Alkalinity of diverse water samples can be altered by mercury preservation and borosilicate vial storage

Benjamin Mos1, Ceylena Holloway1, Brendan P. Kelaher1, Isaac R. Santos1,2 & Symon A. Dworjanyn1

We compared the effects of preservation and storage methods on total alkalinity ($A_T$) of seawater, estuarine water, freshwater, and groundwater samples stored for 0–6 months. Water samples, untreated or treated with $\text{HgCl}_2$, 0.45 µm filtration, or filtration plus $\text{HgCl}_2$, were stored in polypropylene or borosilicate glass vials for 0, 1, or 6 months. Mean $A_T$ of samples treated with $\text{HgCl}_2$ was reduced by as much as 49.1 µmol kg$^{-1}$ (1.3%). Borosilicate glass elevated $A_T$, possibly due to dissolving silicates. There was little change in $A_T$ of control and filtered samples stored in polypropylene, except for untreated groundwater (~ 4.1% reduction at 6 months). $\text{HgCl}_2$ concentrations of 0.02–0.05% reduced the $A_T$ of fresh, estuarine, and ground water samples by as much as 35.5 µmol kg$^{-1}$ after 1 month, but had little effect on the $A_T$ of seawater. Adding glucose as a carbon source for microbial growth resulted in no $A_T$ changes in 0.45 µm-filtered samples. We suggest water samples intended for $A_T$ analyses can be filtered to 0.45 µm, and stored in polypropylene vials at 4 °C for at least 6 months. Borosilicate glassware and $\text{HgCl}_2$ can be avoided to prevent analytical uncertainties and reduce risks related to use of Hg$^{2+}$.

Total alkalinity ($A_T$) is a measure of the capacity of water to buffer against changes in acidity. Interest in alkalinity measurements has increased in recent years as research into the global carbon cycle and anthropogenic climate change has intensified. For instance, alkalinity measurements are required to understand the impacts of ocean acidification on marine organisms1, resolve feedbacks among aquatic and atmospheric carbon pools2, quantify critical processes such as coral reef calcification3, model biological and non-biological responses to global warming and increased CO$_2$ levels4, and assess novel climate adaptation strategies5. Fundamental to the application of $A_T$ is its accurate measurement6.

Accuracy of $A_T$ measurements relies on the methods used to preserve and store samples prior to analysis. These methods are well established for seawater samples7,8. There is, however, a paucity of studies comparing the effectiveness of preservation and storage methods for non-oceanic water samples, particularly for samples collected from groundwater or brackish ecosystems. Only three studies have examined aspects of preservation or storage methods for freshwater $A_T$ samples9–11. It is important that storage and preservation methods are investigated for non-marine water samples given there is growing interest in quantifying the role of estuarine, freshwater, and groundwater systems in the global carbon cycle12,13.

For logistical reasons, water samples are typically collected and stored for hours to months prior to $A_T$ analysis. It is necessary to inhibit biological activity in samples because biogeochemical processes can alter $A_T$14. The conventional method to inhibit biological activity in stored water samples is the addition of a saturated $\text{HgCl}_2$ solution, which was first developed for water samples stored for analyses of N, P, and Si15,16. Arguably, the use of $\text{HgCl}_2$ became established as the primary preservation method for $A_T$ samples after 2007 when standard operating procedures (SOP) for analyses of seawater carbonate chemistry were described7. There is, however, substantial concern about global mercury levels and pollution17 including the use of $\text{HgCl}_2$ for water preservation18, and the applicability of $\text{HgCl}_2$ to samples other than seawater. The toxicity and environmental persistence of Hg$^{2+}$ presents a health risk for researchers and requires substantial costs for safe handling and disposal19,20. In addition, failure to account for the diluting effects of added $\text{HgCl}_2$ solutions on $A_T$ is a potential source of error in analyses7. The
Material, preservation method, and storage period on AT using a fully crossed design (2 material × 4 preservation methods × 3 storage periods), resulting in 24 treatments for each of the four water sources (collected July 2016, Table 1). There were five replicate samples for each treatment combination (24 treatments × 5 replicates per treatment = total 120 independent samples for each water source). The preservation methods were (1) the addition of 100 µL saturated HgCl₂ solution (25 °C), equivalent to 0.2% of the volume of water samples, (2) filtration, (3) treatment with a combination of HgCl₂ and filtration, or (4) the use of borosilicate glass vessels (Table 1).

In this study, we tested the efficacy of practical, low-cost, and safer alternatives to the use of HgCl₂ and borosilicate glass for AT preservation and storage of seawater, groundwater, estuarine water, and freshwater samples (Table 1). We treated water samples using a saturated HgCl₂ solution, 0.45 µm filtration, or the combination of HgCl₂ and filtration. We stored the treated samples and untreated controls for 0, 1, or 6 months in polypropylene vials. To assess the effectiveness of different preservation methods and storage vessels, we compared AT values in all treatments to the respective AT of untreated water from the four sources measured at the beginning of the experiment. To understand whether the amount of HgCl₂ affects AT, we compared concentrations of 0, 0.002, 0.02, 0.05, 0.2, and 0.5% HgCl₂ on the AT of seawater, groundwater, estuarine water, and freshwater samples stored for 0 and 1 months. Finally, to evaluate whether high dissolved organic carbon (DOC) concentrations that promote biological activity influence the efficacy of different preservation methods, we added glucose to treated (HgCl₂, filtration) and control water samples, and measured AT, DOC, pH, and dissolved oxygen (DO) after 0 and 1 months.

Materials and methods

Study sites and sample collection. Water samples from four sources were collected from locations near Coffs Harbour, New South Wales, Australia; hereafter called seawater, estuarine water, freshwater, and groundwater. Commonly reported parameters for each water source are shown in Table 1. Water was collected in a 20-L polyethylene drum triple rinsed with the sample water at each location, and transported to the laboratory for processing within 1 h.

Effects of preservation and storage methods on AT. An experiment tested the effects of storage vessel material, preservation method, and storage period on AT using a fully crossed design (2 materials × 4 preservation methods × 3 storage periods), resulting in 24 treatments for each of the four water sources (collected July 2016, Table 1). There were five replicate samples for each treatment combination (24 treatments × 5 replicates per treatment = total 120 independent samples for each water source). The preservation methods were (1) the addition of 100 µL saturated HgCl₂ solution (25 °C), equivalent to 0.2% of the volume of water samples, (2) filtration.
using a disposable filter (0.45 µm, Sartorius Minisart NML), or (3) filtration followed by the addition of 100 µL saturated HgCl₂ solution (25 °C). The control treatment was not filtered and did not have HgCl₂ added. Treated and control samples were stored in either gas tight glass vials (~44 mL, Thermo Fisher Scientific B7950, Type 1, Class A, 33 expansion borosilicate glass) or polypropylene vials (~38 mL, Techno Plas P8027UU) for 0, 1, or 6 months.

Vials were prepared by cleaning in a 1 M HCl bath for ~24 h, followed by rinsing for ~24 h in Milli-Q water (18.2 MΩ cm⁻¹ resistivity). Glass vials were then wrapped in aluminium foil and placed in a 450 °C muffle furnace for 4 h to remove organic carbon. Polypropylene vials were dried at room temperature. All vials were tripled rinsed with either the filtered or unfiltered water type according to the assigned treatment, before filling. The vials were filled until a convex meniscus formed and then capped. Capped vials containing samples assigned time 0 were analysed within 3 h of capping. The remaining capped vials were stored in a refrigerator (4 °C) for either 1 or 6 months before analysis to look for changes related to the different processing approaches. Aliquots of the seawater, estuarine water, freshwater, and groundwater (10 mL, n = 5) taken directly from the 20-L drums within 90 min of collection, were analysed (Table 1) and used as benchmark controls to assess changes in AT.

**Effects of HgCl₂ concentration on AT.** An experiment tested the effects of the final concentration of saturated HgCl₂ in water samples on AT using a fully crossed design (6 HgCl₂ concentrations × 2 storage periods), resulting in 12 treatments for each of the four water sources (collected October or December 2018, Table 1). There were five replicate samples for each treatment combination (12 treatments × 5 replicates per treatment = total 60 independent samples per water source). All water samples were filtered (0.45 µm, Sartorius Minisart NML) and placed in polypropylene vials (~38 mL, Techno Plas P8027UU) as previously described. Aliquots (1, 10, 25, 100, or 200 µL) of saturated HgCl₂ solution (25 °C) were added, equivalent to 0.002, 0.02, 0.05, 0.2, or 0.5% of the volume of water samples, respectively. A control (0%) treatment did not have mercury added. Initial (0 month) water samples without mercury were used as benchmark controls (water parameters including AT are shown in Table 1). All samples designated time 0 were analysed within 3 h. The remaining vials were stored in a refrigerator (4 °C) for 1 month before analysis.

**Effect of glucose enrichment on the efficacy of preservation methods.** An experiment tested the effects of preservation method, water source, and storage period on AT in the presence of high dissolved organic carbon (DOC) levels achieved by the addition of dissolved glucose. High DOC levels promote microbial activity, particularly respiration, which has the potential to alter the carbonate chemistry of stored water samples. An experiment tested the effects of the final concentration of saturated HgCl₂ in water samples on AT using a fully crossed design (6 HgCl₂ concentrations × 2 storage periods), resulting in 12 treatments for each of the four water sources (collected May or June 2020, Table 1). The preservation methods included the addition of 100 µL saturated HgCl₂ solution (25 °C), equivalent to 0.2% of the volume of water sample, or filtration using a disposable filter (0.45 µm, Sartorius Minisart NML). A control treatment was not filtered and did not have HgCl₂ added. A high DOC treatment was created by adding aliquots of a concentrated glucose solution (10,000 ppm, Sigma-Aldrich G8270) to water samples (seawater 48.2 µL; estuarine water 88.8 µL; freshwater 104.3 µL; groundwater 457.9 µL). This treatment increased DOC by an order of magnitude (~10–15 times) compared to levels measured in untreated benchmark controls (Supplementary Information Table S1). These DOC concentrations are at the extreme upper limit typically measured in diverse water samples. An ambient DOC treatment did not have glucose solution added.

There were eight replicates for each treatment combination (12 treatments × 8 replicates per treatment = 96 independent samples per water source). Five replicates were used to monitor AT, and DOC. To avoid cross-contamination, the remaining three replicates were used to measure pH and dissolved oxygen (DO) at the designated sampling time using a Hach HQ40d multicontroller fitted with a LDO101 DO probe and a PHC301 pH probe calibrated with Metrohm buffers (6.230–7.230). Measurements of pH were recorded on the NIST scale (pHNIST). Treated and control samples were stored in polypropylene vials (~38 mL, Techno Plas P8027UU) for 0 or 1 month, as previously described. Initial (0 month) water samples that were not filtered and did not have mercury or glucose added were used as benchmark controls for AT (water parameters including AT are shown in Table 1). Benchmark controls for DOC, pH, and DO were defined for treated and control water samples (Supplementary Information Table S1). All samples designated time 0 were analysed within 3 h. The remaining vials were stored in a refrigerator (4 °C) for 1 month before analysis.

**Sample analyses.** Each replicate vial was destructively sampled at its assigned sampling time; for instance, replicates assigned to a 1 month storage treatment were not measured again at 6 months. To measure total alkalinity (AT), a 10 mL aliquot from each vial was analysed by potentiometric titration using a Metrohm 888 Titrand® calibrated using certified reference materials (Batch 116 for 2016/17 analyses; Batch 166 for 2018/19 analyses; Batch 170 for 2020 analyses), and titration protocols tailored to each water source developed during previous research. The protocols ensured the titrations generated sufficient data points by, for example, tailoring the rate at which acid was added to a sample. NaCl was added to the HCl titrant to match the respective salinity of the four water sources (Table 1) (SOP 37). Samples were warmed in a 25 °C water bath prior to analysis, and analyses were carried out in a temperature-controlled room (25 °C). At the designated sampling time (0, 1, or 6 months), all samples from a single water source were analysed in a haphazard order within 3 h after reaching ambient temperature (25 °C). To monitor precision and check for drift, certified reference materials (Batch 116, 166, or 170 respectively) were used for the commencement of sample analyses and once every 20th sample (every 1–2 h). Across all analyses of reference material, precision was better than 2.3 µmol kg⁻¹ (n = 3–5). AT values were calculated using the Gran approach, and, where applicable, corrected for dilution by the HgCl₂ solution and/or glucose solution. The Gran approach is endorsed by Dickson et al.’s Guide to Best Practice and...
the US Geological Survey TWRI Book\textsuperscript{35}, is commonly used internationally (e.g.\textsuperscript{36-38}), and is the only method suitable for all of the four water sources examined in this study\textsuperscript{39}. The Gran approach and curve fitting generate similar alkalinity values, often within 0.1\% or 1 \mu mol L\textsuperscript{-1} (e.g.\textsuperscript{39-40}). Any differences between the two calculations are likely less than our error, and would therefore have no material impact on our results or conclusions. Data were used to calculate $\Delta A_T$ for each replicate, the difference between the $A_T$ of the replicate and the mean $A_T$ of the respective benchmark control (see Table 1 for $A_T$ values of benchmark controls). Standard deviations of $\Delta A_T$ for each treatment were calculated according to SOP 23\textsuperscript{3}. 

To measure dissolved organic carbon (DOC), a 3 mL aliquot from each vial was analysed by the wet oxidation method using a OI analytical Aurora 1030 TOC analyser (OI Analytical, USA), with an accuracy of 4\% and precision of 2\%. Where applicable, DOC values were corrected for dilution by the HgCl\textsubscript{2} solution\textsuperscript{7}. Data were used to calculate $\Delta$DOC for each replicate, i.e. the difference between the DOC of the replicate and the mean DOC of the respective benchmark control. Standard deviations of $\Delta$DOC for each treatment were calculated according to SOP 23\textsuperscript{3}.

**Statistical analysis.** Dunnnett's T3 tests were used to determine if $A_T$, DOC, pH, and DO values in temporal treatments, added HgCl\textsubscript{2} volume treatments, or added glucose treatments were significantly different from values measured in their respective benchmark control, using IBM SPSS Statistics (v25.0).

**Results**

**Effects of preservation and storage methods on $A_T$.** The storage vessel and preservation method had significant effects on $A_T$ (Fig. 1). Mean $A_T$ of freshwater and seawater samples stored in glass vials generally increased over time by 1.6–13.6 \mu mol kg\textsuperscript{-1} compared to their respective benchmark control (Fig. 1). There were no significant differences in the $A_T$ of estuarine water samples stored in glass vials compared to the benchmark control, although mean $\Delta A_T$ was generally above (after 0 or 1 month) or below (after 6 months) two standard deviations of the benchmark control (i.e. within $\pm$ 0.8–3.0 \mu mol kg\textsuperscript{-1} respectively) (Fig. 1, Table 1). In contrast, the $A_T$ of seawater, estuarine water, and freshwater samples stored in polypropylene vials for 0, 1, or 6 months were not different than the $A_T$ of their respective benchmark controls, except for mercury and filter + mercury treatments where mean $A_T$ was reduced by 0.9–12.7 \mu mol kg\textsuperscript{-1} compared to the benchmark controls (Fig. 1). For groundwater, the mean $A_T$ of samples held in glass and polypropylene vials generally declined by 7.6–153.0 \mu mol kg\textsuperscript{-1}, except for the filter only treatment where $A_T$ was generally equivalent to the benchmark control (Fig. 1).

There were no significant differences in $A_T$ between control treatments (i.e. no filtration or HgCl\textsubscript{2}) at 0, 1, or 6 months and their respective benchmark control for seawater, estuarine water, and freshwater samples stored in polypropylene (Fig. 1). Conversely, seawater, estuarine water, and freshwater samples stored in glass vials experienced increases in $A_T$ over time in control treatments by up to 13.6 \mu mol kg\textsuperscript{-1} after 6 months. For groundwater samples, $A_T$ in the control treatments declined regardless of the type of material they were stored in, falling by 3.7–4.1\% after 6 months (Fig. 1). For all water sources, mean $\Delta A_T$ of filtered samples were always comparable to the mean $A_T$ of their respective benchmark controls (i.e. within $\pm$ 0.8–3.0 \mu mol kg\textsuperscript{-1} respectively). In contrast, mean $A_T$ for all water sources treated with HgCl\textsubscript{2} or the combination of HgCl\textsubscript{2} and filtration were generally lower than in benchmark controls by < 49.1 \mu mol kg\textsuperscript{-1}, except for freshwater and seawater samples stored in glass vials where mean $A_T$ increased over time by < 11.3 \mu mol kg\textsuperscript{-1} after 6 months (Fig. 1).

**Effects of HgCl\textsubscript{2} concentration on $A_T$.** For estuarine water, freshwater, and groundwater samples, the effects of mercury preservation on $A_T$ differed depending on how much saturated HgCl\textsubscript{2} was added (Fig. 2). Mean $A_T$ of estuarine water was reduced by 9.0–13.2 \mu mol kg\textsuperscript{-1} by the addition of 0.05\% or more HgCl\textsubscript{2} at time 0. After 1 month, estuarine water $A_T$ fell by 11.2–12.2 \mu mol kg\textsuperscript{-1} in 0.2 and 0.5\% HgCl\textsubscript{2} treatments. Mean $A_T$ of freshwater was reduced by 8.1 \mu mol kg\textsuperscript{-1} by the addition of 0.5\% HgCl\textsubscript{2} at time 0, and after 1 month, fell by 13.0–26.8 \mu mol kg\textsuperscript{-1} in all treatments that had HgCl\textsubscript{2} added compared to the benchmark control (Table 1). Mean $A_T$ of groundwater was always reduced by 9.6–44.1 \mu mol kg\textsuperscript{-1} by the addition of 0.02\% or more HgCl\textsubscript{2}, but there was no difference in the $A_T$ of samples with 0.002\% HgCl\textsubscript{2} and benchmark controls. In contrast to the other water sources, mean $A_T$ of seawater samples treated with HgCl\textsubscript{2} were generally not different from the $A_T$ of the benchmark control (initial 0\% treatment, Table 1), although mean $\Delta A_T$ of 0.05, 0.2, and 0.5\% treatments were generally below two standard deviations of the benchmark control (Fig. 2, Table 1). For all water sources, $A_T$ in control treatments without mercury after 1 month were comparable to benchmark controls, except for groundwater where $A_T$ fell by 17.5 \mu mol kg\textsuperscript{-1}.

**Effect of glucose enrichment on the efficacy of preservation methods.** The addition of glucose to increase dissolved organic carbon (DOC) generally had little effect on all types of samples (Fig. 3, Table 1). For seawater, filtered samples had the same $A_T$ as the benchmark control after 0 and 1 month regardless of whether glucose was added (Fig. 3, Table 1). The control treatment also had similar $A_T$ to the benchmark control, except after 1 month in the control/glucose added treatment where $A_T$ fell by 12.2 \mu mol kg\textsuperscript{-1}. Seawater treated with mercury had higher $A_T$ than the benchmark control ($\Delta A_T$ 2.8–7.0 \mu mol kg\textsuperscript{-1}), although this increase was only statistically significant for samples without added glucose (Fig. 3).

For freshwater and groundwater after 0 month, and estuarine water after 0 and 1 month, most treatments had similar $A_T$ to the benchmark control (Fig. 3, Table 1). After 1 month, $A_T$ in the freshwater control treatment without glucose increased by 7.9 \mu mol kg\textsuperscript{-1} (Fig. 3, Table 1). Freshwater with HgCl\textsubscript{2} added had lower $A_T$ than the benchmark control at time 0 ($\Delta A_T$ 4.1–4.8 \mu mol kg\textsuperscript{-1}), although this decrease was only statistically significant for samples without added glucose (Fig. 3). After 1 month, freshwater with added HgCl\textsubscript{2} had either higher
Figure 1. The effects of storage vessel material and preservation method on difference in total alkalinity ($\Delta A_T$) of seawater, estuarine water, freshwater, and groundwater samples stored for 0, 1, and 6 months. All results represent the difference between observations and the mean $A_T$ of untreated samples measured at the beginning of the experiment ($A_T$ values of benchmark controls shown in Table 1). Water samples were treated using one of four methods (no treatment; 0.45 µm filter; 100 µL saturated $\text{HgCl}_2$ solution (25 °C); filter + $\text{HgCl}_2$). Samples were then stored in either polypropylene (white) or borosilicate glass (black) vials at 4 °C for 0, 1, or 6 months. Shaded areas on graphs represent ± 2 standard deviations of the respective benchmark control (Table 1). Asterisks indicate there was a significant difference in $A_T$ of samples in a treatment compared to the $A_T$ of the benchmark control according to Dunnett’s tests, and should not be used to evaluate statistical difference or similarity among treatments. Data are means ± 1 standard deviation. n = 5 except for the seawater 6 months/glass/Control treatment where n = 4. As mean $\Delta A_T$ for the groundwater 6 month/Control treatments were greater than $-100 \mu\text{mol kg}^{-1}$, values are given on the figure (means ± 1 S.D.).
Figure 2. The effects of mercury concentration on total alkalinity ($\Delta A_T$) of seawater, estuarine water, freshwater, and groundwater samples stored for 0 or 1 months. Results represent the difference between observations and the mean $A_T$ of untreated samples measured at the beginning of the experiment ($A_T$ of benchmark controls are shown in Table 1). Shaded areas on graphs represent ±2 standard deviations of the respective benchmark control (Table 1). Asterisks indicate there was a significant difference in $A_T$ of samples in a treatment compared to the $A_T$ of the benchmark control according to Dunnett’s tests, and should not be used to evaluate statistical difference or similarity among treatments. Data are means ±1 standard deviation. $n = 5$ except for the freshwater 0.05% mercury/1 month treatment where $n = 4$. 
HgCl₂ used to preserve water samples can reduce the accuracy of AT measurements, particularly for freshwater groundwater significantly reduced mean AT by anywhere from 0.9% to 4.7% after 1 month. In contrast, AT of on earlier work that focused primarily on seawater samples. Estuarine water, freshwater, seawater, and groundwater preservation and storage of AT water samples collected from a range of aquatic environments.

Discussion

We tested the effects of common storage and preservation methods on the AT of diverse water samples, building on earlier work that focused primarily on seawater samples. Estuarine water, freshwater, seawater, and groundwater samples were significantly altered when stored in borosilicate glass vials or treated with HgCl₂. In contrast, samples filtered to 0.45 µm and/or stored in polypropylene vials for up to 6 months were generally comparable to their benchmark controls. The combination of 0.45 µm filtration and storage in polypropylene vials was the only treatment that consistently prevented changes in AT across most water sources (i.e. within two standard deviations of the benchmark control, ±0.8–3.0 µmol kg⁻¹, respectively), and was equivalent or more effective than HgCl₂ even when samples were enriched in glucose to promote microbial activity. Based on these results, we contend that filtration and polypropylene are viable alternatives to the use of HgCl₂ and borosilicate glass for preservation and storage of AT-water samples collected from a range of aquatic environments.

The use of poisonous mercury may not be necessary when storing water samples for AT analyses. The addition of saturated HgCl₂ was often associated with substantial reductions in the AT of freshwater, estuarine water, or groundwater samples stored for 1 or 6 months. It is unlikely that mercury-resistant bacteria reduced AT in these treatments because AT was reduced to the same extent in filter + mercury treatments and mercury only treatments. Instead, Hg²⁺ may have reduced AT by forming complexes with dissolved organic matter (DOM), a component of AT. DOM interacts strongly with mercury. For example, 45–100% of Hg²⁺ in coastal seawater can be organically complexed with DOM, with the remainder complexed with Cl⁻ or OH⁻ ions. Mercury is more likely to be found in complexes with Cl⁻ than OH⁻ when Cl⁻ levels exceed ~350 mg L⁻¹, although this is dependent on pH. Variability in DOM or Cl⁻ concentrations might therefore explain why the reducing effects of HgCl₂ on AT in our study were smallest in seawater.

The degree to which HgCl₂ reduced AT often depended on the concentration used, but this was not consistent among all water sources. The addition of ≥0.2% HgCl₂ to estuarine water and ≥0.02% HgCl₂ to freshwater and groundwater significantly reduced mean AT by anywhere from 0.9% to 4.7% after 1 month. In contrast, AT of seawater was not consistently altered by any of the HgCl₂ concentrations tested. We are not aware of any studies that have examined the effects of HgCl₂ concentrations on AT, although the concentrations that we tested (0.02–0.05%) are often recommended to preserve samples before AT analysis. Our results demonstrate that standard levels of HgCl₂ used to preserve water samples can reduce the accuracy of AT measurements, particularly for freshwater and groundwater samples, further highlighting the need to identify alternative methods for storing non-oceanic water samples.

Instead of HgCl₂ preservation, the accuracy of AT analyses can be improved by using filtration to inhibit biological activity in water samples. Filtration has added benefits in that it increases safety for researchers and reduces the costs of managing HgCl₂ poisoned samples. There was no effect of 0.45 µm filtration on AT of water samples from across a salinity spectrum. Other studies have also found no effects of filtration on the AT of alpine freshwater, pond water, and seawater. Importantly there were no changes in the AT of filtered samples stored in polypropylene vials for at least 6 months, with the exception of groundwater in two of three experiments, demonstrating the enduring effectiveness of filtration. Although AT was often unchanged for seawater, estuarine water, and freshwater samples that were not treated with HgCl₂ or filtered, these water samples should be filtered before storage to prevent changes in AT due to particulates or microbes. For some types of groundwater, filtration may not be sufficient to prevent changes in AT over time, although our results indicate changes in AT may be small (<0.7%) when AT concentrations are <4000 µmol kg⁻¹. We observed precipitates and substantial declines in alkalinity when groundwater samples with very high alkalinity (>12,000 µmol kg⁻¹) were stored for 1 month prior to analysis. We hypothesise chemical or biological activity were responsible for changes in the AT of filtered or unfiltered groundwater, despite refrigeration. Low temperatures slow, but do not stop, chemical and biological activity, perhaps also explaining why changes in AT in the groundwater control treatments became more apparent over time (e.g. after 6 months, Fig. 1). For groundwater samples, researchers may need to balance the requirements for accuracy and precision of AT measurements against the risks and costs associated with using combined filtration and HgCl₂ preservation. If highly accurate measurements are required, our results suggest 0.002% HgCl₂ can preserve 0.45 µm-filtered groundwater for at least 1 month without substantially
Figure 3. The effects of glucose addition and preservation method on difference in total alkalinity (Δ$A_T$) of seawater, estuarine water, freshwater, and groundwater samples stored for 0 and 1 month. All results represent the difference between observations and the mean $A_T$ of untreated samples measured at the beginning of the experiment ($A_T$ values of benchmark controls are shown in Table 1). Water samples were treated using one of three methods (no treatment; 0.45 µm filter; 100 µL saturated HgCl$_2$ solution (25 °C)), and had a concentrated glucose solution added (black) or no glucose added (white). Addition of the glucose solution increased dissolved organic carbon (DOC) by ~10–15 times compared to ambient levels (Supplementary Information Table S1). Samples were stored in polypropylene vials at 4 °C for 0 or 1 month. Shaded areas on graphs represent ±2 standard deviations of the respective benchmark control (Table 1). Asterisks indicate there was a significant difference in $A_T$ of samples in a treatment compared to the $A_T$ of the benchmark control according to Dunnett’s tests, and should not be used to evaluate statistical difference or similarity among treatments. Data are means ±1 standard deviation. n = 5. Note: scale of Y axes differs for groundwater initial and 1 month.
altering $A_T$, with the exception that waters with extremely high alkalinity should be analysed as soon as practical to avoid physical or chemical changes. Higher concentrations of mercury do not seem to improve preservation.

Polypropylene vials had no measurable effects on the $A_T$ of water samples stored for up to 6 months, adding to growing evidence that plastic vessels are suitable alternatives to glassware storage for $A_T$ analyses. Conversely, some water samples stored in borosilicate glass vials had elevated $A_T$, especially in low pH conditions (i.e. pH of groundwater < river < estuary < ocean; Table 1). This is possibly due to the pH-dependent dissolution of acid neutralising materials from the glass (e.g. borate, silicate, or hydroxyl ions). The glass vials we used are made to the same specifications as the borosilicate glass bottles recommended by Dickson et al., but the glass surface area to water volume ratios are different (our glass vials = 2.0 cm$^2$/mL vs. 1-L narrow-mouth bottle = 0.6 cm$^2$/mL), which may explain the potential release of detectable amounts of alkalinity in our experiments (also see). Huang et al. found soda-lime glassware increased $A_T$, but reported no effect of borosilicate glass vials on seawater stored for up to 47 days. Differences between our results and Huang et al. may be because we (i) tested the effects of borosilicate glass using untreated water standards as our benchmarks, (ii) used different brands/shapes of high quality borosilicate glassware that are produced by different manufacturers, which also have different surface area to volume ratios, or (iii) tested for longer storage periods. For example, we found a minor but detectable effect of borosilicate glass on the $A_T$ of seawater after 6 months, but not at 0 or 1 month (Fig. 1). The effects of borosilicate glass on $A_T$ may also be concealed by the effects of $\text{HgCl}_2$. When tested in isolation, borosilicate glass and $\text{HgCl}_2$ had substantial, but opposing, effects on $A_T$. In contrast, samples treated with $\text{HgCl}_2$ and stored in borosilicate glass vials often had equivalent $A_T$ to benchmark controls, similar to the generally stable $A_T$ of $\text{HgCl}_2$ poisoned seawater certified reference materials stored in borosilicate glass bottles for up to 3 years. These findings highlight the importance of considering the potential for interactive effects when assessing the efficacy of experimental methods.

The prevention of biological activity that could alter $A_T$ is a primary aim of sample preservation methods. However, when we added glucose to samples to promote microbial activity, changes in DOC, pH, and DO that could be indicative of biological activity did not substantially differ among treatments over time, nor directly correspond with changes in $A_T$ in different preservation treatments. Most changes in $A_T$ observed in our experiments were likely due to precipitation, adsorption, flocculation, dissolution, or other chemical reactions. One implication is that preservation and storage methods that are appropriate for stabilising alkalinity may be unsuitable when analysing pH or non-carbonate chemistry parameters where biological activity is a major concern (e.g. DOC, DO). Similar to earlier findings, filtration and plastic storage vessels were not sufficient to prevent changes in DOC, pH, and DO over time. Consequently, methods to preserve and store water samples need to be tailored to the specific parameter of interest.

Overall, our results suggest there is considerable potential for conventional preservation and storage methods to alter the $A_T$ of water samples, particularly from non-marine water sources. To avoid the detectable pitfalls of $\text{HgCl}_2$ and borosilicate glassware, most water samples intended for $A_T$ analysis could instead be filtered to 0.45 µm, and then stored in polypropylene at 4 °C for at least 6 months. Avoiding $\text{HgCl}_2$ preservation not only improves the precision and accuracy of $A_T$ analysis of diverse water types, but also brings environmental benefits, minimises risks to researchers, and ultimately reduces the cost associated with analysis.

Data availability

All data generated or analysed during this study are presented in this published article and its Supplementary Information file. Datasets are available from the corresponding author on reasonable request.

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Author contributions
All authors contributed to conceptualisation and data interpretation, and approved the article for submission. CH and BM collected samples. BM and CH conducted the experiments and ran chemical analyses. BM did statistical analyses, with input from BK. BM and CH wrote the manuscript. SD, IS, and BK provided critical feedback.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to B.M.

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