Accepted Manuscript
Version of record at: https://doi.org/10.1021/acsearthspacechem.2c00220

# Organic Signatures of Surfactants and Organic Molecules in Surface Microlayer and Subsurface Water of Delaware Bay

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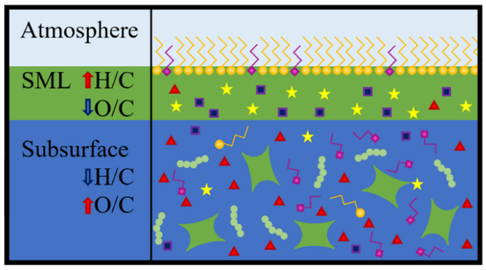
**Abstract**

Surface-active species are present in marine waters and can accumulate in the surface microlayer (SML). Surfactants are amphiphilic organic compounds that reduce surface tension at an interface. Current work is investigating the link between the molecular composition of surfactants in the SML and those in subsurface waters, as well as the differences in surfactant molecular composition across different water types. In this study, subsurface water and SML samples were collected on three sampling days at three sites in the Delaware Bay: the Mouth of the Bay, Mid-Bay, and the marsh-influenced Broadkill River. Organic matter was extracted from the SML and subsurface water using two solid-phase extractions (graphitized carbon and C18) and then analyzed using tensiometry and high-resolution mass spectrometry, in positive and negative ionization modes. Here, we show that molecules with high H/C are preferentially enriched in the SML compared to the subsurface waters. We demonstrate that the measured organic extracts contribute to lower surface tensions in the SML. A rainfall event led to increased terrestrial runoff and mixing that altered the composition of the organic molecules in the SML and subsurface waters of the Broadkill River site, and the composition of the extracted organic molecules varied across sampling days. These results imply that the surfactant compositions are not uniform across sampling regions or from the subsurface to the SML.




**Keywords**: microlayer, surfactants, mass spectrometry, surface tension, Delaware Bay, estuarine water

## 1. INTRODUCTION

Surfactants are a class of organic molecules that have surface-active properties that reduce the surface tension of a solution. Surfactant molecules are composed of both a hydrophilic head group and a hydrophobic tail group. These properties allow surfactants to aggregate and form a monolayer at the air−water interface, altering the surface tension and reducing the free energy at the interface.[1] The influence of individual surfactants on the surface tension of an air−water interface is dependent on both their chemical structure and concentration.[2,3]

Surfactants have been observed in marine environments, such as the open ocean[4−8] and estuarine waters.[9] Sources of surfactants in marine environments include production from phytoplankton[10,11] and bacteria,[12] terrestrial runoff,[13] and discharge of water treatment facilities.[14] The input of natural and anthropogenic surfactants to marine environments can change the chemical composition of the surfactants and can in turn influence biogeochemical cycles, such as the air−sea gas exchange[15−18] and aerosol particle production.[4,5,15,16]

Marine surfactants can influence the ocean surface by aggregating at the sea-surface microlayer (SML),[6,9,19] where they can modulate gas transfer and influence marine aerosol production. Surfactants in the SML can influence the air−sea gas exchange by reducing the transfer velocity of gases,[17,18] such as carbon dioxide[18,20] and oxygen.[21] While some studies have measured surfactants in the SML, their exact influence on air−sea gas fluxes has not been constrained.[12,17] The molecular composition of the surfactants in the SML likely contributes to the properties, such as surface tension depression and the formation of monolayers at the surface, that modulate the air−sea fluxes.[12,20] This in turn influences the ability of models to accurately predict the carbon dioxide budget on a global scale.[20] Surfactants in the SML can also undergo photochemical reactions that produce volatile organic compounds that are emitted into the atmosphere. These compounds can react to form marine secondary organic aerosols[22] or contribute to the aging of aerosol particles in the marine boundary layer.[23,24] The SML can also directly influence primary marine aerosol particles produced through the bubble bursting process.[25,26] Surfactants in the SML can modulate the time a bubble spends on the surface of the ocean before bursting, which affects the thickness of the bubble film and the subsequent size and composition of the aerosol particles produced.[2,27] The organic compounds in the SML can be emitted in the aerosol particles, contributing to surfactant coatings on atmospheric particles.[28,29] The presence of surfactants in the SML can also lead to and stabilize ocean foams, increasing their lifetimes on the ocean surface and further affecting the production of primary marine aerosol particles.[30] Together, these demonstrate the importance of characterizing the molecular composition of surfactants in the SML and subsurface waters with the ultimate goal of understanding their influence on air−sea fluxes.

In recent years, there have been multiple studies focused on characterizing and quantifying surfactants found in marine waters. In some studies, the presence of surfactants in seawater was confirmed by measuring its surface tension using a tensiometer, and surface tension depressions were calculated as the difference between the theoretical surface tension of pure salt water and the measured surface tension of the seawater.[4,31,32] These studies concluded that an increase in surface tension depression was due to the presence of organic surfactants. Voltammetry has also been used to measure the total concentration of surface-active substances in the SML[6,19] and seawater.[6−8,19] Other studies have used colorimetry and ultraviolet−visible (UV−vis) spectroscopy to quantify total surfactant concentrations and their ionic class fractions, including anionic, cationic, and nonionic surfactants, in the SML,[5,9,29] seawater,[4,5] and estuarine water.[9] Additionally, Fourier-transform infrared (FTIR) spectroscopy has been used to measure the functional group composition of surfactants in marine foams.[30] To identify specific surfactant-like compounds in marine waters, recent work has been done using high-resolution mass spectrometry techniques.[33,34]

Solid-phase extraction techniques have been developed that target organic molecules to separate them from the subsurface water and SML for characterization. Common solid-phase extraction techniques include commercially available cartridges with different sorbent materials, such as styrene divinyl benzene polymer (i.e., PPL) or hydrocarbons with 8 or 18 carbons (C8 or C18) bonded to a silica base, with different pore sizes. In a comparison of commonly used solid-phase extraction techniques, PPL was found to be the most efficient at extracting general marine DOM from marine samples, but C18 was found to isolate similar organics, excluding nitrogen-rich groups.[35] Further studies have developed methods to target extractions of specific surfactant classes. Burdette and Frossard[34] used solid-phase extraction and demonstrated high extraction efficiencies of anionic and nonionic surfactants using C18 sorbent material and high extraction efficiencies of cationic surfactants using graphitized carbon sorbent material. Their method was used to extract DOM from estuarine water that had surfactant-like formulas comparable to tentatively identified surfactants from marine sources collected by Cochran, et al.[33]

Mass spectrometric methods have the ability to characterize the molecular compositions of potential surfactants. The combination of tensiometry and mass spectrometry can confirm the presence of surfactants and identify their formulas. Mass spectrometry is commonly used to characterize DOM found in marine environments.[35−38] With the use of high-resolution mass spectrometry, chemical formulas can be assigned to natural DOM signals.[38,39] These formulas have previously been used to broadly classify organic compounds into groups based on hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios.[33,37,40,41] Additionally, using tandem mass spectrometry techniques, ion fragmentation can be used to identify the chemical structures of individual DOM signals. The use of both positive and negative ionization modes contributes unique information about the composition of DOM.[42]

Previous studies using high-resolution mass spectrometry have observed organic compounds in the SML to have high H/C and low O/C values.[33,36,38] Other studies have identified sulfur-containing molecules[36] and fatty acids[33,38] in marine organic compounds that may be surfactant-like.

The Delaware Bay is a marine site that has been extensively monitored, including studies of long-term DOM trends,[43] riverine input into the estuary and Middle Atlantic Bight,[44] and characterizations of DOM along a salinity gradient of the main estuary axis.[45] The estuary has multiple riverine inputs that can contribute terrestrial DOM into the estuary.[46] Photochemical degradation of terrestrial DOM within an estuary can lead to the photoproduction of carbon monoxide and carbon dioxide and alter the chemical structure of remaining DOM.[47,48] However, a different study using ultrahigh-resolution mass spectrometry found that for the Delaware Bay, DOM processing is not significantly impacted by photochemical reactions.[45] While DOM in the estuary has been studied extensively, new studies are starting to focus more on characterizing the chemical composition of the surfactant-like molecules in the Delaware Bay.

In this study, we identify properties of surfactants in SML and subsurface estuarine water in the Delaware Bay. We use a targeted extraction method with two different solid-phase extraction cartridges (graphitized carbon and C18) to collect anionic, cationic, and nonionic surfactant-like organic molecules and characterize them with high-resolution mass spectrometry in both the positive and negative ionization modes. The surface tension of each water and SML sample was used to confirm the presence of surfactants, and a surface tension depression was calculated to normalize surface tensions for different salinities throughout the bay. This combination of targeted extractions, measurements of surface tension, and characterization with high-resolution mass spectrometry has not been previously reported for samples of this type. We compare the surfactant-like properties of organic molecules extracted from the SML and subsurface water samples collected from three different locations within the Delaware Bay estuary over a 3-day period, including variability in rainfall and wind speeds.

## 2. METHOD

**2.1. SML and Subsurface Water Collection.** SML and subsurface water samples were collected from three different locations within the Delaware Bay, as shown in Figure 1. The sampling sites include the Mouth of the Bay (38.76886°N, 74.93893°W), the middle of the bay (38.99203°N, 75.17441°W) referred to here as Mid-Bay, and within the Broadkill River marsh (38.79660°N, 75.17485°W). SML and subsurface water samples were collected daily from each site over a 3-day period from June 12, 2019, to June 14, 2019, onboard the *R/V Joanne Daiber* through the University of Delaware School of Marine Science and Policy. The three sampling days will be referred to as Day 1, Day 2, and Day 3 to emphasize the dates relative to each other. The focus of this study is on Day 1 and Day 3 to highlight the day-to-day differences to the Day 3 samples caused by the wind and rainfall.

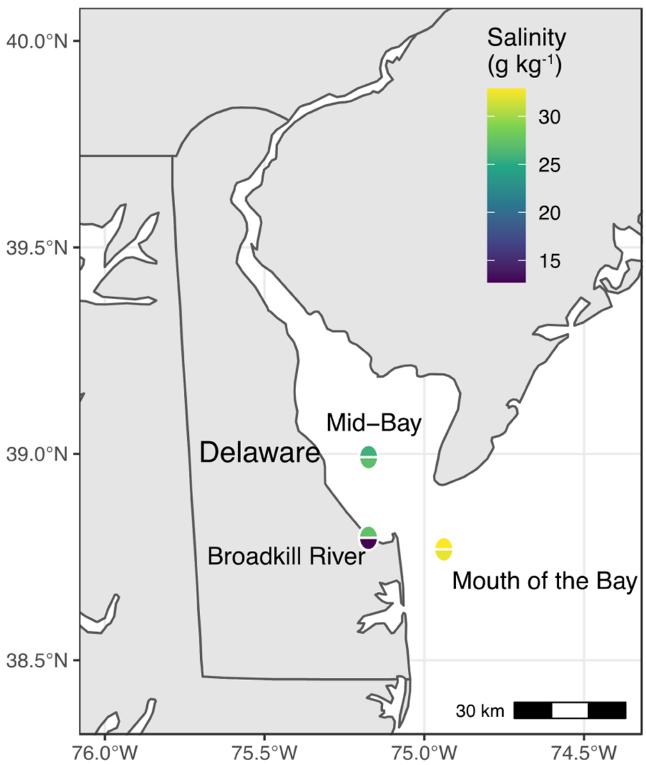



**Figure 1.** Map of the sampling locations in Delaware Bay. The markers represent the measured salinity during sampling with the top representing Day 1 and the bottom representing Day 3.

The SML was sampled using the glass plate method,[49] which collects the top 20−150 μm of the ocean's surface.[16,49] This method is described by Coffey[50] and briefly here. The SML was collected by dipping an acid-washed (1 M HCl) glass plate with a surface area of 1782.86 $cm^2$ into the water vertically until submerged and then pulling it back up vertically. The excess water dripped off the plate initially for a period of ∼5 s, and then an acid-washed Teflon squeegee was used to scrape the organic-rich film of SML into a precleaned 300 mL amber glass bottle.[49] This was repeated until 200−250 mL of SML was collected. Start and stop times (Table 1), as well as the number of glass plate dips for each SML sample, were recorded. On average, SML sampling required about 36 dips for each sample.

Subsurface water samples were collected from the side of the boat in precleaned 300 mL amber glass bottles at a depth of around 10 cm. All SML and subsurface sample bottles were wrapped in aluminum foil and stored frozen before transporting them back to the University of Georgia for analyses. Due to logistical constraints, samples were frozen to preserve the DOM for transportation. Studies have shown that measured concentrations of surface-active substances in seawater may vary due to freezing and thawing[51−53] but acknowledge that freezing the unfiltered sample may be the best storage method when online analyses are not feasible.[53] Previous studies measuring the composition of DOM have used this method of freezing samples for preservation prior to analysis.[54−56] In this study, all samples were treated uniformly and will have the same potential impact of freezing and thawing. Blanks of ultrapure water were collected daily during the sampling period in precleaned 300 mL amber glass bottles and were treated the same as the SML and subsurface water samples.

**2.2. Extraction of Organic Molecules.** SML, subsurface, and blank water samples were prepared and characterized as described by Burdette and Frossard.[34] Briefly, each sample or blank was first thawed to room temperature (∼14 h) and then filtered using 0.45 μm polyethersulfone membrane syringe filters (VWR). Previous work has demonstrated that a small fraction of DOC may be lost to filters due to absorption.[7,57,58] In this study, we consider anything lost to the filter to not be part of the DOC fraction measured here. The water was then split into two 100 g portions in precleaned and pre-weighed amber glass bottles for extraction. Excess water was filtered and stored in vials at 4 °C for surface tension and major ion analyses (Sections 2.4 and 2.5).

Organic molecules were extracted from the two filtered 100 g samples using two separate solid-phase extractions (SPE) cartridges in parallel.[4,34] Envi18 (C18 sorbent material, 0.5 g bed weight, MilliporeSigma) cartridges were used to target anionic and nonionic fractions of surfactants, and EnviCarb (graphitized carbon sorbent material, 0.5 g bed weight, MilliporeSigma) cartridges were used to target cationic surfactants. The resulting organic extracts from the Envi18 and EnviCarb extractions were analyzed separately using mass spectrometry in negative and positive ionization modes, respectively, as described in the next section. Each SPE cartridge was first conditioned with 6 mL of acetonitrile (MilliporeSigma) and was then rinsed with 12 mL of ultrapure

**Table 1. Physical Conditions of the Delaware Bay during Sample Collection**

| date | location | collection time (local) | sample type | salinity (g $kg^{-1}$) | water temp. (°C) | tidal height (cm) | pH |
|---|---|---|---|---|---|---|---|
| 6/12/19 Day 1 | Mouth of the Bay | 9:14−10:08 | SML | 31.79 ± 0.39 | | | |
| | | 10:04 | subsurface | 33.97 ± 0.55 | 16.8 | 12.86 | 7.99 |
| | Mid-Bay | 11:46−12:33 | SML | 24.69 ± 0.77 | | | |
| | | 11:30 | subsurface | 26.72 ± 0.77 | 20.4 | 6.58 | 8.22 |
| | Broadkill River | 16:01−16:52 | SML | 28.07 ± 0.55 | | | |
| | | 15:58 | subsurface | 26.39 ± 0.63 | 25.6 | 116.74 | 7.94 |
| 6/14/19 Day 3 | Mouth of the Bay | 9:00−9:29 | SML | 32.73 ± 0.55 | | | |
| | | 8:58 | subsurface | 31.74 ± 0.63 | NA[a] | 94.37 | NA[a] |
| | Mid-Bay | 11:23−11:51 | SML | 27.34 ± 0.25 | | | |
| | | 11:53 | subsurface | 26.77 ± 0.48 | 19.7 | 6.86 | 8.11 |
| | Broadkill River | 13:25−14:03 | SML | 13.62 ± 0.23 | | | |
| | | 14:04 | subsurface | 11.81 ± 0.21 | 22.3 | 8.99 | 7.09 |

[a]Not recorded due to rough water conditions.

**Table 2. Mean ± Standard Deviations of O/C, H/C, and OM/OC and Percent Aliphatic (% aliph.) and Percent Aromatic (% arom.) of Organic Compounds Extracted from Each Sample from the Delaware Bay Using the Envi18 and EnviCarb Cartridges and Analyzed Using Negative (−ESI) and Positive (+ESI) Electrospray Ionization with the Q-ToF-MS, Respectively**

| | | | −ESI | | | | |
|---|---|---|---|---|---|---|---|
| date | location | sample type | O/C | H/C | OM/OC | % aliph. | % arom. |
| 6/12/19 Day 1 | Mouth of the Bay | SML | 0.313 ± 0.208 | 1.504 ± 0.390 | 1.733 ± 0.351 | 37.4 | 12.8 |
| | | subsurface | 0.322 ± 0.210 | 1.447 ± 0.384 | 1.733 ± 0.352 | 35.4 | 14.5 |
| | Mid-Bay | SML | 0.315 ± 0.203 | 1.485 ± 0.400 | 1.734 ± 0.356 | 34.7 | 13.6 |
| | | subsurface | 0.322 ± 0.216 | 1.476 ± 0.393 | 1.739 ± 0.360 | 34.8 | 13.6 |
| | Broadkill River | SML | 0.305 ± 0.205 | 1.503 ± 0.377 | 1.711 ± 0.347 | 37.3 | 11.9 |
| | | subsurface | 0.331 ± 0.218 | 1.457 ± 0.403 | 1.759 ± 0.372 | 34.3 | 16.6 |
| 6/14/19 Day 3 | Mouth of the Bay | SML | 0.321 ± 0.218 | 1.458 ± 0.415 | 1.728 ± 0.366 | 35.4 | 16.4 |
| | | subsurface | 0.328 ± 0.219 | 1.480 ± 0.378 | 1.752 ± 0.369 | 34.3 | 13.1 |
| | Mid-Bay | SML | 0.295 ± 0.197 | 1.444 ± 0.406 | 1.718 ± 0.357 | 32.1 | 17.8 |
| | | subsurface | 0.332 ± 0.216 | 1.495 ± 0.395 | 1.761 ± 0.368 | 36.0 | 13.7 |
| | Broadkill River | SML | 0.328 ± 0.219 | 1.495 ± 0.393 | 1.754 ± 0.371 | 36.1 | 14.1 |
| | | subsurface | 0.364 ± 0.240 | 1.389 ± 0.430 | 1.828 ± 0.409 | 27.4 | 21.8 |
| | | | +ESI | | | | |
| date | location | sample type | O/C | H/C | OM/OC | % aliph. | % arom. |
| 6/12/19 Day 1 | Mouth of the Bay | SML | 0.337 ± 0.255 | 1.250 ± 0.478 | 1.741 ± 0.405 | 27.9 | 32.2 |
| | | subsurface | 0.271 ± 0.196 | 1.475 ± 0.386 | 1.665 ± 0.335 | 42.3 | 13.5 |
| | Mid-Bay | SML | 0.273 ± 0.205 | 1.463 ± 0.406 | 1.663 ± 0.338 | 38.8 | 14.6 |
| | | subsurface | 0.250 ± 0.196 | 1.533 ± 0.378 | 1.615 ± 0.327 | 41.9 | 11.1 |
| | Broadkill River | SML | 0.278 ± 0.191 | 1.469 ± 0.374 | 1.673 ± 0.330 | 35.5 | 13.1 |
| | | subsurface | 0.272 ± 0.186 | 1.421 ± 0.394 | 1.660 ± 0.319 | 30.2 | 17.0 |
| 6/14/19 Day 3 | Mouth of the Bay | SML | 0.267 ± 0.187 | 1.461 ± 0.382 | 1.657 ± 0.333 | 33.8 | 16.0 |
| | | subsurface | 0.253 ± 0.189 | 1.503 ± 0.382 | 1.622 ± 0.333 | 40.2 | 10.7 |
| | Mid-Bay | SML | 0.261 ± 0.198 | 1.492 ± 0.392 | 1.646 ± 0.357 | 38.9 | 14.0 |
| | | subsurface | 0.244 ± 0.173 | 1.502 ± 0.384 | 1.612 ± 0.300 | 42.6 | 12.4 |
| | Broadkill River | SML | 0.298 ± 0.205 | 1.478 ± 0.379 | 1.708 ± 0.349 | 33.8 | 13.0 |
| | | subsurface | 0.334 ± 0.238 | 1.408 ± 0.407 | 1.767 ± 0.396 | 28.2 | 18.7 |

water. After the 100 g of sample or blank water was processed through the cartridge, 2 mL of 0.1% triethylamine (MilliporeSigma) in ultrapure water was added to the empty bottle to collect any remaining sample and was then transferred to the SPE cartridge. Then, 10 mL of 0.1% triethylamine was added to the SPE cartridge to remove any remaining salt. This rinsing step elutes salt while retaining organic molecules in the solid-phase material.[34] The organic molecules collected within the SPE material were then eluted into vials using 4 mL of acetonitrile. The acetonitrile was evaporated from the vials with dry nitrogen gas (99.998% purity, Airgas). The remaining dried organic films were stored at 4 °C.

To prepare for mass spectrometry analysis, the dried organic fractions from each extraction cartridge were rehydrated separately with a 1:1 mixture of methanol (ACS Grade, Thermo Fisher Scientific) and ultrapure water to a total volume of 1 mL. Two reference standards, Genistein (MilliporeSigma) and Reserpine (SCIEX), were added to each rehydrated sample at concentrations of 5 $\mu$M each, to include a reference peak in the negative and positive ionization modes of the mass spectrometer, respectively.

**2.3. Characterization of Extracted Organic Molecules Using High-Resolution Mass Spectrometry.** The rehydrated organic extracts were analyzed using an electrospray ionization quadrupole time of flight mass spectrometer (ESI-Q-TOF-MS; Impact II, Bruker) at the Proteomics and Mass Spectrometry Facility (PAMS) at the University of Georgia. This instrument has high resolution (20,000 resolving power at 400 Da), high mass accuracy (1.0 ppm), and tandem mass spectrometry capability to induce fragmentation of ions. 3 $\mu$L of the rehydrated organic extracts were injected into the instrument using a loop injection, with a methanol carrier at a flow rate of 160 $\mu$L/h. Each sample extract pair was analyzed in sequence. First, the EnviCarb extract was analyzed in the positive ionization mode, and then the Envi18 extract was analyzed in the negative ionization mode. Calibrations were done before each ionization set using a common set of organic standards. The capillary voltage was set to 4.5 kV for positive ionization and −2.5 kV for negative ionization, and spectra were acquired in a mass-to-charge ratio ($m/z$) range of 50−1500 Da. Each sample was injected twice to confirm reproducibility.

Fragmentation spectra were obtained in a third injection of the sample into the mass spectrometer. Fragmentation was performed using collision-induced dissociation (CID) with applied energies of 10 and −20 eV for positive and negative ions, respectively. Peaks were selected for fragmentation based on meeting an intensity threshold of 20 times the signal-to-noise ratio and not appearing in the sample blank spectrum. Each fragmentation spectrum was analyzed separately and was compared sample to sample for similarities.

The high-resolution mass spectra from the ESI-Q-TOF-MS were initially processed using the instrument software (DataAnalysis, Bruker). The mass accuracy of the spiked reference standards was confirmed to be within 0.5 ppm. To confirm the accuracy of the peak locations, a lock mass was applied to diethylhexyl phthalate[59] and palmitic acid[60] in the positive and negative ionizations, respectively, for some samples. The spectra were then exported and a custom MATLAB code, described by Wozniak, et al.,[40] was used to assign chemical formulas to $m/z$ peaks, with a mass tolerance of 1.0 ppm. Chemical formulas were calculated with elemental ranges of $C_{0-50}$, $H_{0-100}$, $O_{0-30}$, $N_{0-6}$, and $S_{0-2}$, following elemental constraints used previously for similar sample types.[33,34,37] Chemical formulas were also constrained by the rules presented by Stubbins, et al.,[39] as follows: (i) double-bond equivalents (DBE) must be a whole number greater than or equal to zero; (ii) formulas containing an odd number of N have odd nominal mass and those containing an even number of N have even nominal mass; (iii) $C \leq 50$; (iv) $2 \leq H \leq (2{*}C + 2)$; (v) $0 \leq O \leq (C + 2)$; (vi) $O/C < 1.2$; (vii) $0.333 < H/C < 2.25$ (viii) $N/C < 0.5$; and (ix) $S/C < 0.2$. For positive ionization, formulas were assigned assuming ionization through protonation $[M + H]^+$ and/or sodium addition $[M + Na]^+$. For negative ionization, formulas were assigned assuming ionization through deprotonation $[M - H]^-$ and/or the loss of a counterion $[M - Y]^-$. This formula assignment method was also used on the fragmentation spectra. In this study, formulas were identified with $N_{0-5}$. Previous research has shown that mass spectrometers with resolving power below 100,000 at 400 Da cannot reliably resolve and identify formulas containing nitrogen and sulfur.[61,62] Here, we spiked each sample with the reference standard Reserpine, which contains two nitrogen atoms. The Reserpine formula was accurately identified in all of the samples, even with a mass tolerance of ± 1.0 ppm, supporting that our formulas are properly being identified. The lower resolution of this instrument may contribute some uncertainty in the formula assignments. Only formulas that were unambiguously identified are discussed in this study.

Results were exported and further statistical analyses were done using *R*. Ratios of hydrogen to carbon (H/C), oxygen to carbon (O/C), and organic mass to organic carbon (OM/OC) were calculated for the molecular formulas determined from both the negative and positive ionizations. The H/C and O/C ratios were plotted as van Krevelen diagrams to infer patterns in the formulas. OM/OC was used as a measure of the organic carbon content in the overall molecule.

Values were compared using percent change, calculated as the absolute value of the difference between the first value and the second value, normalized by the second value. An unpaired Student's $t$-test was used to compare the measured mean values of the spectra, including H/C, O/C, and OM/OC. The data sets were each compared to determine if there was a significant difference in the measured values across all of the samples at a 95% confidence. The mean and standard deviation of each set of measured data were calculated (Table 2) and were used to calculate the pooled standard deviation and the $t$ values for each comparison.

Cosine similarities[63,64] were calculated to compare the similarities of the signal intensity vectors of the combined mass spectra from the positive and negative ionizations of each sample. To create a single combined spectrum for each sample, one vector was created connecting the start of the positive ionization spectrum to the end of the negative ionization spectrum for each sample. Examples of spectra comparisons with low and high cosine similarity are shown in Figures S1 and S2, respectively.

Principal component analysis (PCA) was used to reduce the dimensionality of the data and separate the data based on the variance.[65] Further description of the inputs and results of the PCA are discussed in Supporting Information Text S1. PCA has previously been used to interpret mass spectra of marine DOM and separate mass spectral results based on the variance of the samples.[41,66]

**2.4. Concentration of Major Ions.** The concentrations of major ions ($Na^+$, $Cl^-$, and $SO_4^{2-}$) were measured using two Dionex Integrion high-pressure ion chromatography (HPIC) instruments (Thermo Fisher Scientific). Calibration curves were created for $Na^+$, $Cl^-$, and $SO_4^{2-}$, as the three largest ion contributors to sea salt. The samples and blanks were diluted by a factor of 1000 to a total volume of 5 mL, and the concentrations of the major ions were measured for each sample and blank. The salinities of the SML and subsurface water samples were calculated by dividing the concentration of each major ion by the known fraction of that ion in sea salt. The estimated salinities from each of the major ions ($Na^+$, $Cl^-$, and $SO_4^{2-}$) were then averaged to calculate the salinity for each sample. Absolute salinity was calculated using the sample density and is reported in g $kg^{-1}$. On Day 1, a refractometer was also used to measure salinities, and this HPIC method produced similar values.

**2.5. Surface Tension Measurements.** The surface tensions of the filtered SML, subsurface, and blank water samples were measured using a pendant drop tensiometer (OCA 15EC, Dataphysics, Germany).[4,67] The samples were equilibrated at room temperature for 1−2 h before measuring the surface tension with tensiometry. The calculated theoretical surface tension of each sample devoid of surface-active organics was calculated using the sample salinity and the room temperature at the time surface tension measurements were taken.[4,68] Surface tension depression for each sample was calculated as the difference between the calculated theoretical surface tension and the surface tension measured with pendant drop tensiometry.

The surface tensions of the sample extracts were also obtained using pendant drop tensiometry. After the Envi18 cartridge sample extracts were analyzed with high-resolution mass spectrometry, they were dehydrated using dry nitrogen gas. The dried organic extracts were rehydrated using ultrapure water through a series of 9 dilution steps, starting with 40 $\mu$L and ending with a final volume of 3840 $\mu$L. The surface tension at the rehydrated step (40 $\mu$L) is referred to here as the minimum surface tension and is the focus of this discussion.

**2.6. Additional Measurements.** During the sampling period, the physical properties of the water were measured and meteorological data were recorded. The water temperature and pH were measured and recorded during this period by the instruments onboard the *R/V Joanne Daiber*. After the cruise period, the tidal height was also recorded from the National Oceanic and Atmospheric Administration (NOAA) measurements.[69] Tidal height is reported here in centimeters above the mean lower low water (MLLW).

## 3. RESULTS

**3.1. Chemical and Physical Properties at Sampling Locations.** The sampling sites and days varied in water temperature, salinity, tidal height, and pH (Table 1). Most of the samples were collected at low tides (tidal height of 6.58 to 12.86 cm above the MLLW). The samples were collected at high tide on Day 1 at Broadkill River (tidal height of 116.74

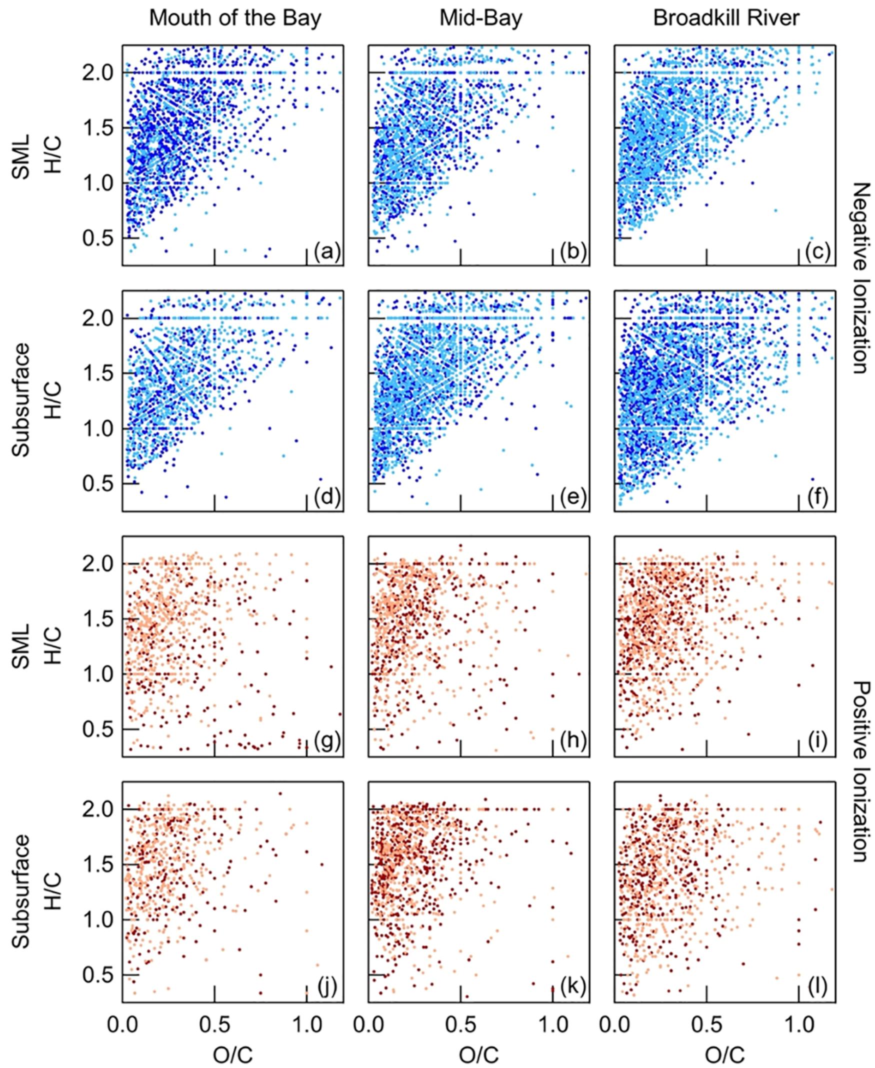



**Figure 2.** van Krevelen diagrams using the H/C and O/C atomic ratios for assigned formulas of ionized organic molecules measured using (a–f) negative ionization and (g–l) positive ionization Q-ToF-MS and extracted using Envi18 and EnviCarb cartridges, respectively. Samples are colored based on the day they were sampled (Day 1 is darker and Day 3 is lighter) and are separated based on the site (horizontally) and sampling method (vertically) for the two ionization modes.

cm above the MLLW) and on Day 3 at the Mouth of the Bay (tidal height of 94.37 cm above the MLLW). On both days, the Mouth of the Bay site had the highest salinity (33.97 ± 0.55 and 31.74 ± 0.63 g $kg^{-1}$, for Day 1 and Day 3, respectively; Figure 1) reflecting its location closest to the ocean endmember. The Broadkill River site, which is downstream from the Great Marsh in Delaware and flanked by salt marshes, had features of a river with low salinity on Day 3 (11.81 ± 0.21 g $kg^{-1}$) and higher salinity on Day 1 (26.39 ± 0.63 g $kg^{-1}$), showing more marine influence when sampling was during high tide and prior to the rainfall that impacted Day 3 (Figure 1). The Mid-Bay site had salinities that fell within the range of the other two sites (26.72 ± 0.77 and 26.77 ± 0.48 g $kg^{-1}$, on Day 1 and Day 3, respectively), reflecting the mixture of fresh water and salt water influences. The water temperature was the lowest at the Mouth of the Bay (16.8 °C, for Day 1) and the highest at the Broadkill River (25.6 and 22.3 °C, for Day 1 and Day 3, respectively). The pH remained relatively consistent throughout the sampling days and sites. The highest pH (8.22) was from Day 1 at the Mid-Bay and the lowest pH (7.09) was from Day 3 at the Broadkill River.

**3.2. Characteristics of Organic Compounds Extracted from SML and Subsurface Waters.** Figure 2 shows van Krevelen diagrams of the organic compounds extracted and identified from the SML and subsurface waters using both the Envi18 and EnviCarb cartridges and analyzed with negative and positive ionization modes, respectively. The mass spectra contained about 20,000 chemical formulas, combined for both ionization methods and all samples. The negative ionization mode generally produced more chemical formulas per sample on average (~1200) than the positive ionization mode (~500), suggesting that more organic compounds were extracted and characterized in the Envi18 extraction and negative ionization.

For both positive and negative ionization modes, the organic formulas are generally clustered in the high H/C (> 1.25) and low O/C (< 0.5) regions with fewer formulas identified in the low H/C (< 1.25) and low O/C (< 0.5) regions, as well as the high H/C (> 1.25) and high O/C (> 0.5) region (Figure 2). With negative ionization, there are no compounds in the low H/C and high O/C regions, while with positive ionization, there are a small number in that region, as discussed later.

**Table 3. Surface Tensions and Calculated Surface Tension Depressions of Collected SML and Subsurface Water, as well as Minimum Surface Tension of Organic Compounds Extracted from Each Sample Using Envi18 Cartridges**

| date | location | sample type | surface tension (mN $m^{-1}$) | surface tension depression (mN $m^{-1}$) | minimum surface tension (mN $m^{-1}$) |
|---|---|---|---|---|---|
| 6/12/19 Day 1 | Mouth of the Bay | SML | 70.43 ± 0.43 | 3.61 | 28.08 ± 0.32 |
| | | subsurface | 70.12 ± 0.57 | 3.81 | 32.65 ± 0.44 |
| | Mid-Bay | SML | 69.65 ± 0.52 | 4.11 | N/A |
| | | subsurface | 70.42 ± 0.57 | 3.06 | N/A |
| | Broadkill River | SML | 70.12 ± 0.32 | 3.69 | 28.63 ± 0.25 |
| | | subsurface | 70.20 ± 0.25 | 3.51 | 32.37 ± 0.34 |
| 6/14/19 Day 3 | Mouth of the Bay | SML | 70.82 ± 0.22 | 3.51 | 29.61 ± 0.54 |
| | | subsurface | 70.16 ± 0.60 | 3.72 | 45.41 ± 0.53 |
| | Mid-Bay | SML | 69.95 ± 0.39 | 3.50 | N/A |
| | | subsurface | 70.60 ± 0.65 | 2.83 | N/A |
| | Broadkill River | SML | 69.78 ± 0.36 | 3.36 | 27.86 ± 0.37 |
| | | subsurface | 70.28 ± 0.42 | 3.05 | 32.28 ± 0.47 |

Overall, 84.5% of the assigned chemical formulas across all of the samples have O/C ratios of < 0.5, and 70.9% of the chemical formulas have H/C > 1.25. Together, this indicates that a large fraction of the identified organic formulas are aliphatic and that these formulas are representative of molecules that are similar in composition, including H/C and O/C, of known surfactants, lipids, and fatty acids.[33,34,38] Previous studies identified aliphatic formulas as those containing H/C greater than 1.0 and a DBE to carbon (DBE/C) ratio of less than 0.3.[39] In this study, average DBE for each sample ranged from 7.36 to 8.41, for both ionization modes, and average DBE/C ranged from 0.31 to 0.47 (median of 0.35). Of the full formula set, about 8000 (40.4% of the total formulas) fall in the range of DBE/C less than 0.3 and can therefore be classified as aliphatic molecules.

The chemical formulas with aromatic and condensed aromatic structures were identified in the full mass spectral data set, including both ionization modes, by ions that had an aromaticity index (AI) of greater than 0.5 and 0.67, respectively.[39,41,70,71] Overall, there were 2959 formulas (15.1% of the total formulas) that were identified as aromatic and 963 formulas (4.9% of the total formulas) that were identified as condensed aromatic compounds. The small overall fractions and the distribution of these formulas throughout the samples caused the average AI values for each sample to be similar. The average AI ranged from 0.203 to 0.257, including both ionization modes, except for the positive ion formulas in the Mouth of the Bay Day 1 SML sample, which had an AI of 0.352 and was statistically different (Student's *t*-test, $p < 0.05$) from the rest of the samples. While this is unexpected for the SML of the most "ocean-like" sample, this is higher than that of the AI for the negative ion formulas for the same sample (0.211). This is consistent with the fact that the EnviCarb cartridge and positive ionization collected and detected different compounds, compared to the Envi18 cartridge and negative ionization, as discussed later.

Overall, the average H/C and average O/C showed consistent trends across the samples, within a given extraction and ionization type. For the Envi18 extraction and the negative ionization, the average H/C was 1.389 to 1.495 for subsurface waters and 1.444 to 1.504 for the SML (Table 2). The average O/C was 0.322 to 0.364 for the subsurface waters and 0.295 to 0.328 for the SML (Table 2). The average OM/OC was 1.733 to 1.828 for the subsurface waters and 1.711 to 1.754 for the SML (Table 2).

For the EnviCarb extraction and the positive ionization, the average H/C, O/C, and OM/OC had generally larger ranges than the Envi18 extraction and the negative ionization. The average H/C was 1.408 to 1.533 for subsurface waters and 1.250 to 1.492 for the SML (Table 2). The average O/C was 0.244 to 0.334 for the subsurface waters and 0.261 to 0.337 for the SML (Table 2). The average OM/OC was 1.612 to 1.767 for the subsurface waters and 1.646 to 1.741 for the SML (Table 2). The trends in the positive ionization mode differ from those in the negative ionization mode and are more unexpected, as discussed in later sections. This may largely be attributed to both the differences in the molecules targeted by the sorbent material in each extraction cartridge, as well as differences in the ionization modes. While the Envi18 cartridge is most efficient at extracting anionic and nonionic surfactants, it misses a fraction of organic molecules, including the cationic surfactants, which are most efficiently extracted with the EnviCarb cartridge.[34] The Envi18 cartridges extract similar compounds as the commonly used PPL cartridges.[35] Comparing the composition of the two extracts and two ionization modes gives further insight into the composition of the total surfactant pool.

The H/C and O/C patterns overall are consistent with the presence of lipid-like (H/C ∼ 2 and O/C ∼ 0.2), protein-like (H/C ∼ 1.8 and O/C ∼ 0.4), amino-sugar-like (H/C ∼ 1.8 and O/C ∼ 0.8), and condensed hydrocarbon-like (H/C ∼ 1 and O/C ∼ 0.2) DOM.[72] With the EnviCarb extraction and positive ionization, some formulas indicate a slight presence of lignin-like (H/C ∼ 1 and O/C ∼ 0.5) molecules within the carboxyl-rich alicyclic molecules (CRAM) region, but that is not present in the negative ionization. Only a small presence of these molecules is likely due to the targeted extraction with the Envi18 cartridge and the analyses with the ESI-Q-ToF-MS. This demonstrates that the separate SPE extractions are able to isolate unique organic compounds based on the selectivity of the different sorbent materials. Additionally, while carbohydrate-like molecules (H/C ∼ 2 and O/C ∼ 1) have been observed previously in marine-derived organic species,[73] carbohydrate-like molecules were not observed in high quantities in these samples. This is likely due to the targeted extraction of large, surface-active organic species that may have low extraction efficiency for smaller, soluble sugars, such as glucose,[34] or the fact that carbohydrates may not ionize as well as carboxylated or as more aliphatic compounds. Previous studies have demonstrated that carbohydrates often do not cluster in one specific region of the van Krevelen diagram and are spread out over larger ranges of H/C and O/C,[72] thus implying that carbohydrate-like molecules may still be present in these samples but not identified with this method.

**3.3. Surface Tension of Water Samples and Extracted Organics.** The surface tension of the subsurface water at the three sampling sites on both sampling days ranged from 70.12 to 70.60 mN $m^{-1}$ (Table 3), indicating relatively similar surface tensions of the subsurface water across the different sampling sites and days. The surface tension of the SML samples ranged from 69.65 to 70.82 mN $m^{-1}$ (Table 3). While this range is slightly larger than that of the subsurface waters, bulk surface tension measurements of both water types are similar and do not vary greatly across the sampling sites or days. These measured surface tensions are significantly (Student's *t*-test, $p < 0.05$) less than the measured surface tensions of pure water at the same temperature (70.75 to 72.23 mN $m^{-1}$, depending on the temperature at the time of measurement) and the calculated theoretical surface tension of seawater devoid of organics (calculated for each sample salinity and temperature). The surface tension depressions ranged from 2.83 to 3.81 mN $m^{-1}$ (median of 3.285 mN $m^{-1}$) for the subsurface water and 3.36 to 4.11 mN $m^{-1}$ (median of 3.56 mN $m^{-1}$) for the SML samples (Table 3). These depressions demonstrate the presence of surfactants in the samples, which cause a decrease in surface tension relative to pure salt water. The surface tension depressions are consistent with those measured and calculated for subsurface seawater in the open ocean (2.40 to 3.72 mN $m^{-1}$).[74]

The minimum surface tensions, measured as the surface tension of the most concentrated extract solution (40 $\mu$L of ultrapure water added to the dry extract), ranged from 32.28 to 45.41 mN $m^{-1}$ for the subsurface waters and 27.86 29.61 mN $m^{-1}$ for the SML. These values are all very depressed compared to the surface tensions of the bulk water (Table 3), demonstrating the selective extraction of organic molecules, including surfactants, through the SPE extraction.

## 4. DISCUSSION

**4.1. Preferential Enrichment of High H/C and Low O/C Organic Compounds in SML.** There are clear differences in the organic species measured in the SML and subsurface waters, both in the individual SML and subsurface sample pairs as well as overall, as indicated by the van Krevelen diagrams (Figure 2), for both the positive and negative ionization modes. Across all three sampling locations and both days (Day 1 and Day 3), the subsurface water samples have more assigned formulas in the negative ionization mode than the SML samples (mean of ~1200 and 1100, respectively). This is especially evident in the Broadkill River site, with the higher density of formulas and the extension to lower H/C values in the subsurface water samples (Figure 2f). The trend is the opposite in the positive ionization data which have higher quantities of assigned formulas in the SML samples (mean of ~510 for the SML and 450 for the subsurface waters). The number of assigned formulas indicates the number and variety of organic compounds measured from the sample extracts. An overall greater number of assigned formulas in the subsurface water samples than in the SML samples supports the idea that specific organic species are preferentially enriched in the SML based on their composition.

The preferential enrichment of specific organic compounds in the SML is also evident in the average compositions of the organic compounds, as indicated by their average ratios of O/C and H/C. In the negative ionization mode, the average O/C of the SML samples is consistently lower than that of the corresponding subsurface water sample, across all sampling days and locations (Table 2). When the SML and subsurface sample pairs are compared, the SML median O/C is 5.3% less than that of the subsurface water. The extent varies by sampling site and day, with the two largest decreases in SML O/C compared to subsurface water O/C on Day 3 at the Broadkill River site (10.1%) and Day 3 at the Mid-Bay site (11.3%), both of which are statistically significant differences (Student's *t*-test, $p < 0.05$). These reductions in O/C from the subsurface to the SML indicate that the SML is depleted in oxygen-containing molecules, compared to the subsurface water and that the organic molecules are less oxidized. Because the Broadkill River has the lowest salinity (Day 3: 11.81 g $kg^{-1}$) and the largest terrestrial influence, this indicates that the terrestrial organics, associated with higher O/C ratios, were not enriched in the SML.

Similarly, the H/C is generally higher for the SML compared to the corresponding subsurface water sample (Table 2). When comparing the SML and subsurface sample pairs, the SML median H/C is 1.9% greater than that of the subsurface water. The extent of the difference varies by sampling site and day, with Day 3 Mouth of the Bay and Day 3 Mid-Bay samples showing lower H/C in the SML than the paired subsurface water (1.4 and 3.3%, respectively). The Day 3 samples were collected after a rainfall event, which may have impacted these trends, as discussed in Section 4.3. The other sites and sampling days have H/C increases of 0.6% (Mid-Bay, Day 1) to 7.6% (Broadkill River, Day 3) from the subsurface to the SML. The higher H/C in most of the SML samples indicates that the organic molecules in the SML have more saturated functional groups, with fewer double bonds and rings than the organic molecules in the subsurface water samples.[39]

Together, the lower O/C and higher H/C values in the SML indicate that those molecules may be more hydrophobic. This is consistent with previous studies of DOM in SML and subsurface waters that found the SML DOM to have more formulas containing H/C > 1.25 and O/C in the range of 0.10−0.40.[38,50] Also, the consistently lower O/C in the SML samples, compared to the subsurface water samples, suggests that there is no additional photochemical processing in the SML beyond that in the subsurface water.[38]

In the negative ionization mode, the OM/OC exhibited a similar trend to the O/C, between the SML and subsurface water samples (correlation of $R = 0.95$ between the O/C and OM/OC across all of the samples). All of the average OM/OC for the SML samples were less than the average OM/OC for the corresponding subsurface water samples. The SML and subsurface pairs had OM/OC for the SML of up to 4.1% (Broadkill River, Day 3, statistically different; Student's *t*-test, $p < 0.05$) less than that of the subsurface water. This slight decrease in OM/OC is an additional indication of a relative decrease in noncarbon atoms bonded in the organic molecules in the SML samples, compared to those in the subsurface water.

The trends in the enrichment of specific organic formulas in the SML compared to the subsurface waters are less apparent in the positive ion formulas from the EnviCarb cartridge extraction (Table 2). Some of this difference is attributed to the targeted extraction of specific organic molecules with the two different SPE extractions, as well as differences in the ionization efficiency of the two modes. The Envi18 cartridges are more efficient at extracting anionic and nonionic surfactants, while the EnviCarb cartridges are more efficient at extracting cationic surfactants.[34] In the positive ionization mode, the O/C values are higher in the SML than in the subsurface water across all sample sites and days, except for the Broadkill River on Day 3. Overall, the O/C of the positive ion formulas are all lower than those of the negative ion formulas (statistically significant, Student's $t$-test, $p < 0.05$) and are on average 14% less for each sample, indicating that the EnviCarb cartridge is less efficient at collecting organic species with high O/C formulas or that the organic molecules with high O/C do not ionize as efficiently with positive ionization. This is also supported by the overall lower number of formulas identified for the positive ionization mode, after the EnviCarb extraction (Figure 2g–l). However, the trends in the H/C values are similar for formulas identified in both the positive and negative ionization modes. The overall difference in H/C between the formulas identified in the two ionization modes for each sample is 1.0% on average and is less than 4% across all of the samples, with no statistical difference between these two means (Student's $t$-test, $p > 0.05$). The one exception is the Day 1 Mouth of the Bay SML sample, which was significantly different from the rest (Student's $t$-test, $p < 0.05$) and had a mean H/C 17% lower, in the EnviCarb extraction and positive ionization. Despite the similar mean H/C ratios, there was a slightly larger range of H/C values for formulas assigned in negative ionization than in positive ionization, with average H/C ranges of 1.85 and 1.80, respectively.

Additional comparisons of the molecular formulas of the organic extracts in the negative ionization mode show variability in both the fraction of aliphatic formulas and the fraction of aromatic formulas identified in the SML and subsurface water samples. The DBE/C varies across samples and is higher in the SML than in the subsurface for the Mouth of the Bay Day 3 and the Mid-Bay Day 3 samples but lower in the subsurface for all other sampling days and sites. As expected, the H/C and fraction of aliphatic molecules are strongly correlated ($R = 0.90$). Combining the DBE/C with the H/C, the overall fraction of aliphatic molecules is higher in the SML (35.5%) compared to the subsurface waters (33.7%) (Table 2). This demonstrates a slight enrichment of aliphatic molecules from the subsurface into the SML. The fraction of aromatic species in the SML samples compared to the subsurface water samples varies across sample pairs. In general, there is a slightly larger fraction of aromatic and condensed aromatic species in the subsurface waters (1.1% greater on average) compared to the SML, except for the Day 3 sample pairs collected at the Mouth of the Bay and Mid-Bay. There are negative correlations between the fraction of aromatic molecules and H/C ($R = -0.93$) and the fraction of aliphatic molecules ($R = -0.90$), as expected.

One specific example of the targeted enrichment of high H/C organic molecules in the SML is at the Broadkill River sampling site (discussed further in Section 4.2). On both Day 1 and Day 3, there were more aliphatic molecules (36.7% compared to 30.9%) and fewer aromatic molecules (13.0% more compared to 19.2%) in the SML than in the subsurface water in the negative ionization mode. The van Krevelen diagram further demonstrates this point showing that the Broadkill River subsurface samples (Figure 2f) have more formulas falling in the high O/C and high H/C region compared to the SML samples (Figure 2c).

The organic compounds from the EnviCarb extraction and positive ionization generally followed similar trends for the aliphatic and aromatic fractions as the Envi18 extraction and negative ionization. The difference between the percent aliphatic and aromatic compounds in the negative and positive ionization modes further demonstrates the differences in the DOM extracted by the two cartridges. The DBE/C is higher in the SML than in the subsurface waters for the Mouth of the Bay and Mid-Bay samples (see Section 4.2) but higher in the subsurface for the Broadkill River sampling site. Combined with the H/C, the overall fraction of aliphatic molecules is lower in the SML (34.8%) compared to the subsurface waters (41.7%), for the Mouth of the Bay and the Mid-Bay but higher in the SML for the Broadkill River (34.6%) than the subsurface (29.2%). This supports the enrichment of different molecules from the subsurface into the SML observed with the negative ionization. For both Day 1 and Day 3 at the Mouth of the Bay and Mid-Bay, the SML had larger fractions of aromatic and condensed aromatic compounds (median of 15.3%) compared to the subsurface waters (median of 11.8%). The opposite trend was observed for the Broadkill River site with larger fractions of aromatic and condensed aromatic compounds in the subsurface waters (median of 17.9%) compared to the SML (13.1%).

Also with the EnviCarb extraction and the positive ionization mode, there is a difference in the aromatic fractions of formulas for the SML and subsurface waters. The most significantly different sample based on formula comparisons is the SML sample from Day 1 at the Mouth of the Bay. This sample has the largest mean AI (0.352), which is statistically different (Student's $t$-test, $p < 0.05$) from all other samples, and the largest fraction of aromatic compounds (32.2%) across all of the samples and both ionization modes (Table 2). The O/C and H/C patterns in this SML sample (Figure 2g) include formulas at low H/C and a range of high O/C that are not observed in other samples, including its paired subsurface sample (Figure 2j). These chemical formulas are also not commonly observed in other environmental samples.[72]

The statistical similarities of the combined positive- and negative-mode mass spectra between the SML and subsurface were compared using cosine similarity (Figure 3). The mass spectra of the SML and subsurface water samples are very similar for the Mid-Bay and Broadkill River sites as indicated by the cosine similarities greater than 0.97 for all of the sample pairs (Figure 3). The mass spectra of the SML and subsurface water samples have more differences for those collected at the Mouth of the Bay. This is shown by a relatively low cosine similarity of 0.86 for subsurface and SML samples collected on Day 1. It is even more evident on Day 3 with a cosine similarity between the SML and subsurface water sample spectra of 0.74. The low cosine similarity of the Mouth of the Bay SML and subsurface water sample pairs indicates that there are unique compounds measured in each type. The similarity of the spectra in the SML and subsurface water sample pairs at the other two locations may be controlled by specific chemical signatures with large abundances, since those samples also had variability in other spectral features, such as O/C and H/C.

Additionally, the differences in the organic species measured in the SML and the subsurface waters are supported by the measured surface tension values. The surface tensions of the SML and subsurface water (both prior to extraction) do not vary much, all falling within ±1% of each other (Table 3). Overall, the calculated surface tension depression is greater in

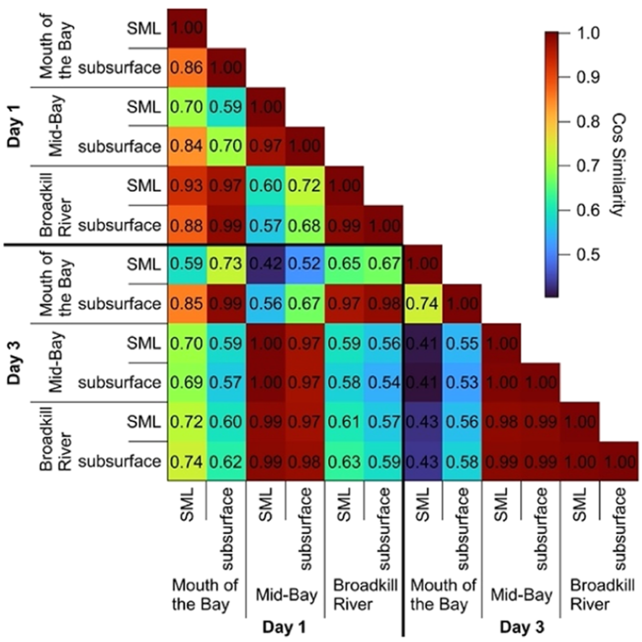



**Figure 3.** Cosine similarities of mass spectra from each site and sample type on Day 1 and Day 3.

the SML (median 3.56 mN $m^{-1}$) than in the subsurface water (3.29 mN $m^{-1}$), except for the paired SML and subsurface water samples from the Mouth of the Bay on both Day 1 and Day 3 (see Section 4.2). The surface tension depressions had moderate negative correlations with OM/OC ($R = -0.52$) and O/C ($R = -0.50$). There was no significant correlation between surface tension depression and mean H/C ($R = 0.21$). Together, these results support the idea that molecules with lower O/C are more surfactant-like and lead to greater surface tension depressions but that the saturation of carbon atoms in the molecules does not play as large of a role in the surface tension depression.

Because the surface tension measurements and calculations of the bulk water samples may not represent the full effect of the surfactants on the surface tension, we also measured the minimum surface tension of the organic extracts of the SML and subsurface water samples. The organic extracts from the Envi18 cartridges were hydrated to an initial volume of 40 $\mu$L dilution (referred to as the minimum surface tension) (Table 3). The minimum surface tensions for the SML are all less than those of the subsurface waters. The minimum surface tensions are 11.6 to 34.8% lower for the SML sample extracts than the subsurface water extracts. This indicates that surfactants are more concentrated in the SML or that the surfactants in the SML are more efficient and have a larger effect on surface tension, compared to the paired subsurface waters. This supports the difference in the molecular composition of the organic species measured in the mass spectra of the SML and subsurface water samples.

In general, the SML organic compounds had higher H/C and lower O/C values than the paired subsurface organic compounds. This indicates the enrichment of high H/C organics in the SML and is consistent with hydrophobic compounds more readily adhering to bubble surfaces to be transported to the SML. The trend with the aliphatic and aromatic compounds is less defined, but in general, the SML has smaller fractions of aromatic compounds and larger fractions of aliphatic compounds than the subsurface water. The larger fractions of aliphatic compounds are consistent with the higher H/C in the SML. Additionally, the SML is more enriched in surfactants than the subsurface waters, as indicated by the lower minimum surface tensions.

**4.2. Differences in Organic Composition Based on Sampling Locations.** There are clear differences in the chemical and physical properties of the water at the three sampling sites (Table 1; Figure 1). The water temperature was lowest at the Mouth of the Bay and highest at the Broadkill River (Table 1). The salinity was the highest at the Mouth of the Bay, with the most ocean-like conditions, and lowest at the Broadkill River, as expected (Table 1; Figure 1). The differences between the properties of the water at the sampling sites can contribute to or indicate differences in the chemical composition of the organic species between the sites.

For both the SML and subsurface waters and both ionization modes, the quantity of assigned formulas varied with the sampling location (Figure 2). Combining the SML and subsurface pairs and both sampling days, the Broadkill River had the largest number of assigned formulas (~8000) and the Mouth of the Bay had the fewest (~5000). This indicates that the Broadkill River site had the most organic species that were extracted and characterized.

Overall, the assigned chemical formulas are similar across all of the sample locations, as indicated by the similarities in average O/C, H/C, and OM/OC (Table 2). For molecular formulas identified in the negative ionization mode, the average O/C and H/C for each site, including both days and sample pairs, varied by less than 4.8 and 0.9%, respectively. Similarly, the OM/OC varied by less than 1.5%. For molecular formulas identified in the positive ionization mode, there is more variability in the O/C (up to 12.9%), but that is mainly influenced by the formulas in the sample pairs on Day 3 from the Broadkill River, which are statistically different (Student's *t*-test, $p < 0.05$) from the other samples (discussed further in Section 4.3). In the positive ionization mode, the average OM/OC and H/C for each site, including both days and sample pairs, varied by less than 4.0 and 5.0%, respectively. Together, this indicates that the sampling location was not a large source of variability in the organic composition.

The cosine similarities show similarities and differences between the combined mass spectra from the different sample sites (Figure 3). Comparing the sampling sites, the highest similarities in spectra are from the subsurface water from the Mouth of the Bay on both days with the Broadkill River SML and subsurface water on Day 1 (> 0.96), as well as the Broadkill River SML and subsurface water on Day 3 with the SML and subsurface water on both days at the Mid-Bay (> 0.97), as shown in Figure 3. The least similarity is in the comparison of the Mouth of the Bay and the Mid-Bay samples for both days and sample types (Figure 3). Figure S1 demonstrates the differences in the spectra of the SML samples from the Mouth of the Bay and the Mid-Bay on Day 3. Similar to the trend with the assigned formulas, the sample location is not the main driver in the differences in the organic species between all of the samples.

While the Broadkill River samples from Day 3 had high similarity to the Mid-Bay samples, the Broadkill River subsurface on Day 3 had significantly different mean organic composition values compared to those of the other samples, across all of the sampling locations and days (Table 2). This Broadkill River subsurface sample from Day 3 has a larger fraction of aromatic compounds (21.0% of the total assigned formulas) compared to the Mid-Bay samples (average of 14.9% of the total assigned formulas). This is consistent with previous work in the Delaware Bay that observed the aromaticity of DOM to be inversely correlated with salinity.[45] The observed difference in the fraction of aromatic compounds is not captured in the cosine similarities since the cosine similarity values only represent intensity values for mass spectra. The aromatic peaks are low in intensity (mean peak height of 1926) compared to the aliphatic compounds (mean peak height of 3788) and do not significantly contribute to the cosine similarity.

Additionally, ion fragmentation was used to compare the chemical structures and identifications of organic species observed in the different sampling locations. Two examples of compounds identified in the sample extracts using fragmentation are oleic acid and sucrose. Oleic acid was identified in the Mouth of the Bay (Day 1 subsurface and SML; Day 3 subsurface) and in the Broadkill River (Day 1 SML and subsurface; Day 3 subsurface). Oleic acid is a naturally occurring fatty acid surfactant that has previously been measured in marine samples.[75−78] Oleic acid may contribute to the reduction in surface tension in these samples, as discussed later. It may also be an indicator of marine influence; however, other sources may contribute to the oleic acid in these samples.[79] Sucrose was identified in the Mid-Bay (Day 1 subsurface; Day 3 SML and subsurface) and the Broadkill River (all samples). Sucrose has previously been observed in samples influenced by terrigenous sources, such as plant pollen and dust.[80−82] Sucrose is not a surfactant and has an unsaturated cyclic structure and therefore does not contribute to reductions in surface tension. These organic molecules observed through fragmentation may be present in other samples but are below detection limits.

In addition to the chemical similarities, the surface tensions of the samples overall did not vary significantly based on the sample location (Table 3). The bulk surface tension was similar across the sample sites, varying less than 2% when comparing single days and sample types, while the associated surface tension depression varied by more than 10%. The largest surface tension depressions were observed at the Mouth of the Bay, indicating that it has stronger surfactants or higher concentrations of surfactants than the other sampling locations. Overall, the minimum surface tensions of the sample extracts were also similar, with more significant variability influenced by the sampling day (Section 4.3). The average minimum surface tensions varied by less than 6% overall for the Mouth of the Bay and the Broadkill River.

Taken together, these results indicate that there may be some distinct chemical species in the Mid-Bay that are not in the Broadkill River or the Mouth of the Bay, and vice versa, implying that there may be some unique sources of organic species to these regions. However, the similarity of the average chemical compositions across the sampling regions indicates that the broad organic pool is similar in composition and has similar sources.

**4.3. Differences in Organic Composition Attributed to Tides, Rainfall, and Wind.** On the night of Day 2, prior to Day 3 sample collection, the sampling region had 14 mm of rainfall over a short time period.[83] Rainfall has been shown to increase terrestrial DOM runoff into bay regions, and the rainfall on the night of Day 2 may have contributed to increased terrestrial DOM in the Delaware Bay on Day 3.[84−86] Additionally, during Day 3 sampling, there were high wind speeds (> 9.0 m $s^{-1}$ with gust speeds > 12.8 m $s^{-1}$). Previous studies have shown that wind speed can influence the SML by disturbing and decreasing its thickness[87,88] and by reducing surfactant enrichment in the SML.[15] Because the sampling procedure was unchanged during periods of high winds, variability in the results can be attributed to differences in the SML. High winds can also lead to increased turbulence in the ocean mixed layer and can lead to sediment remobilization in shallower waters, such as the Broadkill River.[85,86] On Day 3, the tidal heights were also different from Day 1 during sampling at the Mouth of the Bay (low tide on Day 1 and high tide on Day 3) and the Broadkill River (high tide on Day 1 and low tide on Day 3). The difference in tide height may also influence the composition of DOM in the Broadkill River on Day 1 and Day 3, due to runoff and the shallower depth of sampling site.

The rainfall, wind, and tidal heights during sampling contributed to differences in water properties, such as temperature, pH, and salinity, across the sites on Day 1 and Day 3 (Table 1). The Broadkill River site also had a decrease in pH (7.94 to 7.09) and salinity (26.4 to 11.8 g $kg^{-1}$ for the subsurface) from Day 1 to Day 3 that can be attributed to a combination of the addition of fresh water from rainfall and runoff and the lower tidal height on Day 3 at that site. The temperature, salinity, and pH remained relatively constant from Day 1 to Day 3 at the Mouth of the Bay and Mid-Bay sites (Table 1).

The biggest differences in the organic composition observed in the negative ionization mode from Day 1 to Day 3 were at the Broadkill River (Table 2). The O/C increased significantly (Student's *t*-test, $p < 0.05$) by 10.0 and 7.3% in the subsurface and SML, respectively, from Day 1 to Day 3. The H/C decreased significantly (Student's *t*-test, $p < 0.05$) by 4.6% in the subsurface water and stayed relatively constant in the SML, while the OM/OC remained relatively constant, from Day 1 to Day 3. The fraction of aliphatic organic molecules was higher on Day 1 (35.8%) than on Day 3 (31.7%) for these samples. This difference in organic composition at the Broadkill River site could be due to the increase in terrestrial runoff or sediment remobilization on Day 3 caused by the rainfall and wind. Additionally, since Day 1 was sampled at high tide, it may have had more marine influence. The other sites did not have significant variability in elemental ratios and organic composition from Day 1 to Day 3. Since these sites are a greater distance from land, there could be a delay in the effect of increased runoff, or there could be less of an effect due to the deeper water.

The cosine similarity of the combined negative- and positive-mode mass spectra showed more differences from Day 1 to Day 3 (Figure 3) than the elemental ratios. For the Mouth of the Bay, the subsurface samples were very similar (cosine similarity > 0.98), but the SML samples were very different (cosine similarity < 0.60) comparing Day 1 and Day 3 (Figure 3). These differences are likely due to the effects of the rain and wind. As expected at the Broadkill River, based on the elemental ratios, the subsurface and SML mass spectra had low cosine similarities on Day 1 and Day 3 (0.60 and 0.59, respectively), highlighting the notable differences in organic composition. The Mid-Bay samples showed the most similarity between sampling days (cosine similarities > 0.97). The significant overlap in the Mid-Bay SML spectra for Day 1 and Day 3 samples is shown in Figure S2. The high cosine similarities demonstrate that the intensity and composition of organic species in the Mid-Bay were the least influenced by the wind and rainfall for the Day 3 samples. The similarities between sampling days may also be because this site was sampled during low tide on both days, thus removing the influence of the tide.

The differences in organic composition across the sampling days are further demonstrated in the PCA analysis (Figure S3). The PC1 vs PC2 comparison (Figure S3) shows two clusters of samples, the first including all of the Day 1 samples as well as the Day 3 samples from the Mouth of the Bay (subsurface and SML) and the Mid-Bay (SML). The first cluster extends to the bottom left quadrant of the plot, which is associated with the largest surface tension depressions. The other unique cluster contains both Day 3 Broadkill River samples and the Day 3 Mid-Bay subsurface sample. This cluster separation can be attributed to the rainfall and tides that may have increased the mixing in the water column and contributed more terrestrial organic matter for these samples. Because the surface tension depression takes salinity into account, the main difference between the samples is the influence of terrestrial compared to marine organics and the influence of rain and wind, both of which are accentuated at the shallow Broadkill River site. The PC1 axis separates most of the samples based on sampling day (Figure S3). The Day 3 samples mainly project into the negative PC1 axis (5 of the 6 samples, with only the Mid-Bay SML sample having positive PC1 values), while the Day 1 samples mainly project into the positive PC1 axis (4 of the 6 samples, with only the Mid-Bay SML and Mouth of the Bay subsurface samples having negative PC1 values). Additionally, at PC2 values greater than 0, the Day 1 samples all have positive PC1 projections while the Day 3 samples all have negative PC1 projections.

The chemical formulas with significant associations with the PC1 and PC2 axes were also compared (Figure S4). There were significant (Student's *t*-test, $p < 0.05$) differences between the O/C, OM/OC, m/z , and nitrogen count between formulas contributing to the separation along the positive and negative PC1 axes (Figure S4). There are also significant differences between the $m/z$ and sulfur counts along the PC2 axis (Figure S4). The combination of these two axes can be used to determine formulas that contribute to the projections of the Day 3 Broadkill River subsurface and SML samples and the Day 3 Mid-Bay subsurface sample that separate from the rest of the samples and are in top left quadrant (Figure S3). The formulas in this quadrant contain higher O/C, nitrogen counts, and OM/OC ratios, in addition to lower $m/z$ and sulfur counts. This indicates that the organic matter associated with this quadrant is more oxygen-rich and nitrogen-rich. This is consistent with molecules like sucrose, which was identified from fragmentation, that are heavily oxygenated.

Some similar trends are observed in the surface tension comparisons day-to-day at the different sites as the trends with the organic composition. However, the surface tensions have day-to-day differences in the Mid-Bay and Broadkill River samples but are more similar day-to-day in the Mouth of the Bay samples. The largest change in surface tension depression from Day 1 to Day 3 was observed in the Broadkill River samples with a decrease of 13.1% for the subsurface and 8.9% for the SML. Additionally, the surface tension depression decreased in the Mid-Bay samples by 7.5% in the subsurface and 14.8% in the SML from Day 1 to Day 3. This decrease in surface tension depression indicates that the organic species sampled on Day 3 at both sites contributed less to reducing the surface tension and may thus have been less surfactant-like. While the Mouth of the Bay showed no difference in the surface tension depression on Day 1 to Day 3, it had a significant increase in the minimum surface tension from Day 1 to Day 3 in the subsurface. This indicates that the extracted surfactants were weaker or in lower concentrations on Day 3 in the subsurface water. This may be due to the additional mixing caused by the rain and wind on Day 3. Additionally, the lack of difference in the SML minimum surface tension from Day 1 to Day 3 may be due to the accumulation of surfactants in the SML.

This data shows some short-term and location-based environmental variability for organic species in the Delaware Bay. The largest differences in organic composition and surface tension from Day 1 to Day 3 are observed in the samples collected at the Broadkill River site. This is likely because this riverine site was more affected by terrestrial runoff, rainfall, and tidal height than the other two sampling sites. Due to the limited number of samples, it is difficult to assign definitive environmental drivers to these differences.

## 5. CONCLUSIONS

In this study, we characterized the composition and surface tension of organic species in the SML and subsurface water samples collected over three days and at three sites in the Delaware Bay. The combination of surface tension and high-resolution mass spectrometry measurements is powerful for characterizing the chemical composition and interfacial properties of the organic species in the SML and subsurface water samples. The SML samples differed from the subsurface samples in mean chemical characteristics and surface tensions. The SML generally had lower surface tension minimums and more hydrophobic organic molecules. This indicates that the molecules with higher H/C and lower O/C were more enriched in the SML and contributed more to surface tension depression. Based on the limited number of sampling days and sites, less definitive conclusions can be made about the environmental drivers of the observed variability over the range of sampling sites and days. The organic species and their interfacial properties showed fewer differences based on sample collection site. The presence of distinct molecules, such as oleic acid and sucrose, differed based on sampling site, but the overall average organic composition was more similar than compositions at the same sites sampled on different days. In this case, rain, wind, and associated runoff are speculated to have generated the differences in composition and highlight the influence of environmental conditions on surfactant properties. Overall, these results show the enrichment of high H/C molecules in the SML and the influence of terrestrial organic matter on the composition of organic compounds in the SML and subsurface waters of the Delaware Bay estuary.

These results further our understanding of surfactants in estuarine environments, their preferential enrichments in the SML based on composition, and the influence of rainfall and tides on their composition. This work shows that the surfactant composition and resulting surface tension depression are not uniform across sampling conditions. Additionally, the surfactants in the SML vary in composition compared to the surfactants in the subsurface waters demonstrating that direct measurements must be made of SML surfactant composition. Here, the SML was enriched in organic compounds with higher H/C values that contributed to more surface tension depression, compared to the associated subsurface waters.

More work can be done to link the sources of surfactants to the surfactant compositions and related surface tension depressions measured here. Future work using these techniques could also include comparisons of the organic species in the particulate organic carbon and the dissolved organic carbon fractions of the SML and seawater.

## SUPPORTING INFORMATION

The Supporting information is available free of charge at https://pubs.acs.org/doi/10.1021/acsearthspacechem.2c00220

Overlapped mass spectra that have low cosine similarity (Figure S1); overlapped mass spectra that have high cosine similarity (Figure S2); principal component analysis (Text S1); projections of the PCA of the surface microlayer and water samples (Figure S3); and loadings of the principal components (Figure S4) (PDF)

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

Primary financial support for this work was provided by the University of Georgia Investment in Sciences Initiative and Office of Research as well as the National Science Foundation (NSF) through awards to the University of Georgia (OCE-2123368) and the University of Delaware (OCE-2123402). The authors thank Dr. Dennis Phillips in the Proteomic and Mass Spectrometry Facility at the University of Georgia for his assistance with mass spectrometry. They also thank the captain of the *R/V Joanne Daiber* at the University of Delaware for assistance in sample collection.

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