Trends in Analytical Chemistry 60 (2014) 1–15

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Determination of nanomolar levels of nutrients in seawater

Jian Ma [a,*], Lori Adornato [b], Robert H. Byrne [c], Dongxing Yuan [a]

[a] State Key Laboratory of Marine Environmental Science, College of the Environment and Ecology, Xiamen University, Xiamen, Fujian 361102, China
[b] SRI International, Engineering & Systems Division, Marine Technology Program, 450 8th Avenue S.E., St Petersburg, Florida 33701, USA
[c] College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, Florida 33701, USA

ARTICLE INFO

Keywords:
Determination
Flow analysis
Liquid-core waveguide
Liquid waveguide capillary cell
Measurement protocol
Nanomolar concentration
Nutrient
Seawater
Solid-phase extraction
Trace nutrient

ABSTRACT

Nutrients (phosphate, nitrate, nitrite, ammonium and silicate) exert strong controls on oceanic primary productivity. In oligotrophic areas, which cover approximately 40% of the world's oceans, nutrient concentrations can drop to nanomolar levels or lower due to biological uptake, so highly sensitive methods and technologies are urgently needed for nutrient measurements in such areas. In this work, we review procedures for phosphate and nitrite/nitrate analyses published since the review of Patey et al. [32], and procedures for analysis of ammonium and silicate at nanomolar levels. Our review includes aspects of measurement protocols that bear strongly on the quality of analyses of trace nutrients, including contamination of reagents, sample storage, and preparation of nutrient-free seawater. This review excludes methods that have limits of detection greater than 1 μM, and methods that are not specific to seawater.

Contents

* Corresponding author: Tel.: +86 592 2186916; Fax: +86 592 2185889.
E-mail address: jma@xmu.edu.cn (J. Ma).

http://dx.doi.org/10.1016/j.trac.2014.04.013
0165-9936/

1. Introduction

1.1. Trace nutrients in the ocean

Essential macronutrients in aqueous systems include phosphate, nitrate, nitrite, ammonium and silicate. In fresh waters and coastal seawater, excess nutrient inputs lead to eutrophication and degradation of the aquatic system. In contrast, oligotrophic areas in the open ocean are often subject to N/P/Si limitation due to vertical stratification and drawdown from primary production. The importance of N and P and their cycles in the ocean has been comprehensively reviewed [1–6]. While nitrate and phosphate are required nutrients for all phytoplankton, silicate constitutes an additional requirement for siliceous phytoplankton, such as diatoms, which occasionally dominate inorganic carbon sequestration in the upper ocean. Over areas of the tropical and subtropical ocean, silicate in the euphotic zone can be seasonally or chronically depleted to <0.1–0.6 μM. At these levels, silicate can limit diatom productivity and, thereby, the export of carbon from the surface ocean [7,8].

Typical vertical profiles for five nutrients are shown in **Fig. 1** [9]. Normally, in well-lit surface waters, biological uptake reduces nutrients to low-nM levels; while remineralization of sinking particulate matter causes nutrient concentrations (e.g., nitrate and phosphate) to increase to μM levels with depth.

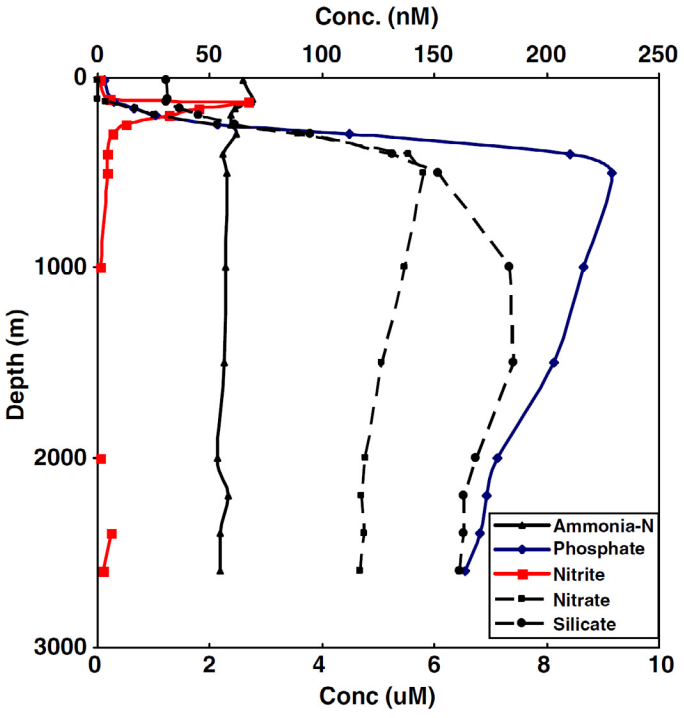

Fig. 1. Depth profile of ammonium, phosphate, nitrite, nitrate and silicate in the eastern Mediterranean [9], with permission from Elsevier.

1.2. Previous reviews of methods for nutrient analysis

Due to the importance of nutrients in marine systems, measurements of nutrient concentrations are among the most commonly performed analyses in oceanographic research. Previous comprehensive reviews of nutrient analyses include: journal publications between 2003 and 2013, including the works of Miró et al. [10], Gray et al. [11] and Worsfold et al. [12]; book chapters on seawater analysis [13–15]; and, the GO-SHIP guideline [16].

Reviews specific to one or two nutrients include the works of: Moorcroft et al. [17] for nitrite and nitrate analysis; Molins-Legua et al. [18] and Gray et al. [19] for ammonium analysis; and, Estela and Cerdà [20], Motomizu and Li [21], Villalba et al. [22] and Berchmans et al. [23] for phosphate analysis.

1.3. Techniques for trace-nutrient analysis

Over much of the world's surface oceans, nutrient concentrations often fall below the limit of detection (LOD) of conventional analytical techniques. Because nutrients play a controlling role in primary productivity and carbon sequestration, it is highly desirable to develop sensitive methods to address this measurement challenge. Applications vary widely and include determinations of the nutrient conditions that control diazotroph distributions [24–26], investigations of plankton-community structure relative to ambient nutrient concentrations [27,28], examination of complex microbial relationships [29], and assessments of the effects of climate change on marine populations [30] and freshwater and estuarine populations [31].

In 2008, Patey et al. [32] ritically reviewed methods for determination of nitrite/nitrate and phosphate in seawater at nanomolar concentrations based on publications before early 2007. Our present article provides an update on techniques for nitrite/nitrate and phosphate analysis, and a review of ammonium and silicate techniques. Measurements of the latter two nutrients (especially ammonium) have proved challenging for both analytical and marine chemists.

Several approaches can be used to lower LODs for nutrient analyses [32]:

- optimization of chemistries – for nutrient analyses, improvement opportunities are very limited because nutrient-analysis chemistries have been investigated comprehensively;
- preconcentration of analytes or analyte-derivatives using liquid-liquid extraction or, more recently, solid-phase extraction (SPE) techniques;
- amplification of the detected spectrophotometric signal (i.e., absorbance) by increasing the path-length of the absorption cell;

Table 1
An overview of recently published methods for nanomolar phosphate analysis in seawater (2007–13)

Detection	Chemistry	Technique	Analytical performance	Comments	Ref.
Colorimetry	PMB	Combination of 5-fold MAGIC concentration and SCFA coupled with 2 m LWCC	LOD: 0.3 nM Range: 1–25 nM r^2: 0.9996	– Very sensitive method – Manual operation is still needed in the MAGIC method – Filter selection is important	[36]
Colorimetry	PMB	Reverse FIA, 2 m LWCC	LOD: 0.5 nM Range: up to 165 nM r^2: 0.9990 RSD: 1.54% (24.7 nM), 1.86% (82.5 nM), n = 9	– rFIA to amplify sensitivity – Silicate and arsenate interferences were evaluated – Potentially useful for shipboard and *in situ* applications	[37]
Colorimetry	PMB	FIA, 2 m LWCC	LOD: 1.5 nM Range: up to 100 nM r^2: 0.995	– Underway system for real time monitoring of phosphate – Introduces procedures for preparation of LPSW	[38]
Colorimetry	PMB	SFA, 2.5 m LWCC	LOD: 0.8 nM Range: up to 250 nM r^2: 0.9870 RSD: 6.1% (5 nM), 0.8% (50 nM), n = 5	– High resolution underway system for continuous analysis – The sensitivity and analytical range is far different with discrete system (shown below)	[39]
Colorimetry	PMB	SCFA, 2 m LWCC	LOD: 0.5 nM Range: up to 700 nM r^2: 0.9992 RSD: 1.8% (10 nM), n = 8; 0.9% (60 nM), n = 9	– System for discrete samples – DI water was used for blank instead of low phosphate seawater – Minimized Schlieren effect by increasing sample injection and wash time	[39]
Colorimetry	PMB	CFA, 50 cm Type I LCW as flow cell	LOD: < 1 nM R: 1–1000 nM Sampling rate at 0.4–0.75 Hz	– *In situ* application in upper 200 m – The only reported high-resolution vertical profile	[40]
Colorimetry	PMB-CTAB	Flow analysis, C18 SPE concentration of PMB-CTAB	LOD: 1.57 nM Range: 3.4 to 515 nM r^2: 0.9978–0.9997 RSD: 4.45% (20.6 nM), 4.73% (82.5 nM), 6.75% (206.2 nM), n = 6	– Shipboard and field applications – Modification of a previous system, but shortcomings of ion-pair formation still exist	[41]
Colorimetry	PMB	Flow analysis, HLB SPE concentration of PMB	LOD: 1.42 nM Range: 3.4–1134 nM r^2: 0.9992–0.9997 RSD: 2.5% (31 nM), measured at different day	– Great improvement relative to previous system – Overcomes the shortcomings of ion-pair formation – DI water was used for blank instead of low phosphate seawater	[42]
Colorimetry	Vanadomolybdate method	Integrated lab-on-a-chip analyzer	LOD: 52 nM Range: 0.1 to 60 μM r^2: 0.9967 RSD: 13.6% (400 nM), n = 4	– Microfluidic analyzer with low reagent requirement and low power consumption – Underway shipboard analysis system – Not sufficiently sensitive for oligotrophic applications	[43]
Amperometry	Oxidation of molybdenum to molybdate, which reacts with phosphate to form phosphomolybdenum complex	Potentiostat μ-Autolab III and electrode	LOD: 120 nM Range: 0.49 to 3.3 μM RSD: 1.7–2.6% (0.33–3.22 μM), n = 10	– First step of *in situ* reagentless electrochemical sensor for phosphate – Sulfuric acid is the only needed liquid reagent – Good agreement (deviation of 2.5%) with colorimetry for seawater sample analysis – Not sufficiently sensitive for oligotrophic applications	[44]
Voltammetry	Oxidation of molybdenum to molybdate, which reacts with phosphate to form phosphomolybdenum complex	Potentiostat μ-Autolab III and modified electrode	LOD: 190 nM Range: 0.65 to 3.01 μM r^2: 0.9897 RSD: 3.7–4.0% (0.65–3.012 μM), n = 10	– Totally free of any liquid reagent and interference from silicate – Good agreement (deviation of 1.3%) with colorimetry – Potentially for *in situ* application with reduced energy consumption and quite fast response – Not sufficiently sensitive for oligotrophic applications	[45]

- use of a highly sensitive detection technique (e.g. utilization of fluorometry instead of spectrophotometry for ammonium analysis); and,
- modification of the optical system to increase the signal-to-noise ratio

2. Methods of analysis of phosphate at nanomolar concentrations

Among published techniques, the modified phosphomolybdenum blue (PMB) method [33] is the most widely used for phosphate determination [20–22,32]. The PMB method is based on the reaction of phosphate and molybdate in acidic medium in the presence of antimony to form phosphomolybdenum yellow (i.e., 12-molybdophosphoric acid), with subsequent reduction to PMB by a reducing agent, such as ascorbic acid, to increase sensitivity. Dissolved reactive phosphorus (DRP) is operationally defined as phosphate that passes through a 0.45-μm membrane [34] and then reacts with the PMB reagents to form the detectable blue species. Defined as such, DRP includes orthophosphate and a small quantity of acid-hydrolysable organic or condensed phosphorus compounds [13,35]. Though several other techniques (e.g., chemiluminescence) were introduced by Patey et al. [32], almost all recent references involving trace-phosphate analysis (after 2007) are based on classic PMB chemistry (see **Table 1**).

2.1. Liquid-core waveguide (LCW) and liquid waveguide capillary cell (LWCC) methods

In recent years, small diameter, long path-length optical cells composed entirely or in part of Teflon AF have been adapted for use in spectrophotometric systems [46,47]. According to Beer's law, the sensitivity of spectrophotometric detection can be improved with longer path-length optical cells. Use of LCWs and LWCCs for long path-length analyses is described in the literature. In this review, the term LCW represents a Type I cell composed entirely of Teflon AF polymer, and LWCC denotes the Type II commercial cell comprising silica with Teflon AF cladding (sold by World Precision Instruments, http://www.wpiinc.com).

Type II cells comprise silica tubing with a Teflon AF cladding to prevent direct contact between sample and polymer. Thus, the LWCC is easier to clean and has better physical stability, but the Type II cell has a slightly shorter effective optical path-length than its physical path-length because of the presence of the additional fused silica layer [47] [and references therein]. Type I cells, which require careful cleaning to extend operational use, are typically less expensive than Type II cells and are more amenable for *in-situ* use since they are flexible and can be coiled into a relatively small footprint. Several techniques based on LCW or LWCC have been reported and applied in land-based laboratories, shipboard analyses, underway systems and *in-situ* monitoring.

Li and Hansell [36] reported experimental results that directly compared the two most commonly used methods for quantifying nanomolar concentrations of phosphate; the Magnesium Induced Co-precipitation (MAGIC) method [48] and the standard PMB technique using a LWCC. The MAGIC preconcentration method involves addition of sodium hydroxide to the water sample to induce precipitation of brucite ($Mg(OH)_2$). Phosphate is quantitatively removed from solution by adsorption on the precipitate. The precipitate is then concentrated by centrifugation, dissolved in a small volume of dilute acid and the standard PMB protocol is then used to determine phosphate. Li and Hansell [37] observed agreement between both methods at phosphate concentrations < 100 nM and found that the combined MAGIC and LWCC techniques produced an LOD as low as 0.3 nM. The authors also evaluated the effects of filtration on a 50-nM standard phosphate solution and determined that there were minimal losses of DRP for both 0.45-μm cellulose nitrate membranes (MFS Membrane, –1.2 ± 1.1 nM in deionized water (DIW) and –3.1 ± 1.4 nM in seawater) and 0.45-μm cellulose ester membranes (Millipore-Membrane, –0.8 ± 1.2 nM in DIW and –4.5 ± 1.4 nM in seawater). The authors further evaluated potential interferences caused by silicate and arsenate. It was found that adjusting the solution pH to <1 precluded silicate interference while no interference was found with arsenate. The authors concluded that the slow rate of arsenomolybdate formation at room temperature prevented any observed interference. In contrast to the typical flow-injection analysis (FIA) system, Ma et al. [37] combined an LWCC with 'reverse' FIA, where reagent is injected into the sample flow. Since the concentration detected in the reagent zone increased with increasing dispersion, determinations carried out with only slight dilution resulted in higher sensitivity. An LOD of 0.5 nM was obtained with a linear dynamic range up to 165 nM. The relative standard deviations (RSDs) for analysis of 24.7 nM and 82.5 nM samples were 1.54% and 1.86% (n = 9). This rFIA-LWCC system has potential for both shipboard and *in-situ* analysis.

Li et al. [38] developed a shipboard dual-LWCC-FIA system for simultaneous underway monitoring of phosphate, and nitrate plus nitrite, at nanomolar concentrations. The authors reported LODs of approximately 2 nM for nitrate plus nitrite and 1.5 nM for phosphate. Using the shipboard underway system, sea water was continuously pumped into a clean plastic vial through a Teflon tube, and then into two separate channels for phosphate and nitrate plus nitrite measurements. A 6-inlet and 1-outlet periodic valve was used to switch between sample, standards, and carrier solution for each channel, thereby allowing regular, automated calibrations to occur. Real-time survey results on the west Florida continental shelf and the oligotrophic Sargasso Sea were presented, as well as results obtained by manual analysis of more than 1000 discrete seawater samples obtained on two cruises in the North Atlantic. Zimmer and Cutter [39] recently developed two systems based on use of LWCCs in conjunction with conventional auto-analyzers. Their first system, capable of automated data logging every 30 seconds for up to 16 consecutive hours in continuous flow mode, provides high resolution, real-time analysis of nanomolar DRP in ocean surface waters. A second system was optimized for measurements of discrete samples. Baseline corrections were facilitated by use of a parallel channel containing DIW and reagents. The authors used DIW rather than low nutrient seawater in order to minimize any effects of background phosphate. Sample and wash times were increased to achieve plateau-shaped peaks after the transient wash/sample mixing period. The two systems showed similar LODs (0.8 vs. 0.5 nM) making them well suited for analysis of oligotrophic waters. The authors also compared results from samples analyzed using a LWCC and the MAGIC technique and found that they compared reasonably well, especially at low concentrations.

A high-resolution *in situ* analyzer, SEAS II (Spectrophotometric Elemental Analysis System, second generation) equipped with an LCW, demonstrated high-resolution quantification of nanomolar phosphate, nitrite and nitrate in oligotrophic waters. With a 50 cm path-length cell, phosphate was accurately measured at concentrations in the range 1 nM–1 μM [40]. The analyte concentration range can be adjusted by changing the LCW cartridge to one with a cell longer or shorter. In addition to measurement of a primary analyte, SEAS II is capable of collecting concurrent auxiliary data from up to four separate instruments, including a CTD, a fluorometer, a PAR sensor, and a second SEAS II instrument. Sampling frequency depends on peripheral instrument selection and is in the range 0.4–0.75 Hz.

2.2. Method based on solid-phase extraction

SPE techniques, which have been widely used in sample treatment for organic pollutants, were applied to concentrate the derivation compounds of phosphate to increase sensitivity. Liang et al. [49] reported an SPE system that used a C18 SPE cartridge to concentrate an ion-pair complex of PMB and cetyltrimethylammonium bromide (CTAB), a cationic surfactant. The complex was subsequently eluted and PMB was detected spectrophotometrically (LOD <2 nM). The authors found no significant difference between results from analysis of two seawater samples using their SPE technique and the MAGIC technique. Furthermore, the SPE technique requires a smaller sample volume and shows a lower LOD than MAGIC.

Ma et al. [41] modified Liang et al.'s system to promote shipboard applications, increase sample throughput, and minimize sample volume. The modified system replaced peristaltic pumps with micro solenoid pumps in parallel, reducing reagent use by up to 80% and sample volume by ~25%. Variations of stopped-flow time and sample-loading time gave three different standard curves, which corresponded to three linear ranges within the range 3.4–515 nM. The modified system has been successfully applied to shipboard determination of trace phosphate in the South China Sea. Statistical *t*-tests indicated no significant differences between the SPE approach and the MAGIC method. However, sample throughput is limited by slow PMB-CTAB ion-pair formation, and longer reaction times are required at low concentrations.

To overcome these issues, Ma et al. [42] successfully used another commercially available sorbent, Waters Oasis hydrophilic–lipophilic balance (HLB), to concentrate PMB directly. With this procedure, requirements for temperature control and long reaction times were

eliminated. Also, because organic solvents were no longer present in the washing solution, Schlieren effects were minimized. As no salinity effect was observed with this technique, DIW can be used as the matrix and the method can serve as a reference method to evaluate procedures for preparation of "phosphate-free" seawater.

Compared with LCW/LWCC-based methods, the SPE method requires 1–2 orders of magnitude greater sample volume, which is not problematic for hydrographic surveys, but might limit its application for the analysis of samples with limited volume (e.g., ice-core samples).

2.3. Microfluidic analyzer

Legiret et al. [43] reported a high-performance autonomous analytical system based on the vanadomolybdate method for shipboard phosphate analysis. The chemistry is based on the rapid direct reaction of phosphate with an acidified vanadomolybdate reagent, producing a yellow-colored complex. The authors chose this "yellow" method over the classical "blue" method because the stability of the reagent mixture was reported to be over 1 year, much longer than that for the PMB method. The authors' "lab-on-a-chip" (LOC) system combined a microfluidic chip manufactured from tinted poly-(methyl methacrylate) with a custom-made syringe pump, embedded control electronics, and on-board calibration standards. With a path-length of 25 mm, the LOC improved the LOD (52 nM) by two orders of magnitude compared to comparable portable systems based on the "yellow" chemistry. LOC systems have been used successfully in coastal and offshore waters where results compared well with those obtained using a reference bench-operated phosphate auto-analyzer. The LOD of this microsensor, while quite suitable for coastal monitoring, is too high for applications in oligotrophic oceans where phosphate concentrations (especially at the surface) are normally much lower than 50 nM.

2.4. Electrochemical detection

Jonca et al. [44,45] recently reported several electrochemical, reagentless techniques that could be incorporated into low power, underwater phosphate sensors. The techniques employed on-board oxidation of molybdenum to form molybdate and protons, which subsequently reacted with phosphate to form the phosphomolybdenum complex that could be detected electrochemically. Two main challenges include preventing interference from silicate, which undergoes the same reaction with molybdate, and identifying an appropriately sensitive electrochemical-detection scheme. The authors reported that maintaining a proton-to-molybdate ratio of 70 precludes silicomolybdate formation, and that the ratio can be achieved through use of a three-cell design that employs a non-proton exchange membrane (DuPont, Nafion PFSA, 180-μm thickness) [45]. Amperometric detection with a rotating gold electrode (LOD 0.11 μM) or pulse voltammetric detection with a static gold electrode (LOD 0.19 μM) can be used for phosphate detection, with the latter more attractive for *in-situ* use due to its power requirement being lower.

3. Methods for analysis of nanomolar concentrations of nitrite/nitrate

Most of the spectrophotometric methods for nitrite/nitrate analyses are based on the Griess approach, which is not subject to interferences in oxygenated seawater [50]. The classic Griess assay typically relies on the diazotization of a suitable aromatic amine by reaction of nitrite with sulfanilamide (SAM) and N-1-naphthylethylenediamine dihydrochloride (NED). The resultant pink-colored azo dye is detectable at 540 nm. Nitrate is usually reduced to the more reactive nitrite using a copper-coated cadmium column, and then determined via the Griess assay [17]. **Table 2** shows a summary of the recent reports on trace nitrite/nitrate analysis.

3.1. LCW and LWCC methods

Using an optical system similar to that described for the phosphate analysis in sub-section 2.1, Adornato et al. [40] configured SEAS II for nitrate determinations in seawater. By utilizing a 15-cm path-length cell and multiple wavelength spectrophotometry, SEAS II offered a 10^4-fold linear dynamic range (2 nM–20 μM) and proved capable of fully ascertaining the distribution of nitrate and nitrite in the upper 200 m of the oligotrophic ocean. Multiple wavelength spectrophotometry extends the detection range by concurrently monitoring the peak and several off-peak wavelengths of the absorbance spectrum of the Griess pink azo dye. Each wavelength corresponds to a particular linear dynamic range, with lower concentrations determined using the peak absorbance and the higher concentrations determined using off-peak wavelengths.

The underway system developed by Li et al. [38] described in sub-section 2.1 was also used for nitrate plus nitrite analysis in coastal and offshore waters. Feng et al. [51] recently developed an rFIA-LWCC system for simultaneous determination of nanomolar nitrate and nitrite. The system eliminated the bubbles typically introduced in segmented continuous flow analysis (SCFA) and offered higher sample throughput and precision than sequential injection analysis (SIA). The nitrite and nitrate analytical channels of the system shared the same detection system, allowing determination of both analytes with a single sample injection. Different reagent injection strategies were investigated to increase sensitivity and improve peak shape. Compared to normal FIA, this rFIA method had lower reagent consumption, less sample dispersion, and higher sensitivity. LODs for both nitrite and nitrate were 0.6 nM. As such, this system could be used for real-time field analyses and underway oceanographic research.

3.2. SPE-based method

Chen et al. [52] developed a sequential injection-SPE method for nitrite determination. The pink azo compound formed by the Griess reaction was quantitatively adsorbed onto a Sep-Pak C18 cartridge and, after prewashing the cartridge with water and ethanol (28%, v/v), eluted with a solution containing ethanol (26.6%, v/v) and acid (0.108 M H_2SO_4). An LOD of as low as 0.1 nM was reached using a 150-mL sample. This method was employed for shipboard analysis during a 1-month cruise in the South China Sea. However, it was found that the seawater matrix led to low recoveries (70–80%) and that different C18 cartridges displayed low batch-to-batch reproducibility. Zhang et al. [53] therefore replaced C18 with an HLB solid phase, which delivered close to 100% recoveries for samples with different salinities. The HLB cartridge could be used for at least 50 samples.

Drawbacks to SPE methods include a requirement for high sample volumes (up to 200 mL required to obtain nanomolar LODs), and the need to replace the reaction vessel for each new analysis cycle. In order to overcome these challenges, Zhang et al. [54] developed a flow system coupled with on-line HLB SPE and LCW analysis to determine nitrate and nitrite simultaneously. For nitrite, the extracted Griess azo-compound was eluted and detected with a 16-cm LCW-based detector. Using a separate portion of sample, nitrate was reduced to nitrite with a Cu-Cd reduction column, and then extracted, eluted and detected in the same manner. The coupled HLB-SPE-LCW technique eliminates matrix interferences and reduces the overall sample volume. However, the system is somewhat complex (requires 4 pumps, 2 ten-port injection valves, a multi-position selection valve and 20 steps).

Table 2
An overview recently published methods for nanomolar nitrate/nitrite analysis in seawater (2007–13)

Detection	Chemistry	Technique	Analytical performance	Comments	Ref.
Colorimetry	Cd column reduction, Griess reaction	FIA, 2 m LWCC	Nitrate: LOD: 2 nM Range: up to 100 nM r^2: 0.995	– Underway system for real time monitoring of nitrate plus nitrite – Application to coastal and offshore seawater	[38]
Colorimetry	Cd column reduction, Griess reaction	CFA, 15 cm Type I LCW as flow cell	Nitrate: LOD: 2 nM Range: 2–20000 nM with multi-wavelength spectrophotometry	– *In situ* application in upper 200 m – The only reported high-resolution vertical profile	[40]
Colorimetry	Griess reaction	CFA, 106 cm Type I LCW as flow cell	Nitrite: LOD: 0.35 nM Sampling rate at 0.4–0.75 Hz	– *In situ* application in upper 200 m – The only reported high-resolution vertical profile	[40]
Colorimetry	Cd column reduction, Griess reaction	Reverse FIA, 1 m LWCC	Nitrate and nitrite: LOD: 0.6 nM Range: 2–500 nM r^2: 0.9997 RSD: 0.08–4.75% (various concentrations), 1.1% (10.5 h continuous sample analysis), n = 41	– rFIA to increase sensitivity – Matrix interference were systematically evaluated – Potentially useful for field and underway applications	[51]
Colorimetry	Griess reaction	Flow analysis, C18 SPE concentration analysis of pink azo form using Griess reaction	Nitrite: LOD: 0.1 nM Range: 0.71 to 429 nM r^2: 0.9999 RSD: 1.44% (14.3 nM), n = 8	– Shipboard analysis and field applications – Improved sample throughput compared with published C18 SPE method	[52]
Colorimetry	Griess reaction	Flow analysis, HLB SPE concentration of pink azo of Griess reaction	Nitrite: LOD: 0.5 nM Range: 1.4 to 85.7 nM r^2: 0.9999 RSD: 4.0% (7.1 nM), 1.0% (28.6 nM), n = 8	– Improved sample analysis recovery compared with C18 – HLB reused for at least 50 times – Complicated system	[53]
Colorimetry	Cd column reduction, Griess reaction	Flow analysis, HLB SPE concentration analysis of pink azo form using Griess reaction, 16 cm Type I LCW as flow cell	Nitrite: LOD: 0.3 nM Range: 2 to 100 nM r^2: 0.9996 RSD: 3.6% (10 nM), n = 7, 2.2% (100 nM), n = 10 Nitrate: LOD: 1.5 nM Range: 5 to 200 nM r^2: 0.9994 RSD: 4.3% (10 nM), n = 7, 2.6% (100 nM), n = 10	– Avoids matrix interference of LCW method – Reduced sample volume – Simultaneous determination of nitrite and nitrate – Complicated system	[54]
Colorimetry	Griess reaction	Preliminary test of a lab-on-a-chip analyzer	Nitrite: LOD: 14 nM Range: 0.050 to 10 μM	– Integrated optical illumination and detection system – No analysis of natural waters	[55]
Colorimetry	Griess reaction	Integrated lab-on-a-chip analyzer	Nitrite: LOD: 15 nM Range: up to 5 μM	– Microfluidic analyzer with low reagent requirement and low power consumption – 57 h in situ deployment – Not sufficiently sensitive for analysis of oligotrophic seawater	[56]
Colorimetry	Cd column reduction, Griess reaction	Integrated lab-on-a-chip analyzer	Nitrite: LOD: 20 nM Nitrate: LOD: 25 nM Range: up to 350 μM	– Microfluidic analyzer with low reagent requirement and low power consumption – 26-day in situ deployment – Not sufficiently sensitive for analysis of oligotrophic seawater	[57]
Chemiluminescence	Chemiluminescence between NO (generated from nitrite and nitrate by UV irradiation) and luminol reagent	Ion pair chromatography, on-line UV irradiation	Nitrite: LOD: 50 nM Range: 0.1 to 20 μM r^2: 1.000 RSD: 2.7% (0.5 μM), n = 7 Nitrate: LOD: 400 nM Range: 1 to 200 μM r^2: 0.9999 RSD: 2.1% (5 μM), n = 7	– Simultaneous determination of nitrate and nitrite in <2 min – Low recovery for seawater analysis – Not sufficiently sensitive for analysis of oligotrophic seawater	[58]
Mass spectrometry	Isotope dilution using ^{15}N	Headspace or SPME for derivatized sample introduction to GC-MS	Nitrite: LOD: 70 nM RSD: 4.0% (3.2 μM), n = 8 Nitrate: LOD: 2 μM RSD: 4.0% (22.6 μM), n = 8	– Complex derivatization procedure – Good agreement with μM level CRM – Not sufficiently sensitive for analysis of oligotrophic seawater	[59]
Mass spectrometry	Isotope dilution using ^{15}N and ^{18}O	Headspace for derivatized sample introduction to GC-MS	Nitrite: LOD: 11 nM Nitrate: LOD: 43 nM	– Complex derivatization procedure – Good agreement with μM level CRM – Not sufficiently sensitive for analysis of oligotrophic seawater	[60]

3.3. Microfluidic sensor

Sieben et al. [55] described the design, the fabrication, the analysis and the performance of a continuous flow, microfluidic absorption cell and detection system, appropriate for nitrite detection, and also for a range of chemistries involving colorimetric analysis {e.g., phosphate [43]}. Based on this microfluidic technique, Beaton et al. developed *in-situ* sensors for nitrite (LOD 15 nM) [56] and, using 15 valves, three absorbance cells and three syringes, both nitrate and nitrite (LODs 25 nM for NO_3^- and 20 nM for NO_2^-) [57]. These highly integrated LOC sensors showed adequate analytical performance and endurance in coastal waters (e.g., 26-day deployment in Southampton waters) and their small size and low power consumption should make them amenable for use on AUVs and gliders.

3.4. Ion chromatography

In order to detect nitrate and nitrite simultaneously with high-sample-throughput, Kodamatani et al. [58] used a short octadecylsilane column to separate the two ions, followed by online UV irradiation and subsequent detection by luminol chemiluminescence. Although this method showed good separation and required only 2 min per sample, the LODs of 50 nM for nitrite and 400 nM for nitrate are high for analysis of oligotrophic seawater. Moreover, the recoveries for seawater analysis were low and the authors indicated "it is clear that the proposed method was influenced by substance in the matrix of the seawater".

3.5. Gas chromatography-mass spectrometry (GC-MS)

Pagliano et al. [59,60] reported use of direct headspace or solid-phase microextraction (SPME) with GC-MS to separate and to detect a derivatized compound of nitrate and nitrite. Isotopically enriched internal standards (^{15}N or ^{18}O) were employed for quantification. Although measured concentrations of nitrate and nitrite agreed well with values in the seawater certified reference material (CRM) for nutrients, the 3.2-μM nitrite CRM and 22.6-μM nitrate CRM concentrations used are far too high to permit evaluation of the approach for nanomolar analyses.

4. Methods for analysis of nanomolar concentrations of ammonium

Several techniques have been reported for the trace ammonium analysis in seawater, and **Table 3** summarizes of these methods.

4.1. Indophenol blue (IPB) method

The IPB method based on Berthelot's reaction has been widely used for the determination of ammonium in seawater. Under alkaline conditions, ammonia and hypochlorite form a monochloramine, which subsequently reacts with two molecules of phenol. Application of this reaction in conjunction with a catalyst provided highly sensitive determinations of ammonia in natural waters [84]. In the Fifth ICES Intercomparison Exercise for the determination of nutrients in seawater, Aminot et al. [84] comprehensively evaluated this method. It was found that accurate determination of ammonium was difficult for the community measuring marine nutrients. For example, the standard deviations were 22–23% for samples at medium and high concentrations, and up to 56% for samples at low concentrations. Application of the IPB method to seawater posed specific problems, such as precipitation and salinity-dependent pH variation of the seawater matrix, as discussed by Pai et al. [85]. Most importantly, the conventional IPB method is insufficiently sensitive for nanomolar determinations of ammonium concentrations [84]. Several approaches have been proposed to increase the sensitivity of this method.

Brzezinkski [61] concentrated IPB by extraction into n-hexanol at low pH with subsequent re-extraction into an aqueous alkaline buffer. This procedure provided a 12-fold improvement in sensitivity and a 26-fold improvement in precision relative to standard aqueous analyses. However, the authors reported that the method was labor intensive and only 30 samples could be processed in an 8-h day. Several improvements were proposed, including utilization of SPE rather than organic solvent to concentrate the IPB. Two such SPE-based methods have been reported. Clark et al. [62] first concentrated IPB on C18 cartridge, and then detected the eluted IPB by GC-MS after derivatization with Sylon TP (25% trimethylsilylimidozole in pyridine). This GC-MS method was applied to studies of ammonium-regeneration rates at three stations in the oligotrophic North-East Atlantic Ocean. However, in addition to the need for high-cost instrumentation and experienced operators, the manual sample-processing procedures (color formation, SPE enrichment and elution, eluent evaporation and derivatization) were complicated and time consuming (>12 h).

As an alternative SPE methodology based on their protocol for analysis of nitrite [52], Chen et al. [63] used preconcentration of IPB with HLB sorbent, elution with 30% (v/v) ethanol and 1.0 mM NaOH, and subsequent spectrophotometric determination at 640 nm. After optimizing parameters, such as extraction conditions, reagent concentrations, reaction times, pH and temperature, an LOD of 3.5 nM was obtained with a linear range up to 428 nM. Compared with GC-MS, this method is faster (20 min sample^{-1}), less expensive and less labor intensive. As a related procedure, a polytetrafluoroethylene (PTFE)-type membrane filter was used to concentrate the ion-pair compound of IPB and Zephiramine [86], but this manual method is labor intensive and time intensive, and no data were obtained for analysis of seawater samples.

The only reported use of a LWCC for trace ammonium analysis was by Li et al. [64]. A large volume auto-sampler was modified to the SCFA-LWCC (2 m) system and used to analyze samples in Biscayne Bay. The flow configuration and the composition of the reagents were comprehensively optimized. This method provided an LOD of 5 nM and rapid analysis, and showed good agreement with results obtained using a conventional auto-analyzer. The authors reported that a combination of EDTA and citrate prevented formation of Ca and Mg hydroxide precipitate in the analytical pH range selected (>11–12). A detailed procedure for producing low ammonium seawater was described (sub-section 6.5 below).

4.2. Orthophthaldialdehyde (OPA)-based method

Fluorometry is attractive for its inherent sensitivity, particularly for methods involving reaction of ammonia with OPA. However, this reaction is not highly selective because primary amines also produce fluorescence with OPA. With a gas-diffusion (GD) step, some interfering compounds (e.g., amino acids) can be removed. The combination of GD and FIA with fluorometric detection has been applied for determination of ammonia in seawater [65] and an *in-situ* sensor based on similar set-up has been reported [66]. The LODs of both methods were approximately 1 nM. Although these methods were employed in field analyses, both have serious salinity effects (e.g., sensitivity in seawater is 1.67 times greater than sensitivity in DIW [65]). In addition to a difference in GD efficiency in different matrices, one possible reason for the salinity effects is that the classic OPA-2-mercaptoethanol chemistry in these two methods is strongly influenced by the presence of sodium or potassium. Zhang and Dasgupta [87] modified the conditions of the OPA reaction, replacing 2-mercaptoethanol by sulfite, and observed no ionic strength effect with sodium chloride and sulfate up to 0.1 M. This modified OPA-sulfite method has subsequently been widely used for

Table 3
An overview of published methods for nanomolar ammonium analysis in seawater

Detection	Chemistry	Technique	Analytical performance	Comments	Ref.
Colorimetry	IPB	Manual sample preparation, solvent extraction	LOD: < 5 nM Range: up to 2000 nM r^2: 0.9991–0.9998 RSD: < 4% (≤50 nM), n = 12	– Requires no specialized equipment – Contains several manual steps and uses significant volumes of organic solvent – Slight salinity effect	[61]
GC-MS	IPB	Manual sample preparation, C18 SPE concentration of IPB, GC-MS detection of derivatized eluent	LOD: < 10 nM Range: 10–100 nM	– Complicated sample treatment procedures – > 12 h for IPB reaction – Application in ammonium regeneration rate studies	[62]
Colorimetry	IPB	Flow analysis, HLB SPE concentration of IPB	LOD: 3.5 nM Range: up to 428 nM r^2: 0.9994 RSD: 5.7% (44.6 nM), n = 8; < 6.0% (52.4–288.7 nM), n = 3–5	– Fully automated method – 20 min sample^{-1} – Elimination of background interference from seawater through SPE	[63]
Colorimetry	IPB	CFA coupled with 2 m LWCC	LOD: 5 nM Range: up to 1000 nM r^2: 0.9997 RSD: < 5% (all range concentration), n = 6	– Modified auto-sampler for large volume samples – Automated method for large number of samples – 1.5 min sample^{-1} – Slight salinity effect	[64]
Fluorometry	OPA-2-mercaptoethanol	Gas diffusion coupled with FIA	LOD: 1.1 nM Range: up to >2000 nM r^2: 0.9979 RSD: 1.8% (250 nM), n = 12	– 2 min sample^{-1} – Large salinity effect – Reaction at 35 °C – Methylamine might interfere	[65]
Fluorometry	OPA-2-mercaptoethanol	Gas diffusion coupled with FIA	LOD: ~ 1 nM	– Modification from Jones 1991 – Large salinity effect – Reaction at 37 °C – Applications in field and underway systems	[66]
Fluorometry	OPA-sulfite	Gas diffusion coupled with FIA	LOD: 7 nM Range: up to 1000 nM r^2: 0.994 RSD: 5.7% (800 nM), n = 12	– 2 min sample^{-1} – No salinity effect – No interference from volatile small molecular-weight amines – Control of contamination was comprehensively discussed	[67]
Fluorometry	OPA-sulfite	SFA	LOD: 1.5 nM Range: up to 250 μM r^2: 0.9991–0.9999 RSD: 0.2–2% (500–5000 nM), n = 2; 0.17% (3000 nM), n = 5	– First application of OPA-sulfite method for seawater analysis – Salinity effect less than 3% – Primary amine interference less than 0.5% – 3 min sample^{-1} – < 1 % carry-over – Very wide analytical range	[68]
Fluorometry	OPA-sulfite	Manual operation	LOD: < 31 nM Range: 31 nM to 50 μM r^2: 0.999	– No specific instrument is required – Single reagent – 3 h reaction time at room temperature	[69]
Fluorometry	OPA-sulfite	FIA	LOD: 30 nM Range: up to 50 μM r^2: 0.999 RSD: ~ 1% (0.5–4 μM), n = 5	– Reaction at 30 °C – ~ 7 min sample^{-1} but, with stopped flow, 3 min sample^{-1} – Salinity effect: for 5–35 less than 2%; below 5, around -9% – Negligible interference from primary amines and Hg^{2+} – Linearly depression from S^{2-} – Potential for *in situ* analysis	[70]
Fluorometry	OPA-sulfite with formaldehyde added	CFA or FIA	LOD: 1.1 nM Range: up to 600 nM r^2: 0.998 RSD: 2.2% (200 nM), 6.7% (1 nM), n = 10	– Remarkable 1 s sample^{-1} with CFA – Shipboard application in underway systems – Long-term stability over 25-days – Amino acid interference on low level ammonium	[71]
Fluorometry	OPA-sulfite with formaldehyde added	Autonomous bath analyzer	LOD: 1 nM Range: up to 25 μM r^2: 0.9990–0.9991 RSD: 0.6% (200 nM), n = 10	– Novel design for rapid mixing and complete reaction – Shipboard application in underway systems – Calibration curve generated by auto dilution	[72]
Fluorometry	OPA-sulfite with formaldehyde added	Portable autonomous bath analyzer	LOD: 10 nM Range: 0.05–10 μM r^2: 0.9930 RSD: 0.3% (2 μM), n = 10	– Improved LED photodiode-based fluorescence detector – Field application in underway systems – Back flushed passive filter for sediment laden coastal waters – Long-term stability over 16-days with RSD of 3%	[73]
Fluorometry	OPA-sulfite	SIA	LOD: 60 nM	– < 0.6 min sample^{-1} – Shipboard application in 3-week cruise – No additional details about analytical performance	[74]

Table (*continued*)

Detection	Chemistry	Technique	Analytical performance	Comments	Ref.
Fluorometry	OPA-sulfite	CFA, dual channel reagents to compensate for background fluorescence	LOD: < 5 nM Range: 0.05–1 μM (linear) 1–25 μM (second-order polynomial) r^2: 0.9997, 0.9999 RSD: 4% (50 nM), 2% (1 μM), 3-day data acquisition	– Sensitive to salinity variations – Signal depression from high amino acid or amine concentrations – 12% signal effect from phytoplankton bloom – Reagent stable for 17 days – Suitable for oligotrophic environment rather than coastal and eutrophic environment	[75]
Fluorometry	OPA-sulfite	Multi-pumping flow analysis	LOD: 13 nM Range: up to 1 μM or 16 μM with two different gains r^2: 0.992–0.999 RSD: < 2% (5 μM), 8-h data	– Miniature system – Single reagent – Reaction at 82°C – Custom made detector – Sensitivity increased linearly with salinity at (0.25% per unit change in salinity – < 2 min sample^{-1}	[76]
Fluorometry	OPA-sulfite	IC for separation of ammonium and detection with OPA chemistry	LOD: 100 nM Range: 0.05–1 μM r^2: 0.997 RSD: < 4% (peak area), <1% (retention time)	– Separation from 2800000 folds of sodium and 28000 folds of amino acids – 16 min sample^{-1} – Post-column reaction at 60°C – Seawater sample needs ten-fold dilution	[77]
Fluorometry	OPA-sulfite	Chemical reaction in disposable 48-well microplate, detection with microplate fluorescence reader	LOD: 5 nM Range: 0.05–10 μM r^2: 0.999–1.000	– No interference from suspended particular matter and colored organic acids – Serious salinity effects – Possible contamination by atmospheric ammonia	[78]
Fluorometry	OPA-sulfite	SPE concentrates the fluorescent compound from OPA-sulfite-ammonium reaction, flow batch analysis	LOD: 0.7 nM (land); 1.2 nM (shipboard) Range: 1.67–300 nM r^2: 0.9912, 0.9935 RSD: 3.5% (20 nM), n = 5	– Increase analytical range by changing reaction time – Sample storage methods were evaluated – High resolution vertical profile in South China Sea – HLB cartridge could be reused > 50 times	[79]
Conductimetry	–	FI-GD-IC	LOD: 20–40 nM Range: up to 2000 nM r^2: 0.99 RSD: 1–6% (1 μM)	– Separation of ammonium and methylamines – 50 mL sample required – Relatively complex system – Shipboard analysis	[80]
Conductimetry	–	Purge-and-trap to isolate ammonium from samples	LOD: 75 nM Range: 0.05–6.0 μM r^2: 0.997 RSD: < 4% (peak area), <1% (retention time)	– Manual operation for sample pretreatment – No salinity effect – Collection efficiency independent of ammonium concentration – 15 min for purge-and-trap, 9 min for IC analysis	[81]
Conductimetry	-	GD, ammonia diffusing through membrane changes the conductivity of the acceptor solution	LOD: 10 nM Range: up to 2.0 μM RSD: < 6% (1 μM), <2.5% (3 μM)	– *In situ* sensor for estuarine, coastal, and shelf waters – Stable for deployment of at least 30 d – Good agreement with IPB method – The acceptor solution must be prepared very carefully	[82]
Indirect spectrometry	Acid-based indictor	FIA-GD, ammonia diffusing through membrane changes the color of the acceptor solution (acid-base indictor)	LOD: 50 nM Range: up to 10 μM RSD: < 2% (2 μM), n = 28	– LED photometer detector – Clogging of membrane, 1% HCl washing is needed every 5 h – GD for ammonia transfer efficiency of 62%	[83]

determinations of ammonium concentrations in seawater without the salinity effect [67].

Two groups have several publications in this field. In 1997, Kérouel and Aminot [68] first combined SCFA with the OPA method for determination of ammonium in seawater. This method is direct, free from primary amine interferences, nearly free of salt effects (less than 3% in the 0–35 salinity range), and has an LOD of 1.5 nM with applicability up to 250 μM by on-line dilution. Additional methods based on the same OPA chemistry include: a manual method with a single working reagent (consisting of OPA, sodium sulfite, and sodium borate) but with a reaction time as long as 3 h at room temperature [69]; and, a method based on replacement of SCFA with more robust FIA that is insensitive to pressure, and therefore potentially useful for *in-situ* analysis [70]. The combination of FIA and fluorometry for ammonium analysis appears promising for use in submersible devices, especially in environments with variable salinity and turbidity. However, the method LOD of 30 nM might be inadequate for applications in the oligotrophic ocean.

Amornthammarong and Zhang [71] achieved both higher sensitivity and a more stable reagent solution by mixing formaldehyde with sulfite in the OPA reagent. Addition of formaldehyde can reduce the influence of potential interfering species, such as amines and amino acids, but application of this method to low-level ammonium measurement still requires corrections for interfering species. The continuous flow FIA shipboard analyzer of Amornthammarong and Zhang [71] has an LOD of 1.1 nM, negligible salt effects, no refractive index effect, and a sample throughput of 3600 h^{-1}. However, severe carry-over problems are inherent to the FIA design, particularly at high sampling rates, and these can adversely affect the accuracy of field data. Moreover, the FIA method consumes a considerable quantity of reagents, needs regular

replacement of pump tubes (ca. weekly) and requires repeated, time-consuming manual calibrations. Through application of a pipette-based mixing chamber, Amornthammarong et al. therefore designed [72] an autonomous batch analyzer to overcome the drawbacks of their previous batch and continuous flow analyzers. This batch analyzer was used for ammonium determinations and then modified for use as a portable analyzer for *in-situ* applications [73]. The two analyzers showed good performance with respect to LOD (1–10 nM), analytical range (up to >10 μM), stability (e.g., RSD of 3% in a 16-day continuous analysis of 660 samples) and prompt sample analysis.

To increase automation efficiency further for trace ammonium determinations based on OPA chemistry, and to reduce sample/reagent consumption, different flow techniques, such as SIA [74], dual-channel continuous flow analysis [75] and miniature multi-pumping flow analysis [76] have been developed. The OPA procedure has also been used as a post-column derivatization technique after ammonium is separated by ion chromatography (IC) [77]. However, because this system exhibited salinity effects, seawater samples had to be diluted 10-fold. Poulin and Pelletier [78] utilized a 48-well microplate for rapid, automated readings of a variety of samples with different fluorescence and matrix effects. Although high loading of suspended particular matter, colored organic acids, and salinity changes did not diminish the accuracy of the authors' ammonium determinations, contamination from atmospheric ammonia may present problems.

Zhu et al. [79] recently combined an SPE technique with fluorometric detection in a flow-batch system to increase sensitivity and to eliminate potential interferences. The OPA reaction product was efficiently extracted onto an HLB cartridge, and then rapidly eluted with ethanol and measured by fluorometry. This method showed LODs of 0.7 nM and 1.2 nM in shore-based and shipboard laboratories. A high-resolution vertical profile of temperature, salinity, nitrate + nitrite and ammonium obtained using this method at the South East Asia Time-Series site (18°N, 116°E) is shown in **Fig. 2**.

4.3. Conductivity-based method

Conductimetric detection is one of the most commonly used techniques in IC. Gibb et al. [80] described a coupled technique using the combination of flow injection, GD and IC for determination of ammonium (LOD ~20–40 nM) and methylamine (LOD 3–5 nM). The technique used alkaline EDTA both to convert the analytes to their non-protonated forms and to preclude formation of calcium and magnesium hydroxide precipitates.

Wang et al. [81] described analysis of volatile organic compounds in aqueous samples that involved a purge-and-trap technique for separation of ammonium from high-salinity water samples and subsequent IC detection (LOD 75 nM).

The LODs of these two IC methods are too high for applications in the oligotrophic ocean but should be useful for coastal monitoring.

Plant et al. [82] demonstrated an *in-situ* analyzer with an LOD of 10 nM that used a GD cell to isolate the analyte from the matrix, and a conductivity detector for analyte detection. A sodium hydroxide/sodium citrate solution was used to convert ammonium to ammonia in the sample while preventing Ca and Mg hydroxide precipitation. Ammonia diffused across the membrane into a receiving solution of dilute hydrochloric acid, and the resulting change in conductivity was related to ammonia concentration. Devices using this technique were recently deployed on coastal moorings, benthic flux chambers and a drifter west of Monterey Bay. The devices can measure five samples per hour for up to 30 days in surface waters (depths ≤ 3 m).

4.4. Indirect spectrophotometry method

Willason and Johnson [83] described a simple method based on FIA-GD (LOD 50 nM, 60 samples per hour) similar to that described in Plant et al. [82]. Using a basic solution, the NH_4^+ in seawater was converted to NH_3, which then diffused through the membrane and converted protonated phenol red to its unprotonated form in an acceptor stream. The color change of the indicator, due to the ammonia-induced pH change, was monitored by a light-emitting diode (LED)-based photometer at 565 nm. The absorbance change was proportional to the concentration of ammonium in the sample.

5. Methods for analysis of nanomolar concentrations of silicate

Spectrophotometric silicate analyses are most commonly based on reactions with molybdenum salts in an acidic medium to form a yellow silicomolybdic complex (SiMY). SiMY can be measured directly or after it has been reduced to silicomolybdenum blue (SiMB).The analytical procedures leading to spectrophotometric detection of SiMB constitute the standard protocol for determination of dissolved silica [13,16]. **Table 4** summarizes the reported methods for trace silicate analysis in seawater.

5.1. LCW-based method

Amornthammarong and Zhang [88] reported LCW spectrophotometric measurements of low-level silicate in natural waters based on the SiMB chemistry. Poly-vinyl alcohol was added to prevent precipitation in the ammonium molybdate solution and improve the stability of the SiMB complex. This method shows no refractive index effects and only a small salinity effect for seawater samples (and that can be corrected). This method was used for shipboard determinations of silicate in Gulf Stream surface seawater in the Florida straits during a cruise between Florida and the Bahamas. However, the analysis time was comparatively lengthy (~15 min sample^{-1}) and, most importantly, the LOD (100 nM) was unexpectedly high for a 2-m LCW.

Recently, Ma and Byrne [89] combined FIA with a custom-made 160-cm LCW system to achieve an LOD of 10 nM for silicate with a sample throughput of 12 h^{-1}. Interference from phosphate was examined and eliminated through addition of oxalic acid. Salinity and seawater matrix effects were investigated and minimized through on-line dilution of samples with reagents. The targeted analytical range of 10 nM–5 μM can easily be extended to higher concentrations without altering the experimental hardware (e.g., by simply changing flow rates or selecting alternative analytical wavelengths), so this experimental set-up can be modified for on-line measurement of trace silicate in ultra-pure water (industrial applications) and shipboard/underway measurements in seawater (marine science applications).

5.2. Solvent extraction

Brzezinski and Nelson [90] described a solvent-extraction method for measuring nanomolar silicate in seawater. The procedure was based on the formation of beta silicomolybdic acid by reaction of silicate and molybdic acids at low pH, extraction of the combined acids into *n*-butanol, and reduction with a mixture of *p*-methylaminophenol sulfate and sulfite. The concentration of the resulting SiMB in the extract was determined colorimetrically at 810 nm using a 10-cm cuvette. The method increased the sensitivity by a factor of 30 and precision by a factor of 14 compared with standard aqueous analyses. However, the procedure is labor intensive, and needs large volumes of organic solvent. Moreover, the

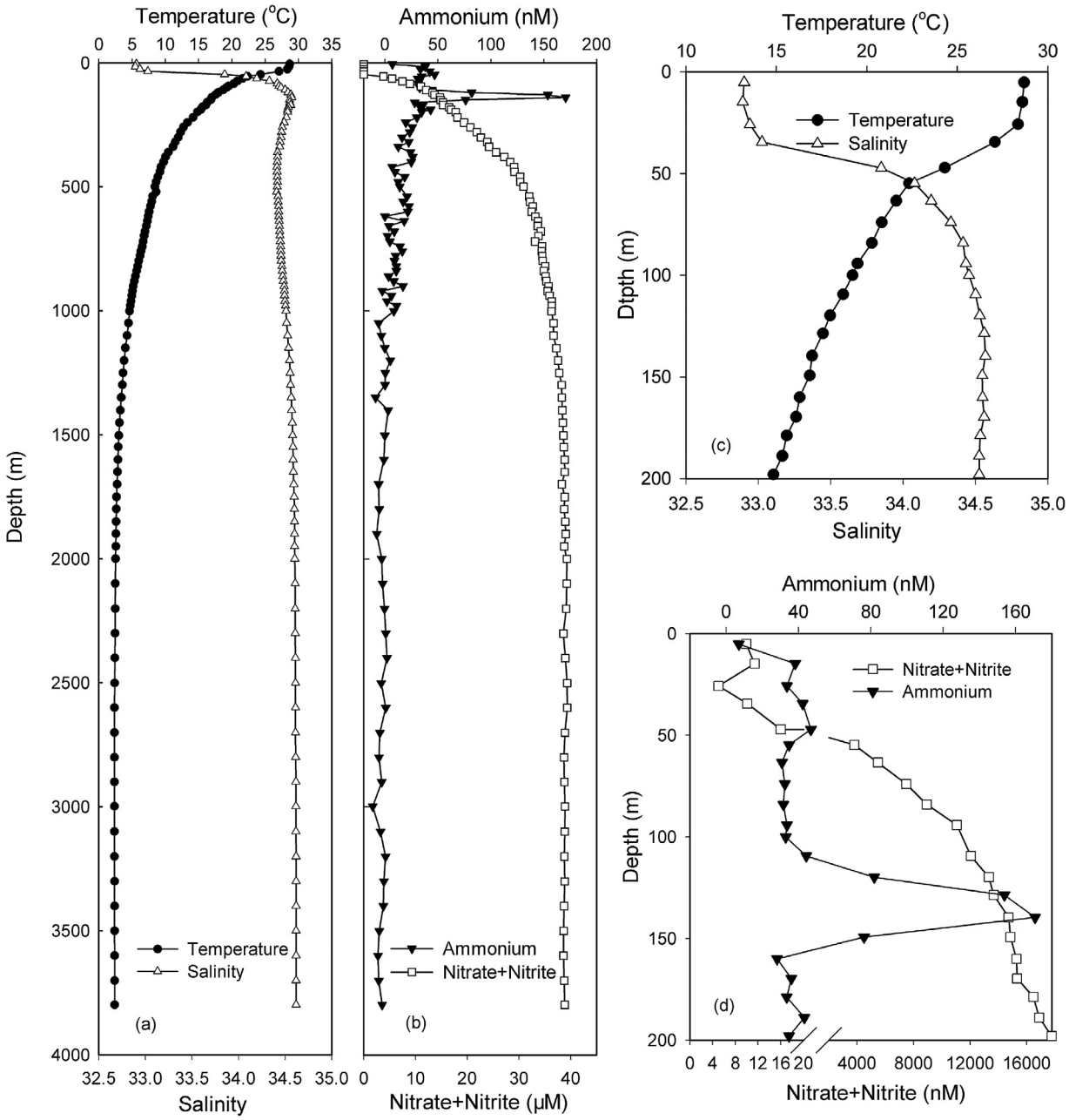

Fig. 2. The vertical profile of temperature, salinity, nitrate + nitrite, and ammonium at the SEATS station (18°N, 116°E) with 92 depth levels: (a) temperature and salinity; (b) nitrate + nitrite and ammonium; (c) enlargement of (a) above the depth of 200 m; and, (d) enlargement of (b) above the depth of 200 m [77], with permission from Elsevier.

sensitivity of the method for measurements in seawater is 70% of that in DIW due to a significant salt effect. In view of the need for salt-effect corrections, this method is cumbersome and has seldom been applied at sea.

5.3. MAGIC method

The MAGIC method, introduced in 1992 by Karl and Tien [37] and one of the most widely used methods for determination of phosphate at nM levels, was adapted by Rimmelin-Maury et al. [91] for silicate analysis. The method involves addition of NaOH to water samples, quantitative removal of silicate from solution by adsorption to $Mg(OH)_2$ precipitate, concentration of the precipitate by centrifugation, dissolution of the precipitate in a small volume of dilute acid, and then determination using the standard SiMB protocol. Although the authors comprehensively demonstrated the efficacy of the method (repeatability, sensitivity and precision), the MAGIC procedure is unavoidably laborious and time consuming. It requires 2 h for color formation in the concentrated silicate solution.

5.4. Ion-exclusion chromatography (IEC)-based method

As an alternative method to complement classic colorimetric methods for silicate analysis, Hioki et al. [92] chose IEC in combination with inductively-coupled plasma mass spectrometry (ICP-MS) as an independent, secondary method for analysis of silicate in a certified seawater reference material. Silicate was separated from chloride and sulfate ions by IEC and measured by highly-sensitive,

Table 4
An overview of published methods for nanomolar silicate analysis in seawater

Detection	Chemistry	Technique	Analytical performance	Comments	Ref.
Colorimetry	SiMB	Hyphenated flow analysis with 2 m LWCC	LOD: 100 nM R: 0.1–10 μM r^2: 0.9968	– Small salinity effect – ~ 15 min sample^{-1} – Shipboard application in underway systems – Relatively high LOD	[88]
Colorimetry	SiMB	FIA, 160 cm Type I LCW	LOD: 9 nM Range: up to 5 μM r^2: 0.9997 RSD: 1.51% (250 nM), n = 7	– 5 min sample^{-1} – No salinity effect – Potentially for on-line or shipboard analysis	[89]
Colorimetry	SiMB	Manual sample preparation, solvent extraction	LOD: < 5 nM Range: up to 500 nM r^2: 0.9995 RSD: < 8% (≤50 nM), n = 12	– Requires no specialized equipment – Contains several manual steps and uses significant volumes of organic solvent – Significant salinity effect	[90]
Colorimetry	SiMB	Manual sample preparation, MAGIC method with 12.5-fold pre-concentration factor, 10-cm cell	LOD: 3 nM Range: 3–500 nM r^2: 0.998 RSD: 2.9% (69 nM), n = 8	– Improved version of the MAGIC procedure – Reaction time is ~ 2 h – Manual operation still needed for MAGIC method	[91]
ICP-MS	–	IEC separation of silicate from other ions in seawater	LOD: 80 nM RSD: 1.4% (20.6 nM), n = 6	– Alternative method for CRM – ~ 20 min sample^{-1} – Not sufficiently sensitive for oligotrophic seawater – Costly instruments needed	[92]
Conductivity	–	IEC separation of silicate from other ions in seawater	LOD: 20 nM Range: 0.1–1000 μM r^2: 0.997 RSD: 2.0% (100 μM), n = 8	– Not applicable for low concentration samples – ~ 14 min sample^{-1} – Not sufficiently sensitive for oligotrophic seawater – Costly instruments needed	[93]

selective ICP-MS. The LOD of the method was 80 nM, fulfilling the authors' requirement for CRM analysis, but not satisfying requirements for oligotrophic ocean analyses.

Li and Chen [93] also utilized IEC to separate silicate from other ions in seawater, but replaced ICP-MS with a conductivity-detection system to reduce the cost of the instrument. The LOD was 20 nM with a linear range of 0.1–1000 μM, but only one seawater sample with a 426 μM concentration was analyzed. Whether detected by ICP-MS or conductivity, these IEC-based systems are both expensive and, in many venues, the experienced operators required for the analyses may not be available.

6. Aspects related to analysis of nanomolar concentrations of nutrients

6.1. Cleaning and maintenance

Cleaning protocols for nutrient-measurement apparatus are not as strict as the protocols for trace-metal analysis [94]. Nevertheless, the importance of cleaning and maintenance, especially when dealing with LWCCs should be kept in mind. Sufficient flushing of measurement cells with diluted HCl, NaOH solution, DIW, and, in some cases, dilute soap solution is essential and should be a routine aspect of measurement protocols [46].

6.2. Sample storage

Analysis of trace nutrients should be conducted as soon as possible after sample collection. When this is not possible, it is of prime importance that samples are efficiently preserved without altering the original concentrations of the species of interest. Patey et al. [95] compared nutrient-concentration measurements for unfiltered and filtered samples. The phosphate and nitrate concentrations of filtered samples were stable for 24 h at 4°C; nitrate changes in unfiltered samples were insignificant, but phosphate concentrations increased significantly after storage for 1 or 2 days at 4°C and after 12 days for frozen samples. The elevated phosphate concentrations of frozen samples were attributed to cell lysis.

Zhu et al. [79] recently investigated the influence of storage on trace-ammonium concentrations in seawater samples. Ammonium was adequately preserved for at least 5 days if samples were immediately frozen and stored at -20°C in clean, high-density polyethylene bottles.

6.3. Sample contamination

Due to the high dissolution rate of atmospheric NOx and ammonia into aqueous solutions, determination of nitrogenous nutrients at low concentrations is extremely challenging. Zhang et al. [53] and Zhu et al. [79] designed special devices to avoid interferences from atmospheric NOx and ammonia. Glass cups were found to be subject to ambient ammonium contamination, possibly due to ammonium adsorption on glass walls. Consequently, polypropylene cups were used for both samples and standards [64].

In addition to contamination from the atmosphere, reagents have been reported to be a significant source of contamination. Clark [62] described contamination in methods that utilize the IPB reaction and outlined techniques to determine their contribution to apparent ammonia concentrations. Ammonia was removed from the low-nutrient seawater used for calibration standards by running seawater continuously (≥24 h) through 2-μm pore Teflon tubing that was immersed in a 10% HCl solution.

Watson et al. [67] described approaches to control contamination for the OPA method. One effective approach involved use of a PTFE diffusion cell with a 10% sulfuric acid receiving solution to remove ammonia from flowing sodium hydroxide reagent. And a nitrogen-flushed glove box was used to prevent atmospheric ammonia from contaminating samples in the laboratory and at sea [67].

Trace-nutrient contamination may be more serious than is generally recognized. For example, through measurements with the FIA-LCW method [89], a glass pH electrode immersed in seawater was observed to produce detectable (nanomolar) levels of dissolved silicate, as shown in the Supplementary Materials in the online version at doi:10.1016/j.trac.2014.04.013.

6.4. Interferences

Flow-based methods may suffer from certain problems, such as refractive index changes (Schlieren effects) whereby salinity differences between samples and washing/carrier solutions create interferences in absorbance measurements [96]. Consequently, the presence of Schlieren effects should always be considered for flow analysis of trace nutrients. Corrections for this effect using dual-wavelength analysis can be applied to nitrate/nitrite analysis and are based on the Griess reaction, but the broad absorption spectra of PMB and SiMB preclude use of a suitable (non-absorbing) reference wavelength.

In an investigation of interferences for nanomolar nitrate and phosphate determinations, Patey et al. [95] suggested that interferences should be systematically assessed in any newly developed analytical system for phosphate analysis. In particular, filtration was noted as having an effect on analytical results, as discussed in sub-section 2.1 [37,48,95].

Utilization of the term "DRP" instead of "phosphate" eliminates from consideration the contributions of DOP. However, since DOP concentrations can be much higher than DRP concentrations in the oligotrophic ocean [48], it is recommended that the influence of hydrolysis be evaluated for different forms of DOP.

6.5. 'Nutrient-free' seawater

In conventional methods with micromolar LODs, low-nutrient seawater (LNSW) at nM levels is used as "blank" and is thereby considered essentially "nutrient free". This assumption cannot be applied to nanomolar nutrient analysis.

For phosphate, Li et al. [38] compared two methods for making "phosphate-free" seawater. The use of $FeCl_3$ additions to remove phosphate was found to be more effective than the MAGIC method and did not alter the seawater matrix. For ammonium, several drops of 1 M NaOH were added to LNSW collected from the surface of the Gulf Stream until a small amount of precipitation was observed. The sample was subsequently swirled and heated to 60°C to drive off gaseous NH_3. The solution was then sealed, passively cooled to room temperature, and finally filtered (0.45 μm) to produce ammonium-"free" seawater [64]. Methods for preparation of nitrate/nitrite-"free" seawater have not been reported.

Procedures for preparing "nutrient-free" seawater are complicated, and time- and labor-consuming, and, most importantly, subject to contamination during the different steps (e.g., filtration), so we recommend that methods that are free of salinity effects (e.g. [42],) are used as "reference" methods to evaluate the quality of "nutrient-free" seawater. Of course, it should be recognized that such methods are based on the assumption that ultrapure DIW is nutrient free.

6.6. Trace-nutrient CRMs

With the development of techniques for trace-nutrient analysis, we highly recommend that trace-nutrient CRMs are used to evaluate the accuracy of new methods and instruments. However, because of problems with sample storage and uncertainties in the preparation of "nutrient-free" seawater, production of CRMs for trace-nutrient analysis will be a substantial challenge for the nutrient-measurement community.

7. Conclusions

The aim of this review was to explore the vast diversity of methods available for detection and analysis of nanomolar nutrients in seawater. The review of Patey et al. [32] has been updated to include new phosphate and nitrate/nitrite publications to the end of 2013; and, ammonium and silicate analysis were comprehensively reviewed to the end of 2013. This review also includes aspects of measurement protocols that bear strongly on the quality of trace-nutrient analyses, including contamination of reagents, sample storage, and preparation of nutrient free seawater. In summary of this review, the use of spectroscopic analysis based on classical nutrient chemistry is highly recommended due to the simplicity of the protocols and the wide availability of required instrumentation (e.g., shipboard and *in situ*).

Several trends can be reported concerning the efficacy of trace-nutrient analysis, e.g.:

- LWCCs have been widely utilized in trace-nutrient analysis; and,
- the combination of classic SCFA and LWCC can be utilized with commercial products.

In addition to the use of LWCCs, recent publications show that the sensitivity of spectroscopic methods can be increased through concentration of derivatized analytes on SPE. *In-situ* microfluidic sensors have been developed and deployed, but the LODs of such sensors may currently be inadequate for oligotrophic oceans.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (41306090) and Fundamental Research Funds for the Central Universities (2012121062). We thank the two anonymous reviewers for helpful comments on the manuscript.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.trac.2014.04.013.

References

[1] A. Paytan, K. McLaughlin, The oceanic phosphorus cycle, Chem. Rev. 107 (2007) 563–576.
[2] J.A. Brandes, A.H. Devol, C. Deutsch, New developments in the marine nitrogen cycle, Chem. Rev. 107 (2007) 577–589.
[3] D.E. Canfield, A.N. Glazer, P.G. Falkowski, The evolution and future of earth's nitrogen cycle, Science 330 (2010) 192–196.
[4] D.G. Capone, D.A. Bronk, M.R. Mulholland, E.J. Carpenter, Nitrogen in the Marine Environment, second ed., Academic Press, 2008.
[5] M. Voss, H.W. Bange, J.W. Dippner, J.J. Middelburg, J.P. Montoya, B. Ward, The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change, Phil. Trans. R. Soc. B 38 (2013) 20130121.
[6] D.M. Karl, Microbially mediated transformations of phosphorus in the sea: new views of an old cycle, Annu. Rev. Mar. Sci. 6 (2014) 279–337.
[7] M.A. Brzezinski, D.M. Nelson, The annual silica cycle in the Sargasso Sea near Bermuda, Deep Sea Res. I 42 (1995) 1215–1237.
[8] J.-C. Marty, J. Chiaverini, M.-D. Pizay, B. Avril, Seasonal and interannual dynamics of nutrients and phytoplankton pigments in the western Mediterranean Sea at the DYFAMED time-series station (1991–1999), Deep Sea Res. II 49 (2002) 1965–1985.
[9] M.D. Krom, E.M.S. Woodward, B. Herut, N. Kress, P. Carbo, R.F.C. Mantoura, et al., Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: results from a phosphorus starved system, Deep-Sea Res. II 52 (2005) 2879–2896.
[10] M. Miró, J.M. Estela, V. Cerdà, Application of flowing stream techniques to water analysis. Part I. Ionic species: dissolved inorganic carbon, nutrients and related compounds, Talanta 60 (2003) 867–886.
[11] S. Gray, G. Hanrahan, I. McKelvie, A. Tappin, F. Tse, P. Worsfold, Flow analysis techniques for spatial and temporal measurement of nutrients in aquatic systems, Environ. Chem. 3 (2006) 3–18.
[12] P.J. Worsfold, R. Clough, M.L. Lohan, P. Monbet, P.S. Ellis, C.R. Quétel, et al., Flow injection analysis as a tool for enhancing oceanographic nutrient measurements-A review, Anal. Chim. Acta 803 (2013) 15–40.
[13] J.D.H. Strickland, T.R. Parsons, A practical Handbook of Seawater Analysis, Second ed., Bull. Fish. Res. Bd. Can., 1972.
[14] A. Aminot, R. Kérouel, S.C. Coverly, Nutrients in seawater using segmented flow analysis, in: O. Wurl (Editor), Practical Guidelines for The Analysis of Seawater, CRC Press, Boca Raton, 2009, pp. 143–178.

[15] H.P. Hansen, F. Koroleff, Determination of nutrients, in: K. Grasshoff, K. Kremling, M. Ehrhardt (Editors), Methods of Seawater Analysis, Wiley-VCH, Weinheim, 1999, pp. 159–228.
[16] D.J. Hydes, A. Aoyama, A. Aminot, K. Bakker, S. Becker, S. Coverly, et al., Determination of dissolved nutrients (N, P, Si) in seawater with high precision and inter-comparability using gas-segmented continuous flow analysers, The GO-SHIP repeat hydrography manual: A collection of expert reports and guidelines, 2010.
[17] M.J. Moorcroft, J. Davis, R.G. Compton, Detection and determination of nitrate and nitrite: a review, Talanta 54 (2001) 785–803.
[18] C. Molins-Legua, S. Meseguer-Lloret, Y. Moliner-Martinez, P. Campíns-Falcó, A guide for selecting the most appropriate method for ammonium determination in water analysis, Trends Anal. Chem. 26 (2007) 914–930.
[19] S.M. Gray, P.S. Ellis, M.R. Grace, I.D. McKelvie, Spectrophotometric determination of ammonia in estuarine waters by hybrid reagent-injection gas-diffusion flow analysis, Spectrosc. Lett. 39 (2006) 737–753.
[20] J.M. Estela, V. Cerdà, Flow analysis techniques for phosphorus: an overview, Talanta 66 (2005) 307–331.
[21] S. Motomizu, Z.-H. Li, Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques, Talanta 66 (2005) 332–340.
[22] M.M. Villalba, K.J. McKeegan, D.H. Vaughan, M.F. Cardosi, J. Davis, Bioelectroanalytical determination of phosphate: a review, J. Mol. Catal. B: Enzym. 59 (2009) 1–8.
[23] S. Berchmans, T.B. Issa, P. Singh, Determination of inorganic phosphate by eletroanalytical methods: a review, Anal. Chim. Acta 729 (2012) 7–20.
[24] M.J.A. Rijkenberg, R.J. Langlois, M.M. Mills, M.D. Patey, P.G. Hill, M.C. Nielsdóttir, et al., Environmental forcing of nitrogen fixation in the Eastern tropical and sub-tropical North Atlantic Ocean, PLoS ONE 6 (2011) e28989.
[25] B.A. Ward, S. Dutkiewicz, C.M. Moore, M.J. Follows, Iron, phosphorus, and nitrogen supply ratios define the biogeography of nitrogen fixation, Limnol. Oceanogr. 58 (2013) 2059–2075.
[26] F. Hashihama, K. Furuya, S. Kitajima, S. Takeda, T. Takemura, J. Kanda, Macro-scale exhaustion of surface phosphate by dinitrogen fixation in the western North Pacific, Geophys. Res. Lett. 36 (2009) L03610.
[27] M.J. Ellwood, C.S. Law, J. Hall, E.M.S. Woodward, R. Strzepek, J. Kuparinen, et al., Relationships between nutrient stocks and inventories and phytoplankton physiological status along an oligotrophic meridional transect in the Tasman Sea, Deep Sea Res. I 72 (2013) 102–120.
[28] N.J. Fuller, G.A. Tarran, D.G. Cummings, E.S. Woodward, K.M. Orcutt, M. Yallop, et al., Molecular analysis of photosynthetic picoeukaryote community structure along an Arabian Sea transect, Limnol. Oceanogr. 51 (2006) 2502–2514.
[29] A. Pitcher, L. Villanueva, E.C. Hopmans, S. Schouten, G.-J. Reichart, J.S.S. Damsté, Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone, ISME J. 5 (2011) 1896–1904.
[30] S.C. Doney, M. Ruckelshaus, J.E. Duffy, J.P. Barry, F. Chan, C.A. English, et al., Climate change impacts on marine ecosystems, Ann. Rev. Mar. Sci. 4 (2012) 11–37.
[31] H.P. Jarvie, T.D. Jickells, R.A. Skeffington, P.J.A. Withers, Climate change and coupling of macronutrient cycles along the atmospheric, terrestrial, freshwater and estuarine continuum, Sci. Total Environ. 434 (2012) 252–258.
[32] M.D. Patey, M.J.A. Rijkenberg, P.J. Statham, M.C. Stinchcombe, E.P. Achterberg, M. Mowlem, Determination of nitrate and phosphate in seawater at nanomolar concentrations, Trends Anal. Chem. 27 (2008) 169–182.
[33] J. Murphy, J.P. Riley, A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta 27 (1962) 31–36.
[34] S. Auflitsch, D.M.W. Peat, I.D. McKelvie, P.J. Worsfold, Determination of dissolved reactive phosphorus in estuarine waters using a reversed flow injection manifold, Analyst 122 (1997) 1477–1480.
[35] P.J. Worsfold, L.J. Gimbert, U. Mankasingh, O.N. Omaka, G. Hanrahan, P.C.F.C. Gardolinski, et al., Sampling, sample treatment and quality assurance issues for the determination of phosphorus species in natural waters and soils, Talanta 66 (2005) 273–293.
[36] Q.P. Li, D.A. Hansell, Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater, Anal. Chim. Acta 611 (2008) 68–72.
[37] J. Ma, D. Yuan, M. Zhang, Y. Liang, Reverse flow injection analysis of nanomolar soluble reactive phosphorus in seawater with a long path length liquid waveguide capillary cell and spectrophotometric detection, Talanta 78 (2009) 315–320.
[38] Q.P. Li, D.A. Hansell, J.-Z. Zhang, Underway monitoring of nanomolar nitrate plus nitrite and phosphate in oligotrophic seawater, Limnol. Oceanogr.: Methods 6 (2008) 319–326.
[39] L.A. Zimmer, G.A. Cutter, High resolution determination of nanomolar concentrations of dissolved reactive phosphate in ocean surface waters using long path liquid waveguide capillary cells (LWCC) and spectrometric detection, Limnol. Oceanogr.: Methods 10 (2012) 568–580.
[40] L.R. Adornato, E.A. Kaltenbacher, D.R. Greenhow, R.H. Byrne, High-resolution *in situ* analysis of nitrate and phosphate in the oligotrophic ocean, Environ. Sci. Technol. 41 (2007) 4045–4052.
[41] J. Ma, D. Yuan, Y. Liang, M. Dai, A modified analytical method for the shipboard determination of nanomolar concentrations of orthophosphate in seawater, J. Oceanogr. 64 (2008) 443–449.
[42] J. Ma, D. Yuan, Y. Liang, Sequential injection analysis of nanomolar soluble reactive phosphorus in seawater, Mar. Chem. 111 (2008) 151–159.
[43] F.-E. Legiret, V.J. Sieben, E.M.S. Woodard, S.K.A.K. Bey, M.C. Mowlem, D.P. Connelly, et al., A high performance microfluidic analyser for phosphate measurements in marine waters using the vanadomolybdate method, Talanta 116 (2013) 382–387.
[44] J. Jońca, V.L. Fernández, D. Thouron, A. Paulmier, M. Graco, V. Garçon, Phosphate determination in seawater: toward an autonomous electrochemical method, Talanta 87 (2011) 161–167.
[45] J. Jońca, W. Giraud, C. Barus, M. Comtat, N. Striebig, D. Thouron, et al., Reagentless and silicate interference free electrochemical phosphate determination in seawater, Electrochim. Acta 88 (2013) 165–169.
[46] L.J. Gimbert, P.J. Worsfold, Environmental applications of liquid-waveguide-capillary cells coupled with spectroscopic detection, Trends Anal. Chem. 26 (2007) 914–930.
[47] R.N.M.J. Páscoa, I.V. Tóth, A.O.S.S. Rangel, Review on recent applications of the liquid waveguide capillary cell in flow based analysis techniques to enhance the sensitivity of spectroscopic detection methods, Anal. Chim. Acta 739 (2012) 1–13.
[48] D.M. Karl, G. Tien, MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments, Limnol. Oceanogr. 37 (1992) 105–116.
[49] Y. Liang, D. Yuan, Q. Li, Q. Lin, Flow injection analysis of nanomolar level of orthophosphate in seawater with solid phase enrichment and colorimetric detection, Mar. Chem. 103 (2007) 122–130.
[50] P. Griess, Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt Ueber einige Azoverbindungen, Berichte der deutschen chemischen Gesellschaft 12 (1879) 426–428.
[51] S. Feng, M. Zhang, Y. Huang, D. Yuan, Y. Zhu, Simultaneous determination of nanomolar nitrite and nitrate in seawater using reverse flow injection analysis coupled with a long path-length liquid waveguide capillary cell, Talanta 117 (2013) 456–462.
[52] G. Chen, D. Yuan, Y. Huang, M. Zhang, M. Bergman, In-field determination of nanomolar nitrite in seawater using a sequential injection technique combined with solid phase enrichment and colorimetric detection, Anal. Chim. Acta 620 (2008) 82–88.
[53] M. Zhang, D. Yuan, Y. Huang, G. Chen, Z. Zhang, Sequential injection spectrophotometric determination of nanomolar nitrite in seawater by on-line preconcentration with HLB cartridge, Acta Oceanol. Sin. 29 (2010) 100–107.
[54] M. Zhang, D. Yuan, G. Chen, Q. Li, Z. Zhang, Y. Liang, Simultaneous determination of nitrite and nitrate at nanomolar level in seawater using on-line solid phase extraction hyphenated with liquid waveguide capillary cell for spectrophotometric detection, Microchim Acta 165 (2009) 427–435.
[55] V.J. Sieben, C.F.A. Floquet, I.R.G. Ogilvie, M.C. Mowlem, H. Morgan, Microfluidic colourimetric chemical analysis system: application to nitrite detection, Anal, Methods 2 (2010) 484–491.
[56] A.D. Beaton, V.J. Sieben, C.F.A. Floquet, E.M. Waugh, S.A.K. Bey, I.R.G. Ogilvie, et al., An automated microfluidic colourimetric sensor applied *in situ* to determine nitrite concentration, Sens. Actuators B 156 (2011) 1009–1014.
[57] A.D. Beaton, C.L. Cardwell, R.S. Thomas, V.J. Sieben, F.-E. Legiret, E.M. Waugh, et al., Lab-on-chip measurement of nitrate and nitrite for in situ analysis of natural waters, Environ. Sci. Technol. 46 (2012) 9548–9556.
[58] H. Kodamatani, S. Yamazaki, K. Saiton, Y. Komatsu, T. Tomiyasu, Rapid method for simultaneous determination of nitrite and nitrate in water samples using short-column ion-pair chromatographic separation, photochemical reaction, and chemiluminescence detection, Anal. Sci. 27 (2011) 187–192.
[59] E. Pagliano, M. Onor, E. Pitzalis, Z. Mester, R.E. Sturgeon, A. D'Ulivo, Quantification of nitrite and nitrate in seawater by triethyloxonium tetrafluoroborate derivatization-Headspace SPME GC-MS, Talanta 85 (2011) 2511–2516.
[60] E. Pagliano, J. Meija, R.E. Sturgeon, Z. Mester, A. D'Ulivo, Negative chemical ionization GC/MS determination of nitrite and nitrate in seawater using extract matching double spike isotope dilution and derivatization with triethyloxonium tetrafluoroborate, Anal. Chem. 84 (2012) 2592–2596.
[61] M.A. Brzezinski, Colorimetric determination of nanomolar concentrations of ammonium in seawater using solvent extraction, Mar. Chem. 20 (1987) 277–288.
[62] D.R. Clark, T.W. Fileman, I. Joint, Determination of ammonium regeneration rates in the oligotrophic ocean by gas chromatography/mass spectrometry, Mar. Chem. 98 (2006) 121–130.
[63] G. Chen, M. Zhang, Z. Zhang, Y. Huang, D. Yuan, On-line solid phase extraction and spectrophotometric detection with flow technique for the determination of nanomolar level ammonium in seawater samples, Anal. Lett. 44 (2011) 310–326.
[64] Q.P. Li, J.-Z. Zhang, F.J. Millero, D.A. Hansell, Continuous colorimetric determination of trace ammonium in seawater with a long-path liquid waveguide capillary cell, Mar. Chem. 96 (2005) 73–85.
[65] R.D. Jones, An improved fluorescence method for the determination of nanomolar concentrations of ammonium in natural waters, Limnol. Oceanogr. 36 (1991) 814–819.
[66] R.T. Masserini Jr., K.A. Fanning, A sensor package for the simultaneous determination of nanomolar concentrations of nitrite, nitrate, and ammonia in seawater by fluorescence detection, Mar. Chem. 68 (2000) 323–333.
[67] R.J. Watson, E.C.V. Butler, L.A. Clementson, K.M. Berry, Flow-injection analysis with fluorescence detection for the determination of trace levels of ammonium in seawater, J. Environ. Monit. 7 (2005) 37–42.

[68] R. Kérouel, A. Aminot, Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis, Mar. Chem. 57 (1997) 265–275.
[69] R.M. Holmes, A. Aminot, R. Kérouel, B.A. Hooker, B.J. Peterson, A simple and precise method for measuring ammonium in marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci. 56 (1999) 1801–1808.
[70] A. Aminot, R. Kérouel, D. Birot, A flow injection-fluorometric method for the determination of ammonium in fresh and saline waters with a view to *in situ* analyses, Wat. Res. 35 (2001) 1777–1785.
[71] N. Amornthammarong, J.-Z. Zhang, Shipboard fluorometric flow analyzer for high-resolution underway measurement of ammonium in seawater, Anal. Chem. 80 (2008) 1019–1026.
[72] N. Amornthammarong, J.-Z. Zhang, P.B. Ortner, An autonomous batch analyzer for the determination of trace ammonium in natural waters using fluorometric detection, Anal, Methods 3 (2011) 1501–1506.
[73] N. Amornthammarong, J.-Z. Zhang, P.B. Ortner, J. Stamates, M. Shoemaker, M.W. Kindel, A portable analyser for the measurement of ammonium in marine waters, Environ. Sci.: Process Impacts 15 (2013) 579–584.
[74] C. Frank, F. Schroeder, Using sequential injection analysis to improve system and data reliability of online methods: determination of ammonium and phosphate in coastal waters, J. Autom. Methods Manag. Chem. 2007 (2007) 49535. Article ID , 6 pages.
[75] S.K.A.K. Bey, D.P. Connelly, F.-E. Legiret, A.J.K. Harris, M.C. Mowlem, A high-resolution analyser for the measurement of ammonium in oligotrophic seawater, Ocean Dyn. 61 (2011) 1555–1565.
[76] B. Horstkotte, C.M. Duarte, V. Cerdà, A miniature and field-applicable multipumping flow analyzer for ammonium monitoring in seawater with fluorescence detection, Talanta 85 (2011) 380–385.
[77] C.-T. Kuo, P.-Y. Wang, C.-H. Wu, Fluorometric determination of ammonium ion by ion chromatography using postcolumn derivatization with *o*-phthaldialdehyde, J. Chromatogr. A 1085 (2005) 91–97.
[78] P. Poulin, É. Pelletier, Determination of ammonium using a microplate-based fluorometric technique, Talanta 71 (2007) 1500–1506.
[79] Y. Zhu, D. Yuan, Y. Huang, J. Ma, S. Feng, A sensitive flow-batch system for on board determination of ultra-trace ammonium in seawater: method development and shipboard application, Anal. Chim. Acta 794 (2013) 47–54.
[80] S.W. Gibb, R.F.C. Mantoura, P.S. Liss, Analysis of ammonia and methylamines in natural waters by flow injection gas diffusion coupled to ion chromatography, Anal. Chim. Acta 316 (1995) 291–304.
[81] P.-Y. Wang, J.-Y. Wu, H.-J. Chen, T.-Y. Lin, C.-H. Wu, Purge-and-trap ion chromatography for the determination of trace ammonium ion in high-salinity water samples, J. Chromatogr. A 1188 (2008) 69–74.
[82] J.N. Plant, K.S. Johnson, J.A. Needoba, L.J. Coletti, NH4-Digiscan: an in situ and laboratory ammonium analyzer for estuarine, costal, and shelf waters, Limnol. Oceanogr.: Methods 7 (2009) 144–156.
[83] S.W. Willason, K.S. Johnson, A rapid, highly sensitive technique for the determination of ammonia in seawater, Mar. Bio. 91 (1986) 285–290.
[84] A. Aminot, D.S. Kirkwood, R. Kérouel, Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C 5 questionnaire, Mar. Chem. 56 (1997) 59–75.
[85] S.-C. Pai, Y.-J. Tsau, T.-I. Yang, pH and buffering capacity problems involved in the determination of ammonia in saline water using the indophenol blue spectrophotometric method, Anal. Chim. Acta 434 (2001) 209–216.
[86] T. Shoji, E. Nakamura, Collection of indonaphthol blue on a membrane filter for the spectrophotometric determination of ammonia with 1-naphthol and dichloroisocyanurate, Anal. Sci. 26 (2010) 779–783.
[87] G. Zhang, P.K. Dasgupta, Fluorometric measurement of aqueous ammonium ion in a flow injection system, Anal. Chem. 61 (1989) 408–412.
[88] N. Amornthammarong, J.-Z. Zhang, Liquid-waveguide spectrophotometric measurement of low silicate in natural waters, Talanta 79 (2009) 621–626.
[89] J. Ma, R.H. Byrne, Flow injection analysis of nanomolar silicate using long path-length absorbance spectroscopy, Talanta 88 (2012) 484–489.
[90] M.A. Brzezinski, D.M. Nelson, A solvent extraction method for the colorimetric determination of nanomolar concentrations of silicic acid in seawater, Mar. Chem. 19 (1986) 139–151.
[91] P. Rimmelin-Maury, T. Moutin, B. Queguiner, A new method for nanomolar determination of silicic acid in seawater, Anal. Chim. Acta 587 (2007) 281–286.
[92] A. Hioki, J.W.H. Lam, J.W. McLaren, On-line determination of dissolved silica in seawater by ion exclusion chromatography in combination with inductively coupled plasma mass spectrometry, Anal. Chem. 69 (1997) 21–24.
[93] H.-B. Li, F. Chen, Determination of silicate in water by ion exclusion chromatography with conductivity detection, J. Chromatogr. A 874 (2000) 143–147.
[94] GEOTRACES standards and intercalibration committee, Sampling and sample-handling protocols for GEOTRACES cruises, Version 1.0, 2010. Available from: http://www.geotraces.org/libraries/documents/Intercalibration/Cookbook.pdf, accessed March 11, 2014.
[95] M.D. Patey, E.P. Achterberg, M.J.A. Rijkenberg, P.J. Statham, M. Mowlem, Interferences in the analysis of nanomolar concentrations of nitrate and phosphate in oceanic waters, Anal. Chim. Acta 673 (2010) 109–116.
[96] I.D. McKelvie, D.M.W. Peat, G.P. Matthews, P.J. Worsfold, Elimination of the Schlieren effect in the determination of reactive phosphorus in estuarine waters by flow-injection analysis, Anal. Chim. Acta 351 (1997) 265–271.