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Oliver Wurl

Louise Zimmer

Gregory A. Cutter
Old Dominion University, gcutter@odu.edu

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Arsenic and phosphorus biogeochemistry in the ocean: Arsenic species as proxies for P-limitation

Oliver Wurl,[1,*] Louise Zimmer,[2] and Gregory A. Cutter

Department of Ocean, Earth, and Atmospheric Sciences, Old Dominion University, Norfolk, Virginia

Abstract

Arsenic and phosphorus are biochemically very similar, and hence arsenate (As^{5+}) is toxic by interfering with the energy metabolism, in particular during P limitation. However, many phytoplankton detoxify As by reducing arsenate to arsenite (As^{3+}), and/or methylating it to mono and dimethyl As. Such As detoxification becomes operative in oligotrophic waters when phosphate concentrations are below those for As; therefore, we evaluated the potential use of these detoxification products as indicators of P limitation by measuring As speciation during the US GEOTRACES North Atlantic transect. The distribution of As^{3+} concentrations in surface waters is similar to that of N : P ratios and alkaline phosphatase activity (APA), two conventional proxies for P-limitation. As^{3+} concentrations have a very similar relationship to phosphate as APA to phosphate, and therefore indicate the potential of As^{3+} as proxy for P-limitation. From the relationship to phosphate we derived threshold values of As^{3+} concentration to indicate moderate and extreme P-limitation. We then applied these threshold values to assess P-limitation with high horizontal resolution in the North Atlantic, improving on the contradictory assessments using the conventional proxies. Our new evaluation is consistent with the general concept that the North Atlantic is moderately to extremely limited in phosphate.

Arsenic and phosphorus are biochemically very similar, so that phytoplanktonic uptake of arsenate (As^{5+}) induces toxic effects due to its substitution in the adenotriphosphate (ATP) cycle, effectively decoupling the energy metabolism (Lehninger 1975). Many phytoplankton species evolved different strategies to ameliorate the toxic effects of As^{5+}, including reduction to arsenite (As^{3+}) and methylation to monomethyl- (MMAs) and dimethylarsenic (DMAs). These detoxification products are less harmful and easier to excrete (Andreae and Klumpp 1979; Sanders and Riedel 1993; Hellweger et al. 2003). Hellweger et al. (2003) proposed a mechanistic model with a limited methylation capacity due to slower kinetics compared with the preceding reduction. Such capacity may be exceeded during luxury P uptake because the chances of taking up As^{5+} increase with higher rates of P uptake. Hellweger et al. (2003) proposed that exceeding methylation capacity leads to the excretion of the reduced As^{3+}. Surface waters of oligotrophic central gyres have particularly high As : P ratios, with As^{5+} concentrations in a range of 10–15 nmol L^{-1} (Cutter et al. 2001; Cutter and Cutter 2006), while phosphate is typically within a range of 0.2–10 nmol L^{-1} (Wu et al. 2000; Ammerman et al. 2003). Some phytoplankton species are better adapted to live in regimes with high As : P ratios than others (Planas and Healey 1978; Sanders and Vermersch 1982). For example, several phytoplankton have evolved a P uptake mechanism that discriminates against As^{5+} transport into the cell (Maher and Butler 1988). Nevertheless, the excretion of detoxification products is potentially marking waters where P limitation is occurring and therefore As^{3+} and methylated As are potential proxies.

Nutrient limitation has been of interest to the marine scientific community for decades due to its importance in the ecology of marine systems. For example, climate change over the past decades has induced stronger stratification in the subtropical Pacific, favoring nitrogen-fixing organisms (Karl et al. 2001). The increase in the nitrogen pool shifts phytoplankton communities toward phosphorus limitation (Karl et al. 2001). It has also been shown that the stoichiometric ratio N : P plays a controlling factor in carbon export in the deep ocean (Broecker 1982; Geider and La Roche 2002). For future studies, the marine science community requires easily applicable and reliable tools to assess the status of nutrient limitation. Beardall et al. (2001) reviewed the benefits and weaknesses of approaches for determining nutrient limitation. For example, the ratio of N : P as a proxy is based on the Redfield ratio, which is a general atomic ratio of carbon, nitrogen, and phosphorus found in plankton throughout the ocean (Redfield et al. 1963). Although the Redfield ratio is a foundation of marine biogeochemistry, modern oceanography recognizes wide diversions from the assumption that the Redfield ratio of N : P = 16 : 1 is constant through the ocean and among different phytoplankton species (Falkowski 2000). Therefore, the N : P ratio as a proxy for nutrient limitation involves uncertainties that make it a qualitative indicator at best. Alkaline phosphatase activity (APA), the activity of the enzyme cleaving phosphate esters from organic matter, is expressed by phytoplankton as response to limitation of inorganic phosphate. APA typically increases under severe P-limitation, but caution in interpretation of APA data is required due to secondary

* Corresponding author: oliver.wurl@io-warnemuende.de

Present address:

[1] Leibniz Institute for Baltic Sea Research Warnemünde, Germany

[2] Present address: Aarhus University, Department of Bioscience, Frederiksborgvej 399 Postboks 358, DK-4000 Roskilde, Denmark

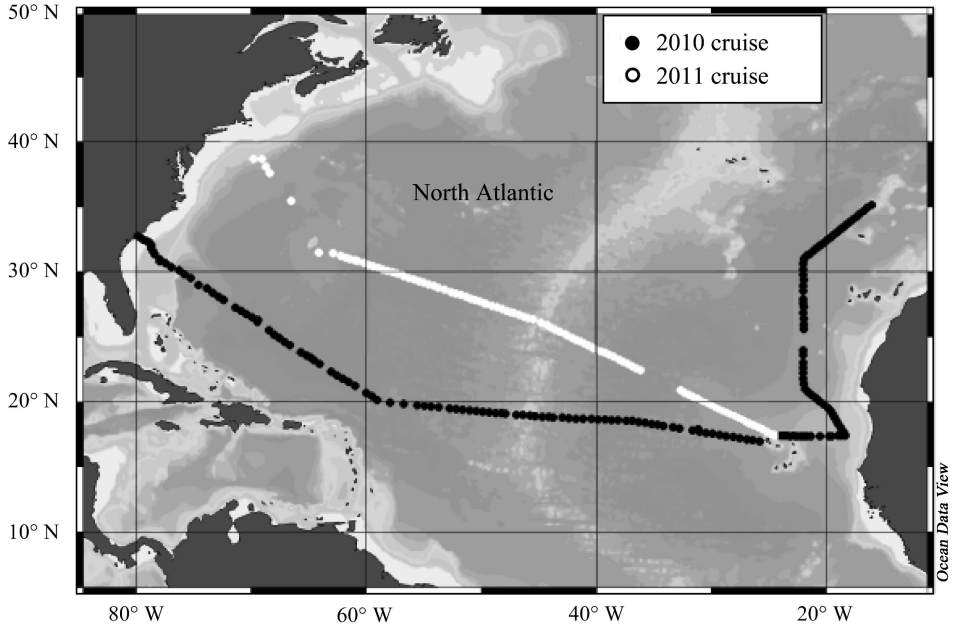

Fig. 1. Station locations and cruise track for the 2010 (black dots) and 2011 (white dots) U.S. GEOTRACES expeditions in the North Atlantic.

N-limitation, contribution from bacterial enzymes, potential release of the enzyme from cells, and the temporal lag in expression (Beardall et al. 2001; Duhamel et al. 2010). The complexities of nutrient uptake and utilization, and ambiguities in existing approaches, caused Jansson et al. (1988) and Beardall et al. (2001) to suggest the use of multiple methods for assessing P-limitation. The development of new proxies for nutrient limitation will contribute to a better understanding of nutrient uptake and cycling in the ocean.

In this paper, we examine the suitability of As^{3+} and methylated As as new proxies for P-limitation and their potential to complement existing assessment methodologies. The various residence times of As^{3+} and methylated As (days to months) determine the period over which these potential proxies may be integrating. We present an extensive data set on the concentrations of As species, phosphate, nitrate, and alkaline phosphate activity (APA) from two trans-North Atlantic U.S. GEOTRACES cruises.

Methods

Study area and sampling methods—We participated in two U.S. GEOTRACES expeditions in the subtropical Atlantic in 2010 and 2011 (Fig. 1). The 2010 expedition used the R/V *Knorr* and left Lisbon, Portugal, on 15 October, stopped in Sao Vicente, Cape Verde Islands, and arrived in Charleston, South Carolina, on 27 November. In 2011, R/V *Knorr* left Woods Hole, Massachusetts, on 06 October and arrived 11 December in Praia, Cape Verde Islands. A total of 180 and 96 surface samples were collected during the 2010 and 2011 expeditions, respectively. Surface samples were collected from an underway clean surface sampling system described in detail elsewhere (Bruland et al. 2005). Briefly, the sampling system was towed ~ 5 m off the starboard, aft quarter of the ship at 1–3 m depth depending on ship's roll. The sampling system included a polytetrafluoroethylene Teflon™ diaphragm pump (Bruiser™, Osmonics) and perfluoroalkoxy Teflon™ tubing to provide contamination-free surface water directly to the ship's analytical laboratory. Surface samples were on-line filtered through 0.2 μm Acropak filter cartridges and collected in fluoroethylenepropylene (FEP) bottles, typically every 4 h on a 24 h basis. Surface samples were also collected for the determination of nitrate, nitrite, and phosphate in the lower nmol L^{-1} range, as well as for APA in 150 μm-screened samples. Salinity, sea-surface temperature, and fluorescence data were also recorded underway through the ship's sensors.

Arsenic measurements—Due to instability of As species during storage, we conducted shipboard determinations of As^{3+}, As^{5+}, MMAs, and DMAs within 6 h of collection. Arsenic species were determined utilizing a selective hydride generation method with cryogenic trapping and gas chromatography-photoionization detection (GC-PID; Cutter et al. 1991). A chromatographic Carbopack B-HT column (2 m length of 3.2 mm inner diameter FEP tubing, 60–80 Mesh) was used for the analysis of As^{3+}. A column filled with 15% OV 3 on Chromasorb W-AW DMCS (4.6 m length of 3.2 mm inner diameter FEP tubing, 80–100 mesh) was used for the analysis of total As (i.e., As^{3+} + As^{5+}), MMAs, and DMAs. Fifty milliliters (mL) of sample treated with sulfanilamide (0.5 mL of 2% solution) to remove nitrite interferences (Cutter and Cutter 2006) were degassed in a stripper for 2 min. As^{3+} was then converted to its hydride by the addition of $NaBH_4$ at a pH of 6.2 (Tris(hydroxymethyl)-aminomethane-HCl buffer), trapped

for 7 min on a cryogenic trap, and re-volatilized and swept into the GC/PID. Similarly, hydride generation in the presence of 0.5 mol L^{-1} HCl yielded the concentrations of total As, MMAs, and DMAs. Two GC-PID systems, one for As^{3+} and one for methyl As, were calibrated daily using the standard additions method with surface seawater. Detection limits for inorganic and methylated As were 0.001 nmol L^{-1} and 0.005 nmol L^{-1}, respectively (Cutter and Cutter 2006). Precision was better than 5% (relative standard deviation) at 0.5 nmol L^{-1}.

Alkaline phosphatase activity (APA)—APA was measured fluorometrically using 4-methylumbelliferyl phosphate (MUF-P, Sigma-Aldrich) as substrate according to Ammerman (1993). Samples for APA were collected through a 150 μm mesh screen to remove large zooplankton from the contamination-free seawater system just prior to or after the collection of As samples. The substrate was added to the samples to a final concentration of 100 nmol L^{-1}, and incubated in the dark at in situ temperature using a flow-through seawater incubator. The tracer assay estimates in situ substrate turnover rates rather than potential activities at saturating substrate levels. We applied the tracer assay to be consistent and relate our measurements to the APA threshold values for nutrient limitation reported by Healey and Hendzel (1979). The tracer assay is also more sensitive because saturating substrate levels result in high background fluorescence. Fluorescence of the product MUF was measured in 3 mL subsamples adjusted to a pH of 10 after various incubation periods (typically 0, 4, 8, 12 h). Fluorescence was read with a fluorometer (Turner Designs Model 10AU) at a wavelength of 455 nm under excitation at 365 nm. Blanks were assessed using boiled seawater, and linearity of the assay was checked weekly with a set of MUF standards (Sigma-Aldrich). Activities were calculated according to Jaeger et al. (2009).

Nutrient determinations—Phosphate, nitrate, and nitrite were measured with a long-path, low volume, liquid waveguide capillary cells (LWCC) linked to a conventional nutrient auto-analyzer. The methods are described in detail elsewhere (Aminot et al. 2009; Zimmer and Cutter 2012). Briefly, surface seawater was filtered through a 0.2 μm hollow fiber filter (MediaKap®-5) and then analyzed on a helium gas-segmented, continuous-flow, nutrient auto-analyzer (Alpkem 300-series). The auto-analyzer was modified with a 200 cm LWCC (World Precision Instruments, Sarasota) and light transmission measured by a light-emitting diode spectrophotometer (LEDSpec, World Precision Instruments). Phosphate was measured at 690 nm based on the formation of a phosphomolybdate complex (molybdenum blue method), and nitrate and nitrite were measured at 540 nm based on sulfanilamide and N-naphthyl-ethylenediamine method with cadmium coil reduction (Aminot et al. 2009). Detection limits were 0.5 nmol PO_4^{3-} L^{-1}, 1.5 nmol NO_3^- L^{-1}, and 0.6 nmol NO_2^- L^{-1}. Precision (as relative standard deviation) was typically below 10% at 5 nmol L^{-1}. Arsenate (As^{5+}) also forms a molybdate complex, and the potential interference of arsenate on phosphate determination has been addressed for the described nutrients analyzer in a recent study (Zimmer and Cutter 2012). Briefly, a phosphate concentration of 10.5 nmol L^{-1} increased by $< 10\%$ after addition of 27 nmol As^{5+} L^{-1}, a concentration exceeding typical levels in the North Atlantic (this study). Continuous-flow analysis has a shorter reaction time (2–7 min) compared with the manual method (90 min), and therefore utilizes the faster formation of the phosphomolybdate complex relative to the arsenomolybdate complex.

Chlorophyll data—We used remotely sensed chlorophyll *a* (Chl *a*) concentrations from the moderate resolution imaging spectroradiometer (MODIS; Carder et al. 2004) instrument onboard the Terra satellite. We obtained Chl *a* concentrations at our sampling locations from the nearest 4 km grid of Level-3 processed MODIS data sets (Feldman and McClain 2012).

Results

Distribution of nutrients and APA—Concentrations of phosphate, APA, and N : P ratios were used to assess nutrient limitation in the study regions and are shown in Fig. 2a–c. Phosphate concentrations exceeded 20 nmol L^{-1} in the West African upwelling region in proximity to the Cape Verde Islands and approaching the U.S. East Coast continental shelf, leading to the highest observed phosphate in the western (60°W to 80°W; mean = 12.7 ± 10.8 nmol L^{-1}) and eastern North Atlantic Ocean (16°W to 35°W; mean = 19.4 ± 17.3 nmol L^{-1}) without a significant difference between the two regions ($p > 0.05$, Kruskal–Wallis test with Dunn's post-comparison). We detected significantly ($p < 0.0001$) lower phosphate concentrations in the central North Atlantic Ocean (35°W to 60°W) with a mean of 7.9 ± 1.6 nmol L^{-1}. We choose the ratio of dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorous (DIP) as a nutrient-based indicator for nutrient limitation. Ptacnik et al. (2010) ranked the ratio DIN : DIP as a well-performing indicator for nutrient status, whereas the ratio of particulate organic nutrients was the worst performing indicator due to biologically recalcitrant nitrogen compounds and non-autotrophic sources. In the following, the ratio N : P refers to DIN : DIP. The mean N : P ratio in the eastern, central, and western North Atlantic Ocean was 4.1 ± 4.4, 4.2 ± 1.8, and 3.9 ± 1.7, respectively. The highest N : P ratios were detected in West African upwelling region of the eastern North Atlantic Ocean (Fig. 2b). The ratio further south within the West African upwelling region was low (N : P < 3; Fig. 2b), and values in this range indicate no P-limitation, but N as the limiting nutrient (Wu et al. 2000). The existence of N limitation is consistent with the lowest APA (0.3 nmol P L^{-1} h^{-1}) and highest phosphate concentrations (70 nmol L^{-1}) measured throughout the study. In 2011, we observed N : P ratios of up to 15 in the subtropical gyre of the central Atlantic Ocean between 45°W and 55°W (Fig. 2b), which indicated a tendency toward more P-limiting conditions in the surface waters due to higher nitrate concentrations (up to 50 nmol L^{-1}).

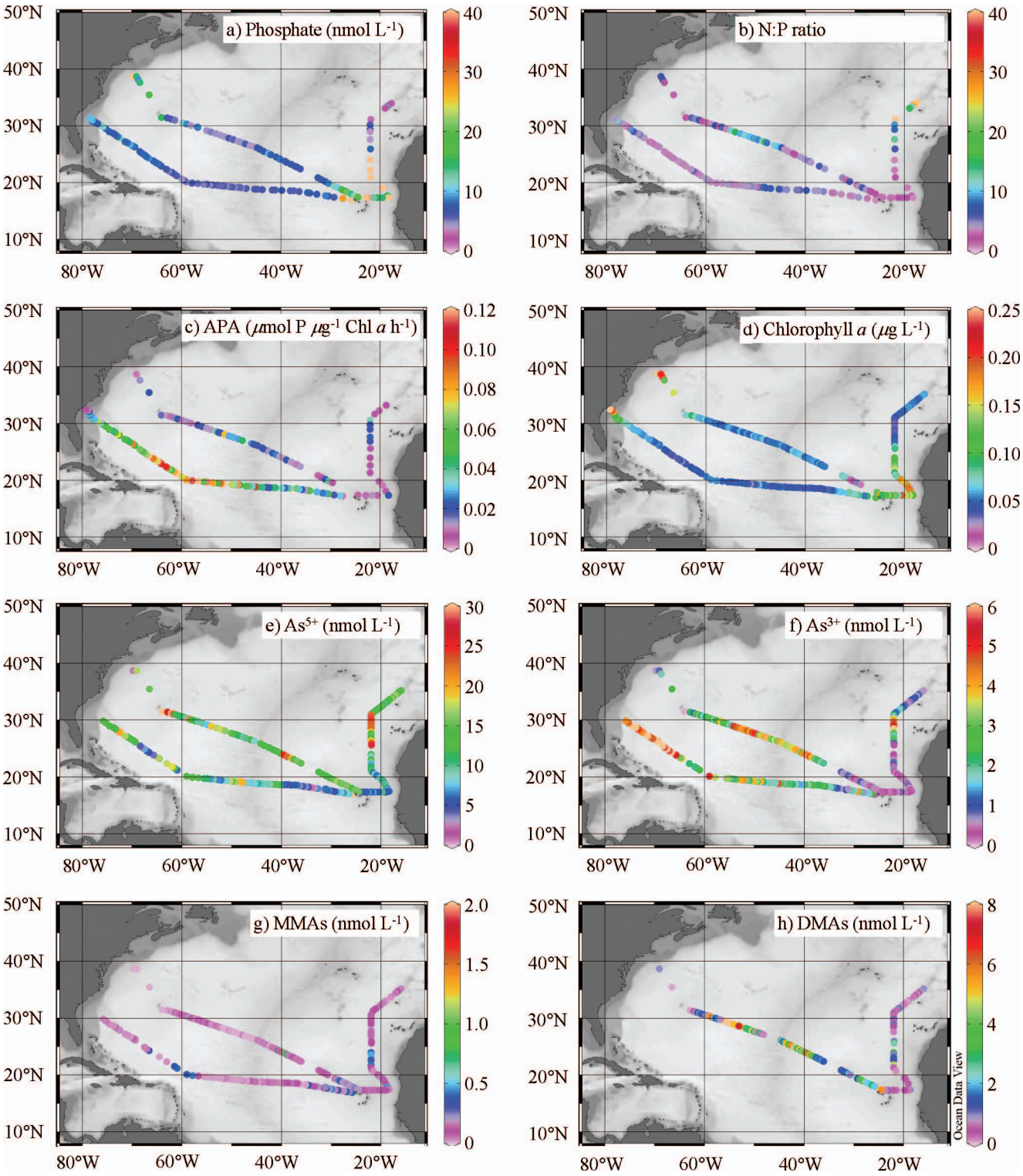

Fig. 2. Distributions of (a) phosphate concentrations, (b) N : P ratios, (c) alkaline phosphatase activities (APA), and concentrations of (d) chlorophyll *a,* (e) As^{5+}, (f) As^{3+}, (g) monomethyl arsenic (MMAs), and (h) dimethyl arsenic (DMAs) in surface waters of the North Atlantic in 2010 and 2011.

Correspondingly, the APA increased in this region from 0.5 nmol P L^{-1} h^{-1} to 2 nmol P L^{-1} h^{-1}. Despite these observations, we did not find a significant correlation between N : P ratios and APA (Table 1).

Arsenic concentrations—Concentrations of As^{5+}, As^{3+}, MMAs, and DMAs are shown in Fig. 2e–h. Our observed mean concentration of total dissolved inorganic arsenic (As^{5+} + As^{3+}) of 14.3 ± 6.7 nmol L^{-1} is comparable to

Table 1. Correlation matrix between determined parameters with spearman coefficients (upper matrix) and *p*-values (lower matrix). Significant *p*-values are in bold. Parameters are arsenite (As^{3+}), arsenate (As^{5+}), total inorganic arsenic ($As^{3+} + As^{5+}$), monomethyl arsenic (MMAs), dimethyl arsenic (DMAs), arsenate to phosphorus ratio (As^{5+}:P), phosphate concentration (PO_4^{3-}), alkaline phosphatase activity (APA), nitrogen to phosphorus ratio (N:P) and chlorophyll a concentration (Chl *a*).

	As^{3+} (nmol L^{-1})	As^{5+} (nmol L^{-1})	$As^{3+} + As^{5+}$ (nmol L^{-1})	MMAs (nmol L^{-1})	DMAs (nmol L^{-1})	As^{5+}:P	PO_4^{3-} (nmol L^{-1})	APA (μmol L^{-1} μg^{-1} Chl *a* h^{-1})	N:P	Chl *a* (μg L^{-1})
As^{3+} (nmol L^{-1})		0.222	0.481	−0.353	−0.033	0.427	−0.316	0.423	0.314	−0.422
As^{5+} (nmol L^{-1})	**0.0008**		0.951	−0.125	0.281	0.570	−0.088	−0.039	0.204	−0.156
$As^{3+} + As^{5+}$ (nmol L^{-1})	**<0.0001**	**<0.0001**		−0.198	0.229	0.647	−0.144	0.092	0.273	−0.248
MMAs (nmol L^{-1})	**<0.0001**	0.0969	**0.0081**		0.147	−0.396	0.420	−0.394	−0.275	0.271
DMAs (nmol L^{-1})	0.7015	**0.0014**	**0.0100**	0.1269		0.072	0.143	−0.369	0.533	0.242
As^{5+}:P	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.4207		−0.797	0.260	0.680	−0.296
PO_4^{3-} (nmol L^{-1})	**0.0001**	0.3171	0.1029	**<0.0001**	0.0809	**<0.0001**		−0.364	−0.633	0.435
APA (μmol L^{-1} μg^{-1} Chl *a* h^{-1})	**<0.0001**	0.6723	0.3129	**<0.0001**	**0.0001**	**0.0138**	**0.0001**		−0.052	−0.491
N:P	**0.0003**	**0.0277**	**0.0029**	**0.0047**	**<0.0001**	**<0.0001**	**<0.0001**	0.6149		−0.251
Chl *a* (μg L^{-1})	**<0.0001**	**0.0245**	**0.0003**	**0.0002**	**0.0038**	**0.0008**	**<0.0001**	**<0.0001**	**0.004**	

earlier studies in the Atlantic (range = 17.6–22.8 nmol L^{-1}, Statham et al. 1987; range = 18.3–20.3 nmol L^{-1}, Middelburg et al. 1988; mean = 12.9 ± 1.8 nmol L^{-1}, Cutter and Cutter 1995; mean = 15.7 ± 1.3 nmol L^{-1}, Cutter and Cutter 1998; mean = 16.3 ± 2.1 nmol L^{-1}, Cutter et al. 2001). However, our observed variation in the total concentration is larger than in the earlier studies cited above. As^{5+} concentrations typically ranged between 12 nmol L^{-1} and 20 nmol L^{-1} (mean = 12.2 ± 6.0 nmol L^{-1}), comparable to earlier studies in the Atlantic (Statham et al. 1987; Middelburg et al. 1988; Cutter and Cutter 1995; Cutter et al. 2001). However, we found lower concentrations between 20°W and 35°W south of 20°N, ranging between 5 nmol L^{-1} and 10 nmol L^{-1}, and occasionally concentrations exceeding 20 nmol L^{-1}. The mean concentration of As^{3+} was lowest in the eastern North Atlantic (1.0 ± 1.1 nmol L^{-1}) and increased in the Central (3.1 ± 1.0 nmol L^{-1}) and western North Atlantic (4.0 ± 1.9 nmol L^{-1}). The mean concentration in the eastern North Atlantic was very significantly lower compared with the central and western part ($p < 0.001$, Kruskal–Wallis test with Dunn's post-comparison). Considering each data set individually (i.e., data from 2010 and 2011), mean concentrations among all three regions were significantly different ($p < 0.05$) in each year. As shown in Fig. 2e, the difference in the two data sets is that in 2010 the highest mean concentration of As^{3+} was observed in the western North Atlantic, whereas in 2011 the highest mean concentration was found in the central North Atlantic. Figure 2 indicates that no arsenic species other than As^{3+} show spatial differences among the three regions. In general, MMAs was uniformly distributed in both years, typically not exceeding 0.30 nmol L^{-1} (mean = 0.11 ± 0.15). The DMAs concentrations found in this study from the Eastern Atlantic Ocean (mean = 0.65 ± 0.82 nmol L^{-1}) are comparable to concentrations found elsewhere in the Atlantic Ocean (Andreae 1979), Pacific Ocean (Cutter and Cutter 2006), and Mediterranean Sea (Cabon and Cabon 2000), but we observed higher and more variable concentrations in the central Atlantic Ocean (mean = 3.2 ± 1.8 nmol L^{-1}).

For the complete data set, no correlation between the different As species was observed (Table 1), indicating a decoupling of detoxification metabolisms within the phytoplankton community in the North Atlantic or longer residence times of the organic species compared with As^{3+}. However, a fundamental observation remains that As^{3+} was the only species spatially distributed at significantly different concentration levels in the North Atlantic. In the following, section we present a discussion of how the spatial distribution relates to potential P-limitation.

Discussion

The overall objective of this study was to investigate whether metabolized As species can serve as proxies for P limitation in phytoplankton due to the biochemical similarities between As and P. This objective then requires an examination of both the existing proxies and the proposed one.

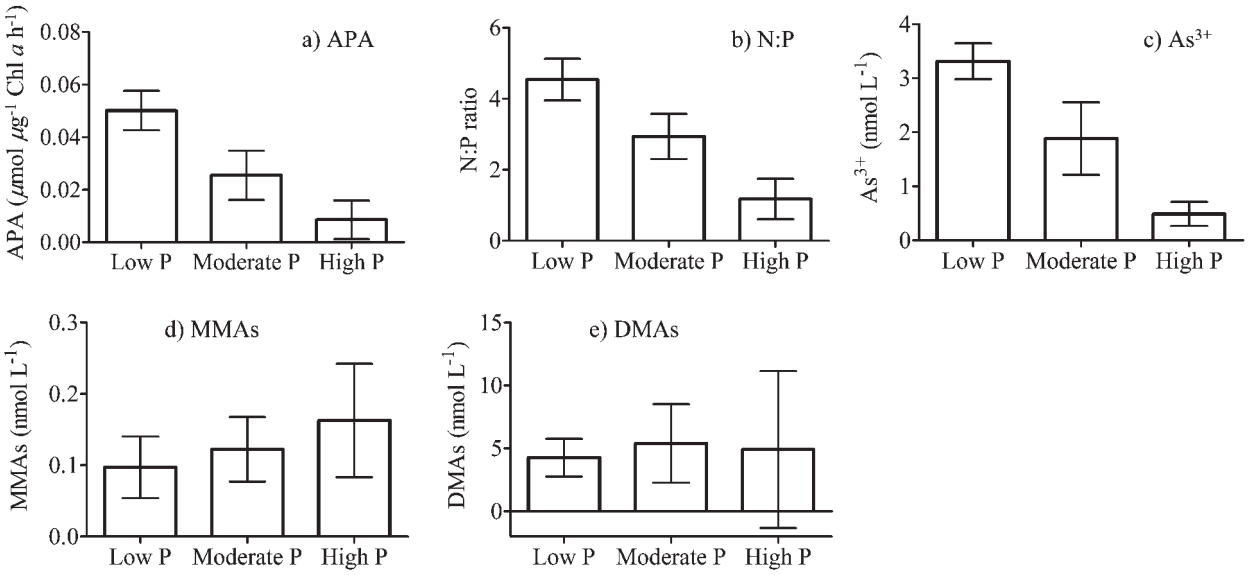

Fig. 3. Mean values for (a) alkaline phosphatase activity (APA), (b) N : P ratio, (c) concentrations of As^{3+}, (d) concentrations of monomethyl arsenic (MMAs), and (e) concentrations of dimethyl arsenic (DMAs) at three phosphate ranges (Low = 1–10 nmol L^{-1}, Moderate = 10–20 nmol L^{-1}, High = 20–70 nmol L^{-1}). The bars represent the 95% confidence intervals of the means.

Distribution of arsenic species—Overall, we found a notable distribution of As^{3+} in the North Atlantic (Fig. 2e) when compared with different concentration ranges of phosphate (Fig. 3). In Fig. 3 we categorized phosphate concentrations in three ranges representing low (1–10 nmol L^{-1}), moderate (10–20 nmol L^{-1}), and high (20–70 nmol L^{-1}) levels based on our observed range and other studies of low-nanomolar phosphate in the North Atlantic (Wu et al. 2000; Mather et al. 2008). We also found that our observed concentrations of As species are similar to earlier studies (Middelburg et al. 1988; Cutter et al. 2001; Cutter and Cutter 2006), except for DMAs.

Cutter and Cutter (2006) made the observation that DMAs concentrations in the North Pacific increased from a background concentration of 0.1–0.2 nmol L^{-1} to 0.8 nmol L^{-1} at stations under the influence of nitrogen-fixing *Trichodesmium* spp. blooms. *Trichodesmium* spp. under P-limiting conditions induces genetic arsenate detoxification strategies that include production of arsenate reductase and genes encoding an efflux pump (Hewson et al. 2009). *Crocosphaera watsonii,* another nitrogen fixer in the North Atlantic, was able to grow and fix nitrogen with the addition of 30 nmol L^{-1} As^{5+} (Dyhrman 2011). Similarly, Henriksson and DaSilva (1978) observed high resistance to arsenic in blue-green algae without inhibition of nitrogen fixation. The subtropical North Atlantic has been reported as an important marine system for nitrogen fixation, including *Trichodesmium* spp., due to elevated deposition of iron-rich dust from arid regions of North Africa (Capone et al. 2005). Such As resistance, and high nitrogen fixation rates in the subtropical North Atlantic, may enhance detoxification processes and potentially lead to the excretion and elevated concentrations of DMAs compared with the North Pacific (Cutter and Cutter 2006) and subarctic North Atlantic (Cutter and Cutter 1998). In addition, methylation depends on phytoplankton species and their physiological status (Sanders and Windom 1980). Relative rates of methylation by phytoplankton and demethylation by bacteria proceed concurrently, and affect the presence of MMAs and DMAs (Sanders 1979). Photochemical oxidation and bacterial degradation studies suggested that methylated As species have much longer residence times (months) in the surface ocean compared with As^{3+} (days; Johnson and Pilson 1975; Cutter 1992; Cutter and Cutter 2006), leading to a more uniformly distributed pattern of the methylated species. For this reason, As^{3+} may have a simpler relationship to P-limitation than MMAs or DMAs.

As^{3+} as proxy for P-limitation—Healey and Hendzel (1979) reported Chl *a*–normalized APA threshold values for no, moderate, and extreme P-limitation of < 0.003, 0.003–0.005, and > 0.005 μmol P μg Chl a^{-1} h^{-1}, respectively. Although these threshold values have been used in the literature to assess P-limitation in natural ecosystems (Guildford and Hecky 2000; Ammerman et al. 2003), they have been derived from limited culture experiments of three freshwater phytoplankton species, which challenges the application to assess P-limitation in natural populations. Indeed, using Healey and Hendzel's (1979) threshold values, 93% of our APA data indicate extreme P-limitation. However, data on N : P ratios indicate that the majority of stations were N-limited (N : P < 5). This inconsistency emphasizes the difficulties in the qualitative assessment of nutrient limitation in the Atlantic Ocean. In the following, we discuss the application of As species as alternative proxies for P-limitation.

We observed a very significant, although weak, negative correlation between APA and phosphate ($r = -0.3160$, $n = 112$, $p = 0.0007$; Fig. 4) similar to Nausch (1998). From Fig. 4a we observe that phosphate concentrations below 12 nmol L^{-1} can trigger the activity of alkaline phosphatase.

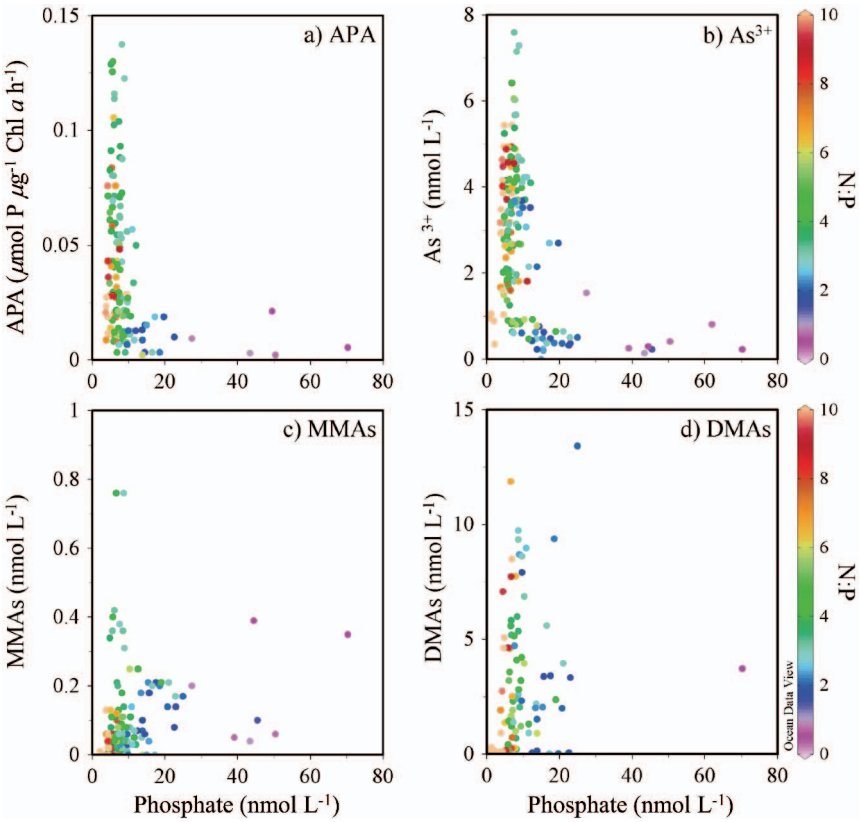

Fig. 4. Relationship of (a) alkaline phosphatase activity (APA), (b) As^{3+} concentrations, (c) MMAs (monomethyl As) concentrations, and (d) DMAs (dimethyl arsenic) concentrations to phosphate concentrations. Color scale represents the N : P ratio.

Although the N : P ratios shown in Fig. 4a do not indicate P-limitation, its trend toward higher values is consistent with lower phosphate concentrations and increasing APA. Similar inverse hyperbolic relationships between phosphate and APA have been reported in the literature (Nausch 1998; Sebastián et al. 2004*a*; Mather et al. 2008), indicating that below a certain level phosphate triggers inducible APA, but also showing the presence of PO_4^{-}-independent APA (Fig. 4a). The published criterion of the regulatory function is variable among ecosystems, and typically is at 100 nmol PO_4^{3-} L^{-1} in coastal and upwelling regions (Nausch 1998; Sebastián et al. 2004*a*; Labry et al. 2005) compared with about 10 nmol PO_4^{3-} L^{-1} in oligotrophic waters (Mather et al. 2008). Our derived criterion of 12 nmol L^{-1} is consistent with Mather et al. (2008), indicating that phytoplankton communities in oligotrophic waters are better adapted to low inorganic phosphate conditions.

To further examine the above findings, we investigated differences in APA, N : P ratios, and As species at different phosphate levels (Fig. 3). We categorized phosphate concentrations in three bins representing low (1–10 nmol L^{-1}), moderate (10–20 nmol L^{-1}), and high (20–70 nmol L^{-1}) phosphate concentrations based on our observed range and other studies on low-nanomolar phosphate in the North Atlantic (Wu et al. 2000; Mather et al. 2008). APA, N : P, and As^{3+} concentrations were significantly higher in the low-phosphate bin (1–10 nmol L^{-1}) compared with the other two categories (Fig. 3a–c; $p < 0.0001$, Kruskal–Wallis test with Dunn's post-comparison). In contrast, MMAs and DMAs were uniformly distributed among all three phosphate categories (Fig. 3d–e). These figures further support our finding that As^{3+}, but not the organic As species, behaves similarly to conventional proxies for P-limitation. We also observed a linear relationship (Model II) between APA and As^{3+} concentrations (Fig. 5), with a y-intercept of 1.04 ± 0.51 nmol L^{-1}, and a slope of 50.76 ± 10.31 nmol As^{3+} L h $nmol^{-1}$ P. This slope is significantly different from zero ($p < 0.0001$). We also found a positive and significant correlation ($r = 0.459$, $p < 0.0001$) between As^{3+} and the ratio of As^{5+} : P (Table 1), a stress indicator of P-limitation or the likelihood to take up As^{5+} instead of P. There is an increasing risk of indiscriminant As uptake when As : P ratios are relatively high (Andreae and Klumpp 1979; Sanders 1979), and the cell transforms As into compounds that are less harmful and easier to excrete (methylated As and As^{3+}). The detoxification products are either excreted or stored innocuously (Andreae and Klumpp 1979; Sanders and Windom 1980; Sanders and Riedel 1993; Hellweger et al. 2003). The fact that we did not observe a correlation between the methylated As and As^{5+} : P ratio is indicative of either the dominance of arsenic reduction in our study compared with methylation as detoxification strategy, or more likely the longer residence times of organic As species in surface waters compared with As^{3+} (Cutter 1992;

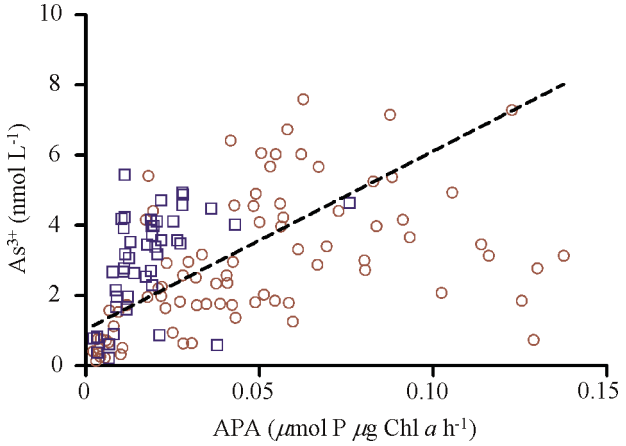

Fig. 5. Linear relationship (Model II) between alkaline phosphate activities (APA) and As^{3+} concentrations. The red circles represent data from 2010, and the blue squares represent data from 2011. The line represents the best fit for all data with an equation of $[As^{3+}] = 50.76 \times APA + 1.04$ ($n = 129$).

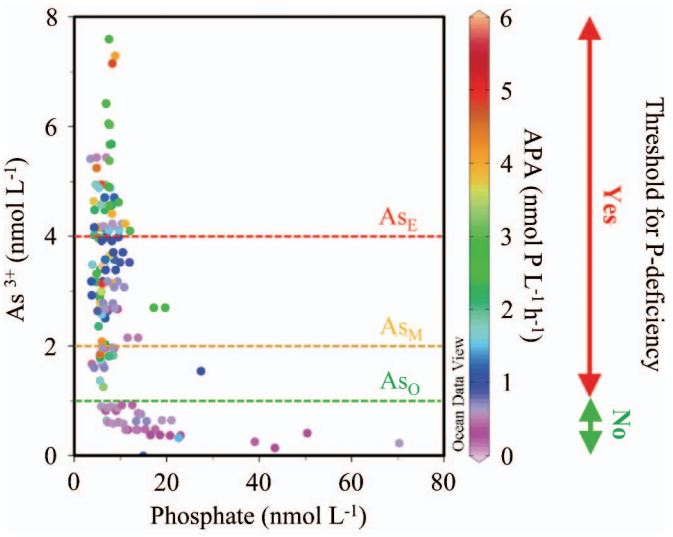

Fig. 6. Relationship of As^{3+} concentrations to phosphate concentrations and derived threshold values for P-limitation (described in text). Color scale represents alkaline phosphate activities (APA).

Cutter and Cutter 2006), thus obscuring the simple correlation between these species and short-term proxies of P-limitation.

If we consider APA to be a suitable proxy for P-limitation (Guildford and Hecky 2000; Ammerman et al. 2003), but recognize the previously cited problems (Beardall et al. 2001), then substituting the concentrations of As species for APA in Fig. 4a can begin to evaluate their suitability as proxies (Fig. 4b–d). The similarities between the plots of APA vs. phosphate (Fig. 4a) and As^{3+} vs. phosphate (Fig. 4b) are remarkable, including the increasing trends of N : P ratio. The phosphate concentration at which we observed an increase in As^{3+} concentrations is 12 nmol L^{-1}, which is consistent with the concentrations at which APA also increased. This similarity indicates that As^{3+} is an alternative proxy for P-limitation. Such a relationship is reported here for the first time, and is consistent with typical trends of As^{3+} during a phytoplankton bloom (Cabon and Cabon 2000), depleting phosphate and subsequently increasing As^{3+}. The corresponding plots for MMAs (Fig. 4c) and DMAs (Fig. 4d) show less similarity with the APA plot (Fig. 4a), and their suitability as proxies may be more limited for the Atlantic Ocean.

A trend of increasing APA with decreasing phosphate and increasing As^{3+} concentrations is shown in Fig. 6. From this figure, we empirically delineated thresholds for P-limitation. (1) As_0 is defined as the upper 95% confidence interval of the average As^{3+} concentration for data points with > 12 nmol PO_4^{3-} L^{-1}, and is 1.0 nmol As^{3+} L^{-1}. The concentration of 12 nmol PO_4^{3-} L^{-1} is the regulatory threshold for APA (Fig. 4a and Mather et al. 2008) and As^{3+} excretion (Fig. 4b). The As_0 threshold is similar to the As^{3+} background concentration indicated as y-intercept of 1.04 ± 0.51 nmol L^{-1} in Fig. 5. Concentrations of As^{3+} below and above this level indicate no and low P-limitation, respectively. The reasoning here is that As^{3+} may exceed a background concentration of 1 nmol L^{-1} through abiotic processes, bacterial degradation of MMAs and DMAs, or reduction of small amounts of As^{5+} that continued to be taken up even in the presence of high phosphate concentrations (Hellweger et al. 2003). (2) As_M (moderate threshold) is defined as twice the As_0 level, or 2.0 nmol As^{3+} L^{-1}. It represents the point of rapid increase of As^{3+} excretion, and is close to the $\log_{10}$ mean (1.5 nmol As^{3+} L^{-1}) of the whole data set. (3) As_E (extreme threshold) is defined as four times the As_0 level, or 4 nmol As^{3+} L^{-1}. At this level, APA typically exceeds 2.0 nmol P L^{-1} h^{-1}. In addition to exceeding the As^{3+} threshold, the APA should exceed 0.5 nmol P L^{-1} h^{-1} for an indication of positive P-limitation.

Based on the biochemical coupling between As and P, the surface-water distributions of As^{3+}, and its observed relationships with conventional proxies, we conclude that As^{3+} is useful as a supportive proxy to complement APA and N : P, and therefore improve the reliability of P-limitation assessments. The organic As species seem to correlate to a lesser extent with the conventional proxies, but different residence times may decouple any simple relationships. However, As^{3+} as single indicator may have limitations just like the conventional proxies (e.g., due to the presence of mixed phytoplankton communities, the effect of secondary nutrient limitation, and the temporal lag between nutrient limitation and the appearance of indicators). In terms of implementing the use of this additional proxy, the analysis of As species in seawater is well-established (Andreae 1979; Cutter et al. 1991), fast (9 min) compared with APA assays, and more affordable than continuous-flow analyzers typically used for nutrients analysis (N : P ratios). Overall, the As hydride technique (*see* Methods section) is easy to learn, simple to handle, and robust for shipboard measurements. There is also a recently developed electrochemical method (Salaun et al. 2012) with sufficiently low detection limits for As^{3+} that could be used.

Improved assessment of P-limitation—Conventional proxies for the assessment of P limitation are N : P ratios

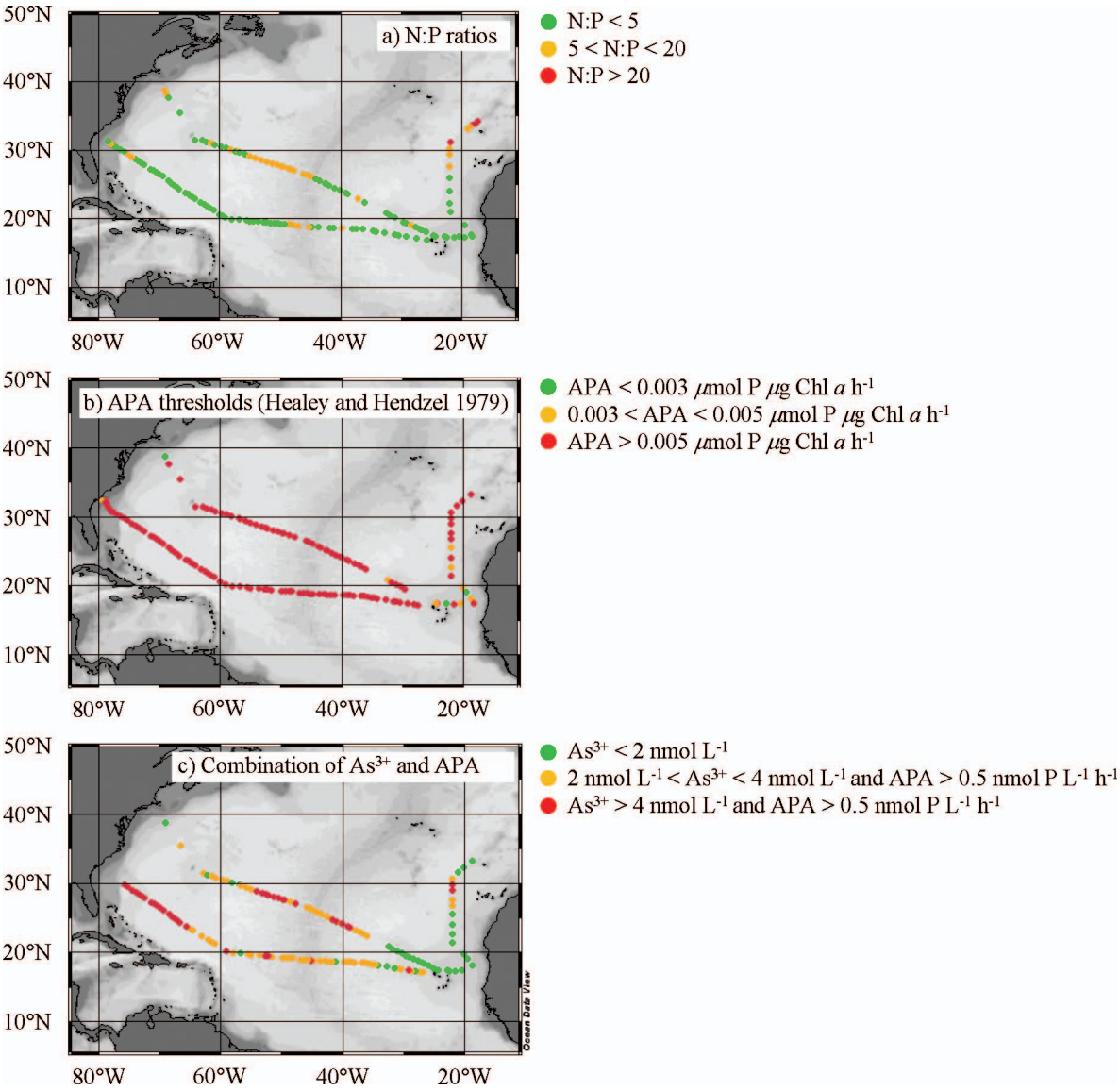

Fig. 7. Assessment of P-limitation in the North Atlantic using (a) N : P ratios, (b) thresholds of alkaline phosphatase activity (APA), and (c) As^{3+} concentrations with APA. Color scale represents no (green), moderate (orange), and extreme P-limitation (red).

and APA, but as noted above, both proxies have issues with their application (Beardall et al. 2001). These issues include the complex composition of plankton communities, deviations from the classic Redfield ratios, luxury uptake of nutrients, APA associated with bacterial communities, and the temporal lag of APA expression. Indeed, we observed strongly contradictory results applying APA and N : P proxies for the North Atlantic (Fig. 7). Based on earlier work on N : P ratios from dissolved nutrients (Wu et al. 2000; Vidal et al. 2003), we consider a ratio of 5 as a conservative threshold for the transition from nitrogen-limited to more balanced conditions. Applying this threshold ratio, Fig. 7a indicates that wide regions of the North Atlantic Ocean are nitrogen-limited. On the other hand, using threshold values of Chl *a*–normalized APA (Healey and Hendzel 1979), the majority of the North Atlantic Ocean is under P-limitation (Fig. 7b). Other studies found similar contradictory results using N : P and APA as proxies to assess P-limitation in the Atlantic Ocean (Vidal et al. 2003; Sebastián et al. 2004*a*). We have already discussed issues with both proxies in the previous section. N : P ratios clearly underestimated known P-limitation in the Atlantic Ocean (Wu et al. 2000; Vidal et al. 2003; Mather et al. 2008), but provide a good indication of no or low P-limitation in the North-western African upwelling system (Vidal et al. 2003; Sebastián et al. 2004*b*). In contrast, threshold values of Chl *a*–normalized APA indicate stronger P-limitation in the upwelling system. P-limitation in upwelling systems may occur through rapid microbial uptake of the abundant supply of nutrients, but high phosphate concentrations in our (Fig. 2a) and earlier studies (Sebastián et al. 2004*a*; Mather et al. 2008) indicate that conditions of P-limitation in the northwestern African upwelling system are unlikely to be correct. We conclude

that the APA thresholds may also overestimate P-limitation in other regions of the North Atlantic Ocean.

To improve the assessment of P-limitation, we have applied our derived thresholds of As^{3+} and APA (Fig. 7c), which means a region is classified as moderately P-limited if As^{3+} concentration and APA exceed 2.0 nmol L^{-1} and 0.5 nmol P L^{-1} h^{-1}, respectively, and extremely P-limited if As^{3+} concentration and APA exceed 4 nmol L^{-1} and 0.5 nmol P L^{-1} h^{-1}, respectively. The new assessment indicates no or low P-limitation in the upwelling system extending west of the Cape Verde islands to 35°W, consistent with literature reports (Vidal et al. 2003; Sebastián et al. 2004*b*). Such an extension may originate from upwelling filaments transporting nutrient-rich waters to the open ocean before utilization by phytoplankton (Sebastián et al. 2004*a*; Zonneveld et al. 2010). Such a feature was not revealed in the assessment of P-limitation using APA threshold values (Fig. 7b). In the Central Atlantic Ocean (35°W to 60°W), P-limitation increases to a moderate level in both 2010 and 2011, contrasting with N : P ratio's underestimations (no to low P-limitation) and APA threshold's overestimations (extreme P-limitation). Our observation of moderate P-limitation is generally consistent with relatively low concentrations of dissolved organic phosphorus (DOP) in the Central Atlantic Ocean ranging from 50 nmol L^{-1} to 120 nmol L^{-1} (Mather et al. 2008). Lower concentrations of DOP are an indication for activities of alkaline phosphate and, therefore, P-limitation. We observed two regions of extreme P-limitation (between 39°W and 43°W, and 50°W and 55°W), the latter located at the boundary to a region with DOP concentrations $<$ 50 nmol L^{-1} (Mather et al. 2008). Despite the more common limitation by iron, nitrogen fixation by *Trichodesmium* spp. in the central Atlantic has also been reported to be phosphorus-limited (Sañudo-Wilhelmy et al. 2001; Mulholland et al. 2002), which contributes to the moderate to extreme P limitation reported here. In 2010, we observed extreme P-limitation in the Sargasso Sea (24°N to 30°N and 67°W to 77°W) consistent with typical DOP concentrations below 120 nmol L^{-1} (Wu et al. 2000; Mather et al. 2008), high APA (Cotner et al. 1997), and P-limitation in bacterial growth (Cotner et al. 1997; Rivkin and Anderson 1997) in this region. P-limited waters of the northern Gulf of Mexico (Dagg et al. 2007) may also be transported into the Sargasso Sea through the Gulf Stream and branches of the North Atlantic Gyre (Schmitz and McCartney 1993).

Clearly, N : P ratio and APA as indicators for P-limitation have problems, in particular under more complex biogeochemical conditions such as N-fixation typically occurring in the North Atlantic (Sañudo-Wilhelmy et al. 2001; Mulholland et al. 2002). We have shown that As^{3+} can be useful in a dual assessment of P-limitation in combination with APA. It further highlights the need for multiple, simultaneous approaches (Jansson et al. 1988; Beardall et al. 2001) to assess nutrient limitation in oceanic regimes.

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