. 30 000 m^2 of Hg-contaminated seabed sediments from a Hg spill from the U864 WWII submarine wreck. By the time of sinking, the submarine is assumed to have been holding a cargo of ca. 65 tons of liquid Hg. Natural organic matter and petroleum hydrocarbons from fuels and lubricants in the wreck are potential sources of organic carbon that could potentially fuel MeHg formation beneath a future cap. The results of our study clearly demonstrated that introduction of algae OC to Hg contaminated sediments, triggered high rates of MeHg production as long a there was sufficient OC. Thus, MeHg production was limited by the amount of organic carbon available. The study results also confirmed that, within the six-month duration of the study and in the absence of bioturbating fauna, a three-centimeter sediment clay cap could effectively reduce fluxes of Hg species to the overlying water and isolate the Hg-contaminated sediments from direct surficial deposition of organic matter that could potentially fuel methylaton.

Keywords: Mercury; methylation; U-864 WWII submarine; microcosm and boxcosm; Fedje, Norway

35 1. INTRODUCTION

36 Mercury (Hg) contamination in natural waters and sediments is a global problem. A
37 number of mitigation strategies have been applied to remediate both Hg-contaminated waters
38 (Herrero et al. 2005, Zhang et al. 2005) and sediments (Hosokawa 1993, Palermo 1998). For
39 remediation of Hg-contaminated sediments, in situ subaqueous capping (ISC) offers a
40 feasible and efficient management option (Palermo 1998). The use of an ISC involves
41 placing a layer of clean fill material at the sediment-water interface to prevent contaminant
42 release and contact with benthic macrofauna and overlying surface water. In situ subaqueous
43 caps have been successfully used to manage Hg-contaminated sediments in Minamata Japan
44 (Hosokawa 1993) and Hamilton harbor Canada (Azcue et al. 1998). The application of an
45 ISC can however, alter the location and extent of biogeochemical zones (Johnson et al. 2010,
46 Randall et al. 2013a) and potentially increase MeHg formation under sulfate reducing
47 conditions if organic carbon (OC) is available (Randall et al. 2013a). There are multiple
48 potential sources of OC to Hg-contaminated sediments including natural organic matter and
49 petroleum hydrocarbons (PH) from natural seeps and oil spill from sunken ship wrecks
50 (Monfils et al. 2006), leakage from underground storage tanks (Boopathy 2004); or
51 purposeful addition of emulsified vegetable oil for reductive bioremediation of aquifer
52 sediments contaminated with chlorinated organic compounds (Borden 2007). This study was
53 therefore carried out to investigate whether; i) introduction of OC to Hg-contaminated marine
54 sediments would affect Hg speciation and cycling, ii) how a combination of OC addition and
55 capping would affect Hg methylation dynamics.

56 Methylmercury is mainly produced in anoxic sediments and soils, primarily by dissimilatory
57 sulfate- and iron-reducing bacteria (DSRB and DFeRB) (Bravo et al. 2015, Gilmour et al.
58 2011) and to some extent methanogens (Gilmour et al. 2013). In addition to Hg and a suitable

terminal electron-accepting process (TEAP) anaerobic microbial MeHg formation usually requires the presence of an appropriate organic substrate i.e. an electron donor (Bravo et al. 2015, Johnson et al. 2010, Randall et al. 2013a).

The relationship between organic matter content in marine sediments and MeHg formation is however complex. A number of studies have reported both a positive (Graham et al. 2012, Mitchell and Gilmour 2008, Schartup et al. 2013) and negative (Driscoll et al. 2012, Hammerschmidt and Fitzgerald 2004, Schartup et al. 2013) correlation between sediment organic matter content and MeHg formation rates. The quality of the organic matter has also been suggested to control MeHg formation in marine sediments (Mitchell and Gilmour 2008). We are aware of only two studies (Johnson et al. 2010, Randall et al. 2013a) that have investigated how the application of an ISC affects the TEAP and MeHg formation in the underlying sediments (Johnson et al. 2010, Randall et al. 2013a) The mesocosm study by Randall et al. (2013a) used freshwater sediments where (unlike in marine systems), DSRB activity can be sulfate limited. The study by Johnson et al. (2010) did not investigate possible limitation of DSRB activity (and hence MeHg formation) by sediment organic matter content.

The aim of this study was therefore to investigate how introduction of allochthonous OC to Hg-contaminated sediments with low autochthonous OC content, would affect methylation of in situ Hg. We hypothesized that laying an ISC over such contaminated sediments would alter the MeHg formation-demethylation balance leading to an increase in MeHg concentration relative to controls without ISC. We designed our boxcosm study to reflect both the current in situ sediment conditions around the U-864 wreck site; and after possible application of an ISC over the sediments. In our boxcosm study, we amended the Hg-contaminated sediment (grainy sand with low native OC content) with varying doses of a

labile OC (chlorella algae; ca. 50 wt % carbon). We believe this is the first study to systematically investigate the effect of OC addition to Hg-contaminated marine sediments addition on; i) MeHg formation and ii) the effect of capping on Hg methylation dynamics beneath an ISC.

2. MATERIALS AND METHODS

2.1 Sampling of Hg-contaminated sediments from U864 site

Surface sediment samples for this study were collected in January 2013 from the seabed wreck site, which lies about two nautical miles (3.7 km) west of the Norwegian North Sea island of Fedje (Figure S1). Sediments were sampled using a Van Geen grab mounted on the arm of a Remotely operated vehicle controlled from the deck of the vessel *Skandi Skolten* docked above the U864 wreck site. The sediment was then transported to the shore lab and stored in the dark at a temperature of 5−10 °C until use. The Hg contaminated sediment area around the wrecks is estimated to be ca. 0.03 km^2, with sediment "hot spots" in the immediate vicinity of the wrecks and decreasing away from the wreck.

2.2 Sediment preparation

The sediments were carefully checked for any ammunition, as they were collected from a site in the proximity of a WWII submarine wreck (Kystverket 2014a, b). All large stones and pebbles were physically removed. The rest of the sediment (which consisted of gravelly sand) was homogenized and aliquoted into eight-6L portions in which 0, 5, 15 and 45 g of pulverized *Chlorella sp* green algae with a carbon content of ca. 47 wt %, was added (corresponding to approximately 0, 25, 75 or 225 $g{\cdot}C{\cdot}m^{-2}$ respectively). Each of the eight sediment portions (i.e. three with added algae and one control for capped and uncapped

106 respectively; Figure S2 and S3) was placed in a wooden tray and set in a freezer at -20 °C for
107 seven days forming frozen three centimeter sediment layers ("frozen sheets") with the same
108 surface area as the boxcosms. An extra four frozen layers were prepared in a similar way but
109 this time with fresh (uncontaminated) marine clay sediment to act as caps for the four capped
110 treatments. The marine clay sediment used for the cap was collected from a reference
111 location in the Outer Oslofjord, remote from any known point source of anthropogenic
112 discharges.

113 *2.3 Boxcosms set up*

114 The boxcosm setup used in this study is depicted in Figure 1 (and Figure S2 in the
115 supplementary information). The set up is a slight modification of the one described by
116 Josefsson et al. (2012) for their study of the efficiency of different capping materials for in
117 situ subaqueous capping of contaminated marine sediments. The eight boxcosms in this study
118 were placed in large concrete tank (L 2 m x W 0.8 m x H 0.6 m, Figure1 and Figure S2) with
119 flow-through seawater pumped from 60 m depth, maintaining a temperature of 8 −10 °C and
120 a salinity of ~34 throughout the six month experimental period. The water level in the tanks
121 was about 1 cm below the rim of the boxes. The same seawater was also pumped into a
122 header tank from which it was distributed to the four uncapped boxes at an average (± 1
123 standard deviation) flow rate of 0.94 ± 0.05 mL min^{-1}, corresponding to a turnover time of
124 ca. 6.4 days for the overlying water in the uncapped boxcosms. An air-diffusing system,
125 consisting of an airstone diffuser placed in a perforated Plexiglas tube in the center of each
126 box, was used for stirring and aerating the water.

127 Figure 1

128

Before adding the various layers, the lids were removed from the eight polycarbonate boxes previously filled to a depth of about 20 cm with fresh uncontaminated clay sediment (same sediment as used for the ISC) leaving about 15 cm of the box filled with seawater (Figure S2 and S3). Each of the eight frozen sheets was removed from the form and carefully sunk onto the sediment surface in each box. After thawing on top of the clean sediment surface, the added layers filled in the entire surface area leaving no open spaces in corners or along the walls of the boxes. The frozen cap clean sediment layer was then similarly placed on top of each of the four capped boxcosms while the other four were left uncapped.

2.4 Water and sediment core sampling

The set up with eight boxcosm treatments and circulating fjord water was allowed to equilibrate for seven days, after which the first water and sediment core samples were taken. Surface water for Hg and MeHg flux measurements was sampled by inserting acid-cleaned silicon tubing into the box under the top cover and siphoning off 250 mL into an acid-cleaned, Teflon-coated polyethylene bottle. The unfiltered water was then acidified to 50 mM HCl using trace metal grade (Merck, Suprapur) HCl. Surface water was only sampled from the four uncapped boxcosms. Sediment cores were extracted from the microcosms using a 5 cm diameter, 25 cm long hollow acrylic cylinder. A 10-12 cm core was then carefully extracted by pulling out the cylinder maintaining ca. 3-5 cm of the surficial sediment water layer. A rubber piston was then used to slowly push the core up the cylinder leaving ca. 3 cm of water covering the top of the sediment. The cylinder was clamped vertically next to a Micromanipulator for the sensor measurements (Figure S4).

153 *2.5 Microsensor profile measurements*

154 After sampling, the cylinder holding the sediment core was carefully moved to the measuring
155 station (Figure S4) for microsensor measurements. The measuring station comprised a single
156 axis automated micromanipulator, potentiostat and 100 or 200 micron tip sensors for O_2, pH
157 and H_2S (all from *Unisense*®, Figure S4). Profiles of dissolved O_2, H_2S and pH in sediment
158 pore water were obtained using respective microsensors at a vertical resolution ranging from
159 100 to 1000 μm. The oxygen microsensor was calibrated using a two-point calibration
160 consisting of fully aerated and N_2-purged seawater for 100% and zero O_2 saturation
161 respectively. The H_2S microsensor was calibrated with freshly prepared N_2-degassed Na_2S
162 standards made up in pH < 4 citrate buffer. The concentration of the stock Na_2S standard
163 solution was also calibrated using the classical Cline spectrophotometric method (Cline
164 1969). The pH microelectrode was calibrated with appropriate pH 4, 7 and 10 buffers. After
165 the sensor measurements the, bottom piston was slowly pushed to bring the core on level
166 with the cylinder top, thus draining off all the surficial water. One centimeter cores were then
167 sliced by pushing the piston upwards. Each sample was put in an acid clean polyethylene vial
168 and immediately put in a freezer at -20 °C until MeHg extraction and subsequent analysis.
169 Microsensor measurements data mainly for H_2S and pH was limited by the glass sensor tip
170 breakage by sand grains in sediment the cores.

171 *2.6 Chemical analysis*

172 *2.6.1 Total Hg in sediments:*

173 All MeHg and total Hg (THg) analysis (except sediment digestion and THg analysis by
174 pyrolysis) was conducted in a filtered air, positive pressure class 1000 trace metal clean lab.
175 The THg in sediments was analyzed using a Lumex RA-915 + Hg Analyzer coupled to a
176 PYRO-915+pyrolyzer (Lumex Ltd., St. Petersburg, Russia). The pyrolyzer temperature was

520-580°C. The instrument was calibrated using PACS-2, marine sediment certified reference material-CRM from the National Research Council-NRC of Canada (having a certified THg concentration of 3.04 ± 0.20 mg/kg). Another marine sediment CRM, MESS-3 (also from NRC) was used for quality control. Sediment samples were analyzed wet. The sediment water content was determined gravimetrically after heating in an oven at 105°C for 12 h. The sediment water content for the original Hg-contaminated sediment was 27 wt %. The total organic matter, estimated from loss on ignition (heating dried sediment in a muffle oven at 550 °C for 12 hr) was ca. 1 wt %. The THg concentration determined by the Lumex Pyro analyzer (mass ± standard deviation, n=3) was 8.9 ± 1.8 mg/kg (dry mass). Liquid Hg droplets have been observed in previous sediments from the site but No Hg droplets were observed in sediments used in this study (although the presence of Hg microdroplets cannot be ruled out). Concurrent measurement of MESS-3 estuarine CRM with a certified THg concentration (mass ± standard deviation) of 0.091 ± 0.009 mg/kg gave a concentration of 0.08 ± 0.014 mg/kg (n=3). The concentrations of Al, Fe, Mn in dried sediment digests (aqua regia digestion) were measured (in helium mode) on an Agilent 7700 quadrapole ICP-MS equipped with a collision cell.

2.6.2 Total Hg in water and MeHg in sediments

Methylmercury was extracted from sediments using a slight modification of one of the methods described in Bloom et al. (Bloom et al. 1997) for the extraction of MeHg from environmental samples. In short, the extraction method involved leaching a weighed (ca. 0.6 g) wet sediment sample with $HNO_3/CuSO_4$ and extraction into dichloromethane (CH_2Cl_2), followed by back extraction into water. The prepared sample was then analyzed for MeHg via distillation, ethylation, purge and trap pre-concentration, GC separation and Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS) detection (Brooks Rand®) according to USEPA method 1630. Each batch of 30 sediment extracts included; three blanks, two marine

sediment certified reference material (CRM) samples and one or two duplicates. The Sediment CRM used was ERM CC 580 from the European Commission`s Institute of Reference Materials and Measurements (MeHg recovery of 85 %).

Total (unfiltered) MeHg in water samples was similarly determined by CVAFS after Distillation, aqueous ethylation, purge and Trap, according to USEPA method 1630. Total Hg in water was determined after sample oxidation with $KBr/KBrO_3$, followed by purge and trap and CVAFS according to USEPA Method 245.7 (rev 2.2, 2005). The method detection limits (3 x standard deviation of blank concentration) were 0.1 ng/L for THg in water. For MeHg detection limits were 0.02 ng/L for water and ca. 0.1 µg/kg for wet sediment. Precision (as relative standard deviation (RSD) of parallel samples) was less than 10 % for both THg and MeHg methods. A summary of metal, organic matter and water concentrations in Hg-contaminated sediment used for the boxcosm experiment is shown in Table S1

3. RESULTS

3.1 Methylmercury formation in Sediments

Figure 2 summarizes the change in MeHg concentration measured in the Hg-contaminated sediment layers from capped and uncapped boxcosms. The MeHg fraction (as a % of THg) in the control sediment boxcosms for both capped and uncapped boxcosms without any added OC, at the start (day 7) was very low (0.02 ± 0.001 and remained so for the entire duration of the experiment (Figure 2). The initial (i.e. day 7) MeHg fraction for all the OC-treated sediments was similarly low, but progressively increased in subsequent months; implying negligible MeHg contribution from the added algae and confirming that methylation of in situ inorganic Hg was the main source of measured MeHg (Figure 2).

225 Figure 2

226

227

228

229 4.0 DISCUSSION

230 *4.1 Capping enhanced methylation and shifted the terminal electron accepting processes*
231 *boundaries*

232 In situ inorganic Hg was methylated at varying extent in all environments where the sediment
233 was both anoxic and had sufficient OC content. Figure 3 shows the sediment MeHg, H_2S and
234 O_2 concentration profiles in the high carbon capped boxcosm after 202 days. The MeHg
235 concentration in the Hg-contaminated layer was also higher in the capped treatments at
236 similar depth and OC concentration, compared to the uncapped treatments (data not shown).
237 In the only other study on MeHg formation in marine sediments under an ISC Johnson et al.
238 (Johnson et al. 2010) observed a 50% increase in MeHg beneath a sediment cap, 2-3 cm
239 higher than in an uncapped sediment, concomitant with an upward shift of anaerobic bacterial
240 activity beneath a sediment cap under anoxic conditions. Figure 3 shows the microsensor
241 depth profiles of O_2, H_2S and pH for the high dose capped and uncapped boxcosms. The ISC
242 ensured that the whole of the Hg-contaminated sediment layer was anoxic (Figure 2) and
243 potentially amenable to anaerobic microbial MeHg formation most likely by DSRB but also
244 by FeRB and/or other Hg methylating bacteria (Gilmour et al. 2013, Parks et al. 2013).
245 Hydrogen sulfide was detected at varying concentrations in all OC-amended capped
246 boxcosms but only in the high OC dose (45 g added algae) uncapped boxcosm. We also
247 observed dense microbial growth on the sediment surface of this high carbon uncapped

248 boxcosm (Figure S5) that lasted for one month. The high biological activity resulted into near
249 complete oxygen depletion at the water-sediment interface and H_2S production just below the
250 sediment surface.

251 Figure 3

252

253

254 *4.2 Capping organic carbon-rich sediments enhanced MeHg formation*

255 The MeHg formation rates for the entire duration of the experiment (denoted as k_{meth}) are
256 shown in Figure 4 and Table S2. The k_{meth} were determined from linear regression
257 analysis of sediment MeHg concentration measurements from the entire three-centimeter
258 Hg-contaminated layer. Thus, the added organic carbon comprised ca. 15 to 55 % of the
259 final total OC concentration in the boxcosms. As seen from the low k_{meth} values in Figure
260 4 and Table S2, the original OC had little effect in methylating the in situ Hg compared to
261 the added OC. This influence of k_{meth} on OM quality has been noted elsewhere for
262 sediments from Chesapeake Bay (Mitchell and Gilmour 2008). Schartup et al. (2013)
263 recently investigated the role of organic matter in sediment MeHg formation from nearly
264 a dozen US estuarine systems. They (Schartup et al. 2013) observed both positive and
265 negative correlation between k_{meth} and sediment organic matter content from different
266 estuarine systems and also attributed the lack of correlation between organic matter and
267 k_{meth} to OC quality. Overall, the capped Hg-contaminated sediments were nearly three
268 times more efficient in methylating the in situ inorganic Hg per gram of added organic
269 carbon, (0.006 and 0.002 % day^{-1} per % added sediment OC) compared to the uncapped
270 sediments (Figure 4). Schartup et al. (2013) recently reported rates of 0.2 % day^{-1} per %
271 LOI; $r^2 = 0.7$), 30 times higher, for seven of the eleven estuarine sediments where they

found a positive correlation between k_{meth} and sediment organic matter content. It is difficult to directly compare our OC dependence on k_{meth} data with their data because; i) Schartup et al. (2013)k_{meth} was determined via isotope spiking, which unlike ours, is a measure of Hg formation "potential" rather than the actual k_{meth} ii) the k_{meth} was based on % LOI (rather than OC) in which the OC content in LOI can range from 30-60 %. Nevertheless, Schartup et al. (2013) rate dependence on OC is within the same order of magnitude (assuming a 40% OC content for LOI). Lambertsson and Nilsson (2006) also reported a positive

Figure 4

linear correlation between estuarine (Bothnian Sea) sediment organic matter content and k_{meth}. (Lambertsson and Nilsson 2006). They reported MeHg formation rates of ca. 0.2 ngg-1day^{-1} per % LOI, similar to our study (0.5 and 0.2 ngg^{-1}day^{-1} per % OC for capped and uncapped respectively).

4.3 Capping was effective in preventing MeHg migration

Despite the enhanced Hg methylation underneath the cap (cf. the uncapped boxcosms), the three-centimeter ISC was effective in containing the methylated Hg beneath it. As Figure 3 and Figure S6 show, there was very little MeHg migration through the cap, even in the capped boxcosm with the highest organic matter (Figure S6). The MeHg concentration in the cap was low except at the cap-contaminated sediment interface, where we occasionally measured high MeHg concentration. These high MeHg concentrations were more likely due to; i) mixing of the Hg-contaminated sediment layer with the capping layer during slicing of the cores and ii) errors in locating the exact location of the interfacial region between the two layers, rather than MeHg migration into the cap layer (Figure S6). The higher methylation rate in the capped boxcosms compared to the uncapped might also be related to the thickness

297 of the contaminated layer. For example, assuming an 8 mm oxygen penetration depth into the
298 three-centimeter sediment layer (Figure 3), the fraction of the total Hg-contaminated
299 sediment area that was potentially amenable to Hg methylation (i.e. was anoxic and had
300 enough bacteria-degradable organic carbon), for the uncapped boxcosms was ca. 30% less
301 compared to the capped boxcosm (cf. with ca. < 3% for a 30 cm Hg-contaminated layer for
302 similar O_2 penetration). The net Hg methylation in the capped boxcosms was however twice
303 as high, despite similar levels of added organic carbon concentration and even anoxia. Other
304 factors (other than sediment surface area) that might account for the difference might include
305 different demethylation rates (as we only measured net MeHg formation) between capped
306 and uncapped boxcosms.

307

308 *4.4 Introduction of organic carbon enhanced sediment to water fluxes of both THg and MeHg*
309 We wanted to investigate how introduction of allochthonous OC to Hg-contaminated
310 sediments with low autochthonous OC, would affect methylation of in situ Hg. This is
311 important since the main function of an ISC is to prevent Hg and MeHg release to the
312 overlying surface water. Figure 5 shows the sediment to water fluxes of THg and MeHg for
313 the uncapped boxcosms. The sediment-water fluxes were calculated from the difference in

314 Figure 5

315 THg or MeHg concentration of surficial seawater entering and leaving the boxcosms (at a
316 flow rate of ca. 1 $mLmin^{-1}$ (Figure 1). The concentration of both THg and MeHg in the
317 influent source water was below the detection limits (0.1 and 0.02 ng/L for THg and MeHg
318 respectively). Addition of OC clearly enhanced the fluxes of THg and MeHg in the OC-
319 amended boxcosms (Figure 5). The sediment-water flux of THg and MeHg species is mainly
320 due to diffusion but other mechanisms that can enhance the flux, such as gas ebullition during

anaerobic OC decomposition (Randall et al. 2013b) cannot be ruled out. The later combined with occasional particle resuspension during sampling could explain some of the abnormally high THg flux measurements in Figure 5 including the low OC dose boxcosm on day 42. The average flux (mean ± standard deviation, n = 4) for the non-OC amended control was 240 ± 21 and 1.0 ± 0.4 ng m^{-2} day^{-1} for THg and MeHg respectively. This mean excludes the last (202 day) water sample (800 ng m^{-2} day^{-1}), which we suspect might have incorporated sediment particles due to disturbance during sampling (Figure 5). The THg fluxes show an initial peak for the first sampling soon after the boxcosms were setup (day 7) and then generally levels off in subsequent months except for the low OC dose boxcosm. The initial peak in THg (but not MeHg) flux is most likely due to sediment resuspension during the boxcosms set up (see methods section in the supplementary information for details). The higher flux of THg in OC-amended sediment was most likely due to increased solubility of Hg species due to organic complexation (Graham et al. 2012, Gu et al. 2011). Gu et al. (2011) recently carried out a study on Hg reduction and complexation by natural organic matter in anoxic environments. They showed that reduced organic matter was capable of both reducing Hg(II) to Hg(0) and also reacting with Hg(0) to form Hg–DOM complexes via ligand-induced oxidative complexation (Gu et al. 2011). The MeHg flux in this study was linearly correlated (R^2 = 0.9) with the underlying sediment OC concentration (Figure 6). The above correlation excludes two abnormally high fluxes of the 23 flux measurements. The average MeHg flux (mean ± standard deviation) for the 202 day duration of the experiment ranged from 1.0 ± 0.4 to 85 ± 13 ng m^{-2} day^{-1} for the control and the high OC dose boxcosm respectively with an overall mean of 23 ± 32 ng m^{-2} day^{-1} for all the boxcosms. They compare well to the flux (52 ng m^{-2} day^{-1}) reported by Randall et al. (2013a) from lake sediments microcosms after 42 days but are higher than those reported by Hollweg et al. (2009) (0.2 ng m^{-2} day^{-1}) for Chesapeake Bay sediments. The enhanced MeHg and THg

346 sediment to water fluxes is consistent with recent findings showing that organic matter can; i)
347 enhance the oxidation of $Hg(0)_{aq}$ to Hg(II) (Colombo et al. 2013, Hu et al. 2013) and ii)
348 enhance the biotic MeHg formation in anoxic environments (Graham et al. 2012, Hollweg et
349 al. 2009, Lambertsson and Nilsson 2006, Mitchell and Gilmour 2008).

350

351 Figure 6

352 5. CONCLUSION

353 The results of this study clearly show that introduction of labile organic carbon (in this case
354 algae) to Hg-contaminated marine sediments (where SO_4^{2-} is abundant) increases both the
355 MeHg formation from in situ sediment-bound inorganic Hg, and fluxes of both THg and
356 MeHg to the overlying water. The increase in methylation of in situ sediment-bound Hg we
357 observed in this study was linearly correlated to the content of the organic carbon introduced
358 and application of an ISC enhanced that methylation (cf. uncapped controls). The findings of
359 this study have important implications on both the fate, and remediation (via ISC application)
360 of Hg-contaminated aquatic sediments in general. Both MeHg formation and MeHg fluxes to
361 overlying waters, in contaminated and anoxic sediments are likely to be enhanced in nutrient-
362 rich eutrophic marine waters that experience high organic matter export due to chronic algal
363 blooms (Cloern 2001). Marine macro algae biomass has been proposed as a suitable medium
364 for the removal of inorganic Hg from contaminated waters (Herrero et al. 2005). Similarly,
365 iron nanoparticles coated with food grade cellulose, have also been suggested for use in
366 immobilizing Hg in contaminated sediments (Xiong et al. 2009). The results of this study
367 suggest that there might be a high risk of MeHg formation if Hg-rich algal biomass is not
368 properly disposed. There is also a need to further investigate the biogeochemical fate of Hg
369 immobilized in cellulose-rich sediments. There is a risk that application of an ISC or porous

surficial cap over such organic-rich sediments, after onset of anoxia, is likely to enhance MeHg formation. Thus the composition and thickness of any cap applied over organic-rich Hg-contaminated sediments should be carefully evaluated; The cap should be; i) thick enough to prevent Hg species leakage to overlying water, ii) able to isolate the contaminated sediments from addition of organic matter and iii) the ISC should have low organic matter content to reduce the potential of MeHg formation.

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473

Figure 1

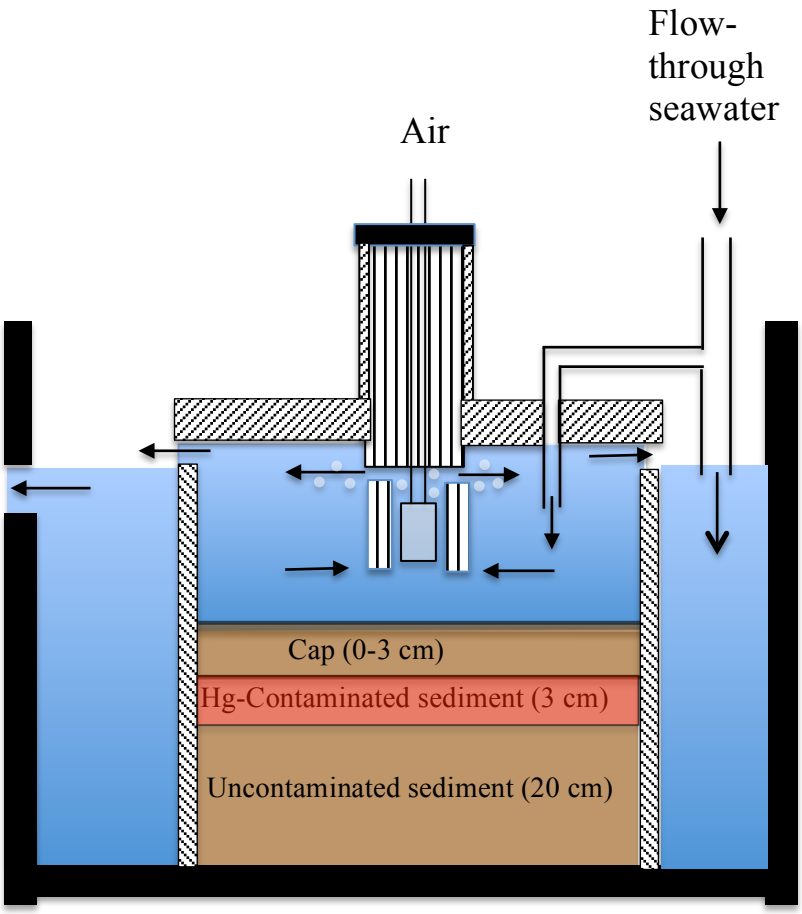

Figure 1. Set-up showing the experimental boxes (boxcosms) used to study the effect of organic carbon and capping on Hg speciation in contaminated marine sediments. The three-centimeter Hg-contaminated sediment layer in three of the four capped and uncapped (control) boxcosms (total of eight boxcosms) was amended with varying levels of organic carbon (as pulverized *chlorella* algae). Both the cap and bottom layers were clay sediment sampled from an uncontaminated site. See text for more details.

Figure 2

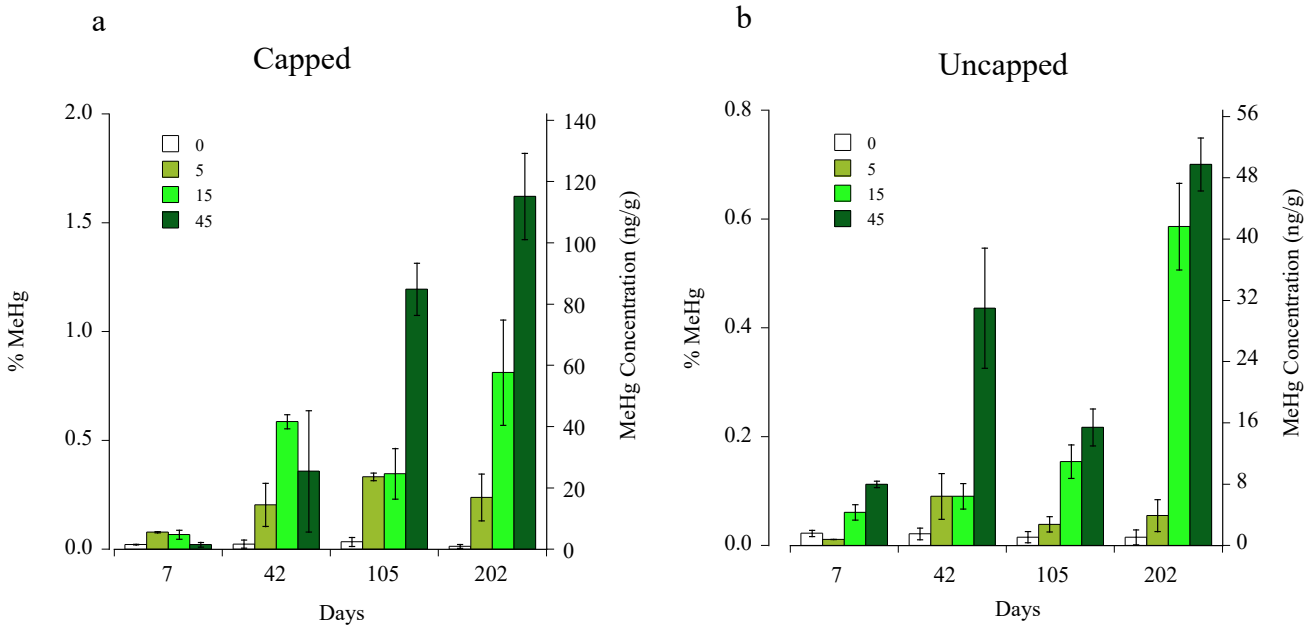

Figure 2. Time series for MeHg concentration in boxcosms containing a) capped and b) uncapped Hg-contaminated sediments (note the different Y-axis scales). The bars represent the average MeHg concentration in the Hg-contaminated layer enriched with 0, 5, 15 and 45 grams of algae per liter of wet sediment. The error bars represent the standard deviation on the MeHg concentration in the three one-centimeter core sections from the contaminated layers in each boxcosm.

Figure 3.

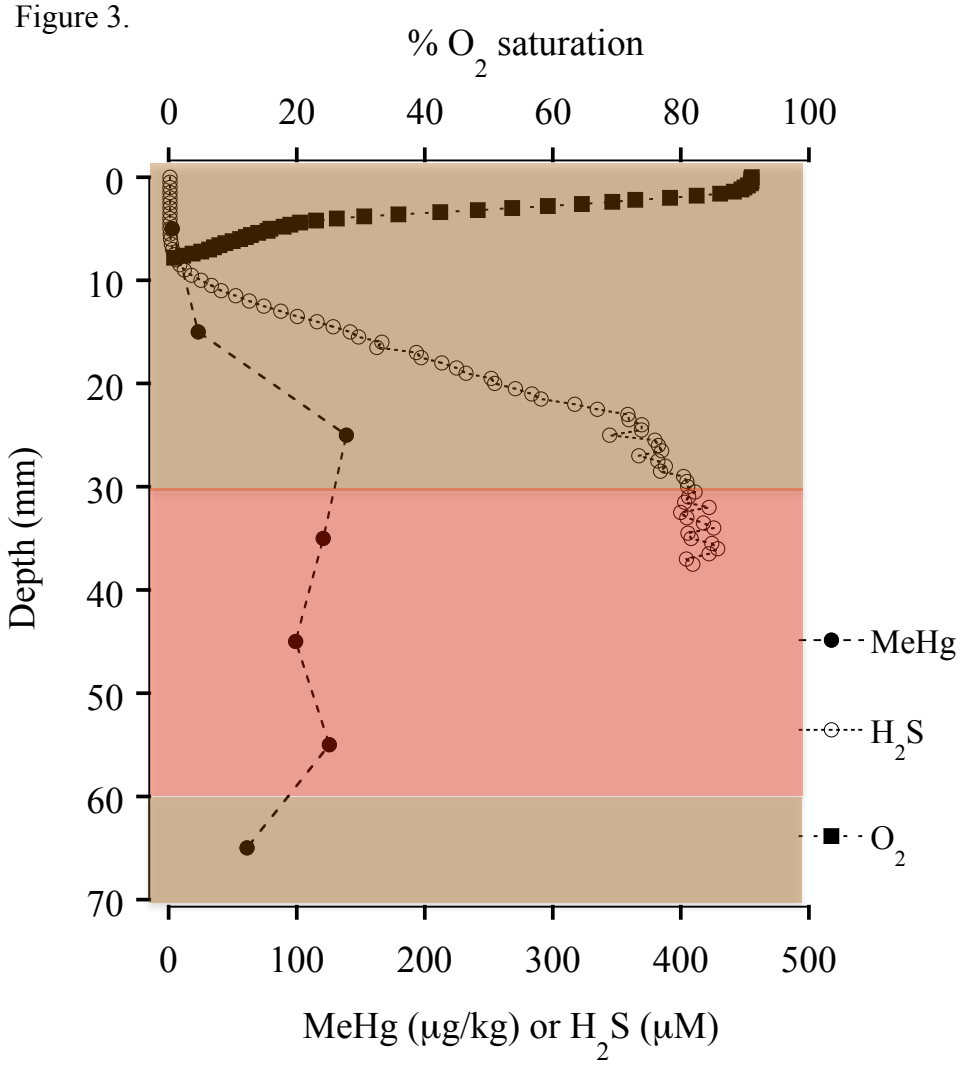

Figure 3. Depth profile MeHg, H_2S and O_2 concentration measurements for the high organic carbon capped boxcosm after 202 days. The H_2S and O_2 measurements were made using respective microsensors. The MeHg concentration was measured from a 10 mm slice from the respective depth. The shading indicates the extent of the cap (grey), Hg-contaminated (red) and bottom (grey) sediment layers. See text for details on other boxcosm profiles

Figure 4

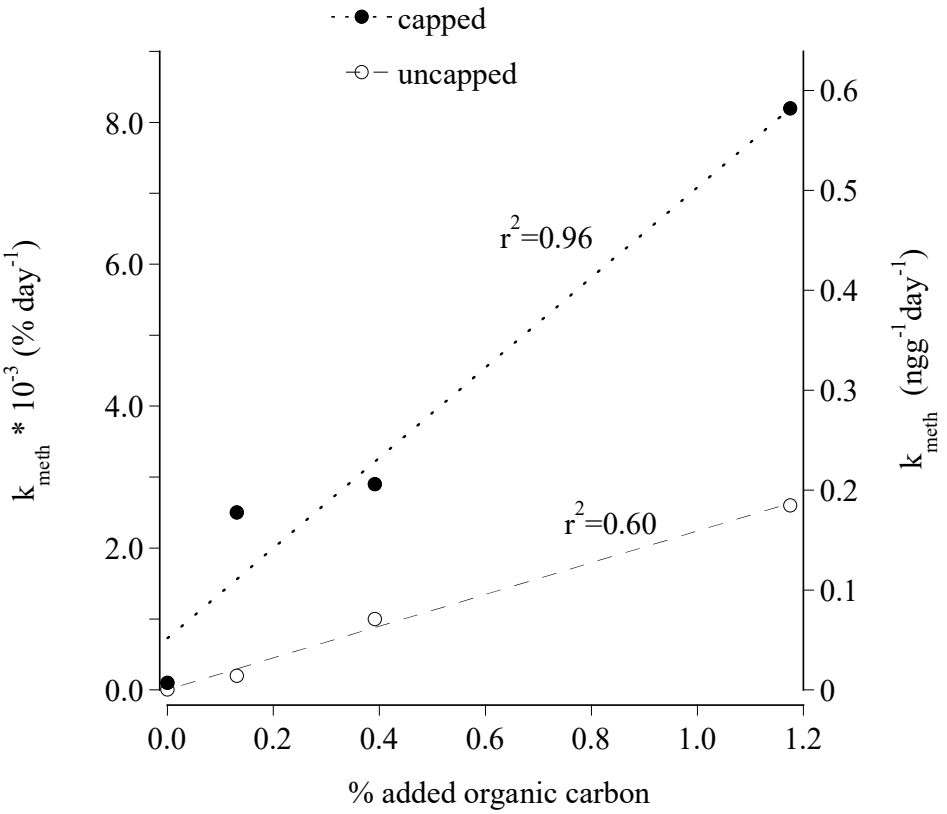

Figure 4. Relationship between MeHg formation rate, k_{meth}, and added organic carbon for capped (filled circles) and uncapped (open circles) Hg-contaminated sediment boxcosms.

Figure 5

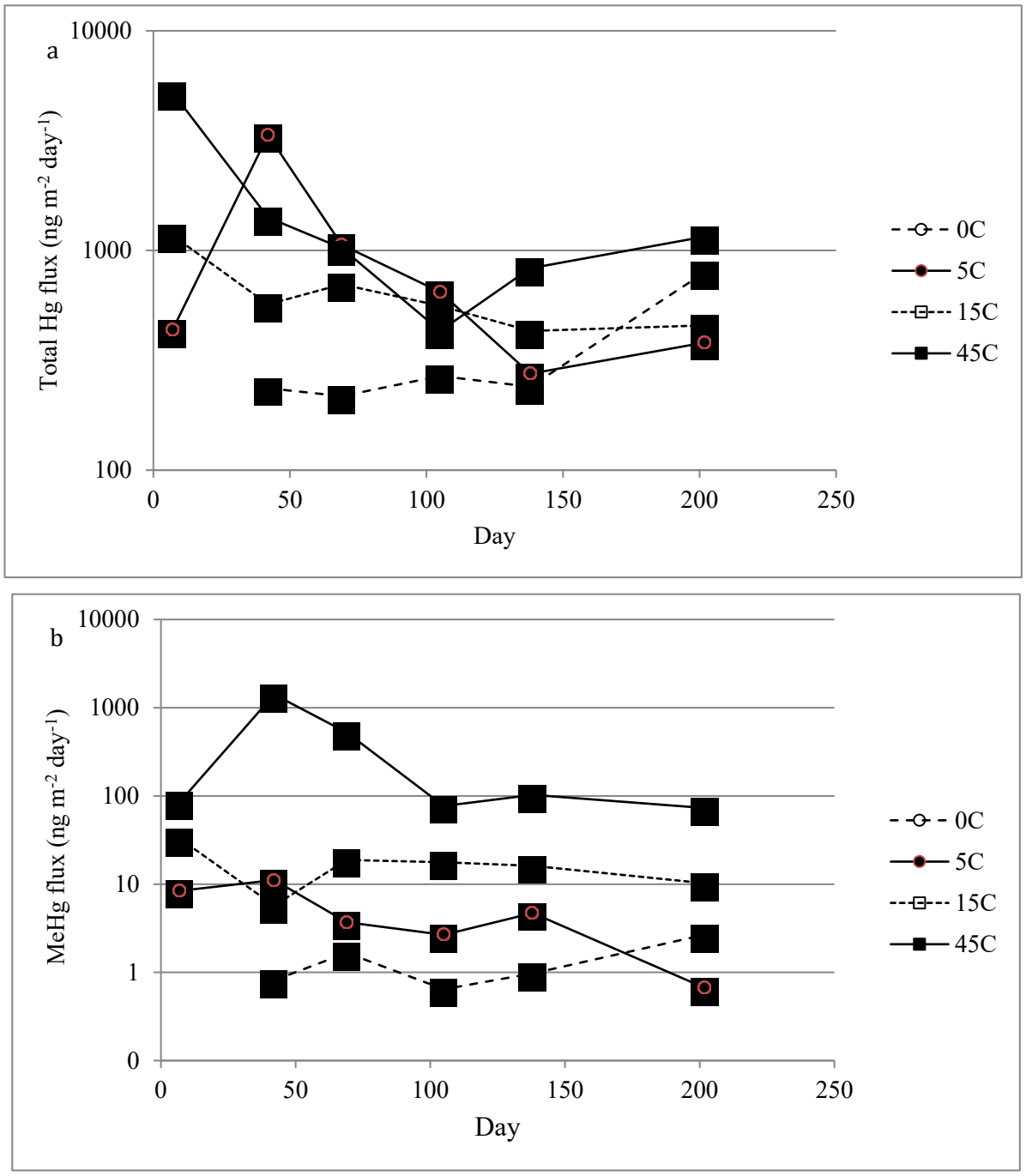

Figure 5. Fluxes of THg (a) and MeHg (b) from sediment to overlying water in uncapped Hg-contaminated sediments amended with 0 (control), 5, 15, and 45 g of pulverized algae. Note the log scale on the vertical axis.

Figure 6.

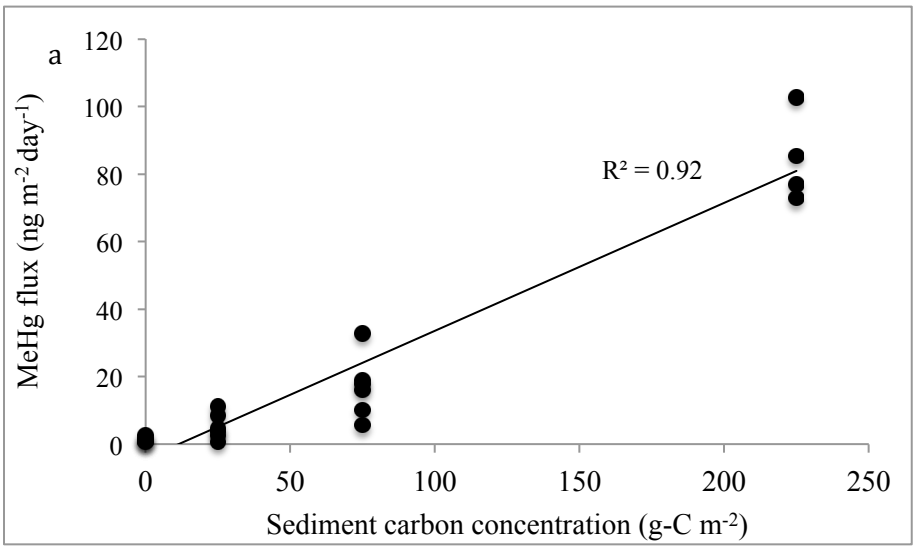

Figure 6. Sediment to water fluxes of MeHg as a function of organic carbon added to Hg-contaminated sediments.

Supporting information

Effects of organic matter addition on methylmercury formation in capped and uncapped marine sediments

Kuria Ndungu, Morten Schaanning and Hans Fredrik Veiteberg Braaten*

Historical Background

In February 1945 the German submarine U-864 was sunk about two nautical miles (3.7 km) west of the Norwegian North Sea island of Fedje (Figure S1). The submarine was torpedoed midship, broke in two and sank, and is located at 150 m depth. U-864 is assumed to have had 67 tons of liquid mercury on board, stored in 1857 carbon steel cans in compartments inside the keel. When torpedoed, the mid-section of the submarine was blown up, and an unknown number of the steel cans were destroyed and mercury was spread to polluting the surrounding seabed. Mercury cans may also have corroded and spilled Hg during the last 75 years (Kystverket 2014a, b)

Figure S1. Map showing location of U-864 wreck where Hg-contaminated sediments for this study were sampled.

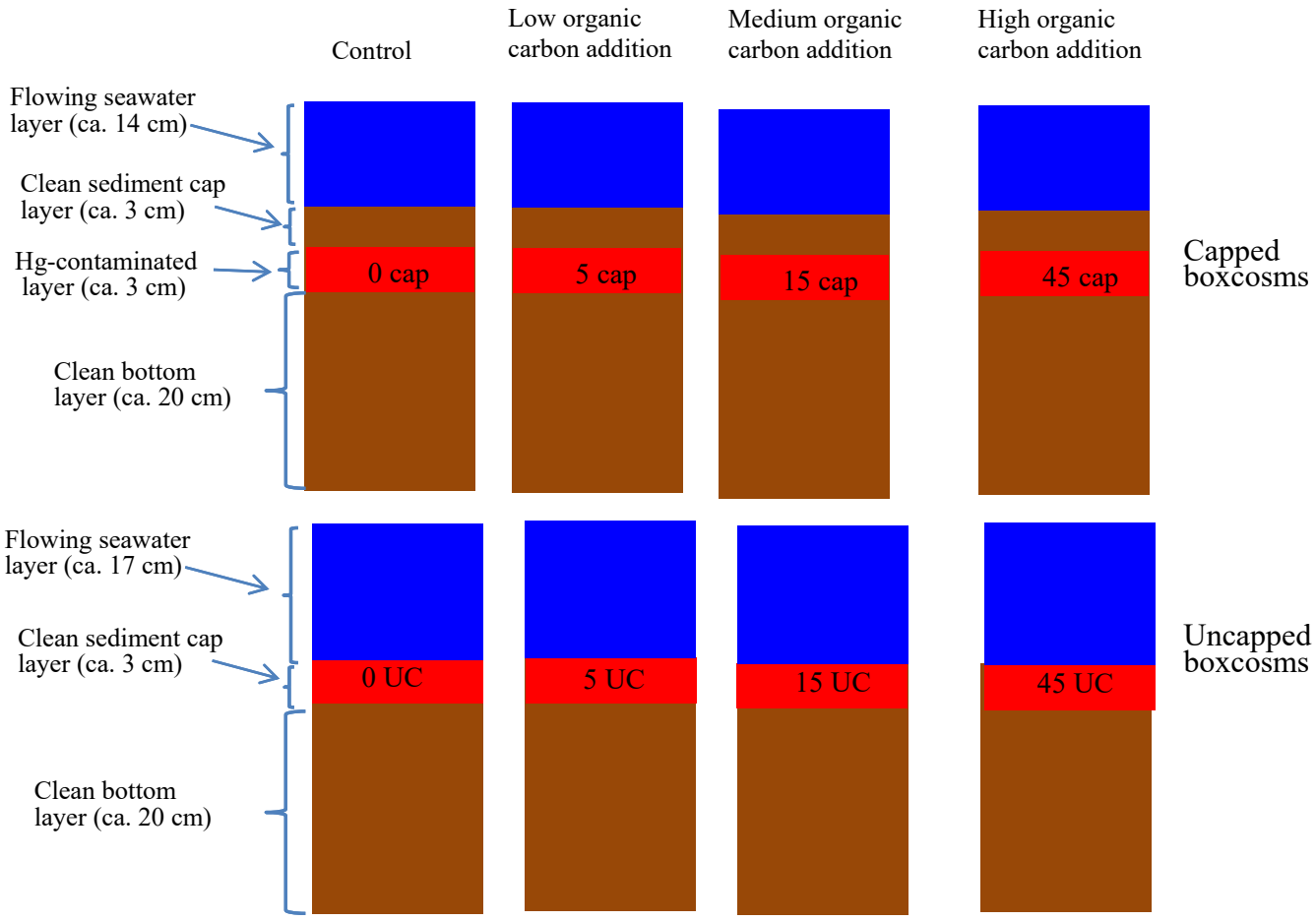

Figure S2. Experimental set up to study the effect of capping and organic matter on Hg speciation in Hg-contaminated sediments sampled from the U-864 seabed site near Fedje. The four Hg-contaminated sediment layers for the capped (top row) and the uncapped (bottom low) were amended with 0, 5, 15 and 45 g of pulverized chlorella algae. See text for details. A photo of the set up is shown in Figure S2.

Figure S3. Photo of the boxcosm experimental setup depicted in the schematic in Figure S1 showing the four uncapped (front row) and capped (back row) sediment boxcosms

Sediment core sampling

Sediment cores were extracted from the microcosms using a 5 cm diameter, 25 cm long hollow acrylic cylinder. The cylinder was driven 10-12 cm into the sediment until the top was just below the overlying water surface level. A second slightly wider (6 cm i.d.) hollow cylinder was also inserted around the first core to ca. 3 cm deeper than the coring cylinder. This second cylinder was used to cover the hole created by the removed core. A tight-fitting rubber cock was then inserted displacing the water in the inner cylinder and thus creating a vacuum. A 10-12 cm core was then carefully extracted by pulling out the cylinder maintaining ca. 3-5 cm of the surficial sediment water layer. The second cylinder was then pushed further into the sediment (up to sediment surface level) and tightly capped with a rubber stopper to cover the hole. The extracted sediment core was immediately transferred (with as little disturbance as possible) to the laboratory at the field station, a few meters from the microcosms. A rubber piston was then used to slowly push the core up the cylinder leaving ca. 3 cm of water covering the top of the sediment. The cylinder was clamped vertically next to the micromanupulator for the sensor measurements (Figure S4).

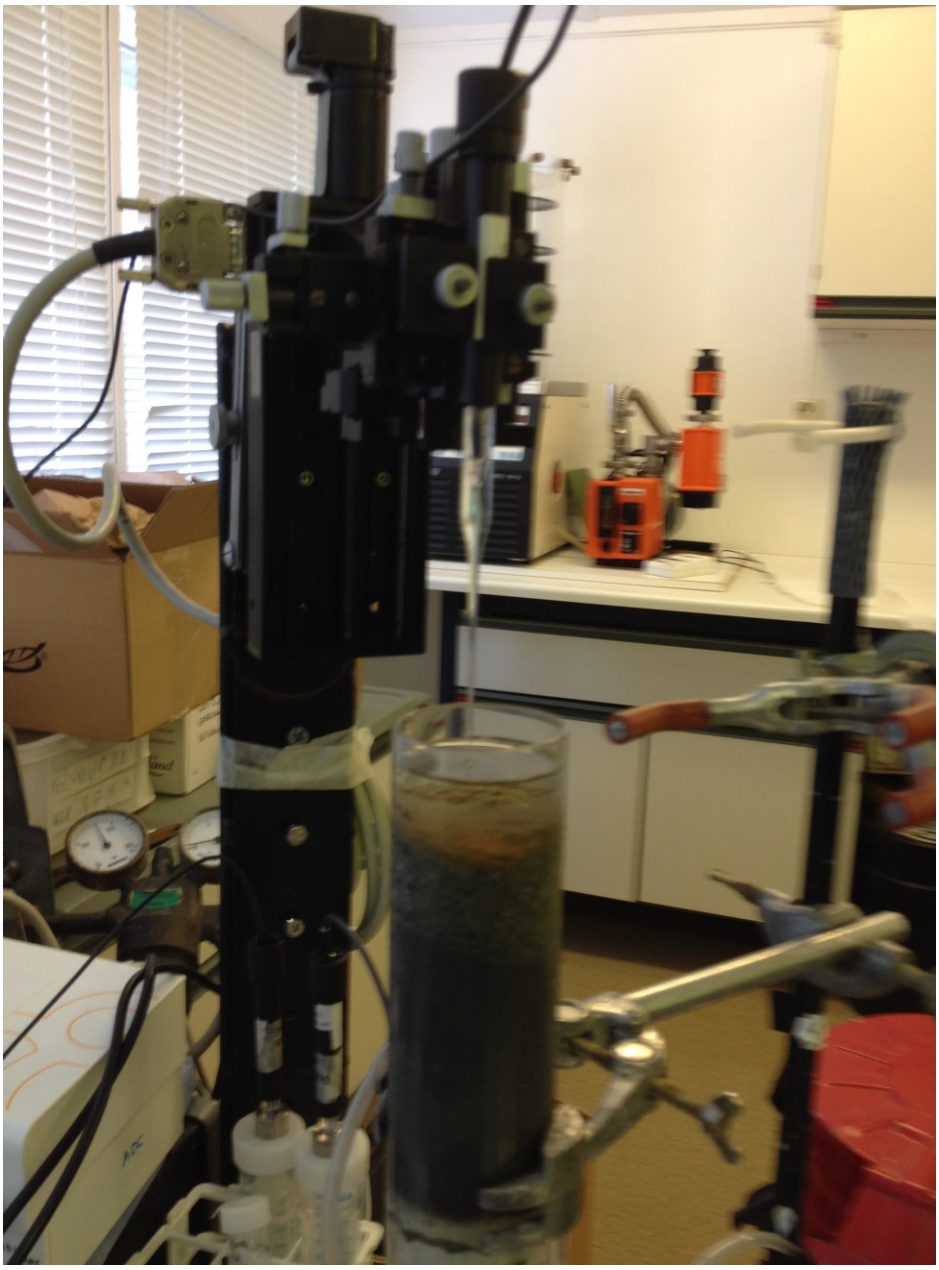

Figure S4. Set up for microsensor depth profile measurements of O_2, H_2S and pH, showing O_2 concentration measurement on a core extracted from one of the boxcosms.

High O_2 utilization in high carbon dose uncapped boxcosm

There was intense sediment-surface microbial activity on the uncapped high carbon dose (45 g added algae) boxcosm. The intense microbial activity resulted in to low and variable surface O_2 saturation (30 to <60%) for that month and almost complete anoxia at the sediment water interface.

Figure S5: Microbial growth observed on the surface of Hg-contaminated sediment core after one month. The core was sampled from the uncapped (open) boxcosm amended with 45 g of pulverized marine algae per liter of sediment. The extensive algae growth was not observed in subsequent months.

The extensive O_2 utilization by aerobic bacteria in the Hg-contaminated sediment layer thus occasioned H_2S production at relatively shallow depth. As shown in Figure S5 above.

Table S1. Summary of metal, organic matter and water concentrations in Hg-contaminated sediment used for the boxcosm experiment. The concentrations shown are mean ± standard deviation of at least three determinations in dry sediment.

H_2O (wt %)	LOI (wt %)	Total Hg (mg kg^{-1})	Fe (g kg^{-1})	Mn (g kg^{-1})	Al (g kg^{-1})
26	1.2	9.0 ± 1.7	7.5 ± 1.3	1.6 ± 0.4	5.3 ± 1.2

Table S2. Mercury methylation rates (k_{meth}) calculated from measurements of mono methyl mercury (MeHg) concentration in sediment cores from Hg-contaminated sediments amended with algae-derived organic carbon.

[a]Organic carbon added (wt % wet sediment)	[b]Methylation rate (k_{meth})					
	[d]Capped boxcosms			Uncapped boxcosms		
	k_{meth} ($ngg^{-1}day^{-1}$)	k_{meth} (% day^{-1})	R^2	k_{meth} ($ngg^{-1}day^{-1}$)	k_{meth} (% day^{-1})	R^2
[c]0	0.01	<0.001	0.97	< 0.01	<0.001	0.05
[c]0.13	0.18	0.002	0.97	< 0.02	<0.001	0.98
0.39	0.21	0.003	0.61	0.19	0.003	0.89
1.18	0.59	0.008	0.93	0.18	0.003	0.84

[a]Organic carbon added as pulverized algae (47wt% C) to ca. 1 L (1.8kg wet mass) boxcosm

[b]Methylation rate (net methylation plus demethylation) calculated for the entire duration of the experiment (202 days)
[c]k_{meth} calculated up to 105 days

Neglegible methylmercury penetration into the capping layer

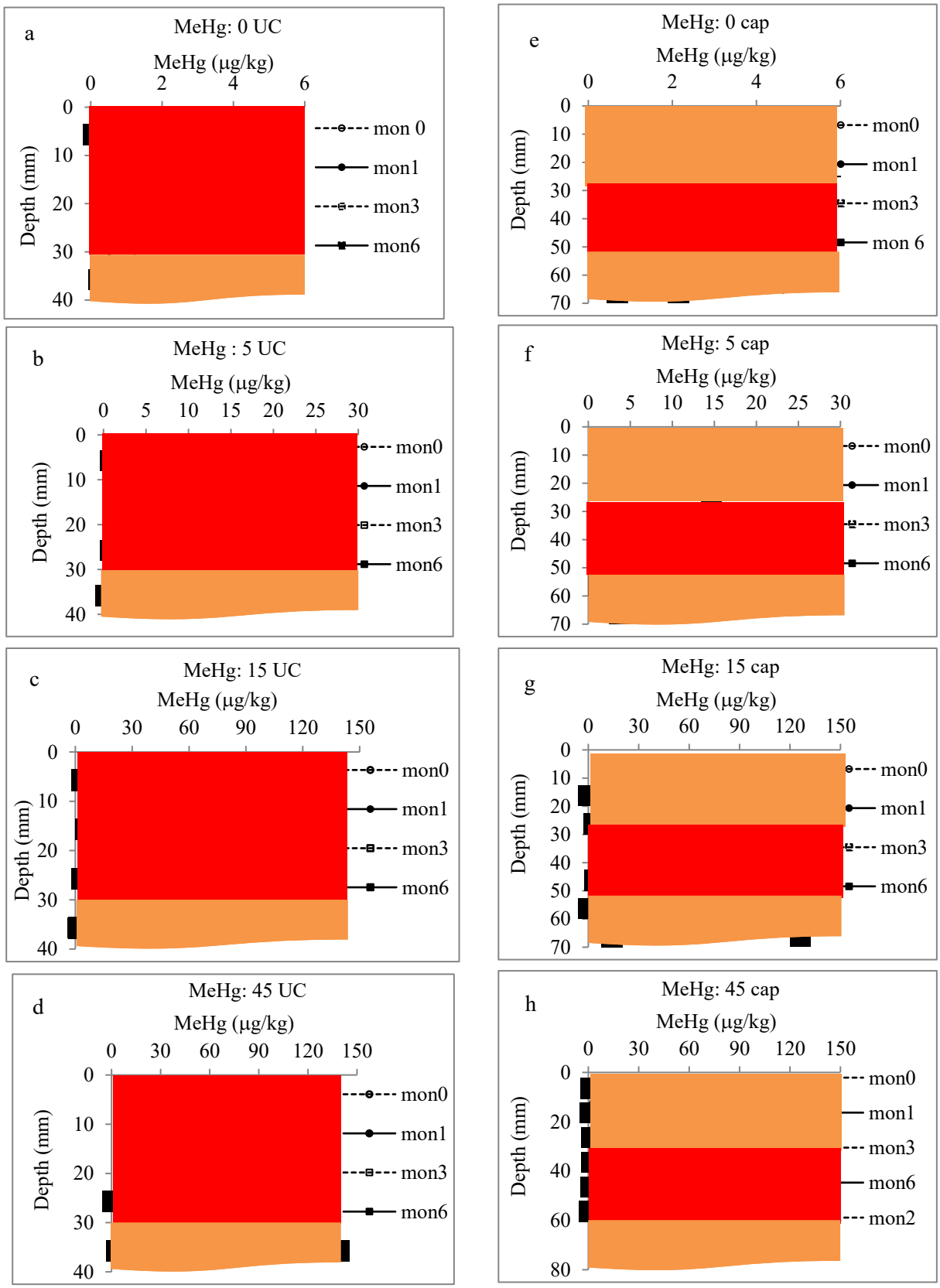

Figure S6. Depth profiles of MeHg concentration in cores from boxcosms: (a-d) uncapped; (e-h) capped. Each concentration is plotted at the mean depth for the respective 10 mm slice. The shading indicates the extent of the cap (grey), Hg-contaminated (red) and bottom (grey) sediment layers.

REFRENCES

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Norwegian Coastal Administration site detailing all activities Kystverket, (2014b) Undersea Images of submarine U-864 http://www.flickr.com/photos/kystverket/sets/72157638343710506/ (acessed 16 Feb. 2016).