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Arsenolipids in Plankton from High- and Low-Nutrient Oceanic Waters Along a Transect in the North Atlantic

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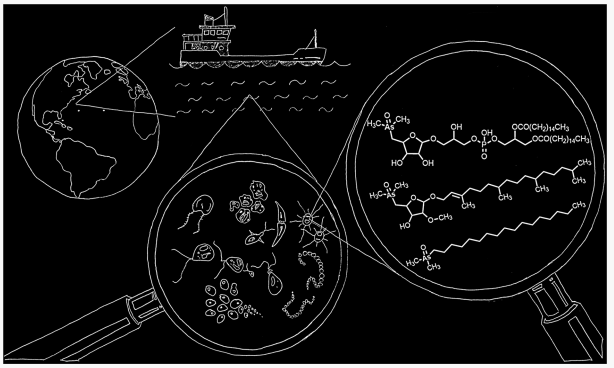
ABSTRACT: Although the natural occurrence of arsenic-containing lipids (arsenolipids) in marine organisms is now well established, the possible role of these unusual compounds in organisms and in the cycling of arsenic in marine systems remains largely unexplored. We report the finding of arsenolipids in 61 plankton samples collected from surface marine waters of high- and low-nutrient content along a transect spanning the Gulf Stream in the North Atlantic Ocean. Using high-performance liquid chromatography (HPLC) coupled to both elemental and molecular mass spectrometry, we show that all 61 plankton samples contained six identifiable arsenolipids, namely, three arsenosugar phospholipids (AsPL958, 10−13%; AsPL978, 13−25%; and AsPL1006, 7−10% of total arsenolipids), two arsenic-containing hydrocarbons (AsHC332, 4−10% and AsHC360, 1−2%), and a methoxy-sugar arsenolipid that contained phytol (AsSugPhytol, 1−3%). The relative amounts of the six arsenolipids showed clear dependence on the nutrient status of the ambient water with plankton collected from high-nutrient waters having less of the arsenosugar phospholipids and more of the three non-P containing arsenolipids compared to low-nutrient waters. By combining these first field data of arsenolipids in plankton with reported global phytoplankton productivity, we estimate that the oceans' phytoplankton transform per year 50 000−100 000 tons of arsenic into arsenolipids.



INTRODUCTION

Naturally occurring arsenic-containing lipids (arsenolipids) are abundant in marine algae as a consequence of the uptake and biotransformation of arsenate naturally present in seawater. Research on the various arsenolipids in algae has mostly been performed with macroalgal species and has shown the presence of two main classes, namely, arsenic-containing hydrocarbons (AsHCs) and arsenosugar phospholipids (AsPLs),[1−7] whereas in cyanobacteria, only AsPLs have been discovered to date.[8,9]

Arsenolipids in unicellular algae and marine cyanobacteria have been much less investigated, and all studies so far have worked with cultured species experimentally exposed to elevated concentrations of arsenate in their growth media.[10−12] These studies have shown that unicellular algae produce the same arsenolipids found in macroalgae in addition to a novel phytol arsenosugar (AsSugPhytol), which has not been reported in macroalgae.[11] There has been, however, no investigation on the arsenolipids in unicellular algae or cyanobacteria under natural conditions, namely, phytoplankton from oceanic waters; this lack of field data reflects the difficulty in measuring arsenolipids when sample masses are small. Recent developments in methods for analyzing arsenolipids, based on high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS), have enabled the use of smaller sample amounts typical of phytoplankton collection.[10,13,14]

The biosynthesis of arsenolipids by phytoplankton is relevant to the biogeochemical cycling of arsenic. It is presumed that algae and cyanobacteria take up arsenate from seawater because their membrane transporters for the essential nutrient phosphate are unable to distinguish the two very similar oxyanions and arsenate is mistakenly incorporated into biosynthetic schemes designed for phosphate.[15−19] A likely outcome of this interaction is that the formation of arsenolipids is influenced by the phosphate/arsenate ratio in the plankton's ambient water, and this has already been demonstrated in two laboratory studies, one using a macroalga[3] and the other using a unicellular alga.[10] The observed effects, however, showed intriguing differences: when the phosphate/arsenate ratio was low, a macroalgal *Ectocarpus* species produced more arsenosugar phospholipids, whereas the

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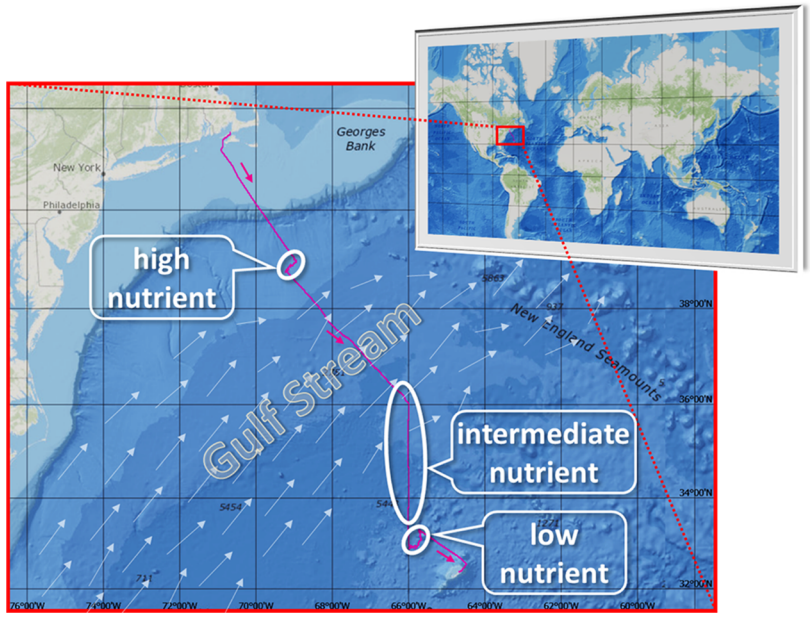

Figure 1. Path of scientific cruise with corresponding 61 sampling sites (high-nutrient sites: HN1−HN27, intermediate-nutrient sites: IN1-IN7, low-nutrient sites: LN1−LN27; numbering from northwest to southeast).

unicellular alga *Dunaliella tertiolecta* produced less. Another clear difference was the dominance of the AsSugPhytol in *D. tertiolecta* and its apparent absence in *Ectocarpus*.

These results are likely to have environmental relevance to the type of arsenolipid found in phytoplankton because in contrast to arsenic concentrations, which are fairly constant at ca. 13−27 nM (1−2 μg As L^{-1}) throughout the world's oceans, phosphate concentrations can vary widely (<0.1−3.2 μmol L^{-1}) with typically lower concentrations (<0.005−1.5 μmol L^{-1}) in surface seawater.[20−22] We hypothesize that the arsenolipid profile for oceanic phytoplankton varies depending on the phosphate concentrations in the water. To test this hypothesis, we collected plankton from marine surface waters of high- and low-nutrient content along a transect spanning the Gulf Stream in the North Atlantic Ocean, and determined their arsenolipid profiles using HPLC-mass spectrometry. The results are discussed in terms of the origin of arsenolipids, their possible biochemical roles, and their significance to the biogeochemical cycling of oceanic arsenic.

MATERIALS AND METHODS

Sample Collection. Seawater and plankton samples were collected during the cruise KN207-01 in the North Atlantic between Woods Hole and Bermuda, which took place from April 21 to May 05, 2012 and covered two distinct maritime regions separated by the Gulf Stream (Figure 1). The first region, northwest of the Gulf Stream, was located in the Labrador Current and is characterized by higher nutrient (HN) levels, resulting in high primary production.[22,23] The second region, the Sargasso Sea, southeast of the Gulf Stream, was subdivided into an intermediate-nutrient (IN) zone at the southeastern boundary of the Gulf Stream featuring seasonal circular systems of water currents and a zone close to Bermuda featuring lower levels of nutrients (LN) with corresponding lower primary production.[22,24] Marine plankton samples were collected at a depth of ca. 5 m by continuously pumping seawater (40−120 L) through four glass fiber filters (Whatman GF/F, 47 mm diameter, 0.7 μm pore size) arranged in parallel (Figure S1). As the plankton built up on the filter surface, the seawater flow continuously decreased from its initial flow of about 175 L h^{-1}; we stopped the sampling when the flow had dropped to about 20 L h^{-1}, which took usually 1−2 h for the HN sites and 3−4 h for the IN and LN sites. Collected filters from each site were frozen (−70 °C), transported to Graz, and stored (−80 °C) until further sample preparation. At the time of analysis, a filter containing the plankton was briefly held at room temperature and by use of a metal punch (12 mm diameter), a subsample from each was taken for total carbon analysis; the remaining filter was used for the determination of arsenic species.

Chemicals and Standards. Water (18.2 MΩ cm) used for all laboratory procedures was obtained from a Milli-Q system (Merck Millipore GmbH, Vienna, Austria). Dichloromethane (≥99.9%, DCM) and methanol (≥99.9%, MeOH) were obtained from VWR (Vienna, Austria). Formic acid (≥98%) and aqueous ammonia (25%, NH_3) were purchased from Carl Roth GmbH (Karlsruhe, Germany), as were the standard solutions (1000 ± 2 mg L^{-1} each) of germanium, indium, and tellurium. For a certified reference material, we used NMIJ CRM 7405-a (Trace Elements and Arsenic Compounds in Seaweed—Hijiki) from the National Metrology Institute of Japan (Tsukuba, Ibaraki, Japan). Six arsenolipids used as standards, namely, AsFA362, AsFA388, AsFA418, AsHC332, AsHC360, and AsHC444 (Figure S2) were synthesized in-house according to Taleshi et al.,[25] and standard solutions of these species, each at 0.1 mM (=7.5 mg As L^{-1}), were prepared in MeOH. Additionally, arsenolipids AsPL958, AsPL986, AsPL1014, AsPL1042, and AsPL1070, which are present in the NMIJ CRM 7405-a, were used as reference species.[26] We also implemented our in-house reference material (*D. tertiolecta*), produced during a previous study in our laboratory in Graz,[11] to further validate the applied methods for those As species absent in CRM Hijiki, namely, AsSugPhytol, AsPL978, AsPL980, AsPL982, and AsPL984. Figure S2 shows the structures for all relevant arsenolipids available as standards in our study.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Instrumentation. Solvents were evaporated on a centrifugal lyophilizer (Christ RVC 2−33 CD plus, Martin Christ GmbH, Osterode am Hartz, Germany). Separation of arsenic species was carried out on an Agilent 1100 series HPLC system prior to post column passive splitting directing 10% of the outflow for online elemental determination to an inductively coupled plasma mass spectrometer (ICPMS; an Agilent 7900 series instrument from Agilent Technologies, Waldbronn, Germany), and the remaining 90% outflow was directed to an electrospray ionization triple quadrupole mass spectrometer (ES-QQQ-MS; Agilent 6460 series instrument) for molecular determination. The ICPMS was equipped with a MicroMist concentric glass nebulizer (Glass Expansion, West Melbourne, Australia) and a Peltier cooled Scott-type double-pass spray chamber with standard Ni interface cones. The ES-QQQ-MS was furnished with a jet-stream atmospheric pressure electrospray ionization source using nitrogen as both nebulizer and drying gas. Measurements were performed in a positive ionization mode. Accurate mass measurements were performed with an electrospray ionization high-resolution mass spectrometer (HR-ESMS; Q-Exactive Hybrid Quadrupole-Orbitrap MS from Thermo Fisher Scientific, Erlangen, Germany) after HPLC on a Dionex Ultimate 3000 series instrument (Thermo Fisher). The HR-ESMS was furnished with a heated atmospheric pressure electrospray ionization source (HESI-II) using nitrogen as both nebulizer and drying gas.

Determination of Nutrients, Biophysical Parameters in Seawater, Chloropigments, and Total Carbon in Biomass. Nutrients were determined in seawater collected along the transect at 18 sites (nine samples at LN, three at IN, and six at HN sites) at depths of 2−8 m and treated as described before.[27] In brief, a portion of filtered seawater, collected in acid-washed bottles, was analyzed for phosphate, silicate, nitrate, nitrite, and ammonia by the Marine Chemistry Laboratory at the University of Washington. The analyses were performed on a colorimetric auto analyzer (Technicon AAII; SEAL Analytical Inc., Wisconsin) following the protocols of the World Ocean Circulation Experiment (WOCE) hydrographic program. In addition, high sensitivity phosphate analyses were conducted at the Woods Hole Oceanographic Institution using the magnesium-induced coprecipitation method (MAGIC) for the IN and LN regions; these data were reported by Martin et al.[22] Additional seawater parameters at 5−11 m depths were determined using in situ instrumentation at 27 HN, 6 IN, and 27 LN sites. Chlorophyll-*a* fluorescence determination was performed using a WET Labs ECO-AFL/FL fluorometer at wavelengths of ex/em = 470/695 nm (Seabird Electronics). Seawater temperature, salinity, and turbidity were measured using a CTD Sea-Bird SBE 911 plus instrument (Seabird Electronics). An SBE 43 sensor (Seabird Electronics) was used to measure dissolved oxygen concentrations. In situ chlorophyll fluorescence measurements were validated by direct measurement of chloropigments in filtered biomass by HPLC-HR-ESMS, as described by Becker et al.[28] Both chlorophyll-*a* and divinyl chlorophyll-*a* were detected in all samples.

As a measure of the total biomass on each filter, the punched-out pieces (12 mm; representing 7.1% of the whole filter surface covered with plankton) were analyzed for their total carbon content. These measurements were performed by the company Bioenergy 2020+ GmbH (Graz, Austria) using a RC612 Multiphase Carbon and Hydrogen/Moisture Determinator. The limit of detection was 0.01 mg C; our measured values ranged from 0.09 to 0.35 mg C. The precision of total C determinations for our samples and experimental method was 0.7−4.7%, evaluated by triplicate measurements of punch outs from three different filters.

Sample Preparation and Arsenolipid Measurements. After total C analysis of the plankton on the punched-out filter piece, the remaining filter from each site was extracted sequentially three times with a mixture of DCM/MeOH (2 + 1, v/v) containing 1.0% ammonia (3 mL, v/w); extracts were filtered (0.2 μm, nylon), combined, and evaporated to dryness under vacuum (25 °C, 10 mbar, 20 h). Prior to analysis by HPLC-ICPMS/ES-QQQ-MS, samples were redissolved in MeOH (250 μL).

Samples were measured by HPLC-ICPMS/ES-QQQ-MS in split mode (passive splitter; fixed ratio), whereby 10% of the HPLC outflow was directed to the ICPMS (Agilent 7900) and the remaining 90% to the ES-QQQ-MS (Agilent 6460). After the splitter, the flow to the ICPMS was augmented by a support flow (water incl. 1 vol % formic acid and 20 μg L^{-1} Ge, In, Te; 0.4 mL min^{-1}) to compensate for the lower flow, as well as to introduce Ge, In, and Te as internal standards. For separation of arsenic species by reversed-phase HPLC, a Shodex Asahipak ODP-50 column (150 × 4.6 mm; 5 μm particle size) was used under gradient elution conditions (mobile phase A: water incl. 0.1 vol % formic acid and B: MeOH incl. 0.1 vol % formic acid): 0−25 min, 60−100% B; 25−32 min, 100% B; 32−32.1 min, 100−60% B; and 32.1−40 min, 60% B. The column was operated at 40 °C; the mobile phase flow was set to 0.5 mL min^{-1}, and the injection volume was 50 μL.

The ES-QQQ-MS was operated in multiple reaction monitoring (MRM) mode where the first quadrupole was adjusted to the precursor ion of relevant arsenolipids and the second quadrupole was set on either m/z 105 or 123 for AsFAs and AsHCs; m/z 111 or 269 for AsSugPhytol; and m/z 97 or 409 for AsPLs (see below for more details). Source parameters for the ionization were: gas temp. 350 °C; gas flow 12 L min^{-1}; nebulizer pressure 25 psi; sheath gas temp. 300 °C; sheath gas flow 10 L min^{-1}; capillary voltage 5.0 kV; and nozzle voltage 2.0 kV. ICPMS was operated under no-gas condition monitoring m/z 75 (^{75}As and $^{40}Ar^{35}Cl$), 53 (^{53}Cr and $^{40}Ar^{13}C$), 77 (^{77}Se and $^{40}Ar^{37}Cl$), 82 (^{82}Se), 74 (^{74}Ge), 115 (^{115}In), and 125 (^{125}Te). Spray chamber temperature was set to −4 °C and gradient compensation was achieved by introducing a solution of water incl. 10 vol % MeOH via the rotatory pump of the ICPMS at a flow of 0.1 mL min^{-1} directly into the spray chamber. Arsenolipids in the samples were quantified from HPLC-ICPMS measurements and external calibration using three synthetic AsHCs (AsHC332, AsHC360, and AsHC444); the data are reported as ng As g^{-1} carbon.

To obtain more details on the identity of arsenolipids present in the plankton samples, we used high-resolution molecular mass spectrometry. HR-ESMS determinations of arsenolipids were performed on a combined algal extract sample to ensure adequate concentrations for accurate mass recording. This extract was prepared by extracting six whole filters with a mixture (15 mL) of DCM/MeOH (2 + 1, v/v) containing 1.0% ammonia (v/w) two times sequentially; combined extracts were filtered (0.2 μm, nylon) and evaporated to dryness under vacuum (25 °C, 10 mbar, 10 h). Prior to analysis by HPLC-HR-ESMS, the sample was redissolved in MeOH (250 μL). We used the same HPLC

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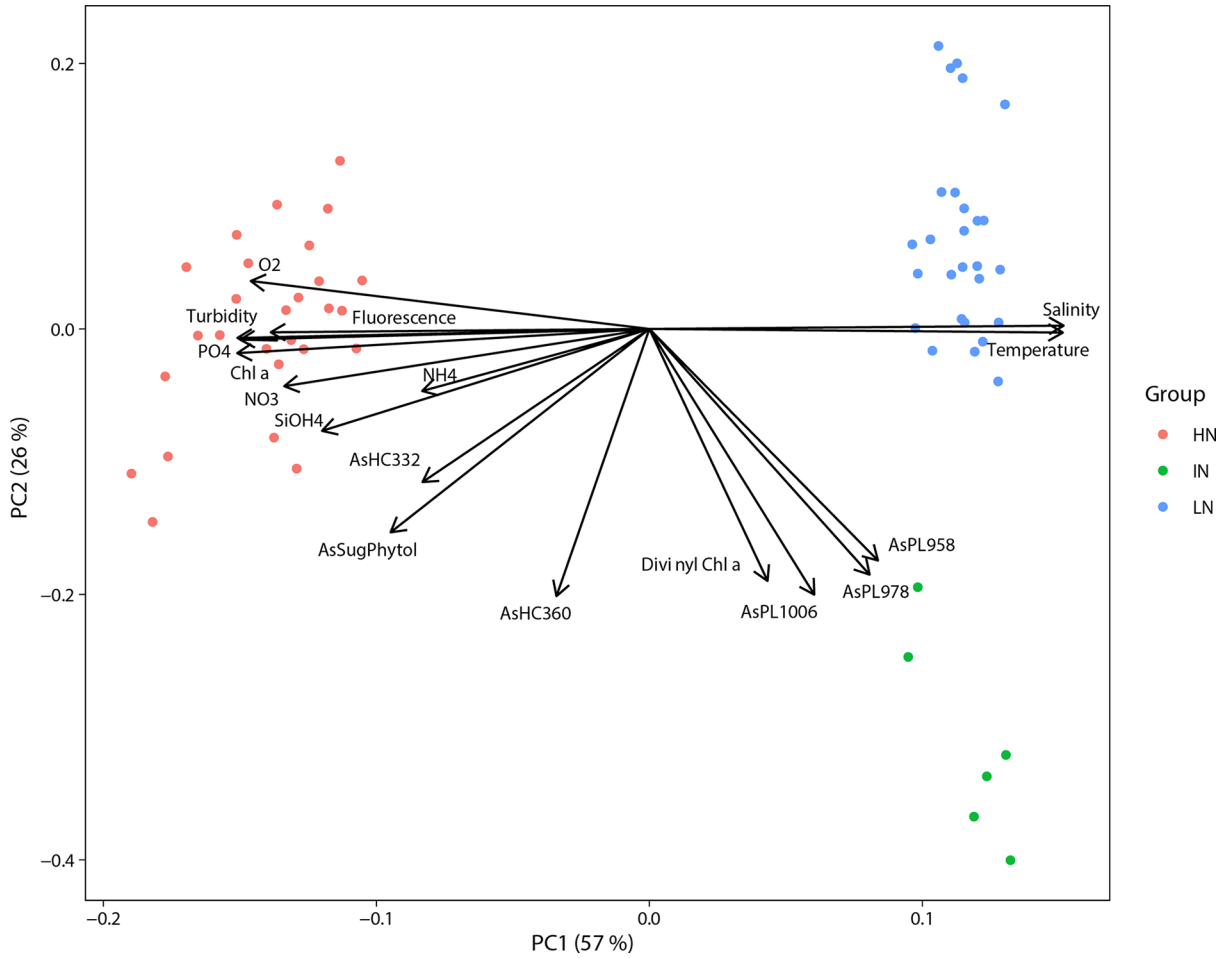

Figure 2. Principal component analysis (PCA) of arsenolipid species with environmental variables. Samples are indicated by dots and colored according to sample site regions that are outlined in the methods section: high-nutrient (HN), intermediate-nutrient (IN), and low-nutrient (LN). Arrows indicate the level of which each variable loaded on principal component 1 and 2 (PC1 and PC2). Latitudinal nutrient and physical water properties were heavily loaded on PC1. High-nutrient characteristics were negatively associated with PC1 and low-nutrient characteristics positively associated with it. PC2 was negatively associated with all arsenolipid species. Notably, all three AsPLs were positively loaded on PC1, opposite to the PO4 concentration, while other arsenolipids were negatively loaded. Furthermore, divinyl chlorophyll-*a* was loaded nearly identically on PC1 and PC2 as the AsPLs.

conditions as described earlier in this work, and directed the entire outflow of the HPLC-column into the mass spectrometer. HR-ESMS measurements were performed in a positive ionization mode, with a drying gas temperature of 350 °C, spray voltage of 4.0 kV, and a resolution of 70 000 (fwhm). The observed mass ranges were set to m/z 100−305, 300−905, and 900−1300 with data-dependent fragmentation at various normalized collision energies (NCE: 15, 30, and 45) and subsequent recording of MS/MS fragment ions. The combined algal extract was also analyzed by HPLC-ICPMS/ES-QQQ-MS, as described earlier in this work.

Statistical Analyses. One-way ANOVA with least significant difference post-hoc test was performed to test differences of the means between sampling sites of different nutrient levels. Significance levels were: $*P < 0.05$ and $**P < 0.001$.

Principal components analysis (PCA) was conducted using the concentration of the six detected arsenolipids, as well as the aforementioned inorganic nutrient, chloropigment, and seawater parameter metadata. The locations and times of the arsenolipid sampling were not exactly coincident with the other seawater metadata. Thus, the arsenolipid data and metadata were paired based on the proximity between where they were collected, no more than 50 km away. Statistical PCA analysis was conducted using the open-source R (4.0.3) "stats" package and "prcomp" method based on correlation. All variables were scaled to have unit variance before analysis, thereby ensuring that no variable dominated the analysis. Additionally, all variables were unit centered. Sample IN1 was excluded from analysis, as incomplete location data prevented pairing with metadata. Graphing was done using the R package "ggplot2."

RESULTS AND DISCUSSION

We divided the transect into three nutrient regions, namely, high-nutrient (HN), intermediate-nutrient (IN), and low-nutrient (LN) sites according to nutrient concentrations determined in the corresponding water bodies (Table S1). Phosphate concentrations dropped from 0.105 ± 0.015 μmol L^{-1} (HN; $n = 27$) to 0.010 ± 0.005 μmol L^{-1} (LN; $n = 27$) after crossing the Gulf Stream; the six IN sites had phosphate concentrations of 0.016 ± 0.004 μmol L^{-1}. Arsenate concentrations, however, remained constant on both sides of the Gulf Stream. For example, our previously reported data for

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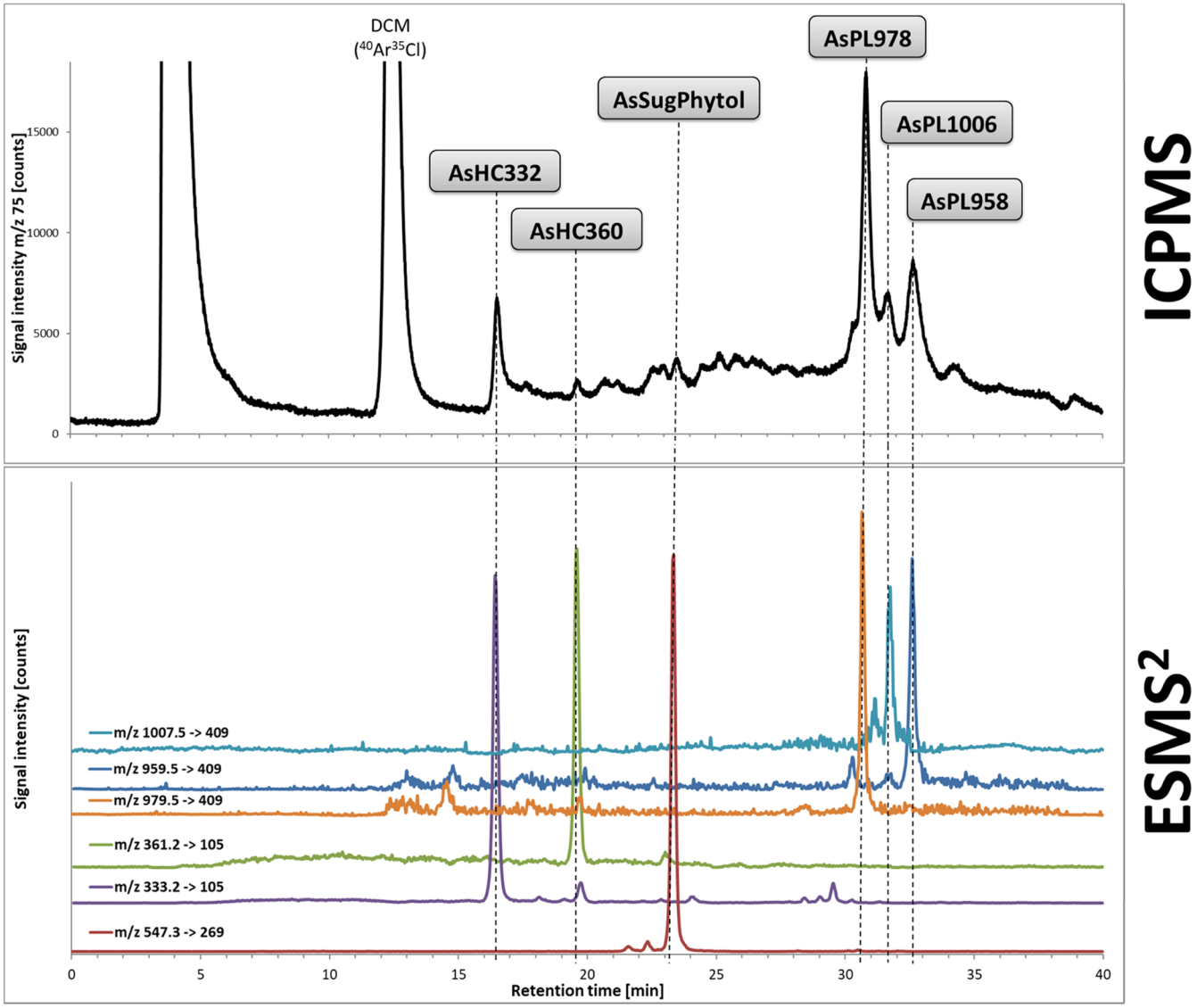

Figure 3. Chromatograms of the combined algal extract showing the six major identified arsenolipids. HPLC conditions: Asahipak ODP-50 (4.6 × 125 mm, 5 μm particles); 40 °C; 50 μL inj. vol.; flow rate 0.5 mL min^{-1}; mobile phase A: 0.1 vol % formic acid in water and B: 0.1 vol % formic acid in MeOH; gradient: 0−25 min, 60−100% B and 25−32 min, 100% B; flow split post column: 90% to ES-QQQ-MS and 10% to ICPMS; sheath flow: 1 vol % formic acid in water at 0.4 mL min^{-1}; and gradient compensation: 10 vol % MeOH in water at 1.0 mL min^{-1}. ES-QQQ-MS detection was performed in positive MRM mode monitoring transitions mentioned above (nitrogen was used as collision gas) with a resolution of 0.3 (fwhm). Arsenic species were identified based on retention time matching with authentic arsenolipid standards and corresponding mass transitions of molecular ions. Furthermore, we used HR-ESMS to determine accurate masses (Δm < 1 ppm) and thereby confirm the identity of individual As species (Table S2). The peak at ca. 12.5 min in the HPLC-ICPMS chromatogram is an artifact of remaining DCM ($^{40}Ar^{35}Cl$) in the sample and was identified by observing a peak at the same RT at m/z 77 ($^{40}Ar^{37}Cl$).

samples collected during the same cruise and from the same waters as the phytoplankton as part of a depth profile sampling showed mean arsenate concentrations of 1.22 ± 0.04 μg As L^{-1} (n = 7) and 1.20 ± 0.05 μg As L^{-1} (n = 8) from high- and low-nutrient areas, respectively.[29] These results were consistent with those reported by Wurl et al. from nearby sites in the North Atlantic showing similar concentrations of arsenate within depth profiles and across sampling sites (1.18 ± 0.21 μg As L^{-1}, reported as 15.7 ± 2.8 nM, n = 8).[30]

Silicate and nitrate featured highest concentrations at HN, intermediate concentrations at IN, and lowest concentrations at LN sites. We obtained further support for our subdivision of the transect by comparing the waters' salinities, temperatures, and chlorophyll-a concentrations (Table S1 and Figure S3). We observed a clear increase of salinity (from 33.7 ± 0.4 PSU to 36.6 ± 0.3 PSU) and temperature (from 12.5 ± 0.4 to 20.2 ± 0.2 °C) from HN to LN sites, whereas chlorophyll-a concentrations dropped from 0.4−1.6 mg m^{-3} at HN sites to 0.05−0.2 mg m^{-3} at LN sites. IN sites featured intermediate salinity and chlorophyll-a values but they had much higher amounts of divinyl chlorophyll-a relative to HN or LN sites (see below). Water temperature was slightly elevated and more variable at IN compared to LN and HN sites, likely due to the influence of the warm Gulf Stream waters. Principal component analysis showed clear delineation of the sites in these three regions (Figure 2). Notably, all three AsPLs were positively loaded on principal component one (PC1), which explained 57% of the variance in the data, while concentrations

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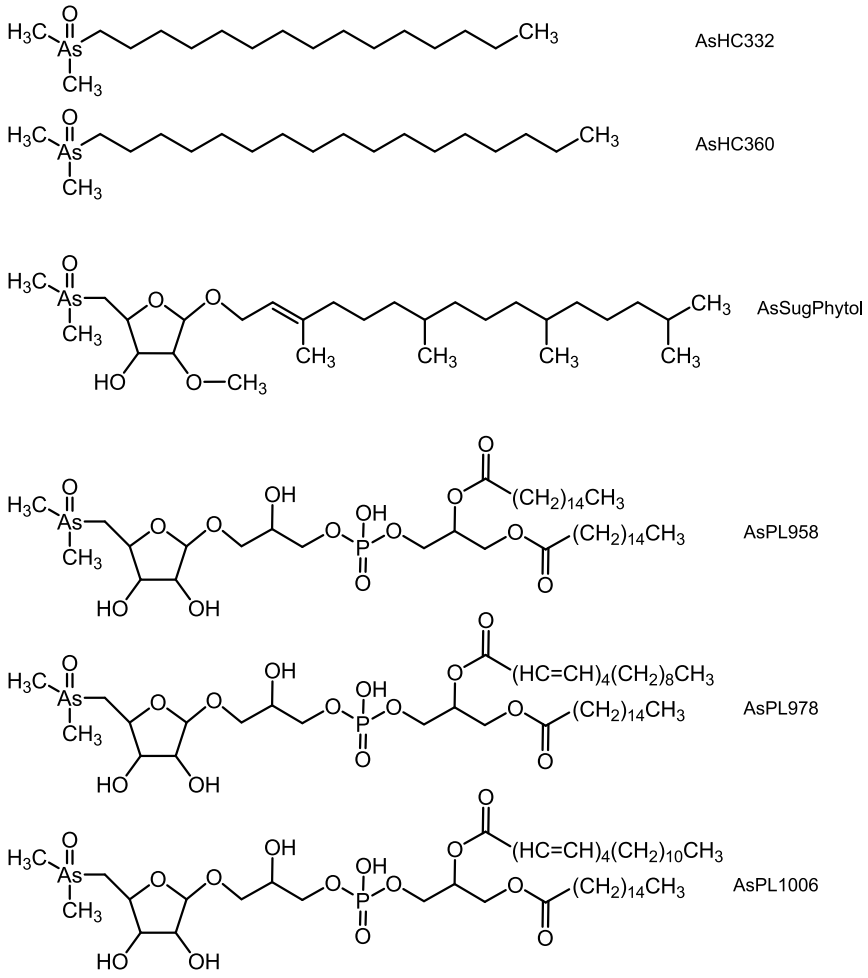

Figure 4. Arsenolipids featured in this study: arsenic hydrocarbons (AsHC332 and AsHC360), arsenosugar phospholipids (AsPL958, AsPL978, and AsPL1006), and phytyl 2-*O*-methylarsenoriboside (AsSugPhytol). The number represents the molecular mass of the arsenolipid; for AsPLs, we show only one possible isomer of the lipophilic sidechains.

of phosphate and the other arsenolipids were negatively loaded.

Analytical Aspects. The quantitative determination of arsenolipids presents analytical challenges because of the multitude of compounds and low concentrations. Our current study provided the additional challenge of handling small sample biomass (1−6 mg C per sample; Table S3) collected on glass fiber filters from oceanic plankton sampling. Because it was not practical to quantitatively scrape off the green biomass deposit from the filter, we decided to use the whole plankton/filter sample and optimize the extraction procedure accordingly. A mixture of DCM/MeOH, previously used to extract arsenolipids from macroalgae,[26] was not successful for the plankton/glass fiber filters owing to a strong interaction between the basic $Me_2As(O)$-group in the compounds and the silica surface of the filters.

These interactions preventing extraction were overcome by adding 1% NH_3 (v/w) to the DCM/MeOH mixture (Figure S4). The efficiency of the method was assessed by spiking three plankton samples with standards of three AsHCs at concentrations comparable to those in the natural samples; returned spike recoveries were 97 ± 5% (AsHC332), 104 ± 9% (AsHC360), and 93 ± 9% (AsHC444) within three replicates each (Figure S5).

Identification of Arsenolipids in Plankton from the North Atlantic. HPLC-MS revealed a multitude of arsenolipids in plankton samples collected from 61 sites encompassing high-, intermediate-, and low-nutrient waters along a transect spanning the Gulf Stream in the Atlantic Ocean (Figure 3). Six of the major compounds present were identified and quantified in all samples, namely, three arsenosugar phospholipids (AsPL958, 10−13%; AsPL978, 13−25%; and AsPL1006, 7−10% of total arsenolipids), two arsenic-containing hydrocarbons (AsHC332, 4−10% and AsHC360, 1−2%), and one phytyl methoxy-arsenosugar lipid (AsSugPhytol, 1−3%) (Figure 4). The compounds were identified based on both retention time matching with authentic standards and reference materials using ICPMS/ES-QQQ-MS and with HR-ESMS confirming the identities with Δm < 1 ppm. Among the identified arsenolipid species, we revealed the presence of a novel arsenosugar phospholipid, namely, AsPL1006 ($C_{49}H_{88}AsO_{14}P$; $[M + H]^+$ = 1007.5200, Δm = +0.6 ppm), an analogue of AsPL978 with an additional ethyl group, using HR-ESMS and subsequently ES-QQQ-MS in MRM mode. There was no indication of the presence of arsenic-containing long-chain fatty acids in any of the samples tested.

Arsenolipid Distribution in Plankton from High-, Intermediate-, and Low-Nutrient Sites in the North

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Atlantic Ocean. We found a similar qualitative profile of arsenolipids for plankton sampled from all nutrient level waters, with the same six compounds predominating and comparable patterns of minor, unidentified compounds (Figure 5). The quantitative data, normalized per g of total C, showed similar levels of total arsenolipids for the HN (1050 ± 270 ng As g^{-1} C) and LN (1160 ± 320 ng As g^{-1} C) sites (Table 1). There were, however, clear and significant differences in the relative amounts of the major individual arsenolipids between the HN and LN sites; plankton from the LN sites had 17−50% higher levels of each of the three arsenosugar phospholipids and correspondingly lower levels of the other three non-P containing arsenolipids (Table 1 and Figure 6; also see Table S3 for complete data set).

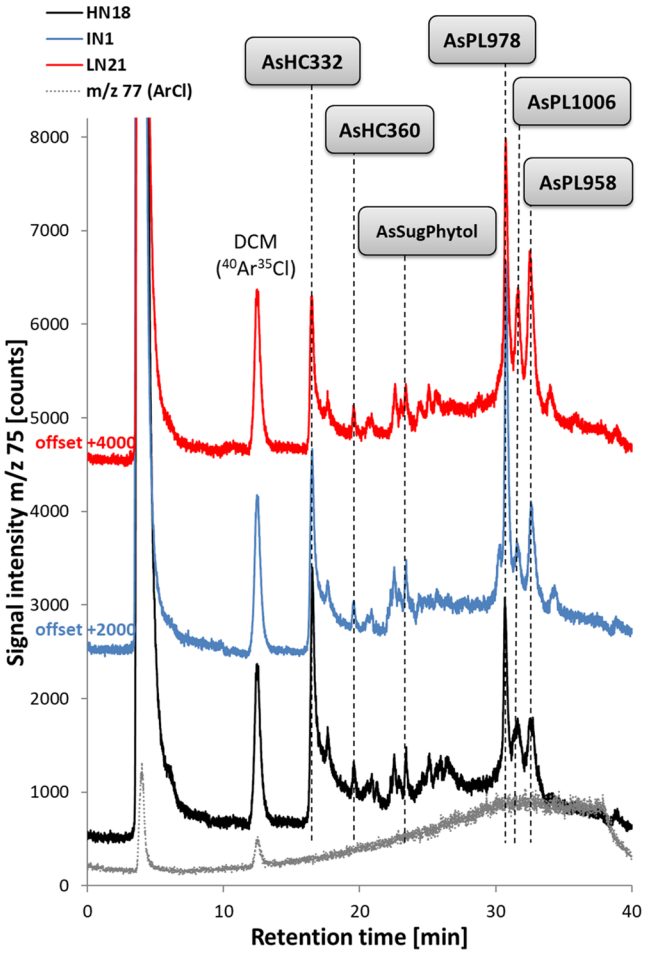

Figure 5. HPLC-ICPMS chromatograms of lipid extracts from plankton collected from typical high-nutrient (3.7 mg total C extracted), intermediate-nutrient (2.5 mg total C), and low-nutrient (3.0 mg total C) sites showing the four identified arsenolipids and the major unidentified ones. HPLC conditions, as described in Figure 2. The peak at 12.5 min is an artifact of remaining DCM ($^{40}Ar^{35}Cl$) in the samples.

Plankton from the IN sites had a 2-fold higher mean concentration of total arsenolipids (2060 ± 320 ng As g^{-1} C) compared to the other two sites. This large difference was almost entirely due to greatly increased levels of all three arsenosugar phospholipids (Table 1). The levels of non-P arsenolipids were also generally higher than those at the HN or LN sites, but the differences, in terms of absolute concentrations, were small, and there was no clear pattern in the data (Table 1 and Figure 6). The IN sites also differed from the HN or LN sites by showing 10-fold higher levels of divinyl chlorophyll-*a*, a biomarker for cyanobacteria of the *Prochlorococcus* genus (Figure S3). In culture experiments, cyanobacteria are known to biosynthesize arsenosugar phospholipids, but other arsenolipids have not been detected.[8,9] *Prochlorococcus* possess a broad complement of arsenic detoxification genes,[31] and these genes are more prevalent in low-phosphate regions of the ocean.[32]

The PCA recapitulated the distinct differences in arsenolipid concentrations between regions, showing that higher concentrations of arsenosugar phospholipids at the IN region contributed significantly to the variance in the overall data set (Figure 2). The amounts of divinyl chlorophyll-*a* loaded in the PCA were almost identical to the arsenosugar phospholipids.

Environmental Considerations. Although our sampling method provided only a combined plankton sample, the pore size of our GF/F filters (0.7 μm) likely allowed most bacterial organisms to pass through, and therefore we assume we collected mainly cyanobacteria and unicellular algae. The collection period corresponded to the spring phytoplankton bloom in the North Atlantic, increasing the likelihood that the major sample components were cyanobacteria and unicellular algae.[24,33] Furthermore, arsenic hydrocarbons and arsenosugar phospholipids, characteristic arsenicals in algae,[6,10,34] were dominant in our plankton samples, whereas arsenic fatty acids (bound and free), commonly reported in marine animals,[35−38] were not present. Also present in all our samples was the recently discovered phytol derivative (AsSugPhytol), which is a product from unicellular algae like *D. tertiolecta*,[10,11] but was also recently found in other marine and hypersaline/hyper alkaline environments.[13,14,39]

While there are no data clearly demonstrating a biological role for arsenic, it is increasingly being reported in molecules that play important roles in biology, for example, bound into phosphatidylcholines as constituents of membrane lipids.[40] The various arsenolipids produced by marine plankton possibly reflect the needs of the organism to discriminate P from other elements and reserve the P for biosynthesis of nucleic acids. Such an interplay between P and other elements (e.g., N and S) in phytoplankton has been reported in a study showing that when oceanic phosphate levels were low,

Table 1. Arsenolipid Concentrations (ng As g^{-1} carbon) Present on Filters Obtained from High-, Intermediate-, and Low-Nutrient Areas in the Atlantic Ocean (Mean ± s.d.)[a]

sample	AsHC332	AsHC360	AsSugPhytol	AsPL978	AsPL1006	AsPL958	total AsLip
HN1−27	105 ± 32	17 ± 4	26 ± 7	135 ± 34	81 ± 20	104 ± 26	1050 ± 270
IN1−7	86 ± 18	29 ± 6	30 ± 5	507 ± 51	188 ± 38	277 ± 68	2060 ± 320
LN1−27	68 ± 22	11 ± 4	10 ± 4	203 ± 61	95 ± 28	151 ± 46	1160 ± 320

[a]For significant statistical differences between sites, see Figure 6.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

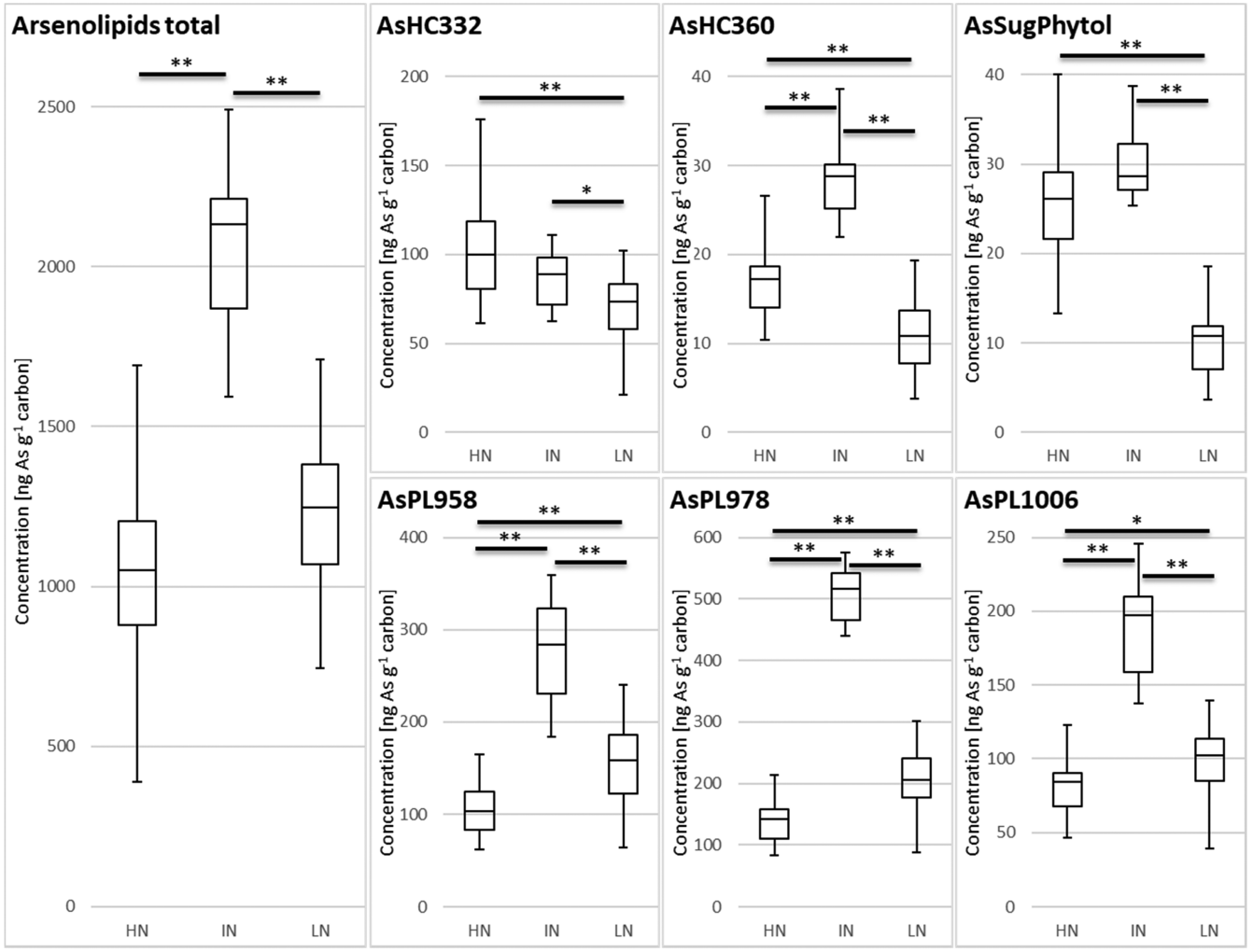

Figure 6. Concentrations of the major arsenolipids occurring in sampled areas; high-nutrient: HN1−HN27 (n = 27), intermediate-nutrient: IN1-IN7 (n = 7), and low-nutrient: LN1−LN27 (n = 27). Quantification based on HPLC-ICPMS data. Significant statistical differences between sites are indicated with *P < 0.05 or **P < 0.001.

phytoplankton produced more S- and N-containing lipids in an effort to preserve essential P for nucleic acid synthesis.[41,42] Based on previous laboratory experiments with *Dunaliella*,[10,11] we expected to find that in low-phosphate waters, non-P arsenolipids, such as AsHCs and AsSugPhytol, would predominate. Our field results, however, show the opposite effect with AsPLs being the major arsenolipids when phosphate levels were low. We note that in our field study, there could be changes in the planktonic community along the transect, especially in the intermediate-nutrient zone, as mentioned above, and as previously reported.[43,44] Indeed, cyanobacteria of the genus *Prochlorococcus* were very abundant (indicated by its biomarker divinyl chlorophyll-*a*) at the IN stations where AsPL concentrations were the highest (Figure S3). Cyanobacteria produce arsenosugar phospholipids to the apparent exclusion of other arsenolipids,[8,9] and thus the relative contributions of cyanobacteria and algae to the plankton biomass could greatly influence the relative amounts of P- and non-P arsenolipids.

The difference in results between the laboratory- and field-based studies with unicellular algae highlights the practical limitations with both types of experiments. The laboratory experiment could precisely control arsenate exposure covering high to low values, but phosphate levels needed to be kept rather high (1 μmol L^{-1}) to maintain algal growth to produce sufficient biomass for subsequent measurements.[10] In the current field study, on the other hand, arsenate concentrations were fairly constant (typical for oceanic waters), and it was not possible to obtain samples covering a wide range of natural arsenate exposures. In contrast to arsenate, phosphate levels can vary widely among oceanic water bodies, and with depth. The Gulf Stream creates a clear division with waters north of the stream (our HN sites, ca. 0.10 μmol L^{-1}) having phosphate levels 10-fold higher than those south of the Gulf stream (LN sites, ca. 0.01 μmol L^{-1}.). Within our HN or LN sites, however, there was little variation in phosphate levels of surface waters, and thus there was no opportunity to investigate changes to arsenolipids at phosphate exposures between ca. 0.01 and 0.10 μmol L^{-1}. We note that Wurl et al. observed, through seawater depth profiles at sites in the North Atlantic, that the relative amounts of small arsenic species, such as methylarsonate and dimethylarsinate, in seawater were influenced by phosphate concentrations.[45]

Our data can be used to provide a first global estimate of arsenolipids for future models of arsenic biogeochemistry. The global phytoplankton productivity has been estimated at 45−50 billion tons of carbon (50 × 1015 g C) per year, a value that represents almost half the world's total primary production

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

including all land plants.[46] Combining this phytoplankton productivity value with our data on arsenolipid content in phytoplankton (Table 1), we estimate that each year, the oceans' phytoplankton incorporate 50 000−100 000 tons of arsenic into arsenolipids. When speculating on the fate of this large quantity of arsenolipids, it is important to consider the rapid turnover rate of phytoplankton in the sea, which results in only about 2% of the total annual production of phytoplankton being alive at any one time.[46] Phytoplankton are constantly being consumed by the oceans' heterotrophs leading to phytoplankton debris and fecal material, which sink slowly through the water column. Microbes may then degrade the arsenolipids to simpler arsenic species that are released into the water.[4] Such a scenario would be consistent with depth profiles of arsenic species in North Atlantic waters, where arsenobetaine and several other small arsenicals were associated mainly with the photic zone but were also found at depths of 200 m and beyond.[29]

In summary, we report that arsenolipids were present in all 61 natural phytoplankton samples collected from areas of high-, intermediate-, and low-nutrient contents in the North Atlantic. The sum of arsenolipids remained fairly constant between samples collected at low- and high-nutrient sites, although the type of arsenolipids produced varied substantially with the seawater's nutrient content. It is most likely that arsenolipids will prove to be widespread among unicellular algae and cyanobacteria in the global oceans, and thus their biosynthesis and degradation, under conditions of varying ocean chemistries, will play a pivotal role in understanding the biogeochemical cycling of arsenic.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06901.

Descriptive text—the optimization of the extraction procedure; nutrient concentrations (Table S1); high-resolution mass spectral data for arsenolipid species (Table S2); organic biomass (mg carbon per filter) and arsenolipids (ng As g^{-1} carbon) present on filters containing plankton (Table S3); the sampling setup used for collecting plankton samples; (Figure S1) arsenolipid standard compounds (Figure S2); surface seawater salinity, temperature, fluorescence (chlorophyll-*a*), and divinyl chlorophyll-*a* from sampling sites (Figure S3); HPLC-ICPMS chromatograms of CRM Hijiki following different extraction procedures (Figure S4); and HPLC-ICPMS chromatograms of an extract of plankton (Figure S5) (PDF)

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Notes

The authors declare no competing financial interest.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Supporting Information

Arsenolipids in plankton from high and low nutrient oceanic waters along a transect in the North Atlantic

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12 pages comprising

One page descriptive text

Three Tables

Five Figures

One reference

Optimization of the extraction procedure

A preliminary attempt to extract the arsenolipids employing DCM/MeOH mixtures was inefficient for the phytoplankton samples because the active silica (SiO_2) surface of the filter interacted with the basic $Me_2As(O)$-group of the arsenolipids and retained them. Thus, the extraction procedure was optimized by testing solvent mixtures with Hijiki CRM alone, and with Hijiki CRM in the presence of glass fiber filter. In each test, 3 mg (weighed to a precision of 0.01 mg) of Hijiki and 3 mL solvent was used, and the extractions were performed in 15 mL polypropylene tubes. The sample containing only Hijiki was extracted with DCM/MeOH (2+1, v/v), whereas the samples containing Hijiki plus filters were extracted with DCM/MeOH (2+1, v/v), or DCM/MeOH (2+1, v/v) containing 0.1 % NH_3 (v/w), or DCM/MeOH (2+1, v/v) containing 1.0 % NH_3 (v/w), as described in the following section. Recoveries were tested by spiking three high nutrient (HN) samples at levels comparable to the natural level of three arsenolipids with standards of AsHC332 (100 ng As g^{-1}), AsHC360 (20 ng As g^{-1}), and AsHC444 (20 ng As g^{-1}) prior to extraction.

Table S1. Nutrient concentrations (µmol L^{-1}), chlorophyll-*a* (mg m^{-3}; fluorescence), divinyl chlorophyll-*a* (10^8 peak area L^{-1}; HPLC/HR-MS)and temperature (°C) in surface seawaters from high nutrient (HN; *n* = 27, except for divinyl Chl-*a*), intermediate nutrient (IN; *n* = 6), and low nutrient (LN; *n* = 27, except for divinyl Chl-a) sampling sites along the transect in the North Atlantic Ocean (mean ± s.d.; adopted from Van Mooy and Rauch[1]).

Site	Phosphate	Silicate	Nitrate	Nitrite	Ammonia	Chlorophyll-*a*	Divinyl-Chl-*a*	Water temp.
HN	0.105 ± 0.015	1.13 ± 0.21	0.084 ± 0.022	0.007 ± 0.003	0.063 ± 0.058	1.10 ± 0.35	2.4 ±1.3 (*n* = 8)	12.5 ± 0.3
IN	0.016 ± 0.004	0.99 ± 0.02	0.043 ± 0.036	0.023 ± 0.005	0.010 ± 0.001	0.27 ± 0.08	36 ± 30 (*n* = 6)	21.2 ± 1.4
LN	0.010 ± 0.005	0.55 ± 0.17	0.016 ± 0.014	0.011 ± 0.004	0.014 ± 0.014	0.10 ± 0.03	2.3 ± 0.6 (*n* = 3)	20.4 ± 0.1

Table S2. High-resolution mass spectral data for arsenolipid species identified in plankton samples collected in the North Atlantic Ocean.

Compound	Elemental composition	Calculated mass $[M+H]^+$	Measured mass $[M+H]^+$	Mass difference [ppm]
AsHC332	$C_{17}H_{37}AsO$	333.2133	333.2133	+0.1
AsHC360	$C_{19}H_{41}AsO$	361.2446	361.2449	+0.9
AsSugPhytol	$C_{28}H_{55}AsO_5$	547.3338	547.3340	+0.4
AsPL958	$C_{45}H_{88}AsO_{14}P$	959.5200	959.5201	+0.1
AsPL978	$C_{47}H_{84}AsO_{14}P$	979.4887	979.4891	+0.3
AsPL1006	$C_{49}H_{88}AsO_{14}P$	1007.5200	1007.5206	+0.6

Table S3. Organic biomass (mg carbon per filter) and arsenolipids (ng As g^{-1} carbon) present on filters containing plankton collected from high (HN), intermediate (IN) and low nutrient (LN) areas in the Atlantic Ocean (*n* = 1).

Sample	Biomass	AsHC332	AsHC360	AsSugPhytol	AsPL978	AsPL1006	AsPL958	Total AsLip
HN1	3.3	111	17	35	136	92	75	1152
HN2	4.1	63	11	26	92	68	78	767
HN3	4.7	83	14	29	132	76	103	964
HN4	5.9	61	10	13	87	56	69	675
HN5	3.7	109	16	35	152	97	115	1287
HN6	4.8	77	12	28	112	73	81	886
HN7	3.6	83	13	29	143	90	108	1068
HN8	3.8	102	14	29	130	83	93	1129
HN9	3.5	176	17	40	214	123	138	1693
HN10	3.3	146	17	39	194	117	141	1624
HN11	4.1	98	13	29	146	89	108	1259
HN12	4.4	71	13	23	82	47	62	669
HN13	3.1	129	19	26	146	84	112	1036
HN14	2.8	155	23	32	169	94	123	1281
HN15	3.1	160	27	28	172	113	165	1357
HN16	2.8	157	23	27	162	89	129	1213
HN17	4.2	113	18	26	159	90	130	1058
HN18	3.7	92	17	23	156	82	127	1078
HN19	3.5	109	18	20	143	90	98	1051
HN20	4.1	120	18	16	109	76	93	926
HN21	2.9	117	22	21	143	88	121	1195
HN22	5.2	74	14	14	83	50	72	689
HN23	3.5	93	20	24	111	60	84	883
HN24	3.9	100	19	21	118	67	89	873
HN25	3.9	82	18	18	102	61	86	866
HN26	5.7	79	14	22	91	51	73	722
HN27	3.7	77	18	26	160	86	125	963

Table S3 continued.

Sample	Biomass	AsHC332	AsHC360	AsSugPhytol	AsPL978	AsPL1006	AsPL958	Total AsLip
IN1	2.5	71	29	32	575	274	274	2061
IN2	2.8	63	22	26	483	138	187	1593
IN3	2.4	72	29	29	530	150	184	1680
IN4	2.0	96	31	33	516	245	360	2223
IN5	1.3	89	23	39	553	208	284	2134
IN6	1.3	101	27	25	440	212	325	2204
IN7	1.2	111	39	28	448	197	321	2491
LN1	2.0	57	14	12	282	134	199	1294
LN2	1.9	59	8	11	244	98	187	1258
LN3	2.3	65	15	11	270	102	199	1435
LN4	3.2	49	9	8	219	97	158	1125
LN5	2.5	73	13	10	222	98	158	1417
LN6	2.0	71	16	14	298	122	182	1480
LN7	1.7	71	13	12	178	89	112	1074
LN8	1.6	84	19	14	235	108	166	1326
LN9	1.7	78	17	10	227	115	187	1327
LN10	4.8	21	4	4	88	39	63	477
LN11	2.4	61	11	7	179	79	108	929
LN12	1.7	74	16	13	267	112	200	1471
LN13	1.5	75	18	19	259	111	192	1427
LN14	5.0	23	5	5	126	58	140	658
LN15	2.7	59	11	10	184	82	147	1066
LN16	4.6	32	6	6	103	49	80	552
LN17	2.6	37	5	5	111	43	63	631
LN18	3.1	43	5	6	91	45	70	579
LN19	2.2	94	13	7	184	102	184	1392
LN20	2.4	84	9	7	175	92	164	1239
LN21	3.0	75	8	11	238	118	186	1255
LN22	1.6	102	10	11	223	116	135	1248
LN23	1.7	92	8	11	201	105	122	1118
LN24	2.0	85	8	6	198	108	134	1185
LN25	2.1	82	11	12	206	124	165	1374
LN26	1.6	102	12	17	301	139	240	1710
LN27	2.1	80	5	13	173	88	124	1162

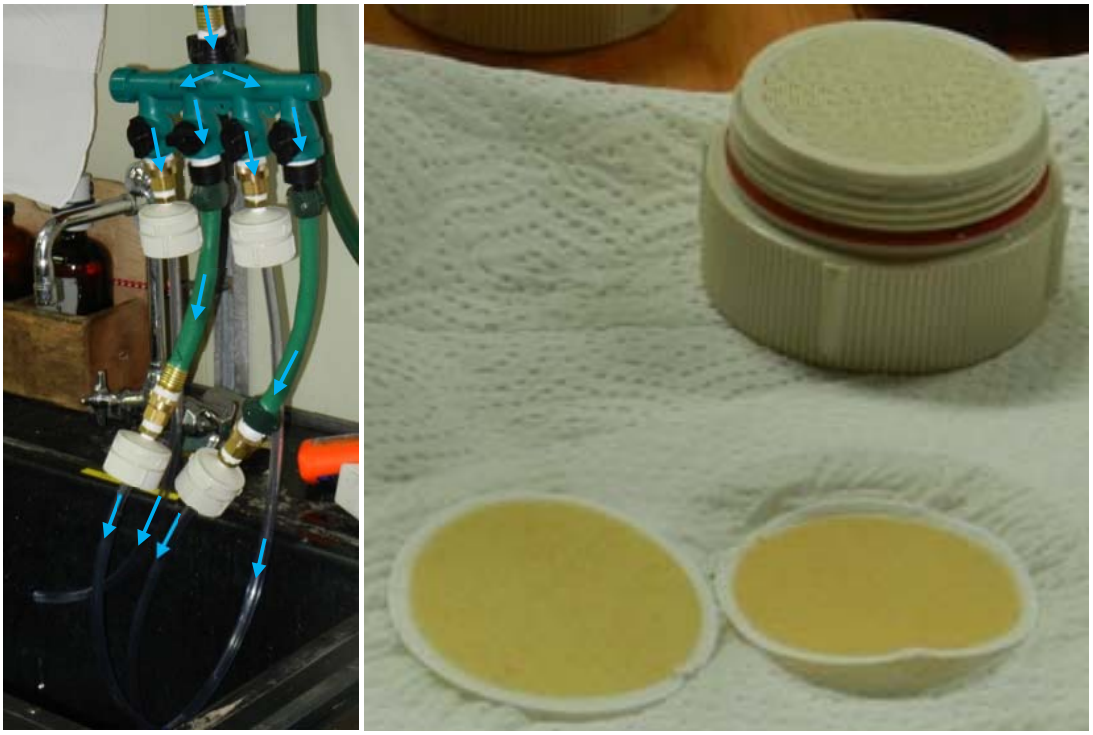

Figure S1. Sampling set-up used for collecting plankton samples onboard the cruise ship (left) and typical plankton containing filters obtained (right). The samples were collected at a depth of ca 5 m by continuously pumping seawater (40-120 L) through four glass fiber filters (Whatman GF/F, 47 mm diameter, 0.7 µm pore size) arranged in parallel.

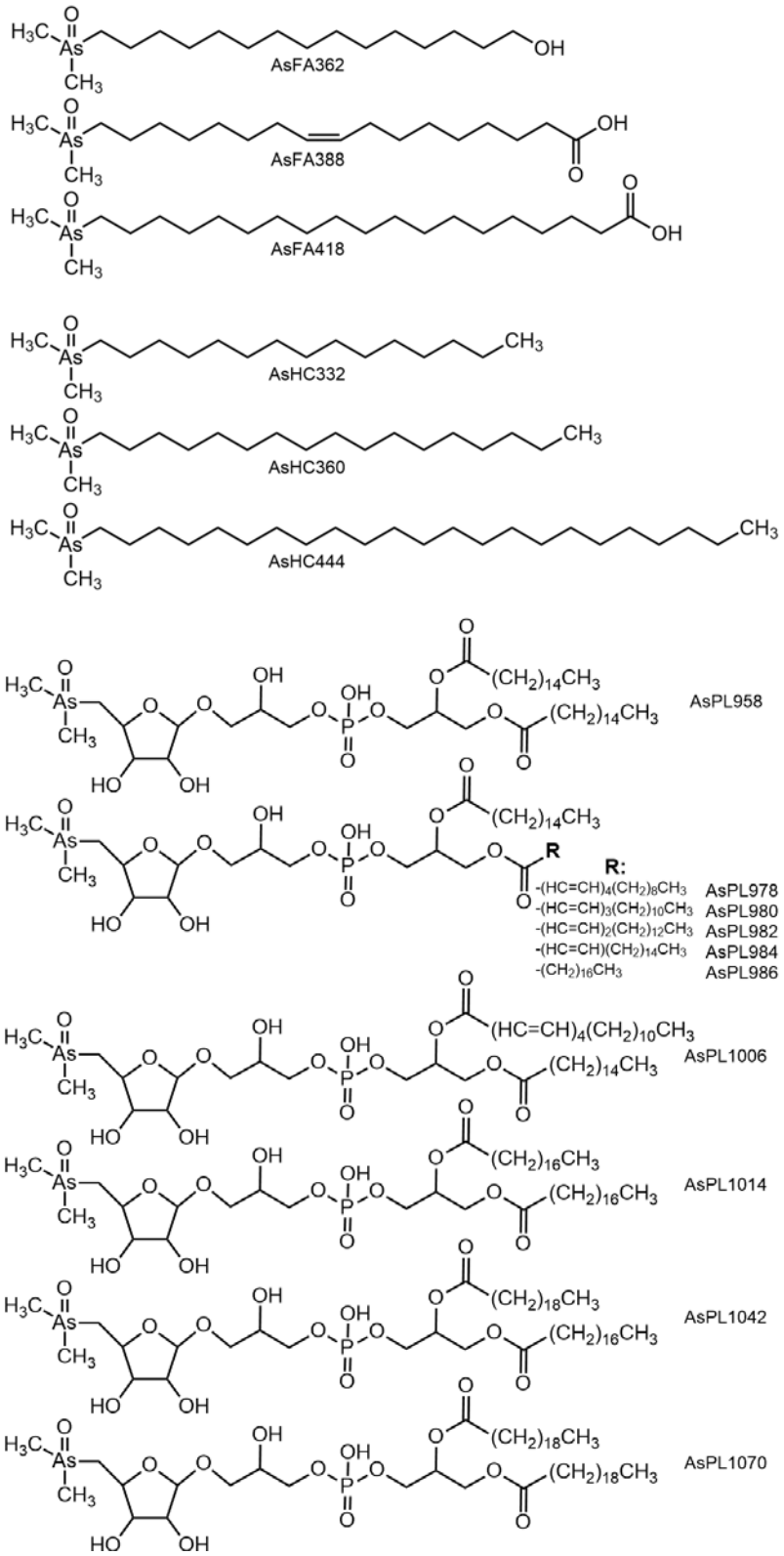

Figure S2. Arsenolipid standard compounds available in this study. AsHCs and AsFAs were synthesized in-house, while AsSugPhytol and AsPLs were previously determined in CRM-Hijiki (NMIJ 7405-a) and our in-house reference material *D. tertiolecta*. Double bond positions of AsSugPhytol, AsPL978, AsPL980, AsPL982, and AsPL984 were not determined; we show one possible isomer for the AsPLs' lipophilic sidechains.

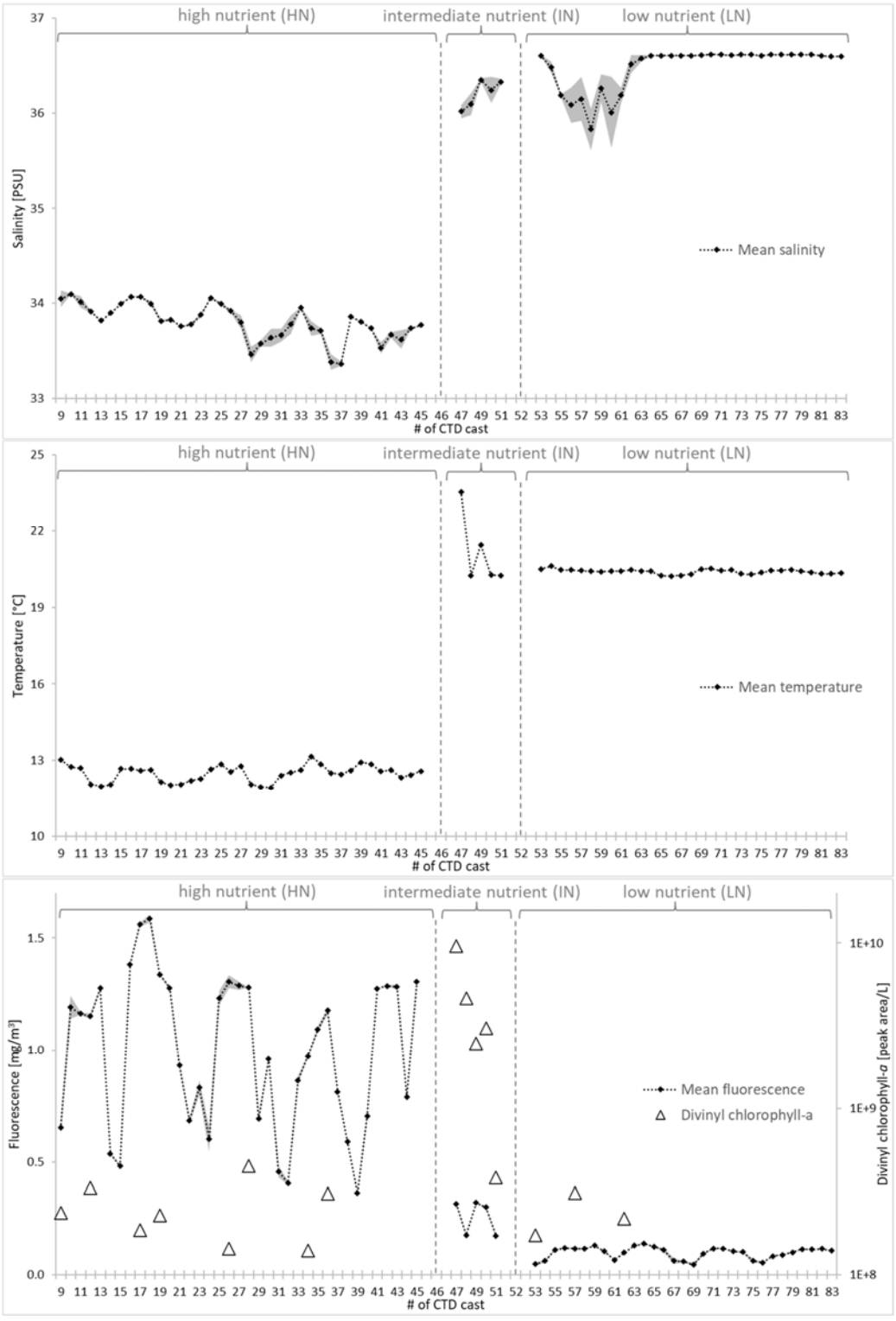

Figure S3. Surface seawater salinity (top), temperature (center), fluorescence (chlorophyll-*a*; bottom), and divinyl chlorophyll *a* from low nutrient (LN), intermediate nutrient (IN), and high nutrient (HN) sampling sites along the transect in the North Atlantic Ocean (mean ± s.d. of $n = 2$; s.d. is represented as grey areas).

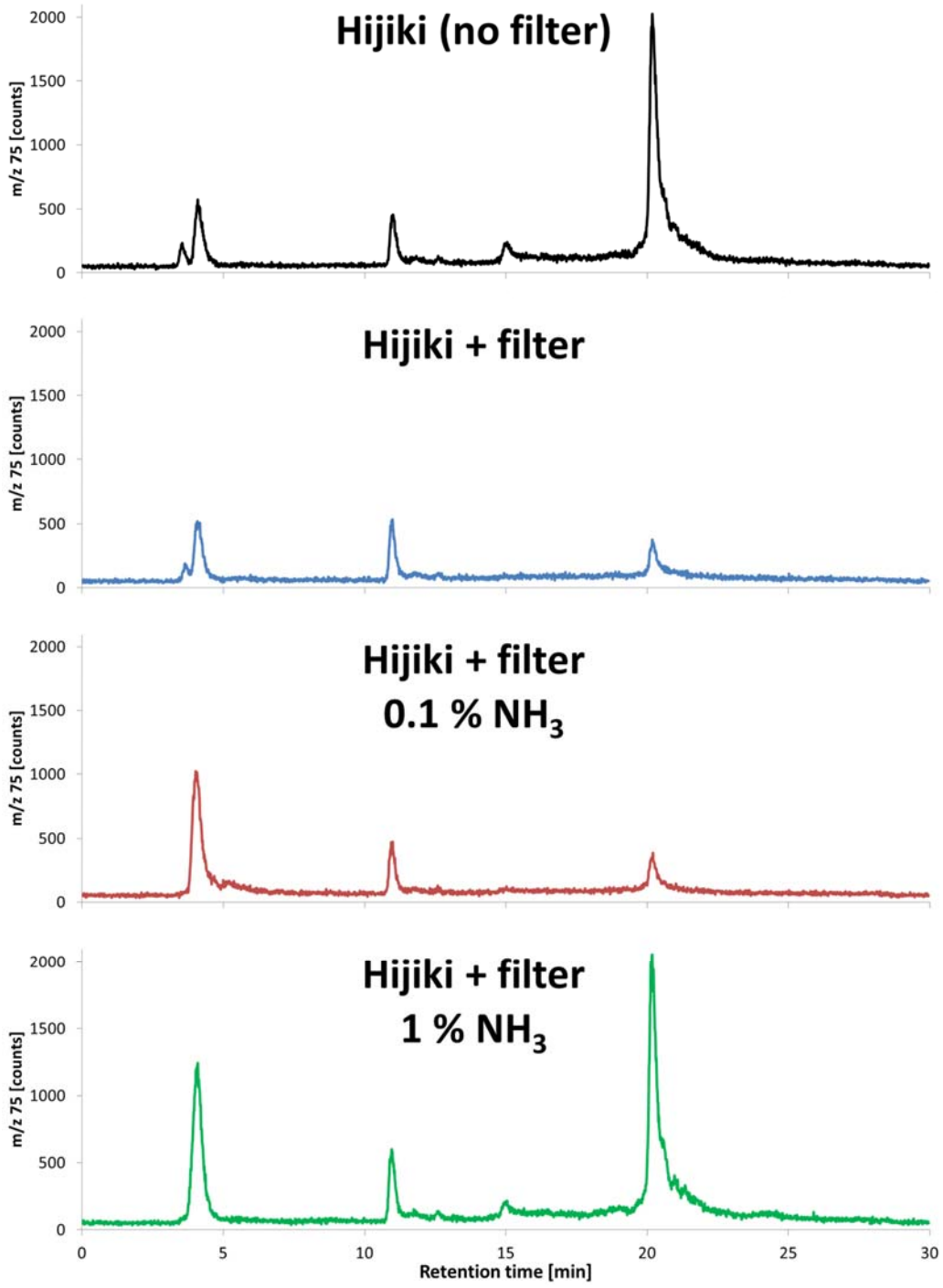

Figure S4. HPLC-ICPMS chromatograms of CRM Hijiki (7405-a). Black: extracted with DCM/MeOH (2+1, v/v); blue: glass fiber filter added, then extracted with DCM/MeOH (2+1, v/v); red: glass fiber filter added, then extracted with DCM/MeOH (2+1, v/v) containing 0.1 % ammonia (v/w); green: glass fiber filter added, then extracted with DCM/MeOH (2+1, v/v) containing 1.0 % ammonia (v/w). Asahipak ODP-50 (4.6 x 125 mm, 5 µm particles); 40 °C; 50 µL inj. vol.; flow rate 0.5 mL min^{-1}; mobile phase A: 0.1 %vol formic acid in water, B: 0.1 %vol formic acid in MeOH; gradient: 0-15 min, 60-100 % B; 15-20 min, 100 % B; 10 % split to ICPMS; sheath flow 1 %vol formic acid in water at 0.4 mL min^{-1}; gradient compensation 10 %vol MeOH in water at 0.1 mL min^{-1}.

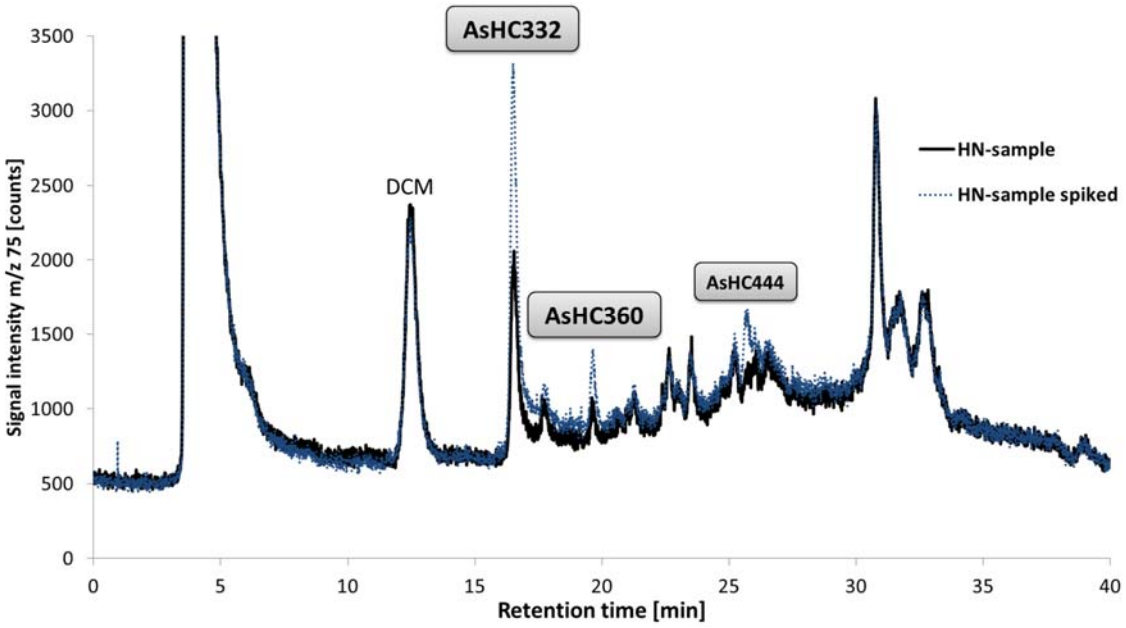

Figure S5. HPLC-ICPMS chromatograms of an extract of plankton from a typical high nutrient site and the same extract spiked with arsenolipid standards AsHC332 spiked at 100 ng As g^{-1}; and AsHC360 and AsHC444 spiked at 20 ng As g^{-1}. HPLC conditions (also described in Figure 2) were: Asahipak ODP-50 (4.6 x 125 mm, 5 µm particles); 40 °C; 50 µL inj. vol.; flow rate 0.5 mL min^{-1}; mobile phase A: 0.1 %vol formic acid in water, B: 0.1 %vol formic acid in MeOH; gradient: 0-25 min, 60-100 % B; 25-32 min, 100 % B; flow split post column. Peak at 12.5 min is an artefact of remaining DCM ($^{40}Ar^{35}Cl$) in the samples.

References

(1) Van Mooy, Benjamin A. S.; Rauch, S. *Inorganic nutrients from R/V Knorr cruise KN207-01 (SargassoSeaLipids project)*. http://lod.bco-dmo.org/id/dataset/3985 (accessed 2021-01-20).