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Measurement of methylamines in seawater using solid phase microextraction and gas chromatography

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Abstract

The methylamines form part of the marine organic nitrogen pool. Although they are ubiquitous in marine systems, little is known about their distribution, production and fate in the marine environment. Analytical methods have been developed for their analysis in marine waters, but these have employed custom-made apparatus, limiting data to a few studies. Here, we report a method developed for the determination of methylamines in seawater which combines headspace solid phase micro-extraction (SPME) and gas chromatography with selective nitrogen detection. Gaseous methylamines were pre-concentrated from water samples (approximately 1 L) at elevated temperature, onto a polydimethylsiloxane/divinylbenzene SPME fibre. The analytes were then thermally desorbed from the fibre in a gas chromatograph containing a CP-Volamine column and a nitrogen-phosphorus detector. The method was routinely calibrated down to 1 nM for all three analytes using matrix-matched external standards. Limits of detection were determined operationally and ranged from 0.4-2.9 nM during two sampling campaigns. The analytes were detected in seawater samples from the English Channel and Southern Ocean where concentrations up to 20 and 5.6 nM were measured, respectively, consistent with previously reported levels. We propose this method for the analysis of methylamines, and other low molecular weight amines, as it is sensitive, robust, and the necessary equipment is widely-available.

Introduction

Nitrogen is a dynamic element in marine systems. It is primarily used as a nutrient by microorganisms, but may also have a role in climate chemistry. Nitrogen-containing osmolytes (N-osmolytes) such as glycine betaine, trimethylamine-N-oxide and choline are produced by phytoplankton to maintain osmotic pressure (Yancey 2005 and Burg 2008); these N-osmolytes can degrade to produce methylamines (Jameson et al. 2016; King 1984; Oremland et al. 1982; Oren 1990). Recently, marine bacteria have been shown to use methylamines as a source of energy and remineralise the nitrogen to ammonium (Lidbury et al. 2015). Further, methylamines are required for a bacterial conversion of the climate relevant sulfur gas dimethyl sulfide to dimethylsulfoxide (Lidbury et al. 2016), firmly establishing them as important components in biogeochemical cycles. The methylamines (MAs) are mono-, di- and trimethylamine (MMA, DMA and TMA, respectively); they are analogues of ammonia with the formula $(\text{CH}_3)_n\text{NH}_{3-n}$. The MAs are ubiquitous in the marine environment, where their reported concentrations vary from micromolar to nanomolar levels in estuarine and oceanic environments, respectively (Fitzsimons et al. 2006; Gibb et al. 1999a). They comprise a small percentage of marine organic nitrogen (Burdige et al. 1995; Lee and Olsen 1984) but can diffuse across the sea-air interface where they may affect atmospheric chemistry as a source of base to the atmosphere, influencing cloud formation (Dall'Osto et al. 2017). Indeed, a recent study demonstrated that atmospheric concentrations of DMA above 65 nmol m^{-3} by volume could enhance particle-formation more than 1,000-fold compared with ammonia, sufficient to account for observed atmospheric particle-formation rates (Almeida et al., 2013). Methylamines are therefore relevant for atmospheric processes as well as biogeochemical cycles.

Analysis of the MAs is challenging due to their polarity, aqueous solubility, volatility and existence at low concentrations in marine environments. Derivatization of the

methylamines to permit their analysis by gas and liquid chromatography has been reported, but methods involved either conversion of TMA to DMA (daCosta et al. 1990) or could not resolve DMA and TMA (Herráez-Hernández et al. 2006; Parshintsev et al. 2015), rendering them unsuitable for the analysis of all three analytes. Methods designed for the analysis of methylamines from marine waters and sediments have exploited the MAs' volatility to facilitate their pre-concentration and measurement (Abdul-Rashid et al. 1991; Yang et al. 1993; Gibb et al. 1995). The technique reported by Abdul-Rashid et al. (1991) involved a pre-concentration step carried out in a specially-adapted Quikfit flask (Cavett flask), with analysis by gas chromatography and nitrogen-phosphorus detection (GC-NPD). Limits of detection achieved using this method were 2-12 nM which, while appropriate for estuarine water and sediment pore-waters (Fitzsimons et al. 2005; Fitzsimons et al. 2006), were likely to be too high for coastal and oceanic waters (Gibb et al. 1995; Gibb et al. 1999a). Gibb et al. (1995; 1999a) employed a coupled flow injection-gas diffusion-ion chromatography system, which was deployed on-board ship to collect the first extensive MA datasets reported for marine waters. However, despite scientific drivers to understand the sources, sinks and distribution of MAs in seawater, this method, and a similar approach from Yang et al. (1993), have not been adopted by the oceanographic community as they require custom-made, engineered apparatus.

Solid phase micro-extraction (SPME) is a simple sample preparation technique which eliminates the need for solvents or complicated apparatus for concentrating volatile or non-volatile compounds from gaseous, liquid or headspace samples (Arthur and Pawliszyn 1990). SPME has been used to measure organic compounds in water and air and is compatible with gas chromatography. It provides a linear response over a wide concentration range (Arthur et al. 1992a; Arthur et al. 1992b) and has been used to measure MAs in wastewater (Abalos et al. 1999), though with high limits of detection (186-869 nM). It has also been used for the

measurement of marine trace gases, including dimethylsulfide (Niki et al. 2004; Vogt et al. 2008; Yassaa et al. 2006).

The aim of this study was to develop a sensitive method for the analysis of MAs in coastal and oceanic waters using widely-available consumables and instrumentation. The SPME technique was tested in order to achieve experimental conditions suitable for the trace measurement of MAs in seawater (Gibb and Hatton 2004; Gibb et al. 1995; Gibb et al. 1999a; Gibb et al. 1999b). The method employed headspace SPME and GC-NPD for analyte pre-concentration and measurement, respectively, and was then used to measure MAs in water samples collected from the English Channel and during a research cruise in the Southern Ocean.

Materials and procedures

Chemicals

Trimethylamine hydrochloride (98%, CAS number 75-50-3), dimethylamine hydrochloride (99%, CAS number 124-40-3), and monomethylamine hydrochloride (99%, CAS number 74-89-5) were purchased from Acros Organics. Sodium chloride (99.5%, CAS number 7647-14-5, analytical reagent grade), sodium hydroxide (AR, CAS number 1310-73-2, analytical reagent grade) and hydrochloric acid (37 %, extra pure; CAS number 7647-01-0) were purchased from Fisher Scientific.

SPME Equipment

The SPME unit comprised a length of fused-silica fibre coated with a polymer, which was bonded to a stainless steel plunger and installed in a holder resembling a modified microliter syringe. The plunger moved the fibre out of a hollow needle and the unit functioned by passing the needle through the septum sealing the flask containing the water sample, then exposing the fibre to the headspace. Following extraction, the needle was introduced into the GC injector using an SPME adaptor, the fibre exposed and the analytes thermally desorbed. The amount of analyte adsorbed by the fibre depended on the thickness of the polymer coating and on the distribution constant for the analyte.

Cleaning procedure

All glassware and plasticware were rinsed with high purity water (HPW) at 18.2 M Ω cm resistivity (Millipore) then soaked in a low nutrient, 1 % detergent solution (Nutracon) for 24 h. Following rinsing with HPW, items were soaked in hydrochloric acid (10 % v/v) for 24 h then rinsed with HPW. Cleaned items were double-bagged in clean polyethylene bags and stored until use.

Sample collection

Surface seawater was sampled at station L4 of the Western Channel Observatory (<http://www.westernchannelobservatory.org.uk/14/>), 10 km offshore in the Western English Channel, from the non-toxic supply aboard the R/V Plymouth Quest. A 10 L sample was collected from which sub-samples of 1 L were dispensed into high density polyethylene (HDPE) bottles, then immediately gravity filtered using 47 mm GF/F filters (0.7 μm pore size) into 1 L HDPE bottles containing 10 mL HCl (37 %) to immediately quench biological activity and shift the MAs equilibrium in favour of the non-volatile, protonated form. Water samples from the Southern Ocean were collected on-board BIO Hespérides at a depth of 4 m using a rosette sampler, which was sub-sampled using 1 L HDPE bottles, filtered into HDPE bottles containing 10 mL HCl then stored at 4 °C until analysis.

SPME

A water sample (0.85 L) was transferred to the volumetric flask then amended with NaCl, which increased the volume to 0.98 L. The solution was then adjusted to pH 13 with 10 M NaOH solution (20 mL) and heated (60 °C), with stirring, for the extraction period (time intervals between 0.5 and 12 hours were tested and a 2.5 hour extraction window chosen, described in detail below). Constant temperature was achieved by immersing the flask in a water-bath comprising a aluminium foil-wrapped Pyrex beaker on a stirrer hotplate (Figure 1). The needle assembly was then clamped in position and inserted into the headspace of the volumetric flask, through the Subaseal. Once in position, the fibre was exposed to the headspace and the exposed neck of the volumetric flask was wrapped in aluminium foil.

The analytical method was calibrated using external, matrix-matched standard solutions containing the three MAs, in accordance with manufacturer guidance (Supelco 2001). Additional seawater was collected to make up matrix-matched standards and prepared by

adjusting it to pH 13, followed by purging with nitrogen gas for 6 hours to remove background MAs and other volatiles. Calibration solutions were prepared by adding NaCl (30% w/v) and MAs (range 0.1-10 nM). Matrix blank extractions were carried out regularly using purged seawater. This also served to ensure there was no contamination from the NaCl and NaOH additions.

Gas chromatography

The MAs were measured using an Agilent 6890 GC fitted with a Agilent CP-Volamine column (part number: CP7447, dimensions 60 m × 0.32 mm) and a NPD. Base-deactivated SPME inlet liners and predrilled septa (Sigma Aldrich) were used in the GC injector. The injector temperature was set to 270 °C and, once injected, the fibre was exposed for 5 minutes; GC conditions are shown in Table 1. The SPME needle depth in the injector was set to 40 mm and a SPME inlet guide was used to prevent contact between the fibre and injector liner, which would degrade the former. The cleanliness of the fibre was monitored throughout the analytical day by injecting it as described above at regular intervals without prior sample extraction. Specifically, the unextracted fibre was injected at the beginning and end of the analytical day and between sample extractions. Data were recorded using Agilent ChemStation software, Version B.03.01.

Limits of detection

Limits of detection were calculated according to Equation 1 (ICH 1996)

$$LoD = 3.3\sigma/S \quad (1)$$

Where σ is the standard deviation of the peak area response calculated from the lowest concentration standard solution, and S is the linear slope of the calibration curve.

Assessment and discussion

GC-NPD as a separation and detection method for MAs

Aqueous injections of the MAs were used to establish chromatographic separation using the CP-Volamine column, and linearity of response to the analytes by GC-NPD. The analytes eluted in the order MMA, DMA, TMA, reflecting relative basicity of the gaseous species (Clayden et al. 2001), and increasing compound interaction with the stationary phase.

Initial tests showed that the CP-Volamine column provided adequate retention, with the three MAs eluting between 11 and 13 minutes. However, the chromatography was poor, with significant tailing causing co-elution of TMA and DMA. MMA gave particularly poor chromatography with two peaks observed. There was also evidence of multiple peaks for both DMA and TMA.

With partial resolution achieved using the CP Volamine column, the use of sodium hydroxide to address adsorption problems was investigated. Addition of NaOH to the sample matrix (water) significantly improved both the chromatography and resolution of the three MAs. A mixed standard sample was injected and three resolved peaks were evident with MMA, DMA and TMA eluting between 8-11 minutes (Figure 2). TMA gave a strong Gaussian peak, while the DMA and MMA peaks tailed slightly without co-eluting. The MAs' response was linear between 0.5 - 10 mmol dm⁻³ ($R^2 = 0.98 - 0.99$).

Solid phase micro-extraction: Fibre selection

SPME has historically been applied to small sample volumes (< 20 mL), based on this fibre performance was assessed using a test sample of 15 mL volume containing 3.2 μM of each of the three MAs (approximately equivalent to a 0.2 μL injection of a 100 mM standard solution in splitless mode). Three SPME fibre-coatings (Sigma-Aldrich, UK) were tested: polyacrylate, carboxen/polydimethylsiloxane (carboxen/PDMS) and polydimethylsiloxane/divinylbenzene

(PDMS/DVB). The polyacrylate fibre (df 85 μm , partially crosslinked phase, needle size 24 ga, product number 57304) was designed for polar semi-volatiles with a relative molecular mass (RMM) range of 80-300 Daltons, the Carboxen/PDMS fibre (df 85 μm , needle size 24 ga, StableFlex fiber, product number 57334-U) was designed for gas analysis and has a RMM range of 30-225 Daltons, and the PDMS/DVB fibre (df 65 μm , needle size 24 ga, StableFlex, product number 57326-U) was designed for volatiles and amines and had a RMM range of 50-300 Daltons. The coating thickness was 65 μm in accordance with manufacturer guidelines advising maximum film thickness for extraction of the most volatile amines. MAs were not detected using the polyacrylate fibre, likely because the analytes were polar, highly volatile and outside the recommended molecular weight range for this fibre (80-300 Daltons). The carboxen/PDMS fibre did produce MA-peaks; however, multiple peaks were observed and chromatography was poor. Though the desorption temperature (270°C) is below the optimal temperature for this fibre, it is the maximum operating temperature for the CP Volamine column. This precludes the use of the carboxen/PDMS fibre. Peaks for all three MAs were observed using the PDMS/DVB fibre (65 μm) despite MMA being outside the RMM range specified. The PDMS/DVB fibre was selected for subsequent method development.

Extraction parameters

SPME is influenced by a number of variables, including pH, ionic strength and temperature (Risticvic et al. 2010). Amines have previously been pre-concentrated in aquatic samples by adjusting the water sample to $\text{pH} \geq 12$ to convert the MAs to their gaseous forms (Abdul-Rashid et al. 1991; Gibb et al. 1995; Yang et al. 1993). In this study, samples were adjusted to pH 13 with NaOH solution prior to extraction. Although precipitation of magnesium hydroxide occurs above pH 12, this does not affect MA recovery (Abdul-Rashid et al. 1991; Gibb et al. 1995; Yang et al. 1993). In addition to volatilization of the analytes, the high pH is known to reduce

ionic interactions between basic compounds protonated on the nitrogen atom and charged functional groups, such as within dissolved organic matter, as the former are converted to gaseous form at pH 13 (Jones, 2005).

Elevated temperature increases headspace capacity and/or the analyte diffusion coefficient, leading to an increase in extraction rate and mass transfer onto the fibre coating (Risticvic et al. 2010). A temperature of 60 °C was selected for this method, based on data reported from static diffusion studies of MAs from water samples to headspace. These techniques achieved recoveries of 83-100 % at 60 °C (Dawit 2006; Fitzsimons et al. 2005; Fitzsimons et al. 2001; Fitzsimons et al. 2006; Yang et al. 1993).

Extraction of MAs in seawater

A number of parameters were considered to ensure that extraction conditions were reproducible, including sample and headspace volumes. A 1 L volumetric flask, sealed with a Subaseal, served as the extraction vessel to obtain a minimal but consistent headspace volume (Zhang and Pawliszyn 1993), while ensuring that the fibre and sample did not come into contact. The phase ratio between the headspace and the sample was optimized to ensure the headspace was small enough to concentrate the gaseous MAs but large enough to accommodate the SPME fibre. It was also necessary to take into account the thermal expansion of the seawater matrix during extraction. The headspace volume of the flask was approximately 0.045 dm³; it was consistent between samples and slight variations in the water volume (which were accounted for) were permitted to achieve this. Heating was performed using a water bath large enough to heat the flask and water sample while excluding the headspace.

Headspace SPME establishes two equilibria: between the water sample and headspace, and between the headspace and fibre. To achieve acceptable reproducibility, both processes had to reach steady-state during the extraction; otherwise small changes in the extraction time

could reduce the amount of analyte extracted (Arthur and Pawliszyn 1990). Four extraction times were tested to confirm that equilibration between the headspace and fibre could be achieved within a reasonable time period, ranging from a typical SPME extraction time of 0.5 h (Risticvic et al. 2010) to 12 h (Figure 3). Extractions were performed in the following order: 2.5, 6, 0.5 and 12 hours. Six extractions were performed at each time interval. Duplicate fibres were used ($n = 3$ per fibre) so that inter-fibre variation could be assessed. The effect of extraction time on MA concentration (Figure 3) was statistically analysed using one-way ANOVA (Table 2), while performance of separate fibres was assessed using a Student's t-test. For MMA and DMA, significantly more analyte was extracted after 2.5 h compared with 0.5 h ($P = 0.018$ and 0.003 , respectively), while no significant difference was observed for TMA. There was no significant difference in the amount of analyte extracted between 2.5 and 6 h for any of the MAs. An extraction time of 12 h extracted significantly more MMA and TMA compared with 2.5 ($P = 0.002$ and < 0.001 , respectively) and 6 h ($P = 0.009$ and < 0.001 , respectively), while no significant difference was observed for DMA (Table 2). The inter-fibre comparison for all extraction times did not reveal a significant difference between the amount of analyte extracted per fibre. As SPME is not used to measure absolute recovery, reproducible measurement of analytes, and sample throughput, were used to choose the optimum extraction time. Although significantly more MMA and TMA was extracted after 12 h, compared to 2.5 h or 6 h (Table 2), this extraction time, while potentially valuable, was impractical for ship-based research where rapid sample throughput is needed. No significant difference was observed between 2.5 h and 6 h, however, so the 2.5 h time interval was selected as the most practical SPME extraction time giving acceptable precision, a key parameter for SPME (Risticvic et al 2010). The results demonstrate that the extraction had reached equilibrium at 2.5 hours, as SPME is an equilibrium technique, reaching an equilibrium position is more important than increasing the concentration of MAs extracted. Once equilibrium is reached, it

is unlikely that additional extraction time would yield statistically higher concentrations. Absolute recovery is not a quality indicator for SPME, but rather acceptable precision using a fixed set of conditions. The extraction time chosen was, therefore, not based solely on the amount of analyte extracted but being able to achieve acceptable calibrations, limits of detection and sample throughput. Although the data in Figure 3 show a large degree of variation, the measurements were made early in the method development process and, with time and practice, variability decreased. This is discussed in more detail in the Method Calibration section.

GC-NPD as a separation and detection method for MAs extracted by SPME

The successful separation and quantification of the MAs using GC-NPD was adapted for use with SPME. Due to the headspace nature of analysis, pH modification with sodium hydroxide was not possible.

The analytes eluted in the order MMA, DMA, TMA, reflecting the relative basicity of the gaseous species. Analyte peaks were consistently absent from the matrix blanks using both HPW and treated seawater. A chromatogram of a SPME-extracted 10 nM standard solution made up in seawater (Fig. 4) and a sample measured from station L4 (Western English Channel) shows the MAs eluting between 8 and 12 minutes. Although peaks exhibited tailing, and complete resolution was not achieved, peaks were reproducible, and consistent integration of all peaks was achieved.

During GC-NPD analysis of SPME-extracted MAs, two peaks were identified for MMA, which was unexpected (Figure 4). A possible explanation is the absence of pH modification, as MMA chromatography was improved by adjusting the pH of aqueous samples to 13 immediately before injection. As analysis with SPME precludes pH modification, this may have affected the MMA peak shape. pH modification reduces the interaction of the MAs

with silanol groups and siloxane bridges in the column, this is particularly important for the low molecular weight MMA where the nitrogen lone pair has the greatest impact upon its chromatographic behavior. The double peak may also be due to a slow injection speed of the SPME fibre in combination with the low boiling point of the MMA. The short retention time of the MMA means it is unlikely to reconstitute at the head of the GC column. This means that the desorption time from the fiber will dictate that shape of the peak on the chromatogram. The most likely reason for the double peak is a delay in exposure of the SPME fibre from the sheathing needle once inside the GC inlet. This gives an apparent double desorption, firstly, when the analyte instantly evaporates off of the fibre whilst inside the needle and secondly, when the plunger assembly is pushed and the fibre is exposed from the sheathing needle. However, the analyte could still be measured quantitatively. As the two peaks appeared consistently within the MMA retention time window, and were quantifiable, both peak areas were included in calculations.

The response of a NPD is proportional to the carbon content of the analyte (Skoog and Leary 1992). For the MAs this increases in the order MMA < DMA < TMA, and these relative responses were confirmed by direct injection of aqueous MA standard solutions. However, SPME extractions of standard MA solutions produced a DMA peak which was consistently larger than that for TMA, indicating that partitioning onto the fibre was more extensive for the former. As gaseous TMA is more basic than both DMA and MMA, a greater affinity for the fibre coating by the former was expected. However, there is a structural necessity for the nitrogen atom in amines to be approximately tetrahedral (sp^3) in order to bond effectively through sorption using its lone pair; for TMA, the 3 methyl substituents of the nitrogen atom may force an increase in the bond angles to a trigonal planar structure. This would leave the lone pair in a pure p -orbital, reducing its effectiveness for basic interactions (Huheey 1983) and presenting as reduced affinity for the fibre. The interconversion of chiral, tertiary amines

between the R- and S-enantiomers is further evidence of the ability of TMA to adopt a less-basic structure (Solomons and Fryhle 2000).

One fibre could be used for up to 200 extractions before signs of degradation, such as greying or crumbling, became visible. Regular replacement of the SPME consumables for injection onto the GC is recommended to optimize fibre performance and lifetime and the SPME-adapted liner and septum were replaced at least every two weeks, corresponding to approximately 50 injections. Fibre lifetime was extended by the use of headspace sampling, which avoids exposure of the fibre to a high pH solution. As the method was calibrated using matrix-matched external standards, full calibrations were performed prior to analyses of all sample sets and frequent check standards analysed to monitor fibre performance, peak areas and extraction reproducibility. Depending on capacity, up to nine samples could be extracted per day, assuming triplicate fibres were used simultaneously.

Method calibration

Calibrations were performed at 1-10 nM in order to assess the method within the expected working range, based on previously reported measurements of MAs in marine waters (Table 3), using standard solutions prepared in pre-purged seawater (Figure 5). A strong correlation to response was achieved, with R^2 values of 0.968 (MMA), 0.987 (DMA) and 0.987 (TMA), and RSDs of 6-14, 8-13 and 2-7 % for MMA, DMA and TMA, respectively (Figure 4). The calibration range was further extended whilst on-board ship during the PEGASO cruise in the Southern Ocean (0.13-13.30 nM), yielding R^2 values of 0.99-1 (Fig. 6).

Calibrations carried out in matrices prepared from HPW, coastal water collected from station L4 and oceanic water collected in the Southern Ocean gave different calibration slopes and x-axis intercepts. This indicates that the sample matrix will have an impact on the extraction efficiency and underlines the need to carry out matrix matched calibrations.

The impact of user variability was also considered; initial experiments carried out showed high variability with relative standard deviations of 31 - 60 %. With time and practice the range decreased to 2 - 12 %. This highlights the need for consistency of approach when carrying out extractions, to optimize precision.

The extrapolation of regression lines for all of the analytes typically resulted in non-zero intercepts (Fig. 5). This is potentially a result of MA analyte being present in the samples but at lower concentrations than the NPD is capable of detecting. However, the calibration was linear in the defined working range; this highlights the importance of defining operational calibration parameters for each sampling event and for the seawater matrix used, as our data suggest that this impacts on the calibration slope and y-axis intercept. Calibration data and graphs are often missing from published research using SPME, but non-zero intercepts have been reported by the manufacturer for a range of analytes using the external standard approach (Supelco 2001). Additionally, calibrations for derivatised amines (Parshintsev et al. 2015) and methylhalides (Yassaa et al. 2009) pre-concentrated by SPME also yielded non-zero intercepts.

Limits of Detection

Limits of detection (LoDs) calculated during field sampling were 0.48-1.88, 1.24-2.91 and 0.38-0.89 nM for MMA, DMA and TMA, respectively. These LoDs were comparable to those previously reported (Table 3) using custom-made equipment (Gibb and Hatton 2004; Gibb et al. 1995; Gibb et al. 1999b), and the data from this study demonstrates the suitability of SPME for measurements of MAs in seawater. The range in LoDs reported demonstrates that their calculation should be a routine operational measurement, in addition to calibration, particularly if analyte concentrations are expected to be close to the LoD.

Sample preservation

Although the MAs are predominantly cationic at seawater pH, diffusion of the gaseous species may occur, making elimination of headspace in sampled water necessary. Furthermore, as low molecular weight organic nitrogen compounds are rapidly consumed by marine bacteria (Middelburg and Nieuwenhuize 2000; Wang and Lee 1995), options for sample preservation were assessed. Specifically, samples were either: 1) immediately filtered on-board ship using 47 mm GF/F filter papers (0.7 μm pore size) and extracted by SPME and analysed the same day or 2) immediately filtered on-board ship using 47 mm GF/F filter papers (0.7 μm pore size) into bottles containing 10 mL of HCl (37 %) which reduced sample pH to 1, then stored in a fridge at 4 °C for extraction by SPME the next day. Bottles were filled to capacity and sealed tightly before storage. The acid served to stop diffusion by converting MAs to cations (MAH^+), and to inhibit microbial activity.

The samples acidified post-filtration contained MA concentrations of 6, 3 and 20 nM for MMA, DMA and TMA, respectively (Table 3); in the non-acidified samples MA levels were below detection. The higher MA concentrations in samples which had been filtered and acidified suggests that the analyte concentrations were preserved by acidification. The water samples were filtered through GF/F filters (nominal pore size of 0.7 μm), which facilitates gravity filtration on a reasonable timescale but could also allow bacteria to pass through. Acidification would kill bacteria in the filtrate, while the low sample pH converts the analytes to their non-diffusing, protonated form (RNH_3^+). Acidification is, therefore, essential to the sampling protocol.

Methylamine concentrations in seawater samples

Concentrations of the MAs in the English Channel (Station L4) ranged from 3-20 nM. The MAs were also measured in water samples collected in the Southern Ocean during the PEGASO research cruise, where concentrations ranged from below detection to 6.9 nM

(Table 3). The concentrations measured for both the English Channel and the Southern Ocean (South Orkney Islands) are comparable to previously reported measurements from these regions (Gibb and Hatton 2004; Gibb et al. 1995). The MA concentrations reported by Gibb and Hatton (2004) were measured in Southern Ocean samples from the Rothera Time Series and were collected at the same time of year (Jan-Feb) as those measured for this study.

Conclusions

The MAs were detected in seawater using an analytical method that combined SPME with GC-NPD. The method is applicable to other low molecular weight amines which might be present in seawater (e.g. ethylated amines). As external calibration with matrix-matched standard solutions of MAs was used, it is important to calibrate the method regularly and monitor fibre performance as well as measuring operational limits of detection. A key advantage of the method is that it is both sensitive and deploys commercially available instrumentation and consumables. Parallel extractions can be carried out if several fibres are deployed and consistent extraction conditions are maintained. The method can be deployed at sea in challenging conditions such as those often present in the Southern Ocean.

The determination of MAs at low nM level using these commercially-available equipment and consumables can potentially facilitate methylamine analysis on a wider scale than previous analytical methods, which were appropriate but involved custom-made apparatus and instrumentation. We propose this method as a widely-accessible approach to analysing LMW amines which is both sensitive and robust, and can be easily used by oceanographers and aquatic scientists. The method will facilitate our understanding of the distribution and cycling of MAs in the marine environment.

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Figure Legends

Figure 1. A diagram of the equipment used for extraction of MAs from seawater by SPME. The Pyrex beaker and the exposed neck of the volumetric flask were wrapped in aluminium foil to efficiently maintain the water temperature at 60 °C. The water sample was stirred during the extraction and the SPME fibre was exposed in the headspace above the sample.

Figure 2. A 10 mM mixed MA standard chromatogram, analysed by GC-NPD using the CP Volamine column (aqueous injection of 1 µL; injector temperature: 270°C, detector temperature: 300°C, oven programme: 40-160°C at 10°C min⁻¹, 160-260°C at 15°C min⁻¹, 5 minute hold at 260°C, samples were pH modified to 13 with NaOH solution)

Figure 3. Average peak areas and standard deviations for MAs resulting from SPME extraction times of 0.5, 2.5, 6 and 12 h. Extractions were performed in the following order: 2.5, 6, 0.5 and 12 hours and 6 extractions were performed at each time interval. Statistical analyses are shown in Table 2.

Figure 4. Chromatograms of (a) 10 nM standard extraction below a seawater sample from Western Channel Observatory station L4, with baseline offset for comparison – the baseline for both samples was approximately 20 picoamps. (b) An expanded chromatogram of the L4 sample around the retention time of MMA. The MAs were extracted using SPME (PDMS-DVB fibre exposed to the headspace of the sample heated to 60°C for 2.5 hours) and analysed by GC-NPD. MMA regularly eluted as two peaks and as data were consistent both peaks were attributed to this analyte and quantified when they occurred.

Figure 5. Calibration graphs comprising mean MA peak areas and standard deviations at the working range used for sample analysis (n = 3 at each concentration). Seawater used to prepare calibration solutions was collected from the sampling site and treated as detailed in the text.

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Table 1. Gas chromatographic conditions used for analysis of methylamines by SPME.

Injection mode	Splitless
Carrier gas	Nitrogen (655 millibar)
Make up gas	Nitrogen (5 mL min ⁻¹)
Detector gases	Hydrogen (2 mL min ⁻¹), air (60 mL min ⁻¹)
Injector temperature	270 °C
Fibre desorption time	5 minutes
Oven temperature 1	50 °C
Hold time 1	2 minutes
Rate 1	10 °C min ⁻¹
Oven temperature 2	180 °C
Hold time 2	0 minutes
Rate 2	15 °C min ⁻¹
Oven temperature 3	260 °C
Hold time 3	5 minutes
Detector temperature	300 °C

Table 2. Statistical comparison, by one-way ANOVA, showing the significance of SPME extraction time for concentration of each MA extracted (n = 6) at the 95 % confidence level. Significant differences are indicated with an asterisk. Mean peak areas and standard deviations are displayed in Figure 2.

Amine	<i>P</i> value for extraction time comparisons			
	0.5 v 2.5 h	2.5 v 6 h	2.5 v 12 h	6 v 12 h
MMA	0.018*	0.426	0.002*	0.009*
DMA	0.003*	0.525	0.129	0.052
TMA	0.103	0.852	< 0.001*	< 0.001*

Table 3. Summary of MA concentrations and analytical figures of merit reported for marine waters.

Location	MMA (nM)	DMA (nM)	TMA (nM)	No. of samples	LoD (nM)	Reference
Plymouth Sound	4-23	13-22	0-13	3	3-5	Gibb et al. (1995)
Mediterranean Sea	4-38	3-15	4-22	30	3-5	Gibb et al. (1995)
Arabian Sea	6-22	2.9-4.2	0.05-0.81	183	0.2-2.3	Gibb et al. (1999)
Western English Channel	3	6	20	2	0.4-2.9	This study
Southern Ocean	bd-2.6	bd-5.4	1.4-6.9	6	0.9-1.9	This study

Figure 1

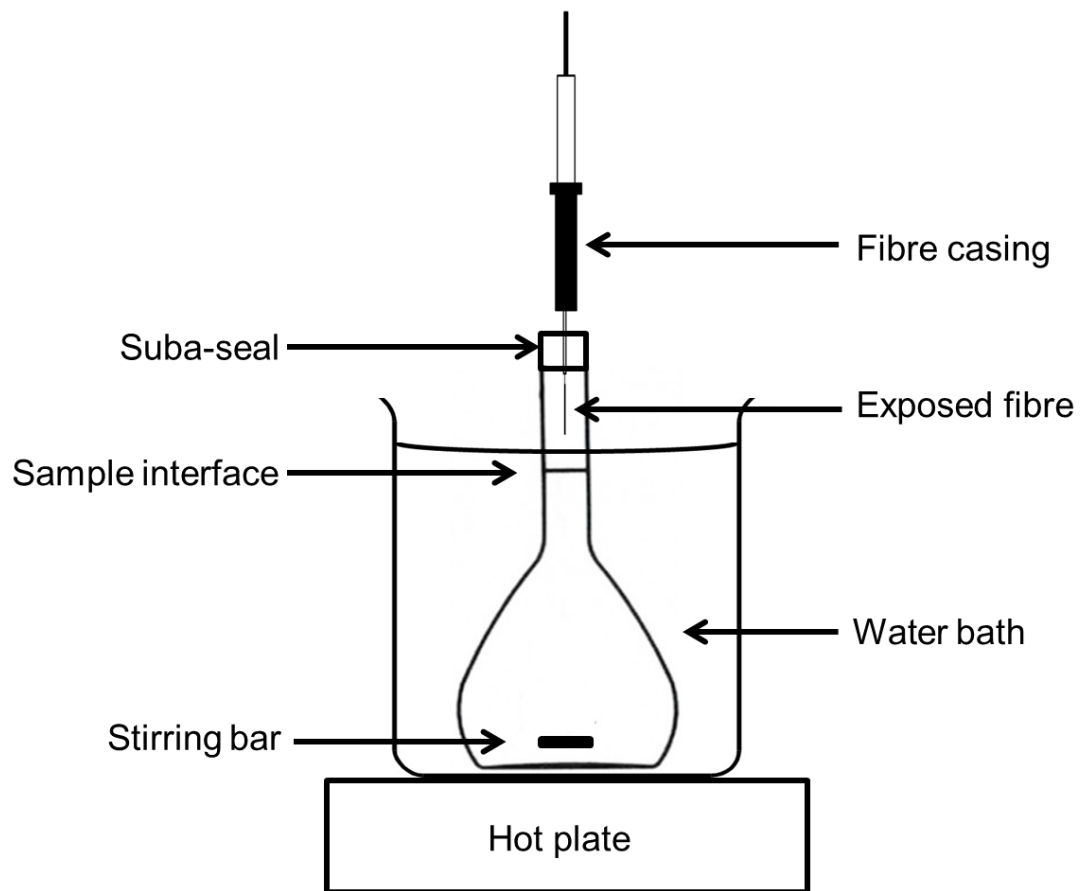


Figure 2.

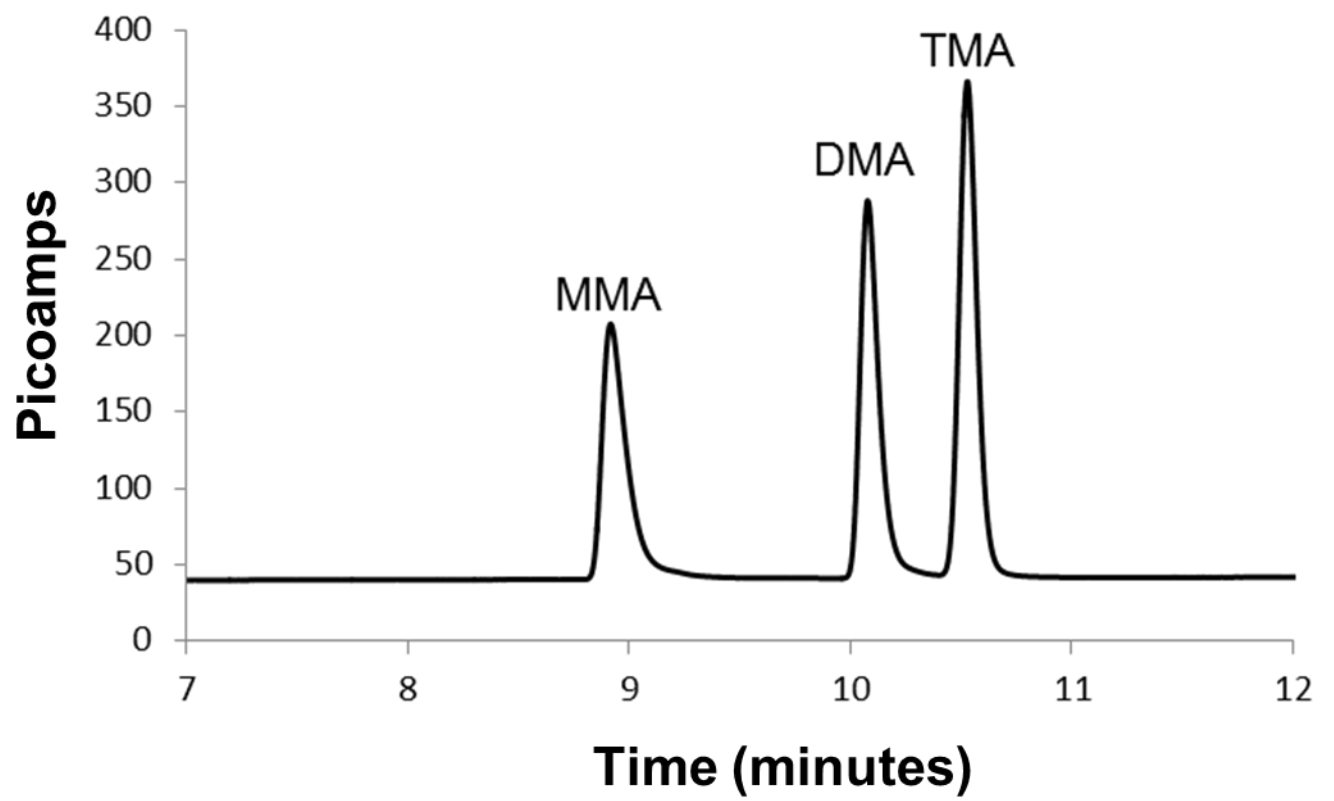


Figure 3

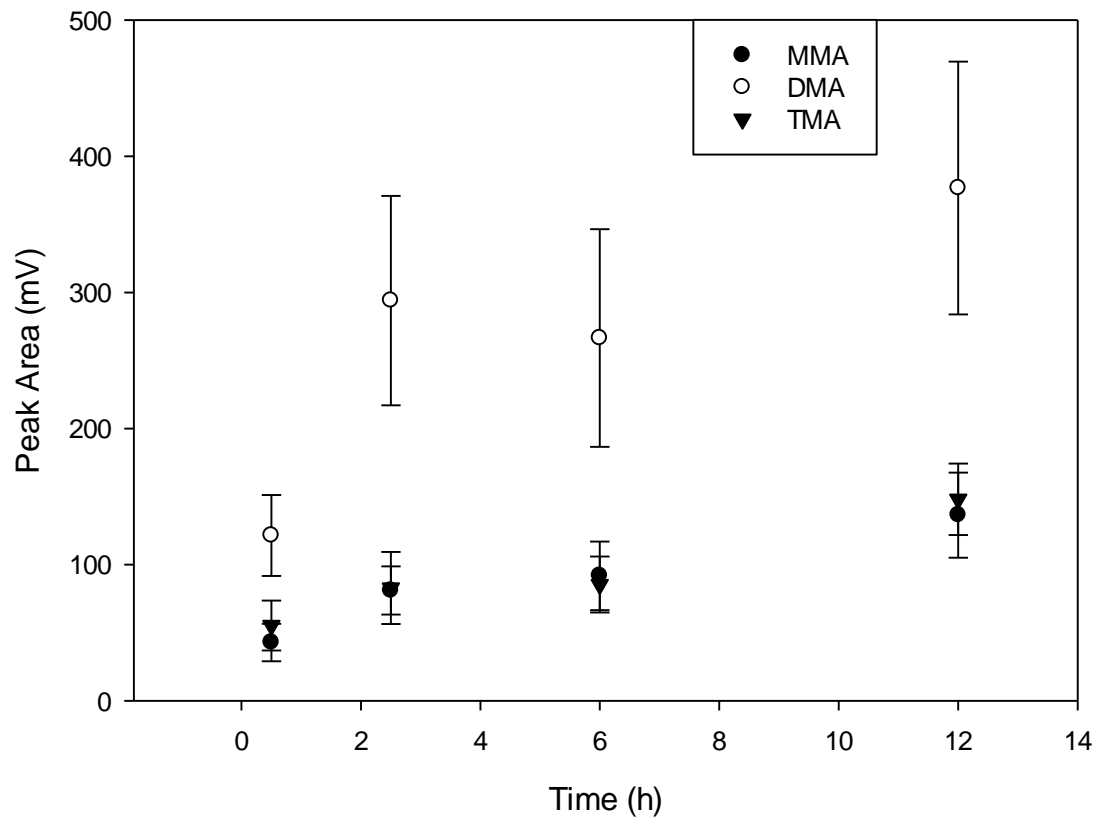


Figure 4

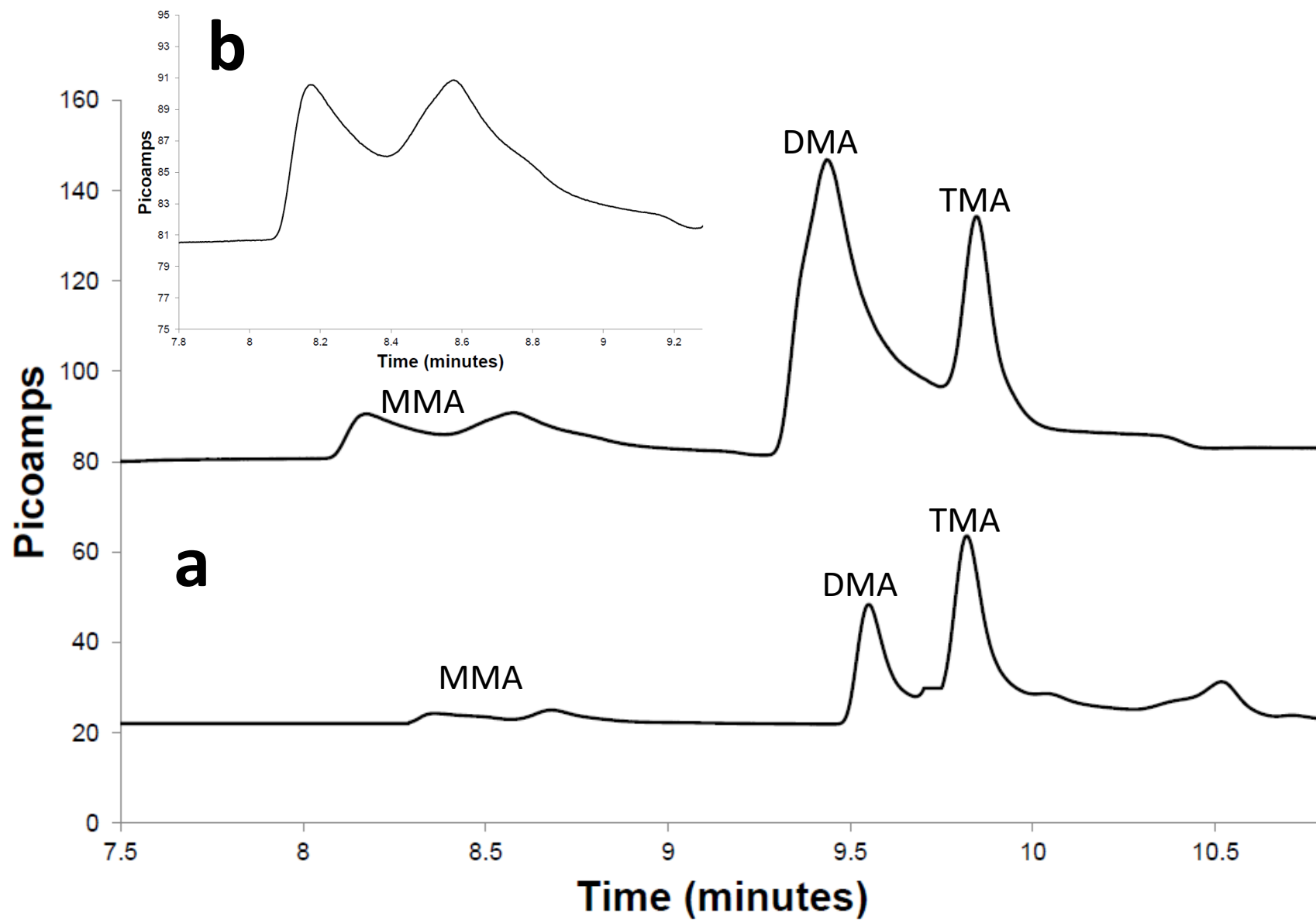
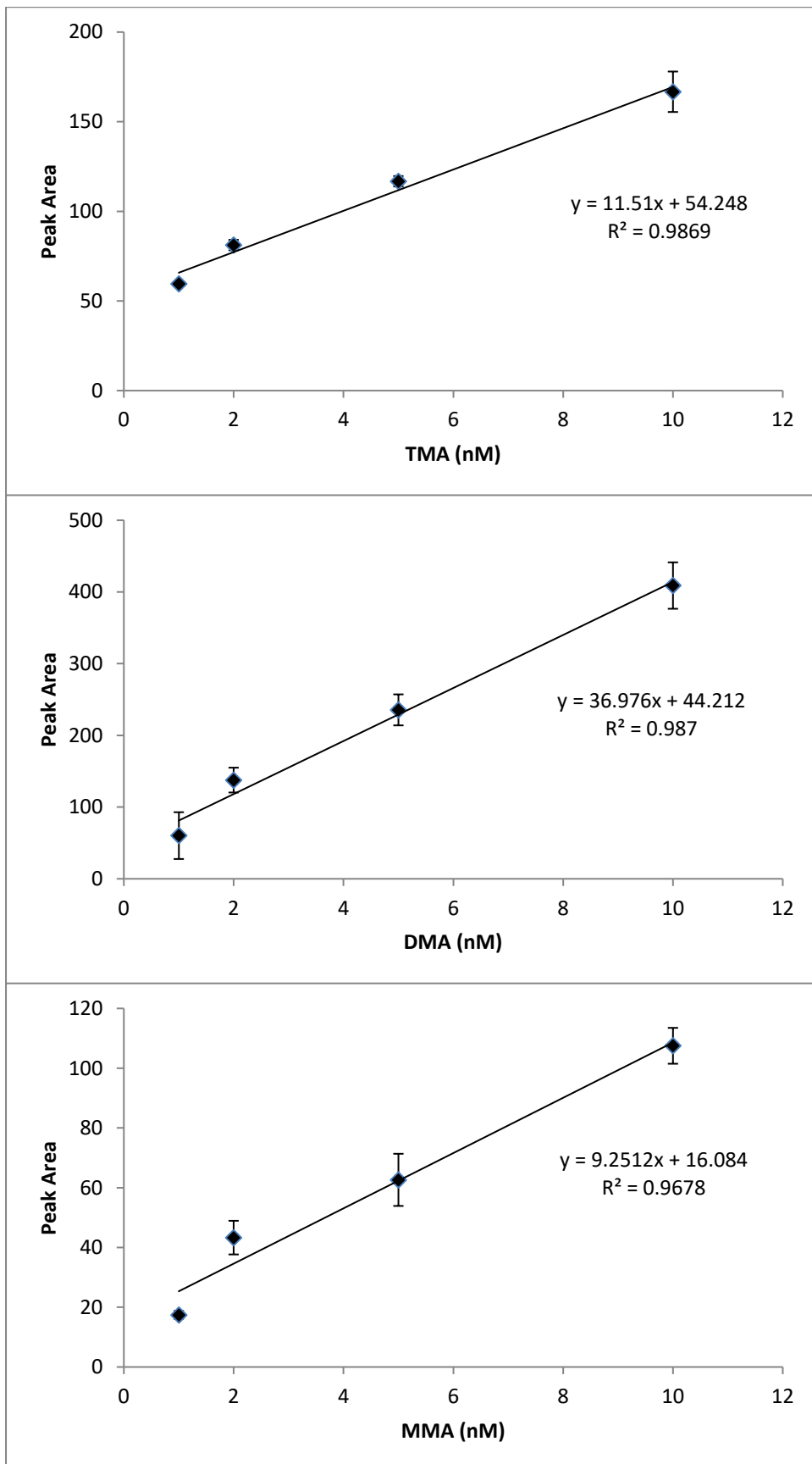


Figure 5



SI Figure 1. Calibration graphs from calibrations carried out on-board ship during field work in the Southern Ocean, comprising mean MA peak areas and standard deviations at the working range used for sample analysis (n = 3 at each concentration). Seawater used to prepare calibration solutions was collected from the sampling site and treated as detailed in the text.

