Comparing Rhizon samplers and centrifugation for pore-water separation in studies of the marine carbonate system in sediments

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Abstract

An accurate description of the carbonate system in pore waters is valuable in studies involving the degradation of sedimentary organic matter, recrystallization of calcium carbonate minerals, calculations of mineral saturation state, and cycling of ions affected by pH. Here, we analyze water chemistry of pore water extracted using centrifugation and Rhizon samplers from hemipelagic sediments in the Gulf of Aqaba, Red Sea, and a shallow salt marsh from Norfolk, England. In both study areas, the data are internally consistent for each pore-water separation technique, but the measured isotopic composition of the dissolved inorganic carbon (δ13C\textsubscript{DIC}) differs between the two techniques. We performed laboratory experiments that show that both Rhizons and centrifugation are prone to degassing of CO2 enriched with 12C. We suggest that during sampling with Rhizons, air fills the voids left by extracted pore water; combined with the membrane’s design to exclude air, some of the aqueous CO2 diffuses into these air bubbles instead of the sampler. Rhizons produce reliable calcium, strontium, manganese, and barium concentration data when soaked in deionized water and then flushed with the sample immediately prior to sampling. However, pore-water extractions with Rhizons are less reliable for analyses of pH and δ13C\textsubscript{DIC}. Centrifugation produces reliable carbonate chemistry and major element data when tubes are fully filled without headspace and sealed tightly. Working in CO2 low/free atmosphere (e.g., N2 glovebox) enhances the chance of losing CO2 from the sample in both sampling techniques due to increased negative gradient of CO2 between the core and its surrounding.

A large portion of all biological and chemical reactions in marine sediments occur within the top few centimeters below the sediment-water interface (Berner 1980). These biological and chemical reactions have a tremendous influence on elemental cycling in the ocean as well as on preservation and modification of the sedimentary record (Froelich et al. 1979; Broecker and Peng 1982). While these biological and chemical reactions can affect the chemical and mineralogical composition of the solids, changes in the chemistry of the interstitial water, i.e., the water filling the voids between sediment grains or pore water, are far more rapid. This is because a relatively small amount of pore water is in direct contact with a large amount of solids and thus responds quickly and dynamically to chemical reactions within the sediment column.

Changes in the chemistry of pore water have been used in many studies both to understand and to quantify rates of processes within sediments (e.g., Burdige 2006; Aller 2014 and references therein). While there has been relative success in measuring the pore-water concentration of major ions, it has been challenging, and yet particularly important, to measure pH and the concentration of dissolved inorganic carbon (DIC). The pH of sedimentary pore waters is a master variable since the concentration of the hydrogen ion is modified by nearly all metabolic reactions and strongly influences the saturation state of many minerals (Jourabchi et al. 2005; Soetaert et al. 2007). The concentration of DIC and its isotopic composition (δ13C\textsubscript{DIC}) are altered by organic matter remineralization as well as CaCO3 dissolution and precipitation (e.g., Martin and Sayles 2006; Bouillon et al. 2007; Hu and Burdige 2007; Rassmann et al. 2016), making DIC and δ13C\textsubscript{DIC} variables of prime interest for any study trying to quantify rates of metabolic and inorganic reactions. The δ13C\textsubscript{DIC} may be used to evaluate organic carbon decomposition, and other processes like identifying zones of methane production and destruction (Sivan et al. 2007). Changes in the δ13C\textsubscript{DIC} may also be recorded in the solid sediment through

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recrystallization of biogenic calcium carbonate or authigenic calcium carbonate precipitation during cementation; the ability to understand changes in the $\delta^{13}$C(DIC) in marine pore waters and how they are incorporated into sedimentary rock may assist in the interpretation of sedimentary carbonate records over geological time (Schrag et al. 2013).

Analyses of the chemistry of pore waters depend on the ability to separate water from the solids while minimally modifying its chemistry during extraction. Many methods have been suggested for pore-water extraction, yet each of these methods has major flaws, leading to lack of consensus regarding the optimal pore-water extraction method. Among the main methods that have been used by the geochemical community, centrifugation allows fairly high-sampling resolution of < 1 cm and can extract a significant quantity of pore water from cohesive sediments. Use of centrifuges is often impractical during ocean research cruises due to a difficulty in balancing the centrifuge, therefore, the method often used in pore-water extraction in the Integrated Ocean Drilling Program (IODP) is high-pressure squeezing (Reagan et al. 2015). Squeezing is suitable for onboard, anaerobic pore-water extraction, however, it is a cumbersome procedure that does not allow high-resolution sampling and may be destructive to the sediment samples (Miller et al. 2014). In recent years, Rhizon samplers are becoming increasingly popular for pore-water sampling from marine and lacustrine sediments (Dickens et al. 2007; Shotbolt 2010). Rhizon samplers are easy to use and less destructive than squeezing or centrifugation. Potential issues with Rhizon samplers are poorer control on fluid migration distance, degassing of dissolved gases, and biases in molecule diffusion through the membrane (Seeberg-Elverfeldt et al. 2005; Schrum et al. 2012; Abbott et al. 2015; Chen et al. 2015).

Past studies comparing different pore-water sampling techniques yielded inconclusive results which highlights that none of the methods available at present is optimal for all cases and all elements of interest. Bufflap and Allen (1995) compared cadmium extraction by centrifugation, squeezing, vacuum filtration, and dialysis in an artificial freshwater medium and concluded that centrifugation and dialysis gave the best results. Based on the ease of use, they recommended using centrifugation for pore-water extractions. Di Bonito (2005) compared soil pore-water extraction of a large number of trace elements by centrifugation, squeezing, Rhizon samplers, and soil suspensions. He concluded that centrifugation and Rhizon samplers produced the best results but that these techniques extract different fractions of the pore fluid: Rhizons preferentially extract water from the most accessible pool while centrifugation averages the soil’s pore water and extracts pore waters from both mobile and less mobile sites. Geibe et al. (2006) compared centrifugation, two suction lysimeters and a zero potential lysimeter for extractions of 26 different analytes from soil pore water. Like Di Bonito (2005), they concluded that centrifugation and Rhizon samplers performed better than other methods but showed considerable and varied differences between the results of these two methods. On IODP cruises, pore-water extractions comparing squeezing and Rhizon samplers have been explored in several studies (Dickens et al. 2007; Schrum et al. 2012; Miller et al. 2014; Miller et al. 2017). Dickens et al. (2007) demonstrated that ammonium and manganese concentrations in waters extracted by these techniques are similar. Schrum et al. (2012) supported this conclusion but found that measured DIC and alkalinity were significantly lower and more scattered in samples extracted using Rhizons samplers. Miller et al. (2014) showed that there is a small but persistent difference between chloride concentrations and $\delta^{18}$O measured using these techniques and suggested that it was caused by water absorption and diffusive fractionation during the filtration process. Miller et al. (2017) suggested that repeat pore-water extractions using Rhizon samplers from the same locations in a core produce internally consistent calcium concentration data.

These studies leave considerable confusion regarding the applicability of centrifugation vs. Rhizon samplers in studies of carbonate chemistry that we address in the present study. We compare high-resolution pore-water extractions for parameters important for carbonate chemistry, using centrifugation and Rhizon samplers from short sediment cores retrieved from two sites in the Gulf of Aqaba, Red Sea, and a shallow salt marsh at the North Sea coast of Norfolk, England. We corroborate these comparisons with experiments in which synthetic sediments are mixed with artificial seawater (ASW) to test the effect of pore-water separations from a homogeneous medium.

Materials and procedures

Sediment sampling

Short sediment cores were retrieved in the northern Gulf of Aqaba, Red Sea, using an MC-400 four barrel multicorer (Ocean Instruments, San Diego). The core tube inner diameter was 9.5 cm and its length was 60 cm. After retrieval, the cores were kept at bottom water temperature and pore-water separations were done within several hours. Locations and water depths of the cores used to compare pore-water extractions using Rhizon samplers and centrifugation are summarized in Table 1. The sediments are predominately composed of quartz, calcite, kaolinite, and albite in the silt grain size and have a porosity of ~0.8 near the sediment-water interface which decreases to 0.6 at 30 cm (Steiner et al. 2016). All cores were kept standing upright during processing by both methods in a room air-conditioned to bottom water temperature at the site of sampling (21°C).

Push cores were subsequently retrieved from a shallow, muddy, iron-rich, salt marsh near Blakeney, Norfolk, England in July 2016. Cores were sealed in the field and taken to the lab for pore-water separations on the following day.

Pore-water separation using centrifugation

Cores for pore-water separation by centrifugation were sectioned every 0.7–2 cm using a core cutter. Sediment samples
were collected from the core cutter using a spatula, inserted into 50 mL tubes and centrifuged for 15 min at 5000 rpm. Following centrifugation, samples were pressure filtered with Millipore 0.2 μm syringe filters.

Gulf of Aqaba pore-water samples analyzed for cation chemistry were gravimetrically diluted 70x with 0.16 M distilled HNO$_3$ immediately after collection. Samples for analyses of DIC and $^{13}$C(DIC) were poisoned with 1% saturated HgCl$_2$ and stored in glass vials until analyses. Samples for pH measurements were stored refrigerated in glass vials until analyses which normally took place on the day following the retrieval of the cores. Norfolk pore water (1.5 mL) was injected into a helium flushed flask containing 100% H$_3$PO$_4$ immediately after pore-water separation for analyses of $^{13}$C(DIC).

**Pore-water separation using Rhizon samplers**

Rhizon samplers (Rhizosphere Research Products R.V., Wageningen) are thin, perforate rods covered by hydrophilic microfiltration membranes composed from a blend of polyvinylpyrrolidone and polyethersulfone with a nominal pore size of 0.12–0.18 μm. Rhizon samplers of 5 cm length (Rhizon CSS 19.21.23F) were horizontally inserted into predrilled holes 2 cm apart in the core liner; these holes were sealed during core retrieval. To minimize the risk of interference between adjacent samplers, we alternately drilled the holes on opposite sides of the core liner. This allowed us to collect water at 1 cm vertical resolution while keeping the distance between each two samplers at 2 cm.

In most cases, the water overlying the sediment core was drained before insertion of the Rhizon samplers (see “Pore waters of cores from natural marine environments” section for discussion on this matter). Thirty minutes after insertion of the samplers, a syringe was connected to each sampler by a luer-lock connection. Pore water was sampled sequentially from top to bottom by creating low pressure in the syringe by means of pulling the plunger back and holding it using a retainer. The first 200 μL of each sample were discarded and the next 1–2 mL collected into the syringe. Collection of each sample took up to 5 min. As part of our experiment, we tested differences between sampling using dry Rhizon samplers and Rhizon samplers that were presoaked in double distilled water for 24 h. We term this “dry” or “wet” Rhizons hereafter. Preservation and treating of separated pore waters was as described in “Pore-water separation using centrifugation” section.

### ASW experiments

ASW was prepared according to the recipe of Kester et al. (1967) with two modifications: we did not add NaF to our ASW and the amount of NaHCO$_3$ was about 25% higher in our ASW to account for the higher alkalinity typically found in pore waters of marine sediments (e.g., Emerson et al. 1982).

ASW was used in two experiments. In the first experiment, we placed the ASW on its own in centrifuge tubes. The tubes were then either centrifuged or water was pulled out of the centrifuge tubes using Rhizon samplers. Later, ASW was mixed with different minerals to create homogeneous synthetic sediments. Mixes included SiO$_2$ (crystobalite) from the size fraction 0.5–10 μm, kaolin, calcite CaCO$_3$ (all purchased from Sigma-Aldrich), and quartz sand sieved to include only the size fraction 150–211 μm. Mixing of the ASW and minerals for preparation of synthetic sediments was conducted in open beakers. Sediment porosities after mixing with ASW were $\phi_{kaolin} = 0.81$, $\phi_{crystobalite} = 0.66$, $\phi_{CaCO_3} = 0.62$, and $\phi_{sand} = 0.39$. In these experiments, 50 mL polypropylene centrifuge tubes were filled with wet synthetic sediment. Pore-water separation was done by inserting a Rhizon sampler into the tube or by sealing the tube, and separating pore water by centrifugation.

To ensure that all samples had identical initial chemical compositions, samples for all experiments were first split into two different centrifuge tubes under lab conditions. ASW samples were tested for the effect of filtration using syringe filters as well as presence of headspace in the centrifuge tubes. Subsequent processing of part of the samples was done in an

| Core   | Date (DD/MM/YYYY) | Water depth (m) | Latitude  | Longitude | Pore-water extraction method |
|--------|-------------------|-----------------|-----------|-----------|-----------------------------|
| C690U  | 27 Jun 2011       | 690             | 29° 27.964'N  | 34° 55.538'E | Centrifugation              |
| C707A  | 30 Aug 2011       | 707             | 29° 27.969'N  | 34° 55.620'E | Centrifugation              |
| C507J  | 01 Jun 2013       | 507             | 29° 29.745'N  | 34° 56.536'E | Centrifugation              |
| R507J  | 01 Jun 2013       | 507             | 29° 29.745'N  | 34° 56.536'E | Rhizon samplers             |
| R720J  | 01 Jun 2013       | 720             | 29° 27.890'N  | 34° 55.653'E | Rhizon samplers             |
| C711N  | 19 Nov 2013       | 711             | 29° 27.973'N  | 34° 55.679'E | Centrifugation              |
| R711N  | 19 Nov 2013       | 711             | 29° 27.973'N  | 34° 55.679'E | Rhizon samplers             |
| Norfolk1 | 07 Jul 2016   | 0.12            | 52° 57.385'N  | 00° 59.900'E | Centrifugation              |
| Norfolk2 | 07 Jul 2016   | 0.12            | 52° 57.385'N  | 00° 59.900'E | Rhizon samplers             |

**Table 1.** Cores utilized in the comparisons between pore-water extraction using centrifugation and Rhizon samplers. C or R in the legend mark pore-water extraction using centrifugation or Rhizon samplers, the three digits are for the water depth, and J or N at the end of the legend state that the cores were retrieved in January 2013 or November 2013, respectively. Bottom water conditions at the Gulf of Aqaba stations were $T = 21^\circC$ and $S = 40.74$ (http://www.meteo-tech.co.il/EilatYam_data/ey_data.asp).
anaerobic chamber filled with N₂ (Coy Laboratory products, Grass Lake, Michigan) to test whether use of glove boxes modifies the outcomes.

Initial experiments with synthetic sediment preparation showed that the chemistry of the ASW changes significantly upon contact with mineral powders. To minimize this, sediments were submerged in ASW and pore water was separated three times over 3–4 d before the experiments began. Several cycles of washing of the minerals prior to the experiment reduced the short-term variability in $\delta^{13}$C(DIC) and brought the composition of the pore-water solution closer to the composition of the ASW (see “Supporting Information”).

Washing was done by mixing the solids with a large volume of ASW, allowing the grains to settle, then centrifuging the sediment and decanting excess pore water for kaolin and cristobalite. Coarse grain quartz sand and CaCO₃ were more efficiently washed by rigorously mixing sediment with water, allowing the sediment to settle and decanting excess water. The sediment was immersed in ASW for 4 h during the first wash, 20 h during second wash, and third wash length was 16 h for CaCO₃ and sand or 40 h for kaolin and cristobalite. Fourth wash is the extraction experiment itself in which sediment was remixed with fresh ASW and separated within 1–2 h.

Analytical chemistry

**Cores from the Gulf of Aqaba**

Analytical measurements of pore waters from the Gulf of Aqaba were carried out at the Hebrew University of Jerusalem. Analyses were performed by a 7500 series inductively coupled plasma mass spectrometer (Agilent Technologies, Santa Clara, California) calibrated with multi element standard IV (Merck-Millipore, Darmstadt) and verified with USGS standards T143, T175, T183, and T199. To minimize artifacts related to instrumental drift, samples were mounted at a random order rather than their depth order and a seawater consistency standard was run every five samples. The average deviation between duplicate measurements of Ca, Sr, Mn, and Ba was 0.6%, 0.5%, 3%, and 6%, respectively.

The measurement of pH was carried out by a PHM64 research pH meter (Radiometer, Copenhagen) at 25°C. pH in pore-water solutions obtained by centrifugation was measured using a Radiometer Analytical PHG201-7 glass pH electrode with a REF201 reference electrode. The electrodes were calibrated using NIST buffer solutions 4.005, 7.000, and 9.180 (Radiometer Analytical) and immersed in a seawater solution for at least 1 h before use. Immersion of the electrode in seawater of similar salinity to the samples equilibrated the liquid junction potential of the electrodes with the solution, allowing internal precision of ≤ 0.005 pH units. All samples from the different Gulf of Aqaba campaigns had the same salinity to within 0.05 PSU yet accuracy of the measurements was likely poorer than the precision due to differences in ionic strengths between standards and samples (Dickson 1993). pH in the pore-water samples obtained using Rhizon samplers was measured with an AMANI-1000 L plastic mini-electrode (Warner Instruments, Hamden, Connecticut) to allow analyses of smaller samples. The AMANI-1000 L electrode had different voltage–pH slopes in distilled water and seawater solutions. To account for this, the AMANI electrode was calibrated relative to the glass electrode by measuring pH in three seawater samples with $pH_{NBS}$ 8.071, 7.352, and 6.736 as measured with a calibrated glass electrode.

Samples for carbon isotope analyses were equilibrated with 100% H₃PO₄ for 24 h before analyses in glass tubes that were preflushed with He. This equilibration turns all DIC into CO₂ which was pushed into a Finnigan-MAT Delta+™ isotope ratio mass spectrometer (IRMS) at the Hebrew University of Jerusalem. The $\delta^{13}$C in the samples was calculated relative to VPDB standard with precision of ± 0.1‰.

DIC was measured in the centrifugation samples using an AIRICA® DIC analyzer (Marianda, Kiel) connected to a Li-6252 IR-CO₂ sensor (LI-COR, Lincoln, Nebraska). A certified reference material provided by Andrew Dickson ( Scripps Institution of Oceanography, La Jolla, California) was run every four samples and used for calibration and drift corrections (Wurgaft et al. 2016). Precision of duplicate measurements of samples from the same vial was ± 3 μmol kg⁻¹ but DIC concentrations measured in replicate samples from the same core and depth separated in different centrifuge tubes often differ by as much as 50–100 μmol kg⁻¹. To minimize water requirements during Rhizon samplings, the concentration of DIC of samples obtained using the Rhizon samplers was calculated based on the 2nd and 4th intensity lines of CO₂ molecules in the IRMS measurement. A calibration curve for this analysis was prepared using NaHCO₃ solutions with a known concentration of DIC. The calibration was verified by measuring the centrifugation samples and Dickson CRM using both the AIRICA and IRMS. Internal precision of the measurement was ± 40 μmol kg⁻¹.

**Cores from Norfolk/synthetic sediments**

Measurements of analytical chemistry on cores from Norfolk and the synthetic sediment experiments were carried out at the University of Cambridge in the Godwin Laboratory for Paleoclimate Research and the Laboratory for Marine Biogeochemistry. For analyses of $\delta^{13}$C(CO₂), 1.5 mL sample was injected into a flushed glass tube filled with helium where the sample reacted with 100% phosphoric acid. After allowing time for equilibration, carbon isotopes were measured with a Thermo Fisher MAT253 IRMS. Analysis of the samples was conducted in dual inlet mode with in house reference gas calibrated relative to VPDB standard. The average measurement precision was ± 0.07‰ based on duplicate analyses.

The pH was measured in ASW and sediment experiments using a Thermo Scientific Orion 3-star pH meter equipped with a Ross pH electrode. The pH meter was calibrated in Thermo Scientific Orion application pH solutions 4.01, 7.00, and 10.01. These experiments were conducted over the course of 3 d. Therefore, filtered water samples from the first 2 d were stored and refrigerated in glass bottles without air bubbles. The stock
solution was sampled every day during the experiment. Prior to analyses, all samples were heated to 25 °C and the electrode was immersed for 30 min in a seawater solution to equilibrate liquid junction potentials of the electrode and solution. Internal precision of the analyses was ± 0.01 pH units.

Total alkalinity was measured in samples from the ASW and synthetic sediment experiments using a Metrohm 848 Titroline Plus autotitrator. Samples were filtered via 0.22 μm PES syringe filters and weighed in the titration vessel. Measurement accuracy was tested using CRM 157 provided by Andrew Dickson (Scripps Institution of Oceanography, La Jolla, California). Dilution of some of the samples with double distilled water was required to achieve large enough sample sizes for the titration. These dilutions do not modify the alkalinity of the samples, since distilled water has zero alkalinity, but they affect the dissociation constants of the carbonate system, yielding higher calculated alkalinities in more dilute samples. To correct for the effect of dilution on the ionic strength of the solution, dilutions were done gravimetrically and corrected using a calibration curve prepared by titrating CRM samples at different dilutions. The average deviation between duplicate alkalinity measurements was 4 μmol kg⁻¹ in water samples and 12 μmol kg⁻¹ in separated pore-water samples.

Major elements were analyzed in samples from the synthetic sediment experiment using an Agilent Technologies 5100 inductively coupled plasma optical emission spectrometer (ICP-OES) after diluting 70x with 0.1 M distilled HNO₃. The instrument was calibrated relative to OSIL seawater standard (Burton 1996) at different dilutions. Concentration of calcium in the OSIL standard was measured by a Triton Plus multicollector thermal ionization mass spectrometer after gravimetrically spiking standard samples with a ⁴²Ca⁴⁸Ca double spike and separating the calcium with AG500W-X8 resin. Strontium concentrations in the OSIL standard were thereafter measured by running an in-house standard with well-known Sr/Ca ratios and calculated using the measured calcium concentration in OSIL seawater. Samples were mounted at a random order and a consistency standard was run every five samples. Using ICP-OES, ratios between elements are more precise than analyses of individual elements since the instrument measures the intensity of all spectral lines at once. All analyses are therefore reported relative to sodium for the spectra lines: Na568.821, Ca422.673, K766.491, Mg279.800, S181.972, and Sr421.552. Standard deviations of 15 repeat measurements of the consistency standard were 0.41%, 0.18%, 0.32%, 0.58%, and 0.41% on Ca/Na, K/Na, Mg/Na, S/Na, and Sr/Na, respectively. Data from these analyses are provided in the Supporting Information.

Assessment and discussion

Pore waters of cores from natural marine environments

Our pore-water profiles from the Gulf of Aqaba show gradual changes with depth and the values obtained for the different geochemical analyses seem reasonable (Fig. 1). However, the measured concentrations and rate of change with depth of several analytes are different when pore waters are extracted using centrifugation and Rhizon samplers. This is particularly true when it comes to measurements of δ¹³C(DIC), which are systematically more negative in the centrifuged samples compared to those obtained using Rhizon samplers (Fig. 1b,d). To test whether there is a genuine problem deriving from the pore-water extraction methods, we measured δ¹³C(DIC) in water extracted using both techniques from salt marsh sediments retrieved from Blakeney, Norfolk, England and found a similar discrepancy (Fig. 2). The δ¹³C(DIC) of salt marsh samples that were sectioned and centrifuged after pore-water extraction by Rhizon samplers was yet higher than δ¹³C(DIC) of pore water initially extracted using centrifuge or Rhizons (Fig. 2). Measured differences in the carbon isotopic composition between pore waters extracted by the two methods thus support the conclusion of Schrum et al. (2012) that pore waters extracted by low-pressure filtration may undergo degassing when CO₂ fugacity increases. It is important to note that water temperature at 700 m in the Gulf of Aqaba is 21°C, easily maintained during processing of the cores, hence temperature cannot account for these differences. Norfolk salt marsh sediments were collected at temperature of ~20°C and from shallow sediments (~12 cm deep) so pressure differences are also not the cause for the different δ¹³C(DIC).

The concentrations of DIC were the same between the extraction methods in the Gulf of Aqaba cores retrieved in the same multicorer quartet (Fig. 1a). pH data show discrepancies between pore waters extracted using Rhizons, and centrifugation from adjacent cores (Fig. 1e). The pH profile obtained from pore water separated by centrifugation was relatively stable in the top ~11 cm. At that depth, pH decreased abruptly and stabilized at 7.5–7.6 on the NBS scale (Fig. 1e). The pH in water extracted using Rhizon samplers appears noisier than pH measured in the centrifuged samples and its gradient with depth is milder, yet observed differences between these cores could possibly be due to natural variability.

Major dissolved element concentrations measured in extracted pore water suggest that centrifugation and Rhizon samplers generally provide comparable results, yet attention needs to be paid to some subtle differences. Figure 3 compares the effect of sampling using centrifugation and Rhizon samplers on a redox sensitive element (manganese), an element whose distribution is associated with redox reactions (barium) and two major elements related to the carbonate system (calcium and strontium). This comparison suggests that sampling for barium is not sensitive to the extraction method. The manganese profiles show some variability between adjacent cores (Fig. 3a,e). This could point to a problem with one of the extraction methods such as water withdrawal from remote sediment layers in the case of Rhizon samplers or oxidation of manganese during processing for centrifugation. However, each of these profiles is internally consistent and the comparison between Fig. 3a,e suggests that in
each case a profile produced by a different method lags after the other, while the peak concentrations are similar for the two extraction methods. Altogether, this suggests that differences between the manganese profiles are likely genuine and result from redox-driven changes over short spatial distances.

On the other hand, in analyses of major elements, where small variations in the absolute concentrations are of interest, the sampling procedure may alter the output. Measured calcium concentrations were higher in samples taken with the Rhizons than in the centrifuged samples, especially in core 507J where the Rhizon samplers were inserted dry (Fig. 3c). In the same core, measured strontium (Fig. 3d), magnesium, and sulfate (not shown) concentrations in pore water extracted using the Rhizon samples were also higher than those of the centrifuged samples. The difference between the results we obtained in the two sampling campaigns (Fig. 3c,d vs. g,h) are probably a result of a modification to the treatment of the Rhizon samplers before sampling. In the January 2013 experiment, we used new dry Rhizon samplers, as received from the manufacturer. Before the November 2013 campaign, the samplers were rinsed with 1 N HCl, thoroughly rinsed with double distilled water and were then partly dried at room temperature before sampling. In both cases, the first 200 μL of the sample was discarded as explained in the methods. It appears that wetting the membrane before sampling satisfied the water absorption capacity of the membrane, which can be significant for a composite polyvinylpyrrolidine and polyethersulfone membrane (Irfan et al. 2014). This supports the claim of

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**Fig. 1.** Carbonate chemistry parameters measured on pore water extracted using centrifugation and Rhizon samplers in cores from the Gulf of Aqaba. The first letter in the core name refers to the extraction method: C for centrifuge and R for Rhizon. Subplots (a, b) compare cores from the same multicorer cast; subplots (c–e) compare cores retrieved in different casts from adjacent locations. Error bars stand for the difference between duplicate analyses of the same sample. Analytical error of centrifuge DIC samples was too small to appear on this scale.

**Fig. 2.** $\delta^{13}$C$_{\text{DIC}}$ in pore waters separated from Norfolk salt marsh sediments using wet Rhizons samplers and centrifuge.
Miller et al. (2014) that water absorption by the polymeric membrane can increase apparent pore-water salinity.

Another issue that may compromise the reliability of samples obtained using Rhizons is the possibility that the Rhizon sampler pulls water from different depths in the core than intended. In our pore-water collection with Rhizon samplers, we found that if there is any overlying water in the core, water is preferentially sampled from above the depth where the Rhizon is situated. This artifact can be seen in the first two samples from core R507J that possess similar water chemistry to the bottom water rather than to sample C507J from the same depths (Fig. 1a,b). To eliminate this artifact, it is essential to completely drain the bottom water above the core before sampling. Once this was done, pore water extracted using Rhizon samplers had the same concentrations as centrifuged samples from the same depths. Centrifugation provides better control over the depth distribution of extracted fluids but typically requires pushing the core out of the core liner and transferring the sediment into centrifugation vials. During this process, there is potential for extensive gas exchange between the ambient air or gas mixture and the sample, a problem of limited importance in Rhizon sampling.

**Sampling of ASW**

Field data presented in the previous section indicate that separations of water from the solid sediment using centrifugation or wet Rhizon samplers produce fairly reproducible results for many dissolved ions. Problems arise when dissolved gases are sampled. Figures 1–2 show that δ¹³C(DIC) in particular may be compromised during either Rhizon or centrifuge sampling but do not indicate which of the methods produce more reliable results. This key question was tested by processing ASW and measuring the pH, δ¹³C(DIC), alkalinity, and dissolved cations with and without treatment by centrifugation or extraction by Rhizon samplers (Fig. 4).

When treating water in the absence of sediments, procedures tested here vary between having minimal impact on water chemistry to causing significant loss of ¹²C rich CO₂ from the solution. Lower pH and lower δ¹³C(DIC) thus are less likely to be artifacts of pore-water separation and more likely to be correct. Control experiments show that δ¹³C(DIC) and pH of the stock solution do not change upon filtration of the water using syringe filters as well as centrifuging a full tube of water without headspace. When a half full tube is centrifuged, pH and δ¹³C(DIC) increase, suggesting that the presence of headspace in the centrifuge tube allows some of the CO₂ and particularly ¹²CO₂ to escape from solution. This is exacerbated when the headspace is filled with N₂. When water is drawn using Rhizon samplers, there is only little loss of CO₂ when sampling with a wet Rhizon but more dissolved CO₂ is missing when sampling using a dry Rhizon or under a N₂ atmosphere. It is thus apparent that while the use of a N₂ atmosphere is beneficial for sampling anaerobic metabolites (e.g., Canfield et al. 1993), it reduces the credibility of carbonate system data. The reason is likely that N₂ atmosphere is devoid of CO₂, creating a much larger gradient with the partial pressure of CO₂ of the pore waters than found in contact with ambient atmosphere.
Artificial sediments

The subsequent samplings of pore water out of artificial sediments prepared by mixing ASW with pure mineral powders are shown in Fig. 5 and tabulated in the Supporting Information. The $\delta^{13}$C(DIC) measured in these experiments had clear dependency on the mineral phase; from a starting $\delta^{13}$C(DIC) around $-2\%_o$, $\delta^{13}$C(DIC) went down to $-6\%_o$ in contact with CaCO$_3$ but went up to $-1\%_o$ to $+1\%_o$ in contact with cristobalite and kaolin, respectively.

The various synthetic sediments also had very different propensities to hold on to their pore water which was evident both anecdotally during the extraction process and in carbonate chemistry of the separated water. The quartz sand and CaCO$_3$ easily yielded water to the Rhizons but yielded little water during centrifugation. The clays released their water when centrifuged yet Rhizon extraction from clays was relatively slow. Water separated from the clays using centrifugation has a lower pH and $\delta^{13}$C(DIC) than water separated by Rhizons (Fig. 5), suggesting that the strong resistance of clay to suction increase degassing rates of CO$_2$ when pore water is extracted using Rhizon samplers.

Alkalinity, $\delta^{13}$C(DIC), and calcium data from the carbonate sediment experiment (Fig. 5; Supporting Information Table S1) suggest that CaCO$_3$ precipitated when ASW was mixed with CaCO$_3$. In this case, concentrations of calcium and alkalinity as well as $\delta^{13}$C(DIC) of the ASW all dropped to well below the initial composition. More precipitation occurred when samples were processed under a N$_2$ atmosphere (Fig. 5). The reason for

Fig. 4. The effect of different sampling techniques on $\delta^{13}$C(DIC) and pH of ASW from identical 50 mL polypropylene tubes. Experiments 1 and 2 differed in the initial pH and $\delta^{13}$C(DIC) of the stock water. Error bars mark the deviation of duplicate measurements from the mean. The label (N2) means that samples were processed in an anaerobic chamber under a nitrogenous atmosphere.
this is that our stock solution was supersaturated with respect to calcite and aragonite, enabling inorganic precipitation from pore waters in contact with seed grains (Wurgaft et al. 2016). We do not find evidence for inorganic CaCO₃ precipitation during sampling in the kaolin, cristobalite, or quartz sand experiments.

Differences between δ¹³C(DIC) in pore waters obtained from natural sediments using Rhizons and centrifuge (Figs. 1b, d, 2) can therefore be explained by artifacts in the separation technique itself. An additional contribution to this inconsistency could come from pore-water migration during sampling. This seems to be of greater concern when sampling using Rhizons as it is never possible to determine the actual path of the solution. Seeberg-Elverfeldt et al. (2005) calculated that within the porosity range 0.5–0.9, the radius of interference of a 10 cm Rhizon sampler collecting 2 mL is 0.27–0.36 cm. This translates to a radius of interference of 0.5–0.7 cm for a 5 cm long sampler. Smoothing of the δ¹³C(DIC) profiles collected with our Rhizon samples suggest that pore-water migration distances are greater than calculated by Seeberg-Elverfeldt et al. (2005) yet the same is not apparent for other chemical species. This issue may be explained by the fact that pore-water extraction using Rhizons forces air into the sediment, replacing extracted water (Fig. 6). The polymeric membrane does not allow air passage into the collection syringe but the penetration of air in between the sediment grains creates an internal headspace. During pore-water extraction, the presence of this gaseous phase favors partitioning of dissolved CO₂ between the liquid and gas phases. This is probably not the case when extracting pore waters using in situ samplers in saturated environments since extracted water is replenished by water, not air.

Evidence in support of this mechanism is found in experiments where the sediment was centrifuged after pore-water extraction using Rhizon samplers (Figs. 2, 5). Pore water obtained following this double treatment consistently had higher pH and δ¹³C(DIC) compared to pore water obtained using centrifugation or Rhizon samplers alone. The higher pH and δ¹³C(DIC) were too high to be explained by larger headspace during centrifugation, suggesting that during pore-water extraction with Rhizons, the remaining pore-water solution

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**Fig. 5.** δ¹³C(DIC) (‰), pH, and total alkalinity (mmol kg⁻¹) measured in pore water extracted from artificial sediments. The experiment was conducted following three cycles of sediment washes with ASW (Supporting Information Table S1). Error bars stand for absolute deviation of duplicate experiments from the mean.
also loses isotopically lower $\delta^{13}$C $\text{CO}_2$. Loss of $^{12}$CO$_2$ enriched DIC from both the extracted and remaining pore water creates a mass balance problem which can be resolved by loss of CO$_2$ from both pools into the more compatible gas phase (Fig. 6).

**Recommendations for use of Rhizon samplers**

While noting drawbacks of Rhizon samplers for pore-water extractions, we do acknowledge that they stand as an attractive alternative to centrifugation and squeezing since they are nondestructive, easy to use and do not require heavy machinery. Adding our data to the findings of previous studies, we can now draw more specific guidelines concerning the use of Rhizon samplers for pore-water separations which may serve as a basis for modifications depending on specific research targets:

1. The membranes of Rhizon samplers tend to absorb a significant amount of water. Prior to using these samplers for pore-water extraction, they should be soaked in distilled water and flushed with the sample. The recommended volume of sample to be discarded in the process is at least 600 $\mu$L in order to make sure that the membrane and tube has been flushed at least twice. Note that this practice does not guarantee the reduction of trace element blanks (Abbott et al. 2015).

2. Schrum et al. (2012) attributed low DIC and alkalinity measured in pore-water samples extracted by Rhizon samplers to inorganic CaCO$_3$ precipitation following CO$_2$ degassing during the extraction process. Our data supports that CO$_2$ is lost from the remaining pore-water solution during sampling as well as from the extracted water. When abundant CaCO$_3$ seed grains are present, this can induce inorganic precipitation of CaCO$_3$. Other minerals seem to be less able to nucleate inorganic precipitation of CaCO$_3$.

3. To minimize water uptake from remote parts of the core, the overlying water must be completely drained first. Attention must also be paid to the orientation of the core during pore-water extraction and samples should be drawn from top to bottom in high-resolution sampling.

4. The need to fully drain overlying water prior to pore-water extraction with Rhizon samplers reduces the reliability of in situ pore-water samplers in muddy sediments based on this or similar extraction methods. In permeable sediments, Rhizons clearly perform better than centrifugation.

5. The manufacturer advises not to reuse Rhizon samplers. Our tests suggest that as long as the membrane remained intact, Rhizons that were thoroughly rinsed immediately after use provide satisfactorily, and in many cases better results than new Rhizons in sampling of major elements. A recommended procedure for washing used Rhizons is: (1) Rinse the outer membrane with a large volume of water immediately after use. Gently wipe remaining sediment grains off the membrane and rinse again until all grains were removed. (2) rinse the Rhizons with MQ-water. (3) Backwash the Rhizons by pushing at least 5 mL MQ-water into each of them using a clean syringe (this step removes solids adsorbed to the membrane and allows verification that the membrane is indeed intact). (4) Place the Rhizons in a beaker full of MQ-water, pull at least 10 mL by each Rhizon sampler. If the Rhizons have been used in anoxic sediments, it may be necessary to rinse them in dilute HNO$_3$ after step (4) to remove any adsorbed Mn and Fe oxides, and to repeatedly rinse them with distilled water.

**Summary**

Our analyses show that both centrifugation and Rhizon samplers produce adequate data for most studied analytes but centrifugation performs slightly better in many cases. In high-
resolution studies of the top sediment layers, centrifugation is clearly a more robust method given the likelihood that Rhizon samplers preferentially pull water from above. For parameters of the carbonate system, pore water extracted using both methods tend to contain less dissolved CO₂ compared to the original solution and particularly seem to have lost isotopically lower δ¹³C O₂, resulting in elevated measured δ¹³C DIC. This problem can be lessened in centrifugation by filling centrifuge tubes to the top and thus minimizing headspace (Fig. 6) but seems to be more severe in pore-water extractions using Rhizon samplers. In the latter case, pore-water advection from other parts of the core cannot be fully controlled and pore-water replacement by air, penetrating during extraction, creates internal headspace, favoring loss of isotopically light CO₂ into the gas phase (Fig. 6). It is important to note that purging N₂ during the extraction operation or working in a N₂ glove box significantly worsens this situation since it increases the gradient between the concentration of CO₂ in pore water and the environment, causing loss of isotopically lower δ¹³C CO₂, regardless of the extraction method used.

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Conflict of Interest
None declared.

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