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ORIGINAL PAPER

# Enhanced Dissolved Organic Matter Recovery from Saltwater Samples with Electrodialysis

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**Abstract** Complexities associated with dissolved organic matter (DOM) isolation from seawater have hampered compositional characterization of this key component of global carbon and nutrient cycles. DOM isolation efficiency by electrodialysis (ED) from salt-containing waters was optimized and evaluated on samples including coastal ocean seawater, open ocean seawater, artificial seawater from axenic cultures of marine phytoplankton, and artificial seawater samples containing standard compounds of different molecular sizes and charge. ED was performed with a system optimized for processing 2–10 L sample volumes. Additionally, the combination of ED and solid-phase extraction, using Bond Elut PPL exchange resin, was evaluated. Using only ED, the following DOC recoveries were achieved: coastal seawater, 71.3 ± 6.5 %; open ocean, 50.5 ± 3.1 %; phytoplankton cultures, 70.3 ± 12.5 %; glucose, 90.2 ± 2.1 %; EDTA, 67.5 ± 9.9 %; and vitamin $B_{12}$, 98.3 ± 1.6 %. With the combination of PPL and ED techniques, an average DOC recovery of 76.7 ± 2.6 % was obtained for coastal seawater, but this recovery was not statistically different from seawater recoveries using only ED. Comparison of C/N ratios and fluorescence excitation emission matrices taken at the beginning and end of the recovery process for coastal samples processed using only ED indicated that the final recovered material was representative of the DOM present in the original samples.

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Typical recoveries using combined PPL and ED exceed those of previous isolation methods.

**Keywords** DOM · Electrodialysis · PPL · DOC recovery

## 1 Introduction

Marine dissolved organic matter (DOM) represents one of the world's largest reservoirs of exchangeable carbon (Hansell and Carlson 2015). Most chemical and spectral characterizations of DOM in seawater are confounded by the high inorganic salt concentrations ($\sim$35 g $L^{-1}$) and comparatively low DOM concentrations ($\sim$0.5 mg $L^{-1}$) (Mopper et al. 2007). Therefore, DOM must be concentrated and isolated before most detailed compositional characterizations can proceed (Repeta 2015). Over the past few decades, many technologies have been developed to isolate DOM from marine samples ranging from selective sorbents to desalting (Green et al. 2014; Repeta 2015).

Solid-phase extraction (SPE) using polystyrene or octadecyl-silica (C-18) resins was one of the first viable methods for isolating DOM from seawater. The number of available SPE sorbents has grown to include cross-linked polystyrene (XAD-2, XAD-4, and XAD-16) and their derivatives (PPL), Isolute ENV, and polyacrylate (XAD-8) (Mopper et al. 2007; Repeta 2015). DOC recoveries and selectivity toward different molecular classes differ among SPE sorbents. Because of this selectivity, samples are sometimes processed with more than one SPE sorbent in series to maximize recovery or to fractionate the sample into different operational classes (Green et al. 2014). Some of the highest DOC recoveries with SPE have been achieved with Bond Elut PPL, averaging approximately 52 % (Table 1; Arrieta et al. 2015; Dittmar et al. 2008; Green et al. 2014; Hertkorn et al. 2013; Stubbins et al. 2012). During PPL processing, samples come into contact with dilute hydrochloric acid (pH 2–2.5) and pure methanol, which could create unknown effects on sample composition (Repeta 2015). Additionally, PPL may select for certain fractions of the DOM pool with a bias toward small and polar compounds (Arrieta et al. 2015). Another method adopted for DOM isolation is cross-flow ultrafiltration, which recovers the high molecular weight fraction (>1 kDa) of DOM most efficiently (Guo et al. 2000). While this method avoids the chemical treatments associated with SPE resin processes, it only recovers up to $\sim$30 % of DOC (Benner et al. 1992; McCarthy et al. 1993; Walker et al. 2011) and selects for larger molecules or molecular aggregates.

Advances in DOM isolation have been achieved through the coupling of reverse osmosis (RO) and electrodialysis (ED) (Chen et al. 2014; Diaz et al. 2008; Green et al. 2014; Gurtler et al. 2008; Helms et al. 2015; Koprivnjak et al. 2006; Vetter et al. 2007; Young and Ingall 2010). In this method, salts are removed from a sample by ED utilizing an alternating series of positive and negative ion-exchange membranes under the influence of an electric potential, while simultaneously reducing sample volume by RO (Vetter et al. 2007). ED/RO DOC recoveries average approximately 68 % (Table 2 for specific values and references). Most ED/RO systems built to isolate DOM from natural seawater samples are designed for large (>50 L) sample volumes and require RO to reduce final sample volumes to sizes practical for freeze-drying (3–5 L) to further concentrate the DOM (Green et al. 2014; Helms et al. 2015). This makes the systems physically large, and therefore unruly to manage. Such systems typically require a minimum volume of

**Table 1** DOC recovery with PPL

| DOC recovery (%) | Number of samples | Sample site | Depth (m) | Volume (L) | References |
|---|---|---|---|---|---|
| 74 ± 2 | 2 | Bermuda Time Series Station | 3000 | 1.507–1.975 | Stubbins et al. (2012) |
| 61 ± 3 | 10 | Natural Energy Laboratory of Hawaii Authority | 674 | 29,523 | Green et al. (2014) |
| 61 | 1 | Natural Energy Laboratory of Hawaii Authority | 21 | 1646 | Green et al. (2014) |
| 62 ± 6 | 14 | North Brazil shelf and coastal zone | Surface | 0.5 | Dittmar et al. (2008) |
| 62 ± 6 | 3 | Apalachicola River and tributaries, Florida | Surface | 1 | Dittmar et al. (2008) |
| 65 ± 6 | 50 | Apalachicola, Florida, salt marshes | Surface and 2 | 1 | Dittmar et al. (2008) |
| 43 ± 2 | 4 | Central Gulf of Mexico | 600 | 4 | Dittmar et al. (2008) |
| 43 ± 5 | 8 | Weddell Sea (surface to bottom) | Surface −1600 | 50 | Dittmar et al. (2008) |
| 37 | 4 | Atlantic ~970 km W of Angola coast | 5 | 50 | Hertkorn et al. (2013) |
| 44 | 4 | Atlantic ~970 km W of Angola coast | 48 | 50 | Hertkorn et al. (2013) |
| 40 | 1 | Atlantic ~970 km W of Angola coast | 200 | 50 | Hertkorn et al. (2013) |
| 43 | 4 | Atlantic ~970 km W of Angola coast | 5446 | 50 | Hertkorn et al. (2013) |
| ~40 | 14 | Tropical Eastern Pacific, Tropical Western Atlantic, and North Atlantic | 1000–4200 | 10 | Arrieta et al. (2015) |

approximately 3 L to prevent pump cavitation, which ultimately limits sample concentration by the RO side of the system.

For this study, a tabletop ED system was designed and constructed to process 0.5–10 L samples from a salinity (conductivity) of approximately 35 ppt (~53 mS) to approximately 0.075 ppt (~0.15 mS) in 3–5 h. This system was designed to be small and maneuverable, with the entire system occupying <1 $m^3$ and having a minimum operating volume of 200 mL. With this system, the 0.5–10 L starting volume is directly freeze-dried

**Table 2** Summary of DOC recoveries with ED/RO

| DOC recovery (%) | Number of samples | Sample site | Depth (m) | Volume (L) | References |
|---|---|---|---|---|---|
| 68 ± 2 | 9 | Natural Energy Laboratory of Hawaii | 674 | 1983 | Green et al. (2014) |
| 63 ± 4 | 5 | Natural Energy Laboratory of Hawaii | 21 | 1102 | Green et al. (2014) |
| 51[b] | 1 | Skidaway Institute of Oceanography dock Savannah Georgia | 1 | 200 | Young and Ingall (2010) |
| 67[b] | 1 | Effingham Inlet (west coast of Vancouver Island in Barkley Sound) | 78 | 120 | Young and Ingall (2010) |
| 58[b] | 1 | Effingham Inlet (west coast of Vancouver Island in Barkley Sound) | 61 | 50 | Young and Ingall (2010) |
| 74[b] | 1 | Gulfstream Off the coast of Savannah Georgia | 5 | 200 | Young and Ingall (2010) |
| 44[b] | 1 | Amundsen Sea ~ 1100 km west of the Antarctic Peninsula | 250 | 200 | Young and Ingall (2010) |
| ~60[b] | 2 | Effingham Inlet (west coast of Vancouver Island in Barkley Sound) | 61 and 78 | 50 and 120 | Diaz et al. (2008) |
| 76 | 1 | Open Atlantic—East of Gulf Stream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 71 | 1 | Open Atlantic—East of Gulf Stream | 860 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 74 | 1 | Open Atlantic—East of Gulf Stream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 63 | 1 | Gulfstream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 95 | 1 | Gulfstream | 322 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 65 | 1 | Gulfstream-CDOM Max[a] | 77 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 59 | 1 | Gulfstream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 68[c] | 1 | Gulfstream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 72 | 1 | Gulfstream | 20 | 394.1 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |

**Table 2** continued

| DOC recovery (%) | Number of samples | Sample site | Depth (m) | Volume (L) | References |
|---|---|---|---|---|---|
| 94 | 1 | Ogeechee River mouth, high tide | 2 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 78 | 1 | Gulfstream | 20 | 103.7 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 60[c] | 1 | Gulfstream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 60[c] | 1 | Gulfstream | 20 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 63[c] | 1 | Gulfstream-CDOM Max[a] | 84 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 51[c] | 1 | Gulfstream-CDOM Max[a] | 84 | 198.4 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 55 | 1 | Ogeechee River mouth, high tide | 2 | 100.5 | Gurtler et al. (2008), Koprivnjak et al. (2006) and Vetter et al. (2007) |
| 77 | 1 | N. Atlantic Upwelling Edge | 5 | 142–473 | Chen et al. (2014) |
| 68 | 1 | N. Atlantic Upwelling Core | 5 | 142–473 | Chen et al. (2014) and Helms et al. (2015) |
| 46 | 1 | N. Atlantic | 415 | 142–473 | Chen et al. (2014) and Helms et al. (2015) |
| 40 | 1 | N. Atlantic | 3000 | 142–473 | Chen et al. (2014) and Helms et al. (2015) |
| 83 | 1 | N. Pacific | 5 | 142–473 | Chen et al. (2014) and Helms et al. (2015) |
| 67 | 1 | N. Pacific | 674 | 142–473 | Chen et al. (2014) |
| 68 | 1 | N. Pacific | 674 | 142–473 | Chen et al. (2014) |
| 67 | 1 | N. Pacific | 674 | 142–473 | Chen et al. (2014) |
| 86 | 1 | N. Pacific | 3500 | 142–473 | Chen et al. (2014) and Helms et al. (2015) |

Note the recovery values below may differ from overall values given in publications because they do not include the fraction desorbed in a strong base rinse (pH = 12) of systems after sample processing. This fraction, although typically included in claimed DOC recoveries, is not typically analyzed because the presence of strong base confounds characterization methods and likely alters DOM composition

[a] CDOM is the chromophoric dissolved organic matter

[b] Assumes that the NaOH rinse fraction comprises 9.9 % of the total DOC recovered (Gurtler et al. 2008) which use the same operational procedure

[c] The electricity was pulsed during the final phase of the ED/RO process

after processing, removing the need for water volume reduction by RO. Thus, DOM losses or alterations that may occur during RO processing are avoided. DOM retention during sample processing with ED was evaluated using coastal ocean seawater, open ocean

seawater, artificial seawater from cultures of marine phytoplankton, and artificial seawater samples containing standard compounds of different molecular sizes and charge. Additionally, various operational parameters such as electrical pulsing, fluid salinity ranges, and sequence of isolation techniques were tested and optimized to improve recoveries. Given the high recoveries and potential recovery of different molecular classes by PPL relative to ED, the two techniques were combined for a subset of natural samples to improve total DOM recoveries and to recover a more representative fraction of the molecular classes present in the original DOM sample.

## 2 Materials and Methods

### 2.1 Electrodialysis

Electrodialysis was performed using a Deukum electrodialysis stack (Deukum GmbH, Frickenhausen, Germany) coupled to a circulation system built in our laboratory (Supplement Fig. S1 & Table S1). The circulation system is composed of three Iwaki WMD water pumps and associated adjustable valves used to control the flow and pressure of the sample (diluate), concentrate (water flow that receives ions from the sample), and electrode rinse (flow that maintains ionic balance between the cathode and anode). In addition to the pumps, the system includes an Oakton Cond 6+ conductivity meter with the electrode mounted in a laboratory-built housing so it could be used inline, and three high-density polyethylene (HDPE) tanks to store the diluate, concentrate, and electrode rinse. A 10-cell-pair electrodialysis stack was constructed consisting of alternating Neosepta anion AMX (strongly basic, anion permeable, high mechanical strength, 2.4 $\Omega$ $cm^2$) and Neosepta cation CMX (strongly acidic, cation permeable, high mechanical strength, 3.0 $\Omega$ $cm^2$) exchange membranes. These membranes are held between opposing flow regulating end blocks of the stack, which, respectively, house a platinized titanium mesh anode and a stainless steel cathode. Two CMX membranes were situated at the end of the stack nearest to the cathode to protect the cathode from corrosion (Pfromm et al. 1999). The membranes are separated by 0.7-mm-thick flexible turbulence promoting spacers and have a cross-sectional active membrane area of approximately 100 $cm^2$. The electric potential across the stack was supplied by a 1.2-kW Sorensen DCS150-8EM1 solid-state power supply. During the ED process, anions are pulled through the AMX membranes toward the anode, while cations are pulled through CMX membranes toward the cathode. As the ions are pulled to their respective electrodes, they move out of the diluate flow channel and into the concentrate flow channel.

#### *2.1.1 ED System Preparation and Sample Processing*

The following procedure was developed for cleaning and preparing the ED system. An electrode rinse solution is made by dissolving 60 g of sodium sulfate ($Na_2SO_4$) into 2 L of Milli-Q water (Pfromm et al. 1999). The electrode rinse tank is filled with the electrode rinse solution and the concentrate tank filled with 24 L of Milli-Q water. With all three channels in circulation, the diluate channel is rinsed 6 times with 1 L of Milli-Q water for 2 min per rinse. After the diluate channel rinses, the concentrate solution is replaced with 24 L of fresh Milli-Q water. Before sample processing, the diluate channel is rinsed with two 300-mL sample aliquots for 2 min per rinse. The pH of the electrode rinse should be

monitored before and after a sample is processed. If the pH falls outside the range of 5.5–7, the electrode rinse flow channel should be rinsed three times with Milli-Q water and the electrode rinse replaced. A pressure of 4.5 psig was maintained in all flow channels during cleaning and sample processing to prevent pressure differences across the membranes.

Based on numerous tests and optimizations, the following procedure was developed for ED processing of samples (Supplement). A sample is placed in the diluate tank and circulated through the system for 2 min to ensure good mixing before the electricity is supplied. At this point, the conductivity of the sample is measured and a 40-mL subsample is collected for DOC and total dissolved nitrogen (TN) analysis (subsample $T_{\text{initial}}$). Once the power is supplied to the system, it is vital that both the conductivity of the sample and the applied amperage are continuously monitored to ensure the electrical current per unit area of membrane does not increase above the limiting current density (LCD). The LCD can be determined by increasing the current across the stack in a stepwise manner and plotting the electrical resistance of the stack as a function of the reciprocal current (Pfromm et al. 1999). If the electrical current exceeds the LCD, ion concentrations drop to near zero at the membrane surface on the diluate side of the membranes (Pfromm et al. 1999). The absence of ions at the membrane surface results in the splitting of water molecules. The hydroxyl ions resulting from the splitting of water pass through the membranes causing a rapid increase in the pH of the concentrate and a corresponding decrease in the pH of diluate (Pfromm et al. 1999).

During sample processing, the conductivity of the concentrate and sample is continuously measured. When the conductivity of the concentrate exceeds that of the sample by approximately 2 mS, the system is briefly shut off and the concentrate is changed. Optimization experiments (Supplement) indicate that sample DOM recoveries are highest when 4 L of concentrate is left in the tank and 20 L of the concentrate replaced with Milli-Q water rather than completely replacing the concentrate with Milli-Q water. After a concentrate change, the system is turned back on and the sample is processed to the desired end point, typically a conductivity of approximately 150 µS (Supplement) which corresponds to an approximate salinity of 0.075 ppt. At the end of the ED process, a 40-mL subsample is collected (subsample $T_{\text{final}}$) to calculate the total DOC and TN recoveries. It should be noted that near the end of the process when sample conductivities are $<400$ µS, the limiting current density is difficult to define. At this point, samples were run at a current of 0.13 amps until the final target conductivity was reached. At these final stages of sample processing, it was found that reducing the electric current below 0.13 amps led to impractically long run times, while not increasing the recovery. At the end of a run, the diluate channel is cleaned by rinsing 6 times with 1 L of Milli-Q water for 2 min per rinse. If the membrane stack is not going to be used to process samples within 1 day, an approximately 100 mS NaCl brine solution is run through the electrodialysis stack to hinder biological growth in the system.

A 3-L sample volume was processed in the majority of experiments. This volume required approximately 4 h to process on the ED system, including system cleaning. The subsequent freeze-drying of this volume required about one week using a laboratory-scale freeze-drying system with a chamber just large enough to accommodate a 3-L beaker.

## 2.2 Bond Elut PPL

Bond Elut PPL, manufactured by Agilent Technologies, is a styrene–divinylbenzene (SDVB) polymer that has been modified with a proprietary nonpolar surface. The cleaning procedures below and the protocol for DOM extraction via SPE using PPL sorbent were

adapted from previous works (Arrieta et al. 2015; Dittmar et al. 2008; Green et al. 2014; Hertkorn et al. 2013; Stubbins et al. 2012) and the manufacturer's guidelines using 6-mL Bond Elut PPL cartridges (columns).

Before DOM can be extracted with PPL columns, the sorbent must first be conditioned and equilibrated (Dittmar et al. 2008). This is a 2-day process with several cycles of sorbent soaking and rinsing. On the first day, the cartridge is filled and drained three times with methanol (LC/MS Grade; 3 cartridge volumes = ~6 mL), then immediately rinsed with three cartridge volumes of Milli-Q water, and again rinsed with three cartridge volumes of methanol. The cartridge is then filled with methanol and left to soak overnight (minimum of 4 h). On the second day of conditioning, a pH 2 Milli-Q solution is made by adding 1 mL of 12 N HCl (ACS grade or higher) to 1 L of Milli-Q water. The cartridge is then rinsed three times with a cartridge volume of Milli-Q water, followed by three cartridge volumes of methanol, and finally three cartridge volumes of the pH 2 Milli-Q solution. The extraction step is immediately preformed after the cartridge final rinse.

Prior to PPL processing, samples are acidified to pH 2 with HCl (ACS grade; 12 N). Acid-washed (pH 2 Milli-Q water) Teflon tubing (ID 2 mm), Luer adaptors, and fittings are attached to the cartridges and used to siphon the sample through the PPL. The flow is adjusted to approximately 10 mL $min^{-1}$. Initial and final weights of the seawater samples are recorded to determine the total processed volumes. Once the entire sample has passed through the column, the PPL columns are rinsed with three cartridge volumes of pH 2 Milli-Q water to remove any remaining salts. Zero-grade air is then passed through the cartridge at approximately 10 psig in an effort to dry the resin before elution. Once the columns are dry, DOM sorbed to the column is eluted with approximately 9 mL of methanol. The volumes of the eluted methanol are measured gravimetrically. Eluted samples are stored at −20 °C until processing.

To calculate final DOM recoveries, a known aliquot of the methanol extract is placed in a vented drying oven at 40 °C for 24 h to dry the sample. The sample is then resuspended in 20 mL of pH 2 Milli-Q water and sonicated for 10 min. Concentrations of DOC and TN are then determined using a Shimadzu TOC-VCSN total organic carbon (TOC) analyzer (see Sect. 2.5 below).

## 2.3 Combined PPL: ED Natural Samples

DOC recovery using the combined PPL/ED technique was evaluated using saltwater samples collected between the dates of 09/03/2014 and 12/10/2014 from the Skidaway River (31°59′24.1″N, 81°01′20.9″W) off the main dock of the Skidaway Institute of Oceanography located near Savannah Georgia, USA. The samples were collected 1 m below the water surface at approximately 1 h after high tide to obtain waters when they are at their maximum salinity during a tidal cycle. The samples, which had initial conductivities between 38 and 47 mS, were filtered through an acid-cleaned Whatman Polycap TC Filter Capsule with pore size 0.2 µm and stored in acid-washed Nalgene 20 L carboys at 4 °C before processing.

Seven samples were collected on different days throughout September 2014 and processed using ED within 3 days of collection. Two approximately 25-L samples were collected on 10/21/14 and 11/25/14 and placed in cold storage in acid-washed carboys. Three separate 2-L aliquots of the samples collected on 10/21/14 were processed on 10/27/14, 11/13/14, and 11/24/14 with solely ED. One 2-L aliquot of the sample collected on 10/21/14 was processed with PPL and subsequently processed with ED on 11/26/14. Three separate 2-L aliquots were taken from the sample collected on 11/25/14 and processed with

PPL and subsequently processed with ED on 11/28/14, 12/10/14 (2 runs). Three separate 2-L aliquots were taken from the sample collected on 11/25/14 and processed solely using ED on 11/30/14, 12/3/14, and 12/7/14. See Table S2 for sample details. Note that the samples processed with PPL then ED were acidified to pH 2 before processing on PPL and not neutralized before ED processing.

## 2.4 Standards and Samples for ED Optimization

Samples of artificial seawater from phytoplankton cultures and open ocean seawater (described below) were processed with ED to evaluate and optimize the DOM recovery of samples presumably representing freshly produced and extensively cycled DOM, respectively. Additionally, to evaluate the recovery of molecules of different size and charge, three standard reagent-grade compounds, including D-(+)-Glucose ($C_6H_{12}O_6$; Sigma-Aldrich $\geq$99.5 %), ethylenediaminetetraacetic acid (EDTA; $(HO_2CCH_2)_2$-$NCH_2CH_2N(CH_2CO_2H)_2$; Sigma-Aldrich, ACS grade, 99.4–100.6 %), and vitamin $B_{12}$ ($C_{63}H_{88}CoN_{14}O_{14}P$; Sigma-Aldrich, $\geq$98 %) were each individually dissolved in artificial seawater (Grasshoff et al. 1999; Kester et al. 1967) and processed with ED.

Two species of marine phytoplankton obtained from the Bigelow National Center for Marine Algae and Microbiota (NCMA) were grown in axenic laboratory cultures and processed with ED. *Thalassiosira pseudonana* (CCMP 1335), a diatom, was cultured in Si replete $^{f}/_{2}$ media (Guillard 1975; Guillard and Ryther 1962) under axenic conditions in artificial seawater (Grasshoff et al. 1999; Kester et al. 1967). The biomass of *T. pseudonana* was harvested via centrifugation at 4500 rpm for 5 min, and the supernatant containing DOM exudates was filtered through a glass fiber filter (Whatman 1825-150 GF/F, pore size 0.7 μm) before processing with ED. *Emiliania huxleyi* (CCMP 371), a coccolithophore, was cultured in $^{L1}/_{25}$ media (Guillard and Hargraves 1993) under axenic conditions in artificial seawater (Grasshoff et al. 1999; Kester et al. 1967). *E. huxleyi* cells were harvested onto a polycarbonate filter (pore size 0.2 μm), and the filtrate containing DOM exudates was frozen until ED processing. These phytoplankton were cultured under different conditions to produce the following treatments: nutrient replete, N-stressed (diatom only) and P-stressed, as part of a different study and sampled in the late logarithmic phase of growth. Three separate cultures were grown for each nutrient treatment and were processed individually with ED.

Open ocean seawater samples were collected using the underway pumping system of the *R/V Savannah* in Atlantic Ocean waters located approximately 100 km east of Savannah, Georgia, on 1/13/14. The samples were filtered through an acid-cleaned Whatman Polycap TC Filter Capsule (0.2 μm) and stored in several 20-L acid-washed Nalgene carboys at 4 °C before processing with the ED. Triplicate aliquots of the open ocean seawater sample and all other samples in this study were processed in accordance with the protocol described above.

## 2.5 Sample Analysis

DOC concentrations were used to monitor DOM recovery during sample processing. DOC concentrations in subsamples (40 mL) were measured as non-purgeable organic carbon using high-temperature catalytic oxidation with a Shimadzu TOC-VCSN or TOC-VCPH analyzer (Benner and Strom 1993; Grasshoff et al. 1999). DOC recoveries were calculated by dividing the mass of DOC at the end of processing (Sample $T_{\mathrm{Final}}$) by the mass of DOC initially supplied (Sample $T_{\mathrm{Initial}}$). Calculated recoveries also account for the removal of

DOC in subsamples taken for measurements (Table S2). The Shimadzu instruments above were also used to measure TN concentrations in the subsamples. During ED processing, >99 % of the dissolved inorganic nitrogen (DIN) was removed from the sample. Thus, TN concentrations determined at the end of the ED process for all sample types more accurately represent dissolved organic nitrogen (DON) concentrations. To calculate initial DON concentrations in seawater and cultured phytoplankton samples, the initial DIN concentrations must be subtracted from the initial TN concentrations. For seawater samples collected at the Skidaway dock, DIN was estimated using mean annual $NO_3^-$ and $NH_4^+$ concentrations of 2.2 and 2.7 μM, respectively, for the Skidaway River estuary (Verity 2002). Initial DIN concentrations for cultured phytoplankton filtrates were measured as $\Sigma NO_x$ using anion chromatography (Metrohm A Supp 5 150.0/$x$ 4.0 mm) coupled with ultraviolet detection (Beckman Coulter DU 720 detector) and a NaCl eluent (Beckler et al. 2014). Initial and final carbon-to-nitrogen (C/N) ratios were calculated by dividing the DOC concentrations by the dissolved organic nitrogen.

$\delta^{13}C$ values for both ED and PPL isolates were determined at the Skidaway Institute Scientific Stable Isotope Laboratory (SISSIL) using a Delta V plus (2007) light stable e mass spectrometer coupled to a ThermoFisher Flash EA (Fry et al. 1996). Freeze-dried aliquots of extracts were weighed into tin combustion cups prior to analysis. Samples were calibrated using an internal laboratory standard of chitin (Sigma-Aldrich), which was in turn referenced to PDB using the USGS 40 isotopic standard. Typical precision on standards was 0.2 ‰ for isotopic composition.

Fluorescence excitation emission matrices (EEM) spectra for Skidaway dock seawater samples were measured on subsamples collected during ED processing in 1-cm quartz cells by a Horiba AquaLog VS140 (CCD1) spectrofluorometer. EEMs are produced from multiple emission spectra collected at successively increasing excitation wavelengths (Fellman et al. 2010; Stedmon and Bro 2008). EEMs can be used to categorize a subset of colored dissolved organic matter known as fluorescent dissolved organic matter (Stedmon and Álvarez-Salgado 2011; Stubbins et al. 2014). The peaks present on an EEM surface correlate to different molecular classes within the sample (Coble et al. 1990, 1998; Fellman et al. 2010; Jaffe et al. 2014; Wagner et al. 2015). Florescence and absorbance measurements for EEMs were processed in similar fashion to previous studies (Coble et al. 1990, 1998; Fellman et al. 2010; Stubbins et al. 2014). However, the Horiba Aqualog and its included software allowed for simultaneous data collection and EEM generation. A Milli-Q water blank EEM was subtracted from each sample EEM to remove Raman scatter peaks (Coble et al. 1998). Rayleigh scattering was removed from EEMs by smoothing with surrounding emission values.

## 3 Results

For the 13 seawater samples collected at the Skidaway dock, DOC recoveries using only ED averaged 71.3 ± 6.5 % (Fig. 1). Two of the seawater samples collected at the Skidaway dock were divided into three 2-L aliquots, with each aliquot processed separately through ED to evaluate reproducibility of DOC recovery. Recovery was 71.1 ± 4.8 % for the sample collected on 11/25/2014 and processed on 11/30/14, 12/3/14, and 12/7/14 and 69.8 ± 11.6 % for the sample collected on 10/21/2014 and processed on 10/27/14, 11/13/14, and 11/24/14. The absence of obvious trends in DOC recovery related to length of

sample storage before processing (Fig. 1) suggests that storage of up to one month at 4 °C is acceptable. A data summary of all ED experiments is shown in Table S2.

Three separate 2-L aliquots taken from the sample collected on 11/25/14 and processed with PPL achieved an average recovery of 39.2 ± 9.7 % (Fig. 2). Subsequent ED processing of these three samples on 11/28/14, 12/10/14 (2 runs) recovered an additional 38.0 ± 4.7 % of the DOC to obtain an overall recovery for these replicate samples of 77.2 ± 3.0 %. For the one 2-L aliquot of the sample collected on 10/21/14 and processed on 11/26/14, PPL processing recovered 34.9 % of the DOC and subsequent processing with ED recovered an additional 40.5 % for an overall DOC recovery of 75.4 %. Overall, the average DOC recovery using solely PPL on four Skidaway dock seawater samples was 38.1 ± 2.3 % (Fig. 2). Subsequent ED of all samples pre-treated with PPL recovered an additional 38.6 ± 3.8 % of the DOC to obtain an overall recovery of 76.7 ± 2.6 % (Fig. 2).

Open ocean samples collected off the coast of Savannah and processed in triplicate with ED had recoveries of 50.5 ± 3.1 %. The average DOC recovery using ED for five individual cultures of *T. pseudonana*, grown under identical nutrient replete conditions, averaged 64.8 ± 14.4 % (Fig. 3). The average DOC recovery using ED for four individual cultures of *T. pseudonana*, grown under identical P-stressed conditions, averaged 64.1 ± 9.1 % (Fig. 3). The average DOC recovery using ED for three individual cultures of *T. pseudonana*, grown under identical N-stressed conditions, averaged 69.1 ± 5.0 % (Fig. 3). The average DOC recovery using ED for three individual cultures of *E. huxleyi*, grown under nutrient replete conditions, averaged 84.4 ± 7.4 % (Fig. 3). The average DOC recovery using ED for three individual cultures of *E. huxleyi*, grown under P-stressed conditions, averaged 74.6 ± 15.3 % (Fig. 3). Overall, the average DOC recovery using ED for all cultured phytoplankton filtrates averaged 70.3 ± 12.5 % (Fig. 3). A summary of ED data for culture experiments is shown in Table S2.

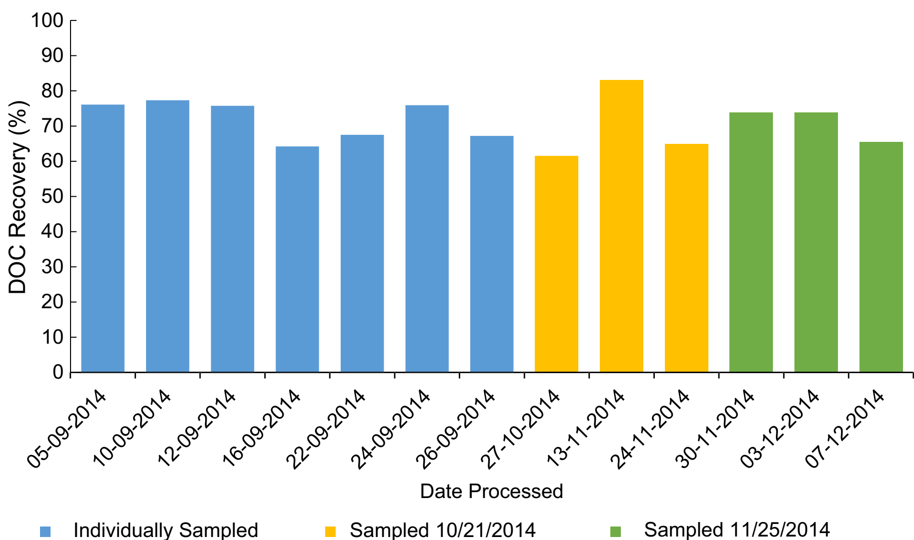


**Fig. 1** DOC recoveries of Skidaway dock seawater samples using ED only. *Blue points* are for individual samples collected September 2014. *Yellow* and *green points* are for triplicate analysis of samples collected on 11/25/2014 and 10/21/2014, respectively

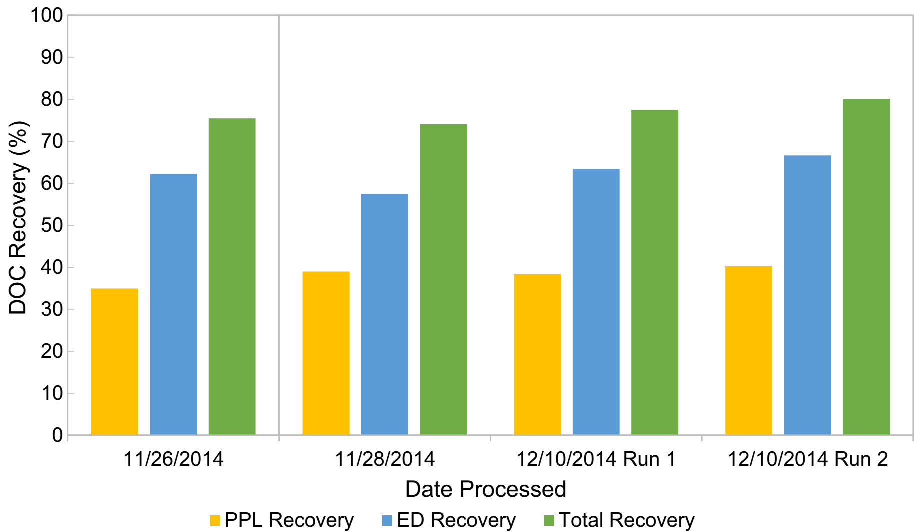


**Fig. 2** DOC recoveries for Skidaway dock seawater samples processed with PPL then ED. The *left side* of the figure displays the single 2-L aliquot of the sample collected on 10/21/14 and processed with PPL and subsequently processed with ED on 11/26/14. The *right side* of the plot displays the three separate 2-L aliquots taken from the sample collected on 11/25/14 and processed with PPL and subsequently processed with ED on 11/28/14, 12/10/14 (2 runs in 1 day)

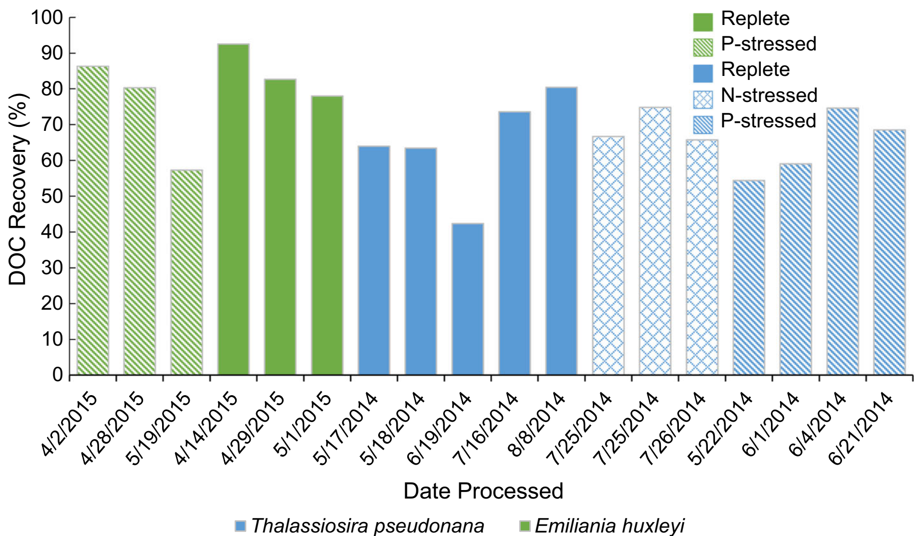


**Fig. 3** DOC recoveries of phytoplankton filtrates cultured under different growth conditions

Standard compounds were processed with ED in triplicate. Glucose, EDTA, and vitamin $B_{12}$ had DOC recoveries of 90.2 ± 2.1, 67.5 ± 9.9, and 98.3 ± 1.6 %, respectively. A summary of ED data for individual compound experiments is shown in Table S2.

Initial C/N ratios before ED processing averaged 19.3 ± 3.6 for Skidaway dock seawater samples and final C/N ratios after ED processing averaged 18.9 ± 1.5, excluding one sample taken on 12/7/2014 (Table 3). Other than the 12/7/2014 sample, DOM C/N initial and final ratios are identical within error (Table 3). Initial and final C/N ratios calculated for *E. huxleyi* are on average within 2.5 % of each other (Table 3). Note TN was not measured on *T. pseudonana* filtrates. Initial and final C/N ratios calculated for the EDTA and vitamin $B_{12}$ standards are on average within 4.8 % of each other (Table 3).

$\delta^{13}C$ values for seawater samples processed with ED only and PPL only average −29.5 ± 1.4 and −25.0 ± 0.0, respectively (Table 4). $\delta^{13}C$ values determined for combined PPL/ED averaged −28.8 ± 1.7 (Table 4). EEMs normalized to their respective maximums for the initial and final subsamples collected during ED processing have peaks with the same proportional intensities that are situated in the same region on the spectral surface (Fig. 4).

## 4 Discussion

DOC recovery, using only ED, was assessed for different DOM types, including samples of natural seawater and filtrates from cultures of two common marine phytoplankton axenically grown under a variety of nutrient conditions. In contrast to both coastal and open

**Table 3** Molar C/N ratios for samples processed solely with ED

| Sample | Date collected | Date processed | DOC recovery (%) | Initial C/N | Final C/N |
|---|---|---|---|---|---|
| P-stressed *E. huxleyi* | – | 5/19/2015 | 57.3 | 9.2 | 8.0 |
| P-stressed *E. huxleyi* | – | 5/1/2015 | 78.0 | 3.5 | 3.1 |
| P-stressed *E. huxleyi* | – | 4/29/2015 | 82.7 | 3.2 | 3.5 |
| Replete *E. huxleyi* | – | 4/14/2015 | 92.5 | 5.9 | 5.3 |
| B12 | – | 11/1/2015 | 100.0 | 3.9 | 4.0 |
| B12 | – | 10/31/2015 | 97.0 | 4.0 | 4.1 |
| B12 | – | 10/30/2015 | 97.9 | 4.1 | 4.1 |
| EDTA | – | 10/28/2015 | 70.4 | 4.6 | 5.0 |
| EDTA | – | 10/26/2015 | 75.6 | 4.7 | 5.1 |
| EDTA | – | 10/24/2015 | 56.3 | 4.8 | 5.6 |
| SKIO Dock | 9/25/2014 | 9/26/2014 | 67.2 | 15.2 | 19.7 |
| SKIO Dock | 9/23/2014 | 9/24/2014 | 75.9 | 14.9 | 17.8 |
| SKIO Dock | 9/22/2014 | 9/22/2014 | 67.5 | 14.5 | 17.8 |
| SKIO Dock | 9/15/2014 | 9/16/2014 | 64.2 | 17.5 | 16.1 |
| SKIO Dock | 9/9/2014 | 9/12/2014 | 75.7 | 18.0 | 18.4 |
| SKIO Dock | 9/10/2014 | 9/10/2014 | 77.3 | 18.1 | 17.7 |
| SKIO Dock | 9/2/2014 | 9/5/2014 | 76.1 | 20.9 | 18.9 |
| SKIO Dock | 11/25/2014 | 12/7/2014 | 65.5 | 61.5 | 32.5 |
| SKIO Dock | 11/25/2014 | 12/3/2014 | 73.8 | 26.5 | 21.2 |
| SKIO Dock | 11/25/2014 | 11/30/2014 | 73.9 | 21.8 | 19.0 |
| SKIO Dock | 10/21/2014 | 11/24/2014 | 64.9 | 21.2 | 19.1 |
| SKIO Dock | 10/21/2014 | 11/13/2014 | 83.1 | 22.0 | 20.2 |
| SKIO Dock | 10/21/2014 | 10/27/2014 | 61.5 | 21.5 | 20.9 |

**Table 4** $\delta^{13}C$ values for Skidaway dock seawater samples processed on ED and PPL

| Isolation technique | Date collected | Date processed | $\delta^{13}C$ (‰) |
|---|---|---|---|
| ED | 9/2/2014 | 9/5/2014 | −29.8 |
| ED | 9/15/2014 | 9/16/2014 | −29.7 |
| ED | 9/22/2014 | 9/22/2014 | −32.1 |
| ED | 9/25/2014 | 9/26/2014 | −28.0 |
| ED | 9/23/2014 | 9/24/2014 | −28.1 |
| PPL | 10/21/2014 | 11/26/2014 | −25.0 |
| PPL | 11/25/2014 | 11/28/2014 | −25.0 |
| PPL | 11/25/2014 | 12/10/14 Run 1 | −25.0 |
| PPL | 11/25/2014 | 12/10/14 Run 2 | −25.0 |
| PPL/ED | 10/21/2014 | 11/26/2014 | −26.9 |
| PPL/ED | 11/25/2014 | 11/28/2014 | −27.8 |
| PPL/ED | 11/25/2014 | 12/10/14 Run 1 | −30.0 |
| PPL/ED | 11/25/2014 | 12/10/14 Run 2 | −30.5 |

ocean DOM, the DOM produced in these axenic phytoplankton cultures is not modified by microbial decomposition and, as such, would likely be compositionally different relative to natural seawater samples. The effect of DOM composition on recovery is not clear. Comparison of the Skidaway dock seawater samples with an average DOC recovery of 71.3 ± 6.5 % to the phytoplankton cultures with average DOC recoveries of 70.3 ± 12.5 % (Table S2) suggests that differing proportions of freshly produced DOM in a sample does not strongly influence overall DOC recoveries. However, comparison of open ocean DOM of 50.5 ± 3.1 % to phytoplankton culture recoveries could indicate compositional influence. There was also no clear relation between nutrient availability during growth of the phytoplankton cultures and DOC recovery (Fig. 3).

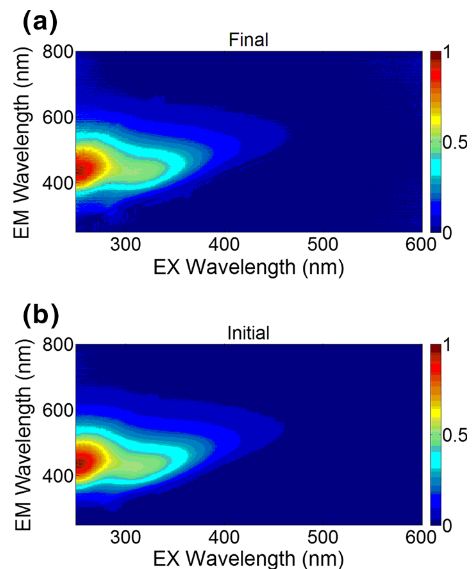


**Fig. 4** Composite average EEM, normalized to their respective maximums of all Skidaway dock seawater samples processed with ED only; **a** initial subsample, **b** final subsample

The recovery of three molecules of differing molecular weight, size, and charge was examined using only ED. A 90.2 ± 2.1 % recovery of glucose (molecular weight 180.2) was achieved using ED. This is in contrast to ultrafiltration techniques, for which such low molecular weight molecules are below membrane size cutoffs (typically 1000 Da) and are therefore expected to pass through the membranes resulting in extremely poor recoveries (Repeta 2015). EDTA, another low molecular weight molecule (molecular weight 292.2), has a charge of −4 at the ED operating pH (pH = 7). The lower recovery of EDTA (67.5 ± 9.9 %) relative to electrically neutral glucose likely results from attractive forces pulling negatively charged EDTA across the membrane toward the anode. Vitamin $B_{12}$ is a larger molecule (molecular weight 1355.4) than both glucose and EDTA, but it is electrically neutral at the pH value maintained during ED processing. The size and charge characteristics of $B_{12}$ are both likely factors in its essentially complete (98.3 ± 1.6 %) recovery during ED. The difference between glucose, EDTA, and $B_{12}$ recoveries suggests that both size and charge play a role in the molecular retention during ED, with small, charged molecules having the lowest recoveries.

Using only ED, the high recoveries achieved on all sample types suggest that, unlike SPE and cross-flow ultrafiltration, preferential size and/or compositional selection is greatly reduced during the DOM isolation process. Reduced compositional and size selectivity during ED relative to other methods is further reflected in the similarity of C/N ratios and EEM spectra of initial and final subsamples. The percent difference between initial and final molar C/N ratios obtained using ED was small in all sample types: Skidaway dock seawater 12.1 %, phytoplankton cultures 2.5 %, EDTA 9.6 %, and $B_{12}$ 0.1 %. This suggests that ED isolation of DOM is not strongly selective in terms of the C/N ratio of molecules retained. The peaks on the initial and final EEMs are situated in the same regions and have the same proportional intensities, suggesting that ED is not selective toward the molecular classes present in the fluorescent DOM fraction characterized by this method (Fig. 4). Examination of two specific excitation wavelengths, 250 and 300 nm, normalized to their respective maximums also shows a high correspondence ($R^2 > 0.99$) of initial and final samples (Supplement Fig. S2). This further confirms the similarities between the initial and final EEM analyses. Average $\delta^{13}C$ values of −29.5 ± 1.4 and −25.0 ± 0.0 for DOC isolated by ED and PPL, respectively (Table 4), suggest a difference in the DOC isolated by the two methods. There has been limited work on the $\delta^{13}C$ composition of different molecular components of DOC (Bauer 2015); however, the lighter isotopic composition of DOC isolated by ED may suggest that it contains higher proportions of isotopically light hydrophobic components like lipids. DOC collected at the Skidaway dock site would be expected to have substantial terrestrial influences given its estuarine location. The average C/N ratio of 19.3 ± 3.6 is consistent with terrestrial influence. It was not possible to determine the $\delta^{13}C$ of the whole water DOC using the method employed. Final $\delta^{13}C$ values for the samples processed with ED are −29 ± 1.4 ‰ and with PPL/ED are −28.8 ± 1.7 ‰, which compare to $\delta^{13}C$ values for freshwater particulate organic matter (−30.4 to −34.5 ‰) from the Savannah River (Flite et al. 2008) and terrigenous DOM in general (−25 to −30 ‰) (Boutton 1991; Williams and Gordon 1970) than to autochthonous, oceanic DOM (−18 to −28 ‰) (Beaupre 2015). As such, the $\delta^{13}C$ values of ED and PPL/ED DOM are consistent with the $\delta^{13}C$ values expected for estuarine samples.

The small increase in DOC recovery from 71.3 ± 6.5 % using only ED relative to the recovery of 76.7 ± 2.6 % (Fig. 2) for the combined PPL, ED process is not statistically significant (Student's $t$ test, $p = 0.13$). Thus, given the time-consuming cleaning and sample processing times associated with PPL techniques, there is little overall benefit in

processing samples with combined ED and PPL. Both ED and PPL/ED recovery values are somewhat higher than the average value for studies applying combined ED/RO, 68 % (Table 2). The small-volume design of the system used in this study eliminates the need for RO to reduce sample volumes for subsequent freeze-drying. Elimination of RO processing greatly reduces the membrane surface area contacted by the sample, which may increase overall recovery and eliminate the need for a base rinse of the system to desorb DOM from RO membranes. It is difficult to fully drain the sample from the system at the end of processing; therefore, the overall sample recovery was likely higher in this study because of the lower system volume, partly achieved through the elimination of RO. Although freeze-drying times could be reduced by using RO, the issues described above counteract the potential benefits of reduced freeze-drying times. Studies using combined ED/RO (Table 2) were performed on systems that were designed to process approximately 200-L samples. With system cleaning, processing of such large-volume samples requires approximately 18 h per sample (excluding freeze-drying). In comparison, the small-volume ED system developed for this study required approximately 4 h to process one sample with cleaning (excluding freeze-drying). This allows for greater analytical replication and for the processing of smaller samples, such as the algal cultures examined in this study. The smaller volume processed by the ED system used in this study also makes it physically much smaller than the ED/RO systems used in previous studies. This greatly facilitates system portability for processing samples on a ship or in the field.

## 5 Conclusions

ED processing of natural and artificial seawater samples in a system optimized for small volumes (0.5–10 L) achieved reproducible DOC recoveries typically greater than those obtained using the best previously applied techniques including PPL resins, cross-flow ultrafiltration, and ED/RO. Sample processing by ED takes 3–5 h, and small starting sample volumes alleviate the need for RO to reduce sample volumes. Similar DOC recoveries of the Skidaway dock seawater samples and axenic phytoplankton culture artificial seawater samples suggest that differing proportions of freshly produced molecules does not influence recoveries. Comparison of initial and final C/N ratios, and EEMs suggest the recovered DOM is compositionally representative of the DOM initially present in the samples.

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