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UNIVERSITY OF CALIFORNIA SAN DIEGO

Efficacy of alternative additives to mercuric chloride for preservation of total alkalinity and total pH in seawater samples stored for later analysis

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Earth Sciences

by

Daniela Nestory

Committee in charge:

Professor Andrew Dickson, Chair
Professor Andreas Andersson
Professor Todd Martz

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University of California San Diego

2026

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ABSTRACT OF THE THESIS

Efficacy of alternative additives to mercuric chloride for preservation of total alkalinity and total pH in seawater samples stored for later analysis

by

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Master of Science in Earth Sciences

University of California San Diego, 2026

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Accurate measurements of seawater total alkalinity (A_T), pH_T and dissolved inorganic carbon (C_T) are crucial to our understanding of biogeochemical phenomena in the global oceans. The standard protocol to preserve seawater carbonate parameters in natural seawaters for later analysis involves adding a small amount of saturated mercuric chloride ($HgCl_2$) solution (0.02%

by volume) to the seawater sample to prevent any biological modifications to inorganic carbon concentrations occurring after the time of collection. However, the environmental risks associated with the handling and disposal of HgCl_2 warrants consideration of alternatives. Here, we assess the suitability of copper sulfate (CuSO_4), zinc chloride (ZnCl_2), silver nitrate (AgNO_3), and benzalkonium chloride (BAC) as potential preservatives of seawater carbonate parameters. Surface seawater samples were collected and treated with one of the following: 20, 40, or 80 $\mu\text{mol kg}^{-1}$ of CuSO_4 ; 10, 80, or 160 $\mu\text{mol kg}^{-1}$ of ZnCl_2 ; 10, 50 or 90 $\mu\text{mol kg}^{-1}$ of AgNO_3 ; and 20 $\mu\text{mol kg}^{-1}$ or 80 $\mu\text{mol kg}^{-1}$ of BAC. A_T and pH_T measurements were analyzed intermittently over 1–4 months. Preservation efficacy was evaluated by comparing the results of each experimental group to control samples treated with 0.02% (55 $\mu\text{mol kg}^{-1}$) HgCl_2 . Results suggest that samples treated with CuSO_4 and ZnCl_2 experienced substantial A_T changes upon treatment, likely due to complexation of the respective metal ions with A_T -contributing molecules while AgNO_3 , and BAC treatments exhibited more subtle A_T changes over time.

INTRODUCTION

Total alkalinity (A_T), dissolved inorganic carbon (C_T) and pH_T are three of four major parameters that describe the seawater carbonate system. When expressed as amount contents (i.e., moles per kilogram of seawater), A_T and C_T are conservative parameters with respect to water mass mixing and changes in temperature and pressure and thus are important in tracking ocean circulation, while all three parameters can be used to understand marine physical and biological processes and determine oceanic responses to anthropogenic perturbations (Sabine et al. 2004; Middelburg et al., 2020; Xue & Cai 2020). Many carbonate chemistry samples are collected in the field and therefore are not analyzed immediately, which introduces the possibility of chemical changes occurring after collection.

Understanding the processes that can alter chemical concentrations within a sample is critical for ensuring proper collection and preservation. Air exchange between a sample and the atmosphere can alter C_T and pH_T values by changing the concentration of dissolved CO_2 within the sample (Emerson & Hedges, 2008). Further, seawater may interact with glassware to release charged ions via dissolution that alters A_T values over time (Zhang et al., 1999; Chopinet et al., 2008; Huang et al., 2012). Standard operating procedures (Dickson et al., 2007) recommend that samples be stored in borosilicate-33 glass bottles, which have proven relatively inert to dissolution, and filled to leave a 1% headspace and sealed using grease and ground glass stoppers to prevent CO_2 exchange. Additionally, samples are treated with 0.02% $HgCl_2$, which serves as a biocide to halt microbial metabolic activity. Cellular processes can alter the speciation of metabolic compounds that affect A_T concentrations, and often require cellular co-transport of protons further influencing chemical makeup. In general, aerobic degradation of organic material decreases A_T , whereas anaerobic processes have the opposite effect (Wolf-Gladrow et al. 2007). Because marine

microbes typically metabolize in accordance with Redfield ratios (Redfield et al., 1963), A_T variability in most oceanic environments is largely driven by nitrogen speciation and the associated co-transport of hydrogen ions in nitrogen-involved reactions (Brewer & Goldman, 1972; Wolf-Gladrow et al., 2007).

Dissolution and precipitation of calcium carbonate (CaCO_3) influences A_T concentrations by respectively releasing and consuming carbonate ions (CO_3^{2-} ; Wolf-Gladrow et al., 2007). CaCO_3 particles present in seawater samples can dissolve during acid dosing stages inherent in C_T and A_T measurement protocols, falsely inflating A_T and C_T results. Studies suggest that the magnitude of this effect depends on the particulate load, and that filtering samples from coastal or highly productive regions may improve data quality (Bockmon & Dickson, 2014; Kim et al., 2006; Chanson & Millero, 2007).

HgCl_2 has a long-standing history as a preservative for carbonate chemistry samples (Argentino et al., 2023). HgCl_2 effectively stops cell metabolism by binding and inhibiting thiol groups essential to enzymatic function thus inducing cell death (Winslow et al., 2001; Mason et al., 2016). Current standard operating procedures (Dickson et al. 2007) recommend preserving seawater carbonate chemistry samples by adding 0.02% by volume of a saturated HgCl_2 solution to the sample (in a borosilicate-33 sample bottle) prior to sealing the bottle. This approach is effective in preserving seawater CO_2 measurements, along with other seawater parameters affected by biogenic activity (G. Emmanuele, personal communication; Takahashi et al., 1970; Kirkwood 1992). Although largely successful, Dickson (2010) reports rare instances where A_T and C_T of Certified Reference Materials (CRMs) changed significantly despite treatment with HgCl_2 . Moreover, it has been shown that HgCl_2 may not be effective in preventing sample degradation in certain environments with low salinity or high organic loads (Argentino et al., 2023).

Growing international concern over the toxicity of mercury to the health of humans, other organisms, and the environment, makes its practical use and disposal increasingly challenging (Langford and Ferner, 1999). The Minamata Convention on Mercury, a global treaty established in 2013 and currently signed by 137 countries, aims to substantially reduce the consumption and disposal of mercury worldwide. Mercury used in seawater samples is diluted to levels ($<0.004\%$ by weight) that are legally permissible for transport under the US Department of Transportation regulations (49 CFR § 172.101(c)(10)); however, countries—such as Norway—prohibit the transport and disposal of solutions containing even lower concentrations of mercury. As stringent mercury regulations become more common, the need for a less hazardous alternative to HgCl_2 as a carbonate chemistry preservative is increasingly apparent.

Perhaps one of the earliest recorded uses of HgCl_2 for preserving carbonate measurements was by Krogh et al. (1904) wherein he describes the two critical reasons for the success of HgCl_2 as a preservative: “... I have not tried any other antiseptic [to HgCl_2], but I consider it extremely probable that, among the existing multitude of these substances, one or more may be found, capable of preventing growth of carbonic-acid-producing organisms but free from any chemical action on the mineral constituents of seawater.” This definition can now be extended to other acid-base producing organisms to reflect its efficacy in preserving A_T samples, a parameter that was not well defined at the time. Krogh further describes two essential qualifications for an additive to be successful; it must 1) effectively stop metabolic activity and 2) remain chemically inert to seawater constituents that contribute to C_T , or pH_T (and now A_T) values.

Here we evaluate the ability of various additive treatments (i.e., copper, zinc, silver, benzalkonium chloride) to preserve A_T and pH_T values in surface seawater over 1–4 months. While other efforts have evaluated the success of other methods, such as filtration and refrigeration, to

preserve carbonate parameters, not all field conditions allow for use of these methods (Mos et al. 2021; Bockmon & Dickson, 2015). Additive treatments are ideal because of their convenience in the field and their ability to initiate preservation shortly after sampling.

Cu^{2+} , Zn^{2+} , and Ag^{+} are metal ions that, like Hg^{2+} , disrupt enzymatic function by replacing essential ions in metabolic pathways, causing oxidative stress and cell death (Winslow et al. 2001; Bielmeyer-Fraser et al. 2014). Benzalkonium chloride (BAC) is a cationic surfactant and widely used household biocide, commonly found as an active ingredient in soaps and disinfectants (Glöel et al., 2015; Pereira and Tagkopoulos, 2019). BAC induces cellular damage by diffusing through the cell membrane and triggering the leakage of cytoplasmic contents (Winslow et al., 2001). To our knowledge, no comprehensive studies have systematically evaluated the preservation effects of alternative biocides on carbonate chemistry samples. However, limited evidence suggests that Cu^{2+} and BAC may be effective at preserving seawater samples for C_T analyses (Glöel et al. 2015; Takahashi et al., 2019).

A greater number of studies have evaluated the effects of preservation treatments (i.e. Cu^{2+} and BAC) on the stable isotopic composition of dissolved inorganic carbon ($\delta^{13}\text{C}-\text{C}_\text{T}$) in samples from a range of aquatic environments. These studies are relevant because, as with A_T and pH_T , microbial activity can substantially alter isotopic ratios following sample collection. Results regarding Cu^{2+} preservation are inconsistent, with some studies reporting effective preservation and others documenting significant isotopic shifts attributed to Cu^{2+} precipitation with carbonate species (Doctor et al. 2008; Taipale et al. 2009). Similarly, studies assessing the effects of BAC on $\delta^{13}\text{C}-\text{C}_\text{T}$ and oxygen-argon ratios (O_2/Ar), another proxy for microbial activity, report conflicting results after storage periods of several weeks (Glöel et al. 2015; Takahashi et al., 2019).

Overall, the success of preservation methods depends on (a) the intended use of the data and (b) the precision with which the relevant parameters can be measured. Here, the success of our experimental treatments is evaluated against two data quality objectives defined by the Global Ocean Acidification Network (GOA-ON). The “climate quality objective” is the more stringent of the two and is defined by uncertainties of $\pm 2 \mu\text{mol kg}^{-1}$ for A_T and ± 0.003 for pH_T , whereas the “weather quality objective” allows more permissive uncertainties of $\pm 10 \mu\text{mol kg}^{-1}$ for A_T and ± 0.02 for pH_T (GOA-ON, 2019; Riebesell et al., 2011). Most laboratories analyzing inorganic carbonate parameters operate within the precision ranges defined by weather-quality objectives since achieving more stringent analytical standards can be prohibitively expensive and often requires highly trained personnel to operate complex instrumentation. Further, studies focused in coastal and estuarine regions do not demand high data resolution since these areas typically experience large diurnal fluctuations in carbonate parameters. Samples treated with HgCl_2 then stored for multiple months before analysis can successfully produce A_T and pH_T measurements within the more stringent climate data objective. While ideally, alternative preservation treatments evaluated here yield samples with similar measurement quality as samples treated with HgCl_2 , laboratories willing to tolerate larger uncertainties may consider weaker biocides as a valuable and less-hazardous alternative to HgCl_2 .

Two of four carbonate chemistry parameters can be used to characterize the carbonate system in seawater samples. Measurement uncertainties and error inherent to associated equilibrium constants vary between alternate parameter pairings causing certain combinations to be more ideal than others. When measured to the state-of-the-art, combining A_T and C_T yields the least propagation error when computing the remaining parameters; however, the ideal combination will ultimately depend on the resources and measurement abilities of the researcher (Zeebe &

Wolf-Gladrow, 2001; Orr et al., 2018, Dickson et al., 2007). In this study we utilized A_T and pH_T however, this study focuses on A_T for the following reasons: 1) the spectrophotometer used to measure pH_T experienced persistent issues that prevented sample analyses on multiple days, 2) A_T measurements demonstrate greater precision and reproducibility over time, as the sources of uncertainty inherent to the open-cell potentiometric titration method employed here are well characterized, and the systems can be reliably calibrated to ensure high data quality (Dickson et al., 2003). Uncertainty relating to elements of spectrophotometric pH_T measurements such as dye purity and light interactions are less well understood and the uncertainty of tris-buffer as a suitable standard is debated (Pratt 2014; DeGrandpre et al., 2014; Takeshita et al., 2020; Clegg et al., 2022).

Analytical uncertainty can result from the following: 1) Errors occurring within the measurement system (i.e. systematic drifts, inaccurate calibrations, aging system components), 2) changes in the external environment that affect measurements (i.e. temperature, pressure, etc.), 3) inconsistent handling of samples by the system operator, 4) variations in chemical composition between duplicate samples, 5) changes in sample carbonate chemistry due to chemical interactions with biocides, 6) sample degradation during storage.

In a recent effort to better understand the contributions of analytical system components on pH_T measurement accuracy, we analyzed multiple CRM batches over several months and saw significant downward pH_T drifts with time across all batches. While downward pH_T drifts are commonly observed in samples stored over months, the magnitudes observed in this study were similar across multiple CRM batches regardless of age, suggesting that errors stemmed within the measurement system. Further data analyses suggested that dye stocks can degrade over time, affecting the accuracy of pH_T measurements. This phenomenon likely affected the precision of the pH_T samples analyzed for this thesis; however, without suitable standards to quality-control system

performance, the presence and impact of this uncertainty on adjacent measurements cannot be characterized.

METHODS

2.1 Experimental Design

This study was conducted during the COVID-19 pandemic, so many components of the experimental design were compromised due to restrictive safety precautions implemented by the university. Operations where multiple people were working closely together were prohibited, thus limiting the ability to collect large volumes of seawater. Further, a rigid analysis schedule was not conceivable due to reduced and unpredictable laboratory access. This led to experiments based on multiple, small volumes of seawater analyzed over different time periods. Further, initial carbonate chemistry values at day 0 were estimated since analyzing samples immediately after bottling would not have been feasible without assistance from others.

A simple preliminary experiment evaluated any immediate changes in A_T resulting from addition of various proposed preservatives to an artificial solution containing only sodium bicarbonate (NaHCO_3) and sodium chloride (NaCl). This allowed problematic treatments to be eliminated from consideration before use in comprehensive, multi-week studies. Furthermore, bicarbonate solution is relatively sterile, was easily made by one person, and is solely comprised of carbonate species, which primarily contribute to A_T concentrations in seawater. Preservatives successful in preliminary tests subsequently advanced to a larger set of experiments, which evaluated A_T and pH_T changes in surface seawater samples over 1–4 months.

2.2 Sample Collection and Processing

Sample water was collected in small batches of 20 L. Since dissolved CO_2 concentrations in seawater change when exposed to ambient air for extended periods of time, collecting larger volumes of seawater was not feasible when only one person is distributing the solution into sample

bottles. Each 20 L batch was recirculated via peristaltic pump for one hour to ensure homogeneity before bottling. Samples were stored in 250 mL borosilicate-33 glass bottles with ~1% headspace and treated with a preservative using an Eppendorf pipette. Bottles were sealed using ground-glass stoppers and grease (Apiezon[®] L grease), then secured using rubber bands and plastic hose clamps.

Preservative stock solutions were prepared by combining preservative compounds with deionized water (Table 2). Each solution was allowed to mix for at least one day before use. All stock solutions, aside from HgCl₂, were not fully saturated to avoid the need to use impractically small pipette volumes. Diluted solutions required volumes between 1–30 μ L per 250 mL sample. Aside from BAC, the concentrations listed refer to the molar amount contents of the metal ion in solution (e.g. 20 μ mol kg⁻¹ refers to [Cu²⁺] not [CuSO₄]). Solution amount contents were calculated using the actual volume contained by the sample bottles used (~280 mL of solution when filled leaving 1% headspace) rather than the bottle's nominal volume of 250 mL.

Samples were analyzed for pH_T spectrophotometrically using our standard laboratory approach (Carter et al., 2013). Samples were measured at 25 °C using purified m-cresol purple indicator dye and data was processed using the equations of Liu et al. (2011). Samples were analyzed for A_T using an open-cell, potentiometric titration as described by Dickson et al. (2003). Dilution corrections were applied to A_T values to account for additions of preservative treatments. CRMs were of limited availability during COVID-19 and thus were not run alongside all titrations.

2.2.1 Bicarbonate Solution

Four batches (B1–B4) of bicarbonate solution were used to test for immediate changes in A_T upon treatment with various preservatives. The solution contained 0.7 mol kg⁻¹ sodium chloride and 2200 μ mol kg⁻¹ sodium bicarbonate, and deionized water. Samples were treated with each of

the following preservatives: 20, 40, or 80 $\mu\text{mol kg}^{-1}$ of Cu^{2+} as CuSO_4 ; 20 or 80 $\mu\text{mol kg}^{-1}$ of Zn^{2+} as ZnCl_2 ; or 10 or 50 $\mu\text{mol kg}^{-1}$ of Ag^+ as AgNO_3 . One concentration of 55 $\mu\text{mol kg}^{-1}$ of Hg^{2+} as HgCl_2 was used a control treatment for all experiments. A_T analyses occurred 1–4 hours after bottling, and average A_T values from samples treated with experimental preservatives were compared against those treated with HgCl_2 .

2.2.2 Surface Seawater

Twelve batches (B5–B16) of surface seawater were collected from Scripps Pier in La Jolla, California between November 2020–July 2021 (Table 1). 60 samples of seawater were created from each batch and analyzed for A_T and pH_T over various lengths of time. Experimental samples were treated using one of the following preservatives: 20, 80 or 160 $\mu\text{mol kg}^{-1}$ Zn^{2+} as ZnCl_2 ; 10, 50 or 90 $\mu\text{mol kg}^{-1}$ Ag^+ as AgNO_3 ; or 30 or 100 $\mu\text{mol kg}^{-1}$ BAC. One concentration of 55 $\mu\text{mol kg}^{-1}$ of Hg^{2+} as HgCl_2 was used a control treatment for all experiments. Most batches were analyzed on three separate occasions within 40 days from time of collection; however, one batch was analyzed after 3 months. Samples were stored in closed crates at room temperature until analyzed. Later in this study, we determined that evaluating A_T and pH_T changes in samples that were not treated with any preservative at all may help to gauge the level of efficacy of preservative treatments. Batches B11 and B13–B16 contained a small number of untreated samples that were analyzed on select days.

2.4 Data processing

The success of treatments in preserving samples over time is based on the following criteria: 1) whether the distribution's uncertainty is within climate and weather data objective goals

(± 2 and $10 \mu\text{mol kg}^{-1}$, respectively) over all analysis days, 2) whether the treatment mean is significantly different from the mean of samples treated with HgCl_2 .

Standard deviations of ΔA_T and ΔpH_T (Eq. 1) were calculated on each analysis day for each treatment according to SOP23 (Dickson et al., 2007). The percentage of measurements meeting the climate and weather data objective before and after two weeks was calculated for each preservation method to assess the stability of treatments over time. T-test procedures outlined in SOP23 compared means between experimental samples and control samples treated with HgCl_2 (Dickson et al., 2007).

Ideally, all experimental values are compared to the mean values of samples treated with HgCl_2 and analyzed after day one. However, due to COVID-19, scheduling days for analyses was highly unpredictable and very seldom occurred on day one. Thus, experimental samples are compared to the mean value of samples measured on the first analysis day.

$$\Delta M_i = M_i - \text{Reference Value} \quad (1)$$

M_i = Measurement of individual sample

Reference Value = Average value of HgCl_2 treated samples measured on the first analysis day

RESULTS

3.1 Immediate effects of preservatives on A_T in sodium bicarbonate solution samples

Two batches of bicarbonate solution (B1, B2) were used to evaluate immediate A_T changes upon treatment with two concentrations of silver ($10, 50 \mu\text{mol kg}^{-1} \text{Ag}^+$) and two concentrations of zinc ($20, 80 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$, Table 1). There were no significant changes for these treatment groups as all mean A_T differences were within $1 \mu\text{mol kg}^{-1}$ of the reference value (Table 3). Standard deviations were below $0.6 \mu\text{mol kg}^{-1}$ which is within the expected range of precision. The remaining batches of bicarbonate solution (B3, B4) were used to evaluate immediate A_T changes upon treatment with three copper concentrations ($20, 40, 80 \mu\text{mol kg}^{-1} \text{Cu}^{2+}$). Concentrations of 40 and $80 \mu\text{mol kg}^{-1} \text{Cu}^{2+}$ yielded high and imprecise A_T values in batch B3 (Table 3). We speculated that these imprecise values might be caused by incompatibility between copper ions within the solution and the pH electrode, thus the experiment was repeated using a different electrode brand. The resulting batch (P4) showed similar A_T decreases and measurement imprecision. However, this imprecision was not consistent between treatment groups. For example, the mean A_T difference of samples treated with $40 \mu\text{mol kg}^{-1} \text{Cu}$ was $-5.8 \pm 5.1 \mu\text{mol kg}^{-1}$ in batch P3 but improved to $-1.5 \pm 0.5 \mu\text{mol kg}^{-1}$ in batch B4 (Table 3). While this supports the notion that a different electrode may yield better measurements, the mean A_T difference in samples treated with $80 \mu\text{mol kg}^{-1} \text{Cu}$ was doubled in batch B4 when compared to P3 (Table 3). Overall, A_T changes and measurement imprecision worsen as Cu^{2+} levels increase. This is evident in both batches, suggesting that the observed measurement quality is not dependent on the electrode brand. For reasons discussed further in another section, we decided to remove copper from the remaining experiments.

3.2 Preservative efficacy on A_T and pH_T in surface seawater samples

3.2.1 $55 \mu\text{mol kg}^{-1} \text{Hg}$

Control samples in 9 of 12 batches had mean ΔA_T values within $\pm 1 \mu\text{mol kg}^{-1}$ over all analysis days and uncertainties, i.e., well within $\pm 2 \mu\text{mol kg}^{-1}$. Exceptions to this include B12 and B13, which showed A_T decreases greater than $15 \mu\text{mol kg}^{-1}$ after one month (Table 4, Fig. 4). Notably, the magnitude of A_T changes in B12 and B13 were not significantly different, as mean ΔA_T values were $2.2 \mu\text{mol kg}^{-1}$ apart after one month. Measurement uncertainties in both batches worsened over time, with standard deviations ranging between $2.5\text{--}5.2 \mu\text{mol kg}^{-1}$ after one month. Separately, A_T of B6 also changed significantly in a short amount of time ($\Delta A_T = -2 \mu\text{mol kg}^{-1}$ after 10 days). These changes were less drastic than batches B12 and B13; however, B6 was not measured again after 10 days, and potentially could have experienced further changes should it been stored for longer time periods.

Batches B6, B12 and B13 showed analogous decreases in pH_T values over time. Mean pH_T of B12 and B13 decreased by similar magnitudes of about -0.070 after one month, with uncertainties of 0.016 and 0.010 , respectively. B6 mean pH_T decreased by -0.014 ± 0.012 after 10 days. In comparison, pH_T of the remaining batches changed by a maximum of 0.003 over all analysis periods, with uncertainties within ± 0.003 .

Drastic changes in A_T and pH_T observed in control samples for batches B12 and B13 are suggestive of contamination via mercury-resistant bacteria, likely due to inadequate sterilization of sampling equipment. Batch B12 and B13 were collected on the same day using the same equipment, further supporting this theory. It's difficult to determine whether the same phenomenon is occurring in batch B6 since this batch was not analyzed after 10 days, which was before drastic A_T and pH_T changes were observed in other batches. It's possible, although unlikely, that drifts in

both A_T and pH_T measurement systems accounted for these small drifts; however, this is difficult to confirm without accompanying CRM data.

3.2.2 10, 50, and 90 $\mu\text{mol kg}^{-1}$ Silver

Treatments of 10 $\mu\text{mol kg}^{-1}$ Ag^+ were assessed using batches B5 and B7. Both batches showed similar changes over time, with A_T increasing between 4–8 days, then stabilizing throughout the next 10 days. Maximum mean ΔA_T values ranged between 1.6–2.1 $\mu\text{mol kg}^{-1}$ and all standard deviations were within $\sim 1 \mu\text{mol kg}^{-1}$ (Table 4). Mean ΔA_T of control samples in batch B7 increased by 1.1 $\mu\text{mol kg}^{-1}$ after 8 days, suggesting that other variables (e.g., system drift) may be contributing to these silver-treated ΔA_T values. Regarding pH_T values, the rate and magnitude of changes varied between batches. Batch B5 decreased two times more than B7 after 18 and 12 days, respectively (Mean $\Delta pH_T = -0.041$ and -0.029 , respectively, Table 4). For both batches, all pH_T values fell outside of the climate data objective range between 2–4 days and outside the weather data objective after 7 days (Fig. Ref). Standard deviations did not exceed 0.002 for any analysis days.

Four batches of seawater (B5, B7, B12, B14) were used to evaluate treatments of 50 $\mu\text{mol kg}^{-1}$ Ag^+ up to a maximum of 35 days. Significant decreases in control A_T and pH_T values observed in batch B12 suggest exposure to mercury-resistant bacteria during bottling. Despite this, A_T of samples treated with 50 $\mu\text{mol kg}^{-1}$ Ag remained stable ($\Delta A_T = 0.1 \pm 1.8 \mu\text{mol kg}^{-1}$ after 35 days, Table 4). Between all other batches, the highest mean ΔA_T value was 2.1 $\mu\text{mol kg}^{-1}$ after 8 days (Batch B7). 94% of A_T samples measured after two weeks were within the climate data objective and 100% of samples were within the weather data objective (Table 5). pH_T measurements in all batches decreased, with most values falling outside the climate data objective in as little as 4 days.

However, pH_T values measured after one day were not significantly different from control values, suggesting treating samples with $50 \mu\text{mol kg}^{-1} \text{Ag}^+$ does not immediately affect pH_T . Batches were affected differently: B5 experienced the highest and most rapid pH_T changes ($\Delta \text{pH}_T = -0.033$ after 12 days, Table 4) while other batches appear to level out just outside the weather data objective range after two weeks (mean $\text{pH}_T = -0.022$ – -0.027 , Table 4). Since batch B5 was not measured again after 12 days, we do not know if its pH_T measurements would also begin to plateau or continue to decrease at similar a similar rate. Overall, 18% of pH_T samples were within the less stringent weather data objective after two weeks (Table 5).

Two batches of seawater (B12, B14) were used to evaluate treatments of $90 \mu\text{mol kg}^{-1} \text{Ag}^+$. Despite significant decreases observed in control samples from B12 (believed contaminated with mercury-tolerant organisms), mean ΔA_T samples treated with silver decreased by only $0.4 \mu\text{mol kg}^{-1}$ after 35 days. pH_T measurements decreased outside of the weather objective range but ΔpH_T values were still less than those of the control samples. Similar patterns were seen in samples treated with $50 \mu\text{mol kg}^{-1} \text{Ag}^+$. Conversely, aside from one set of results analyzed on day 22, mean A_T changes and standard deviations from batch B14 were below $1 \mu\text{mol kg}^{-1}$ (Table 4). Mean ΔpH_T decreased by 0.017 ± 0.003 after 27 days, which is just inside the weather data objective range.

It's apparent there were analytical problems with the alkalinity measurement system on day 21 for batch B14 analyses (Fig. 3). Control samples were scattered, and results for 50 and $90 \mu\text{mol kg}^{-1} \text{Ag}^+$ treatments were decidedly low with more scatter than seen in measurements from other days. Confirming this suspicion, results for all three sets of data were more reasonable on day 26. Further, when considering analogous pH_T measurements, it's possible a few “bad” samples, by way of either contamination or sample degradation further contributed to the variance.

3.2.3 20, 80, and 160 $\mu\text{mol kg}^{-1}$ Zinc

Three batches of seawater (B6, B9, B11) were used to evaluate treatments of 20 $\mu\text{mol kg}^{-1}$ Zn^{2+} . As previously mentioned, it's possible mercury-resistant bacteria were present in batch B6, as both pH_T and A_T in control samples changed dramatically and both measurement systems are unlikely to simultaneously experience drifts of such high magnitudes (mean $\Delta \text{pH}_T = -0.014$ and mean $\Delta A_T = -1.9 \mu\text{mol kg}^{-1}$, after 10 days). A_T and pH_T results of samples treated with 20 $\mu\text{mol kg}^{-1}$ Zn^{2+} varied between the remaining two batches. Samples in batch B9 were first analyzed after day 11 and showed A_T increases averaging 3.3 $\mu\text{mol kg}^{-1}$. Conversely, A_T of batch B11 did not change after four months of storage. Technical issues with the spectrophotometer hindered analyses during various days when measuring batches B9 and B11. However, pH_T of both batches was measured on the final storage day, revealing that B9 had changed almost twice as much as B11 despite only being stored for 23 days ($\Delta \text{pH}_T = -0.052$ and -0.034 , respectively, Table 4).

Four batches of seawater (B6, B9, B11, B16) were used to evaluate treatments of 80 $\mu\text{mol kg}^{-1}$ Zn^{2+} . Batch B9 exhibited the greatest change among all batches, with mean ΔA_T values ranging from 2.1–3.0 $\mu\text{mol kg}^{-1}$ between days 11 and 23 (Table 4). A_T results varied by batch; for instance, Batch B11, which was stored for four months, showed an average change of $-1.6 \mu\text{mol kg}^{-1}$. The pH_T of samples measured after day 1 and 2 was significantly lower than that of samples treated with 20 $\mu\text{mol kg}^{-1}$ Zn^{2+} and measured after the same period which suggests zinc has an adverse effect on sample pH_T . The pH_T of Batch B6 decreased rapidly between days 1 and 6, then stabilized. After six days, ΔpH_T values in all batches appeared to stabilize between 0.040 and 0.062, with no clear worsening over time.

One batch of seawater (B13) was analyzed over one month to evaluate treatments of 160 $\mu\text{mol kg}^{-1}$ Zn^{2+} . Increasing zinc to this extent seems to have a highly adverse effect on both

A_T and pH_T precision. Figure 3 shows that around half of the measurements for both pH and A_T exhibit changes similar to those of samples treated with $80 \mu\text{mol kg}^{-1} \text{Zn}$, while the other half decrease greatly and display significant scatter.

Overall, there doesn't appear to be a significant difference in the preservation efficacy of A_T and pH_T between 20 and $80 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ treatments. However, the presence of either amount seems to worsen measurement precision slightly when compared to control samples. Notably, a threshold appears to exist regarding the concentration of zinc that can be added to samples, as the data quality of those treated with $160 \mu\text{mol kg}^{-1} \text{Zn}$ was clearly compromised. While all A_T samples, regardless of storage period, treated with 20 and $80 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ remain within the weather data objective, the corresponding pH_T samples are outside that objective after just 1 day.

Untreated A_T samples were analyzed alongside Batch 11 and Batch 16 and provide context to results of zinc-treated samples. For instance, Batch 11 exhibited the smallest changes in A_T and pH_T among all batches, despite being stored for the longest period. However, the average A_T of samples treated with $80 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ was comparable to that of untreated samples after four months, suggesting that zinc may not be inhibiting biological activity, but rather that there is insufficient biological activity in the sample to affect A_T , regardless of the treatment. Conversely, untreated samples in batch B16 changed by $\sim 30 \mu\text{mol kg}^{-1}$ after 30 days, which was significantly less than samples treated with $80 \mu\text{mol kg}^{-1} \text{Zn}$.

3.2.4 20 and $80 \mu\text{mol kg}^{-1}$ BAC

Three batches of seawater (B8, B10, B15) were used to evaluate A_T and pH_T changes in samples treated with $20 \mu\text{mol kg}^{-1}$ BAC over one month. Although batches B8 and B10 were

analyzed over similar periods, the average A_T of batch B10 decreased by 4–5 $\mu\text{mol kg}^{-1}$ after day 14, while batch B8 remained unchanged after day 22. A_T decreases of this magnitude were unique to samples treated with 20 $\mu\text{mol kg}^{-1}$ BAC and were not observed in other treatments from this batch. Notably, the corresponding pH_T samples exhibited comparable changes up to day 15 ($\Delta \text{pH}_T = -0.035$ to -0.024 , Table 4). Batch 15 was analyzed between 18 and 31 days and results suggest BAC may lose its efficacy after three weeks. While A_T remained unchanged after day 18, observable decreases began after day 26 and worsen through day 31. pH_T results on corresponding days showed an analogous pattern. Furthermore, standard deviations worsen over time, suggesting the presence of biological activity as its effect is likely to change samples differently leading to high scatter.

Three seawater batches (B4, B6, and B12) were used to evaluate samples treated with 80 $\mu\text{mol kg}^{-1}$ BAC. Across all batches, 96% of A_T samples met the climate data objective range before treatment, decreasing slightly to 90% after two weeks. This performance exceeded that of samples treated with the lower level of 20 $\mu\text{mol kg}^{-1}$ BAC. Higher BAC levels appear to have a significant impact on pH_T upon treatment. Samples treated with 80 $\mu\text{mol kg}^{-1}$ BAC decreased by a similar magnitude across all batches ($\Delta \text{pH}_T = \sim -0.115$), regardless of storage duration. This suggests that, like zinc, BAC alters hydrogen ion concentrations upon application. Notably, pH_T did not exhibit any further changes after this initial downward shift, aside from a potential exception observed in samples measured after day 31. Here, the average pH_T is slightly lower ($\Delta \text{pH}_T = -0.130$) than samples measured on other days with increased variability. While this could be a coincidence, possibly influenced by an outlier with a ΔpH_T of -0.180 , it may also indicate biological activity caused further pH_T decline and increased variability. This suggests that even at

elevated concentrations of $80 \mu\text{mol kg}^{-1}$, BAC may not be sufficient to fully prevent sample degradation due to biological activity.

DISCUSSION

4.1 Design Constraints and Methodological Challenges

Overall, the safety protocols implemented by the university in response to COVID-19 led to complexities when executing the experimental design. For instance, restrictions on working with others in close quarters limited the amount of water that could be collected thereby limiting the scale of production of test samples. This constraint hindered the ability to test all preservatives under identical conditions, reducing the confidence in and accuracy of comparisons between methods. Additionally, quarantine regulations for laboratory personnel exhibiting symptoms made laboratory access unpredictable. Consequently, the confidence of reported A_T and pH_T reference values was affected since these were inferred from the average of control samples analyzed on the first analysis day (Eq. 1), which occurred after one or two weeks for some batches.

The ability of identical treatments to preserve A_T and pH_T varied greatly across multiple batches of seawater. One obvious cause was likely due to the improper sterilization of laboratory equipment, which introduced mercury-resistant bacteria into the sample water, leading to significant decreases in A_T and pH_T in samples treated with $HgCl_2$ in batches B2, B9, and B10. Dickson (2010) reported similar abnormal data patterns when analyzing early batches of certified reference materials for C_T and A_T and suggested that organic acids, produced via the growth of mercury-resistant bacteria, acidified the surrounding solution and titrated alkalinity. These contaminated batches nevertheless provided evidence of the preservation efficacy of silver and zinc. While these also showed some changes, the magnitude of A_T and pH_T changes were significantly lower than for mercury-treated samples, suggesting that these metals may function as

effective biocides in solutions where biological activity is present. Conversely, a limited number of samples not treated with a preservative were collected and analyzed for A_T in later batches and results suggest that biological activity in select batches may have been sufficiently low to not cause significant carbonate chemistry changes even without preservation. For instance, an untreated sample from batch B11 analyzed after four months had only changed by $-2.1 \mu\text{mol kg}^{-1}$, which falls just outside the climate data objective range. Samples treated with $80 \mu\text{mol kg}^{-1}$ Zn showed similar results, which could lead to the false assumption that this method is relatively effective at curbing biological activity unless the results of untreated data are also considered. Unfortunately, untreated A_T samples were not collected from batches B5–B10 and untreated pH_T samples were not collected from any batch, reducing the confidence with which preservation methods tried on these batches can be deemed successful. Future studies should involve collection of an adequate number of untreated samples to verify that the sampled seawater is inclined to change without preservation.

In batches B12–B16, samples not treated with preservatives showed A_T decreases ranging from $5\text{--}60 \mu\text{mol kg}^{-1}$ after three weeks. It's possible the observed disparity between batches is due to seasonal differences in the number of microbes in the collected seawater, as B11 batch was the only batch collected in February while all others were collected in July. A previous study found that the efficacy of BAC as a biocide was dependent on chlorophyll concentration (Gloël et al., 2015), suggesting that particularly high levels of biological activity are not effectively curbed by BAC. This highlights the importance of testing all preservation methods on the same batch of seawater and of analyzing untreated samples, as comparing the preservation effectiveness between different poisons is impossible without eliminating all confounding variables or establishing a benchmark value against which comparisons can be made. Further, this suggests the

importance of using a variety of sources of seawater to test preservation methods, as even seasonal differences from the same geographical source can confound results.

The above limitations should be considered when assessing the potential success of any one preservation method tested in this study. However, it is also valuable to evaluate and identify methods that are clearly problematic and can be eliminated as alternatives to mercuric chloride.

4.2 Copper interacts with seawater constituents

Initial experiments involving immediate analysis of A_T after treating bicarbonate solution showed a clear correlation between A_T decreases and copper concentrations, (Fig. 2) suggesting that copper (II) may alter the solution's carbonate chemistry. When introduced to seawater, metal ions tend to complex with seawater constituents to form various inorganic species which poses complications when identifying inert preservatives (Byrne, 2002; Turner et al. 1981). In this case, copper (II) ions tend to form carbonate and hydroxide compounds (Byrne, 2002). Downward A_T trends observed in Fig. 2 are likely ultimately caused by increased amounts of carbonate and hydroxide precipitating from solution as copper concentrations increase. Theoretically, A_T will be unaffected if the entirety of the precipitate were acidified and redissolved by hydrochloric acid during an alkalinity titration; however, the nature of the measurement process requires titrating only a portion of a typical sample (~ 130 mL). Inconsistent amounts of precipitate transferred into the titration cell explains the imprecise analyses and the observed correlation between A_T and copper concentration, as higher concentrations yield more precipitate that is excluded from the analysis. This cannot be avoided unless the entire sample is acidified. Other studies report similar observations when using Cu^{2+} to preserve dissolved inorganic carbon isotopes in seawater samples (Taipale et al. 2009). Carbonate species account for 90% of A_T concentrations in most oceanic

locations (Emerson & Hedges, 2002), implying that results observed in bicarbonate solution are transferable to field-collected samples. Due to copper's tendency to form a precipitate in bicarbonate solution and its immediate effect on seawater CO₂ chemistry, it should be excluded from consideration in future studies.

4.3 Zinc impacts measurement precision

The addition of zinc to seawater samples significantly increases the scatter of A_T results. This effect is most pronounced in samples treated with 160 µmol kg⁻¹ Zn²⁺, which exhibited standard deviations exceeding 16 µmol kg⁻¹. Data quality of samples treated with lesser concentrations were also affected, albeit to a less obvious extent. There is no clear improvement in preservation efficacy as zinc concentrations increase from 20 to 80 µmol kg⁻¹ Zn²⁺. However, a threshold appears to exist, where increasing concentrations to 160 µmol kg⁻¹ is clearly detrimental to A_T and pH_T data quality. In seawater, zinc (II) mostly chelates with organic ligands (Bruland et al. 2014), though other inorganic species form when zinc exists as free ion in solution. Like Cu(II), Zn(II) tends to complex with A_T-contributing ions (i.e., HCO₃⁻, CO₃²⁻, OH⁻) and the formation of these chemical species indirectly affect pH_T by triggering a release or uptake of protons to balance the carbonate buffering system (Stanley & Byrne 1990). It is possible that at smaller concentrations, zinc is largely consumed by organic ligands, whereas at 160 µmol kg⁻¹ Zn²⁺, the concentrations are high enough for free cations to persist in solution and complex with inorganic species. This theory plausibly explains abnormal data patterns observed in samples treated with high zinc concentrations and underscores the importance of testing various preservative concentrations to account for unforeseen interactions between treatments and

seawater constituents. While concentrations of $160 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ did not cause a visible precipitate to form, these compounds may exist as colloidal material in solution.

Further, samples treated with $80 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ exhibited greater pH_T changes than those treated with $20 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$ when measured after one or two days. The immediacy of these changes and their direct correlation with zinc concentration suggest that these observed pH_T decreases are likely not driven by biological respiration but rather by changes to the chemical composition of the sample due to the following: 1) zinc ions interacting with chemical constituents, or 2) the zinc solution, which has a pH_T dissimilar to that of the sample, affecting the carbonate chemistry upon preservation. Gradual pH_T decreases are observed after the first few days in samples preserved with 20 and $80 \mu\text{mol kg}^{-1} \text{Zn}$ which is suggestive of biological activity degrading the sample. These changes seem to level out or slow down after one week in samples treated with $80 \mu\text{mol kg}^{-1} \text{Zn}$ and suggests either 1) all reactants involved in respiration mechanisms were consumed at this time or, 2) the molecular uptake rate of zinc is slow, and biology didn't completely die until later. Overall, all pH_T samples regardless of zinc concentration fell outside of the weather data objective after 2 days.

4.3 Preservation efficacy of Ag^+ on pH_T and A_T

The efficacy of silver in preserving A_T and pH_T measurements appears to improve at higher concentrations. However, while samples treated with the highest concentration of silver, $90 \mu\text{mol kg}^{-1} \text{Ag}^+$, yielded promising results for A_T , with most measurements falling within the climate data objective, it was only tested using one viable batch (B14), and the low A_T of samples observed after 22 days suggests that instrumental issues may have occurred during analysis, limiting the degree to which its preservation efficacy can be confidently assessed. 94% of A_T

samples treated with $50 \mu\text{mol kg}^{-1} \text{Ag}^+$ fell within the climate data objective after two weeks of storage. However, biological activity occurring within the sample is evident in pH_T measurements, as gradual decreases over time were observed in multiple batches. The mean pH_T of samples from B14 treated with $90 \mu\text{mol kg}^{-1} \text{Ag}^+$ is slightly better than that for samples treated with $50 \mu\text{mol kg}^{-1} \text{Ag}$, again suggesting the possibility that preservation efficacy increases with silver added. Samples treated with $90 \mu\text{mol kg}^{-1} \text{Ag}^+$ were not measured after one or two days and thus it's unsure whether preserving samples with this concentration has an immediate effect on pH_T in a similar manner to $160 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$.

Both batches used to analyze A_T of samples treated with $10 \mu\text{mol kg}^{-1} \text{Ag}^+$ yield positive A_T and negative pH_T changes values after four days. This unlike all other changes seen with other experimental treatments, except for samples collected from B9 and treated with $20 \mu\text{mol kg}^{-1} \text{Zn}^{2+}$, which showed mean A_T increases ranging from $2.6\text{--}3.9 \mu\text{mol kg}^{-1}$ between 11 and 23 days. In future studies, analyzing samples for nutrients may be helpful in identifying which metabolic processes were occurring within the sample and act to confirm some of these suspicions.

Overall, while preservation using 50 and $90 \mu\text{mol kg}^{-1} \text{Ag}^+$ may maintain A_T measurements within the climate data objective for up to one month, preserving pH_T measurements remains more challenging, as none of the samples stayed within the target range after 15 days. It is possible that higher silver concentrations beyond those tested in this study (i.e., $>90 \mu\text{mol kg}^{-1} \text{Ag}^+$) could serve as more effective preservatives for pH_T . However, when AgNO_3 is introduced into seawater, it dissociates, and most free silver ions bind with chloride ions to form AgCl , which precipitates. Although the precipitate did not appear to influence pH_T or A_T measurements observed in this study, increasing silver concentrations further may pose risks. Spectrophotometric pH_T methods rely on light absorbance, and the presence of precipitates can obstruct the light path, potentially

compromising the accuracy of measurements. The likelihood of such interference increases with the amount of precipitate present in a sample.

4.4 Preservation efficacy of BAC on pH_T and A_T

Preservation efficacy of BAC improves with increasing concentration, as samples treated with $80 \mu\text{mol kg}^{-1}$ BAC exhibited smaller mean A_T changes than those treated with $20 \mu\text{mol kg}^{-1}$. Notably, 90% of samples treated with the higher BAC concentration remained within the climate data objective between days 14 and 31. In contrast, results from the $20 \mu\text{mol kg}^{-1}$ treatment varied by batch: batch B10 showed a decrease of approximately $5 \mu\text{mol kg}^{-1}$ after six days, while other batches exhibited no significant changes over similar timeframes. Interestingly, the corresponding pH_T values for batch B10 were comparable to those of other batches, despite the observed differences in A_T .

On average, pH_T of samples treated with 20 and $80 \mu\text{mol kg}^{-1}$ BAC fell by around 0.010–0.017 and 0.150–0.100 after five days, respectively. It's likely that preserving seawater with BAC inherently changes the pH_T since samples treated with higher concentrations experienced larger pH_T changes over identical timeframes from the same batch treated with lower concentrations. After the initial decrease, pH_T in samples treated with $80 \mu\text{mol kg}^{-1}$ BAC appears stable, with no further changes observed throughout the remainder of the month. In contrast, pH_T in samples treated with $20 \mu\text{mol kg}^{-1}$ BAC begins to decline after 25 days, following a period of relative stability. Measurements taken on days 25 and 31 show greater variability compared to earlier time points, which may suggest that respiratory processes were altering the chemical composition at different rates across samples. Gradual pH_T changes observed within the first few weeks suggest that concentrations of $20 \mu\text{mol kg}^{-1}$ BAC is not sufficient to curb metabolic

processes. Interestingly, the most pronounced A_T and pH_T changes occur after several weeks in storage. This contrasts with samples treated with silver and zinc, which exhibit the most significant changes within the first few days. These differences suggest that two distinct processes may be driving the observed carbonate chemistry changes.

As previously speculated, the molecular uptake of zinc and silver may be relatively slow, with their lethal effects on cellular processes only becoming apparent days after treatment. Alternatively, concentrations used may have been insufficient, allowing metabolic activity to continue for several days until key compounds involved in respiration were depleted or biological processes ceased due to prolonged dark storage.

Previous studies evaluating BAC as a preservative for A_T measurements have attributed late-stage A_T declines to spore germination, proposing that spores may be resistant to BAC (Takahashi et al., 2019; Gloël et al., 2015). While this may explain the observed A_T decreases in studies using a single BAC concentration, our results demonstrate a clear relationship between BAC concentration and preservation efficacy, indicating that spore resistance to BAC is unlikely.

4.5 Analogous changes in duplicate samples not treated with $HgCl_2$

Increased regulations and restrictions regarding the purchase and disposal of mercuric chloride have prohibited several research groups from using mercuric chloride and leaving them no choice but to not preserve samples. Ensuring that biological activity has not occurred between the time the sample is collected and when it is analyzed in the lab is difficult, and some labs have resorted to collecting duplicate samples and measuring their agreement to gauge whether the value is accurate (Shangguan et al. 2020). Biological activity occurring within duplicate samples is believed to change the carbonate chemistry concentrations of each sample differently over time,

as biology is heterogeneously distributed throughout the water column and thus within samples, resulting in carbonate chemistry differences between replicates. However, A_T and pH_T results from untreated samples suggest this may not always be the case. For instance, a pair of untreated replicates from B15 decreased by 6–7 $\mu\text{mol kg}^{-1}$ but agreed within 1.2 $\mu\text{mol kg}^{-1}$. Similarly, a pair of untreated duplicates from B16 decreased by more than 40 $\mu\text{mol kg}^{-1}$ yet agreed within 2 $\mu\text{mol kg}^{-1}$. Based on this data, caution should be taken when relying on replicates to authenticate A_T and pH_T values of untreated carbonate chemistry samples.

COMMENTS & RECOMMENDATIONS

University safety restrictions implemented during COVID-19 impacted various aspects of the experimental design and lowered the confidence of select results, particularly regarding successful alternatives to HgCl_2 . However, this study suggests that Cu^{2+} and Zn^{2+} can be eliminated as potential preservation methods for carbonate chemistry samples. Even at low concentrations, Cu^{2+} precipitates with carbonate species immediately upon treatment therefore altering A_T and C_T concentrations. Zn^{2+} may exhibit a similar phenomenon but that is only obvious in higher treatment concentrations, suggesting a clear threshold or “tipping point” that can be crossed as treatment concentrations increase. This limits the scalability of Zn^{2+} to suit more biologically productive waters that may require higher preservation concentrations.

Treatments of $80 \mu\text{mol kg}^{-1}$ BAC and 10 and $50 \mu\text{mol kg}^{-1}$ Ag^+ were the only methods that produced consistent results over multiple batches, with mean A_T and standard deviations at or below $1 \mu\text{mol kg}^{-1}$. Although these treatments show promise, additional testing is required before they can be confidently recommended for routine use. Future experiments should incorporate untreated control samples to assess whether preservation methods are truly preventing changes in carbonate chemistry. In this study, little to no change in A_T was observed in some untreated coastal seawater stored for several months, highlighting the importance of verifying that measurable biogenic alteration occurs regardless of treatment before claiming that a treatment is an effective preservative.

Testing all preservation treatments on identical seawater would facilitate direct comparisons between treatments and eliminate the need to rely on calculated parameters such as ΔA_T . However, variability in results among seawater batches suggests that treatments should also

be evaluated using multiple sources of seawater to assess the influence of organic matter loads on preservation efficacy. Comparisons among batches would further benefit from a standardized analysis schedule.

APPENDIX

Table 1. Collection date, treatments tested, and storage period information for all batches.

Batch ID	Date Bottled	Treatments	Storage Periods (Days)
<i>Batches of NaCl / NaHCO₃</i>			
B1	10/9/20	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Cu 40 $\mu\text{mol kg}^{-1}$ Cu 80 $\mu\text{mol kg}^{-1}$ Cu	—
B2	10/11/20	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Cu 40 $\mu\text{mol kg}^{-1}$ Cu 80 $\mu\text{mol kg}^{-1}$ Cu	—
B3	11/6/20	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Zn 80 $\mu\text{mol kg}^{-1}$ Zn	—
B4	11/7/20	55 $\mu\text{mol kg}^{-1}$ Hg 10 $\mu\text{mol kg}^{-1}$ Ag 50 $\mu\text{mol kg}^{-1}$ Ag	—
<i>Batches of Surface Seawater</i>			
B5	11/16/20	55 $\mu\text{mol kg}^{-1}$ Hg 10 $\mu\text{mol kg}^{-1}$ Ag 50 $\mu\text{mol kg}^{-1}$ Ag	1, 4, 12
B6	11/20/20	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Zn 80 $\mu\text{mol kg}^{-1}$ Zn	1, 6, 10
B7	1/4/21	55 $\mu\text{mol kg}^{-1}$ Hg 10 $\mu\text{mol kg}^{-1}$ Ag 50 $\mu\text{mol kg}^{-1}$ Ag	2, 8, 18
B8	1/12/21	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ BAC 80 $\mu\text{mol kg}^{-1}$ BAC	5, 14, 22

Table 1. Collection date, treatments tested, and storage period information for all batches, continued.

Batch ID	Date Bottled	Treatments	Storage Periods (Days)
B9	1/13/21	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Zn 80 $\mu\text{mol kg}^{-1}$ Zn	11, 20, 23
B10	1/22/21	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ BAC 80 $\mu\text{mol kg}^{-1}$ BAC	5, 14, 17
B11	2/12/21	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ Zn 80 $\mu\text{mol kg}^{-1}$ Zn Untreated	3, 7, 124
B12	7/8/21	55 $\mu\text{mol kg}^{-1}$ Hg 50 $\mu\text{mol kg}^{-1}$ Ag 90 $\mu\text{mol kg}^{-1}$ Ag	4, 10, 35
B13	7/8/21	55 $\mu\text{mol kg}^{-1}$ Hg 80 $\mu\text{mol kg}^{-1}$ Zn 160 $\mu\text{mol kg}^{-1}$ Zn Untreated	5, 7, 36
B14	7/20/21	55 $\mu\text{mol kg}^{-1}$ Hg 50 $\mu\text{mol kg}^{-1}$ Ag 90 $\mu\text{mol kg}^{-1}$ Ag Untreated	5, 22, 27
B15	7/20/21	55 $\mu\text{mol kg}^{-1}$ Hg 20 $\mu\text{mol kg}^{-1}$ BAC 80 $\mu\text{mol kg}^{-1}$ BAC	18, 26, 31
B16	7/24/21	55 $\mu\text{mol kg}^{-1}$ Hg 80 $\mu\text{mol kg}^{-1}$ Zn 160 $\mu\text{mol kg}^{-1}$ Zn Untreated	11, 26, 31

Table 2. Measured A_T results and t-test results for each treatment in experiments using a bicarbonate solution. Values in parentheses represent the number of analyses. A significant t-test result is represented by H=1 ($p = 0.01$).

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean A_T difference ($\mu\text{mol kg}^{-1}$)	t-stat	df	p-value	H
Batch B1						
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2209.8 $\pm$ 0.6 (6)					
10 $\mu\text{mol kg}^{-1}$ Ag	2209.5 $\pm$ 0.6 (6)	-0.3	2.04	10	0.07	0
50 $\mu\text{mol kg}^{-1}$ Ag	2209.7 $\pm$ 0.3 (6)	-0.1	1.72	10	0.12	0
Batch B2						
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2203.0 $\pm$ 0.4 (4)					
20 $\mu\text{mol kg}^{-1}$ Zn	2203.5 $\pm$ 0.6 (6)	0.5	-0.12	8	0.91	0
80 $\mu\text{mol kg}^{-1}$ Zn	2203.7 $\pm$ 0.4 (6)	0.7	-1.02	8	0.34	0
Batch B3						
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2206.4 $\pm$ 0.8 (4)					
20 $\mu\text{mol kg}^{-1}$ Cu	2205.5 $\pm$ 0.6 (4)	-0.9	2.24	6	0.07	0
40 $\mu\text{mol kg}^{-1}$ Cu	2200.5 $\pm$ 5.1 (6)	-5.8	2.36	8	0.05	1
80 $\mu\text{mol kg}^{-1}$ Cu	2202.0 $\pm$ 2.1 (7)	-4.4	1.76	9	0.00	1
Batch B4						
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2204.6 $\pm$ 0.3 (5)					
20 $\mu\text{mol kg}^{-1}$ Cu	2203.4 $\pm$ 1.3 (6)	-1.2	2.58	9	0.03	1
40 $\mu\text{mol kg}^{-1}$ Cu	2203.1 $\pm$ 0.5 (4)	-1.5	6.85	7	0.00	1
80 $\mu\text{mol kg}^{-1}$ Cu	2195.8 $\pm$ 3.5 (3)	-8.7	6.00	6	0.00	1

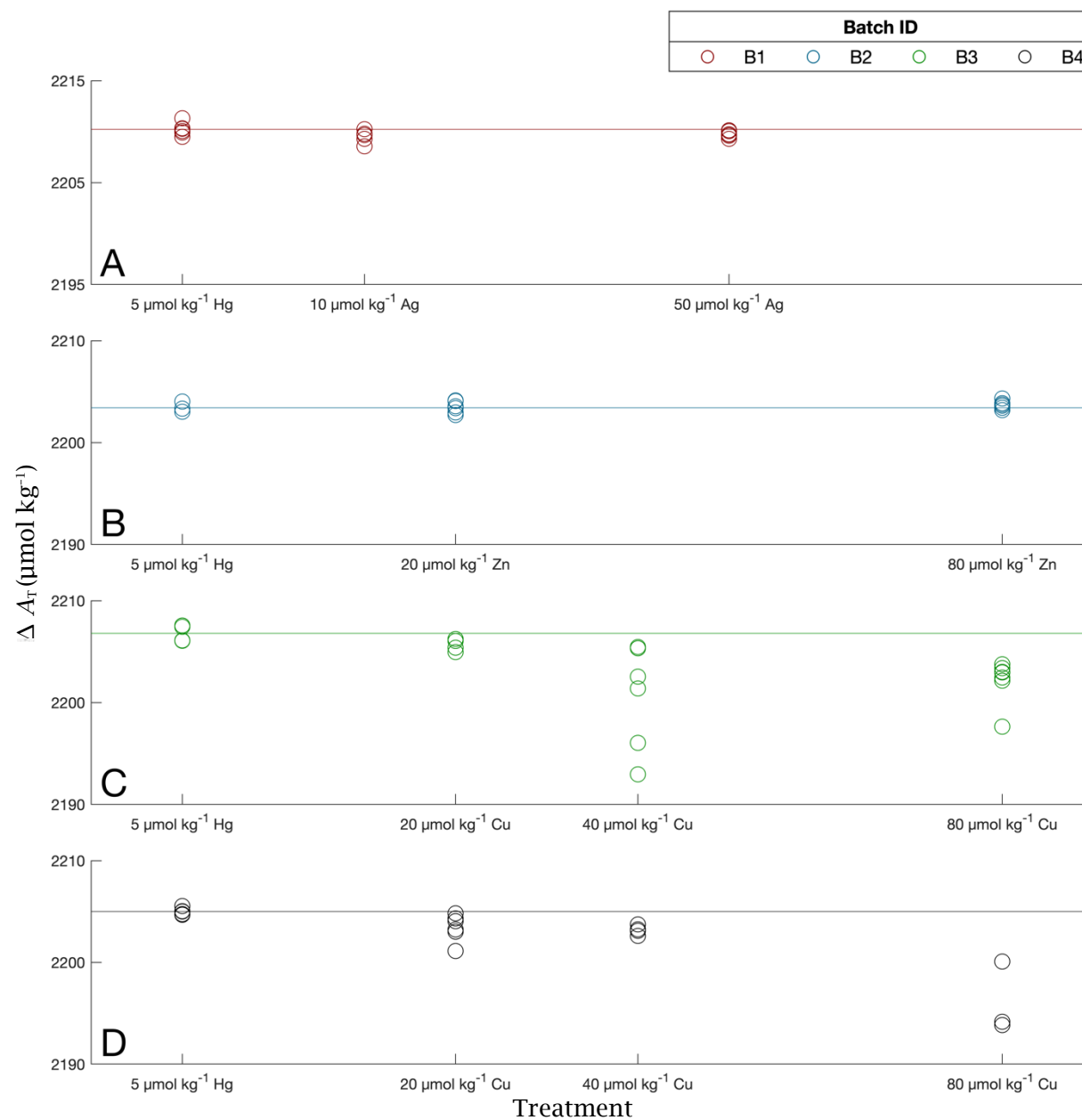


Figure 1. Measured A_T results for Ag, Zn and Cu treatments in bicarbonate solutions. Results were analyzed immediately after preservation.

Table 3. Measured A_T results for all treatments in surface seawater. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Batch B5				
Day 1				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2231.5 $\pm$ 0.4 (6)		7.827 $\pm$ 0.001 (6)	
10 $\mu\text{mol kg}^{-1}$ Ag	2231.6 $\pm$ 0.4 (6)	0.2	7.827 $\pm$ 0.001 (6)	0.000
50 $\mu\text{mol kg}^{-1}$ Ag	2231.2 $\pm$ 0.3 (6)	-0.3	7.826 $\pm$ 0.001 (6)	-0.001
Day 4				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2230.9 $\pm$ 0.4 (6)	-0.6	7.828 $\pm$ 0.001 (3)	0.001
10 $\mu\text{mol kg}^{-1}$ Ag	2232.6 $\pm$ 0.6 (6)	1.2	7.803 $\pm$ 0.002 (4)	-0.024
50 $\mu\text{mol kg}^{-1}$ Ag	2232.6 $\pm$ 1.7 (5)	1.1	7.806 $\pm$ 0.002 (4)	-0.021
Day 12				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2230.6 $\pm$ 0.6 (5)	-0.9	7.824 $\pm$ 0.000 (5)	-0.003
10 $\mu\text{mol kg}^{-1}$ Ag	2233.1 $\pm$ 0.4 (6)	1.6	7.786 $\pm$ 0.002 (6)	-0.041
50 $\mu\text{mol kg}^{-1}$ Ag	2232.0 $\pm$ 0.9 (6)	0.5	7.794 $\pm$ 0.002 (6)	-0.033
Batch B6				
Day 1				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2243.9 $\pm$ 0.4 (6)		7.759 $\pm$ 0.005 (6)	
20 $\mu\text{mol kg}^{-1}$ Zn	2244.3 $\pm$ 1.6 (6)	0.3	7.753 $\pm$ 0.005 (6)	-0.005
80 $\mu\text{mol kg}^{-1}$ Zn	2244.3 $\pm$ 0.4 (6)	0.3	7.732 $\pm$ 0.007 (5)	-0.027
Day 6				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2243.0 $\pm$ 0.5 (6)	-1.0	7.760 $\pm$ 0.004 (5)	0.001
20 $\mu\text{mol kg}^{-1}$ Zn	2242.0 $\pm$ 0.7 (6)	-1.9	7.732 $\pm$ 0.003 (6)	-0.027
80 $\mu\text{mol kg}^{-1}$ Zn	2244.3 $\pm$ 3.0 (6)	-0.1	7.732 $\pm$ 0.008 (6)	-0.062
Day 10				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2242.1 $\pm$ 0.5 (5)	-1.9	7.744 $\pm$ 0.012 (6)	-0.014
20 $\mu\text{mol kg}^{-1}$ Zn	2242.6 $\pm$ 1.6 (6)	-1.4	7.724 $\pm$ 0.002 (5)	-0.035
80 $\mu\text{mol kg}^{-1}$ Zn	2242.6 $\pm$ 2.2 (5)	-1.3	7.703 $\pm$ 0.005 (6)	-0.056
Batch B7				
Day 2				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2240.7 $\pm$ 0.6 (6)		7.818 $\pm$ 0.001 (6)	
10 $\mu\text{mol kg}^{-1}$ Ag	2241.0 $\pm$ 0.5 (6)	0.2	7.809 $\pm$ 0.001 (3)	-0.010
50 $\mu\text{mol kg}^{-1}$ Ag	2240.3 $\pm$ 0.5 (6)	-0.4	7.815 $\pm$ 0.003 (5)	-0.003

Table 3. Measured A_T results for all treatments in surface seawater, continued. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Day 8				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2241.8 $\pm$ 0.9 (6)	1.1	7.816 $\pm$ 0.001 (6)	-0.002
10 $\mu\text{mol kg}^{-1}$ Ag	2242.8 $\pm$ 1.1 (5)	2.1	7.798 $\pm$ 0.002 (4)	-0.020
50 $\mu\text{mol kg}^{-1}$ Ag	2242.8 $\pm$ 1.9 (6)	2.1	7.799 $\pm$ 0.003 (5)	-0.019
Day 18				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2241.2 $\pm$ 0.6 (4)	0.5	7.815 $\pm$ 0.002 (4)	-0.003
10 $\mu\text{mol kg}^{-1}$ Ag	2242.3 $\pm$ 1.0 (5)	1.6	7.790 $\pm$ 0.002 (6)	-0.029
50 $\mu\text{mol kg}^{-1}$ Ag	2241.3 $\pm$ 0.6 (5)	0.6	7.793 $\pm$ 0.001 (5)	-0.026
Batch B8				
Day 5				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2236.0 $\pm$ 0.3 (6)		7.736 $\pm$ 0.003 (6)	
20 $\mu\text{mol kg}^{-1}$ BAC	2236.5 $\pm$ 0.5 (5)	0.5	7.719 $\pm$ 0.004 (6)	-0.017
80 $\mu\text{mol kg}^{-1}$ BAC	2235.9 $\pm$ 0.1 (6)	-0.2	7.624 $\pm$ 0.008 (5)	-0.112
Day 14				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2235.3 $\pm$ 0.8 (6)	-0.7	7.735 $\pm$ 0.002 (6)	0.001
20 $\mu\text{mol kg}^{-1}$ BAC	2235.6 $\pm$ 0.6 (5)	-0.4	7.700 $\pm$ 0.011 (6)	-0.035
80 $\mu\text{mol kg}^{-1}$ BAC	2234.9 $\pm$ 1.2 (6)	-1.1	7.620 $\pm$ 0.005 (6)	-0.115
Day 22				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2235.5 $\pm$ 0.5 (6)	-0.5	7.733 $\pm$ 0.003 (6)	-0.003
20 $\mu\text{mol kg}^{-1}$ BAC	2236.4 $\pm$ 1.0 (6)	0.4	7.695 $\pm$ 0.004 (6)	-0.041
80 $\mu\text{mol kg}^{-1}$ BAC	2235.3 $\pm$ 0.3 (6)	-0.7	7.623 $\pm$ 0.006 (6)	-0.113
Batch B9				
Day 11				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2234.9 $\pm$ 0.4 (6)			
20 $\mu\text{mol kg}^{-1}$ Zn	2238.1 $\pm$ 0.9 (6)	3.3		
80 $\mu\text{mol kg}^{-1}$ Zn	2237.8 $\pm$ 0.7 (6)	2.9		

Table 3. Measured A_T results for all treatments in surface seawater, continued. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Day 20				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2235.0 $\pm$ 0.5 (6)	0.1	7.743 $\pm$ 0.004 (6)	
20 $\mu\text{mol kg}^{-1}$ Zn	2238.8 $\pm$ 1.1 (6)	3.9	7.696 $\pm$ 0.002 (6)	-0.047
80 $\mu\text{mol kg}^{-1}$ Zn	2237.8 $\pm$ 0.8 (6)	3.0	7.683 $\pm$ 0.008 (6)	-0.056
Day 23				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2234.2 $\pm$ 0.9 (5)	-0.7	7.741 $\pm$ 0.001 (5)	-0.002
20 $\mu\text{mol kg}^{-1}$ Zn	2237.4 $\pm$ 0.4 (6)	2.6	7.691 $\pm$ 0.008 (6)	-0.052
80 $\mu\text{mol kg}^{-1}$ Zn	2237.0 $\pm$ 0.5 (6)	2.1	7.686 $\pm$ 0.005 (6)	-0.056
Batch B10				
Day 5				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2232.9 $\pm$ 0.5 (6)		7.846 $\pm$ 0.002 (5)	
20 $\mu\text{mol kg}^{-1}$ BAC	2229.7 $\pm$ 0.9 (6)	-3.2	7.836 $\pm$ 0.005 (7)	-0.010
80 $\mu\text{mol kg}^{-1}$ BAC	2233.3 $\pm$ 0.7 (7)	0.4	7.739 $\pm$ 0.001 (6)	-0.107
Day 14				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2232.1 $\pm$ 0.3 (6)	-0.8	7.845 $\pm$ 0.002 (6)	-0.001
20 $\mu\text{mol kg}^{-1}$ BAC	2227.9 $\pm$ 1.0 (6)	-5.0	7.822 $\pm$ 0.006 (6)	-0.024
80 $\mu\text{mol kg}^{-1}$ BAC	2232.9 $\pm$ 0.3 (6)	-0.1	7.741 $\pm$ 0.005 (6)	-0.105
Day 17				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2233.3 $\pm$ 0.7 (6)	-0.4	7.845 $\pm$ 0.002 (6)	-0.001
20 $\mu\text{mol kg}^{-1}$ BAC	2228.5 $\pm$ 0.2 (5)	-4.4	7.821 $\pm$ 0.005 (5)	-0.025
80 $\mu\text{mol kg}^{-1}$ BAC	2233.0 $\pm$ 0.8 (5)	0.1	7.739 $\pm$ 0.006 (6)	-0.107
Batch B11				
Day 3				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2227.7 $\pm$ 0.9 (6)		7.879 $\pm$ 0.001 (6)	
20 $\mu\text{mol kg}^{-1}$ Zn	2227.7 $\pm$ 1.3 (6)	0.1	7.874 $\pm$ 0.001 (6)	-0.005
80 $\mu\text{mol kg}^{-1}$ Zn	2225.6 $\pm$ 3.5 (6)	-2.1	7.849 $\pm$ 0.007 (6)	-0.030

Table 3. Measured A_T results for all treatments in surface seawater, continued. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Day 7				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2227.1 $\pm$ 0.8 (5)	-0.6		
20 $\mu\text{mol kg}^{-1}$ Zn	2228.3 $\pm$ 0.2 (6)	0.6		
80 $\mu\text{mol kg}^{-1}$ Zn	2228.1 $\pm$ 0.8 (5)	0.5		
Day 124				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2227.3 $\pm$ 0.3 (6)	-0.4	7.879 $\pm$ 0.003 (6)	0.000
20 $\mu\text{mol kg}^{-1}$ Zn	2228.4 $\pm$ 0.8 (5)	0.8	7.845 $\pm$ 0.003 (5)	-0.034
80 $\mu\text{mol kg}^{-1}$ Zn	2226.1 $\pm$ 1.6 (5)	-1.6	7.839 $\pm$ 0.003 (6)	-0.040
Batch B12				
Day 4				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2284.8 $\pm$ 1.3 (6)		7.660 $\pm$ 0.003 (6)	
50 $\mu\text{mol kg}^{-1}$ Ag	2286.7 $\pm$ 0.8 (6)	2.0	7.651 $\pm$ 0.006 (5)	-0.009
90 $\mu\text{mol kg}^{-1}$ Ag	2286.9 $\pm$ 0.7 (6)	2.1	7.649 $\pm$ 0.004 (6)	-0.011
Day 10				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2281.9 $\pm$ 1.2 (6)	-2.9	7.647 $\pm$ 0.006 (5)	-0.013
50 $\mu\text{mol kg}^{-1}$ Ag	2284.5 $\pm$ 0.4 (7)	-0.3	7.641 $\pm$ 0.007 (6)	-0.019
90 $\mu\text{mol kg}^{-1}$ Ag	2284.4 $\pm$ 1.2 (7)	-0.4	7.637 $\pm$ 0.013 (5)	-0.023
Day 35				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2269.4 $\pm$ 5.2 (5)	-15.4	7.590 $\pm$ 0.016 (5)	-0.070
50 $\mu\text{mol kg}^{-1}$ Ag	2284.8 $\pm$ 1.8 (6)	0.1	7.622 $\pm$ 0.011 (6)	-0.038
90 $\mu\text{mol kg}^{-1}$ Ag	2284.3 $\pm$ 3.7 (6)	-0.4	7.624 $\pm$ 0.005 (6)	-0.036
Batch B13				
Day 5				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2281.5 $\pm$ 0.6 (6)		7.673 $\pm$ 0.002 (6)	
80 $\mu\text{mol kg}^{-1}$ Zn	2281.5 $\pm$ 2.7 (6)	0.0	7.640 $\pm$ 0.003 (5)	
160 $\mu\text{mol kg}^{-1}$ Zn	2258.9 $\pm$ 19.7 (6)	-22.6	7.582 $\pm$ 0.048 (7)	

Table 3. Measured A_T results for all treatments in surface seawater, continued. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Day 7				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2282.0 $\pm$ 1.4 (4)	0.5	7.666 $\pm$ 0.008 (4)	-0.010
80 $\mu\text{mol kg}^{-1}$ Zn	2278.1 $\pm$ 7.2 (6)	-3.3	7.634 $\pm$ 0.014 (6)	
160 $\mu\text{mol kg}^{-1}$ Zn	2255.8 $\pm$ 16.9 (5)	-25.7	7.561 $\pm$ 0.035 (6)	
Day 36				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2263.8 $\pm$ 2.5 (6)	-17.6	7.600 $\pm$ 0.010 (6)	-0.070
80 $\mu\text{mol kg}^{-1}$ Zn	2279.9 $\pm$ 5.2 (6)	-1.6	7.616 $\pm$ 0.009 (6)	
160 $\mu\text{mol kg}^{-1}$ Zn	2259.6 $\pm$ 15.1 (5)	-21.7	7.560 $\pm$ 0.031 (5)	

Batch B14

Day 5

55 $\mu\text{mol kg}^{-1}$ Hg (control)	2242.9 $\pm$ 0.8 (6)		7.881 $\pm$ 0.002 (6)	
50 $\mu\text{mol kg}^{-1}$ Ag	2242.4 $\pm$ 0.5 (7)	-0.5	7.872 $\pm$ 0.002 (6)	-0.007
90 $\mu\text{mol kg}^{-1}$ Ag	2241.8 $\pm$ 0.3 (5)	-1.1	7.872 $\pm$ 0.004 (6)	-0.007

Day 22

55 $\mu\text{mol kg}^{-1}$ Hg (control)	2243.0 $\pm$ 1.1 (6)	0.1	7.877 $\pm$ 0.002 (6)	0.004
50 $\mu\text{mol kg}^{-1}$ Ag	2241.3 $\pm$ 1.6 (5)	-1.6	7.852 $\pm$ 0.014 (6)	-0.027
90 $\mu\text{mol kg}^{-1}$ Ag	2238.8 $\pm$ 1.7 (6)	-4.1	7.858 $\pm$ 0.008 (6)	-0.020

Day 27

55 $\mu\text{mol kg}^{-1}$ Hg (control)	2242.9 $\pm$ 0.5 (6)	0.0	7.878 $\pm$ 0.002 (5)	-0.003
50 $\mu\text{mol kg}^{-1}$ Ag	2242.5 $\pm$ 0.8 (6)	-0.4	7.856 $\pm$ 0.004 (4)	-0.022
90 $\mu\text{mol kg}^{-1}$ Ag	2242.3 $\pm$ 0.8 (6)	-0.6	7.862 $\pm$ 0.003 (6)	-0.017

Batch B15

Day 18

55 $\mu\text{mol kg}^{-1}$ Hg (control)	2237.2 $\pm$ 0.2 (5)	0.2	7.897 $\pm$ 0.002 (6)	
20 $\mu\text{mol kg}^{-1}$ BAC	2237.9 $\pm$ 1.0 (6)	0.9	7.860 $\pm$ 0.003 (6)	-0.037
80 $\mu\text{mol kg}^{-1}$ BAC	2238.5 $\pm$ 0.3 (6)	1.5	7.785 $\pm$ 0.009 (6)	-0.112

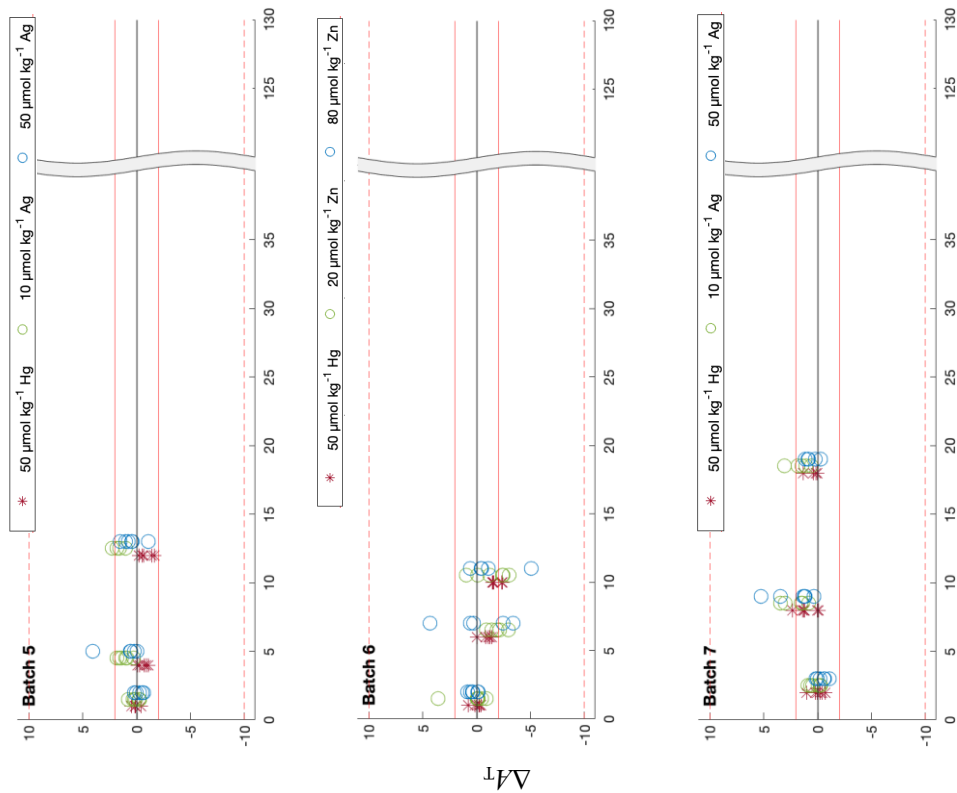
Day 26

55 $\mu\text{mol kg}^{-1}$ Hg (control)	2237.2 $\pm$ 1.0 (6)	0.2	7.896 $\pm$ 0.002 (6)	-0.001
20 $\mu\text{mol kg}^{-1}$ BAC	2235.9 $\pm$ 1.9 (6)	-1.1	7.820 $\pm$ 0.050 (6)	-0.076
80 $\mu\text{mol kg}^{-1}$ BAC	2238.3 $\pm$ 1.2 (6)	1.3	7.779 $\pm$ 0.007 (6)	-0.118

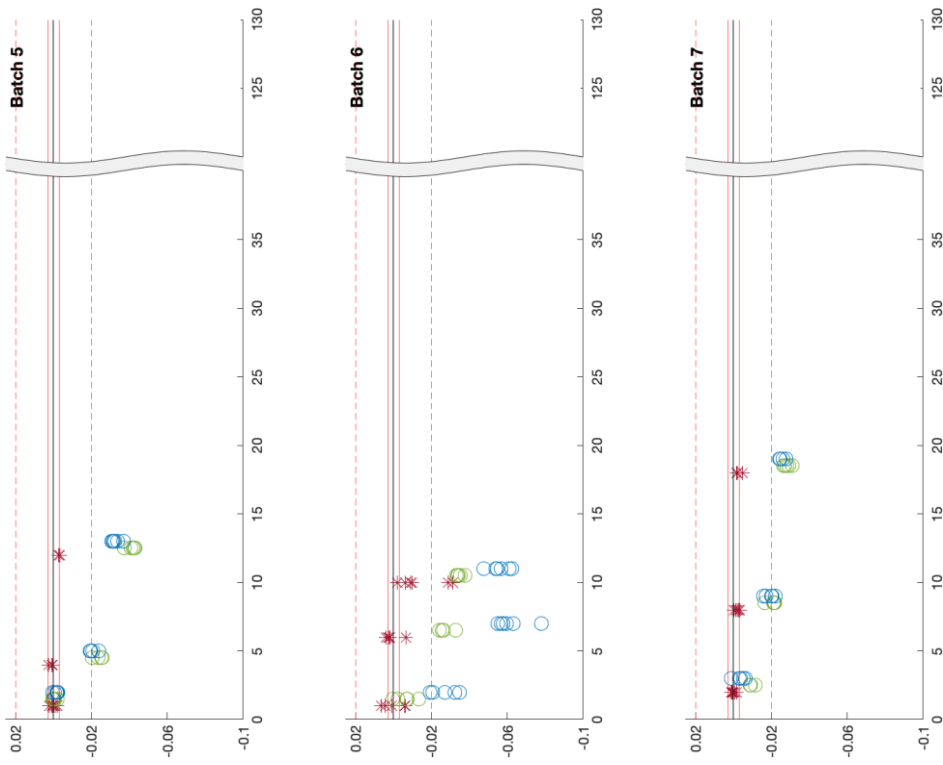
Table 3. Measured A_T results for all treatments in surface seawater, continued. Values in parentheses represent the number of analyses.

Treatment	Mean A_T ($\mu\text{mol kg}^{-1}$)	Mean ΔA_T ($\mu\text{mol kg}^{-1}$)	Mean pH_T	Mean ΔpH_T
Day 31				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2236.7 $\pm$ 1.5 (6)	-0.3	7.897 $\pm$ 0.002 (6)	0.000
20 $\mu\text{mol kg}^{-1}$ BAC	2234.7 $\pm$ 1.0 (6)	-2.3	7.762 $\pm$ 0.050 (7)	-0.134
80 $\mu\text{mol kg}^{-1}$ BAC	2237.7 $\pm$ 1.4 (6)	0.7	7.767 $\pm$ 0.018 (6)	-0.130
 Batch B16				
Day 11				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2261.5 $\pm$ 0.8 (5)		7.793 $\pm$ 0.002 (6)	
80 $\mu\text{mol kg}^{-1}$ Zn	2262.1 $\pm$ 0.7 (4)	0.6	7.746 $\pm$ 0.008 (6)	-0.044
160 $\mu\text{mol kg}^{-1}$ Zn	2257.7 $\pm$ 10.5 (4)	-3.8	7.682 $\pm$ 0.075 (6)	-0.111
Day 26				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2260.8 $\pm$ 0.5 (6)	-0.7	7.792 $\pm$ 0.001 (6)	-0.001
80 $\mu\text{mol kg}^{-1}$ Zn	2262.5 $\pm$ 1.7 (6)	1.0	7.743 $\pm$ 0.008 (4)	-0.050
160 $\mu\text{mol kg}^{-1}$ Zn	2255.7 $\pm$ 16.4 (5)	-5.8	7.704 $\pm$ 0.050 (6)	-0.089
Day 31				
55 $\mu\text{mol kg}^{-1}$ Hg (control)	2261.5 $\pm$ 1.0 (6)	0.0	7.793 $\pm$ 0.002 (5)	0.000
80 $\mu\text{mol kg}^{-1}$ Zn	2263.8 $\pm$ 0.3 (6)	2.3	7.748 $\pm$ 0.003 (6)	-0.045
160 $\mu\text{mol kg}^{-1}$ Zn	2252.6 $\pm$ 11.9 (5)	-8.9	7.671 $\pm$ 0.064 (5)	-0.122

Figure 2. ΔA_T and ΔpH_T measurements over time for all treatments, separated by batch. The red solid and dashed lines represent the climate and weather data objectives, respectively. For comparison, measurements from control samples treated with $55 \mu\text{mol kg}^{-1}$ Hg are also included.



ΔpH_T



Days since collection

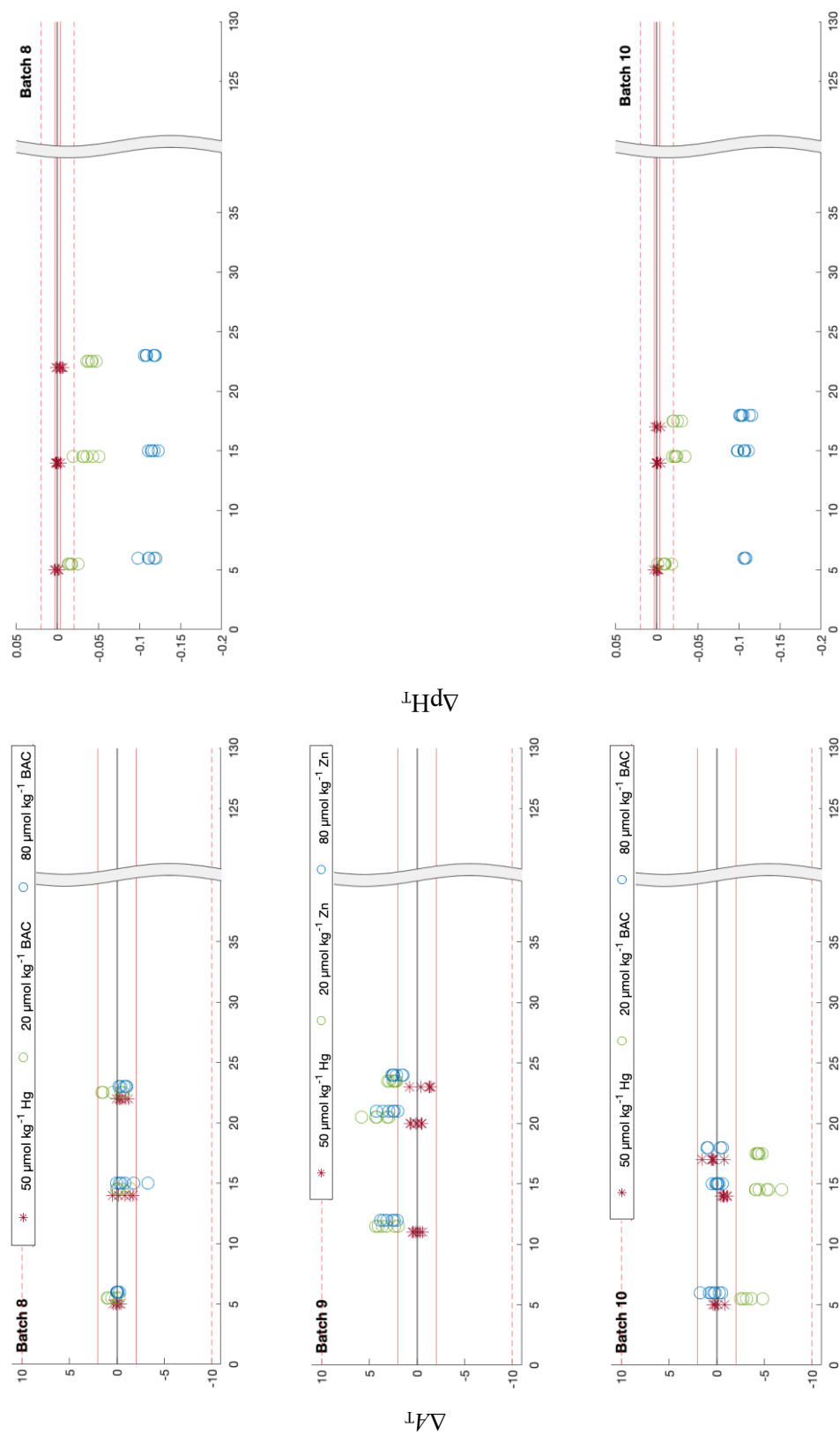


Figure 2 continued

Days since collection

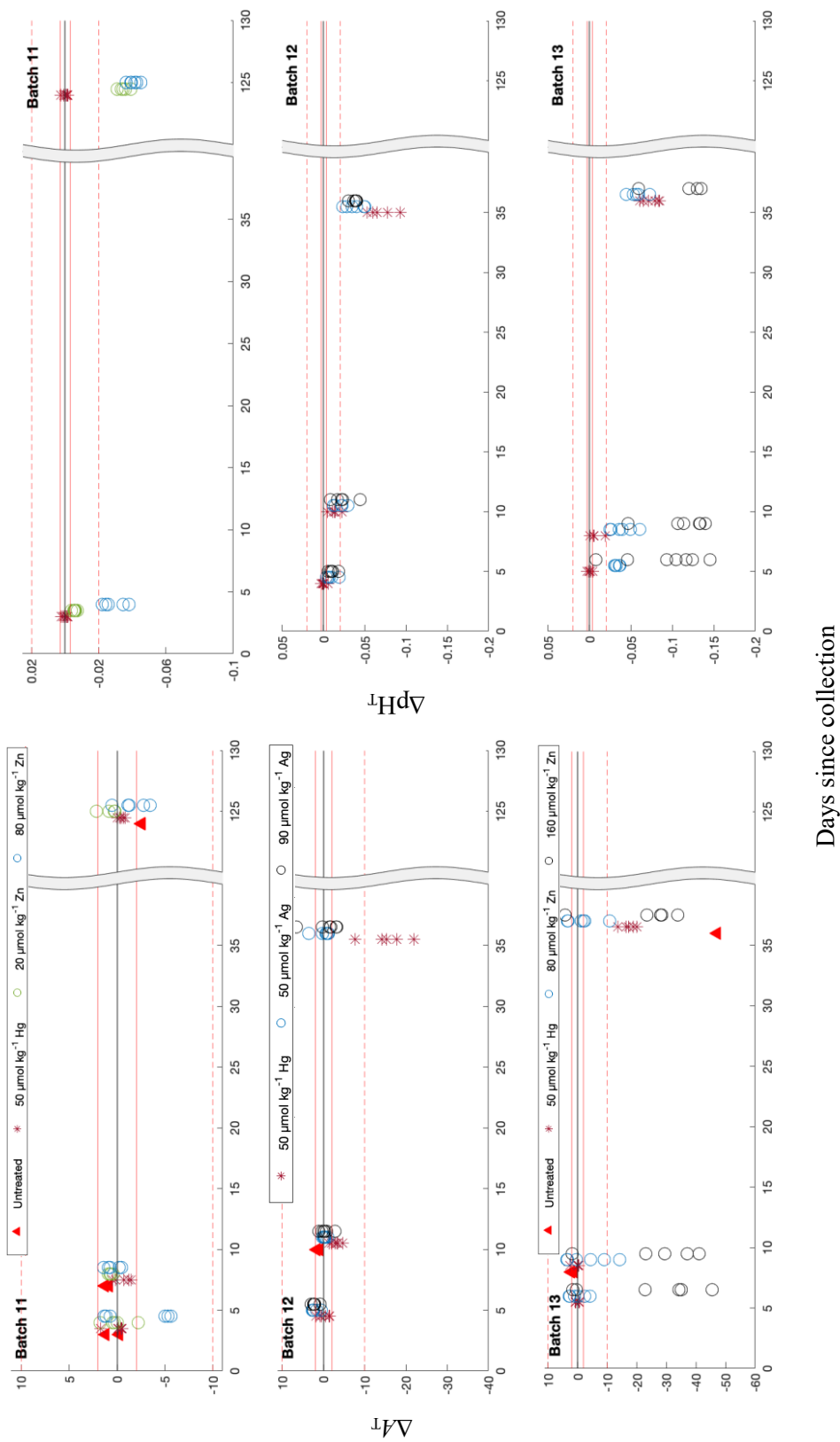


Figure 2 continued

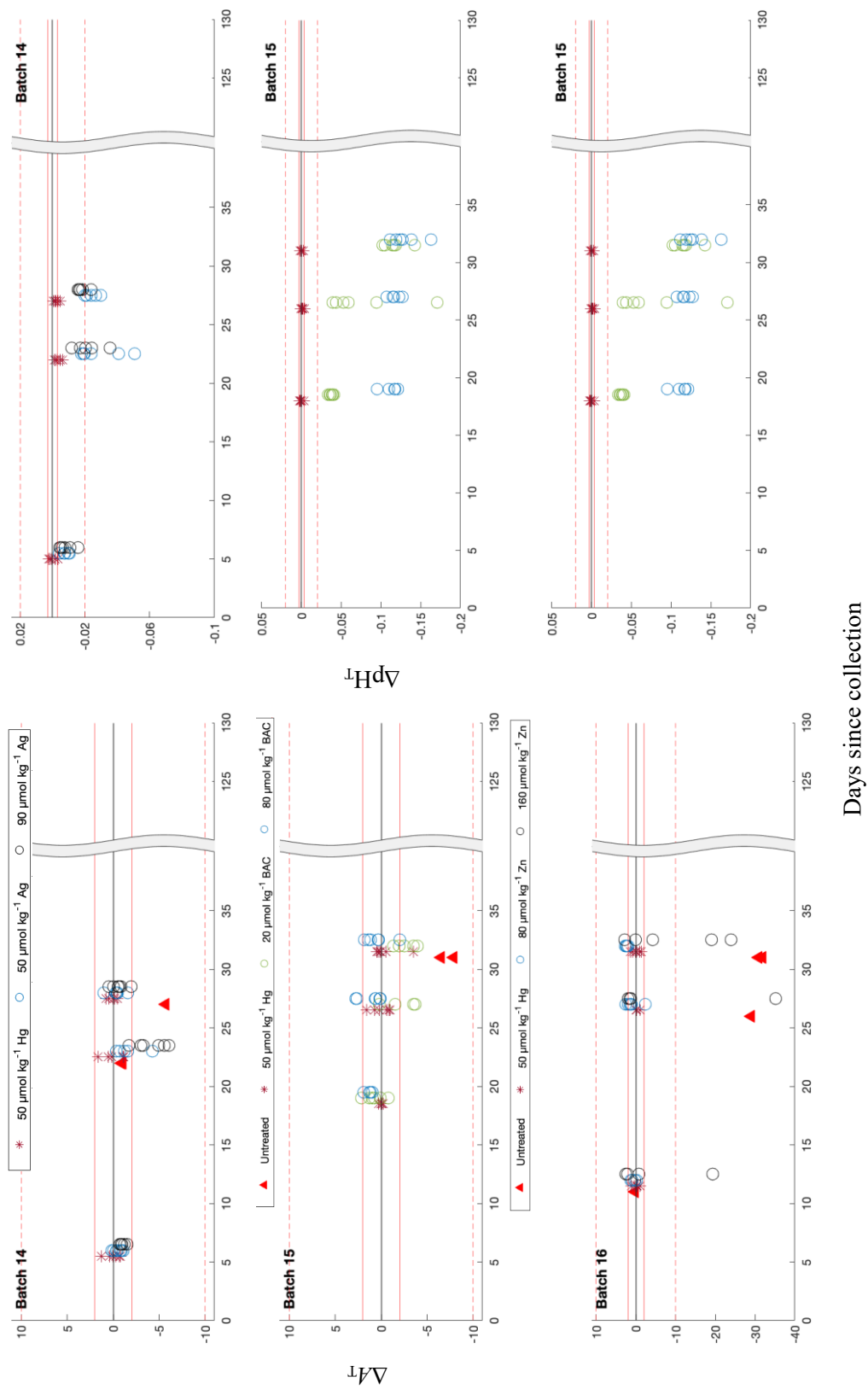


Figure 2 continued

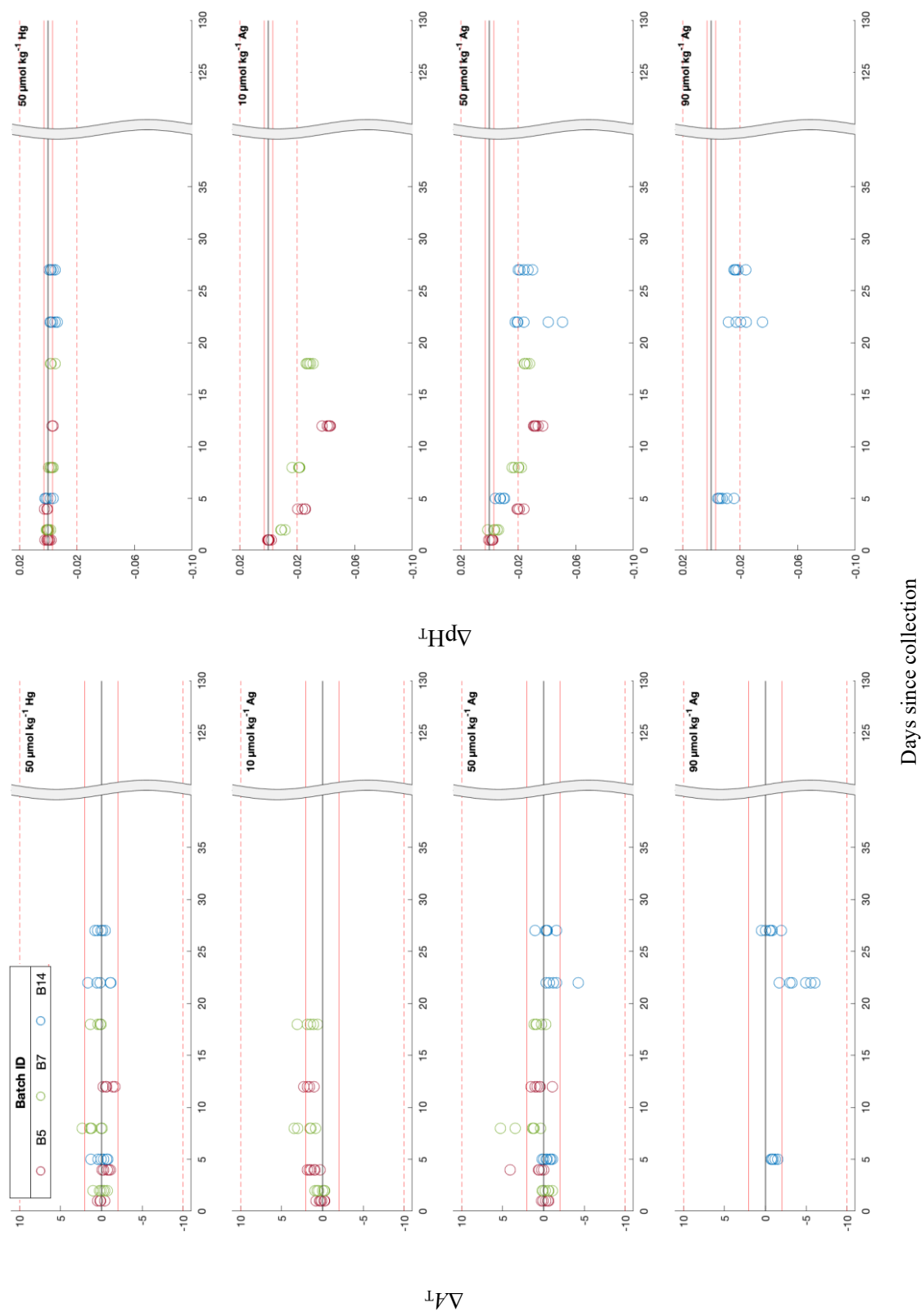


Figure 3. Combined batch ΔA_T and ΔpH_T measurements over time for treatments with 10, 50 and 90 $\mu\text{mol kg}^{-1}$ Ag. The red solid and dashed lines represent the climate and weather data objectives, respectively.

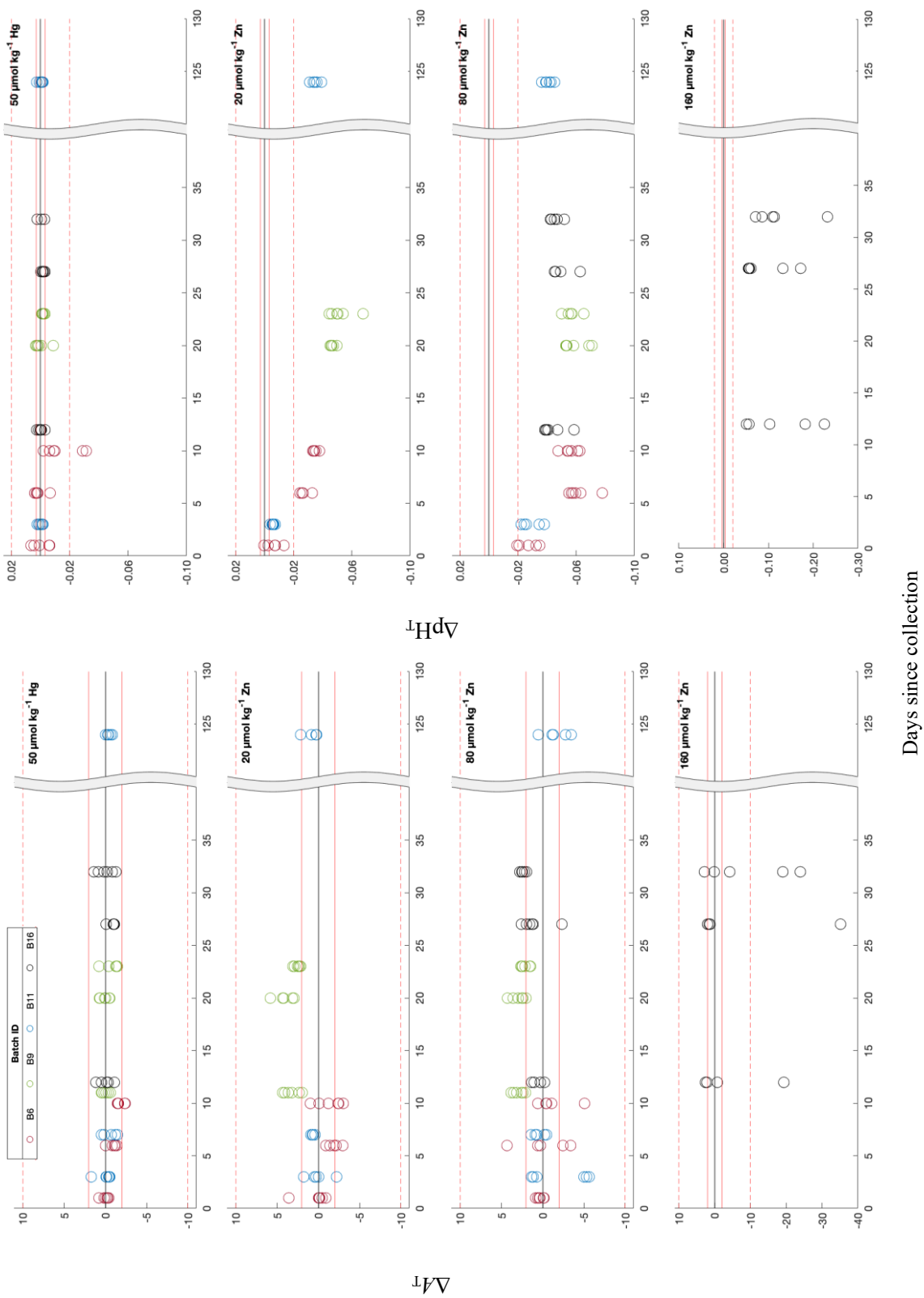


Figure 4. Combined batch ΔA_T and ΔpH_T measurements over time for treatments with 20, 80 and 160 $\mu\text{mol kg}^{-1}$ Zn. The red solid and dashed lines represent the climate and weather data objectives, respectively.

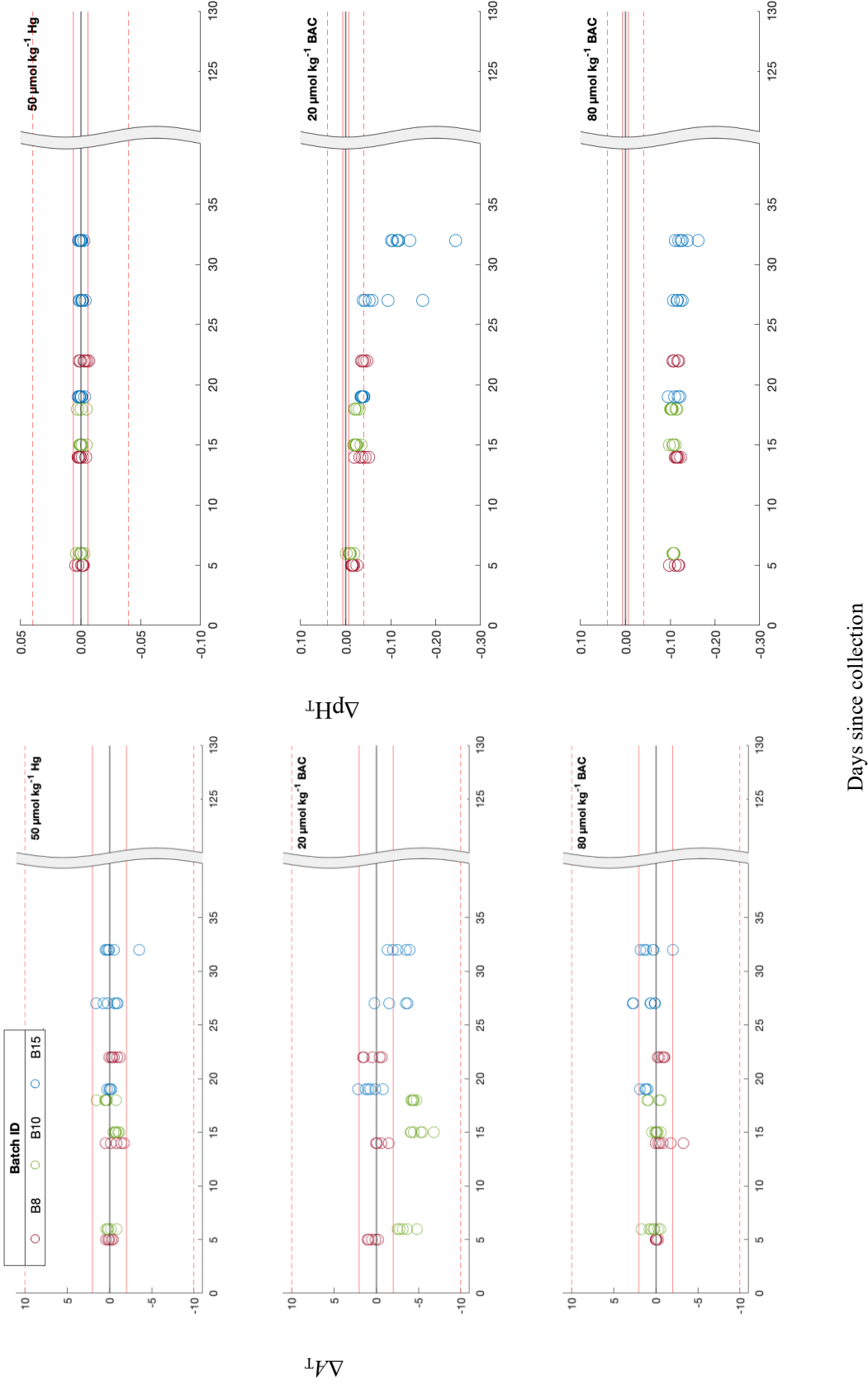


Figure 5. Combined batch ΔA_T and ΔpH_T measurements over time for treatments with 20 and 80 $\mu\text{mol kg}^{-1}$ BAC. The red solid and dashed lines represent the climate and weather data objectives, respectively.

Table 4. Percentage of ΔA_T or ΔpH_T measurements from all batches within climate ($\pm 2 \mu\text{mol kg}^{-1}$) and weather ($\pm 10 \mu\text{mol kg}^{-1}$) data objective ranges between 1–14 days and after 15 days. Results are listed in the format X%, Y% (n), where X = % of measurements within climate objective, Y=% of measurements within weather objective, and n = number of measurements. Measurements from batches B12 and B13 are not included in these results due to their unstable control values.

Preservation Method	1–14 days		15+ days	
	AT	pH	AT	pH
10 $\mu\text{mol kg}^{-1}$ Ag	90%, 100% (29)	26%, 57% (23)	80%, 100% (5)	0%, 0% (6)
50 $\mu\text{mol kg}^{-1}$ Ag	92%, 100% (36)	22%, 66% (32)	94%, 100% (16)	0%, 18% (17)
90 $\mu\text{mol kg}^{-1}$ Ag	100%, 100% (5)	0%, 100% (6)	58%, 100% (12)	0%, 58% (12)
20 $\mu\text{mol kg}^{-1}$ Zn	64%, 100% (36)	13%, 52% (23)	24%, 100% (17)	0%, 0% (5)
80 $\mu\text{mol kg}^{-1}$ Zn	65%, 100% (37)	0%, 3% (29)	35%, 100% (29)	0%, 0% (16)
160 $\mu\text{mol kg}^{-1}$ Zn	25%, 75% (4)	0%, 0% (6)	50%, 70% (10)	0%, 0% (11)
20 $\mu\text{mol kg}^{-1}$ BAC	45%, 100% (22)	4%, 60% (25)	62%, 100% (29)	0%, 3% (30)
80 $\mu\text{mol kg}^{-1}$ BAC	96%, 100% (25)	0%, 0% (23)	90%, 100% (29)	0%, 0% (30)

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