Assessment of methods for collection, preservation, and storage of water samples for dissolved methane and nitrous oxide concentration measurements

Anagha Payyambally[1], Josie L. Mottram[1], Julie Granger[1], and Cara C. M. Manning[1]

1. University of Connecticut, Department of Marine Sciences, Groton, CT, USA

Corresponding author: Cara Manning, cara.manning@uconn.edu

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Abstract

Nitrous oxide (N_2O) and methane (CH_4) are potent greenhouse gases produced and consumed in freshwater and marine systems. Their measurement in water is challenging due to contamination risks at various sampling, storage, and analysis stages. Samples that are stored before analysis are commonly preserved with mercuric chloride ($HgCl_2$), which is highly toxic. To date, no studies have conducted a thorough assessment or comparison among methods for sample collection, preservation and storage, for concurrent analysis of CH_4 and N_2O. This study systematically compares sampling and preservation methods in aqueous samples. Estuarine water samples at 4–11 ºC were collected in 120 mL serum vials without a headspace, preserved, and stored at room temperature for up to 6 months before analysis. Preservation with hydroxide salts at pH 10–12 proved equally effective to preservation with $HgCl_2$. Sample collection and septum insertion while the bottle is submerged minimized contamination with air, improving the accuracy of CH_4 measurements. Depressurization of samples after collection proved necessary to prevent distention of septa (and associated gas transfer) in saltwater samples collected without a headspace, preserved with hydroxide, and stored at a significantly warmer temperature than the in situ water temperature. Two-leg and three-leg septa yielded the most stable concentrations. The number of piercings of the septa and storage orientation had no effects on gas concentrations. Air transportation of the samples did not significantly affect CH_4 and N_2O concentrations. This study will help to improve accuracy of CH_4 and N_2O measurements in water and reproducibility between research groups.

Introduction

Methane (CH_4) and nitrous oxide (N_2O) are two potent greenhouse gases whose concentrations in the atmosphere are increasing due to human activities (Tian et al. 2024; Saunois et al. 2025). Approximately 25% of global N_2O emissions originate from the ocean (Tian et al. 2024), a result of biological transformations in seawater and sediments. Inland freshwaters and wetlands are the largest natural sources of methane on earth, together representing approximately 30% of total natural and anthropogenic CH_4 emissions (Saunois et al. 2025). Concentrations of N_2O and CH_4 in aqueous samples are often measured in tandem using a single instrument, a multi-detector gas chromatograph (Wilson et al., 2018). Both gases are challenging to measure accurately and precisely in aqueous media as concentrations approach equilibrium with the atmosphere, currently in the range of 2–5 nmol kg^{-1} for CH_4 and 6–20 nmol kg^{-1} for N_2O, depending on water temperature and salinity. A recent intercalibration study demonstrated large inter-laboratory differences in N_2O and CH_4 concentration measurements in seawater for sets of identical samples that were collected following a single procedure (Wilson et al., 2018). This study demonstrated that variability is introduced from differences in analytical protocols and provided recommendations to improve inter-comparability, such as routine analysis of air-equilibrated water samples (Wilson et al., 2018). However, differences in sample collection and preservation methodologies between laboratories are also likely to contribute to variability between labs. To further improve the data quality of these crucial measurements, there is a need to systematically compare and evaluate sample collection, preservation, and storage methods for both N_2O and CH_4 to provide recommendations to the community.

A primary challenge with collecting and preserving aqueous samples for dual analysis of N_2O and CH_4 arises due to their contrasting solubilities. The dimensionless Ostwald solubilities (defined as the volume of gas that dissolves per volume of liquid at 1 atm partial pressure of the gas) are approximately 0.028

for CH_4, 0.524 for N_2O, and 0.727 for CO_2 at a salinity of 35 g kg^{-1} and temperature of 20 °C (Wanninkhof et al. 2009). During sampling of either N_2O or CO_2 (and/or dissolved inorganic carbon) some researchers opt to leave a small headspace of air in the vial prior to sealing (0.6–1% headspace) to allow for expansion of the liquid as the sample warms (Dickson et al. 2007; Frame et al. 2014; Frey et al. 2024). The small amount of atmospheric gas introduced to the sample from the headspace is negligible compared to that dissolved in the sample, and corrections for the headspace can be applied if required based on the analytical protocol. In contrast, aqueous samples of lower solubility gases such as CH_4 are typically collected with no headspace (Magen et al., 2014; Wilson et al., 2018) because the molar quantity of CH_4 gas contained in a 1% headspace is significant relative to the quantity dissolved in the water. For example, for air-equilibrated seawater with a temperature of 15 °C and a salinity of 35 g kg^{-1}, a 1% air headspace would increase the total CH_4 content of the vial by 35% but only increase the N_2O content by 0.3%. In this example, a small error in the volume of air, such as leaving a headspace of 1.3 mL instead of 1.0 mL, would lead to a 10% error in the estimated concentration of CH_4, but a negligible error in the concentration of N_2O. Similarly, the accidental introduction of an air bubble into the sample vial during collection would cause a significant error in the calculated concentration of CH_4 and other low solubility gases, but a negligible impact on the concentration of N_2O and other high solubility gases. Collection of samples for dual analysis of both N_2O and CH_4 thus requires that there be no headspace and that the potential for introduction of air bubbles be minimized.

Aqueous gas samples collected into glass vials without a headspace entail additional considerations stemming from the thermal expansion of the sample water after collection, which can lead to septum bulging. For example, as seawater warms from 4 to 24 °C, its density decreases by 0.4%, corresponding to a volume increase of 0.4 mL in a 100 mL vial. Some rubber septa can stretch to accommodate the added volume, which could potentially increase the gas permeability across the septum. If samples are

not depressurized following thermal expansion, the septa can tear, or the glass may shatter. Expansion may be exacerbated by pressure changes induced by air transport of samples and/or transporting the samples in a non-temperature-controlled environment. Depressurization of the septa can be performed by piercing the septum after the water has warmed up, a step that introduces the possibility of air contamination by impacting the integrity of the seal. Certain designs of septa containing a thicker body exhibit reduced bulging, but these septa are more difficult to insert and pierce with needles; the inability to stretch in response to thermal expansion also increases the risk of glass breaking. Thus, a means of avoiding over-pressurization that does not compromise the integrity of septa must be devised to ensure sample integrity. Some investigators performing analysis via headspace equilibration create an inert headspace by adding high purity gas and removing an aliquot of sample shortly after sampling as part of the depressurization step to prevent further bulging of the septa (Magen et al. 2014). However, this method requires access to a high purity compressed gas cylinder in the field. In this study we focused on methods that would not require compressed gases to simplify the collection procedure.

To maintain in situ gas concentrations during storage, samples are preserved to halt biological activity. Mercuric chloride ($HgCl_2$) is the most widely-used preservative for water samples to be measured for N_2O and CH_4, as well as many other dissolved gases and inorganic carbon species (Emerson et al. 1999; Dickson et al. 2007; Bourbonnais et al. 2021; Frey et al. 2024). However, due to its acute toxicity, the United States and European Commission adopted restrictions on the use of mercury (ICC 2018), and some research vessels have prohibited its use (Frey et al. 2024). Additionally, when conducting sampling with less trained individuals (e.g., citizen scientists) and when working on smaller vessel and/or in rough conditions, the addition of a highly toxic substance creates additional risks and barriers to sample collection (Gonski et al. 2021). Furthermore, disposal costs for water containing $HgCl_2$

preservative are very high. Therefore, there is a need to identify more sustainable preservatives that are equally effective for dissolved gas measurements.

Several alternatives to $HgCl_2$ have been explored for preserving CH_4 and N_2O in seawater, including zinc chloride ($ZnCl_2$), copper sulfate ($CuSO_4$), and hydroxide salts including potassium hydroxide (KOH) and sodium hydroxide (NaOH). Benzalkonium chloride, a quaternary ammonium disinfectant, has also been tested for short-term preservation of CH_4 and O_2/Ar samples, but to our knowledge its efficacy for storage periods longer than 2 weeks has not been demonstrated (Gloël et al. 2015; Osaka et al. 2024). Among the tested preservatives, hydroxide preservation appears most promising for long-term storage, as addition of $ZnCl_2$, $HgCl_2$, and $CuSO_4$ can trigger abiotic N_2O production from NO_2^- (Kieskamp et al. 1988; Zhu-Barker et al. 2015; Ostrom et al. 2016; Frey et al. 2024). Hydroxide preservative is comparatively less hazardous than $HgCl_2$, and the transport and disposal of water samples containing this chemical is less cumbersome. Recently, Frey et al. (2024) demonstrated that samples preserved with NaOH at pH of 12 yielded reliable and reproducible results for N_2O concentration and isotopic composition for storage periods up to 6 months. An additional advantage of hydroxide ion preservation for N_2O analysis is that it reduces CO_2 levels in the sample and headspace by shifting the equilibrium to be dominated by carbonate and bicarbonate ions, minimizing CO_2 interference during N_2O analysis by gas chromatography or isotope ratio mass spectrometry (both gases have the same nominal mass of 44 g mol^{-1} and a similar elution time). With regard to CH_4, a study has reported the use of hydroxide salts for preservation of water samples for CH_4 but did not perform comprehensive experiments to evaluate stability of preserved samples with CH_4 concentrations near equilibrium and did not systematically compare hydroxide against $HgCl_2$ (Magen et al. 2014). Moreover, studies on the potential effect of sample pH after preservation (quantity of hydroxide added) on N_2O or CH_4 concentrations have not been conducted. Hydroxide ions help stabilize nitrite (NO_2^-) by maintaining a

high pH and preventing its protonation, thereby reducing abiotic N_2O production during storage, and also cause the precipitation of metals that, when dissolved, can contribute to abiotic N_2O production through chemodenitrification (Frey et al., 2024). Conversely, acidification can trigger abiotic N_2O production from NO_2^- under anoxic conditions (Zhu-Barker et al. 2015; Visser et al. 2020). Whether pH has effects on CH_4 preservation is unclear.

To arrive at standardized sampling protocols for dual CH_4 and N_2O analyses and increase reproducibility, we evaluated the impact of sample collection method, preservation method, transport, and storage on the integrity of CH_4 and N_2O concentrations over a storage period of up to 6 months. In the first experimental set, we evaluated whether preservation with $HgCl_2$ and hydroxide salts (to pH 12) gives equivalent results and stable concentrations over periods of 6 months. Second, we evaluated the influence of hydroxide-dependent preservation pH (pH 10, 12, and 13) on sample integrity. In a third set of experiments, we evaluated sample integrity with regard to different collection and preservation protocols: we compared nonsubmerged sample collection with submerged sample collection; we compared samples that were depressurized with to nondepressurized samples; and we compared three different types of butyl rubber septa (straight plug, 2-leg, and 3-leg). Finally, in a fourth experiment we evaluated whether the number of septum piercings inherent to respective collection methods impacts sample integrity and concurrently tested the impact of upright versus inverted storage for sample longevity. A set of samples was shipped and returned by air freight cross-country to evaluate the effect of air transportation on measured sample concentrations. From the resulting observations, we formulate recommendations for CH_4 and N_2O sample collection, preservation, and storage.

Materials and Procedures

Materials

Water samples were collected into glass serum bottles with a total volume of approximately 120 mL and a mouth outer diameter of 20 mm and inner diameter of 13 mm (Manufacturer: DWK Life Sciences; part number 223747; lot number 50417). Three designs of halogenated butyl rubber septa were tested: a 2-leg lyophilization septum made of gray siliconized bromobutyl rubber (DWK Life Sciences; part number W224100-406; lot number not recorded); a 3-leg lyophilization septum made of gray siliconized bromobutyl (DWK Life Sciences; part number W224100-407; lot number 62084), and a straight-plug butyl septum (DWK Life Sciences; part number 20-0025; lot number: 32918). The septum types were selected based on manufacturer specifications for low gas permeability and suitability for multiple piercings. The 2-leg and 3-leg septa had a durometer of 46, and the straight plug had a durometer of 50, indicating comparable rigidity of the polymers. Septa were used as received from the manufacturer without any further treatment. Previous research demonstrated that the grey halogenated butyl rubber septa display very limited leaching of chemicals from untreated septa (Niemann et al. 2015). For samples that were preserved before crimping (precrimping method), the preservative was added using a volumetric pipette. For samples preserved after crimping (postcrimping method), preservatives were added, and excess water was vented, using 22-gauge needles (1″ or 1.5″ long). Aluminum crimp seals (DWK Life Sciences; part number 22417801; lot number 67570) were used for bottle sealing.

Samples were preserved with $HgCl_2$, KOH, or NaOH. $HgCl_2$ powder (≥99.5%; Millipore Sigma; Product number: 1.04419) was used to prepare a solution at approximately 50% saturation by dissolving 3.8 g of $HgCl_2$ in 100 mL of deionized water. For $HgCl_2$ preservation, we added 100 µL of this solution to each 120 mL sample, corresponding to 0.08% of the total sample volume. This preservative volume is

in line with procedures used for preservation of dissolved inorganic carbon samples, where 0.04 to 0.1% of the total sample volume is typically used for a 50% saturated aqueous $HgCl_2$ solution (Dickson et al. 2007).

KOH pellets (≥85%; Millipore Sigma; Product number: 221473) and NaOH pellets (≥98%; Millipore Sigma; Product number: S5881) were used to prepare respective 8 M hydroxide solutions. As the preservative dissolved, the solution was stirred in an ice bath due to the reaction being exothermic. Hydroxide preservative solutions were prepared at least 24 hours before use to allow for equilibration with atmospheric gases, given previous reports that these pellets can contain trapped CH_4 (Magen et al. 2014). In a first set of experiments, we used 8 M KOH solution following Magen et al. (2014) who found the KOH pellets they tested contained less trapped CH_4 as compared to NaOH. In subsequent experiments, we switched to NaOH because it is available in a higher purity (>98% as compared to 85% for KOH), allowing us to have more confidence in the quantity of base added. We added sufficient hydroxide to bring the pH to 10, 12, or 13, depending on the experiment. We did not test pH 11 because we were not able to obtain a consistent pH of 11 due to the buffering properties of saltwater. Preservative volumes ranged from 1.1 to 5.0 mL (0.9 to 4.2% of the sample volume), depending on the experiment and target pH.

Sample collection and preservation procedures

In all experiments, sample bottles were filled using flexible PVC and silicone tubing affixed to a peristaltic pump to transfer the water. We allowed at least three times the sample volume to flow through the bottle before sealing it. After sample collection and sealing, bottles were rinsed with fresh water to prevent corrosion of the septum from salt water and/or hydroxide exposure. The water temperature, salinity, and pH were recorded at the time of collection. Throughout the manuscript, salinity is reported on the unitless practical salinity scale (PSS-78).

Nonsubmerged, precrimping sample preservation

During sampling by this method, the exterior of the bottle and the mouth remain in contact with air. We flushed the serum bottle with water, allowing the excess water to overflow onto the ground, and then slowly removed the tubing, ensuring the bottle remained filled to the top. Preservative was added using a volumetric pipette. The septum was inserted into the top of the serum bottle and crimp sealed.

Submerged, precrimping sample preservation

Prior to starting the flow of water, serum bottles were placed inside an empty cup (~300 mL), which was taller than the sample bottle. As the bottle was flushed, the excess water overflowing the bottle filled the cup to the brim and then overflowed the cup. The sample tube was carefully removed from the sample bottle while leaving the bottle fully submerged. The preservative was added to the bottle using a volumetric pipette while it remained submerged in the bottle, taking care to ensure that no bubbles adhered to the interior of the bottle. The butyl septum was submerged into the cup surrounding the bottle, ensuring that no bubbles adhered to the bottom of the septum, and then inserted the septum into the bottle. The serum bottle was then removed from the cup while holding the septum in place and sealed with an aluminum crimp seal.

Submerged, immediate postcrimping sample preservation

As with the previous method, the sample bottle was filled inside a cup, the overflowing water was collected into the cup, the septum was inserted underwater, and the sample was sealed after removing it from the cup. The preservative was added immediately after crimp sealing the bottle. Specifically, the preservative solution was injected into each bottle using a 22-gauge 1.5-inch needle attached to a 5 mL plastic syringe, while excess sample overflow was ejected through a vent needle connected to a 5 mL

syringe barrel without a plunger. Prior to insertion, the vent needle was filled with a small amount of water (gently dripping out of the needle) to prevent air from entering the bottle through the vent needle. The vent needle was inserted just below the septum, while the injection needle was placed deeper in the bottle to avoid the preservative being ejected. After piercing, both needles were removed and the sample bottle was rinsed with fresh water, and the exposed septum was covered with silicone grease (DuPont; 4109788, Molykote 111 compound) to help seal the puncture, then the septum and seal were covered with Parafilm to prevent the grease from being wiped off during storage. For samples where the in situ temperature is colder than the storage temperature, this method slightly depressurizes the samples and reduces septum bulging, but the septa nevertheless tend to slightly expand as the sample continues to warm, with the extent of bulging depending on the amount of warming and the preservative used. This procedure results in two needle punctures in the septum.

Submerged, delayed postcrimping sample preservation

This method is identical to the one above, except that we waited until 2 to 3 h after crimping to add the preservative. In this way, simultaneous preservation and venting served to remove water to compensate for the volume of preservative added and thermal expansion. This step results in two needle punctures in the septum but tends to reduce the septum bulging compared to the samples from the method described in the section above (immediate postcrimping).

Delayed depressurization

In all of the above collection and preservation methods – except the delayed postcrimping method – the samples with in situ temperatures below room temperature will continue to warm and expand after collection. During experiment 3, we tested a method where preservative was added prior to sealing (the submerged, precrimping method described above), and a delayed depressurization step was performed 2

to 3 h after preservation, after allowing the samples to warm enough to bulge the septa moderately. We inserted a vent needle connected to a 5 mL syringe barrel without a plunger that was partially filled with water into the sample. After depressurizing, the exposed septum was covered with silicone grease to help seal the puncture in the seal and then the septum and seal were covered with Parafilm to prevent the grease from being wiped off during storage. This method results in one needle puncture in the septum.

Sample storage

After sealing and rinsing, all samples were stored in the dark in an opaque plastic case with custom foam inserts to ensure secure positioning of each bottle. Samples are stored in the dark to prevent photochemical reactions (Li et al. 2020; Leon-Palmero et al. 2025). All samples were stored inverted (septum on bottom), except in Experiment 4, where inverted and upright storage were compared. The potential advantage of inverted sample storage is that it prevents any gas bubble that forms inside the bottle from being in contact with the septum; gas diffuses much faster across rubber than glass, which could increase the rate of sample contamination. The potential advantage of upright storage is that it prevents placing additional pressure on the septum, which could result in leakage of sample water.

Experiments

All experiments were conducted using water from the Long Island Sound estuary collected at the University of Connecticut Avery Point campus. In the first experimental set, bottles were filled directly at the dock. In remaining experiments, water collected at the dock was recirculated and equilibrated with air at a prescribed temperature. In all experiments, the order of time points (analysis dates) was randomized after collection. However, in Experiments 1 and 3, the order of treatments was not randomized (i.e., in Experiment 1, all hydroxide samples were collected, followed by all $HgCl_2$ samples). In each experiment, variables related to preservative, septum, collection, and storage methods

were tested (Table 1), and samples were stored for between 1 day and 6 months before analysis. Each experiment is described in more detail below.

To interpret the results of each experiment (see Assessment section), we used two-way ANOVA to determine whether there were statistically significant differences between samples due to method differences, storage time, and/or interactions between method and storage time, with a significance threshold of $p \leq 0.05$. Post hoc tests were conducted when statistically significant differences were found to identify which specific groups differ. Experimental data is publicly available on Figshare (Payyambally et al. 2026).

Table 1. Summary of preservative, septum, and collection methods tested in each experiment (indicated by X).

Expt	**Preservative**					**Septum**[a]			**Collection**[b]				
	None	$HgCl_2$	pH (NaOH/KOH)			2-leg	3-leg	SP	nonsubmerged		submerged		
			10	12	13				PreC	IPostC	PreC	IPostC	DPostC
1		X		X		X			X				
2	X	X	X	X	X	X					X		
3	X			X		X	X	X	X	X +DD	X	X +DD	
4				X		X					X +DD		X

[a] For septum, SP = straight plug.
[b] PreC = precrimping, IPostC = immediate postcrimping, and DPostC = delayed postcrimping preservative addition; +DD = delayed depressurization.

Comparison of preservation with $HgCl_2$ versus hydroxide (Experiment 1)

We evaluated whether preservation with $HgCl_2$ and hydroxide gives equivalent results for both CH_4 and N_2O, and whether the measured concentration is stable during storage periods up to 6 months. We selected a pH of ~12 for Experiment 1 because it is the most common pH recommended in the literature for gas sample preservation (Magen et al. 2014; Frey et al. 2024). In our samples, the pH was 12.6; we targeted an initial pH slightly above 12 to ensure that the pH remained ≥12 throughout the storage period. Estuary water sampled directly from the dock had a salinity of 25.7–26.0, a temperature of 11.3–11.4 ºC, and a pH of 7.9.

We used 2-leg stoppers and the nonsubmerged, precrimping sample preservation method. No subsequent depressurization was performed. Each sample was preserved with either 100 μL of 50% saturated $HgCl_2$ or 2.5 mL of 8 M KOH. Samples were stored inverted prior to collection. Five replicate samples for each preservation method were collected for each of 8 time points. Analysis occurred the next day, then after approximately 1 week of storage, and then after 1, 2, 3, 4, 5, and 6 months of storage.

Comparison of hydroxide preservation at pH 10, 12, and 13 (Experiment 2)

To test the effect of the sample pH following hydroxide preservation on the measured gas concentrations and stability over time, we sampled estuary water (salinity of 29.0 and a pH of ~7.7) that was recirculated for 24 h through a chiller set to 10 ºC prior to sample collection. This procedure was implemented starting with Experiment 2 because the large number of samples required extended collection times. Collecting samples directly from the tidal estuary over a prolonged time could lead to variability in the gas concentration in the samples.

For this experiment, we used 2-leg stoppers and the submerged, precrimping preservative addition method to reduce air contamination in the samples (see the assessment of experiment 3 below). No subsequent depressurization was performed. We added 1.1 mL of 8 M NaOH to achieve pH 10, 1.8 mL to achieve pH 12, and 5.0 mL to achieve pH 13. We also collected samples preserved with 100 µL $HgCl_2$ and non-preserved samples. Samples were stored inverted prior to collection. Five replicates of each preservation method were collected to analyze the day after collection and after approximately 1, 2, 4, and 6 months of storage. However, for $HgCl_2$ preservation we only collected and analyzed samples after 1 day and 1 month, due to limited water volume in the chilling system and because we concluded from Experiment 1 that preservation with $HgCl_2$ and hydroxide to pH 12.6 gives equivalent results over storage periods up to 6 months.

Comparison of sample collection, preservation, and septum type (Experiment 3)

We evaluated the impact of sample collection, preservation method, and septum design on the measured CH_4 and N_2O concentrations over 6 months. We collected estuary water (salinity of 27.1 and a pH ~7.7), which was recirculated for 24 h through a chiller set to 4 ^oC prior to sample collection. Each sample was preserved with 2.5 mL of 8 M NaOH to achieve a final pH of around 12.

We collected the NaOH-preserved samples in four different ways: 1) the submerged, precrimping method, without depressurization, 2) the submerged, immediate postcrimping, delayed depressurization method, 3) the nonsubmerged, precrimping method, without depressurization, and 4) the nonsubmerged, immediate postcrimping, delayed depressurization method. For collection methods 1 and 2, three types of septa were tested (straight plug, 2-leg, and 3-leg). For methods 3 and 4, we only used the 2-leg septa. Delayed depressurization occurred within 3 h after collection. We also collected a set of non-preserved samples with 2-leg septa using the submerged method without depressurization. Samples were stored

inverted prior to collection. Five replicates of each method (collection method and septum type, where applicable) were collected to analyze at each time point. We analyzed samples after 1 day, and approximately 1 week, and 1, 2, 4, and 6 months of storage.

Comparison of depressurization approaches, storage methods, and the effect of air shipment (Experiment 4)

We evaluated the influence of different depressurization approaches to determine if repeated puncturing influences sample stability, and we also assessed whether upright or inverted storage of sample bottles provides better stability. In parallel, we evaluated whether pressure and temperature changes during air transport affected the measured CH_4 and N_2O concentrations, and whether they caused additional septa tearing or bubble formation compared to samples that were not shipped. All samples were collected using the submerged method, using estuary water (salinity of 30.0 and pH of ~7.7), which was chilled to 4 ^oC for 24 h prior to sample collection. Four replicates were collected per time point and treatment combination (number of piercings, orientation, and shipping status).

Two preservation approaches were tested: 1) the submerged, precrimping, delayed depressurization method and 2) the submerged, delayed postcrimping method. The delayed depressurization and delayed postcrimping preservation both occurred within 3 h after sampling. We collected these samples to evaluate whether the number of piercings and the storage orientation influenced sample stability, under conditions with and without shipping. Samples were analyzed the day after collection. Half of the samples were shipped via air freight on a round trip between Connecticut and California, USA (6 total days in shipping). The samples were shipped between the last week of October and the first week of November in 2025 and therefore would not have been subject to the high temperatures that may occur in

summer months and in warmer climates. The shipped and non-shipped samples were then analyzed after approximately 1, 2, 4, and 6 months of storage.

Sample analysis

Samples were analyzed in the Gas Biogeochemistry Laboratory at the University of Connecticut, using headspace equilibration, following Manning et al. (2026). To create the headspace, 22 mL of water was removed from the sample using a peristaltic pump and added ultrahigh-purity N_2 simultaneously. The sample with headspace was then equilibrated on an orbital shaker tray for ~3 h at room temperature. Using the peristaltic pump, a 20 mL volume of brine (105 g NaCl L^{-1}) was then introduced into the equilibrated sample with a 22 gauge, 3" long needle that reached the bottom of the bottle, whilst the headspace was simultaneously collected into a preevacuated 12 mL Labco Exetainer vial using a gas-tight zero dead volume union with 22 gauge needles on both ends to transfer the sample from the headspace to the vial. The vial contained dried KOH, which acted to remove carbon dioxide from samples not preserved with hydroxide to avoid interference on the N_2O measurements. On each day of sample analysis, we also analyzed samples of air-equilibrated water at room temperature as a quality assurance check (Wilson et al., 2018). Compared to expected results based on atmospheric gas concentrations for CH_4 and N_2O, the average error (difference between measured and expected concentration for air-equilibrated water) across all experiments and analysis days was 0.2 nmol kg^{-1} (2%) for N_2O and 0.3 nmol kg^{-1} (10%) for CH_4, and the standard error of the air-equilibrated water results (n = 21) was 0.1 nmol kg^{-1} for N_2O and 0.2 nmol kg^{-1} for CH_4.

Before sample analysis and after every 40 to 60 samples, a set of standards containing known concentrations of CH_4 and N_2O were run. Standards were prepared from an Airgas certified standard tank containing 9.82 ppm of CH_4 and 7.97 ppm of N_2O in N_2 by dilution with pure N_2 using Alicat mass

flow controllers and injecting the standards into evacuated Exetainer vials. Four to five standards with different gas concentrations, including a pure N_2 standard, were prepared, with the number of standards depending on the sample concentrations in the run. The N_2O and CH_4 concentrations in the Airgas standard cylinder were calibrated to an accuracy of 1% by comparison with a NOAA standard air cylinder, which was calibrated to the WMO scale (Dlugokencky et al. 2020a, 2020b). All samples were analyzed using an xyzTek Bandolero autosampler connected to an SRI 8610 gas chromatograph with two loops in series transferring the sample to a flame ionization detector for CH_4 measurement and an electron capture detector for N_2O measurement, as described in Manning et al. (2026).

Assessment

Preservation with $HgCl_2$ versus hydroxide (Experiment 1)

Samples for this experiment were collected at an in situ temperature of 11 °C and preserved using the nonsubmerged precrimping preservation method without any depressurization or piercing of septa. We noticed bubbles in almost all the samples (<0.5 cm diameter) immediately after sample collection and sealing; bubble size remained stable for the entire 6 months of storage. The bubble was trapped during pipette and septum insertion because a small amount of water was removed from the neck of the bottle during the removal of the sample tube and pipette, making it more difficult to avoid trapping air beneath the septum. The septa of samples preserved with hydroxide were moderately bulged after a day to 6 months, whereas septa of $HgCl_2$-preserved samples did not bulge. The exothermic reaction between the water and hydroxide solution causes a slight and temporary warming of the sample, and this may contribute to increased stretching of the septum for preservation with hydroxide compared to $HgCl_2$. Moreover, adding concentrated hydroxide solution to saltwater samples triggers chemical precipitation. This chemical precipitation results in a slight increase in the total volume inside the bottle: the

precipitated salt and water take up more volume than the same quantity of salt fully dissolved in water. We noted that complete precipitation and settling to the bottom of the bottle took around 5 minutes, and that precipitated salts persisted for the entire storage period. In subsequent experiments, we found that the quantity of precipitate formed and time to complete the precipitation varied depending on the quantity of hydroxide added and the salt content of the water sample. The expected thermal expansion would have corresponded to a density decrease of 0.24% for warming from 11 to 22 ºC, however, the water likely warmed up during collection, slightly reducing the thermal expansion (see the assessment of Experiment 2 below).

The in situ pH was 7.9 and the following preservation, the pH of the samples was stable at 12.6 for hydroxide preservation and 7.9 for $HgCl_2$ preservation over storage periods from 1 to 6 months (Table S1).

CH_4 concentrations in samples preserved with either $HgCl_2$ or hydroxide (pH = 12.6) were stable over time, averaging 20.8 ± 1.6 nmol kg^{-1} across all samples (Fig. 1a; Table S2). The two-way ANOVA test including all time points indicated that there was no statistically significant difference in CH_4 concentrations associated with preservative type across sampling times (p = 0.063; Table 2), with the hydroxide-preserved samples averaging 21.2 ± 1.4 nmol kg^{-1} and the $HgCl_2$-preserved samples averaging 20.6 ± 1.7 nmol kg^{-1} (Table S2). However, statistically significant differences in CH_4 concentrations were observed among sampling time points (p = 0.023; Table 2) – although there was no significant interaction between the preservative and the storage time (p = 0.908; Table 2). A post hoc comparison indicated that measurements after 1 week and 3 months of storage differed significantly from each other (p = 0.030; Table 2), but no other significant differences between time points were found. These differences could be explained by the relatively high analytical precision of the measurements, which made statistical tests sensitive to inter-batch differences in calibration. The

variability in the measured CH_4 concentration of replicate samples was higher for storage times ≥ 1 month (standard deviation 1.0 to 2.5 nmol kg^{-1}) compared to shorter (≤ 1 week) storage periods (standard deviation 0.4 to 0.8 nmol kg^{-1}), a result observed in subsequent experiments (Table S4, S6, S8), demonstrating the value of replicate samples when extended storage is necessary.

With regard to N_2O (Fig. 1b; Table S2), concentrations averaged 12.0 ± 0.4 nmol kg^{-1} across all samples and time points (12.1 ± 0.3 nmol kg^{-1} for hydroxide preservation and 11.9 ± 0.3 for $HgCl_2$ nmol kg^{-1}, Table S2). A two-way ANOVA including all time points indicated that N_2O concentrations differed between preservative treatment (p = 0.006; Table 3) and that concentrations differed significantly among storage times (p = 0.007) – although there was no significant interaction between the preservative and the storage time (p = 0.179). A post hoc analysis revealed that the sets of $HgCl_2$- and hydroxide-preserved samples did not differ significantly at any individual time point, but that overall, the two preservatives yielded significantly different concentrations. For all storage periods except 6 months, the average N_2O concentration was slightly higher in the hydroxide-preserved samples (Table S2). In Experiment 1, the samples were collected directly from the estuary, with all $HgCl_2$-preserved samples collected, followed by all hydroxide-preserved samples. The order of time points was analyzed after the samples were collected. Therefore, it is possible that temporal variability in the in situ conditions may have contributed to the apparent differences between the two preservatives. The hydroxide-preserved samples analyzed after 6 months displayed lower concentrations than all prior time points, and the $HgCl_2$-preserved samples analyzed after 6 months were also slightly lower than the average over the prior 5 months, though the $HgCl_2$-preserved 6 month samples also displayed the largest standard deviation of all prior months (Table S2). This apparent decrease in month 6 may reflect slight calibration differences between months, rather than a storage effect, given that the trend of decreasing N_2O concentration was not observed progressively over multiple months.

We conclude from Experiment 1 that preservation with hydroxide (pH = 12.6) maintains stable concentrations as well as with $HgCl_2$ for at least 6 months for CH_4 and 5 months for N_2O, given an initial sample water temperature of ~11 °C and storage at room temperature with the precrimping method with no depressurization. The CH_4 concentrations by both preservation methods were statistically equivalent, while for N_2O, the concentrations were statistically different, on average 2% higher in the hydroxide-preserved samples, but the cause of this apparent discrepancy is unclear. Hydroxide preservation resulted in more substantial septa bulging, an issue we addressed in Experiments 3 and 4 (see below).

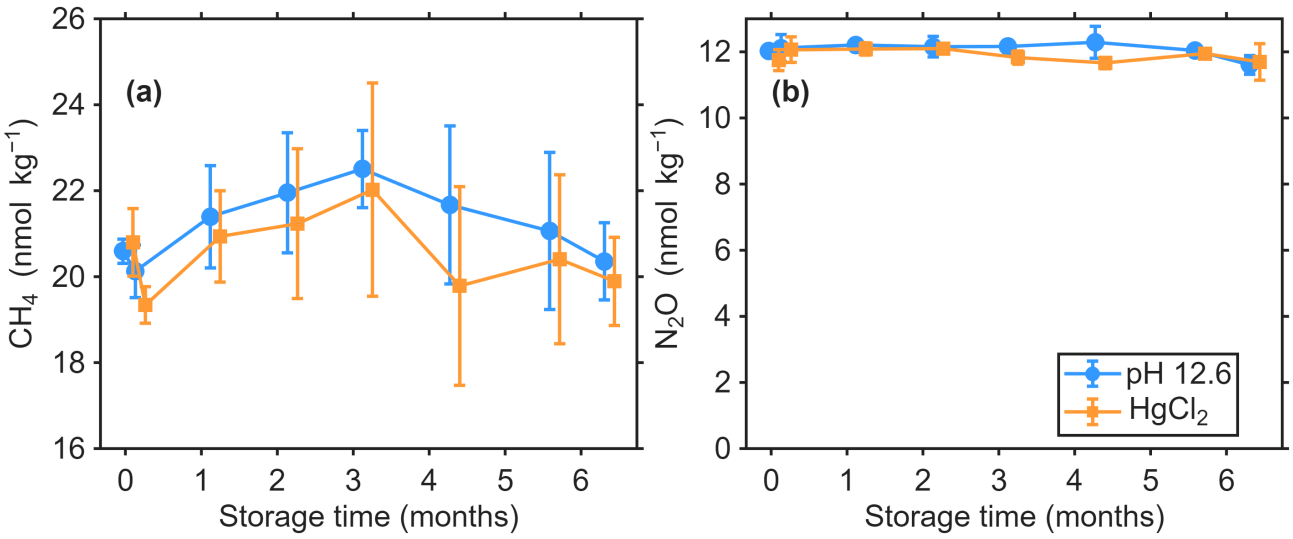

Fig. 1. Experiment 1: CH_4 (**a**) and N_2O (**b**) concentrations measured over successive preservation periods. Blue circles and solid lines represent samples preserved to pH 12.6 using KOH and orange squares and solid orange lines represent samples preserved with $HgCl_2$. All Experiment 1 samples were collected using 2-leg stoppers and the nonsubmerged, precrimping preservative addition method without depressurization, and were stored inverted. Data points for each preservative are slightly offset along the x-axis for visual clarity, to avoid overlap of error bars, which represent the standard deviation of replicate samples for each treatment at each time point.

Table 2. Methane statistical table: two-way ANOVA results for Experiments 1–4. Post hoc results are summarized when there was a significant effect of method and/or time. Sig. diff. refers to results that were significantly different ($p \leq 0.05$).

Expt	Variables tested	Common conditions	Method effect	Storage time effect	Method-storage time interaction	Post hoc results
1	1. $HgCl_2$ 2. pH 12.6	2-leg, nonsub. PreC, nondepr.	$F_{1,59} = 3.58$, $p = 0.063$	$F_{7,59} = 2.54$, $p = 0.024$	$F_{7,59} = 0.38$, $p = 0.908$	*Time:* 1 week and 3 month are sig. diff. from each other ($p = 0.030$)
2A	1. pH 10 2. pH 12 3. pH 13	2-leg, PreC, submerg. nondepr.	$F_{2,53} = 0.81$, $p = 0.449$	$F_{4,53} = 4.84$, $p = 0.002$	$F_{8,53} = 0.71$, $p = 0.678$	*Time:* 1 day is sig. diff. from 1, 4, and 6 month samples ($p \leq 0.020$)
2B	1. pH 10 2. pH 12 3. pH 13 4. nonpres.		$F_{3,73} = 0.85$, $p = 0.470$	$F_{4,73} = 7.61$, $p < 0.001$	$F_{12,73} = 0.67$, $p = 0.770$	*Time:* 1 day is sig. diff. from all other time points ($p < 0.001$)
2C	1. pH 10 2. pH 12 3. pH 13 4. nonpres. 5. $HgCl_2$		$F_{4,37} = 2.18$, $p = 0.091$	$F_{1,37} = 30.00$, $p < 0.001$	$F_{4,37} = 0.88$, $p = 0.484$	*Time:* 1 day is sig. diff. from 1 month ($p < 0.001$).
3A	1. depr. 2. nondepr.	pH 12	$F_{1,214} = 5.63$, $p = 0.019$	$F_{4,214} = 13.15$, $p < 0.001$	$F_{4,214} = 5.66$, $p < 0.001$	*Time:* 6 month is sig. diff. from all other time points ($p \leq 0.003$); 1 month is sig. diff. from 4 months ($p = 0.032$).
3B	1. SP 2. 2-leg 3. 3-leg	pH 12, submerg., IPostC +DD	$F_{2,60} = 2.99$, $p = 0.058$	$F_{4,60} = 0.66$, $p = 0.620$	$F_{8,60} = 1.00$, $p = 0.444$	–
3C	1. sub. 2. nonsub.	2-leg, pH 12, IPostC	$F_{1,40} = 31.53$, $p < 0.001$	$F_{4,40} = 1.00$, $p = 0.421$	$F_{4,40} = 1.41$, $p = 0.249$	*Method:* two methods are sig. diff ($p < 0.001$)
4A	1. PreC +DD 2. DPostC	2-leg, pH 12, submerg.	$F_{1,114} = 1.27$, $p = 0.263$	$F_{4,114} = 1.48$, $p = 0.212$	$F_{4,114} = 1.36$, $p = 0.252$	–
4B	1. inverted 2. upright		$F_{1,114} = 0.32$, $p = 0.572$	$F_{4,114} = 1.54$, $p = 0.194$	$F_{4,114} = 0.43$, $p = 0.785$	–
4C	1. shipped 2. nonship.		$F_{1,105} = 0.28$, $p = 0.596$	$F_{3,105} = 2.31$, $p = 0.081$	$F_{3,105} = 1.34$, $p = 0.266$	–

[a]Nonpres. = nonpreserved; depr. = depressurized; nondepr.: nondepressurized; sub. = submerged; nonsub. = nonsubmerged; nonship = nonshipped. Sig. diff. refers to results that were significantly different ($p \leq 0.05$).

Table 3. Nitrous oxide statistical table: two-way ANOVA results for Experiments 1-4. Post hoc results are summarized when there was a significant effect of method or time. Abbreviations follow Table 1 and 2.

Expt	Variables tested	Common conditions	Method effect	Storage time effect	Method-storage time interaction	Post hoc results
1	1. $HgCl_2$ 2. pH 12.6	2-leg, nonsub. PreC, nondepr.	$F_{1,61} = 8.03$, $p = 0.006$	$F_{7,61} = 3.11$, $p = 0.007$	$F_{7,61} = 1.52$, $p = 0.179$	*Method*: $HgCl_2$ and KOH are not sig. diff. at any time point. *Time:* 6 month concentration is sig. diff. from 1 month and 2 months ($p \leq 0.011$).
2A	1. pH 10 2. pH 12 3. pH 13	2-leg, PreC, submerg. nondepr.	$F_{2,59} = 36.14$, $p < 0.001$	$F_{4,59} = 35.06$, $p < 0.001$	$F_{8,59} = 1.12$, $p = 0.367$	*Method:* pH 13 is sig. diff. from pH 10 and 12 ($p < 0.001$). *Time:* 6 month is sig. diff. from all other times ($p < 0.001$).
2B	1. pH 10 2. pH 12 3. pH 13 4. nonpres.		$F_{3,78} = 19.74$, $p < 0.001$	$F_{4,78} = 27.61$, $p < 0.001$	$F_{12,78} = 13.01$, $p < 0.001$	*Method:* non-preserved is sig. diff. from pH 10, 12, 13 ($p < 0.001$). *Time:* 6 month is sig. diff. from all other times ($p < 0.001$).
2C	1. pH 10 2. pH 12 3. pH 13 4. non-pres. 5. $HgCl_2$		$F_{4,37} = 18.47$, $p < 0.001$	$F_{1,37} = 65.91$, $p < 0.001$	$F_{4,37} = 3.13$, $p = 0.026$	*Method*: pH 13 is sig. diff. from all other methods ($p < 0.001$). *Time*: 1 day and 1 month are sig. diff ($p < 0.001$)
3A	1. depr. 2. nondepr.	pH 12	$F_{1,213} = 6.00$, $p = 0.015$	$F_{4,213} = 7.09$, $p < 0.001$	$F_{4,213} = 7.03$, $p < 0.001$	*Time:* 6 month is sig. diff. from all other time points ($p \leq 0.027$); 4 month is sig. diff. from 1 and 2 months ($p \leq 0.030$)
3B	1. SP 2. 2-leg 3. 3-leg	pH 12, submerg., IPostC +DD	$F_{2,60} = 17.06$ $p < 0.001$	$F_{4,60} = 5.42$ $p < 0.001$	$F_{8,60} = 3.06$, $p = 0.006$	*Method:* SP is sig. diff. from 2-leg and 3-leg ($p < 0.001$). *Time:* 2 month is sig. diff. from 1, 4, and 6 months ($p \leq 0.028$)
3C	1. sub. 2. nonsub.	2-leg, pH 12, IPostC	$F_{1,40} = 2.94$, $p = 0.094$	$F_{4,40} = 3.83$, $p = 0.010$	$F_{4,40} = 0.37$, $p = 0.830$	*Time:* 2 month is sig. diff. from 4 month ($p = 0.013$).
4A	1. PreC +DD 2. DPostC	2-leg, pH 12, submerg.	$F_{1,114} = 2.91$ $p = 0.091$	$F_{4,114} = 74.51$, $p < 0.001$	$F_{4,114} = 1.88$, $p = 0.119$	*Time:* All time point pairings are sig. diff. except for 1 day and 4 month ($p \leq 0.005$)
4B	1. inverted 2. upright		$F_{1,114} = 0.02$, $p = 0.885$	$F_{4,114} = 66.16$, $p < 0.001$	$F_{4,114} = 0.43$, $p = 0.790$	*Time:* All time point pairings are sig. diff. except for 1 day and 4 month ($p \leq 0.007$)
4C	1. shipped 2. nonship.		$F_{1,104} = 2.00$, $p = 0.160$	$F_{3,104} = 87.89$, $p < 0.001$	$F_{3,104} = 2.11$, $p = 0.103$	*Time:* All time point pairings are sig. diff. ($p \leq 0.004$).

Preservation with NaOH (pH = 10, 12, and 13) and with $HgCl_2$ (Experiment 2)

Samples were collected using the submerged precrimping preservation method with no depressurization. Because the samples were not depressurized and the in situ water temperature was 10 ºC, all the septa bulged, and some septa tore by the time the analysis occurred. We noticed that the septa of samples preserved with hydroxide bulged almost twice as much as those in Experiment 1, even though the in situ temperature was similar (10 ºC in Experiment 2 and 11 ºC in Experiment 1). In contrast to Experiment 1, none of the intact samples in Experiment 2 contained bubbles since the pipette and stopper were inserted underwater (submerged). The trapped air bubbles in Experiment 1 provided space for thermal expansion of the water, reducing the septum bulging relative to Experiment 2. Moreover, Experiment 1 samples likely warmed up more during filling due to not being in an overflow cup of water and the air temperature being warmer than the in situ temperature. Compared to samples preserved with hydroxide, the septa of the non-preserved and $HgCl_2$-preserved samples in Experiment 2 were less bulged, analogous to observations in Experiment 1 where $HgCl_2$ septa did not bulge. Additionally, among samples preserved with hydroxide, pH 10 exhibited the least bulging, and pH 13 the most, because a larger quantity of precipitate is formed in the samples with the highest pH. The bulging was most severe in the samples analyzed after 6 months, likely because the heating and ventilation system in the storage room malfunctioned just prior to the sixth month analysis date, causing the storage room temperature to temporarily increase by several degrees. Samples with torn septa that showed clear air contamination upon analysis were not included in the statistical results reported in this paper.

The pH of samples decreased by 0.1 to 0.4 units in the preserved samples and 0.4 units in the non-preserved samples over 6 months after sample collection; $HgCl_2$-preserved samples for pH measurement were not collected due to limited sample water in the collection vessel. As discussed, the $HgCl_2$-

preserved samples were only analyzed after 1 day and 1 month since the Experiment 1 results had already shown comparable results between $HgCl_2$ and pH 12.6 (Table S3).

CH_4 concentrations appeared insensitive to preservation pH, with sample means averaging 5.6 ± 0.8 nmol kg^{-1} across all pH 10–13 samples (Fig. 2a, Table S4). A two-way ANOVA revealed no significant effect of preservation pH on CH_4 concentrations (p = 0.449; Table 2, Experiment 2A). CH_4 concentrations differed significantly among storage times (p = 0.002) but showed no significant interaction between the preservation pH and the storage time (p = 0.678). A post hoc comparison revealed that the CH_4 concentrations were significantly different for the 1 day samples, as compared to the 1 month, 4 month, and 6 month samples ($p \leq 0.020$), but the 2 month samples were not significantly different from the next day (p = 0.998), and no other significant differences between time points were found for the hydroxide-preserved samples.

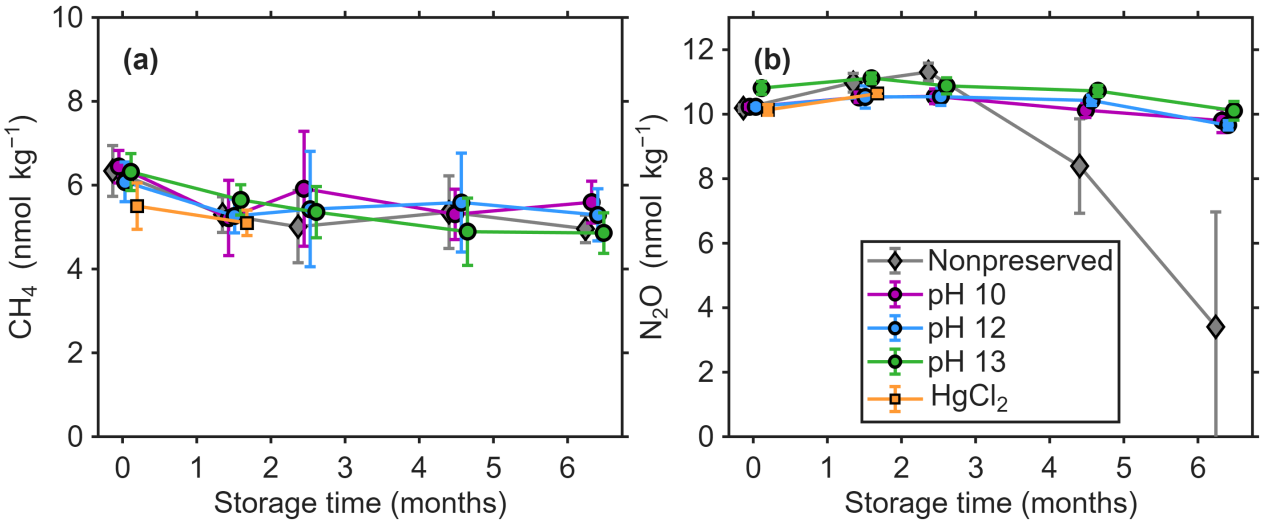

Fig. 2. Experiment 2: CH_4 (**a**) and N_2O (**b**) concentrations in nmol kg^{-1} measured over successive preservation periods. Grey diamonds represent nonpreserved samples, and orange squares represent samples preserved with $HgCl_2$. Purple, blue, and green circles represent samples preserved with NaOH to pH 10, 12, and 13, respectively. All Experiment 2 samples were collected using 2-leg stoppers and the

submerged, precrimping preservative addition method without depressurization, and were stored inverted. Data points for each preservative are slightly offset along the x-axis for clarity to avoid overlapping of error bars, which represent the standard deviation of replicate samples for each treatment at each time point (n = 3–5).

In the non-preserved samples, CH_4 concentrations remained surprisingly stable. Using a two-way ANOVA, it was found that the non-preserved CH_4 samples were statistically indistinct from the pH 10, 12, and 13 treatments ($p = 0.470$; Table 2, Experiment 2B), but that there was a significant effect of time ($p < 0.001$) and no significant interaction between preservative and storage time ($p = 0.770$). A post hoc test revealed that the 1 day samples were statistically different from all other time points ($p < 0.001$).

For samples preserved with $HgCl_2$, the 1 day and 1 month mean concentrations of CH_4 were similar to concentrations with the other preservatives at the same time point (Table S4); $HgCl_2$-preserved samples were not analyzed after longer storage durations due to limitations on sample water in the recirculating tank. We used a two-way ANOVA to evaluate the data from these two time points (Table 2, Experiment 2C) and found that for CH_4, there was no significant effect of preservative ($p = 0.091$) and no significant interaction between preservative and storage time ($p = 0.484$), but there was a significant effect of storage time ($p < 0.001$).

Overall, the CH_4 results, in particular the consistent differences in the 1 day samples as compared to other time points, regardless of preservative, are consistent with batch-specific differences in calibration rather than a systematic drift in the sample concentration over time, The CH_4 concentration in samples analyzed after 1 to 6 months was stable regardless of preservative (Table S4). In all, CH_4 remained comparable among pH treatments and the non-preserved samples over six months of storage.

N_2O concentrations remained fairly stable over six months of storage in all pH treatments (Fig. 2b and Table S5), averaging 10.5 ± 0.5 nmol kg^{-1} across all pH 10–13 samples and sampling times (n = 74) – although the month 6 values for all three pH levels appeared slightly lower than in prior months (mean 9.9 ± 0.3 nmol kg^{-1}, n = 15). A two-way ANOVA revealed significant effects of preservation pH and storage time on N_2O ($p < 0.001$ for pH and $p < 0.001$ for storage time), but there was a significant interaction between preservation pH and storage time on N_2O ($p < 0.001$; Table 3, Experiment 2A). A post hoc analysis revealed that the pH 13 samples were significantly different from pH 10 and 12 samples ($p < 0.001$), but that the pH 10 samples were not significantly different from pH 12 ($p = 0.940$). A post hoc test of storage time found that the 6 month samples were significantly different from all other time points ($p < 0.001$), along with significant differences between several other time point pairings (Table 3, Experiment 2A). Overall, the N_2O concentrations samples were slightly higher in samples preserved to pH 13 (mean 10.7 ± 0.4 nmol kg^{-1}, $n = 25$) as compared to pH 10 and 12 (mean 10.3 ± 0.4 nmol kg^{-1}, $n = 49$; Table S5). In the non-preserved samples, N_2O concentrations began a steep decrease in concentration after more than 2 months of storage; concentrations after 6 months were 3.4 ± 3.6 nmol kg^{-1}, a 67% decline compared to the initial concentration, representing a significant difference from the pH 10, 12, and 13 treatments ($p < 0.001$, Fig. 2b, Table 3, Experiment 2B).

To evaluate the $HgCl_2$ preservative, we performed a two-way ANOVA on all 1 day and 1 month samples and found that there was a significant effect of preservative ($p < 0.001$; Table 3, Experiment 2C) with the pH 13 samples being significantly different from all other preservatives (including non-preserved), and that the 1 day and 1 month time points were significantly different from each other ($p < 0.001$). However, given that only two points were analyzed, this result could be due to calibration differences between runs, as discussed for CH_4. The result for N_2O at pH 13 is consistent with the results

over 6 months, which showed that the differences in for pH 13 as compared to pH 10 and 12 persisted throughout longer storage.

In all, N_2O concentrations were statistically equivalent in the pH 10, 12, and $HgCl_2$-preserved samples, and concentrations remained stable in the pH 10 and 12 samples for at least 4 months of storage, while the pH 13 samples were consistently higher by 4% on average (Table S5). There was a slight decrease in the 6 month concentrations for pH 10, 12, and 13, which could potentially be explained by the increase in room temperature that occurred just prior to the 6 month sample analysis. Results of Experiment 2 indicate that preservation is necessary for multi-month storage of samples being analyzed for N_2O.

Overall, combining the results from both CH_4 and N_2O for Experiment 2, we conclude that preservation with $HgCl_2$ gives comparable results to pH 10–13 for CH_4 and pH 10 and 12 for N_2O and that concentrations are stable for CH_4 for at least 6 months and for N_2O for at least 4 months in samples that are collected with the submerged technique at an in situ temperature of 10 ºC, not depressurized, and stored at room temperature.

Sample collection, preservation, and septum type (Experiment 3)

In this experiment, we evaluated how methods of sample collection, preservative addition, as well as the septum type influence gas concentrations over a 6-month storage period. Given that prior experiments demonstrated comparable sample preservation by hydroxide to $HgCl_2$, all samples were preserved with NaOH. A pH of 12 was selected because it is the most common pH reported in the literature (Magen et al. 2014; Frey et al. 2024). Additionally, a set of non-preserved samples were collected as a control. Sample bottles were filled using Long Island Sound water that was recirculated for 1 day at a temperature of ~4 ºC (comparable to deep ocean conditions) prior to collection and samples were stored at room temperature (~22 ºC), resulting in a density decrease of approximately 0.33%. Samples subject

to precrimping preservation methods that were not depressurized (both submerged and nonsubmerged preservative addition) resulted in bulged septa, with SP stoppers exhibiting the greatest bulging (Fig. 3a). Despite the large thermal expansion and related septa bulging, none of the bulged septa tore. No bubbles were immediately present in the samples collected and preserved using the submerged methods, whereas most of the samples that were collected using the nonsubmerged methods had small bubbles, presumably introduced during injection of the preservative (<0.5 cm diameter). In samples preserved with the postcrimping method (both submerged and nonsubmerged sample collection), immediate postcrimping preservation and delayed depressurization of the bottles prevented bulging of septa (Fig. 3b). Small bubbles (~0.5 cm diameter) were observed in most of the depressurized samples after ~24 h and remained comparable in size throughout the 6 month storage period.

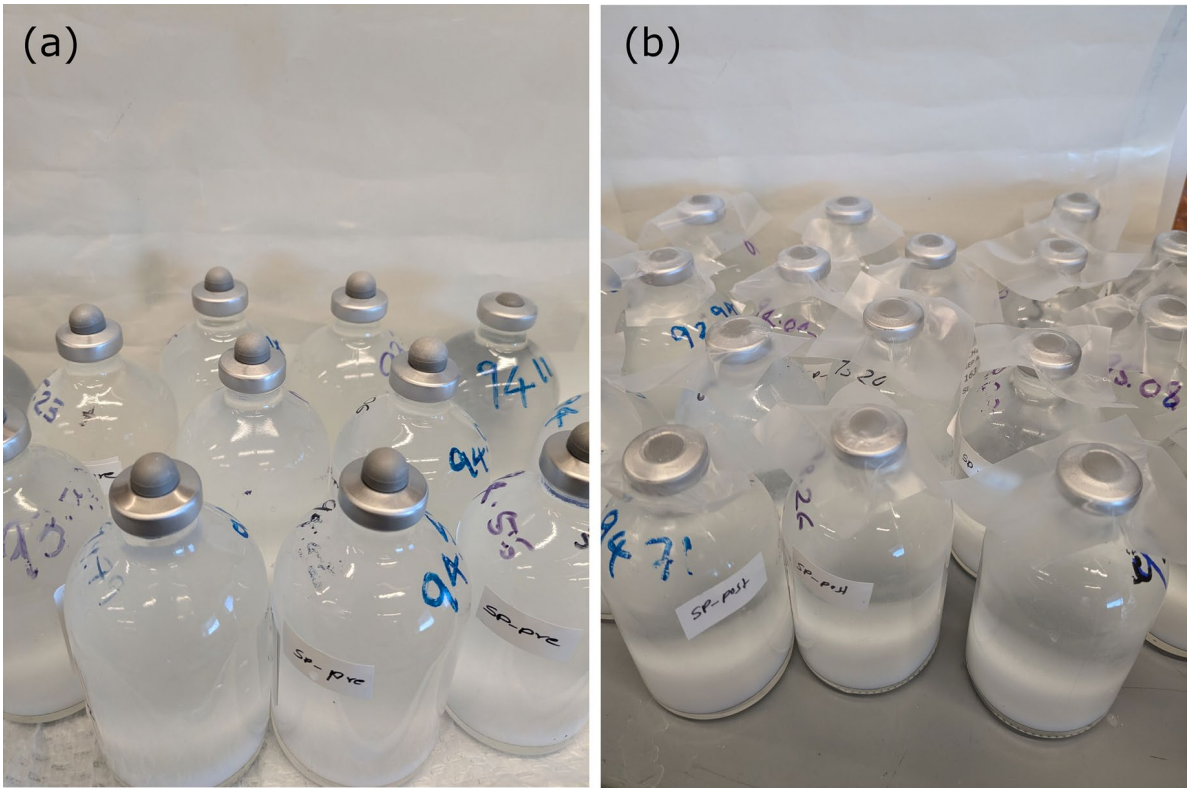

Fig. 3. NaOH-preserved samples collected at an in situ temperature of 4 °C and stored at room temperature, using precrimping preservation without depressurization (**a**) and immediate postcrimping preservation with delayed depressurization) (**b**), during Experiment 3.

CH_4 and N_2O concentrations decreased over time in samples that were preserved to pH 12 and collected with the precrimping method and not depressurized, especially for the SP septa—which incidentally had the highest bulging. Across all sample treatments, the mean day 1 CH_4 concentration was 7.5 ± 0.4 nmol kg^{-1} and after 6 months the CH_4 concentration decreased to 5.6 ± 1.4 nmol kg^{-1}, 6.7 ± 0.4 nmol kg^{-1}, and 6.9 ± 0.9 nmol kg^{-1} for the submerged, nondepressurized SP, 2-leg, and 3-leg septa, respectively (Table S6). The N_2O concentrations were 11.7 ± 0.3 nmol kg^{-1} on day 1 across all treatments and were

10.6 ± 0.6 nmol kg^{-1}, 11.3 ± 0.3 nmol kg^{-1}, and 11.5 ± 0.3 nmol kg^{-1} after 6 months for the submerged, precrimping, nondepressurized SP, 2-leg, and 3-leg septa, respectively (Table S7). A two-way ANOVA revealed that CH_4 and N_2O concentrations in samples that were not depressurized were significantly different from those that were depressurized ($p = 0.019$ for CH_4; $p = 0.015$ for N_2O; Table 2 and 3, Experiment 3A), and that there was a significant effect of time and a significant interaction between depressurization and storage time for both gases ($p < 0.001$).

When evaluating the effect of septum type to determine the optimal septum, we only included samples that were depressurized (immediate postcrimping with delayed depressurization), given that the SP septa showed substantial CH_4 and N_2O concentration decreases in the nondepressurized samples after 4-6 months (Fig. 4b, d). A two-way ANOVA and a post hoc analysis revealed that the N_2O results were significantly different for the SP septa as compared to the 2-leg and 3-leg septa ($p < 0.001$; Table 3, Experiment 3B) and there was a significant effect of time ($p < 0.001$) and significant interaction between time and septum type ($p = 0.0059$). A post hoc analysis concluded that the 2 month data were significantly different from 1, 4, and 6 months ($p \leq 0.028$), likely reflecting calibration differences, rather than a systematic drift over time. The SP septa displayed lower N_2O concentrations on average particularly at longer storage times. Pooling the data from the 2, 4, and 6-month samples, the mean N_2O concentration was 11.3 ± 0.4, 11.7 ± 0.3, and 11.3 ± 0.2 nmol kg^{-1} for the SP, 2-leg and 3-leg septa, respectively. Conversely, for CH_4, there were not statistically significant differences between the three septa ($p = 0.058$; Table 2, Experiment 3B), nor significant differences associated with storage time ($p = 0.620$) or a significant interaction between septum type and storage time ($p = 0.444$). For CH_4, the relative standard deviation was larger as compared to N_2O, which made it more difficult to distinguish systematic methodological differences from random error. As discussed above, the SP septa that were

not depressurized showed the largest CH_4 concentration decrease out of all treatments in this experiment.

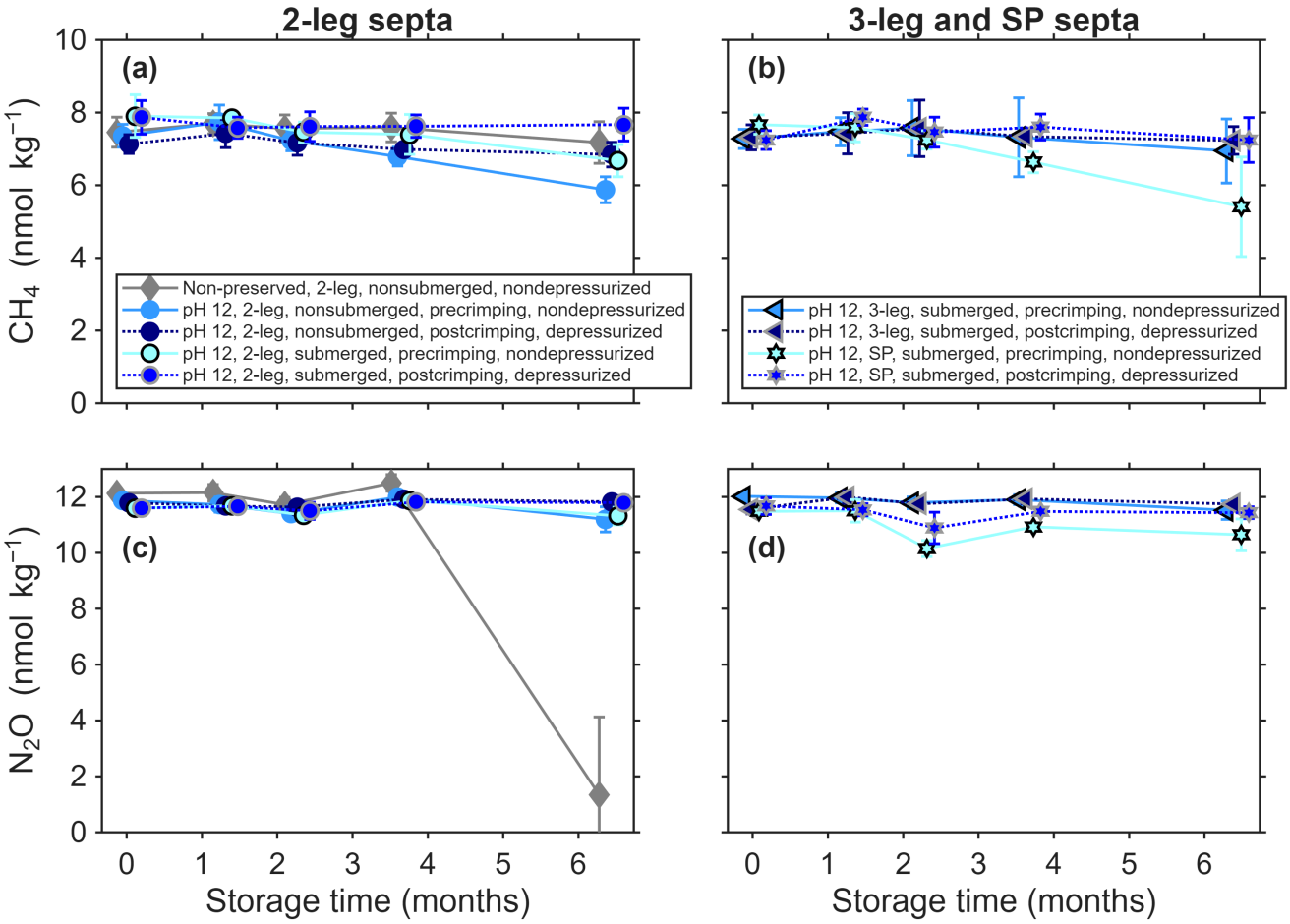

Fig. 4. Experiment 3: CH_4 (**a**–**b**) and N_2O (**c**–**d**) concentrations in nmol kg^{-1} measured over successive preservation periods for 2-leg septa (**a**, **c**) and 3-leg and SP septa (**b**, **d**). Nine different treatment combinations were tested and are shown with different symbols and colors: grey diamonds represent non-preserved samples, and pH 12 samples are shown in various shades of blue with circles, left-pointing triangles, and hexagrams representing 2-leg, 3-leg and SP stoppers, respectively. Cyan and light blue represent precrimping preservation, while royal blue and navy blue represent postcrimping preservation. Symbols with black or grey outlines reflect submerged sample collection, while symbols

without an outline represent nonsubmerged sample collection. Solid lines indicate treatments without depressurization and dashed lines represent treatments with depressurization.

For samples with 2-leg septa that were preserved to pH 12 and depressurized (immediate postcrimping with delayed depressurization), CH_4 concentration displayed significant differences between the submerged and nonsubmerged sample collection methods ($p < 0.001$, Table 2, Experiment 3C), with the submerged samples yielding higher concentrations on average (mean 7.9 ± 0.3 nmol kg^{-1} for submerged and mean 7.3 ± 0.4 nmol kg^{-1} for nonsubmerged). However, there was not a significant effect of time ($p = 0.421$), nor a significant interaction between collection method and storage time ($p = 0.249$), indicating that concentrations were stable over time by both methods (Table 2, Experiment 3C). Conversely, for N_2O there was not a significant difference between submerged and nonsubmerged samples collected with 2-leg stoppers and depressurization ($p = 0.094$; Table 3, Experiment 3C). Comparing the depressurized and nondepressurized samples, there was a significant effect of storage time ($p = 0.010$; Table 3, Experiment, 3C), but a post hoc test revealed that only the 2 month and 4 month samples were significantly different from each other ($p = 0.013$), which likely reflects batch-specific differences in calibration rather than a systematic drift. There was not a significant interaction between method and storage time ($p = 0.830$).

We conclude from Experiment 3 that the SP septa are most susceptible to storage drift, and this issue is exacerbated by bulged septa. We conclude that when saltwater samples are collected with no headspace, preserved to pH 12, and stored at a temperature significantly warmer than the in situ temperature, the samples should be depressurized to ensure accurate measurement of CH_4 and N_2O after long-term storage. The submerged method with depressurization is required for accurate CH_4 measurements, given

the consistent offset between submerged and nonsubmerged samples throughout the 6-month storage period. CH_4 and N_2O concentrations were stable in depressurized samples stored up to 6 months.

Number of piercings, storage orientation, and transport (Experiment 4)

Based on results of Experiment 3 showing the importance of submerged collection and depressurization, we evaluated two different methods of depressurizing the samples following submerged collection: 1) precrimping preservation with delayed depressurization, which resulted in a single piercing of each septum, and 2) delayed postcrimping preservation, where we added preservative 2–3 hours after crimping and simultaneously vented out excess sample with the second needle, which led to double piercing of each septum. We evaluated whether sample integrity differs between these treatments. We also evaluated the effect of storage orientation (upright or inverted) and the effect of air transport on the measured sample concentrations. All samples were collected using the 2-leg septa and submerged technique based on results of Experiment 3. The water was recirculated for 24 h at a temperature of ~4 ^{o}C; cold water was utilized to increase the tendency for thermal expansion.

After one month, most of the samples in this experiment had developed bubbles ranging from 0.5 to 0.8 cm, with no clear differences in bubble size across sample collection methods (precrimping with delayed depressurization and delayed postcrimping or storage orientations). We noticed that bubbles in the non-shipped samples were smaller on average (0.5 to 0.7 cm, compared to 0.5 to 0.8 cm for shipped samples), with fewer than five shipped samples having 1 cm bubbles. In samples stored for multiple months before analysis, the bubble size typically remained stable after formation. As with the samples from Experiment 2, the air handling system in the storage room malfunctioned just prior to the 6-month analysis date causing the storage room temperature to temporarily increase by several degrees, which altered the sample density; however, unlike in Experiment 2, all samples were depressurized so the

bulging due to warming at 6 months was less severe. Experiments 2 and 4 were initiated about 1 week apart, and many of the samples were run together on the GC. Additionally, three of the four replicates were lost for the 4 month delayed post crimping upright samples, and 6 month precrimping with delayed depressurization inverted samples, leading to uncertainty in the final results for these time points.

Whether septa were pierced once or twice for preservation and depressurization did not manifest detectable effects on CH_4 and N_2O concentrations (Table 2 and 3, Experiment 4A; $p \geq 0.212$; Fig. 5). The preservation approach and its interaction with storage time had no significant effect on CH_4 and N_2O concentration ($p \geq 0.09$). However, there were significant differences in N_2O concentration associated with storage times ($p < 0.001$); a post hoc test indicated that there were significant differences between all time point pairings except for the 1 day and 4 month samples ($p \leq 0.005$). This result suggests that batch-specific calibration differences were larger than the variability within an individual run, as there was not a consistent trend in concentration over time. The N_2O concentrations were slightly lower for the 6 month samples as compared to all prior months, a result that was also observed in the Experiment 2 samples that were analyzed on the same day as these samples (Table S5 and S9).

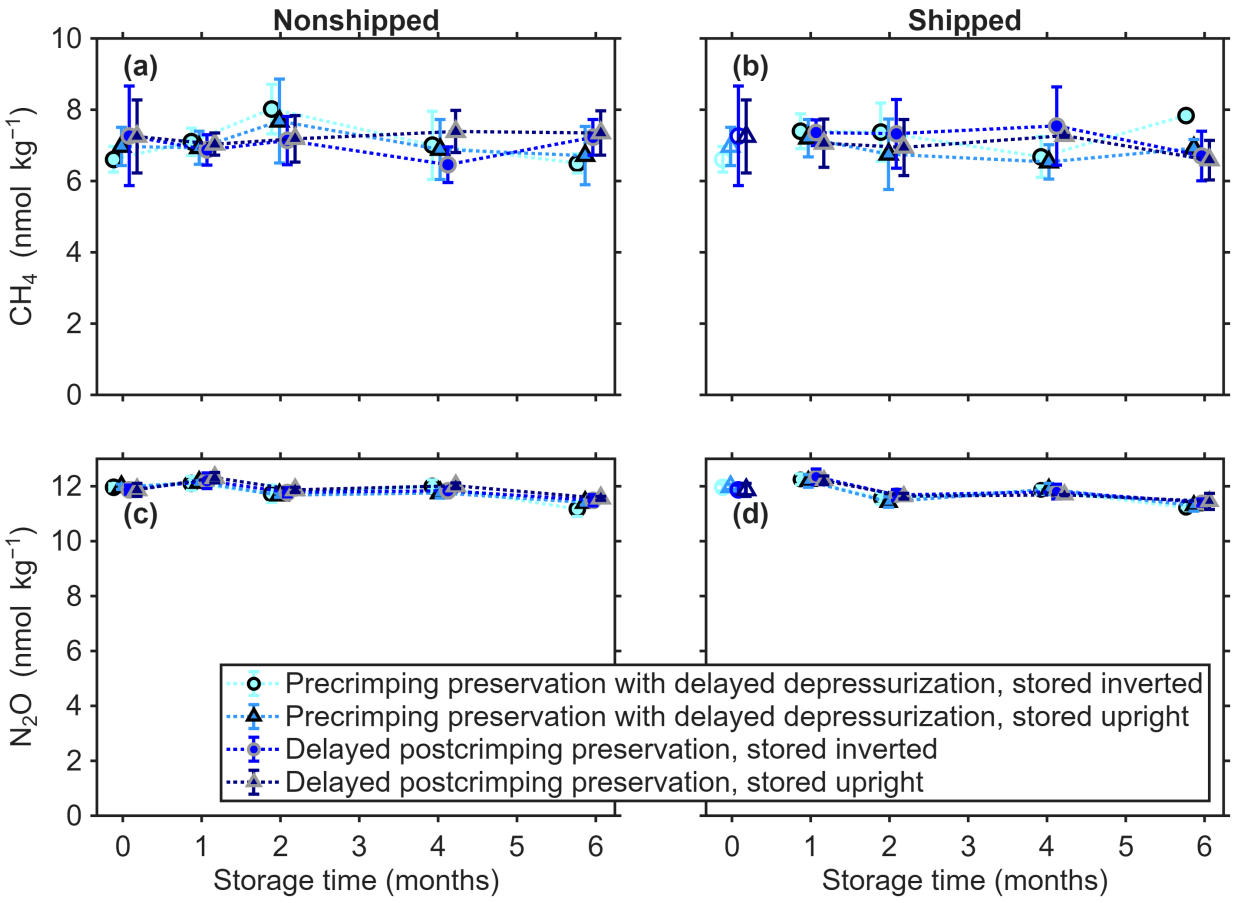

Fig. 5. Experiment 4 results: CH_4 (**a** and **b**) and N_2O (**c** and **d**) concentrations in nmol kg^{-1} for non-shipped (**a** and **c**) and shipped (**d** and **d**) samples measured over successive preservation periods. Cyan and light blue represent precrimping preservation, while royal blue and navy blue represent postcrimping preservation. Circles represent inverted storage and upward triangles represent upright storage. The day 1 results shown in panels **b** and **d** are based on the non-shipped samples and are indicated with non-filled symbols. All samples were collected using 2-leg stoppers and were depressurized (as indicated by the black or grey outline on each symbol and the dashed line, respectively), and samples were preserved to a pH of 12 with NaOH. Average and standard deviation are shown for each treatment (n = 4 typically, but occasionally samples were lost).

Storage orientation, storage time, and their interaction had no significant effect on CH_4 concentration ($p \geq 0.19$, Table 2, Experiment 4B). Sample orientation and its interaction with storage time had no significant effect on N_2O concentrations ($p \geq 0.79$, Table 3, Experiment 4B). Consistent with the results when the samples were separated by piercing, the post hoc test concluded that all time point pairings were significantly different for N_2O concentration, with the exception of the 1 day and 4 month pairing ($p \leq 0.007$).

Finally, shipping, storage time, and their interaction had no significant effect on CH_4 concentrations in depressurized samples ($p \geq 0.08$, Table 2, Experiment 4C). The preservation approach and interaction with storage time similarly time had no significant effect on N_2O concentration ($p \geq 0.10$, Table 3, Experiment 4C). Consistent with the other results from this experiment, there were statistically significant differences in N_2O concentration between each pair of time points (we note that the 1 day samples were not included in this analysis since the shipping took more than 1 day).

We conclude from these results that the number of piercings, storage orientation, and shipping status have no effect on CH_4 and N_2O concentration. The differences observed between time points for N_2O, without a systematic trend, likely reflect that the variability between runs was larger than the variability within runs.

Discussion

To our knowledge, this is the first study comparing the effectiveness of preservatives, sample collection, preservation, and storage method on water samples for simultaneous analysis of CH_4 and N_2O concentrations.

Foremost, our study demonstrates that hydroxide salts are an effective preservative for samples that will be stored for up to 6 months prior to analysis for CH_4 and up to 5 months prior to analysis for N_2O concentrations and yield results that are comparable to $HgCl_2$. Our findings agree with those Frey et al. (2024) who reported that NaOH at a pH of 12 effectively preserves N_2O concentrations in samples collected from an estuary or a lake for periods of 6 months. Researchers currently using $HgCl_2$ for CH_4 and N_2O sample preservation can thus switch to hydroxide salts with confidence.

Our experiments did not reveal an optimum preservation pH for combined CH_4 and N_2O analysis. For future sample preservation, we currently intend to maintain the pH between 10 and 12. In Experiment 2, the results with pH 10, pH 12 and $HgCl_2$ preservative were statistically equivalent for both gases, while pH 13 preservation gave higher N_2O concentrations than any other preservative. Previous publications employing hydroxide preservation have focused on pH 12, which some researchers stated was necessary to ensure that samples are fully lysed (Sigman et al. 2001; Magen et al. 2014; Frey et al. 2024)—although no direct evidence of this notion is presented in these studies. At pH 10, the samples incur less volume expansion after crimping and the volume of preservative is reduced compared to pH 12. We note that the non-preserved samples in Experiment 2 did not display a statistically significant change in CH_4 concentration over 6 months. For preservation of samples with high levels of in situ methane production/consumption, further research on the preservation effectiveness of different pH levels may be warranted. Without additional data, as a precaution, pH 12 could be selected to increase the likelihood of halting biological activity.

Tests of different sampling protocols demonstrated that samples collected for CH_4 analysis should be submerged during collection and preservation because CH_4 concentrations are sensitive to air that is inadvertently introduced during the nonsubmerged method. The observations also made clear that depressurization of saltwater samples is essential for preserving CH_4 and N_2O concentrations for

extended periods when the submerged method and hydroxide preservative is used and the samples are stored at a temperature significantly warmer than the in situ temperature. Samples that were not depressurized resulted in bulging septa, leakage and even bottle breakage. Septum bulging occurs due to thermal expansion of the water after sealing, volume expansion due to chemical precipitation following the NaOH addition, and from the exothermic dissolution of NaOH. Concentrations in bottles that were not depressurized and had bulging septa tended to decrease over months of storage, most dramatically with SP septa. This result may be due to the lower thickness of SP septa compared to the 2-leg and 3-leg stoppers. Another possible explanation is that the legs of the 2-leg and 3-leg stoppers may help prevent them from distending. Somewhat surprisingly, the number of septum piercings associated with depressurization (one versus two) did not alter the stability of measured concentrations over time. Taken together, our experimental results indicate that the septa can be pierced to add preservative and/or depressurize the samples without altering the measured concentration. We used grease on all punctured septa to seal the puncture holes after collection and did not test the effect of not greasing the septa.

In Experiment 3 we observed a statistically significant difference in CH_4 and N_2O concentration for depressurized versus nondepressurized samples preserved to pH 12, and we attribute this difference to gas transfer across the bulged septa of the nondepressurized samples, which warmed from an in situ temperature of 4 ^oC to room temperature after collection. In Experiment 3, CH_4 and N_2O concentration decreased over time in the nondepressurized samples only. Differences between the depressurized and nondepressurized samples were most apparent in month 6 and for the SP septa. In contrast, in Experiment 1 and 2, all samples were nondepressurized and the CH_4 concentrations were stable for 6 months in both experiments, and the N_2O concentrations were stable for at least 5 months in Experiment 1 and 4 months in Experiment 2. In both cases, the 6 month showed lower concentration; the cause of which could not be determined, and may relate to batch-specific calibration differences or a temporary

increase in the laboratory temperature resulting from a malfunction in the heating and ventilation system. In Experiment 1 we used the nonsubmerged technique to collect the samples, which typically leaves a small bubble in the sample, allowing space for thermal expansion, reducing bulging. In Experiment 2, we used the submerged technique without depressurization, and bulging was most extensive in the pH 13 samples and least severe in the $HgCl_2$-preserved samples. In Experiment 1 and 2, the initial sample temperature was 10 to 11 ºC, which would have caused less thermal expansion (a density decrease of 0.24% for warming from 11 to 22 ºC) compared to the samples in Experiment 3 that were filled with 4 ºC water (a density decrease 0.33% for warming from 4 to 22 ºC). We conclude that the importance of depressurizing samples increases as in situ water temperatures get colder, when the submerged technique is used, as the storage period gets longer, and for hydroxide preservation and as the preservation pH increases.

Our study also demonstrates the different tendencies for bubbles to appear in samples depending on the collection method, and the consequent impact of these bubbles on the interpretation of the measured concentrations. Sampling involving nonsubmerged collection methods resulted in the immediate appearance of bubbles, which we conclude were due to air trapped during pipette removal and/or septum insertion, and in Experiment 3 the nonsubmerged technique was associated with statistically significant decreases in measured CH_4 concentrations relative to submerged collection – but no measurable change in the N_2O concentrations. The lower solubility of CH_4 compared to N_2O renders the former more sensitive to air contamination, which is why CH_4 samples must be collected without a headspace, but N_2O samples are sometimes collected with a headspace. Bubbles also appeared following submerged sample collection and subsequent depressurization, but they took a day to appear. We cannot confirm the exact composition of these bubbles, but we presume that they are likely to form under vacuum due to water temperature changes and/or chemical reactions associated with hydroxide addition that impact the

density of the water and internal volume of the bottle. If the sample bottle expands slightly, or the volume occupied by the water and precipitate decreases, these changes can cause a bubble to form under vacuum as the liquid is no longer able to fill the bottle. If these bubbles otherwise consisted of air, we presume they would have caused measurable changes in CH_4 concentrations in proportion to bubble size – a dynamic we did not observe. We conclude that bubble formation following depressurization of samples does not significantly alter the CH_4 or N_2O concentrations.

Sample storage orientation, whether upright or inverted, did not result in detectable differences in gas preservation. Nevertheless, we propose that the inverted storage orientation is preferred to keep any bubble that forms in the samples from being in contact with the septum, which is more permeable for gas than glass, particularly because the rate of gas transfer will increase if the bubble has a lower pressure than ambient air.

Air transportation had no detectable effect on sample preservation, although larger bubbles on average emerged in the shipped samples relative to non-shipped samples. We note, however, that we shipped the samples in October–November and that temporary thermal expansion during transport would be likely be most severe in summer when air temperatures are warmer.

Finally, our results demonstrate that the use of preservatives is necessary to maintain stable N_2O concentrations in samples stored for more than 2 months. In contrast, the measured CH_4 concentration did not significantly change in non-preserved samples that were stored for up to 6 months. Given that our samples were collected in aerobic waters, we anticipate that the rate of biological CH_4 production or consumption was low; incubation techniques quantifying CH_4 oxidation rates in oxygenated seawater typically require spiking samples with labeled isotopes or increasing the concentration significantly in order to determine rate constants (Mau et al. 2013; Uhlig and Loose 2017). Additionally, previous

studies have shown that butyl rubber septa can leach chemicals that are toxic to some CH_4 oxidizers and other organisms, but this issue appears to be much less significant for the pharmaceutical-grade halogenated butyl rubber stoppers used in this study, in contrast to other types of butyl rubber (Niemann et al. 2015). We are not aware of studies explicitly testing the impact of rubber chemical leaching on N_2O production/consumption. However, since we observed significant N_2O consumption in the non-preserved samples, we conclude that the septa used in this study are not toxic to N_2O consumers.

Recommendations

From the experiments we conducted, these are the recommendations for other researchers collecting samples for CH_4 and N_2O in order for the measured gas concentrations to accurately reflect the in situ concentrations after storage.

1. We recommend the use of aqueous hydroxide solution for sample preservation; it is highly effective, simplifies transport of samples, and reduces costs for waste disposal compared to $HgCl_2$. We suggest using NaOH rather than KOH since the former is available in higher purity. The solution should be prepared in advance and equilibrated with air (Magen et al. 2014). Appropriate safety precautions should be taken while preparing and handling this corrosive solution, including wearing gloves and protective clothing.
2. We recommend preserving samples with NaOH to a pH between 10 and 12; these pH values gave equivalent results for both gases in the samples tested over storage periods up to 6 months. At pH 10, the samples incur less volume expansion after crimping and the volume of preservative is reduced.

However, pH 12 may be selected as a precaution to increase the likelihood of halting biological activity. We do not recommend using pH 13 since it yielded the most extensive septum bulging and the N_2O concentrations were at pH 13 were significantly different from pH 10, 12, and $HgCl_2$.

3. The submerged method should be used to collect CH_4 samples. The nonsubmerged method can result in trapping of atmospheric air bubbles inside the bottle during preservative addition or septum insertion, which can alter the concentration measurements of CH_4. N_2O samples can be collected using the submerged or nonsubmerged method since this gas has higher solubility and the measured concentrations are insensitive to the trapping of small air bubbles.

4. We recommend the use of 2-leg bromobutyl septa (DWK part number W224100-407). The straight plug (SP) septa are not recommended as they bulged more readily than the other septa tested, and they exhibited the largest change in CH_4 and N_2O concentration over 6 months. We found that the 3-leg butyl septa bulged the least and gave equally reliable results to the 2-leg septa, but it was more difficult to insert the needles, making our manual headspace creation and transfer steps more challenging. The 3-leg septa may be appropriate for other analytical methods involving the use of autosamplers rather than manual sampler transfer.

5. NaOH can be added before crimping, using a pipette, or after crimping, by piercing the septum to inject the preservative and venting excess sample through a second needle. Regardless of how the preservative is added, if saltwater samples are collected with no headspace and will be stored at a temperature that is significantly warmer than the in situ temperature, it is critical to depressurize the samples prior to long-term storage when NaOH is used as preservative. We found that the CH_4 and N_2O concentrations drifted over 6 months in the 4 ^{o}C nondepressurized samples collected by the submerged method, especially for SP septa. If the precrimping preservative addition method is used, depressurization using a single needle should be performed within a few hours of crimping, once the

septum has bulged and the sample has warmed to close to the storage temperature. If the postcrimping preservative addition is used, we recommend the delayed postcrimping method, where the preservative is added, and sample is depressurized at the same time, after the septum has bulged and the sample has adjusted to the storage temperature. This approach is less time-consuming and reduces the number of times the septum is punctured, in comparison to the immediate postcrimping with delayed depressurization method.

6. Following depressurization, samples must be rinsed with fresh water and dried with a towel to prevent corrosion of the seal from contact with the sample water and preservative. Additionally, the septa should be coated with silicone grease to help seal puncture holes.

7. We obtained comparable results for both upright and inverted storage for up to 6 months and similar bubble size. We recommend storing inverted to prevent any bubble that forms in the bottle from being in contact with the septum, which is more gas permeable than glass.

8. Air transport across the continental USA in October–November did not measurably alter the concentrations of CH_4 or N_2O in the samples. Use of opaque shipping cases with custom foam for the bottles is recommended to avoid light exposure, insulate the bottles, and reduce the temperature change experienced by the water during transport.

9. The above sample collection and preservation recommendations will enable stable gas concentrations for a storage time of at least 6 months for CH_4. For N_2O, we found a decline in its concentration in samples analyzed after 6 months in three of the four experiments. This apparent change may be due to various experimental factors including storage temperature increases, calibration uncertainty, and/or non-randomization of samples rather than a systematic drift. We note that a recent study by Frey et al. (2024) found that N_2O concentrations were stable for up to 6 months with hydroxide preservation to pH 12.

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Authors' Contribution Statement

Anagha Payyambally: conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing – original draft

Josie Mottram: investigation, writing – review and editing

Julie Granger: formal analysis, funding acquisition, resources, supervision, writing – review and editing

Cara Manning: conceptualization, data curation, formal analysis, funding acquisition, resources, supervision, methodology, visualization, validation, writing – original draft, writing – review and editing

Data Availability Statement

The data that support the findings of this study are openly available at Figshare (https://doi.org/10.6084/m9.figshare.33975700).

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Supporting Information for:

Assessment of methods for collection, preservation, and storage of water samples for dissolved methane and nitrous oxide concentration measurements

Anagha Payyambally[1], Josie L. Mottram[1], Julie Granger[1], and Cara C. M. Manning[1]

1. University of Connecticut, Department of Marine Sciences, Groton, CT, USA

Corresponding author: Cara Manning, cara.manning@uconn.edu

The tables below include summaries of experimental results for the measured pH and the means, standard deviations, and number of replicates for different treatments. The results for individual sample replicates are provided in our dataset, which is publicly available at Figshare (https://doi.org/10.6084/m9.figshare.33975700).

Table S1. Experiment 1: Measured pH of preserved samples. We tested one sample each during each storage period. We were not able to measure the pH of some of the samples. The in situ pH was 7.9.

Preservative	Next day	1 week	1 month	2 month	3 month	4 month	5 month	6 month
KOH	–	–	12.6	12.6	12.6	12.6	12.6	12.6
$HgCl_2$	–	–	7.9	7.6	7.9	–	–	7.9

Table S2. Experiment 1 results, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. The treatments in Experiment 1 were preservation with KOH to pH 12.6, and with $HgCl_2$. All Experiment 1 samples were collected using 2-leg stoppers and the non-submerged, pre-crimping preservative addition method without depressurization, and were stored inverted.

		CH_4 (nmol kg^{-1})		N_2O (nmol kg^{-1})	
Storage period	Day	pH 12.6	$HgCl_2$	pH 12.6	$HgCl_2$
Next day	1	20.6 ± 0.3 *n* = 5	20.8 ± 0.3 *n* = 5	12.0 ± 0.1 *n* = 5	11.8 ± 0.3 *n* = 5
1 week	6	20.1 ± 0.6 *n* = 3	19.3 ± 0.4 *n* = 4	12.1 ± 0.4 *n* = 3	12.1 ± 0.4 *n* = 4
1 month	36	21.4 ± 1.2 *n* = 5	20.9 ± 1.1 *n* = 5	12.2 ± 0.2 *n* = 5	12.1 ± 0.2 *n* = 5
2 month	67	22.0 ± 1.4 *n* = 4	21.2 ± 1.7 *n* = 5	12.2 ± 0.3 *n* = 4	12.1 ± 0.1 *n* = 5
3 month	97	22.5 ± 0.9 *n* = 5	22.0 ± 2.5 *n* = 5	12.2 ± 0.2 *n* = 5	11.8 ± 0.2 *n* = 5
4 month	132	21.7 ±1.8 *n* = 5	19.8 ± 2.3 *n* = 5	12.1 ± 0.3 *n* = 5	11.7 ± 0.2 *n* = 5
5 month	172	21.1 ± 1.8 *n* = 5	20.4 ± 2.0 *n* = 5	12.0 ± 0.1 *n* = 5	11.9 ± 0.1 *n* = 5
6 month	194	20.4 ± 0.9 *n* = 5	19.9 ± 1.0 *n* = 4	11.6 ± 0.3 *n* = 5	11.7 ± 0.6 *n* = 4
All storage times	variable	21.2 ± 1.4 *n* = 37	20.6 ± 1.7 *n* = 38	12.1 ± 0.3 *n* = 37	11.9 ± 0.3 *n* = 38

Table S3. Experiment 2: Measured pH of preserved samples. We tested one sample each during each storage period. The in situ pH was 7.7.

Preservative	Next day	1 month	2 month	4 month	6 month
Nonpreserved	7.8	7.6	7.5	7.4	7.4
NaOH (pH 10)	9.9	9.8	9.7	9.7	9.5
NaOH (pH 12)	12.0	12.1	12.0	11.9	11.8
NaOH (pH 13)	13.0	13.0	12.9	12.9	12.8

Table S4. Experiment 2 results for CH_4, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. The treatments in Experiment 2 were preservation with NaOH to pH 10, pH 12, and pH 13, preservation with $HgCl_2$, and non-preserved samples. All Experiment 2 samples were collected using 2-leg stoppers and the submerged, pre-crimping preservative addition method without depressurization, and were stored inverted.

Storage period	Day	Nonpreserved (nmol kg^{-1})	pH 10 (nmol kg^{-1})	pH 12 (nmol kg^{-1})	pH 13 (nmol kg^{-1})	$HgCl_2$ (nmol kg^{-1})
Next day	1	6.3 ± 0.6 n = 5	6.4 ± 0.4 n = 5	6.1 ± 0.5 n = 5	6.3 ± 0.4 n = 5	5.5 ± 0.6 n = 4
1 month	46	5.3 ± 0.4 n = 5	5.2 ± 0.9 n = 5	5.3 ± 0.4 n = 4	5.7 ± 0.4 n = 5	5.1 ± 0.3 n = 4
2 month	77	5.0 ± 0.9 n = 5	5.9 ± 1.4 n = 4	5.4 ± 1.4 n = 3	5.4 ± 0.6 n = 5	–
4 month	139	5.4 ± 0.9 n = 5	5.3 ± 0.6 n = 5	5.6 ± 1.2 n = 5	4.9 ± 0.8 n = 4	–
6 month	195	5.0 ± 0.3 n = 5	5.6 ± 0.5 n = 4	5.3 ± 0.6 n = 5	4.9 ± 0.5 n = 4	–
All storage times	variable	5.39 ± 0.79 n = 25	5.7 ± 0.9 n = 23	5.6 ± 0.8 n = 22	5.5 ± 0.7 n = 23	5.3 ± 0.5 n = 8

Table S5. Experiment 2 results for N_2O, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. The treatments in Experiment 2 were preservation with NaOH to pH 10, pH 12, and pH 13, preservation with $HgCl_2$, and non-preserved samples. All Experiment 2 samples were collected using 2-leg stoppers and the submerged, pre-crimping preservative addition method without depressurization, and were stored inverted.

Storage period	Day	Nonpreserved (nmol kg^{-1})	pH 10 (nmol kg^{-1})	pH 12 (nmol kg^{-1})	pH 13 (nmol kg^{-1})	$HgCl_2$ (nmol kg^{-1})
Next day	1	10.2 ± 0.1 n = 5	10.2 ± 0.1 n = 5	10.2 ± 0.1 n = 5	10.8 ± 0.2 n = 5	10.1 ± 0.2 n = 4
1 month	46	11.0 ± 0.3 n = 4	10.5 ± 0.2 n = 5	10.5 ± 0.3 n = 5	11.1 ± 0.2 n = 5	10.6 ± 0.1 n = 4
2 month	77	11.3 ± 0.3 n = 5	10.6 ± 0.2 n = 5	10.5 ± 0.3 n = 4	10.9 ± 0.2 n = 5	–
4 month	139	8.4 ± 1.5 n = 5	10.1 ± 0.2 n = 5	10.4 ± 0.2 n = 5	10.7 ± 0.2 n = 5	–
6 month	195	3.4 ± 3.6 n = 5	9.8 ± 0.4 n = 5	9.7 ± 0.2 n = 5	10.1 ± 0.3 n = 5	–
All storage times	variable	8.8 ± 3.4 n = 24	10.3 ± 0.4 n = 25	10.3 ± 0.4 n = 24	10.7 ± 0.4 n = 25	10.4 ± 0.3 n = 8

Table S6. Experiment 3 results for CH_4, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. The treatments in Experiment 3 were preservation with NaOH to pH 12 with various collection methods including submerged and nonsubmerged, precrimping without depressurization (PreC, nonDP) and immediate postcrimping with delayed depressurization (IPostC, DD), and 2-leg, 3-leg and straight plug (SP) septa. Additionally, nonpreserved samples (nonpres.), collected with the nonsubmerged, nondepressurized method, were collected as a control.

		Non-submerged			Submerged					
		2-leg			2-leg		SP		3-leg	
Storage period	Day	Nonpres, nonDP	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD
1 day	1	7.5 ± 0.4 *n* = 3	7.4 ± 0.3 *n* = 5	7.1 ± 0.3 *n* = 5	7.9 ± 0.6 *n* = 5	7.9 ± 0.5 *n* = 5	7.7 ± 0.3 *n* = 5	7.2 ± 0.3 *n* = 5	7.3 ± 0.3 *n* = 5	7.3 ± 0.3 *n* = 5
1 month	40	7.7 ± 0.3 *n* = 5	7.7 ± 0.5 *n* = 5	7.4 ± 0.4 *n* = 5	7.9 ± 0.1 *n* = 4	7.6 ± 0.3 *n* = 5	7.6 ± 0.4 *n* = 5	7.9 ± 0.2 *n* = 5	7.5 ± 0.4 *n* = 5	7.4 ± 0.6 *n* = 5
2 months	69	7.6 ± 0.4 *n* = 5	7.2 ± 0.3 *n* = 5	7.2 ± 0.3 *n* = 5	7.5 ± 0.3 *n* = 5	7.6 ± 0.4 *n* = 5	7.2 ± 0.3 *n* = 5	7.5 ± 0.4 *n* = 5	7.6 ± 0.8 *n* = 5	7.6 ± 0.8 *n* = 5
4 months	112	7.6 ± 0.4 *n* = 5	6.8 ± 0.3 *n* = 5	7.0 ± 0.2 *n* = 5	7.4 ± 0.5 *n* = 5	7.6 ± 0.3 *n* = 5	6.6 ± 0.3 *n* = 5	7.6 ± 0.4 *n* = 5	7.3 ± 1.1 *n* = 5	7.3 ± 0.2 *n* = 5
6 months	196	7.2 ± 0.6 *n* = 5	5.9 ± 0.4 *n* = 5	6.8 ± 0.3 *n* = 5	6.7 ± 0.4 *n* = 5	7.7 ± 0.5 *n* = 5	5.4 ± 1.4 *n* = 5	7.2 ± 0.6 *n* = 5	6.9 ± 0.9 *n* = 5	7.2 ± 0.4 *n* = 5
All	All	7.5 ± 0.4 *n* = 25	7.0 ± 0.7 *n* = 25	7.1 ± 0.3 *n* = 25	7.4 ± 0.6 *n* = 24	7.7 ± 0.4 *n* = 25	6.9 ± 1.0 *n* = 25	7.5 ± 0.4 *n* = 25	7.3 ± 0.7 *n* = 25	7.4 ± 0.5 *n* = 25

Table S7. Experiment 3 results for N_2O, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. Sample treatments and abbreviations follow Table S6.

		Nonsubmerged			Submerged					
		2-leg			2-leg		SP		3-leg	
Storage period	Day	No pres., nonDP	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD	PreC, nonDP	IPostC, DD
1 day	1	12.1 ± 0.2 *n* = 3	11.9 ± 0.2 *n* = 5	11.8 ± 0.2 *n* = 5	11.6 ± 0.2 *n* = 5	11.6 ± 0.2 *n* = 5	11.5 ± 0.1 *n* = 5	11.7 ± 0.3 *n* = 5	12.0 ± 0.1 *n* = 5	11.6 ± 0.2 *n* = 5
1 month	40	12.2 ± 0.3 *n* = 5	11.7 ± 0.3 *n* = 5	11.7 ± 0.2 *n* = 5	11.7 ± 0.2 *n* = 4	11.5 ± 0.2 *n* = 5	11.7 ± 0.2 *n* = 5	11.5 ± 0.2 *n* = 5	12.0 ± 0.2 *n* = 5	12.0 ± 0.2 *n* = 5
2 months	69	11.7 ± 0.3 *n* = 5	11.4 ± 0.2 *n* = 5	11.6 ± 0.2 *n* = 5	11.3 ± 0.1 *n* = 5	10.9 ± 0.6 *n* = 5	11.5 ± 0.3 *n* = 5	10.9 ± 0.6 *n* = 5	11.8 ± 0.2 *n* = 5	11.8 ± 0.1 *n* = 5
4 months	112	12.5 ± 0.3 *n* = 5	12.0 ± 0.1 *n* = 5	11.9 ± 0.1 *n* = 5	11.9 ± 0.2 *n* = 5	11.5 ± 0.1 *n* = 5	11.8 ± 0.1 *n* = 5	11.5 ± 0.1 *n* = 5	11.9 ± 0.1 *n* = 5	11.9 ± 0.2 *n* = 5
6 months	196	1.3 ± 2.8 *n* = 5	11.2 ± 0.4 *n* = 5	11.8 ± 0.1 *n* = 5	11.3 ± 0.2 *n* = 5	11.4 ± 0.2 *n* = 5	11.8 ± 0.2 *n* = 5	11.4 ± 0.2 *n* = 5	11.8 ± 0.1 *n* = 5	11.7 ± 0.1 *n* = 5
All	All	10.0 ± 4.6 *n* = 25	11.6 ± 0.4 *n* = 25	11.8 ± 0.2 *n* = 25	11.6 ± 0.3 *n* = 24	11.4 ± 0.4 *n* = 25	11.7 ± 0.2 *n* = 25	11.4 ± 0.4 *n* = 25	11.8 ± 0.2 *n* = 25	11.8 ± 0.2 *n* = 25

Table S8. Experiment 4 results for CH_4, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. In Experiment 4, all samples were preserved to pH 12 using the submerged method and 2-leg septa, and we compared the impacts of precrimping with delayed depressurization (PreC with DD) and delayed post-crimping (DPostC), inverted and upright storage, and shipped versus non-shipped samples.

		Shipped				Non-shipped			
Storage period	Day	PreC with DD (1 piercing)		DPostC (2 piercing)		PreC with DD (1 piercing)		DPostC (2 piercing)	
		Inverted	Upright	Inverted	Upright	Inverted	Upright	Inverted	Upright
1 day	1	–	–	–	–	6.6 ± 0.4 *n* = 3	7.0 ± 0.5 *n* = 3	7.3 ± 1.4 *n* = 3	7.2 ± 1.0 *n* = 2
1 month	31	7.4 ± 0.5 *n* = 4	7.2 ± 0.5 *n* = 4	7.4 ± 0.4 *n* = 4	7.1 ± 0.7 *n* = 4	7.1 ± 0.4 *n* = 4	6.9 ± 0.5 *n* = 4	6.9 ± 0.4 *n* = 4	7.0 ± 0.3 *n* = 4
2 months	62	7.4 ± 0.8 *n* = 4	6.8 ±1.0 *n* = 4	7.3 ± 1.0 *n* = 4	6.9 ± 0.8 *n* = 4	8.0 ± 0.7 *n* = 4	7.7 ± 1.2 *n* = 3	7.1 ± 0.7 *n* = 4	7.2 ± 0.6 *n* = 4
4 months	124	6.7 ± 0.6 *n* = 4	6.5 ± 0.5 *n* = 4	7.5 ± 1.1 *n* = 3	7.3 *n* = 1	7.0 ± 1.0 *n* = 3	6.9 ± 0.8 *n* = 3	6.5 ± 0.5 *n* = 2	7.4 ± 0.6 *n* = 2
6 months	180	7.8 *n* = 1	6.9 ± 0.2 *n* = 4	6.7 ± 0.7 *n* = 4	6.6 ± 0.6 *n* = 3	6.5 ± 0.3 *n* = 4	6.7 ± 0.8 *n* = 4	7.2 ± 0.5 *n* = 4	7.3 ± 0.6 *n* = 4
All	All	7.2 ± 0.7 *n* = 13	6.9 ± 0.6 *n* = 16	7.2 ± 0.8 *n* = 15	6.9 ± 0.6 *n* = 12	7.1 ± 0.7 *n* = 17	7.2 ± 0.8 *n* = 18	6.8 ± 0.5 *n* = 16	7.4 ± 0.5 *n* = 16

Table S9. Experiment 4 results for N_2O, reported as mean ± standard deviation (nmol kg^{-1}) for each time point and treatment. The exact number of days and replicates is reported. Sample treatments and abbreviations follow Table S8.

		Shipped				Non-shipped			
Storage period	Day	PreC with DD (1 piercing)		DPostC (2 piercing)		PreC with DD (1 piercing)		DPostC (2 piercing)	
		Inverted	Upright	Inverted	Upright	Inverted	Upright	Inverted	Upright
1 day	1	–	–	–	–	12.0 ± 0.1 *n* = 3	12.0 ± 0.1 *n* = 3	11.9 ± 0.2 *n* = 3	11.9 ± 0.2 *n* = 3
1 month	31	12.3 ± 0.2 *n* = 4	12.2 ± 0.2 *n* = 4	12.3 ± 0.3 *n* = 4	12.2 ± 0.2 *n* = 4	12.1 ± 0.2 *n* = 4	12.1 ± 0.1 *n* = 4	12.2 ± 0.3 *n* = 4	12.3 ± 0.2 *n* = 4
2 months	62	11.6 ± 0.2 *n* = 4	11.4 ± 0.2 *n* = 4	11.7 ± 0.2 *n* = 4	11.6 ± 0.1 *n* = 4	11.7 ± 0.3 *n* = 4	11.7 ± 0.1 *n* = 3	11.8 ± 0.2 *n* = 4	11.9 ± 0.1 *n* = 4
4 months	124	11.9 ± 0.1 *n* = 4	11.9 ± 0.2 *n* = 4	11.8 ± 0.3 *n* = 3	11.7 *n* = 1	12.0 ± 0.2 *n* = 3	11.8 ± 0.2 *n* = 3	11.8 ± 0.0 *n* = 2	12.0 ± 0.1 *n* = 2
6 months	180	11.2 *n* = 1	11.3 ± 0.2 *n* = 4	11.4 ± 0.1 *n* = 4	11.5 ± 0.3 *n* = 3	11.2 ± 0.3 *n* = 4	11.4 ± 0.1 *n* = 4	11.5 ± 0.2 *n* = 4	11.6 ± 0.1 *n* = 4
All	All	11.8 ± 0.4 *n* = 13	11.7 ± 0.4 *n* = 15	11.8 ± 0.4 *n* = 15	11.8 ± 0.4 *n* = 12	11.7 ± 0.5 *n* = 17	11.8 ± 0.3 *n* = 18	11.8 ± 0.3 *n* = 16	11.9 ± 0.3 *n* = 17