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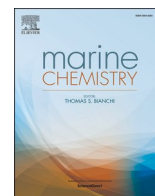
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HgCl₂ addition to pore water samples from cold seeps can affect the geochemistry of dissolved inorganic carbon ([DIC], δ¹³C_{DIC})

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ABSTRACT

The conventional use of mercuric chloride (HgCl₂) as an antimicrobial agent in water samples for dissolved inorganic carbon (DIC) analysis poses health and environmental risks related to its handling and disposal. Even though there is an increasing interest in quantifying pore water DIC contribution to the ocean carbon cycle and deep-sea acidification, the paucity of comparative studies on marine pore waters prevents the modification of sampling protocols and HgCl₂ still remains widely used. Here, we compared DIC concentrations and δ¹³C_{DIC} composition in pore water samples from methane seepage areas in the Barents Sea and offshore N. Svalbard. Samples were extracted using 0.15 μm rhizon filters and split into two aliquots with 2–3 replicates each. Only one aliquot was treated with 10 μL saturated HgCl_{2(aq)}} and all samples were stored in the dark at 4 °C, prior to measurements ~30 days later. The samples yielded extremely wide ranges of DIC concentrations, from 1.8 mM to 20.1 mM, and δ¹³C_{DIC} values, from −36.0‰ to −1.6‰ (VPDB), due to variable contributions of methane-derived DIC to the pore water system. Overall, we obtained lower [DIC] (77% samples; N = 26) and heavier δ¹³C values (79% samples; N = 42) in Hg-treated samples. Isotopic and concentration differences larger than the uncertainties on the mean of replicated measurements (±0.2–0.5‰; ± 0.5 mM) and analytical precision (0.15‰; 0.71 mM) represent the 38% and 19% of the samples, with offsets of up to 7.4‰ and 1.9 mM, respectively. The largest offsets are in agreement with our CO₂-degassing model, suggesting an interaction between mercuric chloride and dissolved hydrogen sulfide released by sulfate-driven methane oxidation. We therefore caution against further use of HgCl₂ for DIC studies of marine pore waters from cold seeps.

1. Introduction

Anthropogenic global warming is already altering the carbon fluxes across the Earth's reservoirs, i.e. geo/bio/atmo/hydro/biosphere (Joos et al., 1999; Canuel et al., 2012). Tracing these variations is of fundamental importance to reduce the uncertainty of carbon cycle models and obtain accurate long-term predictions of global temperatures (Quetin et al., 2020). Carbon dioxide is continuously exchanged between the atmosphere and the upper ocean, and the seawater CO₂-carbonate system performs an effective buffer mechanism for climate change through equilibrium reactions involving the four inorganic carbonate species CO₂, H₂CO₃, HCO₃⁻, CO₃²⁻. Investigating the spatial and temporal variations in distribution and concentration of the dissolved carbonate species previously mentioned, known as dissolved inorganic carbon

(DIC), is key to understand the anthropogenic impacts on the carbon cycle and associated carbon fluxes in/out of the ocean at present-day and in future scenarios.

An increasing number of studies highlighted the occurrence of significant DIC fluxes from sediment pore waters, especially in areas of hydrocarbon seepage where anaerobic oxidation of methane (AOM) (Boetius et al., 2000) and/or the degradation and respiration of petroleum in the subsurface release large amounts of isotopically-depleted carbon (Zhang et al., 2019; Feng et al., 2020; Garcia-Tigreros et al., 2021; Xu et al., 2021). Hydrocarbon seepage systems are widespread along continental margins (Suess, 2018; Phrampus et al., 2020), and there is increasing interest in quantifying the contribution of sediment pore water discharge to the marine carbon cycle (Taniguchi et al., 2019) and deep-ocean acidification (Garcia-Tigreros et al., 2021). Therefore, it

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is particularly important to assess the conventional sampling techniques for DIC measurements of marine pore waters in these peculiar settings in light of recent instrumental advances which enable high-resolution molecular and isotopic determinations of aqueous components.

To preserve the original DIC geochemical composition of marine pore water samples prior to analyses, it is common practice to add a chemical fixative to stop microbial degradation of organic compounds which would otherwise lead to changes in DIC concentration and $\delta^{13}\text{C}_{\text{DIC}}$ during storage (Kroopnick, 1974; McCorkle et al., 1985). Sample fixation is conducted onboard soon after pore water extraction and it requires the use of antimicrobial agents such as mercuric chloride (HgCl_2), copper sulfate (CuSO_4) and benzalkonium chloride (BAC) ($\text{C}_9\text{H}_{13}\text{ClNR}$), firstly tested in pathology and microbiology fields and then established for the treatment of seawater and freshwater matrices for environmental studies. An alternative method involves injection of the sample into a pre-acidified 12 mL Exetainer containing H_3PO_4 in which the dissolved DIC is converted to free CO_2 and the resultant low sample pH inhibits microbial activity. This approach removes the need of toxic antimicrobial agents and provides reliable isotopic results over 6 months (Taipale and Sonninen, 2009) but it increases the overall costs due to the large volumes of acid used. After the publication in 2007 of standard operating procedures (SOPs) for the analysis of seawater

carbonate parameters (Dickson et al., 2007), the use of HgCl_2 saturated solution became the conventional preservation method and is still the most widely used fixative in marine biogeosciences (Gloël et al., 2015; Olack et al., 2018; Cheng et al., 2019; Mos et al., 2021). Mercuric chloride is very toxic to humans and other animals, affecting the central nervous system, gastrointestinal tract, and the kidneys (Vaidya and Mehendale, 2014). It is rapidly absorbed via skin and mucous membranes, and it is slightly volatile at room temperature, thus requiring cautious handling, wearing full personal protective equipment and dedicated fume hoods. DIC sample fixation using HgCl_2 poses serious health risks to the researchers and is costly in terms of safe handling and proper disposal (Mos et al., 2021). Due to its acute toxicity, environmental persistence (Kim and Zoh, 2012) and bioaccumulation (Morel et al., 1998), finding safer and environmental friendly alternatives is of great interest to biogeoscientists.

A recent comparative study indicated that filtration of water samples from different matrices, i.e. seawater, estuarine water, freshwater, and groundwater, through $0.45\ \mu\text{m}$ disposable filters without HgCl_2 treatment can provide reliable Alkalinity values over 6 months of storage time (Mos et al., 2021). The authors also evaluated whether the amount of HgCl_2 affects total Alkalinity by comparing different concentrations ranging from 0.002% to 0.5%, during a month of storage, and they

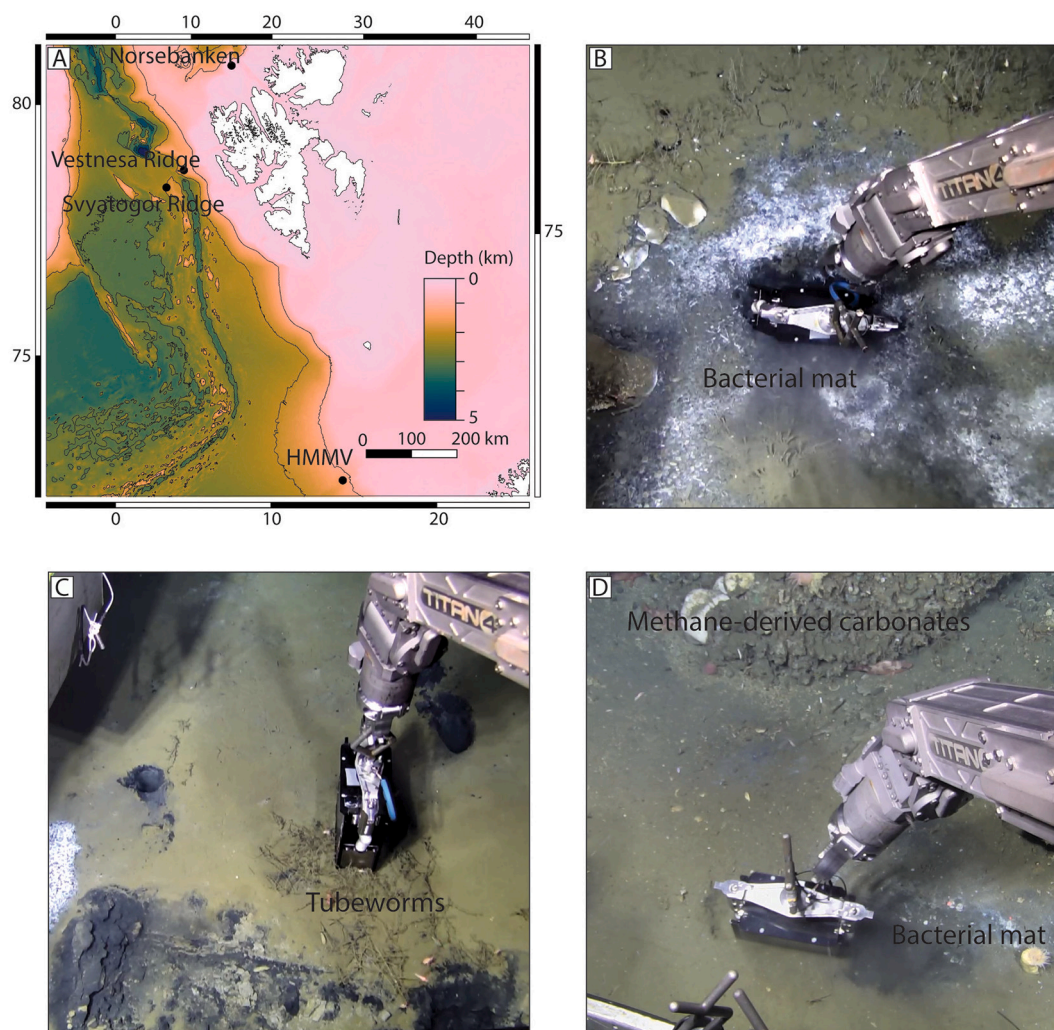


Fig. 1. Location of the sampling sites and seafloor imagery from the methane seepage areas. A) This study used sediment pore waters from four areas distributed along the western Barents Sea margin and offshore Svalbard. B) Blade core CAGE22–2-KH-04_Dive09-Blac-01 was collected from bacterial mats. C) Blade core CAGE22–2-KH-04_Dive11-Blac-04 was collected from a tubeworm habitat close to bacterial mats (bottom left corner). D) Blade core CAGE20–7-KH-01_Dive11-Blac-04 was collected from a bacterial mat. ROV imagery for CAGE22–2_Dive24 is not available. The map in (A) and all figures in this paper use a color scale accessible to people with color vision deficiencies (Cramer et al., 2020).

found that higher concentrations do not seem to improve the preservation of filtered samples. Another study on freshwater samples from ponds and a karst spring system suggested that filtration at 0.2 μm is equally effective as acidification with H_3PO_4 or addition of BAC and to a lesser extent HgCl_2 for preservation of $\delta^{13}\text{C}_{\text{DIC}}$ (Wilson et al., 2020). Similar observations were also reported from lake samples in the Shan'xi Province, China (Li and Liu, 2011), whose DIC carbon isotopic values remained stable for up to 90 days in samples filtered through 0.4 μm and stored at 0–4 °C. In that study, $\delta^{13}\text{C}_{\text{DIC}}$ in water samples treated with HgCl_2 became more positive with increased storage time possibly due to some residual bacterial activity. Following this suite of observations, it is needed to continue testing and comparing the preservation methods on samples from different environments, including marine pore waters which are still under-represented in the literature, to fully evaluate the possibility of the cessation of the mercury-poisoning practice in marine biogeochemical studies or to set limits of applicability.

This paper evaluates the effect of HgCl_2 addition on DIC concentration and $\delta^{13}\text{C}$ composition of marine pore water samples collected from 4 methane seepage locations in the Barents Sea and Arctic Ocean offshore N. Svalbard (Fig. 1A). Cold seeps offer the opportunity to investigate fractionation processes over a wide range of DIC concentrations and isotopic compositions, generated by the release of methane-derived carbon into pore waters. In this study, pore water samples were extracted from marine sediments from active seepage areas hosting chemosynthetic habitats (Fig. 1B, C, D), and then split into 2 experimental conditions, one of which was treated with HgCl_2 . The results after 1 month of storage show an evident negative effect of HgCl_2 addition on the pore water DIC geochemistry under certain conditions and caution against the use of HgCl_2 in biogeochemical studies of cold seep pore waters, to reduce analytical uncertainties and the health and environmental risks associated with the use of this toxic compound.

2. Materials and methods

2.1. Sediment sampling

Marine pore water samples were collected from sediment cores during research expeditions CAGE20–7 (2020), CAGE21–1-AKMA (2021) and CAGE22–2-AKMA (2022) on the research vessel Kronprins Haakon (Table 1). Sediment cores were obtained via gravity coring, multicoring and remotely-operated vehicle (ROV) blade and push coring. Gravity core CAGE22–2-KH-03-GC-03, hereafter named GC-03, was collected using a 6 m long corer. A plastic liner with an inner diameter of 10 cm was inserted into the steel barrel prior to operations and blocked inside with a core catcher and cutter. After sampling, the core liner was pulled off the barrel and cut into 1 m sections. Sections were then taken to a chill room at 4 °C for pore water extraction. Multicores CAGE20–7-KH-01-MC-05 and CAGE22–2-KH-03-MC-01, named MC-05 and MC-01, were collected using a KC Denmark DK8000 (KC Denmark A/S) hosting six parallel 70 cm long tubes with a diameter of 10 cm. After sediment sampling, a liner dedicated to pore fluid samplings was taken out of the sampling device, capped and moved to the chill lab. We used the ROV

Ægir6000 to collect blade cores CAGE20–7-KH-01_Dive13-BlaC-01, CAGE22–2-KH-04_Dive09-BlaC-01, CAGE22–2-KH-04_Dive11-BlaC-04, and push cores CAGE21–1-KH-06_Dive24-PusC-07 and CAGE21–1-KH-06_Dive24-PusC-09, hereafter named Dive13-BlaC-01, Dive09-BlaC-01, Dive11-BlaC-04, Dive24-PusC-07 and Dive24-PusC-09, respectively (Table 1). Blade corers have a rectangular base of 25 cm \times 10 cm and collect a volume of sediment up to $\sim 8000\text{ cm}^3$. Push corers have a round section of 8 cm diameter and contains up to $\sim 2800\text{ cm}^3$ of sediment. Push corer and blade corer liners were pre-drilled with a resolution of 1–2 cm prior to operations to facilitate a rapid pore water extraction.

2.2. Pore water extraction

Pore waters were extracted in a chill lab at 4 °C immediately after core recovery, and processed within 30 min from sampling (Fig. 2). Samples were collected from 2 to 3 core depths in cores Dive13-BlaC-01, Dive09-BlaC-01, Dive11-BlaC-04, GC-03 and MC-01, whereas a higher down-core sampling resolution was applied to MC-05, Dive24-PusC-07 and Dive24-PusC-09. Pore waters were extracted using rhizon soil moisture samplers type CSS (article number 19.21.23F; rhizosphere.com). Rhizons consist of a 5-cm long filter made of inert polymer with average pore size of 0.15 μm , flat tip and outer diameter of 2.5 mm, connected to a PE/PVC tubing. The rhizon is wetted in MilliQ® water prior to use and inserted into the sediment core through 3 mm holes drilled on the liners. Water samples are extracted by the vacuum created using 10 mL HSW Norm-Ject® sterile syringes attached to the rhizon and kept open with a wooden stopper. For core MC-05, samples were collected using 5 mL PET BD Vacutainers®. The vacuum inside of the tubes facilitates the drawing of liquids from the sediments and eventually collected in the tubes. In that case, samples for *No-Hg* and *Hg* were collected separately, removing the tube from the rhizon and re-attaching a new one for the second sampling. The second aliquots were treated with HgCl_2 . We are aware of the fact that rhizon samplers can cause some loss of $^{12}\text{CO}_2$ enriched DIC in the extracted pore waters due to degassing (Schrum et al., 2012; Steiner et al., 2018). For this experiment this does not affect the main interpretations since this process would affect both *No-Hg* and *Hg* aliquots of each sample in the same way. We minimized any potential effect on consecutive vacutainer samplings as well as other syringe samples by pre-soaking the rhizons (Steiner et al., 2018). After sample collection, pore water samples were transferred into 2 mL glass scintillation vials and sealed with screw cap with butyl rubber septa. Vials were filled to the top to avoid CO_2 outgassing which would cause DIC loss as well as carbon isotopic fractionation (Wilson et al., 2020). We added 10 μL of HgCl_2 saturated solution to *Hg* samples. The HgCl_2 solution was prepared in advance by dissolving $\sim 10\text{ g}$ of mercuric chloride crystals into 100 mL distilled water. For each *No-Hg* and *Hg* aliquot, we prepared 2–3 replicates depending on the recovered volume of water, except for samples collected from MC-05, Dive24-PusC-07 and Dive24-PusC-09 due to low extracted volumes. All samples were stored in the dark at 4 °C.

Table 1

Location of sediment cores included in this study.

Core	Expedition	Area	Latitude (DD)	Longitude (DD)	Water depth (m)	Comments
Dive09-BlaC-01	CAGE22–2-AKMA	Svyatogor Ridge	78.3925	5.0827	1933	Bacterial mat
Dive11-BlaC-04	CAGE22–2 AKMA	Svyatogor Ridge	78.3920	5.0843	1941	Tubeworms
GC-03	CAGE22–2 AKMA	Vestnesa Ridge	78.7008	7.0148	1392	Gas hydrates
MC-01	CAGE22–2 AKMA	Vestnesa Ridge	78.7219	7.0322	1380	reference
Dive24-PusC-07	CAGE21–1- AKMA	HMMV	72.0180	14.7035	1262	reference
Dive24-PusC-09	CAGE21–1-AKMA	HMMV	72.0180	14.7034	1263	reference
MC-05	CAGE20–7	Norsebanken	80.5922	14.4276	175	reference
Dive13-BlaC-01	CAGE20–7	Norsebanken	80.5923	14.4269	174	Bacterial mat

HMMV = Håkon Mosby mud volcano; BlaC = Bladecore; GC = gravity core; MC = multi-core; PusC = puscore. Gravity core GC-03 hosted gas hydrates in the core catcher upon recovery. All the cruise reports are available at cage.uit.no.

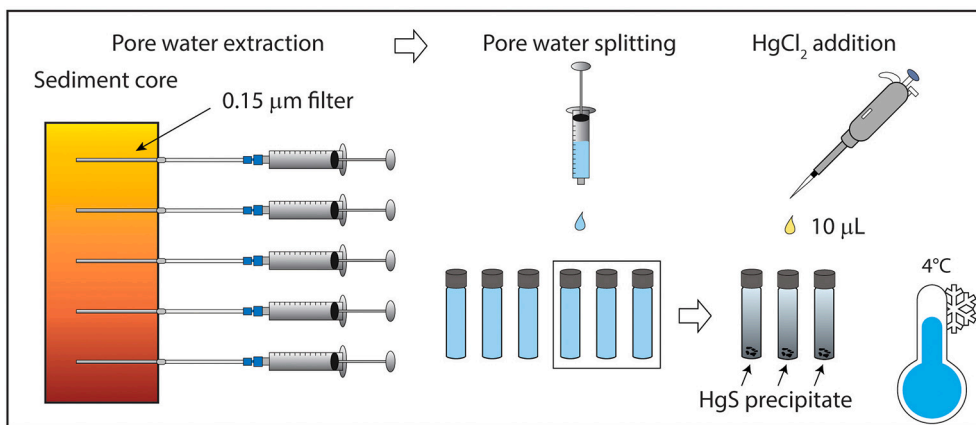


Fig. 2. Schematic representation of the pore water extraction and fixation using mercuric chloride in this study. Samples are extracted using rhizons soil moisture samplers with average pore size of 0.15 μm and then split into 2 aliquots (2 to 3 replicates each). 10 μL are added to one aliquot and induce precipitation of HgS in sulfide-rich samples (see text for explanation). All samples are stored at 4 °C until analyses conducted a month later.

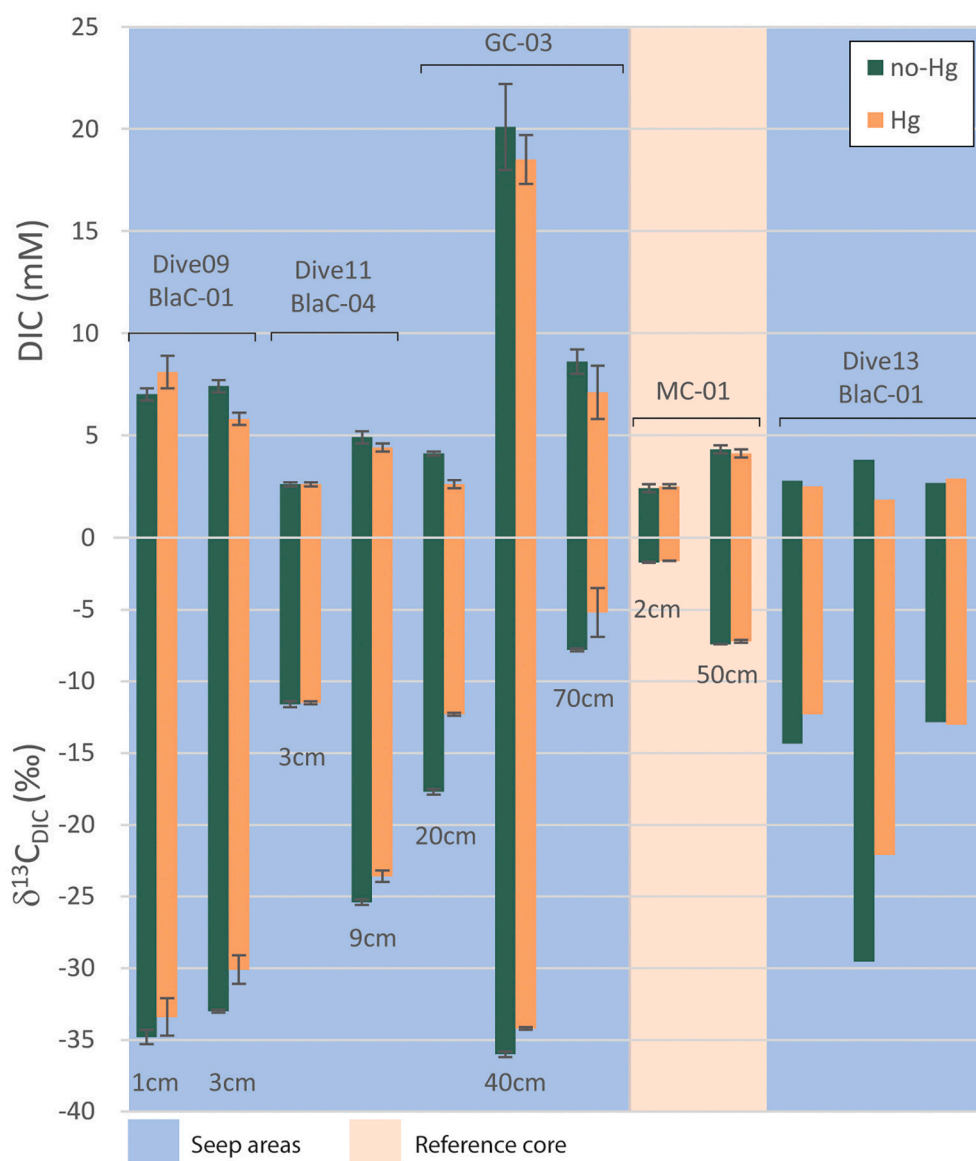


Fig. 3. Comparison of the DIC concentration and carbon isotopic composition ($\delta^{13}\text{C}_{\text{DIC}}$) of pore water samples with HgCl_2 addition (*Hg*) and without it (*no-Hg*). Error bars correspond to the 1 standard deviation of replicates.

2.3. Pore water analysis

Analyses of samples collected from CAGE20–7 and CAGE22–2-AKMA expeditions were conducted at the Stable Isotope Laboratory-SIL at the Centre for Arctic Gas Hydrate, Environment and Climate (CAGE) located at UiT-The Arctic University of Norway, in Tromsø, Norway. 5 drops of water-free phosphoric acid (H_3PO_4) were added to 4.5 mL vials and the vials were then flushed with helium. 150–300 μL of sample was transferred into the vial using a syringe and let equilibrate at 27 °C for >18 h prior to analysis. Measurements of the isotopic composition of released CO_2 were conducted on a Thermo Scientific MAT253 IRMS coupled to a Gasbench II. Three in-house calcite standards covering a range of $\delta^{13}\text{C}$ values from -48.95‰ to 1.96‰ and calibrated against international standards were used for normalization to Vienna Pee Dee Belemnite standard (VPDB) and provided analytical precision better than 0.15‰ (1SD; $N = 5$). DIC concentration was calculated by comparing the IRMS peak areas for the samples with 12 points calibration curves made from two $\text{NaHCO}_3(\text{aq})$ stock solutions. Linear regression of the calibration curves had R-squared values >0.99 for each batch. Concentration predictions can be estimated to have a precision better than 0.71 mM (1SD; $N = 23$). Samples from CAGE21–1 were measured at the Facility for advanced isotopic research and monitoring of weather, climate and biogeochemical cycling (FARLAB) at UiB-University of Bergen. DIC concentration was not determined and therefore does not appear in this study. DIC isotopic composition was determined on a Thermo Scientific Delta V plus connected to a Gasbench II and analytical precision (1SD) based on replicate measurement of in-house standards was better than 0.10‰ ($N = 9$). In this study, the precision associated with the mean of replicate samples is expressed as one standard deviation. Two-sample *t*-test was used to determine whether the addition of HgCl_2 in pore water samples resulted in statistically significant differences in DIC concentration and isotopic composition $\delta^{13}\text{C}$ compared to non-Hg treated samples. Statistical processing was performed on IBM SPSS Statistics v28.0.

3. Results

3.1. Dissolved inorganic carbon geochemistry ([DIC], $\delta^{13}\text{C}_{\text{DIC}}$)

The DIC concentrations and isotopic compositions in the investigated datasets range from minimum values of 1.8 mM (Dive13-BlaC-01 5 cm

Hg) and -36.0‰ (GC-03 40 cm *no*-Hg) to maximum values of 20.1 mM (GC-03 40 cm *no*-Hg) and -1.6‰ (MC-01 2 cm Hg), respectively. The differences between Hg-treated and filtered-only samples are shown in Figs. 3 and 4. Overall, the DIC concentrations in samples containing HgCl_2 are lower and the $\delta^{13}\text{C}_{\text{DIC}}$ values are heavier than in their Hg-free counterparts. The value measured at 2 cm in PusC-09 *no*-Hg is associated with an offset of 7.6‰ from the paired Hg sample. Although this offset is comparable to the ones observed in Dive13-BlaC-01 at 5 cm (7.4‰) and in GC-03 at 20 cm (5.4‰), the inconsistency with the other samples and with the general down-core trend (Fig. 4) make us consider this value as an outlier not included in the statistical discussion. The mean absolute difference of [DIC] values between *No*-Hg and Hg samples based on 26 samples is $0.465 \pm 0.691 \text{ mM}$. The *t*-test run on [DIC] data yielded a $t = 3.431$ which is higher than the tabulated *t* value for 25 degrees of freedom (2.060), demonstrating a statistically evident effect of HgCl_2 addition on DIC concentration. This value is associated with a two-tailed $p = 0.002$ and Cohen's $d = 0.673$, pointing toward a significant difference and medium-high effect on DIC concentration. Cohen's samples effect size coefficient indicates how large an effect is and the relative importance as: small effect $d = 0.2$; medium effect $d = 0.5$; large effect $d = 0.8$. The mean difference of $\delta^{13}\text{C}_{\text{DIC}}$ between *No*-Hg and Hg samples based on 42 samples is $-0.736 \pm 1.533\text{‰}$ (Table 2). Two-sample *t*-test yielded an absolute *t* value of 3.112 which is higher than the tabulated *t* value for 41 degrees of freedom (2.020), indicating a statistically significant difference. The corresponding two-tailed $p = 0.003$ is below the 0.005 threshold value for a 95% confidence interval indicating that the results are replicable. For the *t*-test on $\delta^{13}\text{C}_{\text{DIC}}$ we obtained an absolute $d = 0.480$, suggesting a medium effect of HgCl_2 addition to the measured $\delta^{13}\text{C}_{\text{DIC}}$ differences.

Table 2

Results from two-sample *t*-test on $\delta^{13}\text{C}$ and [DIC] absolute differences of *No*-Hg and Hg samples.

Parameter	MD	SD	df	t	Two-sided p	Cohen's d
$\delta^{13}\text{C}_{\text{DIC}}$	-0.736	1.533	41	-3.112	0.003	-0.480
[DIC]	0.465	0.691	25	3.431	0.002	0.673

MD = mean absolute difference; SD = standard deviation; df = degrees of freedom.

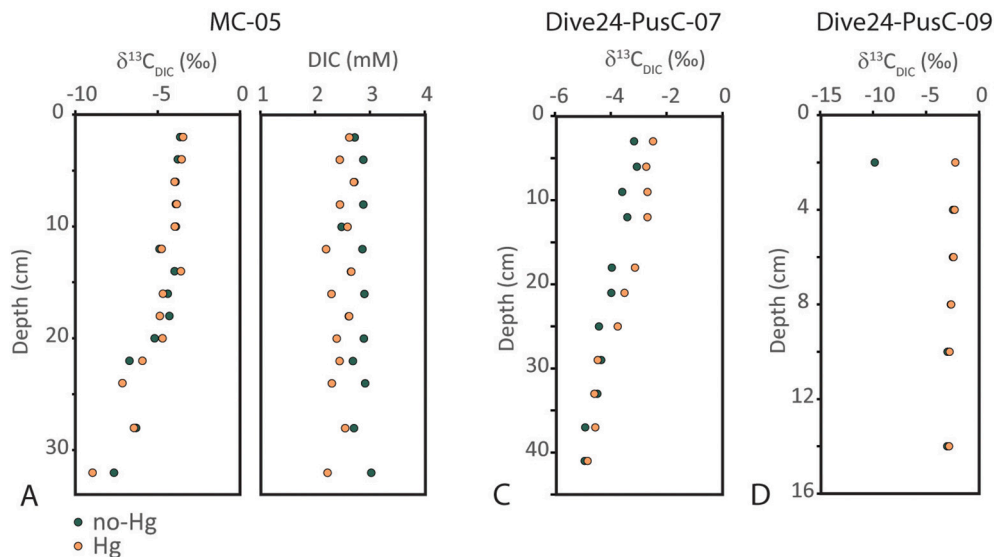


Fig. 4. Comparison of down-core [DIC] and $\delta^{13}\text{C}_{\text{DIC}}$ profiles of pore water samples treated (Hg) and not treated (*no*-Hg) by mercuric chloride addition. A) Sediment core MC-05, offshore N. Svalbard. B) Push core Dive24-PusC-07, Håkon Mosby mud volcano; C) push core Dive24-PusC-09, same location as (C). DIC concentration is not available for PusC-07 and PusC-09.

4. Discussion

4.1. Biogeochemical interpretations of pore water DIC data

The investigated pore water samples were collected from methane seepage environments identified based on echosounder observation of water-column gas flares. It is well known that sub-seafloor AOM (Boetius et al., 2000) releases large amounts of ^{13}C -depleted carbon into pore waters, stable in the form of HCO_3^- , affecting bulk DIC concentration and isotopic composition around the depth at which sulfate encounters the upward migrating methane (Akam et al., 2020), the sulfate-methane transition zone (SMTZ) (Jorgensen and Kasten, 2006). When the methane-derived carbon from oxidation of microbial and/or thermogenic gas ($\delta^{13}\text{C}_{\text{DIC}} < \sim -30\text{‰}$) (Whiticar, 1999) enters the pore water system, it mixes with other DIC sources characterized by heavier $\delta^{13}\text{C}$ signatures, i.e. $\delta^{13}\text{C}\text{-DIC}_{\text{seawater}} \sim 0\text{‰}$, $\delta^{13}\text{C}_{\text{DIC}}$ from mineralization of organic matter ($\sim -26\text{‰} < \delta^{13}\text{C} < \sim -20\text{‰}$) (Judd and Hovland, 2007), leading to more negative bulk $\delta^{13}\text{C}_{\text{DIC}}$ values than background sediments. In our study, the reference cores have $\delta^{13}\text{C}$ values consistently heavier than -10‰ , while the most ^{13}C -depleted isotopic compositions are found in cores Dive09-BlaC-01 ($\delta^{13}\text{C}_{\text{DIC}} = -34.8\text{‰}$; *no-Hg*), Dive11-BlaC-04 ($\delta^{13}\text{C}_{\text{DIC}} = -25.4\text{‰}$; *no-Hg*), Dive13-BlaC-01 ($\delta^{13}\text{C}_{\text{DIC}} = -29.5\text{‰}$; *no-Hg*), GC-03 ($\delta^{13}\text{C}_{\text{DIC}} = -36.0\text{‰}$; *no-Hg*). Those values are associated with the highest DIC concentrations as well (Figs. 3, 4), indicating variable incorporation of ^{13}C -depleted HCO_3^- deriving from methane oxidation. Strong reducing subseafloor conditions are highlighted by the occurrence of black sediment and seafloor chemosynthetic communities typical of methane seepage environments (Fig. 1). The upward flux of hydrogen sulfide released by AOM sustains thiotrophic communities at the seafloor with bacterial mats inhabiting the seafloor areas marked by steepest geochemical gradients (Argentino et al., 2022). Overall, pore water samples from the reference cores display typical marine trends marked by a progressive down-core increase in DIC fraction from organic matter respiration and no isotopic evidence of methane oxidation.

4.2. HgCl_2 addition affected the DIC geochemistry of pore water samples

A total of 33 pore water samples (79%) out of 42 treated with mercuric chloride show heavier mean $\delta^{13}\text{C}_{\text{DIC}}$ than samples only passed through $0.15\ \mu\text{m}$ filtration (Fig. 5A), and 20 (77%) out of 26 have lower DIC concentration (Fig. 5B). There is statistically significant difference

between $\delta^{13}\text{C}_{\text{DIC}}$ and [DIC] of *no-Hg* and *Hg* samples, with the latter aliquot displaying, on average, $\delta^{13}\text{C}_{\text{DIC}}$ values 0.736‰ heavier and [DIC] concentrations $0.465\ \text{mM}$ lower than *no-Hg* treated counterparts (Table 2). Samples with $\delta^{13}\text{C} < -10\text{‰}$ and $\text{DIC} > 3.5\ \text{mM}$ appear to carry larger offsets (Figs. 5A, B). Isotopic and concentration differences larger than the uncertainties on the mean of replicated measurements ($\pm 0.2\text{--}0.5\text{‰}$; $\pm 0.5\ \text{mM}$) and analytical precision (0.15‰ ; $0.71\ \text{mM}$) represent the 38% and 19% of the samples and show offsets of up to 7.4‰ and $1.9\ \text{mM}$, respectively. These values correspond to samples with $\text{DIC}_{\text{no-Hg}} \geq 3.8\ \text{mM}$. Significant loss of ^{12}C -enriched DIC from pore water samples is required to reconcile the observed mass and isotope datasets. To understand the process capable of altering the DIC composition in our samples we need to consider the geochemical environment from which they were collected. AOM in marine sediments consumes SO_4^{2-} as an electron acceptor, generating local sulfidic conditions maintaining high fluxes of hydrogen sulfide toward the seafloor inhibited by chemosynthetic communities (Argentino et al., 2022). The highest sulfide concentration is typically found in the SMTZ, where sulfate-driven AOM rates are the highest (Jorgensen and Kasten, 2006). In cold seep sediments, the addition of HgCl_2 might initiate a reaction with dissolved hydrogen sulfide (H_2S) derived from sulfate-driven AOM (Liu et al., 2020): $\text{SO}_4^{2-} + \text{CH}_4 = \text{HS}^- + \text{HCO}_3^- + \text{H}_2\text{O}$. That process causes the dominant dissolved carbon species composing DIC at seawater conditions, i.e. bicarbonate (HCO_3^-), to pass into the gaseous phase as carbon dioxide (CO_2) following the equations: (1) $\text{H}_2\text{S} + \text{HgCl}_2 = \text{HgS}_{(\text{s})} + 2\text{HCl}^-$; (2) $\text{HCl} + \text{HCO}_3^- = \text{Cl}^- + \text{CO}_2 + \text{H}_2\text{O}$. Subsequent CO_2 degassing from the pore water sample would generate a net decrease in DIC concentration and isotopic fractionation with preferential loss of ^{13}C -depleted carbon. The precipitated HgS is visible macroscopically (Supplementary Video 1) and allows a first qualitative evaluation of the samples' closeness to the SMTZ at the sampled location. This fractionation process has been previously mentioned by Liu et al. (2020) to explain DIC losses up to 90% and $\delta^{13}\text{C}$ increase of up to 1.8‰ in two pore water samples from cold seep sediments of the Makran continental shelf. Although we do not have sulfide concentration datasets for our samples, we witnessed a clear color change in the samples upon mercuric chloride addition (Supplementary Video 1) associated with a strong smell of hydrogen sulfide. Based on the measured datasets, seafloor imagery confirming the occurrence of thiotrophic communities and knowing the biogeochemical processes occurring in the sediments at the investigated methane seepage areas, we believe that the trends observed in our study reflect an interaction between the mercuric chloride antimicrobial agent

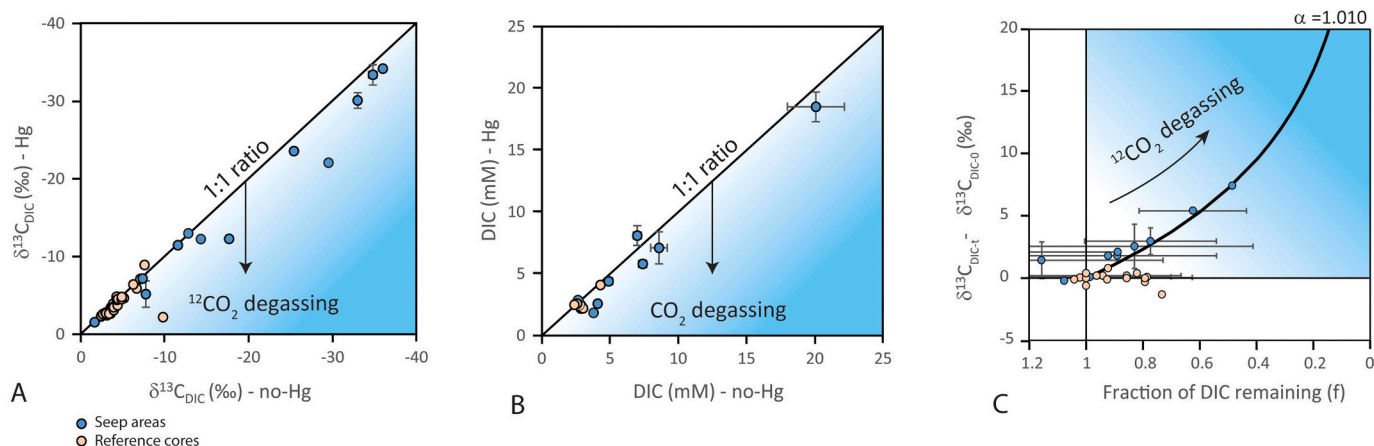


Fig. 5. Comparison of *no-Hg* and *Hg* samples from all the investigated sediment cores. A) Carbon isotopic composition obtained from *Hg*-treated and untreated samples. B) Dissolved inorganic carbon concentration plot of *Hg*-treated and untreated samples. C) Rayleigh distillation model of CO_2 degassing of pore water samples showing the enrichment in $\delta^{13}\text{C}$ value ($\delta^{13}\text{C}_{\text{DIC-t}} - \delta^{13}\text{C}_{\text{DIC-0}}$) of the remaining DIC fraction (f) at increasing DIC losses, with an isotopic fractionation factor $\alpha = 1.010$ at $4\ \text{°C}$ (Mook et al., 1974). $\delta^{13}\text{C}_{\text{DIC-t}}$ values correspond to samples treated with mercuric chloride, measured 30 days after sampling. The $\delta^{13}\text{C}_{\text{DIC-0}}$ values for the analyzed samples are assumed to correspond to *no-Hg* sample values. This model explains the largest isotopic and DIC concentration differences in our dataset. Error bars in (C) are propagated uncertainties (standard deviation, 1SD).

and the hydrogen sulfide produced by AOM in the sediment. The samples collected from AOM-impacted sediment intervals (lightest $\delta^{13}\text{C}_{\text{DIC}}$) display the largest offsets in both the carbon isotopic composition and DIC concentration between the two pore water aliquots (*Hg* vs *no-Hg*).

We attempted to simulate the carbon isotope effect of CO_2 degassing using a simple closed-system kinetic fractionation model using Rayleigh equation: $R = R_0 f^{(\alpha-1)}$, where R_0 is the initial isotopic composition of the substrate ($\delta^{13}\text{C}_{\text{DIC},0}$), f is the fraction of remaining DIC characterized by a composition $R = \delta^{13}\text{C}_{\text{DIC},t}$. The equilibrium isotopic fractionation factor (α) for the couple $\text{HCO}_3\text{-(aq)}-\text{CO}_2\text{(g)}$ is equal to 1.010 at 4 °C (Mook et al., 1974). We assumed: (1) negligible contribution of H_2CO_3 and CO_3^{2-} to DIC pool, (2) bicarbonate decomposition via dehydration (3) no carbonate precipitation. The result of the fractionation model is given in Fig. 5C and indicates that CO_2 degassing can cause a shift in the isotopic composition of remaining DIC pool of $\sim 7\%$ for a 50% DIC loss. Our measured dataset is plotted as enrichment in $\delta^{13}\text{C}$ value ($\delta^{13}\text{C}_{\text{DIC},t}-\delta^{13}\text{C}_{\text{DIC},0}$) against remaining DIC fraction (f), where the initial DIC isotope composition $\delta^{13}\text{C}_{\text{DIC},0}$ corresponds to *no-Hg* sample values, the final composition ($\delta^{13}\text{C}_{\text{DIC},t}$) at time (t) = 30 days is equal to the value measured in *Hg* samples and $f = [\text{DIC}]_{\text{Hg}}/[\text{DIC}]_{\text{no-Hg}}$. Our model successfully reproduces the composition of samples displaying the larger isotopic and DIC concentration offsets, thus supporting our interpretations. Some *Hg* samples having higher DIC concentration and/or lighter $\delta^{13}\text{C}$ composition of *no-Hg* samples (Figs. 5A, B) plot off the fractionation line but lie within the propagated uncertainties (Fig. 5C). While for the sample showing a 16% increase in DIC upon HgCl_2 addition the easiest explanation is that the non-treated counterpart was affected by CO_2 degassing into some headspace left in the vial, the samples lying below the fractionation line might have undergone isotopic re-equilibration after degassing.

The differences between *Hg*-treated and non-*Hg* treated samples of the magnitude reported in our study can significantly affect DIC geochemical datasets from cold seeps at DIC concentrations exceeding ~ 3.5 mM when accompanied by hydrogen sulfide. These findings might also have implications for studies on modern euxinic basins, e.g. Baltic Sea (Neubert et al., 2008), or sulfidic sediments (Wortmann et al., 2001; Antler et al., 2013), where the reaction with dissolved sulfide is expected to be the largest. It must be noted that high availability of reactive iron in the sediments, i.e. Fe(III) (hydr)oxides, can readily remove dissolved sulfides from the pore water system through precipitation of iron sulfide minerals (Formolo and Lyons, 2013), thus preventing any post-sampling alteration of DIC geochemistry by mercuric chloride addition. Even though new studies including pH and H_2S determination and time series data are necessary for further experimental validation of this model and to better constrain the dynamic of this process, our study demonstrates that HgCl_2 addition to marine pore waters can significantly affect the accuracy of DIC datasets at cold seeps. It is therefore suggested to filter the water samples from seep environments with a fine mesh and preserve them under chilled conditions, in agreement with previous observations from other settings (Li and Liu, 2011; Wilson et al., 2020; Mos et al., 2021). We discourage further use of HgCl_2 aboard vessels and in laboratories, to improve the analytical accuracy of DIC measurements and reduce the health risks for researchers and damages to the environment.

5. Conclusions

The use of HgCl_2 as an antimicrobial agent in water samples poses health and environmental risks related to its handling and disposal. Here, we investigated the effect of HgCl_2 addition on the DIC geochemistry ($\delta^{13}\text{C}_{\text{DIC}}$, concentration) of marine pore water samples collected from methane seepage areas in the Barents Sea and offshore N. Svalbard. Pore water samples ranged in [DIC] from 1.8 mM to 20.1 mM and show $\delta^{13}\text{C}$ values from -36.0% to -1.6% . These extremely wide ranges are related to anaerobic oxidation of methane releasing ^{13}C -depleted HCO_3^- into pore waters. A total of 34 pore water samples (79%)

out of 43 treated with mercuric chloride showed heavier mean $\delta^{13}\text{C}_{\text{DIC}}$ than samples only passed through 0.15 μm filtration, and 20 (77%) out of 26 had lower DIC concentration. There is statistically significant difference between mean values of $\delta^{13}\text{C}$ and [DIC] of *no-Hg* and *Hg* samples ($\sim 1.0\%$; ~ 0.6 mM), with larger differences for samples with $\delta^{13}\text{C} < -10\%$ and $\text{DIC} > 3.5$ mM. This trend is consistent with our CO_2 degassing model predictions and is interpreted to reflect the interaction of mercuric chloride with dissolved hydrogen sulfide from AOM, leading to variable loss of $^{12}\text{CO}_2$ from the sample. Although the majority of the offsets lie within replicate and instrumental uncertainties, we found that the 38% and 19% of the differences in $\delta^{13}\text{C}_{\text{DIC}}$ and DIC concentration values exceed them and correspond to samples with $\text{DIC}_{\text{no-Hg}} \geq 3.8$ mM and more influenced by AOM. We therefore caution against further use of HgCl_2 for DIC studies of marine pore waters from cold seeps and other sulfidic sediments which can be susceptible to alteration.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marchem.2023.104236>.

Author contributions

C.A., D.K., M.L. and G.P. conceived the study. C.A. and D.K. and M.L. conducted the pore water samplings. M.L. conducted the dissolved inorganic carbon analyses. C.A. did statistical analyses and wrote the initial manuscript. D.K., M.L. and G.P. contributed to the discussion and improvement of the manuscript and approved the article for submission.

Declaration of Competing Interest

None.

Data availability

The datasets used for this study can be found in Dataverse.NO at <https://doi.org/10.18710/3WLEGZ>.

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References

- Akam, S.A., Coffin, R.B., Abdulla, H.A.N., Lyons, T.W., 2020. Dissolved inorganic carbon pump in methane-charged shallow marine sediments: state of the art and new model perspectives. *Front. Mar. Sci.* 7, 58–60. <https://doi.org/10.3389/fmars.2020.00206>.
- Antler, G., Turchyn, A.V., Rennie, V., Herut, B., Sivan, O., 2013. Coupled sulfur and oxygen isotope insight into bacterial sulfate reduction in the natural environment. *Geochim. Cosmochim. Acta* 118, 98–117. <https://doi.org/10.1016/j.gca.2013.05.005>.
- Argentino, C., Savini, A., Panieri, G., 2022. Integrating fine-scale habitat mapping and pore water analysis in cold seep research: A case study from the SW Barents Sea. In: *World Atlas of Submarine Gas Hydrates in Continental Margins*. Springer International Publishing, Cham, pp. 505–514. https://doi.org/10.1007/978-3-030-81186-0_43.
- Boetius, A., Ravensschlag, K., Schubert, C.J., Rickert, D., Widdel, F., Gieseke, A., et al., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–626. <https://doi.org/10.1038/35036572>.
- Canuel, E.A., Cammer, S.S., McIntosh, H.A., Pondell, C.R., 2012. Climate change impacts on the organic carbon cycle at the Land-Ocean Interface. *Annu. Rev. Earth Planet. Sci.* 40, 685–711. <https://doi.org/10.1146/annurev-earth-042711-105511>.
- Cheng, L., Normandeau, C., Bowden, R., Doucett, R., Gallagher, B., Gillikin, D.P., et al., 2019. An international intercomparison of stable carbon isotope composition

- measurements of dissolved inorganic carbon in seawater. *Limnol. Oceanogr. Methods* 17, 200–209. <https://doi.org/10.1002/lom3.10300>.
- Crameri, F., Shephard, G.E., Heron, P.J., 2020. The misuse of colour in science communication. *Nat. Commun.* 11, 1–10. <https://doi.org/10.1038/s41467-020-19160-7>.
- Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. *Guide to Best Practices for Ocean CO₂ Measurements in PICES Special Publication*.
- Feng, J., Li, N., Luo, M., Liang, J., Yang, S., Wang, H., et al., 2020. A quantitative assessment of methane-derived carbon cycling at the cold seeps in the northwestern South China Sea. *Minerals* 10, 256. <https://doi.org/10.3390/min10030256>.
- Formolo, M.J., Lyons, T.W., 2013. Sulfur biogeochemistry of cold seeps in the Green Canyon region of the Gulf of Mexico. *Geochim. Cosmochim. Acta* 119, 264–285. <https://doi.org/10.1016/j.gca.2013.05.017>.
- Garcia-Tigreros, F., Leonte, M., Ruppel, C.D., Ruiz-Angulo, A., Joung, D.J., Young, B., et al., 2021. Estimating the impact of seep methane oxidation on ocean pH and dissolved inorganic radiocarbon along the U.S. Mid-Atlantic Bight. *J. Geophys. Res. Biogeosci.* 126 <https://doi.org/10.1029/2019JG005621>.
- Gloël, J., Robinson, C., Tilstone, G.H., Tarran, G., Kaiser, J., 2015. Technical note: could benzalkonium chloride be a suitable alternative to mercuric chloride for preservation of seawater samples? *Ocean Sci.* 11, 947–952. <https://doi.org/10.5194/os-11-947-2015>.
- Joos, F., Plattner, G.-K., Stocker, T.F., Marchal, O., Schmittner, A., 1999. Global warming and marine carbon cycle feedbacks on future atmospheric CO₂. *Science* (80-.) 284, 464–467. <https://doi.org/10.1126/science.284.5413.464>.
- Jorgensen, B.B., Kasten, S., 2006. Sulfur cycling and methane oxidation. *Mar. Geochem.* 2, 271–310.
- Judd, A.G., Hovland, M., 2007. Seabed Fluid Flow: The Impact of Geology, Biology and the Marine Environment. <https://doi.org/10.1007/s00254-004-1086-0>.
- Kim, M.-K., Zoh, K.-D., 2012. Fate and transport of mercury in environmental media and human exposure. *J. Prev. Med. Public Health* 45, 335–343. <https://doi.org/10.3961/jpmph.2012.45.6.335>.
- Kroopnick, P., 1974. The dissolved O₂-CO₂-13C system in the eastern equatorial pacific. *Deep-Sea Res. Oceanogr. Abstr.* 21, 211–227. [https://doi.org/10.1016/0011-7471\(74\)90059-X](https://doi.org/10.1016/0011-7471(74)90059-X).
- Li, X., Liu, W., 2011. Effect of preservation on the $\delta^{13}\text{C}$ value of dissolved inorganic carbon in different types of water samples. *Isot. Environ. Health Stud.* 47, 379–389. <https://doi.org/10.1080/10256016.2011.598934>.
- Liu, W., Wu, Z., Xu, S., Wei, J., Peng, X., Li, J., et al., 2020. Pore-water dissolved inorganic carbon sources and cycling in the shallow sediments of the Haima cold seeps, South China Sea. *J. Asian Earth Sci.* 201, 104495 <https://doi.org/10.1016/j.jseas.2020.104495>.
- McCorkle, D.C., Emerson, S.R., Quay, P.D., 1985. Stable carbon isotopes in marine porewaters. *Earth Planet. Sci. Lett.* 74, 13–26. [https://doi.org/10.1016/0012-821X\(85\)90162-1](https://doi.org/10.1016/0012-821X(85)90162-1).
- Mook, W.G., Bommerson, J.C., Staverman, W.H., 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth Planet. Sci. Lett.* 22, 169–176. [https://doi.org/10.1016/0012-821X\(74\)90078-8](https://doi.org/10.1016/0012-821X(74)90078-8).
- Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. *Annu. Rev. Ecol. Syst.* 29, 543–566. <https://doi.org/10.1146/annurev.ecolsys.29.1.543>.
- Mos, B., Holloway, C., Kelaher, B.P., Santos, I.R., Dworjanyn, S.A., 2021. Alkalinity of diverse water samples can be altered by mercury preservation and borosilicate vial storage. *Sci. Rep.* 11, 9961. <https://doi.org/10.1038/s41598-021-89110-w>.
- Neubert, N., Nägler, T.F., Böttcher, M.E., 2008. Sulfidity controls molybdenum isotope fractionation into euxinic sediments: evidence from the modern Black Sea. *Geology* 36, 775. <https://doi.org/10.1130/G24959A.1>.
- Olack, G.A., Colman, A.S., Pfister, C.A., Wootton, J.T., 2018. Seawater DIC analysis: the effects of blanks and long-term storage on measurements of concentration and stable isotope composition. *Limnol. Oceanogr. Methods* 16, 160–179. <https://doi.org/10.1002/lom3.10235>.
- Phrampus, B.J., Lee, T.R., Wood, W.T., 2020. A global probabilistic prediction of cold seeps and associated SEAFloor Fluid Expulsion Anomalies (SEAFLEAs). *Geochem. Geophys. Geosyst.* 21 <https://doi.org/10.1029/2019GC008747>.
- Quetin, G.R., Bloom, A.A., Bowman, K.W., Konings, A.G., 2020. Carbon flux variability from a relatively simple ecosystem model with assimilated data is consistent with terrestrial biosphere model estimates. *J. Adv. Model. Earth Syst.* 12 <https://doi.org/10.1029/2019MS001889>.
- Schrum, H.N., Murray, R.W., Gribsholt, B., 2012. Comparison of rhizon sampling and whole round squeezing for marine sediment porewater. *Sci. Drill.* 47–50 <https://doi.org/10.2204/iodp.sd.13.08.2011>.
- Steiner, Z., Lazar, B., Erez, J., Turchyn, A.V., 2018. Comparing Rhizon samplers and centrifugation for pore-water separation in studies of the marine carbonate system in sediments. *Limnol. Oceanogr. Methods* 16, 828–839. <https://doi.org/10.1002/lom3.10286>.
- Suess, E., 2018. Marine cold seeps: background and recent advances. In: *Hydrocarbons, Oils and Lipids: Diversity, Origin, Chemistry and Fate*. Springer International Publishing, Cham, pp. 1–21. https://doi.org/10.1007/978-3-319-54529-5_27-1.
- Taipale, S.J., Sonninen, E., 2009. The influence of preservation method and time on the $\delta^{13}\text{C}$ value of dissolved inorganic carbon in water samples. *Rapid Commun. Mass Spectrom.* 23, 2507–2510. <https://doi.org/10.1002/rcm.4072>.
- Taniguchi, M., Dulai, H., Burnett, K.M., Santos, I.R., Sugimoto, R., Stieglitz, T., et al., 2019. Submarine groundwater discharge: updates on its measurement techniques, geophysical drivers, magnitudes, and effects. *Front. Environ. Sci.* 7 <https://doi.org/10.3389/fenvs.2019.00141>.
- Vaidya, V.S., Mehendale, H.M., 2014. Mercuric Chloride (HgCl₂). In: *Encyclopedia of Toxicology* (Elsevier), pp. 203–206. <https://doi.org/10.1016/B978-0-12-386454-3.00330-4>.
- Whiticar, M.J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.* 161, 291–314. [https://doi.org/10.1016/S0009-2541\(99\)00092-3](https://doi.org/10.1016/S0009-2541(99)00092-3).
- Wilson, J., Munizzi, J., Erhardt, A.M., 2020. Preservation methods for the isotopic composition of dissolved carbon species in non-ideal conditions. *Rapid Commun. Mass Spectrom.* 34, 1–11. <https://doi.org/10.1002/rcm.8903>.
- Wortmann, U.G., Bernasconi, S.M., Böttcher, M.E., 2001. Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step microbial sulfate reduction. *Geology* 29, 647. [https://doi.org/10.1130/0091-7613\(2001\)029<0647:HDBIES>2.0.CO;2](https://doi.org/10.1130/0091-7613(2001)029<0647:HDBIES>2.0.CO;2).
- Xu, C., Wu, N., Sun, Z., Zhang, X., Geng, W., Cao, H., et al., 2021. Assessing methane cycling in the seep sediments of the mid-Okinawa trough: insights from pore-water geochemistry and numerical modeling. *Ore Geol. Rev.* 129, 103909 <https://doi.org/10.1016/j.oregeorev.2020.103909>.
- Zhang, Y., Luo, M., Hu, Y., Wang, H., Chen, D., 2019. An areal assessment of seafloor carbon cycling in cold seeps and hydrate-bearing areas in the northern South China Sea. *Geofluids* 2019, 1–14. <https://doi.org/10.1155/2019/2573937>.