Iron (Fe) is an essential micronutrient for photosynthesis and nitrogen fixation in the ocean (Morel et al. 2003), and because of its low seawater concentrations, Fe controls primary production in a large portion of the global ocean (Martín and Fitzwater 1988; Moore et al. 2009; Moore et al. 2002). Fe(III) is the thermodynamically favored redox state in oxygenated seawater, yet Fe(III) solubility is suppressed to biologically limiting concentrations of < 0.1 nM in artificial seawater (Kuma et al. 1996; Liu and Millero 1999). In natural seawater, however, electrochemical methods indicate that > 99.9% of dissolved Fe (dFe) is bound to organic Fe-binding ligands that increase Fe’s solubility (Rue and Bruland 1995; Van Den Berg 1995; Wu and Luther 1995). These organic ligands comprise a portion of the dissolved organic carbon (DOC) pool but have a high binding capacity for Fe. Like DOC, little is known about the chemical composition and structure of these Fe ligands (reviewed in Gledhill and Buck 2012). Whereas a small subset of natural siderophores with hydroxamate binding groups have recently been isolated and identified in marine waters (Mawji et al. 2008; Velasquez et al. 2011), they only compose up to 5% of the Fe-binding ligand pool at any location, and thus bulk electrochemical measurements of ligand concentration and binding strength provide our best understanding of the effects of Fe speciation on marine Fe biogeochemistry.

Since Fe is organically bound, however, the characteristics of DOC can provide clues about the binding environment of Fe. For instance, like marine DOC (Guo and Santschi 1997), oceanic dFe is partitioned between both truly dissolved and colloidal species (Wu et al. 2001). In fact, it is thought that the broad size distribution of dFe through the colloidal range stems from Fe’s organic binding environment instead of its potential for inorganic hydrolysis, since other hydrolyzable metals such as aluminum and titanium do not exhibit a significant colloidal phase in marine waters (Dammshauser and Crook 2012). The size fractionation of dFe has been investigated globally (Fig. 1), and the colloidal contribution to dFe has been found to range

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**Assessment and comparison of Anopore and cross flow filtration methods for the determination of dissolved iron size fractionation into soluble and colloidal phases in seawater**

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**Abstract**

The two most frequently used methods for determining the size fractionation of dissolved iron (dFe) in seawater, 0.02 μm pore size Anopore membrane filtration and cross flow filtration using a 10 kDa regenerated cellulose filter, were evaluated and compared. Anopore filtration was found to produce consistent soluble Fe (sFe) concentrations in the filtrate even after > 1 L unfiltered seawater was filtered, indicating that clogging is not an issue for typical open ocean filtration volumes. Cross flow filtration (CFF) only achieved a 70% to 75% mass balance regardless of flow rate and seawater preconditioning; however, Fe losses arose largely from the colloidal (not soluble) size fraction, and Fe loss was constrained to Fe clogging in/on the CFF membrane. Both Anopore membrane and cross flow filtration methods reproducibly size fractionated dFe in seawater samples. Additionally, sFe arising from these two filtration methods were compared for the first time using seawater samples from the North Pacific and Atlantic Oceans. sFe from CFF was always lower than sFe from Anopore filtration, with the ratio of sFe in CFF/Anopore filtration averaging 68 ± 23% (n = 23). This sFe difference is attributed to a combination of the smaller effective pore size of the regenerated cellulose CFF system and the spatial variability in the concentration and composition of dFe in the 10 kDa - 0.02 μm size fraction. Finally, the advantages and disadvantages of each method were reviewed to offer future users a suite of factors with which to choose their ideal ultrafiltration method.

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from 0% in the shallow South Atlantic and Southern Oceans (Bergquist et al. 2007; Boye et al. 2010) to ~90% in the surface ocean of the tropical North Atlantic (Fitzsimmons and Boyle 2014). The spatial and depth resolution of this size distribution is important to understand because the soluble (low molecular weight) and colloidal (high molecular weight) dFe fractions can have very different bioavailabilities and residence times in seawater. In the few bioavailability studies completed on the two size fractions to date, the soluble fraction of dFe was generally preferred over the colloidal fraction (Chen et al. 2003; Chen and Wang 2001; Wang and Dei 2003), although certain forms of colloidal Fe have been found to be highly bioavailable such as Fe bound to exopolymer saccharides (Hassler et al. 2011; Hassler and Schoemann 2009), and it is known that bioavailability varies widely with microorganism identity (Hutchins et al. 1999). Additionally from a residence time perspective, if colloidal Fe is actively aggregating (Honeyman and Santschi 1989), it might have a shorter residence time than stable, strongly bound soluble Fe species, although the relative stability of sFe and cFe has not yet been experimentally tested.

When discussing the size distribution of Fe and organic material, the aquatic chemistry community assigns a “classical” interpretation of the soluble and colloidal size classes (Wells 2002). Soluble compounds are truly dissolved species that cannot be differentiated from the surrounding solvent molecules with a barrier; in fact, in many cases soluble species are chemically bound to the solvent directly. Thus the “ideal” size cutoff separating soluble from colloidal material is the dimension at which a compound is large enough to have a surface separating it from the rest of the surrounding solvent. At the largest colloid dimensions, the “ideal” size cutoff separating colloidal from particulate material is the dimension at which the colloid is large enough to incur gravitational settling. Therefore “classical” colloids are actually particles (because they are differentiated from the solvent milieu by a boundary), but these colloidal particles are simply so small that they do not sink. Only after aggregation might they become large enough to sink, in which case they would fall into the particulate phase.

Ultimately, however, the separation of these size fractions during measurement requires an operational size cutoff set by the filtration technique chosen, and this operational definition does not necessarily match our “classical” perception of the two size fractions. Historically, marine colloids have been
counted using a combination of ultracentrifugation and transmission electron microscopy (Wells and Goldberg 1991, 1992, 1993) or atomic force microscopy (Santschi et al. 1998), as well as light-scattering methods including photon correlation spectroscopy (Chin et al. 1998) and nanoparticle tracking analysis (Tatarkiewicz et al. 2012). Separations of the two size fractions for elemental quantification has been completed by a myriad of techniques including ultrafiltration methods such as cross flow filtration (Buesseler et al. 1996; Nishioka et al. 2001) and Vivaspun ultracentrifugation (Schlosser et al. 2013), small-pore size membrane filtration (Wu et al. 2001), flow field flow fractionation (Baalousha et al. 2011; Stolpe et al. 2010), and various chromatography techniques (Burgess et al. 1996; Chin and Gschwend 1991).

In the last decade, two methods have emerged as the dominant systems used for quantitative size-partitioning studies of marine trace metals because of their ease of use and relatively fast filtration rates: cross flow filtration and membrane ultrafiltration using Anopore filters. A growing number of studies have used one of these two methods to quantify open ocean distributions of both soluble and colloidal Fe (Fig. 1), yet the two methods have never been compared on the same samples. Because there is no reason to expect that the two systems should use the same operational separation of soluble and colloidal dFe, it is difficult to make comparisons between literature studies using the two methods, and thus our global understanding of dFe size partitioning is limited to regions where the same filtration method has been used. In this study, we seek to evaluate these two most common methods for collecting size-fractionated dissolved Fe samples, and we outline the advantages and disadvantages of each, with the ultimate goal of offering a recommendation for when it may be ideal to use one method over the other. We will also compare the two filtration methods on identical seawater samples to examine their differences both spatially and with depth, which might be used to a first approximation to compare literature data using the two ultrafiltration techniques.

**Methods**

**Sample collection**

Samples used in this filtration assessment/intercalibration were collected and analyzed from five different cruises: 1) the U.S. GEOTRACES North Atlantic GA03 cruise Leg 1 in late October-November 2010 (Station 8 at 20.59°N, 22.0°W; Station 12 at the TENATSO time-series station at 17.0°N, 24.0°W), 2) the HOT-231 cruise at Station ALOHA (22.75°N, 158.0°W) in the North Pacific subtropical gyre in April 2011, 3) the U.S. GEOTRACES North Atlantic GA03 cruise Leg 2 in November 2011 (Station 24 at the TENATSO time-series station), 4) the HOE-DYLAN cruise led by the Center for Microbial Oceanography: Research and Education (C-MORE) program at Station ALOHA in July 2012, and 5) the HOE-Phor-II cruise also led by C-MORE at Station ALOHA. On all cruises, samples were handled in ISO-5 rated flow benches (previously Class 100), and all cleaning was completed using only trace metal clean water (first de-ionized by reverse osmosis, then purified through a Barnstead ultrapure deionizing cartridge, and finally distilled in a Corning Vycor still). All hydrochloric (HCl) acid used for cleaning, filtration, and acidification was reagent grade hydrochloric acid distilled four times in a Vycor still that was checked for adequately low Fe blanks before use.

On both GEOTRACES cruises, trace metal-clean seawater was collected using the U.S. GEOTRACES GO-FLO carousel (Cutter and Bruland 2012) and was pre-filtered using 0.2 μm Pall Acropak-200 Supor capsule filters. Filtration protocols were identical to those described by Fitzsimmons and Boyle (2012), except that the Acropak filters were pre-cleaned by soaking overnight in filtered surface seawater that had been acidified to pH 2 using clean HCl, after which they were flushed with 5 L unacidified surface seawater and stored empty in the refrigerator until use. On the Station ALOHA cruises, trace metal-clean seawater was collected using the MITESS/Vanes system (Moored In situ Trace Element Sampler System). This is an autonomous sampler whose exterior is made entirely of metal-free ultra-high molecular weight polyethylene and contains an internal electronics board and motor that are used to open and close a sample bottle at a designated time at depth (Bell et al. 2002). The deployment of MITESS in the Vanes mode, as well as the subsequent pre-filtration of the seawater using 0.4 μm Nuclepore polycarbonate track-etched (PCTE) filters, are described in Fitzsimmons and Boyle (2012). It should be noted that only in the Anopore Clog and Anopore Blank experiments from the Station ALOHA cruises was seawater not pre-filtered with a 0.4 μm PCTE filter so that potential clogging and contamination of the membrane was maximized. All samples were acidified at sea to pH 2 with 6 M HCl.

**Anopore filtration**

Anopore filter membranes (Whatman) are manufactured electrochemically by the anodic oxidation of aluminum, and they contain 0.02 μm pores composed of a single-layer honeycomb structure (Furneaux et al. 1989)/Operationally defined “soluble Fe” (sFe < 0.02 μm) passes through the pores, while colloidal Fe is retained on the filter (0.02 μm < cFe < 0.4 μm) and can be calculated as

\[ cFe = dFe – sFe \] (1)

where dFe is the iron passing through a 0.4 μm (or sometimes 0.2 μm) filter (see “Sample Collection”). Anopore filters are available as individual membranes (called Anodisc membranes) that are mounted on a filtration rig, as in this study, or as syringe filters (called Anotop filters). In both of these cases, a traditional filtration geometry is employed where fluid is pushed parallel to (through) the membrane pores by differential pressure (Fig. 2). Using scanning electron microscopy and deuterium nuclear magnetic resonance, the internal pore walls
of Anopore filters have been shown to be very smooth and uniform in comparison to polycarbonate track-etch filter pores (Crawford et al. 1992), which should decrease the likelihood that soluble compounds would sorb to or get caught in Anopore filter cavities.

The methods we employed to collect soluble Fe samples through Anopore filtration are summarized in Fitzsimmons and Boyle (2012). An untreated 47 mm Anodisc 0.02 μm filter was loaded into an acid-cleaned PFA filter rig (Savillex) for vacuum filtration at ~ 0.4 atm vacuum pressure. Anopore filter cleaning was completed immediately before sample filtration instead of in advance of the cruise because we have found that acid treatment decomposes the alumina filter in the week(s) following cleaning (in accordance with Whatman recommendations for Anopore use with fluids above pH 3.5 only). Thus, immediately before sFe filtrate was collected, each Anodisc filter was first rinsed with > 50 mL of pH 1.5 HCl, followed by a rinse of > 50 mL clean water, and finally a conditioning rinse of > 50 mL seawater sample. This rinsing took ~10 min, and after a single bottle rinse the soluble Fe filtrate was collected in duplicate 30 mL HDPE bottles that were precleaned using the bottle cleaning procedures outlined in Fitzsimmons and Boyle (2012). In total, only about 150 mL total seawater was needed to collect two 30 mL sFe samples (including rinsing volume), and filtration took ~20 min, including the 10 min of rinsing. To avoid Fe sorption to the bottle walls of the seawater sample before Anopore filtration (Fitzsimmons and Boyle 2012), filtration of all samples was completed within 3 h of sample collection.

Cross flow filtration

Cross flow filtration (CFF) was completed using a Millipore Pellicon XL (PLCGC) filter made of regenerated cellulose with a nominal molecular weight cutoff of 10 kDa (surface area of 50 cm²) and was pumped using a Cole Parmer Masterflex peristaltic pump fed with FEP tubing. Feed solution flow rate was typically calibrated at 12 mL min⁻¹, half of the maximum recommended flow rate of 25 mL min⁻¹ through the Pellicon XL filter. The feed solution bottle, permeate bottle, and retentate bottle were held in an ISO 5 flow bench and were fed by FEP tubing that was pre-rinsed in pH 2 HCl before coming into contact with new solutions. Permeate and retentate flow rates were calibrated identically at 6 mL/min each.

New Pellicon filters arrive preloaded with glycerine and preservatives that were washed out before use at sea with several liters of trace metal-clean water, followed by 4 L of 0.25N HCl. After initial cleaning, the low blank of the system was verified by filtering ~ 800 mL of 0.4 μm filtered seawater that had been acidified to pH 2, and then filtering the resulting permeate fraction again as a “new” sample (see methods for treatment of new samples below). The two resulting permeate solutions were then analyzed for their Fe concentration to ensure that the solution filtered twice ([Fe] = 0.31 ± 0.04 nmol/kg, n = 3) did not acquire additional contaminant Fe after its first filtration ([Fe] = 0.29 ± 0.02 nmol/kg, n = 4). These two solutions did not have statistically different Fe concentrations (P = 0.61, Student t test, two-tailed), and thus any CFF processing blank must have been less than the precision of the Fe measurements.

Fig. 2. Anopore and cross flow filtration (CFF) geometries. The filter membrane is shown in black, assuming an equal pore size in the two filtration types (note that CFF actually has a smaller pore size than Anopore membranes in this comparison). Anopore filtration uses a traditional filtration geometry where the solution flows parallel to (through) the membrane pores; colloidal/particulate material accumulates at the membrane surface. CFF uses a tangential geometry where the solution enters perpendicular to the membrane pores; soluble material flows through the pores into the permeate, whereas both soluble and colloidal material exit perpendicular to the pores in the retentate solution.
At sea, CFF was completed on 0.2 or 0.4 μm prefiltered seawater samples as quickly as possible (always within ~ 3 h) after sample collection. Previous experiments have shown that a fraction of dFe sorbs to bottle walls on as short a timescale as 3 h depending on the surface area-to-volume ratio of the bottle (Fitzsimmons and Boyle 2012), and thus CFF filtration was accomplished as quickly as possible to avoid this Fe loss. At the start of any day of CFF, 1 L of pH 1.5 HCl was flushed through the system as an initial rinse, and flow rates were calibrated. Before any new seawater was cross flow filtered (including between samples), 500 mL of pH 1.5 HCl was used to rinse the system. Then 300-350 mL of pre-filtered sample seawater was used to condition the system before permeate and retentate solutions were collected in duplicate 30 mL HDPE bottles after a single rinse each. Relative permeate/retentate flow rates were typically again recorded after sample collection as a secondary check. The system was stored in pH 2.5 HCl after flushing with the pH 1.5 HCl rinse solution. About 500 mL seawater are needed to collect two 30 mL soluble Fe samples through the CFF system, and total filtration time (including the 500 mL acid rinse before sample processing) was ~ 1.3 h.

Using CFF, it is possible to obtain Fe concentrations from the permeate, retentate, or total dissolved (feed solution) fractions. In our CFF system, sFe is equal to the concentration of Fe in the permeate fraction (sFe < 10 kDa). cFe is operationally defined as the fraction between 10 kDa and the 0.2 or 0.4 μm filter used to prefilter the seawater samples. In addition to cFe, the retentate fraction also contains a portion of sFe that is carried along in the solvent; this is monitored using the concentration factor (CF; Buesseler et al. 1996):

\[
CF = \frac{\text{initial sample volume}}{\text{final retentate volume}} = \frac{\text{permeate volume} + \text{retentate volume}}{\text{retentate volume}}
\]

Thus, in our static CFF system where permeate and retentate volumes are identically calibrated, the CF is equal to 2.0. Under ideal permeation conditions where the membrane does not preferentially retain any soluble compounds (permeation coefficient = 1, Schlosser and Croot 2008), the amount of Fe in both permeate and retentate solutions should be the same in a sample with no colloidal Fe. However, in solutions containing cFe, the Fe concentration in the retentate solution must be corrected for the presence of sFe and the degree of concentration to calculate the true cFe concentration:

\[
cFe = \frac{[\text{Fe}]_{\text{retentate}} - [\text{Fe}]_{\text{permeate}}}{\text{CF}}
\]

This calculation of cFe only involves Fe determination in the permeate and retentate fractions (not the total dissolved feed solution). Alternatively, the total dissolved feed solution Fe concentration can be determined in place of the retentate fraction, and Eq. 1 can be used to calculate colloidal Fe. Under 100% mass balance in the CFF system and ideal membrane permeability, the cFe calculated using Eqs. 1 and 3 should be identical, and both have been used in the literature to define cFe concentrations (see “Assessment” for more information on choosing which colloidal Fe equation to use). The recovery of Fe in a measured mass balance can be calculated as

\[
\text{Recovery} = \frac{\text{sFe} + \text{cFe}}{\text{dFe}} \times 100\%
\]

where sFe is the concentration in the permeate solution, cFe is calculated using Eq. 3, and dFe is the concentration of Fe in the sample fed into the CFF system.

**Nuclepore 0.05 μm membrane filtration**

A third ultrafiltration method, a Nuclepore 0.05 μm polycarbonate track-etched (PCTE) membrane ultrafiltration, was tested on the August 2013 HOE-PhoR-II cruise at Station ALOHA at two depths: 15 m and 800 m. The Nuclepore filters were precleaned in the laboratory in advance of the cruise by placing them individually in a container of 1M trace metal clean HCl overnight in an oven at 60°C. Afterward, they were rinsed 6 times with clean water, after which they were soaked in clean water overnight in an oven at 60°C to remove any acid residue from the pores, after which they were rinsed 6 times again with clean water; they were ultimately stored in water. At sea, a clean filter was loaded on the same acid-cleaned PFA filter rig (Savillex) as described in the Anopore filtration section for vacuum filtration at ~ 0.4 atm pressure. Each filter was first rinsed with > 50 mL of pH 1.5 HCl, followed by a rinse of > 50 mL clean water, and finally > 50 mL of 0.4 μm filtered seawater sample to pre-condition the filter. This rinsing took ~ 20 min, and after a single bottle rinse the soluble Fe filtrate was collected in duplicate 30 mL HDPE bottles that were precleaned using the bottle cleaning procedures outlined in Fitzsimmons and Boyle (2012). In total, only about 150 mL total seawater was needed to collect two 30 mL sFe samples (including rinsing volume), and filtration of each sample took a total of ~ 40 min, including the ~ 20 min of rinsing.

**Fe analyses**

Seawater samples were analyzed in triplicate for their Fe concentration by isotope dilution-inductively coupled plasma mass spectrometry (ID-ICP-MS) with a hexapole collision cell Micromass IsoProbe multiple collector-ICP-MS. The ID-ICP-MS method employs a 54Fe-spike and batch preconcentration with nitritotriacetate resin (Lee et al. 2011). Values were only accepted if they were reproduced at least once. Procedure blanks during the seven analytical sessions ranged from 0.023-0.058 nmol/kg, and the detection limit (three times the standard deviation of the procedure blanks for each analytical session) averaged 0.031 nmol/kg. Comprehensive lab analyses of SAFe D2 standard for dFe during the period of these analyses averaged 0.99 ± 0.03 nmol/kg (Bottle 242, ± 1 SD, n = 10) and 0.92 ± 0.01 nmol/kg (Bottle 446, ± 1 SD, n = 9), in close agreement with the current consensus value of 0.933 ± 0.023 nmol/kg (updated May 2013; http://www.geotraces.org/science/intercalibration).
**Assessment**

The two most commonly used filtration methods for measuring the size partitioning of dFe in the open ocean were evaluated in this study: membrane ultrafiltration using Anopore filters with a pore size of 0.02 μm and cross flow filtration using a regenerated cellulose membrane with a 10 kDa molecular weight cutoff. These two filtration mechanisms have inherently different filtration geometries (Fig. 2), where cross flow filtration is assumed to be a less prone to clogging and sampling biases because of its tangential filtration geometry. Anopore filtration, in contrast, has a traditional direct-flow geometry that is more likely to suffer filter fouling but requires much lower sample volumes. The experiments executed on each of these two filter types, as well as a comparison between them, are discussed below.

**Anopore filtration**

Anopore filters were first used to examine the marine distribution of dFe size fractions by Wu et al. (2001), who modified this microfiltration approach for open-ocean dFe size partitioning after it was initially used for Fe solubility studies (Kuma et al. 1996; Liu and Millero 1999). In open-ocean studies, Anopore membrane filtration has two significant advantages over other methods. First, it requires very little seawater volume for filtration and allows for faster filtration times than CFF when small volumes are filtered. Second, Anopore filters can be used on the same filter rig that is used for the 0.2-0.4 μm prefiltrations or in a small syringe and thus are less cumbersome to set up and operate at sea than CFF systems. These two benefits have allowed for sFe to be measured across several ocean basins in moderately high spatial resolution using Anopore filtration (Fig. 1).

Anopore filtration employs a traditional filtration geometry where particles accumulate at the membrane surface, and as a result, it potentially suffers from partitioning artifacts caused by membrane clogging. At the filter surface, particles form a “polarization layer” that changes the basic characteristics of the filter in several ways (Buffel et al. 1992). First, the effective pore size of the filter membrane can be decreased at high particle loadings, changing the pore size cut-off over time. Second, a “concentration polarization” builds up at the membrane surface when the particle concentration at the filter membrane is greater than that in solution, creating an osmotic barrier that results in a decreased flow rate through the membrane (Bowen and Jenner 1995). Furthermore, aggregation and coagulation of colloids is a second-order reaction with respect to particle concentration (Stumm and Morgan 1996), and elevated particle concentrations at the membrane surface may induce the aggregation/coagulation of colloids that bias the quantitative partitioning (Buffel and Leppard 1995). Finally, the presence of enhanced particle loading in the polarization layer can promote adsorption of soluble Fe to the accumulated colloids, which could also bias the partitioning.

Considering that these filtration artifacts amplify with decreasing pore size, a test of the effect of increasing sample volume on soluble Fe concentrations in the Anopore filtrates was completed in the Anopore Clog test. In the first experiment in April 2011, > 1 L unfiltered seawater from 1000 m (dFe = 0.96 nmol/kg) at Station ALOHA was fed through an Anodisc filter over 2.5 h. Unfiltered water was strategically used to maximize potential clogging effects by the particulate load. The results (Fig. 3, squares) show that the initial 30 mL sample (collected after 115 mL seawater had passed through) had sFe of 0.67 nmol/kg, somewhat higher than the ~0.61 nmol/kg sFe sampled after 712 and 1214 mL seawater had been flushed. An initial high-sFe sample could be interpreted in one of two ways: either filter clogging changed the measured dFe size partitioning by decreasing the effective pore size, or filter-derived elutable Fe was not sufficiently rinsed off of the Anodisc filter by the time the first sample was collected, resulting in an anomalously high dFe concentration in the first sample. Statistically, the first sFe sample collected was significantly higher in concentration than the second and third sFe collected (P = 0.025 and 0.019, respectively: Student t test, two-tailed), but practically an external error of at least 0.05 nmol/kg is common in marine Fe analyses (contributed by random sample handling/bottle contamination and analytical uncertainties), and thus the Anopore Clog experiment was repeated to verify whether the initial enhanced sFe was a filter blank or a clogging artifact.

The Anopore Clog experiment was repeated at Station ALOHA in July 2012 with unfiltered seawater collected from...
800 m (dFe = 0.78 nmol/kg). A similar total volume was filtered over 2 h, but sub-samples were collected over smaller volume steps to increase the resolution. In contrast to the initial experiment, the results (Fig. 3, circles) did not show a decreasing sFe trend with increasing volume filtered, despite the initial sample being collected earlier in the filtration process (90 mL seawater filtered) than in the April 2011 study (115 mL filtered). Thus, > 1 L unfiltered deep ocean seawater was insufficient to noticeably reduce flow rate through the filter or cause a drop in the sFe concentration due to a decrease in the effective pore size of the Anodisc filter. This makes Anopore filtration a promising tool for quantitative estimates of dFe partitioning in unfiltered deep-water samples, since modern analytical techniques for trace metal concentration measurement require much less than 1 L volume for sample analysis (Biller and Bruland 2012; Lee et al. 2011; Milne et al. 2010; Obata et al. 1993).

However, if larger soluble Fe sample volumes are required (for instance, for metal isotopes or metal-binding ligand measurements) or samples are collected with significantly higher particulate/colloidal loading (estuarine or coastal waters), the Anopore Clog experiment should be repeated to prove that filter clogging is not biasing the measured partitioning. Although not explicitly tested in this study, prefilttering the seawater samples with a coarse 0.2 or 0.4 μm filter to first separate the dissolved from particulate phases is also recommended before Anopore filtration to reduce particle loadings on the filter that could promote filtration artifacts. However, the decision to pre-filter must be weighed against total filtration time, which should be minimized (in this study kept to < 3 h) because dFe sorbs significantly to bottle walls in as short a time as 3 h (dependent on bottle surface area to volume ratio, Fitzsimmons and Boyle 2012), and Fe speciation/partitioning can change in the < 4 h before filtration (Wen et al. 1996).

Untreated Anopore membranes contain significant Fe from manufacturing, so it was also important to investigate the degree of acid cleaning required to yield uncontaminated samples. In the Anopore Blank experiment, ten replicate filters were rinsed with increasing volumes of pH 1.5 HCl before processing identical volumes of unfiltered seawater from 800 m at Station ALOHA. The findings show that any contaminant Fe from the filter itself is fully removed with as little as a 20 mL rinse with pH 1.5 HCl clean water (Fig. 4), with rinse volumes up to 125 mL not substantially changing the filtrate Fe concentrations (800 m Station ALOHA water sFe averaged 0.480 ± 0.022 nmol/kg). It must be noted, however, that this acid rinsing only removes potential contamination from the filtrate, while particulate Fe contamination from manufacturing (that no longer leaches Fe into the filtrate after the membrane acid rinse) may still be present. In fact, a closer inspection of Anopore filters by synchrotron Fe-XANES analysis (Brandy Toner pers. comm.) showed that an acid-rinsed Anopore filter maintained the same particulate Fe inclusions as an unrinsed filter. As a result, digestion of Anopore filters for direct colloidal Fe quantification would likely suffer a large Fe blank, and thus these filters cannot be used for direct colloidal Fe measurements (and Anopore mass balance cannot be directly assessed).

From the Anopore Blank experiment, there was also no indication that the relatively strong pH 1.5 HCl rinse sufficiently attacked the aluminum oxide filters such that the effective pore size increased, because no increases in sFe concentrations were observed with as much as 130 mL acid rinse. However, longer storage under acidic conditions is not recommended. It is also important to note that the results of this blank evaluation highlight the excellent sFe reproducibility over the 10 individual Anodisc filters, with multiple membranes yielding very uniform sFe concentrations from the same seawater sample (Fig. 4).

Cross flow filtration

Cross flow filtration (CFF) is a well-established technique that has been used for years to separate high- and low-molecular weight DOC phases (reviewed in Buesseler et al. 1996; Guo and Santschi 1997; Wells 2002). As shown in Fig. 2, the sample stream of a CFF system flows tangentially to the membrane pores, so flow of soluble compounds through the pores into the permeate solution is driven by the pressure difference across the membrane, and the colloids are continually swept into the retentate solution. The major advantage of this filtration geometry is that concentration polarization effects at the membrane surface are reduced because fluid shear strips colloids away from the membrane surface, reducing the likelihood of clogging relative to filtration systems of traditional geometry (such as Anopore filtration). Furthermore, extraction efficiencies of CFF systems are typically high, filtration of
large volumes is relatively rapid compared with other filtration systems, and the colloidal phase is concentrated in the retentate solution of recirculating CFF systems for easy analysis (Guo and Santschi 1997), making this the most popular method for directly measuring colloidal species.

However, an evaluation of these CFF systems was made during the “Colloid Cookout” in the mid-1990s (Buesseler et al. 1996), where several groups used their own pretreatment and sample processing methods to size fractionate identical seawater samples using identically rated CFF membranes (1kDa molecular weight size cutoff polysulfone filters, although different manufacturers’ membranes were allowed). The DOC results showed both variable blanks across CFF systems (indicating that system cleaning protocols are critical) and that the retention ratings used by various manufacturers is not uniform, since even membranes rated at the same molecular weight cutoff did not have the same “effective pore sizes” (Gustafsson et al. 1996). Reitmeyer et al. (1996) reported the results of the aluminum (Al) and Fe intercalibration from this experiment, which showed that most systems had high contamination for Al and significant (up to 80%) scavenging/loss of Fe to the CFF system. Although there have been very few analyses of the size partitioning of marine Al since, size partitioning studies of Fe by CFF have continued (as seen in Fig. 1), with surprisingly little consideration for establishing an Fe mass balance. Notable exceptions to this are the Fe fertilization study by Wells (2003), where mass balance > 90% was achieved (Filtron polysulfone membrane, 1 kDa size cutoff), and the Fe solubility study by Schlosser and Croot (2008), where nearly 100% of the Fe was recovered after a dilute hydrochloric acid rinse (Vivaflow polyethersulfone membrane, 10 kDa size cutoff).

For the cross flow filtration evaluation in this study, several steps were taken to optimize the mass balance for Fe. First, regenerated cellulose was chosen as an alternative membrane material to the typical polysulfone in the hope that it might be less likely to sorb Fe compounds, and a small membrane surface area (50 cm²) was selected despite the slower flow rate to reduce the area to which Fe could sorb. The CFF system was also run in static mode (where retentate is collected immediately after filtration and is not sent back to the sample feed) instead of the more classical recirculation mode (where retentate is recycled through the CFF system multiple times) to avoid potential Fe contamination, compounded sorptive losses, or any potential size partitioning changes during recirculation. Finally, the CFF system was conditioned by processing ~ 350 mL sample seawater before sample collection to minimize sorptive losses of Fe to the system walls during sample collection.

The mass balance results from the Station ALOHA tests are shown in Fig. 5 and indicate that, despite these efforts, only 75.0 ± 2.5% of the Fe was recovered from the CFF system. The 25% Fe lost might reflect sorption to the walls of the CFF system (tubing or membrane walls), accumulation on the CFF membrane (i.e., concentration polarization), or retention of colloidal fragments within the pores of the CFF membrane. To determine which of these was the major Fe loss, filtrate sFe samples were collected at multiple time points during the processing of a large sample volume (Fig. 5a). It was hypothesized that if Fe sorption to the walls of the CFF system was responsible, then Fe recovery would improve with prolonged filtration as the active sites became saturated with Fe. The mass balances, however, did not significantly improve with volume filtered (Fig. 5a), indicating that the pre-conditioning of the
system with ~350 mL seawater was likely sufficient to saturate sorption sites with Fe, and the 25% Fe loss is instead occurring in or on the CFF membrane.

Schlosser and Croot (2008) used an acid rinse to liberate retained Fe and achieve a 100% mass balance in their polysulfone CFF system. We used an identical 60 mL 0.06M HCl rinse in clean water under our typical CFF operating methods to liberate membrane-associated Fe, but only 20% of the lost Fe was recovered, ~75% of which appeared in the permeate solution. It is possible that we did not similarly achieve mass balance after acid rinsing because of the difference in our membrane materials; perhaps at this acid concentration, more acid must be cycled through the system to rinse out all of the sorbed/trapped Fe in our regenerated cellulose depth filter. We have noted that no Fe blank has built up upon continual use (i.e., colloidal Fe becomes entwined in the depth membrane), the Fe loss may also reflect soluble Fe species sorbed to a limited number of membrane surface sites or to the trapped colloidal species themselves. The data in the next comparison section suggest that it is only the colloidal Fe fraction that is lost.

Comparison of membrane filtration with CFF

As Fig. 1 shows, marine studies of dFe size fractionation have traditionally used two filtration systems (CFF and Anopore filtration) that have a range of nominal pore size cutoffs, which makes a quantitative comparison of sFe or cFe concentrations from different studies impossible unless the same filter type was used. Only one published paper includes a comparison between the two methodologies. An endnote in the Wu et al. (2001) study reported a comparison of cFe separated by Anopore and CFF filtration on one seawater sample and found them to be identical within error. It must be emphasized, however, that the cFe concentrations discussed were only ~0.1 nM, too low a concentration to detect significant changes between filtration methods, especially given the reported 0.03 nM error on replicates analyses. Thus, a more extensive comparison was necessary.

A key objective for a comparison of colloid separation methods is an assessment of the accuracy, precision, and reproducibility of the nominal pore size cutoffs both within a given filter and among replicate filters. The anodizing voltage of the electrochemical fabrication method for Anopore membranes generates a very reproducible pore size, even at small dimensions, and a high pore density, both of which are desirable for synthetic filters. The pore size distribution of Anopore filters was measured by atomic force microscopy to cover a small range of only 0.0119-0.0278 μm, averaging 0.0188 ± 0.0035 μm over 108 replicates (Bowen et al. 1996), demonstrating that the nominal 0.02 μm pore size defined by Whatman is both accurate and precise. The reproducibility of the Anopore-filtered sFe concentration using 10 different Anodisc filters in this study (Fig. 4) also reflects the precision of sFe through different Anopore filters.

Most CFF filters, in contrast, are depth filters that contain a wider range of effective pore sizes. As a consequence, in some cases retention of compounds smaller than the nominal molecular weight size cutoff occurs, whereas in other cases, compounds larger than the size cutoff pass through the permeate (for example, long and skinny compounds that pass through on their smallest side). The nominal molecular weight cutoff is rated by the manufacturers using standard compounds of a known molecular weight, and they report the percentage of those molecular weight standards that are retained by the filter (for example, a rating requirement might be that 90% of 10 kDa compounds are retained by a 10 kDa membrane). The variability in manufacturers’ ratings, tested under different conditions with different standards, is what largely contributed to the variable permeation behavior of the nominally identical filters used in the previous CFF intercomparison effort (Buesseler et al. 1996). Moreover, the standard compounds chosen for the membrane ratings are optimized for biomedical or industrial water treatment applications (the most frequent users of CFF membranes), and thus proteins or polysaccharides are frequently chosen. There are no equivalent standards for dilute solutions composed of natural compounds. In any filtration, molecular shape, electrostatics, and other physicochemical characteristics of the compounds in solution allow for intermolecular or membrane interactions...
that can change the retention characteristics of different molecules. Thus, at best, CFF can only be nominally defined, and individual filters (especially those from different manufacturers or separate fabrication batches) may act differently than others that are identically rated.

Acknowledging these inherent differences in membrane types, the soluble Fe filtered through Anodisc membranes was compared with the sFe filtered through our 10 kDa CFF membrane (results shown in Fig. 6). Assuming a conversion of 1 kDa = 1 nm and that molecular weight increases nonlinearly faster than molecular size (Verdugo et al. 2004), the nominal molecular weight cutoff of an Anopore filter should be at least 20 kDa (and perhaps much greater), overall larger than the nominal 10 kDa size cutoff of our CFF. Thus, it was expected that the sFe concentration collected through the Anodisc filter would be greater than that filtered through the 10 kDa CFF membrane, and this was true at every location studied (Fig. 6).

In contrast, a study by Schlosser et al. (2013) found the effective pore size of Anotop 0.02 μm filters was smaller than that of their 5 kDa polyethersulfone membranes (Vivaspin), and another study by Chen et al. (2004) similarly reported that the actual molecular weight cutoff of Anopore filters determined using standard molecules was approximately 3 kDa. The results of our comparison, however, do not support these conclusions; our results suggest instead that the pore size of Anopore filters are at least greater than the 10k Da nominal molecular weight cutoff of the regenerated cellulose CFF filter membrane used in this study. We attribute the smaller effective pore size of our 10 kDa CFF filter compared with the 10 kDa filters used in the aforementioned studies (as calculated by a comparison to Anopore membranes) to their different membrane material: regenerated cellulose filters in this study versus polyethersulfone filters in Chen et al. (2004) and Schlosser et al. (2013). This hypothesis is supported by previous evidence.

![Fig. 6. Comparison of the soluble Fe collected using cross flow filtration (CFF; white bars) and Anopore filtration (black bars) on identical seawater samples, shown as a function of increasing water depth. Seawater was collected at Station ALOHA on the HOT-231 cruise (Apr 2011) and the HOE-PhoR-II cruise (Aug 2013), as well as on the U.S. GEOTRACES North Atlantic GA03 cruise in 2010 at Station 8 (USGT10-08: 20.59°N, 22.0°W) and in 2011 at Station 24 (USGT11-24: 17.0°N, 24.0°W) at several depths. Error bars represent 1σ standard deviations on replicate analyses of the same sample. The bold, italicized percentages (upper) indicate the percent of sFe collected using Anopore filtration that was also collected using CFF, whereas the non-bold, italicized percentages (lower) indicate the Fe recovery of the CFF system for that sample.](image-url)
that the effective pore sizes of CFF filters with the same nominal molecular weight cutoff varies due to both membrane material and manufacturers’ pore size rating systems (Buesseler et al. 1996). Additionally, organic compounds such as those binding Fe in the ocean can interact variably with different membranes material (Buffe and Leppard 1995), and thus the polyethersulfone membranes may also have promoted more surface coagulation (and thus colloidal retention) than the regenerated cellulose filters used in this study.

The CFF-Anopore comparison in Fig. 6 indicated that CFF sFe concentrations were 68 ± 23% (n = 23 depths) of those measured in the Anodisc filtrate. Much of this difference in sFe concentrations can be attributed to a difference in the pore sizes of the two filtration systems, with smaller 10 kDa CFF pores allowing less sFe to pass than the larger 0.02 μm Anodisc pores. However, the 17% to 99% spread in sFe ratio between CFF/Anopore is also a function of the spatial variability in the concentration and composition of marine colloidal Fe. For instance, if some of the Fe colloids are composed of loosely associated gels, they could fall apart during the 0.4 atm pressure Anopore filtration and thus be measured as sFe, whereas these same compounds may stay composed during the CFF filtration and be measured as colloidal.

There is also likely natural variability in the spatial distribution of 10 kDa - 0.02 μm colloidal Fe, both geographically and with depth in the water column. The intercomparison data in Fig. 6 is plotted as a function of depth, and the CFF/Anopore sFe ratio above ~ 100 m (taken as an approximate of the depths of deep chlorophyll maxima, DCM, at these stations) was lower (36 ± 16%) than that deeper than 100 m (80 ± 12%), indicating that there may be a greater contribution of colloids in the 10 kDa - 0.02 μm fraction above the DCM that contributes to a change in the CFF/Anopore sFe ratio. This result cannot be a function of higher cFe loss to the CFF system in shallower samples because there is not a consistently lower CFF Fe recovery at shallower depths (Fig. 6). Additionally, there is evidence of spatial variability in the distribution of the 10 kDa - 0.02 μm colloids: at 235 m in the North Atlantic the CFF/Anopore sFe ratio was a very different 84% at USGT10-08 (dissolved oxygen of 141 μmol/kg) than the 51% ratio at the same depth at USGT11-24 (more in the tropical North Atlantic OMZ with dissolved oxygen concentration of 78 μmol/kg), despite being < 500 km apart. In the Pacific at Station ALOHA at a similar depth of 250 m, the CFF/Anopore sFe ratio was higher (94%) than at either North Atlantic stations. Thus, although a 68 ± 23% comparison factor may be a reasonable first-order approximation for reconciling CFF and Anopore datasets in the literature, it is clear that a wider intercomparison across different water masses and biogeochemical gradients is necessary to evolve more predictable patterns. Additionally, given the poor comparison of our 10 kDa CFF filters with the 10 kDa CFF filters of other studies (Chen et al. 2004; Schlosser et al. 2013), it is clear that comparing data between CFF filter types is not a straightforward function of the rated molecular weight cutoff, and CFF data in the literature collected using different pore sizes or filter types will likely not ever be able to be quantitatively compared. Authors should make an effort to compare their chosen CFF filter type with existing filter types when publishing new data to attempt to reconcile their data with literature values.

Although not a comparison of identical seawater samples, two separate samplings at the TENATSO time-series station a year apart (Nov. 2010 and Dec. 2011) provided an additional opportunity to assess the reproducibility of these methods and the consistency of their differences (Fig. 7). sFe was collected at six identical depths in both years using the same CFF system, and the concentrations were statistically identical at all depths except 235 m (P values of the Student t test are mostly > 0.05, Fig. 7a). This finding demonstrates that there were no significant natural changes in Fe concentration or partitioning at TENATSO during the 2 years, making it a good comparison site (the full water column hydrography and dFe concentrations are compared over the 2 years in Hatta et al. pers. comm.). Additionally, this shows that the CFF membrane used in this study provides reproducible sFe concentrations over time, despite the potential CFF artifacts mentioned above.

Furthermore, following the results of the direct ultrafiltration comparison, the TENATSO station the sFe concentrations measured in Anopore filtrates were substantially higher at almost all depths than in CFF permeates in 2010 and 2011 (Fig. 7b). The average CFF/Anopore sFe ratio was 75 ± 22% for the 23 TENATSO samples despite their sampling a year apart, which is statistically identical to the 68 ± 23% CFF/Anopore sFe in the 23 direct filtration comparisons in Fig. 6. Only three TENATSO depths had statistically identical Anopore and CFF sFe concentrations: 185 m, 2750 m, and 3200 m (P values = 0.87, 0.31, and 0.23, respectively, using a Student t test, twotailed). A treatment of the implications of this data comparison on the natural size partitioning of dFe in the North Atlantic will be made elsewhere (Fitzsimmons et al. Accepted).

In the deepest 4 samples between 2750-3500 m, there was close agreement between the sFe concentrations from both filter types. To a first degree, this suggests that there were negligible contributions of colloidal Fe in the 10 kDa–0.02 μm size fraction at these depths. However, this agreement in sFe would be impossible if there was significant sorptive loss of soluble Fe to the CFF membrane. In fact, there were never samples where the sFe from CFF was higher than that through the Anopore filter, which might be true in a location with no 10 kDa–0.02 μm cFe component if sFe was sorbed to the CFF system. The results in Fig. 7 then support the hypothesis that the 20% to 30% Fe loss to the CFF system (as described in the “Cross Flow Filtration” section) is due to trapping/sorption of colloidal Fe alone, and accordingly it is reasonable to interpret permeate Fe from this CFF system as equivalent to sFe.

Ideally, CFF systems should attain 100% recovery of Fe in the permeate and retentate solutions; however, the results of this study and all other studies using CFF to measure dFe size
partitioning in seawater (Fig. 1) have shown that this is nearly impossible without lengthy and aggressive treatments (such as post-filtration acid leaching that renders the remaining colloidal material useless for further characterization). To escape the consequences of poor mass balance, we demonstrate that the Fe recovery was very reproducible for a single sample using our CFF system (Fig. 5), and we have strong evidence to support that all of the retained Fe was colloidal. This has implications for the ideal equation to use to calculate true cFe concentrations by CFF. As discussed in the methods section, the cFe can be calculated using the permeate and retentate concentrations (Eq. 3) or using the permeate and total dissolved (feed solution) concentrations (Eq. 1). Any difference between the cFe calculated by these two methods depends entirely on the CFF recovery. An example of the resulting range in cFe concentrations using the two equations is shown in Table 1 as a function of recovery. We recommend the use of Eq. 1 for our CFF system because it attributes all of the lost Fe to the colloidal fraction. Eq. 3, in contrast, entirely ignores any Fe that is lost to the CFF system, which can falsely bias high the % sFe when cFe contributes most of the Fe loss. For other CFF systems where it is not clear which fraction of Fe is being lost or where it is expected that permeation is non-ideal, Eq. 3 might be a better estimate, although every effort should be made to constrain which phase of Fe is lost and/or the permeation coefficient.

Finally, at two depths on the HOE-PhoR-II cruise at Station ALOHA, we directly compared a third ultrafiltration method, membrane filtration through a 0.05 μm Nuclepore PCTE filter, to the two more commonly used methods described above. We were motivated to try this membrane to find a membrane filtration method with which we could analyze the colloidal fraction directly without a significant filter blank, and polycarbonate filters have been shown to have very low filter blanks (Cullen and Sherrell 1999). The Nuclepore PCTE filtration method employs a traditional filtration geometry (Fig. 2a), and to our knowledge, no marine trace metal size partitioning study has ever employed this membrane.

As can be seen in the results of Fig. 8, the effective pore size of the 0.05 μm fell predictably between the 0.02 μm Anopore membrane effective pore size and the 0.4 μm dFe pore size cutoff. At 15 m in the North Pacific, the dFe was 16% sFe < 10 kDa, 37% was 10 kDa < cFe < 0.02 μm, 17% was 0.02 μm < cFe < 0.05 μm, and 30% 0.05 μm < cFe < 0.4 μm. In contrast, at 800 m
depth at Station ALOHA, the dFe < 0.4 μm and the Fe < 0.05 μm were identical, indicating that there was no 0.05 μm < cFe < 0.4 μm. Instead, much more of the dFe was truly soluble: 55% was sFe < 10 kDa, 11% was 10 kDa < cFe < 0.02 μm, and 34% was 0.02 μm < cFe < 0.05 μm. This suggests that there is large variability with depth in the size distribution of dFe that warrants further study and explanation, especially with regard to its controls on the biogeochemical fate of the marine dFe.

We note that despite its potential advantages in low trace metal filter blanks (which were not directly measured here), the 0.05 μm Nuclepore PCTE filters had very slow flow rates, requiring nearly 45 min to clean and filter a 120 mL sample seawater. This was nearly twice the time required for identical Anopore membrane filtration, making it less tenable to use this filter type for routine quantitative trace metal size partitioning measurements, since filtration must be completed rapidly to avoid physicochemical Fe transformations and wall sorption effects (as discussed above). We also tested 0.015 μm Nuclepore PCTE filters by identical methodology, and we were unable to achieve sufficient flow through these filters. Thus, unless the colloidal fraction is to be analyzed directly on a filter membrane, we recommend the use of one of the other two filter types for size partitioning analyses.

Discussion

Generally, it was found that both CFF and Anopore filtration methods could reproducibly size fractionate dFe in open ocean seawater, but the two methods produced different sFe concentrations from the same seawater sample, likely due to differences in their effective pore sizes. We note that the size separations defined here are valid only for the conditions outlined in this study, and we did not verify the integrity of these methods for other marine trace metals. Furthermore, the dFe size separations by the two systems are ultimately quite arbitrary, because they cannot be verified by any independent method, and the use of standard compounds of known molecular weight is not appropriate because they do not match seawater compound composition, characteristics, or conditions. Despite these seemingly overwhelming limitations, the operational size separations explored here provide a useful insight into the broader issues of trace metal biogeochemistry, especially those involving the composition of dissolved trace metals, and are the state-of-the-art methods. Furthermore, the dFe size separations by the two systems are ultimately quite arbitrary, because they cannot be verified by any independent method, and the use of standard compounds of known molecular weight is not appropriate because they do not match seawater compound composition, characteristics, or conditions. Despite these seemingly overwhelming limitations, the operational size separations explored here provide a useful insight into the broader issues of trace metal biogeochemistry, especially those involving the composition of dissolved trace metals, and are the state-of-the-art methods. In this section, we will review the advantages and disadvantages for each method (summarized in Table 2) and offer a framework with which the best filtration method for a given scientific question and sampling plan can be chosen.

First, as discussed in the “Introduction,” it would be ideal to match the “operational” definition of soluble/colloidal material to the “classical” definition that is used to interpret the oceanographic significance of the observed partitioning. Prioritizing this, CFF would be the better filtration method...
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Table 2. Summarized recommendations for when to use Anopore or CFF to generate size fractionated samples of dissolved elements.

<table>
<thead>
<tr>
<th>Anopore filtration is recommended when</th>
<th>CFF is recommended when</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only a small seawater volume is available</td>
<td>“Ideal” soluble/colloidal definitions are desired (CFF has smallest pore size)</td>
</tr>
<tr>
<td>Only unfiltered seawater is available</td>
<td>Large volume sFe samples are required</td>
</tr>
<tr>
<td>Easy filtration setup is desired</td>
<td>Colloidal material is desired in the aqueous phase/preconcentrated</td>
</tr>
<tr>
<td>Rapid filtering times (for small volumes) is desired</td>
<td>An automated filtration system is desired, or little attention can be paid to filtration</td>
</tr>
<tr>
<td>Colloidal material is desired in solid phase</td>
<td></td>
</tr>
<tr>
<td>Highly sorptive elements are of interest</td>
<td></td>
</tr>
</tbody>
</table>

because it has a smaller pore size. Very tiny colloids can pass through an Anopore filter and be measured as truly soluble, distorting oceanographic interpretations of the resulting partitioning data.

However, we have shown that 25% to 30% of dFe is lost from the CFF system, and there remain many open questions about how this Fe loss changes with colloidal composition and abundance. Although we believe the CFF Fe loss to come entirely from the colloidal fraction in our system, we cannot prove that this is always true (for example, is this valid in high-colloid surface waters? in all regions?), and for these reasons, we would recommend Anopore filtration whenever possible for quantitative investigations of size partitioning, as it appears to be the method with fewest unknowns. CFF also requires a rigorous cleaning procedure and can show extreme variability in colloidal concentrations as a function of both cleaning and conditioning procedures (Reitmeyer et al. 1996), and the various retention ratings of these filters leaves open the potential for batch-to-batch variability in effective filter pore size. Thus, for the best comparison between laboratories and users, Anopore filtration is likely to be the more reliable method.

There are also basic sampling restrictions that might preclude the use of CFF and favor Anopore filtration. For instance, if only small seawater volumes are available, Anopore filtration is ideal because CFF requires at least 300 mL seawater to condition the filter, increasing the seawater volume requirement to ~500 mL to generate just 60 mL of sFe sample. Anopore filters only require ~150 mL seawater to collect the same sized sFe sample. If small sFe samples are adequate, Anopore filtration is also faster than CFF (~30 min cleaning/processing time for 2 × 30 mL sFe samples using Anopore filtration compared to ~1 h of cleaning/processing time using CFF for the same sample size) and is thus better suited for high-throughput work, such as that undertaken by the GEOTRACES program. Additionally, unfiltered samples cannot be processed through CFF systems because large particles have the potential to clog the pumped system, raising the back-pressure and causing the pressure fittings to fail; thus, if pre-filtering the samples is inconvenient, Anopore filtration would be the better filtration choice. Finally, the metal-loss issues with CFF might be fatal for size partitioning studies of particle-reactive elements such as thorium and protactinium (e.g., Dai and Benitez-Nelson 2001), and thus low-surface area Anopore filtration might be preferable in these cases.

However, if larger sample volumes (1 L or more) are required, CFF is the only practical method because it takes too long to filter a large volume sample through an Anopore filter, and it is not clear that the effective pore size would remain unchanged after a >1 L sample was flushed through a single Anopore membrane. Additionally, CFF are semi-automated, and thus multiple systems can be used at once to filter more samples at a time (cutting down on the average time required to filter a single sample). Furthermore, certain chemical characterization methods might require the colloidal material to be in the aqueous phase, in which case a CFF system is the only option.

Finally, several miscellaneous factors can influence the decision of which filtration method to choose. The first of these factors is cost: CFF methods have a high initial cost (~$2000 for a complete system) but are reusable, reducing spending over the long term, while Anopore filters are individually less expensive (~$15 per filter) but accumulate expense quickly when many samples require filtration, since each filter can only be used once. Second, the laboratory bench space required for each of these two methods is quite different. Anopore filtration can be completed in a syringe filter with a straightforward for a less experienced user. CFF can also be run unattended for some periods of time, which may be beneficial for multi-tasking users, while Anopore filtration requires constant attention.

In summary, there is no straightforward recommendation for a single filtration system, as there are many reasons why either filtration system might be preferable (summarized in Table 2). The major conclusion from this study, however, is that either filtration method evolves reproducible data and is scientifically robust, and thus the choice between the two filtration methods is truly a matter of the question being asked, technical requirements in the field sampling, and/or convenience.

Conclusions and recommendations

Our work aimed to evaluate the popular Anopore and cross flow filtration techniques for size fractionation of seawater dFe samples. Anopore filtration has the advantage of small seawa-
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Our CFF system was designed to minimize Fe sorption by having a low filter surface area, running without recirculation, and conditioning with seawater before the size-fractionated filtrate was collected. Nonetheless, ~20% to 30% of the Fe was consistently lost to the CFF system, despite altering sample volumes and flow rates. A major portion of this Fe was not released from the membrane even with a small volume of 0.06N hydrochloric acid. However, results from laboratory testing, and more importantly, the close agreement between sFe concentrations in the permeate and Anopore filtrates in near-bottom waters, implies that the majority of the CFF loss is due to retention of colloidal material rather than simple sorption of soluble Fe species (although this cannot be verified in waters with higher cFe concentrations that contain a 10 kDa - 0.02 μm component). We thus recommend calculation of cFe concentration as the difference between the measured dFe and sFe concentrations, instead of using the retentate Fe concentration that neglects the lost Fe phase, although we can recommend this only for users of our same hardware and sample handling procedures. In any system where sFe can be lost or permeation is non-ideal, an analysis of Fe in the retentate solution is required. In general, we note that low CFF system blanks and proper seawater conditioning of the filter before sample collection are critical to obtaining reproducible sFe samples by CFF, and we recommend quantification of recovery on any CFF system used.

A comparison of sFe on identical seawater samples from 23 depths across the Atlantic and Pacific Oceans showed that sFe collected using CFF was 68 ± 23% of that collected using Anopore filtration, a result reflecting the larger effective pore size of Anopore filters as well as the natural variability of the 10 kDa - 0.02 μm size distribution across the two oceans. Whereas this CFF/Anopore sFe ratio could be used to first approximation to compare the bodies of literature on dFe size partitioning using the two methods, we note that there is still significant variability between filter types (especially for CFF filters) and also spatial/depth variability in the size distribution of dFe that likely makes a true resolution of existing datasets from the literature nearly impossible unless identical filtration methods were used. Only a more spatially extensive estimate of the size distribution of dFe (perhaps through an extended intercomparison effort of the types of filters described here) will ever begin to address this problem.

Finally, we recommend that Anopore filtration is perhaps the better filtration method for quantitative estimates of dFe size partitioning, given the substantial caveats and unknowns in the CFF method. Nonetheless, a full suite of factors must be considered when choosing the ideal filtration method, and we reviewed the advantages and disadvantages of each. With this final note, we emphasize that careful trace metal clean techniques were used in the generation of all data in this study, and our results demonstrate that reliably low filter blanks and the timely completion of ultrafiltration immediately after sample collection (Fitzsimmons and Boyle 2012) are critical to the reproducibility of the data.

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