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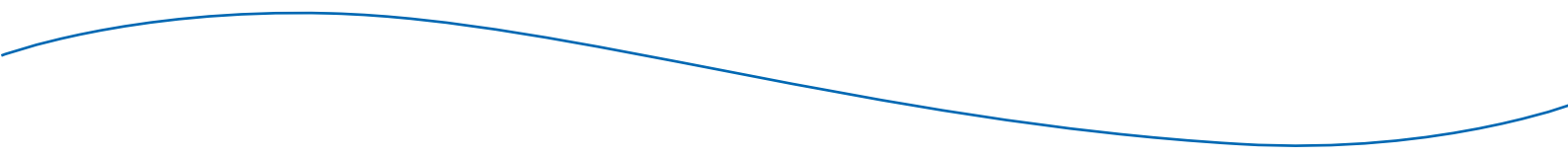
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Abstract

A mesocosm experiment was performed on methylation of mercury in marine sediments from the wreck site at Fedje. The mercury contaminated sediment was enriched with zero, low, medium or high dose of labile organic carbon from pulverized algae and either capped with a thin layer of clay or left uncapped exposed to the seawater flowing through the mesocosm. The small fraction of methyl mercury (MeHg) of 0,006% of the total concentration (TotHg) in the batch of sediment shipped form Fedje indicated that *in situ* methylation at the wreck site is low. In the boxcosms, no significant methylation was observed in sediments with no addition of algae. In the low dose treatment, initial methylation rates were found to be 0.2-0,3 ng MeHg m^{-2} day^{-1}, but the process ceased after the first month, presumably due to depletion of the reservoir of available algae carbon added. In medium and high dose treatments the process proceeded throughout the experimental period at apparently constant rates proportional with the amount of algae added. Methylation was observed in both capped and uncapped Fedje sediments, but the highest rates of methylation were observed in the capped sediments, in which up to 0.6 ng m^{-2} day^{-1} of methyl mercury (MeHg) accumulated during the six months experimental period. Fluxes of TotHg and MeHg to the seawater were only measured in the uncapped sediments. High fluxes were observed in all treatments. The six months median fluxes showed a four-fold increase of TotHg from zero to high algae addition, whereas the corresponding fluxes of MeHg increased 100x from 0,97 to 94.1 ng MeHg m^{-2} day^{-1}. The flux of MeHg could only account for a small fraction (in general less than 10% of the total flux) indicating the formation of soluble organic complexes other than MeHg. A more long-term experiment using petrogenic hydrocarbons is recommended to improve assessment of the risks of Hg methylation at Fedje and at Hg-contaminated wreck sites in general.

4 keywords, Norwegian	4 keywords, English
1. Metylkvikksølv	1. Methylmercury
2. Sediment	2. Sediment
3. Tildekking	3. Capping
4. Mesocosmos	4. Mesocosm

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A mesocosm experiment on methyl mercury formation after capping of U-864 sediments enriched with powdered algae

Preface

This report summarizes the findings of NIVA project O-14161 entitled Investigating the methylation risk of capping Hg-contaminated sediments at the U-864 seabed wreck site near the island of Fedje. The project was financed by The Norwegian Coastal Administration (Kystverket) on a sub-contract from DNV-GL where Jens Laugesen was the contact person. At NIVA, the project was managed by Kuria Ndungu and Morten Schaanning. Mercury contaminated sediment samples were collected by Sigurd Øxnevad at NIVA during the survey in January 2014.Morten Schaanning and Kuria Ndungu carried out the experimental work of setting up, sampling, microsensor analyses of ancillary parameters at Solbergstrand Marine Research Station and processing of sediments prior to MeHg analysis. Total mercury and methyl mercury concentration measurements in water and sediment extracts at the NIVA laboratory in Oslo was performed by Hans Fredrik Veiteberg Braaten. We are grateful to Joachim Tørum Johansen and Jasek Konecki at Solbergstrand Marine research station for help with collecting the capping sediment and setting up the boxcosms, and to Malene Dimmen for help in digesting sediments prior to MeHg analysis.

Oslo, 01.10. 2015

Kuria Ndungu

Contents

Summary

In situ subaqueous capping of the mercury (Hg) containing wrecks of the U-864 submarine and the surrounding Hg-contaminated sediments with clean sediment and (or other material) has been proposed as a viable mitigation measure. The aim of this project was therefore to carry out boxcosm experiments to simulate a sediment capping and study the effect capping might have on the production of methyl mercury (MeHg). We were particularly interested in investigating if the introduction of organic carbon (OC), e.g. from diesel and other petroleum hydrocarbon oils potentially trapped underneath the cap, would enhance the rate of in situ Hg methylation in the low organic matter, Hg-contaminated sediments surrounding the wreck. Sulphur reducing bacteria (SRB) are known to be involved in the process, but with limited knowledge on methylation rates, it was decided to use an easily degradable carbon source to ensure proliferation of this group of bacteria within the time constraints of experimental work. We set up boxcosm experiments in which we amended the Hg-contaminated sediments with zero, low, medium and high doses of OC (as pulverized algae) corresponding to 0, 25, 75 and 225 grams carbon per square meter of sediment respectively. One series of OC-amended Hg-contaminated layer in the boxcosms was capped with clean marine clay layer and a second identical series of layers was left uncapped.

Sediment cores were sampled from all treatments one week after boxcosm set-up and then again after one, three and six months. In addition, the overlying water was sampled each month from boxcosms with uncapped sediments for determination of the leakage of total mercury (TotHg) and MeHg to the overlying water.

The in situ TotHg concentration in the batch sediment from Fedje was 6 481 $\pm$ 747 ng g^{-1} wet sediment (8 892 ng g^{-1} $\pm$ 1002 ng g^{-1} dry sediment). The corresponding in situ MeHg concentration was 0,43 $\pm$ 0,38 ng g^{-1} wet sed. or 0,007% of TotHg, which showed a very low degree of methylation in the source sediment.

No significant Hg methylation was observed in the Hg-contaminated sediments without added OC (both capped and uncapped). However, in all boxcosms where OC was added, we observed extensive increase in MeHg concentration. Thus, six months after the start of the experiment, the MeHg concentration in the high carbon dose boxcosms increased by two orders of magnitude; reaching a maximum concentration of 45 ng MeHg g^{-1} and 120 ng MeHg g^{-1} in the uncapped and capped sediments respectively. Oxygen was observed to only penetrate 1-2 mm into the uncapped sediments and hydrogen sulphide proliferated below 10 mm in the high-dose boxes. The lower accumulation of MeHg in the uncapped sediments could probably be attributed to a number of factors including, inhibition of the methylation process near the sediment water interface, demethylation and loss of MeHg to overlying water.

Mercury methylation rates were calculated by linear regression analyses of the change in MeHg

concentration with time in various depth layers of the sediments. The methylation rates determined as the slopes of the regression lines, varied from <0.0005 μg kg^{-1} day^{-1} in the control boxes without added algae, to 0.16-0.52 μg kg^{-1} day^{-1} in the medium and high dose boxes. Typical rates were 0.1-0.3 μg kg^{-1} day^{-1} in uncapped and 0.3-0.6 μg kg^{-1} day^{-1} in capped layers.

In the treatments with low dose of added algae, initial methylation rates were 0.2-0.3 μg kg^{-1} day^{-1}, but dropped drastically later in the experiment. Thus the low dose treatment indicated that Hg methylation is crucially dependant on the total amount of available OC. For the whole experimental period, linear relationships were found between Hg methylation rate and the amount of OC added with linear correlation coefficients (R^2) of 0.85 and 0.47 for capped and uncapped sediments respectively.

Addition of OC also increased the fluxes of both TotHg and MeHg to the surficial water in the uncapped boxcosms. In the control box without added algae, the median TotHg flux was 240 ng m^{-2} day^{-1} while the corresponding MeHg flux was 0.97 ng m^{-2} day^{-1}.

The results from this study indicate that methylation of in situ inorganic Hg in the seabed sediments from the U864 submarine wreck site is likely to occur if highly degradable OC is introduced. The Hg-contaminated sediments from the site in this short study were amended with a relatively labile form of OC (pulverized *Chlorella s.p.* algae). We were therefore able to observe microbial-aided Hg methylation by SRB (and possibly other Hg methylating bacteria) within a relatively short period of time. No significant amounts of fresh algae material is likely to be trapped during a capping operation at Fedje but SRB can also degrade petroleum hydrocarbon oils. It would therefore be interesting to repeat the study using varying sediment Hg concentration and a less labile OC source such as diesel oil or other petroleum hydrocarbons similar to those potentially present in the U864 wreck. The findings of such a study would be of high general interest to anyone involved in risk assessments of oil-bearing shipwrecks lying on Hg-contaminated coastal sediments.

Sammendrag

Tildekking med rene masser er foreslått som tiltak for å stoppe spredning av kvikksølv (Hg) fra ubåten U-864 og de omkringliggende sedimentene. Målet med dette prosjektet var å undersøke om tildekking kan påvirke produksjonen av metylkvikksølv (MeHg) i sedimentene, særlig dersom det tilføres organisk karbon, f.eks. fra utlekking av diesel eller andre oljeprodukter.

Det er kjent at svovelreduserende bakterier (SRB) har en viktig rolle i metyleringsprosessen, men lite er kjent om hvor raskt metyleringen skjer og hvor raskt den kommer gang ved miljøendringer. For å sikre tilstrekkelig bakterieforøkning i et tidsavgrenset eksperimentelt oppsett valgte vi å benytte en lett-tilgjengelig karbonkilde i form finknust algemateriale. Eksperimentet ble satt opp med forurenset sediment og sjøvann i plastbokser («boxcosms») med varierende mengde tilsatt organisk karbon (tilsvarende 0, 25, 75 og 225 gram karbon per kvadratmeter sediment). For å vurdere forskjellen med og uten tildekking ble det kjørt to parallelle serier: én med og én uten tildekking.

Det ble prøvetatt sedimentkjerner fra alle boxcosms én uke etter oppstart av eksperimentet og deretter etter én, tre og seks måneder. I tillegg ble det tatt månedlige vannprøver fra serien som ikke var tildekket, for å sjekke utlekking Hg og MeHg til de overliggende vannmasser.

Konsentrasjonen av totalkvikksølv (TotHg) i sedimentet fra Fedje brukt i eksperimentet var 6 481 $\pm$ 747 ng g^{-1} sediment våtvekt (8 892 ng g^{-1} $\pm$ 1 002 ng g^{-1} tørrvekt). Konsentrasjonen av MeHg var 0,43 $\pm$ 0,38 ng g^{-1} sediment våtvekt, eller 0,007%. Dette viser at metyleringsgraden i utgangspunktet var svært lav.

I eksperimentet observerte vi ingen signifikant metylering i sedimentene uten tilsatt organisk karbon (hverken i tildekket eller åpen), men betydelig økning i MeHg konsentrasjonen i alle boxcosms hvor organisk karbon var tilsatt. Konsentrasjonen økte med to størrelsesordener, med makskonsentrasjoner på 45 ng MeHg g^{-1} for det utildekkede sedimentet og 120 ng MeHg g^{-1} i det tildekkede sedimentet med høyest dose og etter seks måneder. Oksygen ble observert å trenge 1-2 mm inn i det utildekkede sedimentet og H_2S ble målt dypere enn 10 mm ved høyest dose organisk karbon. Dette antyder at både hemming av metyleringsprosessen nær sedimentoverflaten, demetylering og utlekking av MeHg til vannsøylen kan ha bidratt til lavere akkumulering av MeHg i de utildekkede sedimentene.

Metyleringsratene ble beregnet ved lineære regresjonsanalyser og varierte fra <0,0005 μg kg^{-1} dag^{-1} i boxcosms uten tilsetning av organisk materiale til 0,16 - 0,52 μg kg^{-1} dag^{-1} i boxcosms med medium og høye doser. Typiske metyleringsrater var 0,1-0,3 μg kg^{-1} dag^{-1} for de utildekkede sedimentene og 0,3-0,6 μg kg^{-1} dag^{-1} for de tildekkede.

Ved lav dose tilsatt organisk materiale var metyleringsraten tidlig i eksperimentet 0,2-0,3 μg kg^{-1} dag^{-1}, men falt til null senere i forsøket. Lavdosebehandlingen viste dermed at den totale metyleringen er avhengig av den totale mengden av tilgjengelig organisk karbon. For hele forsøksperioden ble det funnet lineære sammenheng mellom metyleringsrate og mengde tilsatt karbon (korrelasjonskoeffisienter (R^2) på 0,85 for tildekket og 0,47 for utildekket).

Eksperimentet viste også at tilsetning av organisk karbon økte fluks av både THg og MeHg til vannsøylen fra de utildekkede sedimentene. I kontrollboksen uten tilsatt organisk materiale var fluksene (median) 240 ng TotHg m^{-2} d^{-1} og 0,97 ng MeHg m^{-2} d^{-1}.

Resultatene fra denne studien indikerer at metylering av uorganisk Hg i sediment rundt vraket av ubåten U-864 sannsynligvis vil øke dersom lett nedbrytbart organisk karbon kommer til. I dette forsøket benyttet vi lett nedbrytbart organisk materiale for å få en tydelig respons innenfor den relativt korte tidsperioden eksperimentet varte. Det er lite sannsynlig at betydelige mengder av et tilsvarende lettnedbrytbart algemateriale vil kunne fanges under massene som vil brukes ved tildekning ved Fedje. Derfor vil det være interessant å gjenta forsøket med mindre nedbrytbart organisk materiale som med større sannsynlighet vil kunne forekomme ved U-864, for eksempel diesel aller andre typer petroleumsprodukter. Resultatene fra en slik studie vil være av stor generell interesse for alle som er involvert i risikovurderinger av skipsvrak inneholdende olje i områder med kvikksølvkontaminerte sediment.

3. Introduction

The German submarine U-864 was sunk by, a British submarine on 9 February 1945, about two nautical miles west of the Norwegian North Sea island of Fedje. During its sinking, U864 was carrying about 67 metric ton of liquid mercury, believed to be stored in more than 1800 steel canisters in its keel. Since the discovery of the wreck by Royal Norwegian Navy in March 2003, the Norwegian Coastal Administration (NCA) has commissioned several studies, to monitor and assess the pollution risk posed by the Hg (Skei 2004, Uriansrud et al. 2006, Uriansrud et al. 2005, Uriansrud et al. 2005, Uriansrud et al. 2005). That risk involves pollution from Hg-contaminated sediments in the vicinity of the bow and stern wreck pieces and both loose (corroding) Hg canisters that were separated from the ship during impact and the bulk of the rest of Hg canisters still thought to be in the keel and whose condition/integrity is unknown (Kystverket 2014).In addition to metallic Hg, the wreck might still contain unknown quantities of diesel fuel and possibly other hydrocarbon oils (1000 liters of oil have already been emptied from the wreck) (Kystverket 2014). The Norwegian Institute for Water Research-NIVA has participated in sampling and chemical measurements during these assessment/monitoring expeditions to the U-864 wreck site (Øxnevad and Beylich 2013, Skei 2004, Uriansrud et al. 2006, Uriansrud et al. 2005, Uriansrud et al. 2005, Uriansrud et al. 2005).

In situ subaqueous capping of the U-864 wrecks and the surrounding Hg-contaminated sediments with clean sediment and/or other material has been proposed as a viable mitigation measure. The aim of this project was therefore to carry out boxcosm experiments to study the potential effect capping the contaminated sediment would have on Hg speciation, and in particular if and how the presence of organic carbon affects Hg methylation. It was hypothesized that in situ subaqueous capping of the Hg-contaminated sediments (with clean impervious clay sediment) in the presence of organic matter will alter the methylation-demethylation balance leading to an increase in situ MeHg concentrations relative to uncapped and non-carbon amended controls. That is; i) organic carbon would encourage the activity of sulfate reducing bacteria (and other bacteria that aid Hg methylation) at shallow depths beneath (un)capped Hg-contaminated sediments and ii) that Hg methylation will be more pronounced in organic-rich capped compared to uncapped controls.

Our experimental setup was designed to simulate conditions and processes in an in situ subaqueous capping setting within the limitations of a boxcosm experiment and time constraints. We therefore utilized pulverized algae (*Chlorella s.p.*) as a carbon source instead of hydrocarbon oils. Pulverized algae is a more labile carbon source and is degraded by microorganisms much faster (within the six-month study duration) than long chain hydrocarbon oils in the actual seabed wreck site. We did however, use Hg-contaminated sediments sampled from the site (see Experimental section below).

4. Materials and methods

4.1 Sampling of Hg-contaminated sediments from U864 site

Surface sediment samples were collected with a Van Geen grab mounted on the arm of a Remote operated vehicle (ROV) (Øxnevad and Beylich 2013). The ROV was controlled from the deck of a ship from the U864 wreck site. The sediment was transported to the Solbergstrand Marine Research Station (Drøbak, Norway) 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. 30 000 m² (Uriansrud et al. 2005), with sediment "hot spots" in the immediate vicinity of the wrecks and decreasing (Uriansrud et al. 2006, Uriansrud et al. 2005, Uriansrud et al. 2005) . The sediments were carefully checked for any ammunition, as they were collected from a site in the proximity of a WWII submarine wreck (Kystverket 2014).

4.2 Sediment preparation

All large stones and pebbles were physically removed. The rest of the sediment (which consisted of gravelly sand) was homogenized with a large handheld concrete mixer. The homogenized sediment was then subsampled into four equally large 6L portions; a, b, c and d. Then 0, 10, 30 and 90 g of the pulverized green algae was added to each of *a, b, c and d* respectively. No organic matter was added in portion *a*. After algae addition, each sediment portion was again well homogenized before being carefully poured into two 3cm deep, 0.1 m² trays. The trays then contained 0, 5, 15 and 45 g of algae, corresponding to approximately 0, 25, 75 and 225 gC m⁻² respectively (assuming a ca. 47 wt % carbon content in the algae (Phukan et al. 2011). The trays containing the sediments were then placed in a freezer at -20 °C for seven days to provide frozen 3 cm thick sediment layers ("frozen sheets") with the same surface area as the microcosm boxes. An extra four frozen layers were prepared in a similar way, but this time with fresh (uncontaminated) marine clay sediment to act as caps for the four capped treatments. This cap sediment was a left-over of subsurface, marine clay sediments from a previous experiment with sediments collected at a reference location in the Outer Oslofjord, remote from any point source of anthropogenic discharges.

4.3 Boxcosm set up

Eight boxcosms were placed in large tank (L 2 x W 0.8 x H 0.6 m) with flow-through seawater pumped from 60 m depth, maintaining a temperature of 8−10 °C and a salinity of ~34 throughout the six month experimental period. The water level in the tanks was about 1 cm below the rim of the boxes. The 60 m seawater was also pumped into a header tank from which it was distributed to the four uncapped boxes at an average (± 1 standard deviation) flow rate of 0.94 ± 0.05 mL min⁻¹, corresponding to a turnover time of ca. 6.4 days for the overlying water in the uncapped boxcosms. An air-diffusing system, consisting of an airstone diffuser placed in a perforated Plexiglas tube in the centre of each box, was used for stirring and aerating the water.

Before adding the various layers, the lids were removed from the eight polycarbonate boxes (0.32 m x 0.28 m x 0.40 m) (*Figure 1* and *Figure 2*) previously filled to a depth of about 20 cm with fresh uncontaminated clay sediment (same sediment as used for the caps) leaving about 15 cm of the box filled with seawater. Each of the eight carbon enriched frozen sheets was removed from the form and carefully sunk onto the sediment surface in each box. Excess edges was removed with a scrape and after thawing on top of the sediment surface the added layers were carefully moulded

to fill completely into corners and cracks along the walls of the boxes. The frozen sheets for capping were then similarly placed on top of the four capped treatments while the other four were left uncapped.
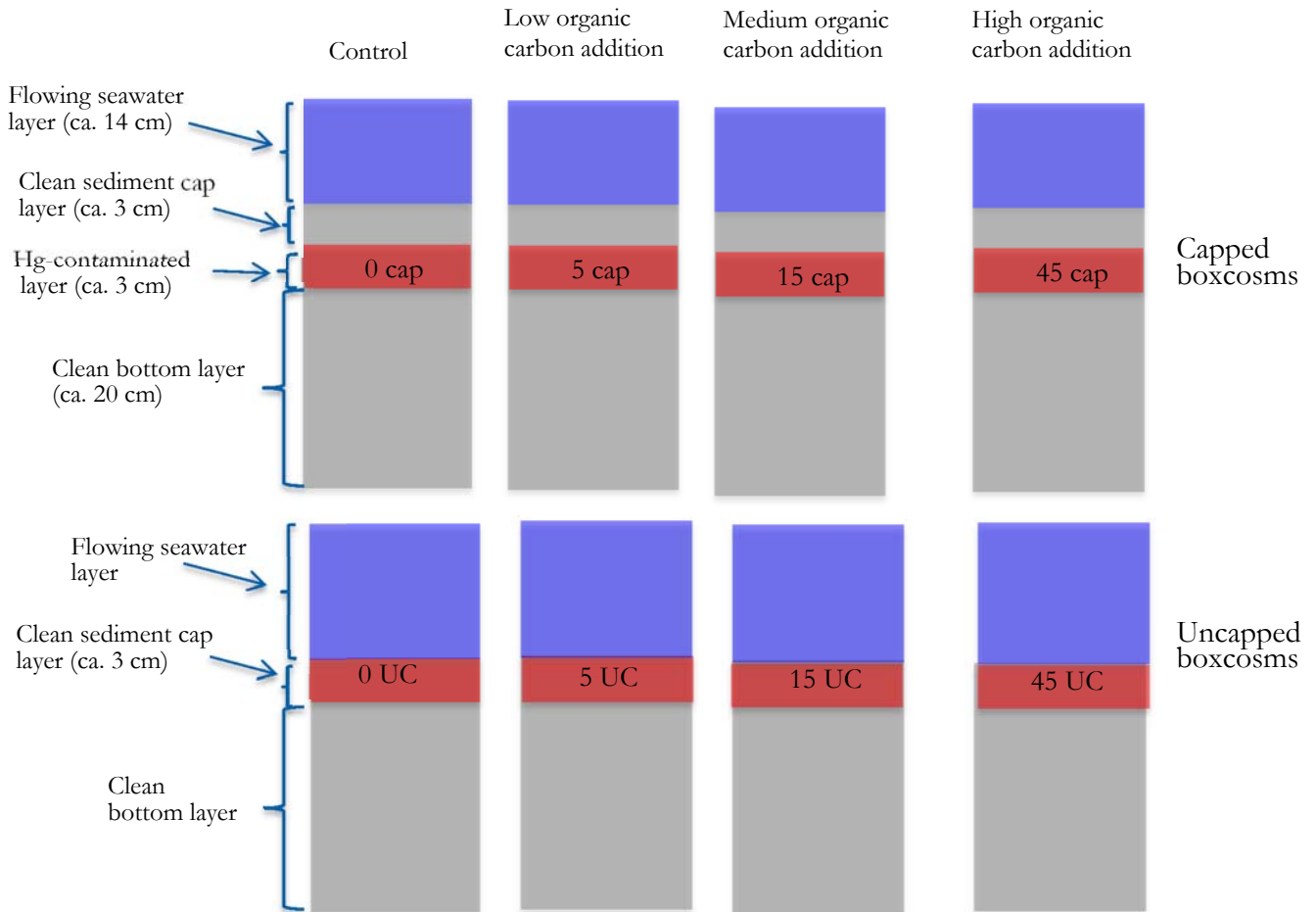

Figure 1. Experimental set up. The four Hg-contaminated sediment layers for the capped (top row) and the uncapped (bottom row) were amended with 0, 5, 15 and 45 g of pulverized chlorella algae. See text for details. A photo of the set up is shown in Figure 2.

4.4 Water and sediment sampling

The set up with eight boxcosm treatments and circulating fjord water was allowed to equilibrate for one week after which water and sediment core samples were taken for month 0. After one week, the biodegradation of the added algae is likely to proceed at high rates.

Figure 2. Photo of the boxcosm experimental setup depicted in the schematic in Figure 1 above

Surface water for Hg and MeHg flux measurements was sampled by inserting a silicon tubing into the box under the top cover and siphoning off 250 mL into an acid-cleaned, Teflon-coated polyethylene bottle containing pre-added trace metal grade (Merck, suprapur) HCl to acidify the water to 50 mM. Surface water was only sampled from the four uncapped boxcosms as sediment to water fluxes of Hg from boxcosms capped with clean sediment cap was expected to be minimal .

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 hollow cylinder with a 6 cm diameter (i.e. slightly larger than the coring cylinder) was inserted around the first core to ca. 3 cm deeper than the coring cylinder. A tight-fitting rubber cock was then inserted displacing the water 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 core was then pushed further into the sediment (to surface level) and then capped with a tight fitting rubber stopper to cover the open end. Thus, the purpose of the second cylinder was to prevent sediment collaps and to cover the hole created by the removed core. 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

Figure 3. Set up for microsensor depth profile measurements of O_2, H_2S and pH, showing O_2

for the sensor measurements (Figure 3).

4.5 Microsensor profile measurements

The measuring station comprised a single axis automated micromanipulator, potentiostat and 100 micron tip sensors for O_2, pH and H_2S (all from Unisense, Figure 3). Profiles of dissolved O_2, H_2S and pH in sediment pore water were obtained using the respective microsensors at a vertical resolution ranging from 0.1 to 1 mm.

The oxygen sensor was calibrated using a two-point calibration consisting of fully aerated and N_2-purged seawater for 100% and zero O_2 saturation respectively. The zero O_2 saturation was alternatively determined by inserting the microsensor a few millimetres into an anoxic sediment with overlying seawater).

The H_2S microsensor was calibrated with freshly prepared N_2-degassed Na_2S standards made up in pH < 4 citrate buffer. The concentration of the stock Na_2S standard solution was also calibrated using the classical Cline spectrophotometric method (Cline 1969).

The pH microelectrode was calibrated with appropriate pH 4, 7 and 10 buffers. After the sensor measurements, the bottom piston was slowly pushed to bring the core on level with the cylinder top thus draining off all the surficial water. One centimeter core section samples were then sliced by pushing the piston upwards. Each sample was put in an acid clean PE vial and immediately put in a freezer at -20 C until MeHg extraction and subsequent analysis.

4.6 Chemical analysis

4.6.1 Total Hg in sediments

Total Hg in sediments was analysed using a Lumex RA-915+ Hg Analyser coupled to a PYRO-915+pyrolyzer (Lumex Ltd., St. Petersburg, Russia). The pyrolyzer temperature was 520-580°C. The instrument was calibrated using PACS-2, marine sediment reference material-CRM (National Research Council-NRC, Canada), having a certified total Hg 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 analysed 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 % (wet wght.). The total organic carbon, estimated from loss on ignition (heating dried sediment in muffle oven at 550 °C for 12 hr) was 1.2 % (dry wght.). Previous studies from the same site have reported water content in the surface sediments of between 26 and 30% and TOC content of between 0.2 and 1.8 % (dry wght.) (Uriansrud et al. 2005).

The total Hg concentration in the sediment batch shipped from Fedje was 8.9 ± 1.8 mg/kg dry wght. (conc. ± 1 stdev, n=3). Liquid Hg droplets have been observed in previous analysis of sediments from the site (Øxnevad and Beylich 2013, Skei 2004, Uriansrud et al. 2005). No Hg droplets were observed in this study but their presence cannot be ruled out. Concurrent measurements of MESS-3 estuarine CRM (Certified Reference Material) with a certified total Hg concentration of 0.091 ± 0.009 mg/kg gave a concentration of 0.08 ± 0.014 mg/kg (n=3). Thus, the relative variation (standard deviation) in total Hg concentration was similar in the analysis of Fedje sediment and the reference material.

4.6.2 Sediment extraction of MeHg

Methylmercury (MeHg) 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 CH_2Cl_2, followed by back extraction into water.

4.6.3 Analyses of total and MeHg in water samples and sediment extracts

The prepared sample was then analysed for MeHg via distillation, ethylation, purge and trap pre-concentration, GC separation and CVAFS detection according to USEPA method 1630. Each batch of 30 sediment extracts included three blank and two marine sediment certified reference material (CRM) samples. The Sediment CRM used was ERM CC 580 from the European Commission`s Institute of Reference Materials and Measurements (Geel, Belgium).

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 by $KBr/KBrO_3$ oxidation, purge and trap and CVAFS according to USEPA Method 245.7 (rev 2.2, 2005).

The method detection limits (3 standard deviations of blanks) are 0.1 ng/L for total Hg 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) is less than 10 % for both total Hg and MeHg methods.

5. Results and Discussion

5.1 Main results on MeHg and sediment profiles of O_2 and H_2S

Figure 4 summarizes the change in MeHg concentration in the most contaminated sediment layers. The figure shows a very clear increase in the MeHg concentration with time and with increasing dose of organic carbon in both capped and uncapped boxcosms. The concentrations reach higher levels in capped than in uncapped sediments.

Most of the MeHg was found within or close to the Hg-contaminated layer (Figure 5), but high concentrations were occasionally also observed above or below the contaminated layers. This was most probably a result of inaccuracies in sediment preparation, including swelling or consolidation after thawing of frozen sheets, core sampling and core sectioning.

The micro-electrode measurements were frequently hampered by electrode damage due to the fragile tips of the electrodes breaking against hard grains of sand and gravel in the Fedje-sediment. Therefore the data-series are incomplete. Nevertheless, Figure 6 shows the depth profiles of O_2, H_2S and pH for the uncapped and capped boxcosms with highest organic carbon addition. These were also the boxcosms with the highest levels of MeHg (Figure 5) and the electrode measurements are suitable for documentation of the chemical environment.

Figure 6 shows that oxygen never penetrated more than a few millimetres into the sediments, neither in capped or uncapped treatments. Thus the lower part of the Hg-contaminated sediment in the uncapped and the whole of the Hg-contaminated layer in the capped layers were anoxic.

Hydrogen sulphide was detected in all capped boxcosms with added algae. The highest concentrations were observed in the high organic carbon treatment. In the uncapped boxcosms, H_2S was only detected in the boxcosms with the highest organic carbon content, but because of incomplete series the presence of H_2S also at lower carbon doses cannot be ruled out.

pH decreased from 7.5-8.0 near the sediment-water interface to frequent minima of 6.2-6.8 at sediment depth 10-20 mm. The minima appeared most strongly developed in the caps, possibly due to upwards diffusion of reduced species from the algae enriched layer.
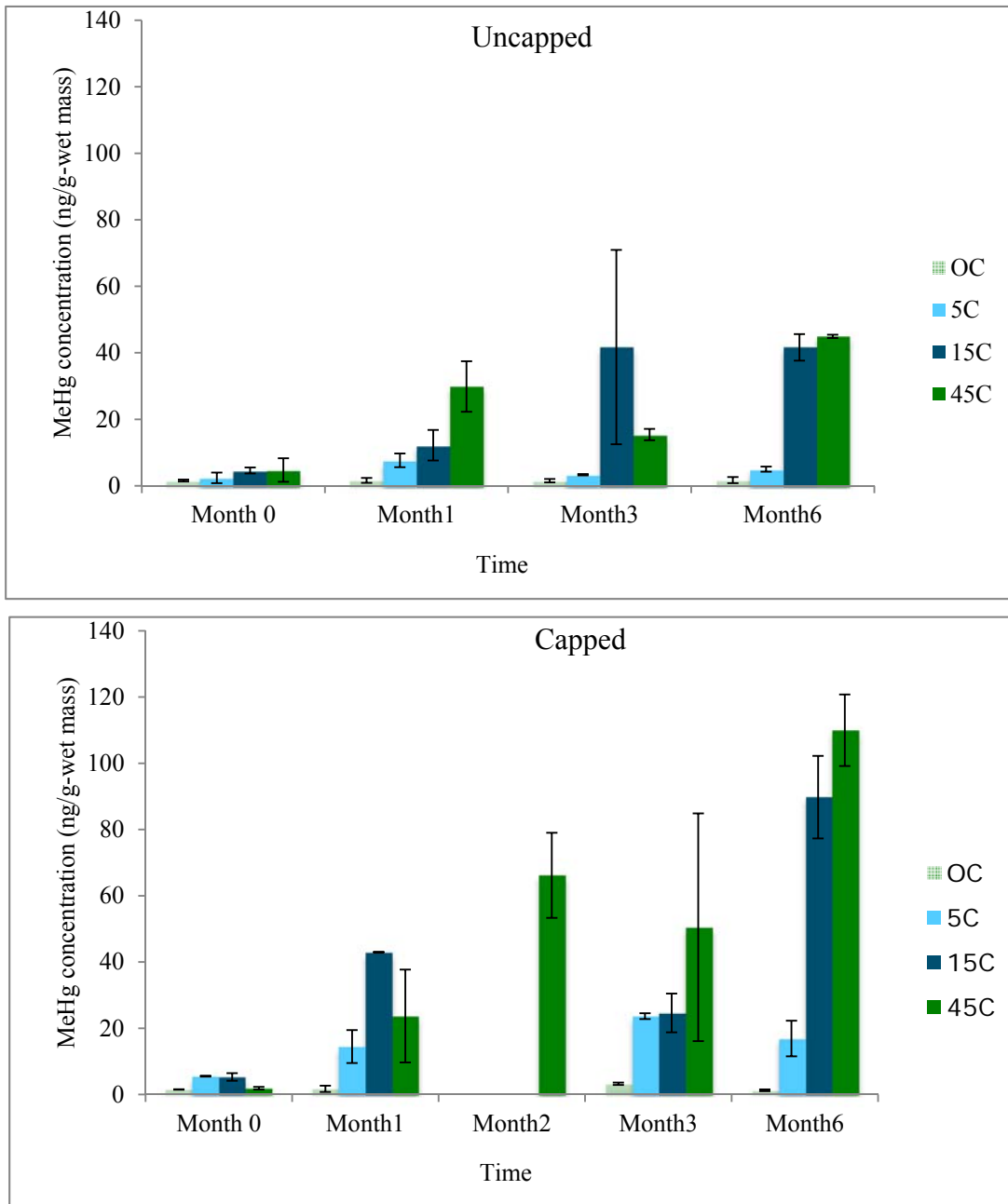
Figure 4. Time series for MeHg concentration in boxcosms containing a) uncapped and b) capped Hg-contaminated sediments. The bars represent the average of the two highest concentrations determined in the contaminated layers in each box. The whiskers show the range of these two determinations.
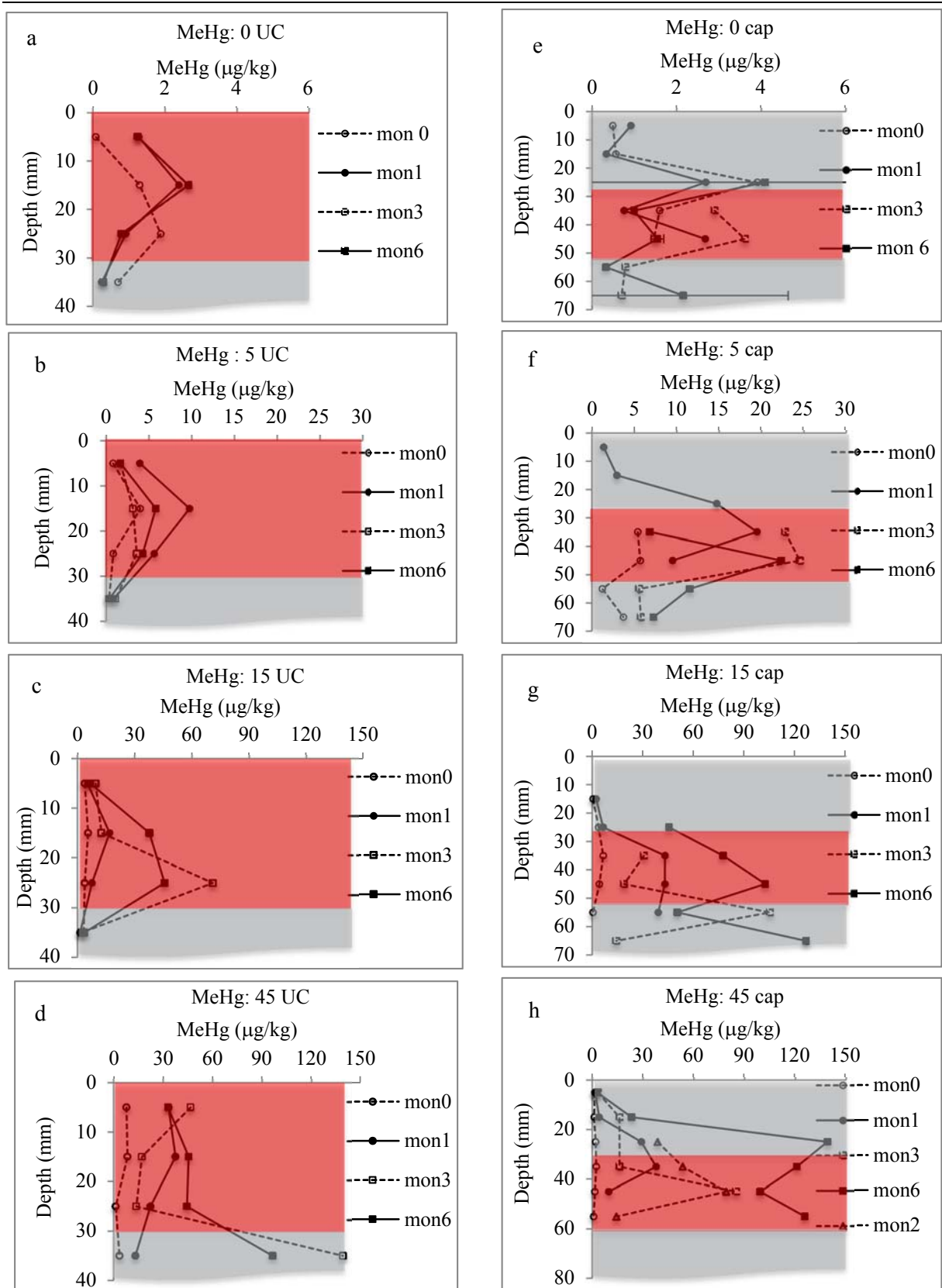
Figure 5. Depth profiles of MeHg concentration in cores from boxcosms: (a-d) uncapped; (e-h) capped. Each concentration is plotted at the mean depth for the respective 10 mm slice. The shading indicates the extent of the cap (grey), Hg-contaminated (red) and bottom (grey) sediment layers.
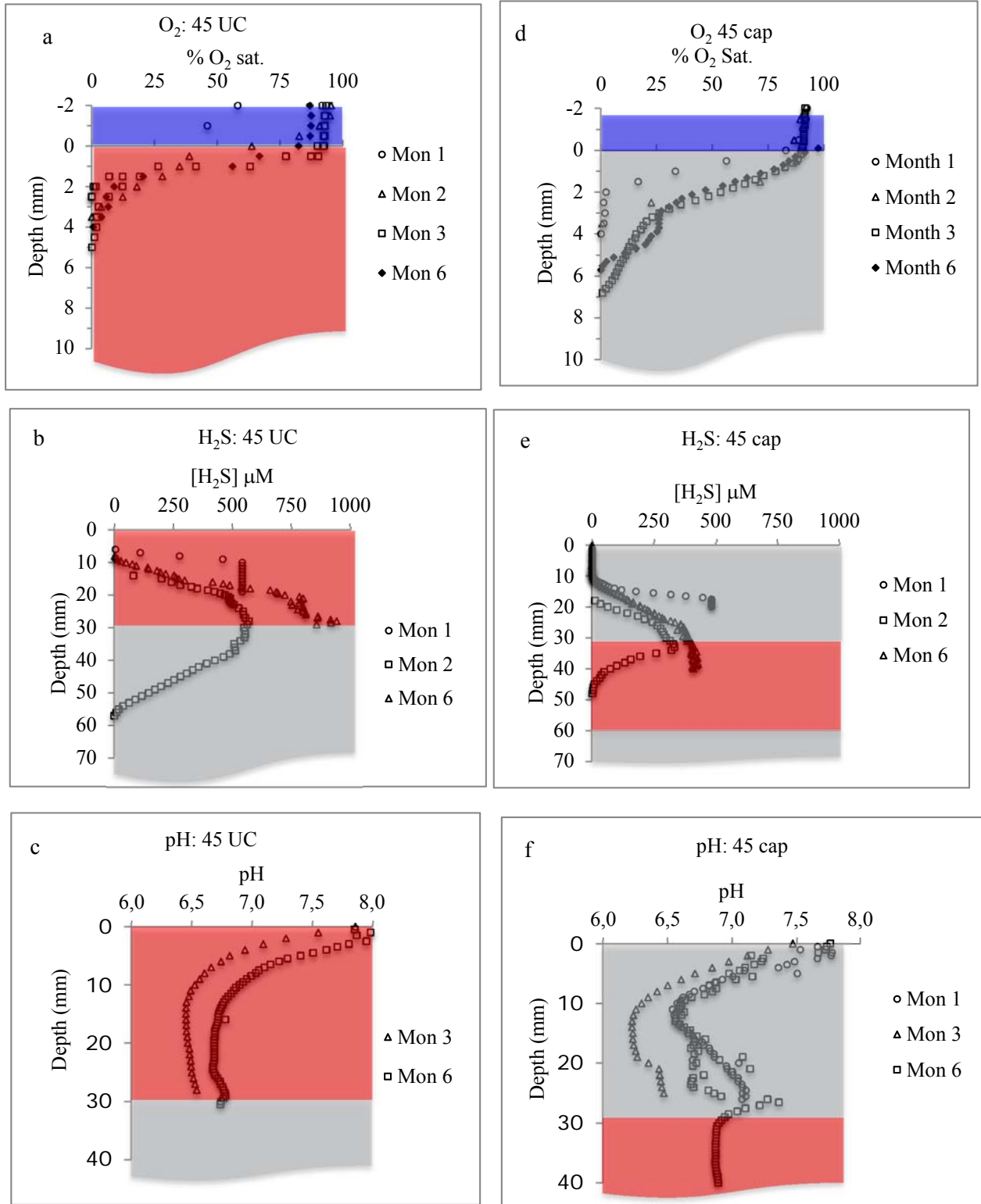
Figure 6. Depth profile microsensor concentration measurements of O₂, H₂S and pH from high carbon dose boxcosms: (a-c) uncapped; (d-f) capped. The blue shading on the O₂ profile indicates the concentration in the overlying water during measurement. Other shading indicates the approximate extent of: red, the Hg-contaminated sediment layer); gery, the cap and bottom sediment layers.

5.2 Mercury methylation rates

The methylation rates in the boxcosms were determined from linear regression analyses of the change with time of MeHg concentration in each boxcosm. In fig.7 regression lines, curve equations and correlation coefficients are shown for the same data as those presented in fig. 4, i.e. the average of the two highest MeHg concentrations in each boxcosm. In the capped boxcosms (fig. 7b), correlation coefficients (R^2) of 0.501, 0.812 and 0.912 for the low, medium and high carbon dose treatments, respectively, appeared to confirm a linear model, at least for medium and high dose. A similar result was obtained for the uncapped treatments, with correlation coefficients of 0.831 and 0.701 for the medium and high dose, respectively (Figure 7a).

The methylation rates determined as the slopes of the regression lines, varied from ± 0.0005 µg kg^{-1} day^{-1} in control boxes without algae additions to 0.16-0.52 µg kg^{-1} day^{-1} in the medium and high dose boxes. The highest rates were found in the capped treatments and a second regression between the calculated methylation rates and the carbon doses (fig. 8) showed good fit for the capped treatments ($R^2 = 0.848$), but less good for the uncapped treatments ($R^2 = 0,467$). Two factors may be inferred to contribute to less accumulation in the uncapped sediments. These are loss of MeHg by diffusion to the overlying water and slower methylation due to the presence of O_2 and/or absence of H_2S.

The lack of correlation between MeHg concentration and time for the low dose treatments ($R^2 \leq$ 0,36 and the low methylation rates ($\leq 0,053$) estimated for the whole six months period (fig.7), does not necessarily show that methylation was negligible in the low dose treatment. Considering the first six weeks only, mercury methylation rates of 0.2 µg kg^{-1} day^{-1} ($R^2 =$ in uncapped and 0.3 µg kg^{-1} day^{-1} in capped sediment can be seen from Figure 7. These rates were not much different from the rates derived for the whole period in the medium and high dose treatments. However, after the first six weeks further accumulation of MeHg was low in the low dose treatments and it appears that the low carbon dose was to small to sustain methylation for more than a few weeks.

In both low carbon dose treatments, the available organic matter (ca. 25 g carbon per meter squared of sediment) was able to methylate ca. 8 and 13 µg kg^{-1} of MeHg which corresponded to 0.1 and 0.2% of the total Hg (ca. 7 mg kg^{-1}). In the high dose treatments, however, the methylation continued throughout the experimental period to reach MeHg fractions of 0.5% and 1.5% for the uncapped and capped boxcosms respectively (Figure 7). Thus the nine times higher carbon dose appeared to provide 5-8 times higher concentrations of MeHg, which suggests that, given sufficient time for the process to proceed until the carbon is "burnt out" (labile fractions degraded), the production of MeHg will be roughly proportional to labile carbon dose.

The dependence of methylation on availability of labile organic carbon may also explain the difference between the capped and the uncapped boxcosms. Aerobic degradation is generally much faster than anaearobic degradation and rapid O_2 driven degradation in the uncapped sediments, may have reduced the availability of labile carbon for the methylation process.
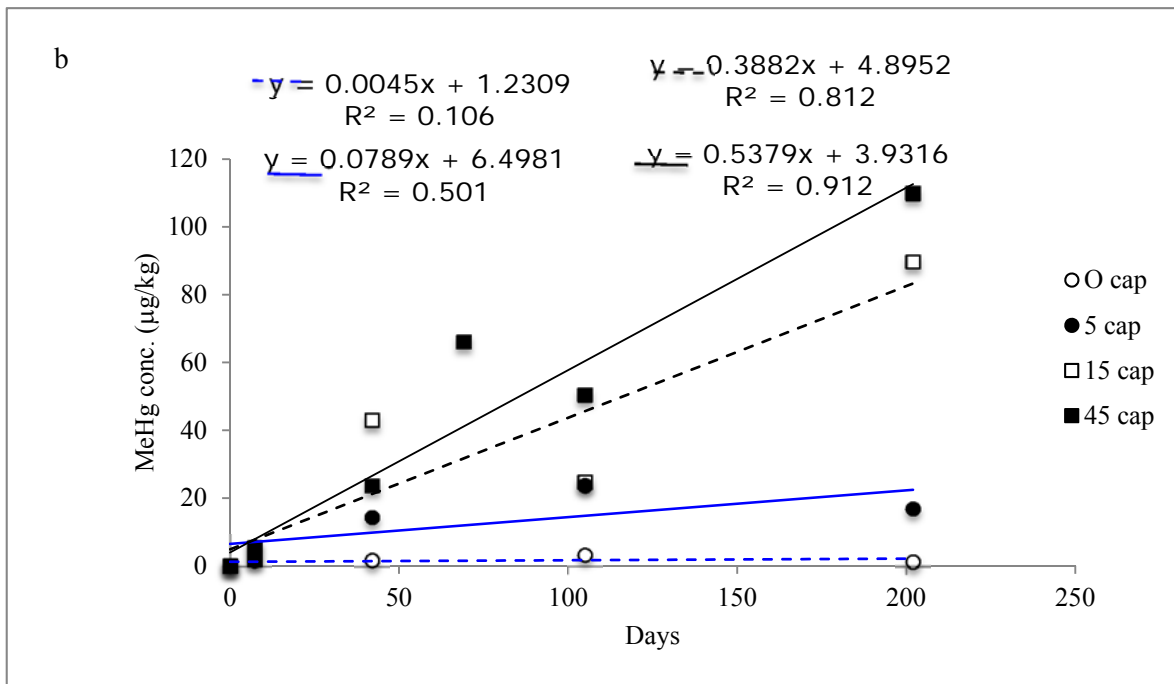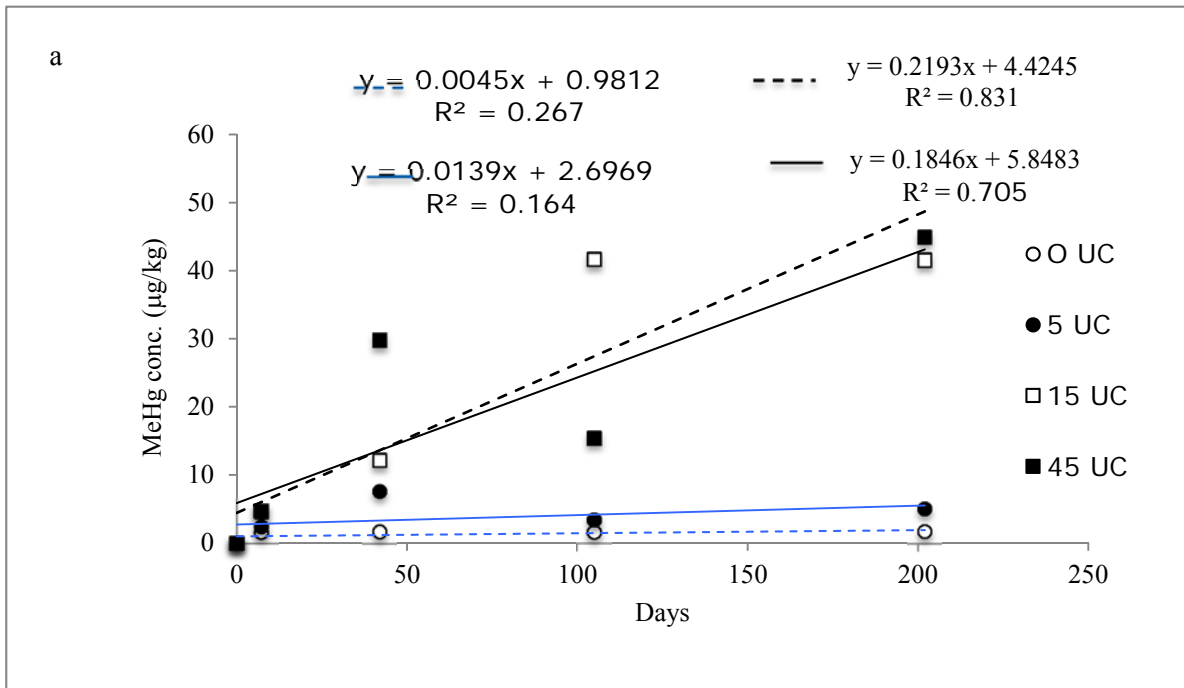
Figure 7. *Methyl Hg concentration change with time: (a) uncapped; (b) capped Hg-contaminated sediments. As in Figure 4, the MeHg concentration was chosen to be the mean of the two highest concentrations observed in each box.*
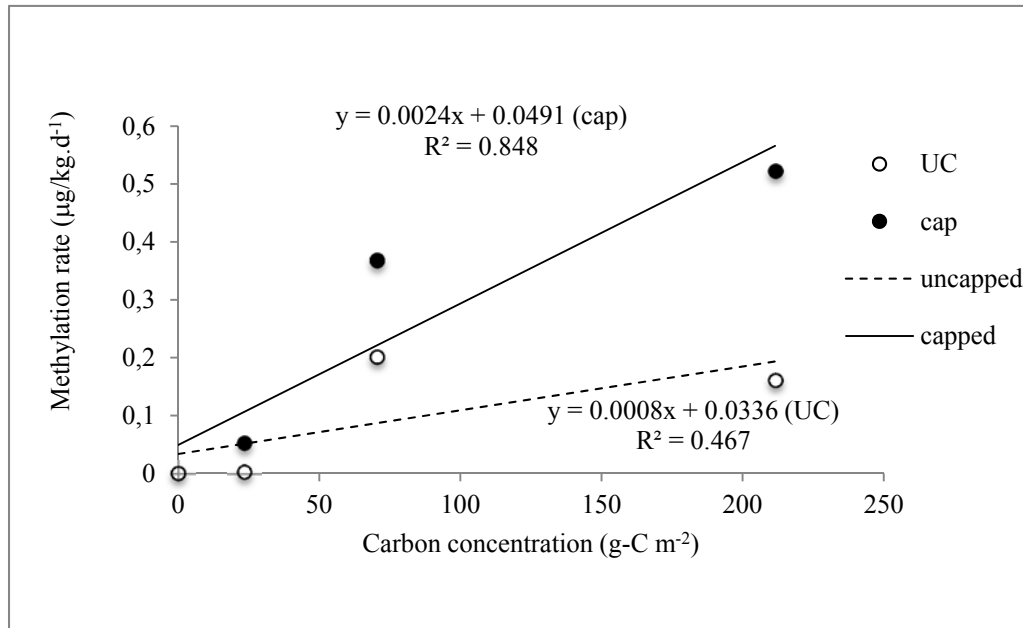
Figure 8. Mercury methylation rates for the six months experimental period as a function of organic carbon dose for capped and uncapped boxcosms. Results of linear regression analyses are shown by line equations and correlation coefficients (R^2).

5.3 Variation of methylation rate with depth

Mercury methylation rates were calculated for each separate depth interval based on the entire six months experimental period. The results showed that the highest rates generally occurred within or near the treated sediment layers and significantly higher in medium and high dose than in zero and low dose boxes (Figure 9). The low rates calculated for the low dose treatment may have been underestimated as a result of labile carbon being depleted after the first 1-2 months (ref. discussion above). Spurious high rates were observed at 3-4 cm depth in high carbon uncapped treatment, at 6-7 cm depth in the medium carbon capped treatment and at 2-3 cm depth in the high carbon capped treatment. Detailed interpretation of these single points on the vertical profiles is not recommended.
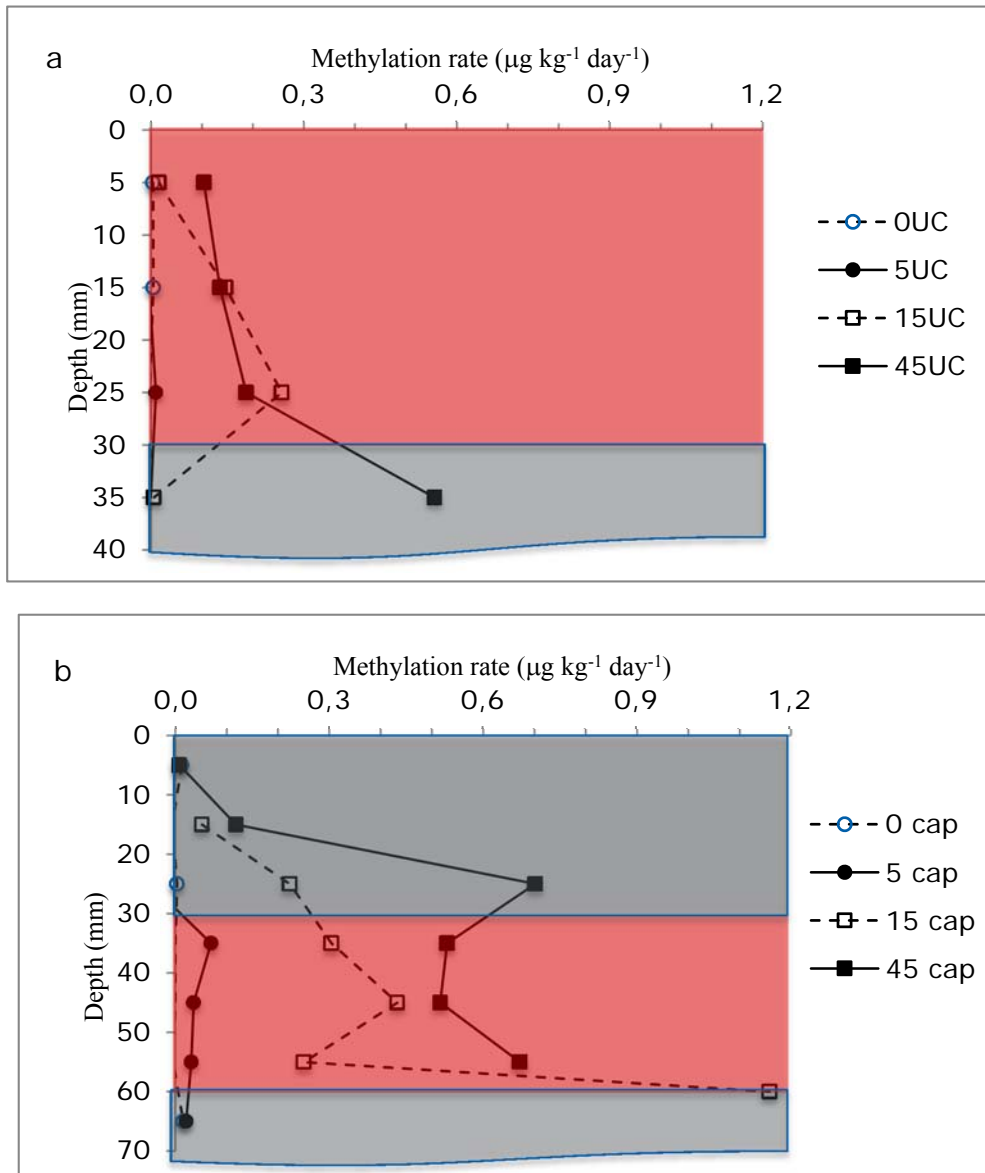
Figure 9. Depth profiles of MeHg formation rates: (a) uncapped; (b) capped boxcosms. The grey shading indicates the approximate extent of the cap and bottom layers; red, the Hg-contaminated sediment layer

5.4 Sediment to water fluxes of Hg and MeHg

The sediment-water fluxes were calculated from the difference in total Hg and/or MeHg concentration of surficial seawater entering and leaving the boxcosms at a flow rate of ca. 1 mLmin[-1] (see Figure 1). Both the total Hg and MeHg concentration were below the detection limits of 0.1 and 0.02 ng/L for total and MeHg respectively, in the source seawater (Outer

Oslofjord, 60 m depth), but clearly higher than detection limits in all samples drawn from the overlying water in the boxes.

In control box without algae added the fluxes of TotHg as well as MeHg were low compared to the boxes in which algae had been added. TotHg varied between 217 and 268 ng m^{-2} d^{-1} throughout most of the experimental period (fig. 10) with an increase at the last sampling. MeHg varied between 0.6 and 2.7 ng m^{-2} d^{-1}. Compared to the fluxes recently measured in sediments from Gunneklevfjorden, another location heavily contaminated with mercury (sediment in class V) (32-62 ng totHg m^{-2} d^{-1} and 0.10-0.32 ng MeHg m^{-2} d^{-1}) (Schaanning m.fl., 2015), the fluxes from the unamended Fedje sediment was approximately 5x higher.

Addition of organic carbon increased the sediment to water fluxes compared to the control (Figure 10). In the boxes with added algae the fluxes of Tot.Hg tended to decrease during the first three months, but in the low dose treatment this trend was perturbed by an increase between month 0 and month 1. The fluxes of meHg were less variable during the experimental period, but in all treatments the highest fluxes were observed in the beginning of the experimental period, i.e. 0-1 month after set-up.

For MeHg, the correlation coefficient (R^2) of 0.925 showed that the increase of the flux was closely correlated with the dose of labile algae carbon (figure 11). Omitting two extreme high values (high dose, 1 and 2 months after set-up) and the two lowest, this correlation was based on 19 of 23 fluxes of MeHg determined. The flux of TotHg also increased significantly ($p<0.05$) with increasing addition of algae carbon, but the correlation was less accurate ($R^2=0.366$).

MeHg:TotHg flux ratios are shown in Table 1. Compared to the ratio of 0,006% in the original Fedje sediment, all flux ratios were high, which is reasonably explained by the difference in water solubility of the two species. A very high ratio (100%) was observed in the water sample drawn one month after set-up and the subsequent ratios declining gradually to 51% on month 2, 18% on month 3, 12% on month 4 and 6.3% on month 6 indicated a major difference between the high dose and the other treatments which was also supported by the appearance of bacteria colonies on the sediment surface indicating a sediment heavily overloaded with labile organic carbon. The release of MeHg from the sediments and the dependence on the dose of labile carbon available was consistent with increased rates of methylation observed in the uncapped sediments (figure 8 and 9). However, the increased flux of MeHg could by far account for the increased flux of TotHg. In control, low and medium dose treatments MeHg:TotHg flux ratios varied between 0.17 and 3,8% with a median ratio of 0.3-2,7% (Table 1). Thus the addition of labile algae carbon must have lead to the production of a relatively soluble Hg-specie other than MeHg. Complexation by natural, dissolved organic matter can increase the solubility of mercury under anoxic, dark conditions (Baohua et al., 2010) and a similar process appears to have occurred in this sediment. The exact nature of this specie cannot be derived from the present study.
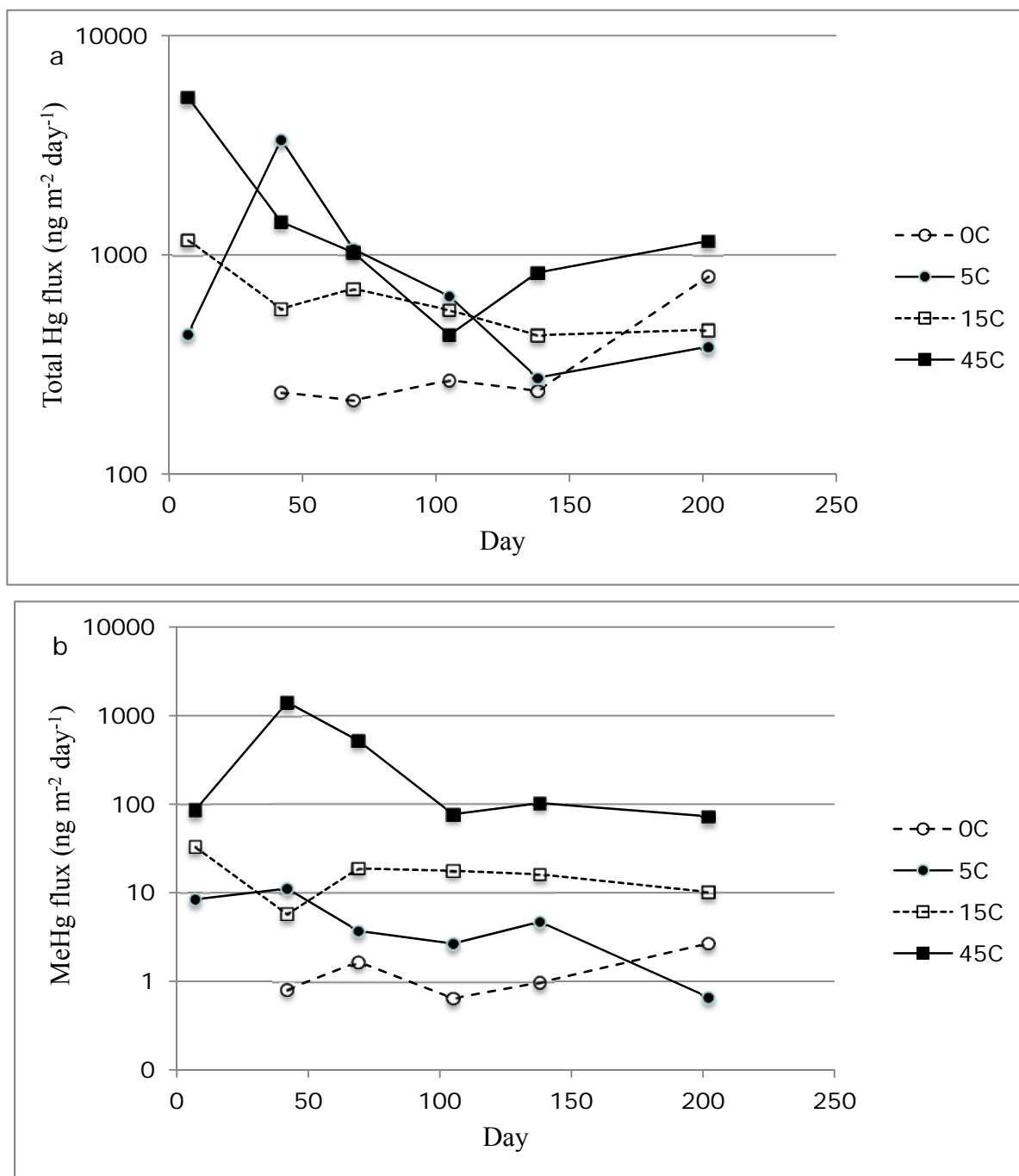
Figure 10. *Fluxes of total Hg (a) and MeHg (b) from sediment to overlying water in uncapped Hg-contaminated sediments amended with 0 (control), 5, 15, and 45 g of pulverized algae. Note that flux axis are in log scale.*
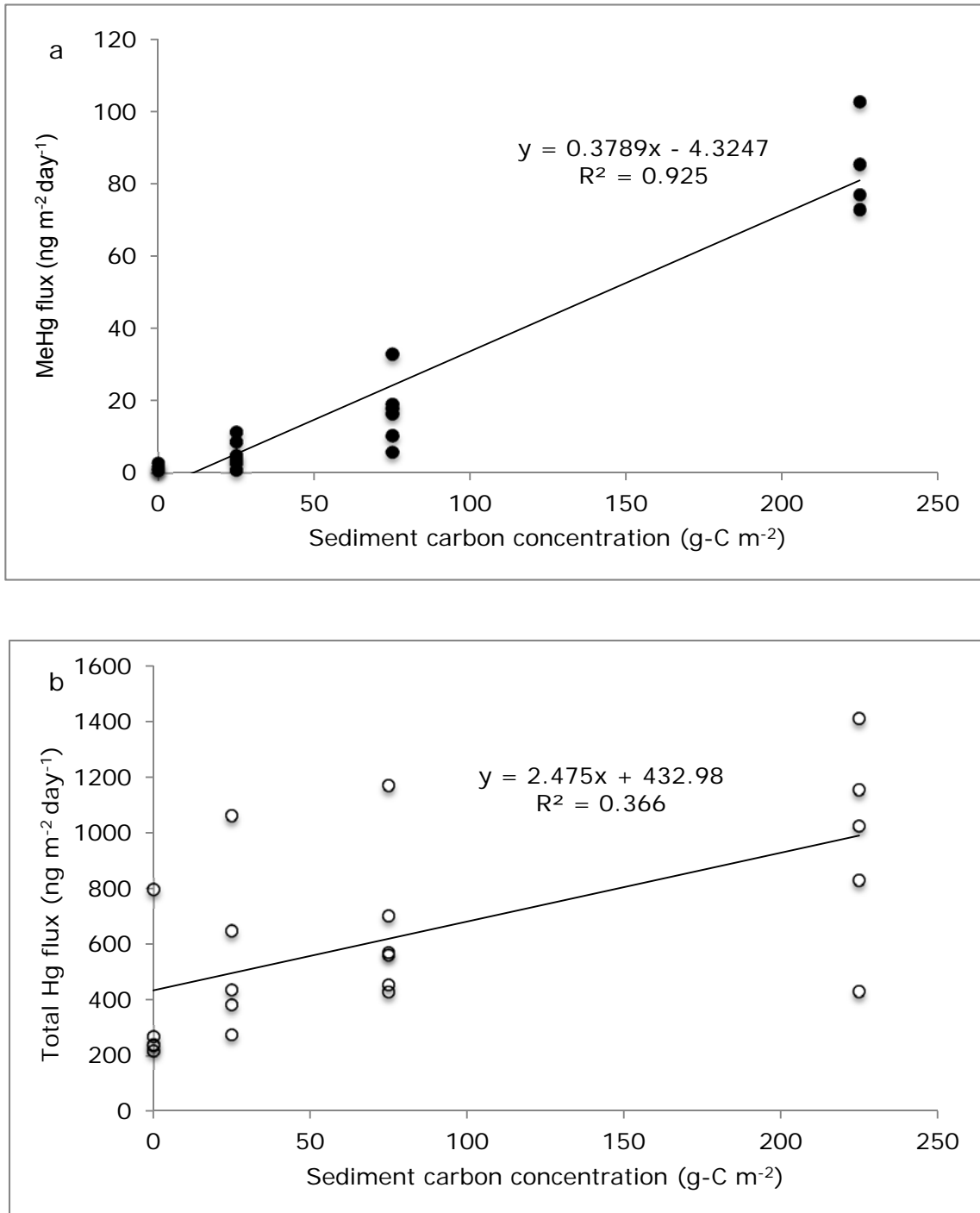
Figure 11. Fluxes of MeHg (a) and total Hg (b) from Hg-contaminated sediments to surficial water from uncapped boxcosms as a function of added organic carbon. See Table 1 and text for details.

Table 1. MeHg:TotHg flux ratios in uncapped Fedje sediments with 0-225 g m^{-2} of labile algae carbon mixed into the 0-3 cm top layer.

Days/g TOC	0	25	75	225
7	-	1,9 %	2,8 %	1,6 %
42	0,3 %	0,3 %	1,0 %	100,1 %
69	0,8 %	0,3 %	2,7 %	51,0 %
105	0,2 %	0,4 %	3,2 %	17,9 %
138	0,4 %	1,7 %	3,7 %	12,4 %
202	0,3 %	0,2 %	2,2 %	6,3 %
Median	0,3 %	0,4 %	2,7 %	15,1 %

6. Conclusions and proposed follow up studies

6.1 Conclusions

We observed extensive net methylation in all boxcosms where organic carbon was added (as pulverized chlorella algae) to Hg-contaminated sediments collected from the U864 wreck site. Methylation of in situ Hg in the contaminated sediment layer was observed in both open (uncapped) boxcosms and in boxcosms that were capped with clean clay sediment layer. Capping of the Hg-contaminated layer increased both the extent and rate of Hg methylation (compared to uncapped controls). No significant methylation of Hg was observed in Hg-contaminated sediments without any added organic carbon (both capped and uncapped). There was extensive anoxia and hydrogen sulphide build up under high carbon dose capped and uncapped boxcosms strongly suggesting that Hg methylation was aided by SRB.   Addition of organic carbon also increased the flux of both total Hg and MeHg to the surficial water in the uncapped boxcosms. The fluxes of both total and MeHg to the surficial water were linearly correlated to the organic carbon concentration in the Hg-contaminated Sediment layer.

6.2 Proposed follow up studies

Tentative results from this study indicate that net methylation of in situ inorganic Hg in the seabed sediments from the U864 submarine wreck site is possible if organic carbon is introduced. The Hg-contaminated sediments from the site in this short study were amended with a relatively labile form of organic carbon (pulverized *Chlorella s.p.* algae). We were therefore able to observe microbial-aided Hg methylation by SRB (and possibly other Hg methylating microbes) in a relatively short experimental period. It would therefore be interesting to repeat the study over a longer time period using hydrocarbon and/or diesel oil similar to the one in the U864 wreck and also vary the Hg concentration. The findings of such a study would be of high general interest for anyone involved in risk assessments on oil-bearing shipwrecks in any coastal sediment contaminated with Hg.

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