



Technical note: Preventing CO₂ overestimation from mercuric or copper(II) chloride preservation of dissolved greenhouse gases in freshwater samples

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Abstract. The determination of dissolved gases (O₂, CO₂, CH₄, N₂O, N₂) in surface waters allows the estimation of biological processes and greenhouse gas fluxes in aquatic ecosystems. Mercuric chloride (HgCl₂) has been widely used to preserve water samples prior to gas analysis. However, alternates are needed because of the environmental impacts and prohibition of mercury. HgCl₂ is a weak acid and interferes with dissolved organic carbon (DOC). Hence, we tested the effect of HgCl₂ and two substitutes (copper(II) chloride – CuCl₂ – and silver nitrate – AgNO₃), as well as storage time (24 h to 3 months) on the determination of dissolved gases in low-ionic-strength and high-DOC water from a typical boreal lake. Furthermore, we investigated and predicted the effect of HgCl₂ on CO₂ concentrations in periodic samples from another lake experiencing pH variations (5.4–7.3) related to in situ photosynthesis. Samples fixed with inhibitors generally showed negligible O₂ consumption. However, effective preservation of dissolved CO₂, CH₄ and N₂O for up to 3 months prior to dissolved gas analysis was only achieved with AgNO₃. In contrast, HgCl₂ and CuCl₂ caused an initial increase in CO₂ and N₂O from 24 h to 3 weeks followed by a decrease from 3 weeks to 3 months. The CO₂ overestimation, caused by HgCl₂ acidification and a shift in the carbonate equilibrium, can be calculated from predictions of chemical speciation. Errors due to CO₂ overestimation in HgCl₂-preserved water, sampled from low-ionic-strength and high-DOC freshwater, which is common in the Northern Hemisphere, could lead to an overestimation of the CO₂ diffusion efflux by a factor of > 20 over a month or a factor of 2 over

the ice-free season. The use of HgCl₂ and CuCl₂ for freshwater preservation should therefore be discontinued. Further testing of AgNO₃ preservation should be performed under a large range of freshwater chemical conditions.

1 Introduction

The determination of dissolved gases by gas chromatography from water samples collected in the field allows the estimation of biological processes in aquatic ecosystems such as photosynthesis and oxic respiration (O₂, CO₂), denitrification (N₂, N₂O), and methanogenesis (CH₄). This technique is also useful to test the calibration of in situ sensors in long-term deployment. However, the accuracy of this approach largely depends on the effectiveness of sample fixation. In fact, the partial pressure of the dissolved gases will continue to evolve in the water sample from the time of collection to the time of analysis unless biological activity is prevented. This is an issue when field sites are far from laboratory facilities and when samples need to be stored until the end of the field season for more efficient processing in large batches. Hence, before using a given chemical to preserve water samples, it must be ensured that it is efficient in inhibiting biological activity without changing the sample's chemistry.

Mercury(II) chloride (HgCl₂) has been widely used as an inhibitor of the above-mentioned biological processes to preserve water samples for the determination of dissolved CO₂ in seawater (e.g. Dickson et al., 2007) and several dissolved

gases in natural and artificial freshwater bodies (e.g. O₂, CO₂, CH₄, N₂ and/or N₂O; Guérin et al., 2006; Hessen et al., 2017; Hilgert et al., 2019; Okuku et al., 2019; Schubert et al., 2012; Xiao et al., 2014; Yan et al., 2018; Yang et al., 2015) because it proved effective at very low concentrations compared to other reagents (e.g. Horvatić and Peršić, 2007; Hassen et al., 1998). Worldwide efforts have sought to reduce the use of mercury because it is considered toxic to the environment and exposure can severely affect human health (Chen et al., 2018). Therefore, alternative preservation techniques to HgCl₂ treatment have been tested for dissolved inorganic carbon (DIC) and δ¹³C-DIC such as acidification with phosphoric acid (Taipale and Sonninen, 2009) or a combination of filtration and exposure to benzalkonium chloride or sodium chloride (Takahashi et al., 2019). Previous studies have shown that simple filtration (and cooling), fixation (precipitation) or acidification was effective in preserving water samples (Wilson et al., 2020). An alternative to using preservatives is to collect in situ water samples, extract the headspace in the field and analyse the headspace in a laboratory (e.g. Cole et al., 1994; Karlsson et al., 2013; Kling et al., 1991). However, these techniques were not tested for the simultaneous determination of several dissolved gases, including CH₄, which is subject to rapid degassing during handling or storage if samples are not preserved because of its low solubility in water (Duan and Mao, 2006). In addition, some of the existing alternatives, such as filtration or field headspace equilibration, are difficult to operate in remote areas in the field under harsh weather conditions and are prone to potential ambient air contamination. Solutions for water sample preservation should therefore involve a minimum of manipulation steps in the field to avoid gas exchange with ambient air. Preservative amendments into sealed water bottles appears to comprise of the most efficient methods. Copper(II) chloride (CuCl₂) and silver nitrate (AgNO₃), the most toxic form of silver, are relevant alternatives to HgCl₂ given their known toxicity (e.g. Ratte, 1999; Amorim and Scott-Fordsmand, 2012) and wide application in water treatments and water purification (Larrañaga et al., 2016; Nowack et al., 2011; NPIRS, 2023; Ullmann et al., 1985). Nevertheless, the efficiency of these alternative preservatives has never been tested for dissolved gas sample preservation.

The addition of HgCl₂ to water is known to produce hydrochloric acid through hydrolysis (Ciavatta and Grimaldi, 1968) and to form complexes with many environmental ligands, both inorganic (Powell et al., 2005) and organic (Tipping, 2002; Foti et al., 2009; Liang et al., 2019; Chen et al., 2017). The complexation of Hg⁺ with the carboxyl or thiol groups of dissolved organic carbon (DOC) in oxic environments could further increase the concentration of H⁺ (Khwaja et al., 2006; Skyllberg, 2008). This acidification can be an issue in poorly buffered water (low ionic strength) with a high concentration of DOC where a shift in the pH and carbonate equilibrium can be induced. In that case, the estimated CO₂ concentration would be higher after HgCl₂ fix-

ation than the in situ concentration and, if the shift in pH is not accounted for, can result in an overestimation of dissolved CO₂ and bicarbonate concentrations. A similar acidification effect is also expected with CuCl₂ treatments (Rippen et al., 2021) but not with AgNO₃ treatments. Such effects would not be expected in marine water due to the high ionic strength of the water (Chou et al., 2016) or freshwater with low pH (< 5.5), under which conditions nearly all dissolved inorganic carbon is CO₂ (Stumm and Morgan, 1981). Thus, there are clear limits of the application of HgCl₂, and possibly CuCl₂, for freshwater sample preservation given its risk of leading to overestimation of CO₂ and bicarbonate concentrations, in addition to exposing field workers to the risks of its high toxicity.

Here we combine data from (i) laboratory experiments and (ii) fieldwork to illustrate risks of misestimation of dissolved gas concentrations in freshwater with some preservatives and provide recommendations for best practices in the field. First, we (i) performed some short-term and long-term incubations of water from a typical heterotrophic unproductive boreal lake with circumneutral pH, low ionic strength (poor buffering capacity) and high DOC concentration to test the effect of storage time and different preservative treatments on the determination of five dissolved gases (O₂, CO₂, CH₄, N₂ and N₂O) by headspace equilibration and gas chromatography. The preservatives were mercuric chloride (HgCl₂) and two alternative inhibitors, chosen for their wide and effective application in water treatments and water purification (copper(II) chloride – CuCl₂ – and silver nitrate – AgNO₃; Xu and Imlay, 2012; Rai et al., 1981). Unamended water samples, where only ultrapure water was added, were also included for comparison. In addition, we (ii) analysed dissolved CO₂ concentration data obtained from a typical productive boreal lake using two independent methods, one by gas chromatography following HgCl₂ fixation and one through dissolved inorganic carbon determination without fixation. We show that the overestimation of dissolved CO₂ concentrations caused by HgCl₂ fixation can be predicted based on chemical equilibria.

2 Methods

The detailed experimental procedures for investigating (i) the effects of storage time and different inhibitors on dissolved gas concentrations as well as (ii) the effects of HgCl₂ on dissolved CO₂ analyses over a range of pH values are summarized in Fig. 1 and described below.

2.1 Effects of storage time and inhibitors on the quantification of dissolved gases

Study site and sampling

Surface water was collected from Lake Svartkulp (59.9761313° N, 10.7363544° E; southeast Norway)

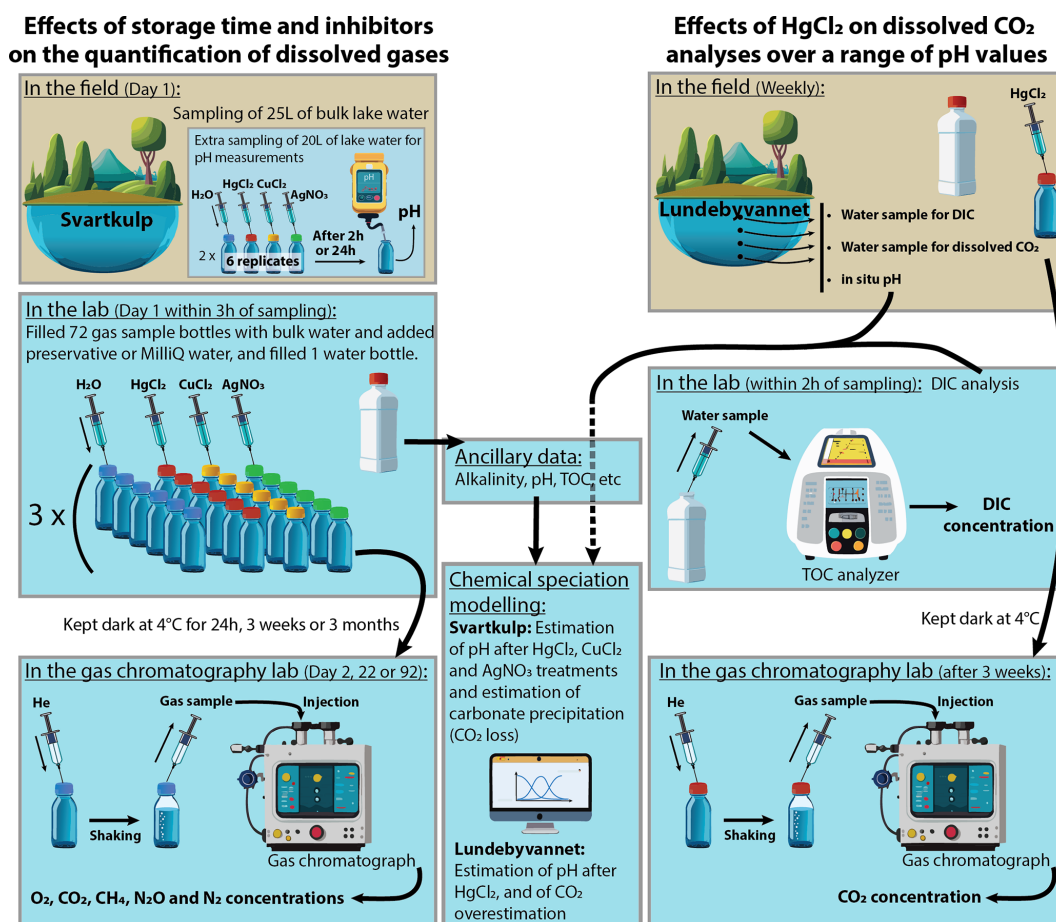


Figure 1. Overview of experimental procedures. Several graphic items in this figure have been generated with the help of Adobe® Firefly™ artificial intelligence generator.

north of Oslo, Norway, on 4 September 2019. A 5 L plastic bottle was gently pushed into the water and progressively tilted to let the water flow into the bottles without bubbling. The bottle aperture was covered with a 90 µm plankton net to avoid sampling large particles. This procedure was repeated five times to yield a total water volume of 25 L. The 5 L water bottles were immediately brought back to the lab. Upon arrival at the laboratory, after temperature equilibration, water from the 5 L bottles was slowly poured, to limit gas exchange with the ambient air, into a 25 L tank to provide a single bulk sample to start the incubation experiment. Filtration, e.g. with 0.45 or 0.2 µm filters, was avoided to minimize changes in dissolved gas concentrations (e.g. Magen et al., 2014). The mixed water sample (25 L) was sub-sampled (0.5 L) for the determination of alkalinity (127 µmolL⁻¹), pH (6.73), ammonium (3 µgNL⁻¹), nitrate (5 µgNL⁻¹), total N (230 µgNL⁻¹), phosphate (1 µgPL⁻¹), total P (9 µgPL⁻¹) and total organic carbon (TOC; 8.9 mgCL⁻¹), all analysed by standard methods at the accredited Norwegian Institute for Water Research (NIVA) lab (see Table S1 in the Supplement). The in situ

temperature of the lake water was measured with a handheld thermometer and was 18.5 °C. Note that particulate organic carbon is a negligible fraction of TOC in Norwegian lake waters, representing on average less than 3 % (de Wit et al., 2023).

Lake Svartkulp was selected for this experiment because it is representative of low-ionic-strength Northern Hemisphere lakes, typically found in granitic bedrock regions in northeast America and Scandinavia. It is a typical low-productivity, heterotrophic, slightly acidic to neutral, moderately humic lake. Similar lakes are found in southern Norway (de Wit et al., 2023), large parts of Sweden (Valinia et al., 2014), Finland, Atlantic Canada (Houle et al., 2022), Ontario, Quebec and northeast USA (Skjelkvåle and de Wit, 2011; Weyhenmeyer et al., 2019).

Laboratory incubation experiment with different preservatives and storage times

The experimental design involved incubating 72 borosilicate glass bottles (120 mL) filled with lake water from our 25 L bulk sample subjected to four different treatments: addition

of 240 µL of a preservative solution of (i) HgCl₂, (ii) CuCl₂ or (iii) AgNO₃ or addition of 240 µL of (iv) Milli-Q water. The bottles amended with Milli-Q water are hereafter referred to as “unfixed”. The 72 bottles were divided into three groups which were incubated cold (+4 °C) and dark for 24 h, 3 weeks or 3 months, respectively, before being processed for dissolved gas analysis by gas chromatography. These incubation times were selected to represent situations where samples are processed directly upon return to the laboratory (24 h) or after medium-term (3 weeks) to long-term (3 months) storage. At each time point and for each treatment, a group of six bottles were further processed for dissolved gas analysis. Concentrations of O₂, N₂, N₂O, CO₂ and CH₄ were determined by gas chromatography (see below) using the headspace technique following Yang et al. (2015). The pH was not measured at the end of the storage period.

In detail, within 3 h of lake water sampling, the 120 mL bottles were gently filled with water from the mixed sample (25 L). Each 120 mL bottle was slowly lowered into the water and progressively tilted to let the water flow into the bottle without bubbling. The bottle was then capped under water with a gastight butyl rubber stopper after ensuring that there were no air bubbles in the bottle. The bottles were randomized prior to preservative or Milli-Q treatment. The preservative or Milli-Q amendment was pushed in each bottle with a syringe and needle through the rubber septum. To avoid overpressure, another needle was placed through the septum at the same time, at least 2 cm above the other needle, to allow an equivalent volume of clean water to be released.

Stock solutions of HgCl₂, CuCl₂ and AgNO₃ were prepared according to Table 1 using high-accuracy chemical equipment (e.g. high-accuracy scale, volumetric flasks). The Ag (silver nitrate EMSURE[®] ACS; Merck KGaA, Germany) Cu (copper(II) chloride dihydrate; Merck Life Science ApS, Norway) and Hg (mercury(II) chloride; undetermined) salts were dissolved in Milli-Q ultrapure water (> 18 MΩ cm). For measurement of CO₂ in seawater samples, the standard method involves poisoning the samples by adding a saturated HgCl₂ solution in a volume equal to 0.05 %–0.02 % of the total volume (Dickson et al., 2007). We used this as a starting point and added 0.02 % saturated HgCl₂ solution to 18 bottles (240 µL of HgCl₂ 10× diluted saturated solution), resulting in a sample concentration of 14 µg HgCl₂ mL⁻¹ (51.6 µM; Table 1). Based on estimated toxicity relative to Hg (Deheyn et al., 2004; Halmi et al., 2019), the silver and copper salts were added in molar concentrations equal to 2 and 3 times the molar concentration of HgCl₂, respectively (Table 1), although this varies between species of microorganisms and environmental matrices (Hassen et al., 1998; Rai et al., 1981).

Additional 24 h incubation experiment with different preservatives for pH measurements

Since pH was not measured at the end of the first incubation experiment, we performed an additional experiment to document any potential rapid (within 24 h) impacts of preservative on pH. A total of 48 borosilicate glass bottles (120 mL) filled with lake water were subjected to the same four different treatments as the first experiment described above: HgCl₂, CuCl₂, AgNO₃ or Milli-Q water amendments. To this end, a 20 L water tank was filled with surface water from Lake Svartkulp on 14 December 2023. The water tank was immediately returned to the laboratory and left for 24 h to equilibrate to the room temperature. On 15 December, 120 mL bottles were gently filled with water from the bulk 20 L sample, as described above. The bottles were randomized prior to preservative or Milli-Q treatment performed as described above. The bottles were then incubated at room temperature for 2 or 24 h. The pH was measured in the initial unamended lake water, in 24 bottles opened after 2 h incubation, and in 24 bottles opened after 24 h incubation. The pH measurements were performed with a WTW Multi 3620 pH meter calibrated using a two-point calibration at pH 4 and 7. All pH measures were corrected for temperature. The water temperature of the water samples during pH measurements ranged between 19.1 and 21.2 °C.

2.2 Effects of HgCl₂ on dissolved CO₂ analyses over a range of pH values

Study site and sampling

Water samples were collected from Lake Lundebyvannet located southeast of Oslo (59.54911° N, 11.47843° E; south-east Norway). Two sets of samples were taken from 1, 1.5, 2 and 2.5 m depth using a water sampler once or twice a week between April 2020 and January 2021 for the determination of (i) dissolved CO₂ by gas chromatography (GC) analysis following fixation with HgCl₂ and (ii) DIC analysis with a TOC analyser. Samples for GC analysis were filled into 120 mL glass bottles (as described above for the 72 incubation bottles), which were sealed with rubber septa under water without air bubbles. Samples for GC analysis were preserved in the field by adding a half-saturated (at 20 °C) solution of HgCl₂ (150 µL) through the rubber seal of each bottle using a syringe, as described above the 72 incubation bottles, resulting in a concentration of 161 µM similar to previous studies (Clayer et al., 2021; Hassen et al., 2017; Yang et al., 2015). Samples for DIC analysis were filled without bubbles in 100 mL Winkler glass bottles that were sealed airtight directly after sampling. These samples were not fixed in any way and were analysed by a TOC analyser within 2 h. Note that estimation of dissolved CO₂ concentrations from pH and DIC is the least uncertain method of indirect CO₂ concentration with estimated relative error of 6 % or less (Golub et al.,

Table 1. Stock and sample concentrations of HgCl₂, CuCl₂ and AgNO₃.

Salt	Stock solution	Sample concentration	Rationale
HgCl ₂	70 g L ⁻¹ (saturated)	14.0 ± 0.01 µg mL ⁻¹ (51.6 µM)	Dickson et al. (2007)
CuCl ₂	131.9 g L ⁻¹	26.4 ± 0.02 µg mL ⁻¹ (154.7 µM)	3 × Hg
AgNO ₃	87.6 g L ⁻¹	17.5 ± 0.02 µg mL ⁻¹ (103.1 µM)	2 × Hg

2017). Lake water temperature and pH were measured in situ using HOBO pH data loggers placed at 1, 1.5, 2 and 2.5 m (Elit, Gjerdrum, Norway).

Lake Lundebyvannet has a surface area of 0.4 km² and a maximum depth of 5.5 m. It often experiences large blooms of *Gonyostomum semen* over the summer between May and September (Hagman et al., 2015; Rohrlack et al., 2020). The lake water is characterized by high and fluctuating concentrations of humic substances (with DOC concentrations ranging from 8 to 28 mg CL⁻¹), ammonium (5 to 100 µg NL⁻¹), nitrate (20 to 700 µg NL⁻¹), total N (average of 612 µg NL⁻¹), phosphate (2 to 4 µg PL⁻¹), total P (average of 28 µg PL⁻¹; Rohrlack et al., 2020; Hagman et al., 2015), a fluctuating pH (from 5.5 to 7.3), weak ionic strength with alkalinity ranging between 30 and 150 µmol L⁻¹, and electric conductivity varying from 40 to 70 µS cm⁻¹. For more details, see Rohrlack et al. (2020).

Lake Lundebyvannet was selected for this experiment because it is representative of productive, low-ionic-strength Northern Hemisphere lakes typically found in the southern part of granitic bedrock regions in northeast America and Scandinavia.

2.3 Analytical chemistry

Gas chromatography

Headspace was prepared by gently backfilling sample bottles with 20–30 mL helium (He; 99.9999 %) into the closed bottle while removing a corresponding volume of water. Care was taken to control the headspace pressure within 5 % of ambient air, and a slight He overpressure was released before equilibration. The bottles were shaken horizontally at 150 rpm for 1 h to equilibrate gases between the sample and headspace. The temperature during shaking was recorded by a data logger. Immediately after shaking, the bottles were placed in an autosampler (GC-Pal, CTC, Switzerland) coupled to a GC instrument with He back-flushing (model 7890A, Agilent, Santa Clara, CA, USA). Headspace gas was sampled (approx. 2 mL) by a hypodermic needle connected to a peristaltic pump (Gilson MINIPULS 3), which connected the autosampler with the 250 µL heated sampling loop of the GC instrument.

The GC instrument was equipped with a 20 m wide-bore (0.53 mm) PoraPLOT Q column for separation of CH₄, CO₂ and N₂O and a 60 m wide-bore Molsieve 5 Å PLOT column

for separation of O₂ and N₂, both operated at 38 °C and with He as the carrier gas. N₂O and CH₄ were measured with an electron capture detector run at 375 °C with Ar/CH₄ (80/20) as makeup gas and a flame ionization detector, respectively. CO₂, O₂ and N₂ were measured with a thermal conductivity detector (TCD). Certified standards of CO₂, N₂O and CH₄ in He were used for calibration (AGA, Germany), whereas air was used for calibrating O₂ and N₂. The analytical error for all gases was lower than 2 %. For the Lake Lundebyvannet time series, CO₂ was separated from other gases using the 20 m wide-bore (0.53 mm) PoraPLOT Q column, while the other gases were not measured.

The results from gas chromatography give the relative concentration of dissolved gases (in ppm) in the headspace in equilibrium with the water. For the lab experiment with Svartkulp samples (Sect. 2.1), the concentration of dissolved gases in the water at equilibrium with the headspace was calculated from the temperature-corrected Henry constant in water using Carroll et al. (1991) for CO₂, Weiss and Price (1980) for N₂O, Yamamoto et al. (1976) for CH₄, Millero (2002) for O₂, and Hamme and Emerson (2004) for N₂. For the Lake Lundebyvannet time series (Sect. 2.2), the concentration of CO₂ in the water samples was determined using temperature-dependent Henry's law constants given by Wilhelm et al. (1977). The quantities of gases in the headspace and water were summed to find the concentrations and partial pressures of dissolved gases from the water collected in the field as follows:

$$[\text{gas}] = \frac{p_{\text{gas}} H V_{\text{water}} + \frac{p_{\text{gas}} V_{\text{headspace}}}{RT}}{V_{\text{water}}}, \quad (1)$$

where [gas] is the gas aqueous concentration, p_{gas} is the gas partial pressure, H is the Henry constant, V_{water} is the volume of water sample during headspace equilibration, $V_{\text{headspace}}$ is the headspace gas volume during equilibration, R is the gas constant and T is the temperature during headspace equilibration (recorded during shaking). The calculations were similar to those of Yang et al. (2015).

DIC analyses

DIC analysis was performed for the Lake Lundebyvannet time series using a Shimadzu TOC-V CPN (Oslo, Norway) instrument equipped with a non-dispersive infrared (NDIR) detector with O₂ as a carrier gas at a flow rate of 100 mL min⁻¹. Two to three replicate measurements

were run per sample. The system was calibrated using a freshly prepared solution containing different concentrations of NaHCO_3 and Na_2CO_3 , and standards were measured in between each sixth sample. CO_2 concentrations in water samples ($[\text{CO}_2]$) were calculated on the bases of temperature, pH and DIC concentrations as follows (Rohrlack et al., 2020):

$$[\text{CO}_2] = \frac{[\text{H}^+]^2 C_T}{Z}, \quad (2)$$

where $[\text{H}^+]$ is the proton concentration ($10^{-\text{pH}}$), C_T is the dissolved inorganic carbon concentration and Z is given by

$$Z = [\text{H}^+]^2 + K_1[\text{H}^+] + K_1 K_2. \quad (3)$$

Here K_1 and K_2 are the first and second carbonic acid dissociation constants adjusted for temperature ($\text{p}K_1 = 6.41$ and $\text{p}K_2 = 10.33$ at 25°C ; Stumm and Morgan, 1996).

2.4 Data analysis

$p\text{CO}_2$ and saturation deficit

Lake Lundebyvannet CO_2 concentrations provided by GC and DIC analyses were converted to $p\text{CO}_2$ (in μatm) as follows:

$$p\text{CO}_2 = \frac{[\text{CO}_2]}{0.987 \times K_H P_{\text{atm}}}, \quad (4)$$

where K_H is the Henry constant for CO_2 adjusted for the in situ water temperature (Stumm and Morgan, 1996) and P_{atm} is the atmospheric pressure in bar approximated by

$$P_{\text{atm}} = (1013 - 0.1 \times \text{altitude}) \times 0.001. \quad (5)$$

Here altitude is the altitude above sea level of Lake Lundebyvannet (158 m). Finally, the CO_2 saturation deficit (Sat_{CO_2} in μatm) was given by

$$\text{Sat}_{\text{CO}_2} = p\text{CO}_2 - [\text{CO}_2]_{\text{air}}, \quad (6)$$

where $[\text{CO}_2]_{\text{air}}$ is the $p\text{CO}_2$ in the air ($416 \mu\text{atm}$ for 2020 in southern Norway retrieved from the EBAS database; NILU, 2022; Tørseth et al., 2012). Sat_{CO_2} gives the direction of CO_2 flux at the water–atmosphere interface, and its product with gas transfer velocity determines the CO_2 flux at the water–atmosphere interface, i.e. whether lake ecosystems are sinks ($\text{Sat}_{\text{CO}_2} < 0$) or sources ($\text{Sat}_{\text{CO}_2} > 0$) of atmospheric CO_2 .

Statistical analyses

The effect of storage time and treatment on five dissolved gases (O_2 , N_2 , CO_2 , CH_4 , N_2O) from the Lake Svartkulp samples was tested with a two-way ANOVA at an alpha level adapted using the Bonferroni correction for multiple testing;

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i.e. $\alpha = 0.05/5 = 0.01$. To evaluate the impact of Hg fixation on Lake Lundebyvannet samples, $[\text{CO}_2]$ values determined by headspace equilibration and GC analysis of HgCl_2 -fixed samples were compared with those calculated from DIC measurements of unfixed samples with a paired t test.

A regression analysis was performed to describe the overestimation of CO_2 concentrations caused by HgCl_2 fixation in Lake Lundebyvannet samples as a function of pH. The total CO_2 concentration in the HgCl_2 -fixed samples ($[\text{CO}_2]_{\text{HgCl}_2}$) can be expressed as

$$[\text{CO}_2]_{\text{HgCl}_2} = [\text{CO}_2]_{\text{i}} + [\text{CO}_2]_{\text{ex}}, \quad (7)$$

where $[\text{CO}_2]_{\text{i}}$ is the initial CO_2 concentration prior to HgCl_2 fixation, i.e. CO_2 concentration in the unfixed samples, and $[\text{CO}_2]_{\text{ex}}$ is the excess CO_2 concentration caused by a decrease in pH following HgCl_2 fixation. The relative CO_2 overestimation (E in %) is given by

$$E = \frac{[\text{CO}_2]_{\text{HgCl}_2} - [\text{CO}_2]_{\text{i}}}{[\text{CO}_2]_{\text{i}}} = \frac{[\text{CO}_2]_{\text{ex}}}{[\text{CO}_2]_{\text{i}}}. \quad (8)$$

The impact of pH (or $[\text{H}^+]$) on E was mathematically described by running a regression analysis using MATLAB[®]. The *fminsearch* MATLAB function from the Optimization Toolbox was used to find the minimum sum of squared residuals (SSR) for functions of the form of $E = A/[\text{H}^+]$ or $E = A \times 10^{-B \times \text{pH}}$. For each optimal solution, the root-mean-square error (RMSE) and coefficient of determination (R^2) were calculated against observed values of E , i.e. values of E determined empirically from observed $[\text{CO}_2]_{\text{i}}$ and $[\text{CO}_2]_{\text{ex}}$.

Chemical speciation, saturation index calculations and prediction of CO_2 overestimation

The speciation of solutes and saturation index (SI) values of selected minerals were calculated with the program PHREEQC developed by the USGS (Parkhurst and Appelo, 2013), neglecting the effect of dissolved organic matter. This was used to assess the impact of the addition of preservative on pH and shifting the carbonate equilibrium as well as dissolved inorganic carbon losses due to carbonate mineral precipitation. PHREEQC is commonly used to calculate the speciation of inorganic carbon and the SI of carbonate minerals and to help estimate the fate of inorganic carbon in carbon-cycling studies (Atekwana et al., 2016; Clayer et al., 2016; Klaus, 2023). For each PHREEQC simulation, two files, the database (with input reactions) and input files, respectively, were used to define the thermodynamic model and the type of calculations to perform. The database of MINTQA2 (i.e. minteq.dat; Allison et al., 1991) was used to describe the chemical system because it includes, inter alia, reactions and constants for Ag, Cu and Hg complexation with Cl, NO_3 and carbonates.

Three PHREEQC simulations were run representing the addition of each preservative solution to sample water from

Lake Svartkulp. The input files described the composition of two aqueous solutions: (i) the preservative solution assumed to contain only the preservative (i.e. HgCl₂ solution) and (ii) sample water from Lake Svartkulp with observed major element concentrations (pH, Al, Ca, Cl, Cu, Fe, Mg, Mn, N as nitrate, K, Na, S as sulfate, Zn; Table S1) and Hg and Ag natural concentration assumed to be 10⁻⁵ mg L⁻¹. The output file provided the activities of the various solutes in the preserved samples, i.e. simulating the mixing of 120 mL of lake water with 240 μL of the AgNO₃, CuCl₂ and HgCl₂ preservative solutions, as described in Sect. 2.1. This procedure allows the estimation of the pH of the preserved samples as well as the SI for various mineral phases. The SI is calculated by PHREEQC comparing the chemical activities of the dissolved ions of a mineral (ion activity product, IAP) with their solubility product (*K_s*). When SI > 1, precipitation is thermodynamically favourable. Note however that PHREEQC does not give information about precipitation kinetics.

Similarly, PHREEQC was used to estimate the decrease in pH caused by adding 150 μL of a half-saturated HgCl₂ solution to Lake Lundebyvannet samples prior to GC analyses, as described in Sect. 2.2. In the absence of data on the chemical composition of Lake Lundebyvannet, we assumed that it had the same composition as Lake Svartkulp water samples. This assumption is supported by the fact that waters from both lakes have circumneutral pH, low ionic strength (poor buffering capacity) and high DOC concentration and would therefore behave similarly in the presence of acids. Briefly, for each 0.1 pH value between a pH of 5.4 and 7.3, the carbonate alkalinity was first adjusted by increasing HCO₃⁻ concentrations in the input files for PHREEQC to confirm that the water was at equilibrium at the given pH value. Then, the effect of adding 150 μL of a half-saturated HgCl₂ solution was simulated as described above for Lake Svartkulp. Knowing the new equilibrated pH, after addition of HgCl₂, the overestimation of CO₂ concentration in Hg-fixed samples relative to unfixed samples (*E*, described in Eq. 8 above) can be predicted as described below.

Adapting Eq. (2), we obtain

$$[\text{CO}_2]_{\text{HgCl}_2} = \frac{[\text{H}^+]_{\text{HgCl}_2}^2 C_T}{Z_{\text{HgCl}_2}} \quad (9)$$

and

$$[\text{CO}_2]_i = \frac{[\text{H}^+]_i^2 C_T}{Z_i}, \quad (10)$$

where [H⁺]_i is the proton concentration measured in the initial water samples prior to HgCl₂ fixation and [H⁺]_{HgCl₂} is the proton concentration estimated by PHREEQC following HgCl₂ fixation, and a similar definition applies for Z_i and Z_{HgCl₂} from Eq. (3). Combining Eqs. (7), (9) and (10), we

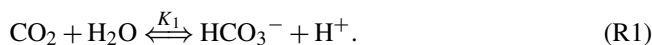
obtain

$$[\text{CO}_2]_{\text{ex}} = C_T \left(\frac{[\text{H}^+]_{\text{HgCl}_2}^2}{Z_{\text{HgCl}_2}} - \frac{[\text{H}^+]_i^2}{Z_i} \right). \quad (11)$$

Hence

$$E = \frac{[\text{CO}_2]_{\text{ex}}}{[\text{CO}_2]_i} = \frac{\left(\frac{[\text{H}^+]_{\text{HgCl}_2}^2}{Z_{\text{HgCl}_2}} - \frac{[\text{H}^+]_i^2}{Z_i} \right)}{\frac{[\text{H}^+]_i^2}{Z_i}}. \quad (12)$$

Alternatively, *E* can also simply be predicted based on the carbonic acid dissociation:



At equilibrium, we have

$$K_1 = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{CO}_2]}. \quad (13)$$

When pH is decreased upon addition of HgCl₂, a fraction (*α*) of the initial bicarbonate concentration [HCO₃⁻]_i is turned into CO₂. This fraction, expressed as [CO₂]_{ex} in Eq. (7) above, can be estimated with Eq. (13) as follows:

$$[\text{CO}_2]_{\text{ex}} = \alpha [\text{HCO}_3^-]_i = \frac{\alpha K_1 [\text{CO}_2]_i}{[\text{H}^+]_i}. \quad (14)$$

Introducing the expression of [CO₂]_{ex} from Eq. (14) into Eq. (8) yields

$$\frac{[\text{CO}_2]_{\text{ex}}}{[\text{CO}_2]_i} = E = \frac{\alpha K_1}{[\text{H}^+]_i}. \quad (15)$$

When the decrease in pH, or acidification, is greater than the buffering capacity of the water, *α* = 1. The value of *α* cannot exceed 1 because the amount of CO₂ produced by a decrease in pH cannot exceed the amount of HCO₃⁻ initially present. In all the other cases, we have *α* < 1. For both predictions of *E*, i.e. with Eqs. (12) and (15), the root-mean-square error (RMSE) and coefficient of determination (*R*²) were calculated.

Finally, additional sources of CO₂ overestimation were investigated by analysing the residuals of the model described by Eq. (12), i.e. the difference between *E* predicted with Eq. (12) and *E* determined empirically with Eq. (8). Briefly, residuals were plotted against pH and in situ temperature. Residuals were separated into two groups based on the empirical value of [HCO₃⁻]_i - [CO₂]_{ex}; i.e. the first group had values of [HCO₃⁻]_i - [CO₂]_{ex} ≥ *a*, while the second group had values of [HCO₃⁻]_i - [CO₂]_{ex} ≤ -*a*, where different values for *a* were used: 20, 10 or 5 μM. The justification for separating residuals into two groups is that (i) the first group represents samples for which bicarbonate alkalinity in the original sample is, as expected, higher than CO₂ overestimation after HgCl₂ fixation, while (ii) the second group represents samples for which bicarbonate alkalinity is not sufficient to explain CO₂ overestimation after HgCl₂ fixation.

CO₂ diffusion fluxes from Lake Lundebyvannet

The diffusive flux of CO₂ (F_{CO_2} in mol m⁻² d⁻¹) from Lake Lundebyvannet surface water was estimated according to

$$F_{\text{CO}_2} = \frac{k_{\text{CO}_2} ([\text{CO}_2] - [\text{CO}_2]_{\text{eq}})}{1000}, \quad (16)$$

where k_{CO_2} is the CO₂ transfer velocity (md⁻¹), $[\text{CO}_2]$ is the surface water CO₂ concentration (μM), 1000 is a factor to ensure consistency in the units, and $[\text{CO}_2]_{\text{eq}}$ is the theoretical water CO₂ concentration (μM) in equilibrium with atmospheric CO₂ concentration calculated with Eq. (3) and a $p\text{CO}_2$ of 416 μatm (see above).

The CO₂ transfer velocity (k_{CO_2}) was estimated as follows (Vachon and Prairie, 2013):

$$k_{\text{CO}_2} = k_{600} \left(\frac{600}{Sc_{\text{CO}_2}} \right)^{-n}, \quad (17)$$

where k_{600} is the gas transfer velocity (md⁻¹) estimated from empirical wind-based models and Sc_{CO_2} is the CO₂ Schmidt number for in situ water temperature (unitless; Wanninkhof, 2014). We used n values of 0.5 or 2/3 when wind speed was below or above 3.7 ms⁻¹, respectively (Guérin et al., 2007). Empirical k_{600} models included those from Cole and Caraco (1998; $k_{600} = 2.07 + 0.215U_{10}^{1.7}$), Vachon and Prairie (2013; $k_{600} = 2.51 + 1.48U_{10} + 0.39U_{10}\log_{10}LA$), and Crusius and Wanninkhof (2003; power model: $k_{600} = 0.228U_{10}^{2.2} + 0.168$ in cm h⁻¹). U_{10} and LA refer to mean wind speed at 10 m in ms⁻¹ and lake area in km², respectively. Sub-hourly U_{10} data for 2020 were retrieved from a weather station of the Norwegian Meteorological Institute located 1.5 km west of Lake Lundebyvannet (station name: E18 Melleby; ID: SN 3480; 59.546° N, 11.4535° E) using the Frost application programming interface (Frost API, 2022). Daily, monthly and yearly (only covering the ice-free season: April–November) F_{CO_2} was estimated using Eq. (12). Daily $[\text{CO}_2]$ was interpolated from weekly data using a modified Akima spline (makima spline in MATLAB[®] based on Akima, 1974). This interpolation method is known to avoid excessive local undulations.

3 Results

3.1 Effects of preservatives and storage time on dissolved gases

In the unfixed samples from Lake Svartkulp, the concentration of O₂ declined while CO₂ increased over time in a close to 1 : 1 molar ratio, likely reflecting the effect of microbial respiration activity and mineralization of organic matter (Fig. 2 and Table S2 in the Supplement). The concentration of O₂ in the unfixed samples decreased from near 300 to below 200 μM (Fig. 2). In the presence of inhibitors, O₂

concentrations tended to be slightly higher at $t = 24$ h and remained constant or declined only slightly over time to generally remain at or above saturation (280 to 300 μM). Thus, the inhibitors were effective in reducing oxalic respiration.

The concentration of CO₂ in the presence of AgNO₃ at $t = 24$ h was not significantly different to the unfixed at $t = 0$ (Fig. 2; paired t test, $P > 0.1$). At $t = 24$ h, CO₂ concentrations were however much higher in the presence of HgCl₂ (135 μM) or CuCl₂ (131 μM) than in the unfixed (89 μM; Fig. 2 and Table S2). The CO₂ further increases from 130 to ~160 μM after 3 weeks in both sample sets preserved with HgCl₂ and CuCl₂, while a decrease in O₂ is less pronounced for samples fixed with CuCl₂ and completely absent for samples fixed with HgCl₂. Overall, the addition of HgCl₂ or CuCl₂ following sampling increased CO₂ concentrations by 47 % after 24 h compared to the unfixed and caused further changes over the 3-month storage time, while preservation with AgNO₃ yielded CO₂ concentrations consistent with the unfixed and caused negligible changes over time (Fig. 2; paired t test, $P > 0.1$).

The concentration of CH₄ across all samples ranged between 0.017 and 0.377 μM (Fig. 2), as expected 2 orders of magnitude smaller than CO₂. At $t = 24$ h, the concentration of CH₄ was over 0.2 μM in the presence of inhibitors, while it was below saturation in the unfixed (0.03 μM; Fig. 2). CH₄ oversaturation in the preserved samples persisted after 3 weeks and 3 months of storage, and CH₄ concentration remained unchanged (Fig. 2 and Table S2).

The concentration of N₂O ranged between 9.8 and 12.7 nM with only samples preserved with AgNO₃ showing negligible changes over time (Fig. 2; paired t test, $P > 0.1$). All the other samples showed consistent patterns with storage time. N₂O concentrations initially increased within the first 3 weeks, followed by a decrease after 3 months.

The changes in N₂ were likely within handling and analytical errors and not different in the presence or absence of inhibitors (Fig. 2 and Table S2; paired t test, $P > 0.1$).

3.2 Effects of preservatives on pH

In the samples amended with ultrapure water or AgNO₃, the pH did not show any significant changes after 2 or 24 h. In contrast, both groups with HgCl₂ and CuCl₂ treatments show significant decreases in pH after 2 h, −0.12 and −0.19, respectively, and 24 h, −0.16 and −0.21, respectively. In addition, they showed a significant decrease in pH from 2 to 24 h. Samples amended with CuCl₂ show the strongest decrease in pH.

3.3 Contrasting impacts of HgCl₂, CuCl₂ and AgNO₃ on dissolved CO₂ estimation revealed by chemical speciation modelling

The PHREEQC simulation of unpreserved samples, based on concentrations of all major elements (Table S1), predicted a

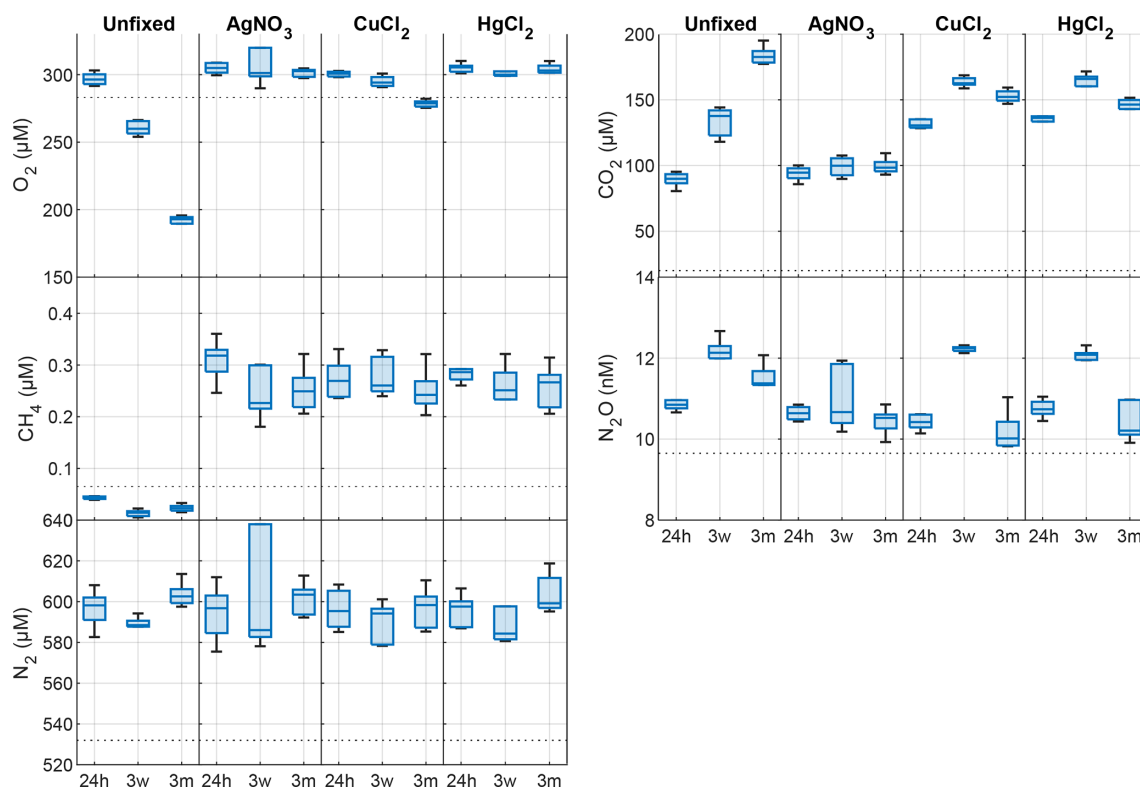


Figure 2. Changes in dissolved O₂, CO₂, CH₄, N₂O and N₂ concentrations (nM or μM) in the absence (unfixed) and presence of different preservatives (AgNO₃, CuCl₂, HgCl₂) at three different times (24h, 24h after incubation start; 3w, 3 weeks after collection; 3m, 3 months after collection). The horizontal dotted line is the saturated gas concentration corresponding to 100 % gas saturation at in situ lake temperature. Box plots show the median and 25th and 75th percentiles, and the whiskers display the minimum and maximum.

pH of 6.72 (Table 2), which is very close to the measured pH of 6.73 (Table S1). This suggests that chemical information provided to PHREEQC is likely sufficient to describe the system, without having to invoke more complex reactions with dissolved organic matter. The addition of HgCl₂ and CuCl₂ both caused a significant decrease in pH to 6.40 and 6.45, respectively (Table 2), which is similar to the decrease observed at the end of the 24 h short-term incubation (Fig. 3).

In the absence of preservatives, none of the common carbonate minerals, including calcite, were associated with a saturation index higher than 1; i.e. dissolution was thermodynamically favourable for all these minerals and no DIC loss was expected (Table 2). However, upon addition of HgCl₂ or CuCl₂, some carbonate minerals, e.g. HgCO₃ or malachite and azurite, respectively, were expected to spontaneously precipitate given their relatively high saturation index values.

3.4 Effects of HgCl₂ on dissolved CO₂ concentration under a range of pH values

CO₂ concentrations in unfixed water samples from Lake Lundebyvannet were significantly lower than in the HgCl₂-fixed samples (mean difference: 52 μM; paired *t* test; *P* < 0.0001; Table 3). Fixation with HgCl₂ caused a general over-

estimation of CO₂ concentration and the saturation deficit (Fig. 4), thus missing out events of CO₂ influx (carbon sink) under conditions of high photosynthesis activity (high pH; Fig. 4). In parallel, PHREEQC predicted a decrease of 0.6 to 1.8 units of pH related to HgCl₂ addition (Fig. S1 in the Supplement).

The pH value of water samples from Lake Lundebyvannet varied between 5.4 and 7.3 (Figs. 4 and 5), mainly due to marked variations in phytoplankton photosynthetic activity (Rohrback et al., 2020). The relative overestimation of CO₂ (*E*) follows an exponential increase with pH and is well reproduced by a simple exponential function ($2.56 \times 10^{-5} \times 10^{1.015 \times \text{pH}}$, RMSE = 44 %, *R*² = 0.81, *p* < 0.0001; Fig. 5).

4 Discussion

Prior to using dissolved gas concentrations in freshwater to estimate the magnitude of biological aquatic processes such as photosynthesis and oxic respiration, denitrification, and methanogenesis, we must ensure that biological activity between sampling and laboratory analyses was efficiently inhibited without significant impacts on the sample's chemistry. Here we report a unique dataset on the impact of three

Table 2. The pH and saturation indices of selected carbonate minerals estimated by PHREEQC for the unpreserved and preserved samples.

Preservatives	pH	Saturation indices			
		HgCO ₃	Cu ₂ (OH) ₂ CO ₃ – malachite	Cu ₂ (OH) ₂ CO ₃ – azurite	Ag ₂ CO ₃
Unfixed	6.72	−2.31	−4.96	−8.71	−16.42
HgCl ₂	6.40	3.64	−5.89	−10.10	−17.20
CuCl ₂	6.45	−2.55	2.26	2.11	−17.44
AgNO ₃	6.71	−2.31	−4.97	−8.73	−4.33

Table 3. CO₂ concentrations ([CO₂], μM) and diffusion fluxes (F_{CO_2} , mol m^{−2} d^{−1}) from Lake Lundebyvannet estimated from HgCl₂-fixed and unfixed samples following Cole and Caraco (1998). The ice-free season spans April to November. Data are also shown in Fig. 6.

Preservatives		Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Ice-free season
[CO ₂]	None	45	39	19	68	59	85	123	120	67
	HgCl ₂	68	75	74	133	130	149	179	178	121
	Diff (%)	+50	+93	+296	+96	+119	+75	+45	+49	+82
F_{CO_2}	None	0.10	0.07	0.01	0.15	0.11	0.23	0.37	0.48	0.17
	HgCl ₂	0.20	0.21	0.16	0.34	0.29	0.47	0.57	0.77	0.35
	Diff (%)	+97	+188	+2163	+130	+162	+99	+55	+62	+108

preservatives on water samples from a typical low-ionic-strength, unproductive boreal lake to give information on potential risks of misestimation of dissolved gas concentrations. We further show, using CO₂ concentration data from a typical productive boreal lake, that using HgCl₂ can lead to negligence of the role of photosynthesis in lake C cycling.

4.1 Best preservative for the determination of dissolved gas concentrations

Given that none of the four treatments (unfixed, HgCl₂, CuCl₂ or AgNO₃) applied to Lake Svartkulp water samples during the 3-month incubation offer an independent control, a first challenge is to determine which of the treatments represent the most realistic dissolved gas concentrations close to real conditions. For CO₂ and O₂, a few studies have used unfixed samples (only preserved dark at +4 °C) up to 48 h after sampling to determine CO₂ or DIC concentrations (e.g. Sobek et al., 2003; Kokic et al., 2015). Therefore, the CO₂ and O₂ concentrations in the unfixed samples collected after 24 h incubation are the most representative of the initial real concentrations. Biological activity might have had an impact, but this is likely negligible over the first 24 h. In addition, the fact that the CO₂ and O₂ concentrations in the samples fixed with AgNO₃ after 24 h, 3 weeks and 3 months are equal to those from unfixed samples after 24 h (Fig. 2) confirms that the unfixed samples after 24 h can be used as a control. In fact, only samples fixed with AgNO₃ are reliable given the expected toxicity of Ag, the absence of an impact on pH (Fig. 3) and unchanged concentrations over the 3-month experiment for all gases. Similarly, N₂O and N₂ concentrations in the unfixed samples after 24 h can be used as a control.

However, for CH₄, Fig. 2 shows that after only 24 h, the CH₄ concentration in the unfixed samples is below atmospheric saturation while it is consistently much higher in all three sets of fixed samples. Boreal lakes are typically oversaturated with respect to CH₄ (Valiente et al., 2022), and it is very unlikely that CH₄ could have been produced in lake water incubated under high concentrations of oxygen and toxic preservatives. Hence, unfixed samples do not represent real CH₄ concentrations. These observations are all consistent with the fact that the three preservatives were effective in preserving CH₄ from oxidation. Even over 24 h, preservatives need to be added to oxic water samples to preserve CH₄ from oxidation. In fact, oxic methanotrophy typically shows rates on the order of μM d^{−1} (Thottathil et al., 2019; van Grinsven et al., 2021). Hence, a CH₄ consumption of 0.3 μM within 24 h in the unfixed water samples is realistic (Fig. 2).

In summary, preservation with AgNO₃ is the only method that offered robust determination of all five dissolved gases with negligible changes in concentration over time.

4.2 Risks of misestimating dissolved gas concentration with HgCl₂ and CuCl₂ preservation

Both sets of samples preserved with either HgCl₂ or CuCl₂ showed CO₂ concentrations that were much higher than the unfixed (after 24 h) or the AgNO₃-fixed samples. This is due to an acidification of the poorly buffered (alkalinity 127 μM) and near-neutral water (pH = 6.73), shifting the carbonate equilibrium from HCO₃ to CO₂ as also shown by Borges et al. (2019). In fact, a rapid decrease in pH was observed upon HgCl₂ and CuCl₂ treatments (Fig. 3). The increase in CO₂ from about 130 to ~160 μM after 3 weeks in both sample

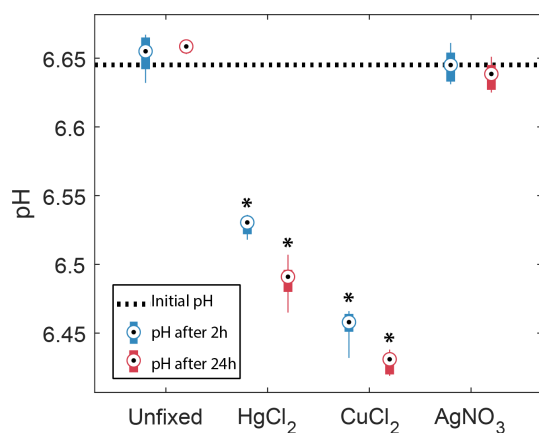


Figure 3. Observed changes in pH in the absence (unfixed) and presence of different preservatives (AgNO₃, CuCl₂, HgCl₂) at two different times, 2 and 24 h after the start of the incubation. The horizontal dotted line represents the initial pH of the bulk water sample. Box plots show the median and 25th and 75th percentiles, and the whiskers display the minimum and maximum of the six replicates. Stars indicate groups that are significantly different from each other and from the initial pH (two-way ANOVA).

sets preserved with HgCl₂ and CuCl₂ is not mirrored by a similar decrease in O₂ (Fig. 2). This suggests that oxidic respiration is not the main source for this additional 30 μM of CO₂ but rather points towards additional acidification of the samples caused, for example, by kinetically controlled complexation of Hg²⁺ with dissolved organic matter (Miller et al., 2009). In fact, the relatively slow complexation of Hg²⁺ with organic thiol groups can release two protons (Skylberg, 2008) and up to three, with some participation of a third weak-acid group (Khwaja et al., 2006). The transient nature of acidification caused by HgCl₂ and CuCl₂ is also evident in the pH impacts showing higher acidification after 24 h than after 2 h incubation (Fig. 3). The following decrease in CO₂ after 3 months (down to ~145 μM) points to other processes. The precipitation of Hg and Cu carbonates, given their high saturation index values (Table 2), would be consistent with the decrease in CO₂ concentrations observed between 3 weeks and 3 months. Calcite precipitation is typically observed in supersaturated solutions within 48 h (Kim et al., 2020). Hence, it is realistic to assume that Hg and Cu carbonate precipitation influenced the CO₂ concentration within the preserved samples over the 3 months of storage time. Impacts of Hg or Cu carbonate precipitation are not evident after 3 weeks, likely because of slow but persistent CO₂ production in the presence of HgCl₂ and CuCl₂ related to acidification as described above (Fig. 2). However, after 3 weeks, this production likely weakens and is counterbalanced by increasing carbonate precipitation.

Overall, the addition of HgCl₂ or CuCl₂ following sampling increased CO₂ concentrations by 47 % within the first 24 h compared to the unfixed, consistent with the acidifica-

tion of −0.16 to −0.21 pH units observed over the same time in the pH incubation experiment (Fig. 3) and the pH estimated with PHREEQC without the interaction with dissolved organic matter (Table 2). In fact, introducing pH and CO₂ concentration values of 6.40–6.45 and 130 μM, respectively, for the samples preserved with HgCl₂ and CuCl₂ into Eqs. (1) and (2) yields DIC concentrations (C_T) of about 270 μM at $t = 24$ h. These DIC concentrations are almost equal to those calculated for the unfixed samples and those preserved with AgNO₃ at $t = 24$ h, i.e. with a pH of 6.73 and CO₂ concentration of 88 μM. Interestingly, the concentration of CO₂ in the samples preserved with HgCl₂ and CuCl₂ continues to increase up to ~160 μM after 3 weeks. Given that oxidic respiration is inhibited (Fig. 2), this additional CO₂ is believed to originate from the progressive release of protons following the relatively slow complexation of Hg²⁺ with dissolved organic matter (Khwaja et al., 2006; Miller et al., 2009; Skylberg, 2008). Note however that this process could not be predicted with PHREEQC given that it neglected the effect of dissolved organic matter.

Unlike the AgNO₃-fixed samples, all the other samples showed an initial increase in N₂O concentration from 24 h to 3 weeks, followed by a decrease from 3 weeks to 3 months. Similar patterns of net N₂O production followed by net consumption were also reported in short-term incubations of seawater from the high-latitude Atlantic Ocean, although over much shorter timescales, i.e. 48 and 96 h (Rees et al., 2021). The large difference in kinetics between the latter experiment (Rees et al., 2021) and our incubation might be attributable to differences in incubation temperature, where the seawater from the high-latitude Atlantic Ocean was incubated at ambient temperatures while our samples were kept at +4 °C. Other differences in the experimental setup might have also played a role. The lack of inhibition of N₂O production and consumption in the samples preserved with HgCl₂ and CuCl₂ can be attributed to the fact that N₂O production tends to increase under increasing acidic conditions (Knowles, 1982; Mørkved et al., 2007; Seitzinger, 1988). In fact, the mole fraction of N₂O produced during denitrification increases compared to N₂ as pH decreases (Knowles, 1982).

4.3 Using PHREEQC to estimate acidification caused by HgCl₂ in samples from Lake Lundebyvannet

As for the samples from Lake Svartkulp as described above, the overestimation of CO₂ concentration in the samples from Lake Lundebyvannet fixed with HgCl₂ (161 μM added; Fig. 4) likely stems from the acidification shifting the carbonate equilibrium from bicarbonate to CO₂. In fact, PHREEQC predicted a decrease of 0.6 to 1.8 units of pH related to HgCl₂ addition in these samples (Fig. S1).

The relative overestimation of CO₂ (E in Fig. 5) followed a typical exponential increase reflecting the decrease in absolute CO₂ concentration with increasing pH (Stumm and Morgan, 1981), caused here by phytoplankton photosynthe-

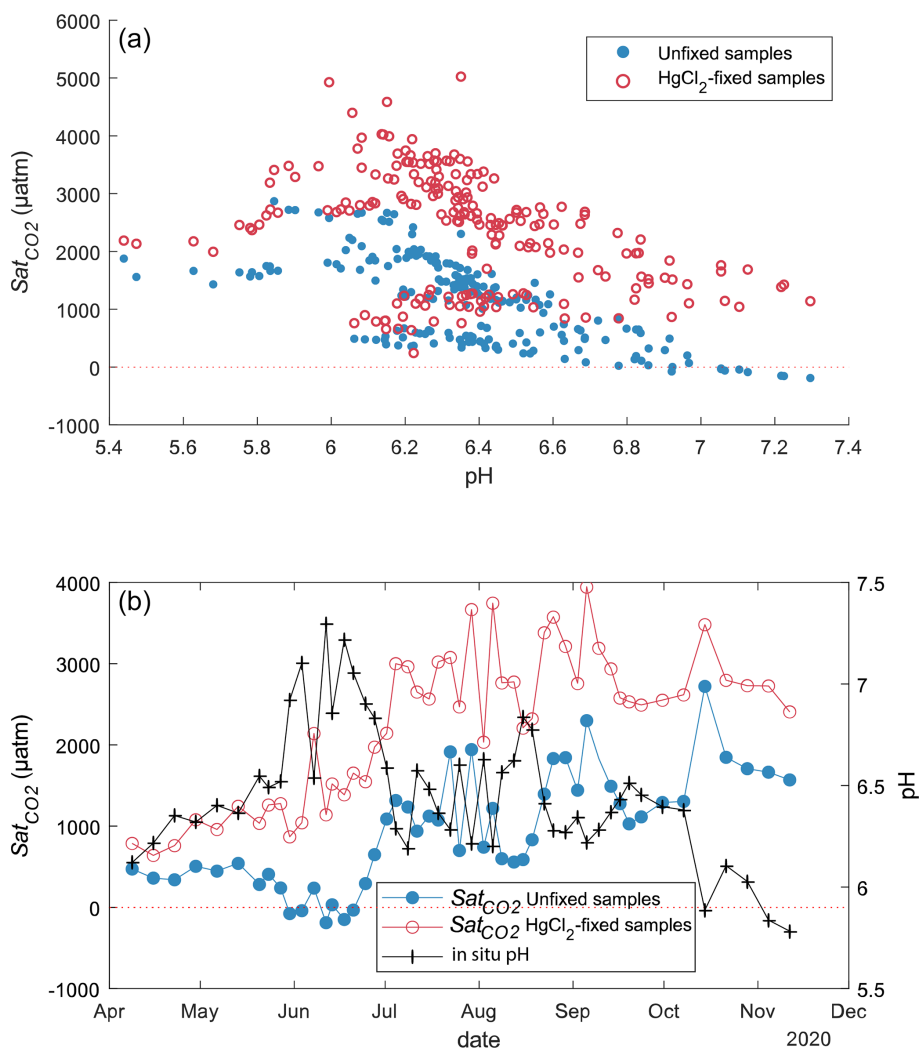
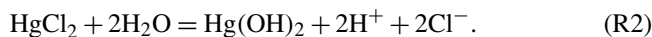


Figure 4. CO₂ saturation deficit (Sat_{CO₂}) in Lake Lundebyvannet as a function of in situ pH for all unfixed (obtained from DIC analysis) and HgCl₂-fixed (obtained from GC analysis) samples (a). Time series of the pH and CO₂ saturation deficit of surface water (1 m deep) for unfixed and HgCl₂-fixed samples (b).

sis. In fact, the exponential increase in CO₂ overestimation is easily predicted by Eq. (9) with an equivalent level of accuracy to the optimized exponential function (Fig. 5). Consistently, the relative overestimation of CO₂ (E) shows an inverse decrease with [H⁺] that is well reproduced by a simple inverse function ($3.25 \times 10^{-5}/[\text{H}^+]$; RMSE = 44 %, $R^2 = 0.81$, $p < 0.0001$; Fig. 5) and predicted by Eq. (15), with an α value of 1. Combining Eqs. (8) and (15) and solving this with pH values estimated from PHREEQC (Fig. S1) for α yields values ranging between 0.72 and 0.89 with an average of 0.85. Unexpectedly, this average α value is almost equal to the ratio of the inverse function coefficient and K_1 ; i.e. $\frac{3.25 \times 10^{-5}}{K_1} = 0.87$. Hence, the relative overestimation of CO₂ (E) caused by HgCl₂ fixation is easily predicted by the change in bicarbonate equilibrium knowing the proton release from HgCl₂ addition, here estimated with PHREEQC.

Hence, PHREEQC can be used to predict a decrease in pH caused by HgCl₂ fixation if sufficient knowledge is gathered on the ionic water composition. Proton release during HgCl₂ fixation can be represented by the following reaction:



From Reaction (R2), it becomes evident that the initial concentration of chloride in the water samples will likely limit HgCl₂ dissociation and proton release. This is a mechanism that is likely to occur in seawater where HgCl₂ has been shown to cause a decrease in pH, although at a negligible level with a maximum decrease in pH of -0.01 (Chou et al., 2016).

Figure 5 shows that a range of water samples were associated with a relative CO₂ overestimation (E) that substantially deviated from the overestimation predicted with Eq. (12) (red

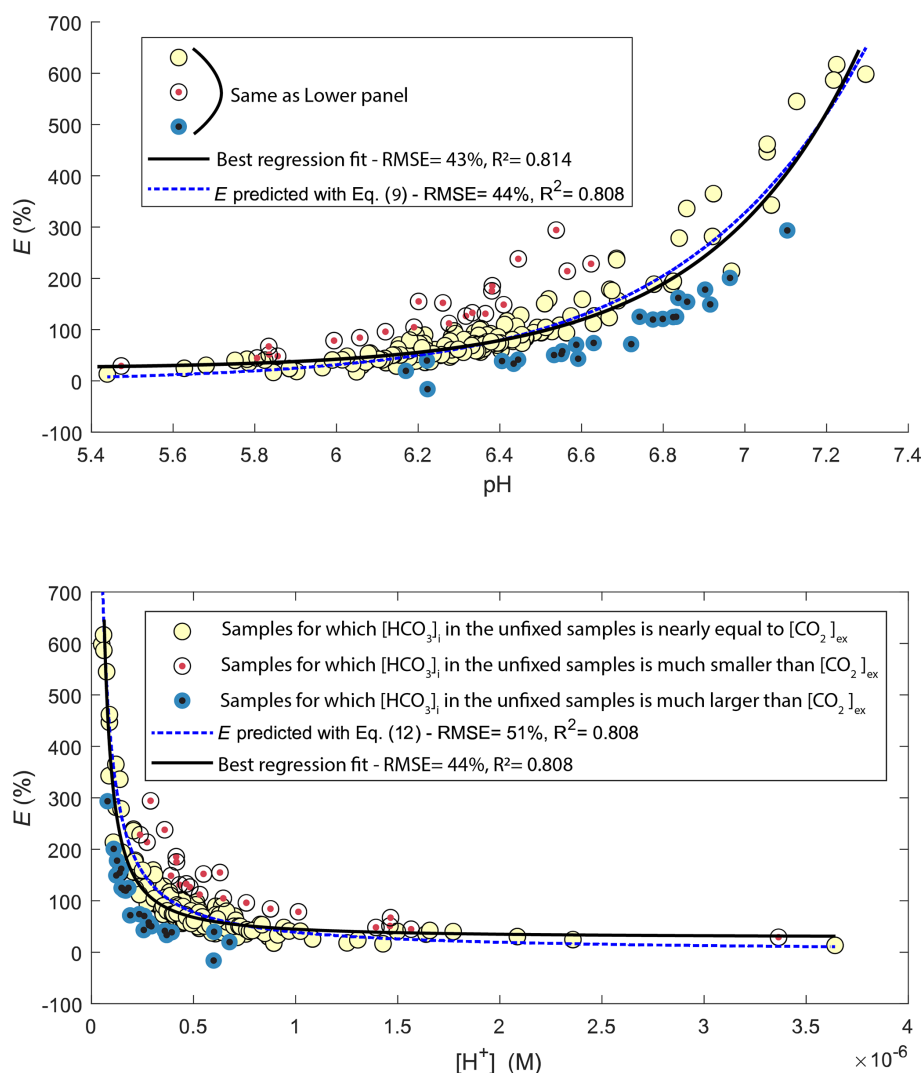


Figure 5. Comparison of observed (circles) and predicted (blue line) relative overestimation (E) of CO₂ concentrations caused by HgCl₂ fixation in Lake Lundebyvannet samples as a function of pH (a) or proton concentration (b). The black line shows the best fit of the regression analysis. Yellow symbols represent samples for which the bicarbonate concentration in the unfixed samples ($[HCO_3^-]_i$) is nearly equal to CO₂ overestimation ($[CO_2]_{ex}$), i.e. $\pm 20 \mu\text{M}$ (equivalent to a pH error of 0.05), while red and blue symbols represent samples for which initial bicarbonate concentration was lower and higher, respectively, than the CO₂ overestimation.

and blue symbols in Fig. 5). In fact, some samples had a higher initial bicarbonate content ($[HCO_3^-]_i$) than the excess CO₂ concentration ($[CO_2]_{ex}$), while others showed the opposite. The former case (blue symbols in Fig. 5) can easily be explained by a higher buffering capacity of the sampled water, i.e. a higher pH after HgCl₂ fixation than that predicted by PHREEQC related to a different water composition. Indeed, the concentration of major elements in the water from Lake Lundebyvannet may vary significantly over time, and in the absence of data, we considered that the water composition, except for DIC, pH and HgCl₂, was constant over time. By contrast, samples associated with $[CO_2]_{ex}$ being larger than $[HCO_3^-]_i$ are more enigmatic. In order to shed light on possible explanations, we visually inspected trends between

empirical deviations from predictions, i.e. residuals, and in situ temperature or pH. Absolute values of residuals showed a progressive increase with pH and in situ temperature, which is in agreement with decreasing precision of the headspace method with increasing temperature and pH (Koschorreck et al., 2021). In fact, CO₂ is less soluble at higher temperature; hence there can be more gas evasion during sampling, and thus the error increases with in situ temperature. In addition, at higher pH, CO₂ concentration decreases, and consequently the absolute error in CO₂ quantification becomes larger relative to measured CO₂ concentration. Interestingly, many of the high residual values were not evenly distributed across the year or across the summer and instead were associated with only a few specific sampling events during

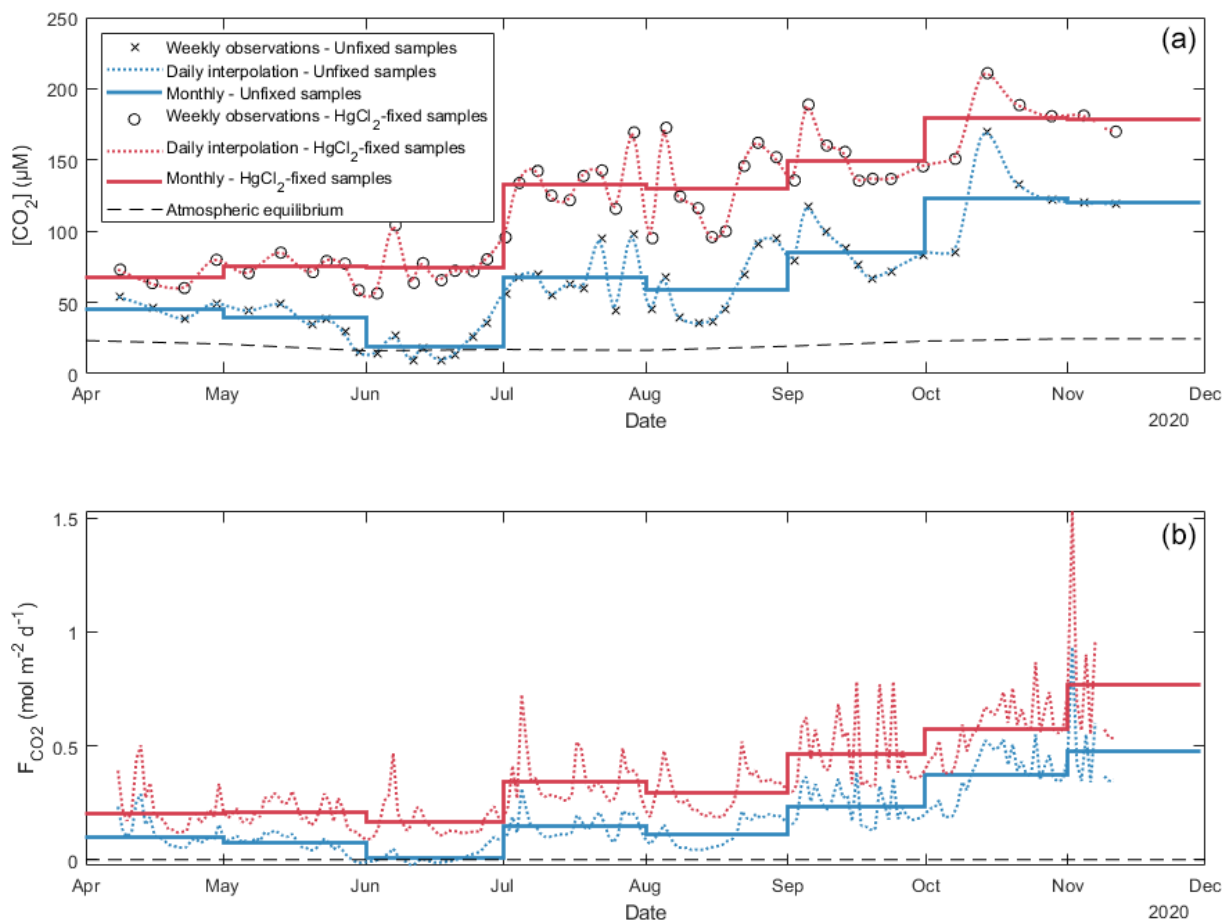


Figure 6. Daily and monthly surface CO₂ concentrations ([CO₂]; **a**) and diffusion fluxes (F_{CO_2} ; **b**) at the water–atmosphere interface from Lake Lundebyvannet (also in Table 3). Unfixed samples were obtained by DIC analysis. Daily [CO₂] was interpolated from weekly data using a modified spline (see text for details). Diffusion fluxes were calculated following Cole and Caraco (1998).

summer (Fig. S2 in the Supplement). This suggests that degassing could have occurred due to high ambient temperature in the field. Water associated with [CO₂]_{ex} being larger than [HCO₃⁻]_i (red symbols in Figs. 5 and S4 in the Supplement) could have been subject to larger degassing in the samples collected for DIC analysis than the samples for GC analysis. On the other hand, degassing was likely larger for samples for GC analysis than for DIC analysis for water associated with [HCO₃⁻]_i being larger than [CO₂]_{ex} (blue symbols in Figs. 5 and S2). In addition to degassing and temperature effects, errors in pH measurements can also cause a large misestimation of CO₂ concentration from DIC analysis, and this error increases exponentially with pH following the shift in the carbonate equilibrium. In summary, our analysis is consistent with that of Koschorreck et al. (2021), showing that errors in the determination of CO₂ concentrations are smaller at lower pH values and lower temperature (Fig. S2).

4.4 Implications for the estimation of lake and reservoir C cycling and recommendations

Using HgCl₂ or CuCl₂ to preserve dissolved gas samples in poorly buffered water samples has large impacts on CO₂ concentrations with considerable risk of leading to incorrect interpretations. The risk of misestimating CO₂ concentration due to HgCl₂ and CuCl₂ preservation is the highest when the pH of the unfixed water is close to the first carbonic acid dissociation constant ($pK_1 = 6.41$ at 25 °C; Stumm and Morgan, 1996). This implies that any small shift in pH will have a significant impact on the carbonate equilibrium between bicarbonate and CO₂. The risk is also the highest in the lowest-ionic-strength waters. In that respect, low-ionic-strength, slightly acidic to neutral, moderately humic lakes commonly found in Norway (de Wit et al., 2023); large parts of Sweden (Valinia et al., 2014); Finland and Atlantic Canada (Houle et al., 2022); and Ontario, Quebec and northeast USA (Skjelkvåle and de Wit, 2011; Weyhenmeyer et al., 2019) are the most prone to errors in CO₂ concentrations related to HgCl₂ or CuCl₂ preservation. Through a preliminary lit-

erature search, we found several studies not only from boreal lakes (Jonsson et al., 2001; Urabe et al., 2011; Yang et al., 2015; Hessen et al., 2017) but also from circumneutral-pH sub-tropical to tropical aquatic environments (Jeffrey et al., 2018; Webb et al., 2018; Ray et al., 2021) where preservation with HgCl₂ may have caused biases in the quantification of CO₂ concentrations as was the case for samples from the Congo River (Borges et al., 2019). A significant part of the low-ionic-strength boreal lakes are becoming increasingly sensitive to changes in nutrients with strong impacts on their role in carbon cycling (Myrstener et al., 2022). In this context, it is crucial to avoid misestimation of CO₂ concentrations and thus avoid use of HgCl₂ or CuCl₂ to ensure a robust understanding of the role of autotrophic processes in lake C cycling. Below we describe the implications for the lake C budget of Lundebyvannet as an example of a misestimation of the role of photosynthesis in a typical productive boreal lake.

In Lake Lundebyvannet, over the ice-free season, average CO₂ concentrations determined following HgCl₂ fixation and GC analysis were 82 % higher than those obtained from DIC analyses (Table 3; Figs. 6 and S3 in the Supplement). CO₂ concentrations obtained from HgCl₂-fixed samples created the illusion that Lake Lundebyvannet was a steady net source of CO₂ to the atmosphere over the ice-free season with a large CO₂ saturation deficit (Fig. 4), while, in reality, the lake switched from being a net source in May to a net sink over a few weeks in Jun, and to a net source again in July (Figs. 6 and S3). Indeed, monthly CO₂ overestimation related to HgCl₂ fixation reached about 300 % in June (Table 3). Propagating this overestimation into the estimates of CO₂ diffusion fluxes with typical wind-based models yields an overestimation of CO₂ fluxes of 108 %–112 % over the ice-free season and up to 2100 % in June (Tables 3 and S3 in the Supplement). Hence, interpreting CO₂ data without correcting for CO₂ overestimation caused by HgCl₂ fixation leads to negligence of the role of photosynthesis in lake C cycling, with major implications for current and future predictions of lake CO₂ emissions.

The use of HgCl₂ to preserve water samples prior to dissolved gas analyses is part of current guidelines for greenhouse gas measurements in freshwater reservoirs (Machado Damazio et al., 2012; UNESCO/IHA, 2008, 2010). Hence, there is a risk of overestimating CO₂ concentrations and emissions, in the absence of the discrete measurement of emissions, from hydropower reservoirs, with consequences for the present and expected greenhouse gas footprint from hydroelectricity. To ensure precise estimation of greenhouse gas concentration and, possibly, emission from hydropower, the use of HgCl₂ should therefore be discontinued.

5 Conclusion

Mercury is a potent neurotoxin for humans and toxic for the environment, and its use should be discouraged, notably following the Minamata Convention on Mercury, a global treaty ratified by 126 countries (16 December 2020) to protect human health and the environment from the adverse effects of mercury. This study further questions the use of HgCl₂ for preservation of poorly buffered (low-ionic-strength) water samples with high DOC concentration for analysis of dissolved gases in the laboratory. Although CuCl₂ is less toxic, it behaved similarly to HgCl₂ and cannot be recommended. In fact, both chlorinated inhibitors caused a significant decrease in pH, shifting the carbonate equilibrium towards CO₂, and are also suspected to promote carbonate precipitation over long-term storage. The only promising inhibitor tested in this study was AgNO₃, notably for dissolved CO₂, CH₄ and N₂O. Silver nitrate is a suitable substitute for HgCl₂ in low-ionic-strength waters; further tests should be carried out with a range of inhibitor concentration and more diverse water samples. The use of chemical inhibitors may not be the best approach. Alternatives exist, such as directly measuring gas concentrations in situ with sensors; sampling the headspace out in the field; and bringing back gas samples (e.g. Cole et al., 1994; Karlsson et al., 2013; Kling et al., 1991; Valiente et al., 2022), rather than water samples, to the lab for gas chromatography analyses. However, care must be taken to know the exact equilibration temperature (Koschorreck et al., 2021) and to avoid gas exchange with the atmosphere as well as to use a clean background gas during headspace equilibration, which can be challenging in remote environments under harsh meteorological conditions.

We further advise against interpretation of CO₂ concentration data from low-ionic-strength, circumneutral water samples preserved with HgCl₂ or CuCl₂. The overestimation of CO₂ concentration caused by HgCl₂ can mask the effect of photosynthesis on lake carbon balance, creating the illusion that lakes are net CO₂ sources when they are net CO₂ sinks. Our analysis from Lake Lundebyvannet shows that HgCl₂ fixation led to an overestimation of the CO₂ concentration by a factor of 1.8, on average, but approaching a factor of 4 during the peak photosynthetic period. An even larger impact is expected on CO₂ diffusive fluxes, which were overestimated by a factor of 2 on average and up to a factor of > 20 during peak photosynthesis. Interpreting such data would have underestimated the current and future role of aquatic photosynthesis.

Data availability. All data supporting this study are available at <https://doi.org/10.4211/hs.436be40748a246269102b20211b49762> (Clayer et al., 2024).

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