Master Thesis

Optimization of hydroxylamine measurements in seawater and its application at the Boknis Eck Time Series Station in the Baltic Sea

By

Tim Jonathan Paulus

Kiel, 2020

Helmholtz Centre for Ocean Research Kiel

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The following thesis was written in the research division of Marine Biogeochemistry in the working group of Prof. Dr. H. W. Bange, which is part of the research unit Chemical Oceanography at the GEOMAR Helmholtz Centre for Ocean Research Kiel from the 02nd of September 2019 to the 20th of May 2020.

Date of submission: 20th of May 2020

1. corrector: Prof. Dr. H. W. Bange

2. corrector: Dr. A. Kock

Declaration of Authenticity

I declare that all material presented in this work is my own work or fully and specifically acknowledged wherever adapted from other sources.

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Kiel, 20th of May 2020 Tim Jonathan Paulus

Abstract

The hydroxylamine (NH_2OH) analytics for seawater samples was introduced by Von Breymann et al. (1982), improved by Butler and Gordon (1986a) and further enhanced by Kock and Bange (2013), hence standard method. This method was used from 2011 on, to investigate NH_2OH concentrations at the Time Series Station Boknis Eck (Baltic Sea). High fluctuations of the recovery from NH_2OH to nitrous oxide (N_2O) with iron(III) were observed during the Time Series. In this master's thesis the pH dependence of this conversion reaction was investigated. Six different sample acidification methods were tested under laboratory conditions in deionized water and seawater from Boknis Eck (BE). The sample pH adjustment was tested using acetic acid (three methods), hydrochloric acid (two methods) and sulfuric acid (one method). The samples' pH conditions ranged from pH 1.4 to pH 3.2 (deionized water) and from pH 1.2 to pH 3.4 (BE). The Recovery Factors for the NH_2OH conversion to N_2O ranged from 21 to 88 % (deionized water) and from 0 to 83 % (BE). Acidification with hydrochloric acid and sulfuric acid were no improvement towards the standard method. In samples prepared with water from Boknis Eck the three methods with acetic acid yielded to higher recoveries (70 to 83%) than the standard method during this thesis. NH_2OH and background N_2O sampling was conducted at the Boknis Eck Time Series Station at six depths (1 m, 5 m, 10 m, 15 m, 20 m and 25 m) monthly from May to August and in October and December 2019. Method validation experiments at Boknis Eck were conducted for the two most promising methods in October and December 2019. The question, if the validated methods are an improvement or not towards the standard method, could not finally be answered. Therefore, the methods must be compared for a longer period with the standard method at Boknis Eck, as the standard method showed a high variability in recovery from month to month. Additionally, a time series of NH_2OH concentrations (BE) from 2011 to early 2017 was revised.

Zusammenfassung

Die Analytik von Hydroxylamin (NH_2OH) in Meerwasserproben wurde durch Von Breymann et al. (1982) etabliert, von Butler und Gordon (1986a) weiterentwickelt und von Kock und Bange (2013) optimiert, im Folgenden als Standardmethode bezeichnet. Seit 2011 wird diese Methode zur Erforschung der NH_2OH-Konzentrationen an der Zeitserienstation Boknis Eck (Ostsee) verwendet. Die Umsetzungsraten von NH_2OH zu Distickstoffmonoxid (N_2O) mit Eisen(III) unterlagen während der Zeitserienmessungen großen Schwankungen. In dieser Masterarbeit wurde der Einfluss des pH-Wertes auf die Umsetzungsrate untersucht. Sechs verschiedene Ansäuerungsmethoden wurden mit Meerwasser von Boknis Eck (BE) sowie deionisiertem Wasser unter Laborbedingungen getestet. Die pH-Werte wurden mit Essigsäure (drei Methoden), Salzsäure (zwei Methoden) und Schwefelsäure (eine Methode) angepasst. Bei den unterschiedlichen Methoden lagen die pH-Werte der Proben zwischen pH 1,4 und pH 3,2 (deionisiertes Wasser) sowie pH 1,2 und pH 3,4 (BE). Die Umsetzungsraten von NH_2OH zu N_2O waren im Bereich von 21 bis 88% (deionisiertes Wasser) und von 0 bis 83% (BE Wasser). Es wurde keine Verbesserung gegenüber der Standardmethode mit Salzsäure und Schwefelsäure erzielt. Die drei Methoden mit Essigsäure in Boknis Eck Wasser erzielten während der Laborexperimente bessere Umsetzungsraten (70 bis 83%) als die Standardmethode während dieser Arbeit. Probennahmen für NH_2OH und den N_2O Hintergrund erfolgten aus sechs verschiedenen Tiefen (1 m, 5 m, 10 m, 15 m, 20 m, 25 m) an der Zeitserienstation, jeden Monat von Mai bis August 2019 sowie im Oktober und Dezember. Methodenvalidierungsexperimente wurden mit den zwei besten Methoden an Boknis Eck im Oktober und Dezember 2019 durchgeführt. Die Frage, ob diese Methoden eine Verbesserung gegenüber der Standardmethode darstellen, konnte final nicht geklärt werden. Dafür müssten die Methoden wegen der starken Unterschiede in den monatlichen Umsetzungsraten bei der Standardmethode über einen längeren Zeitraum an Boknis Eck verglichen werden. Außerdem wurden die NH_2OH Konzentrationen der BE Zeitserie von 2011 bis Anfang des Jahres 2017 überarbeitet.

Abbreviations

AcOH	acetic acid
ADD	addition
AMO	ammonia monooxygenase
AOA	ammonia oxidizing archaea
AOB	ammonia oxidizing bacteria
Ar	argon
ATU	acetylene methanol allylthiourea
BE	Boknis Eck
C_2H_2	acetylene
CO_2	carbon dioxide
Cu^{+II}	cupper(II)ions
DIC	dissolved inorganic carbon
DIL	dilute
DISS	dissolved
DNRA	Dissimilatory Reduction of Nitrate to Ammonia
Exp.	experiment
FAS	ammonium iron (III) sulfate/ ferric ammonium sulfate
Fe^{+II}/ Fe^{+III}	iron(II)ions/ iron(III)ions
GC-ECD	gas chromatograph with an electron capture detector
HACl/ NH_2OH*HCl	hydroxylamine hydrochloride
HAO	hydroxylamine oxidoreductase
HCl	hydrochloric acid

He	helium
HNO_2	nitrous acid
$H_2N_2O_2$	hyponitrous acid
H_2SO_4	sulfuric acid
Me/ CH_4	methane
$MgSO_4$	magnesium sulfate
N	nitrogen
N_2	nitrogen gas
NaCl	sodium chloride
NH_3	ammonia
NH_4^+	ammonium
NIR	nitrite reductase
N. maritimus	Nitrosopumilus maritumus
NH_2OH	hydroxylamine
NO	nitric oxide
N_2O	nitrous oxide
NO_2^-	nitrite
NO_3^-	nitrate
NOR	nitric oxide reductase
R	Recovery Factor
SA/ $C_6H_8N_2O_2S$	sulfanilamide
SOL	solution
Std.	standard
Std. M.	standard method

Contents

1 Introduction

1.1 Hydroxylamine in seawater

Nitrogen (N) is essential for life itself. As a nutrient for organisms and a crucial element for proteins, it is basal for biochemical processes and occurs in a grand variety of chemical forms. The N cycle involves N in different oxidation states; an overview of the complexity of the N cycle is presented in Figure 1. (Francis et al., 2007)

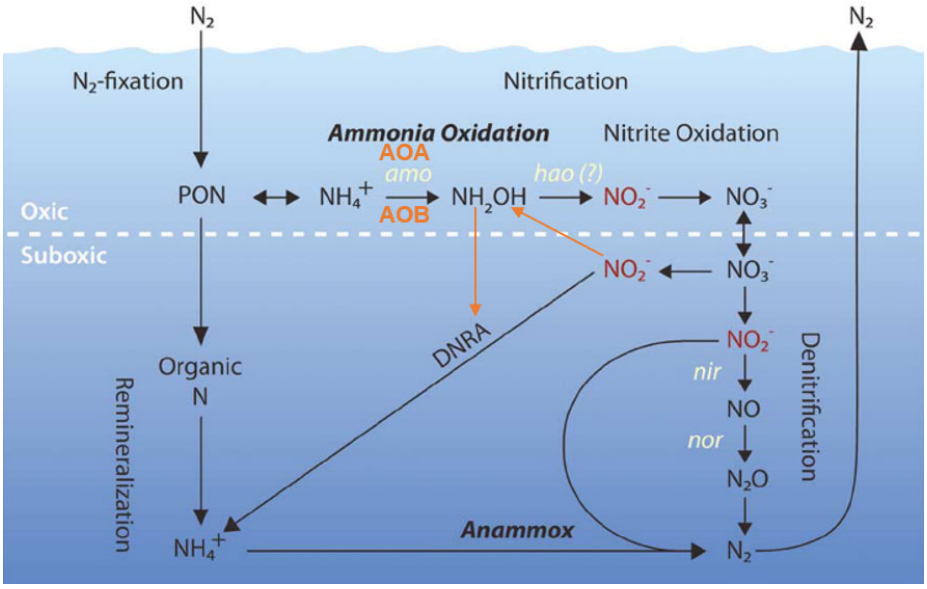

Fig. 1:N cycle overview seawater modified in orange derived from (Francis et al., 2007) with information from (Einsle et al., 2002) and (Wuchter et al., 2006)

The most important forms of N are nitrogen gas (N_2), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). N_2 is accountable for 78 % of the atmospheric N amount. N_2-fixation by microorganisms is crucial for the use by further organisms. The greatest reduced chemical form of N is NH_4^+. It occurs in organisms, although nitrification to NO_3^- is a fast process. Nitrification requires oxic conditions. Hydroxylamine (NH_2OH) is an highly reactive intermediate in N cycle. It is formed

from NH_4^+ during nitrification and is degraded to NO_2^-. (Francis et al., 2007) Nitrification can be conducted by ammonia oxidizing bacteria (AOB) (Arp and Stein, 2003) and by ammonia oxidizing archaea (AOA) (Francis et al., 2007). NH_2OH has long been identified as an intermediate in AOB (Francis et al., 2007), (Arp and Stein, 2003). The first step of AOA nitrification, like in AOB over is ammonia monooxygenase (AMO); thus NH_2OH is probably also an intermediate in AOA. (Wuchter et al., 2006). Dentification of NO_3^- to N_2 occurs under suboxic conditions (Francis et al., 2007).

The bacterial Dissimilatory Reduction of Nitrate to Ammonia (DNRA) is an anerobic process and occurs therefore only in anoxic environments. (Tiedje, 1988). DNRA reduces NO_3^- to NH_3 (NH_4^+), with NO_2^- and NH_2OH as intermediates. NO_2^- has a high proton affinity. The reduction of NO_2^- to NH_2OH requires conditions below pH 2.7. (Einsle et al., 2002) Therefore, DNRA occurs probably not naturally in seawater, but it could be relevant under an acetic microenvironment (Kock and Bange, 2013).

Figure 2 shows the enzymatic production and uptake of NH_2OH over the AOB pathway. The NH_2OH formation is catalyzed by ammonia monooxygenase (AMO) with dissolved oxygen as oxidant, where ammonia (NH_3) is oxidized. The next step is the hydroxylamine oxidoreductase HAO-catalyzed NH_2OH oxidation to nitrite (NO_2^-). As side products from the incomplete oxidation of NH_2OH, nitric oxide (NO) and nitrous oxide (N_2O) can be formed, or from nitrifier-denitrification, the reduction of NO_2^- by the denitrifying enzymes nitrite reductase (NIR) and nitric oxide reductase (NOR). (Arp and Stein, 2003)

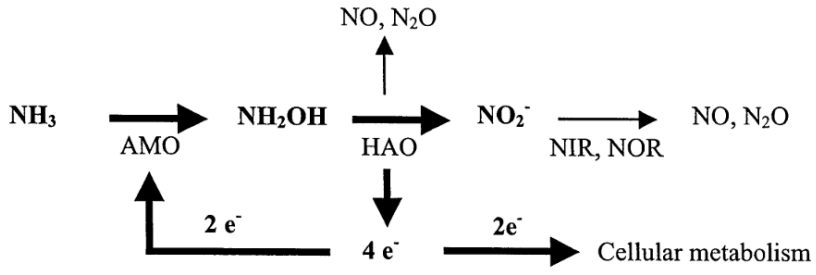

Figure 2: NH_2OH producing and uptake.(Arp and Stein, 2003)

Wuchter *et al.* (2006) indicated that the AOA has 1 to 2 times higher abundance in the ocean than AOB. Vajrala *et al.* (2013) conducted experiments using the archaeon Nitrosopumilus maritimus (N. maritimus) and found that it produces and consumes NH_2OH during NH_3 oxidation to NO_2^-. This is a strong indication that NH_2OH is an intermediate in archaeal nitrification Their results propose that N. maritimums reacts similar to N. europaea. Neither acetylene methanol allylthiourea (ATU) nor acetylene (C_2H_2) inhibited the NH_2OH consumption. This includes both AOB inhibitors and C_2H_2 AOA inhibitor. (Vajrala et al., 2013)

However, whereas the NH_2OH oxidation in AOB is enzymatically catalyzed by HAO, this is probably different for the AOA pathway. The question of which enzyme and gene would be accountable for the NH_2OH oxiodoreductase during AOA remains open. In N. maritimus, no homologue to HAO was identified (Stahl and de la Torre, 2012). Thaumarchaea oxidizes NH_4^+ with NH_2OH and nitrous oxide (NO) as intermediates, the further reduction of these is non-enzymatic (Kozlowski et al., 2016). It was discussed that N_2O can be produced in soils abiotically, under neutral pH conditions by interactions of iron, manganese and organic compounds with intermediates like NH_2OH (Zhu-barker *et al.*, 2015), (Liu et al., 2017). It is not clear yet if conditions in seawater would favor these reactions in the same way, however.

Transition-metal cations such as iron(III) (Fe^{+III}) or cupper(II) (Cu^{+II}) can react with NH_2OH. It has furthermore been shown that NH_2OH reacts with a number of other oxidants, such as nitrous acid, peroxides and oxygen. Oximes condensates when ketones or aldehydes are in the presence of NH_2OH. This reaction is reversible. (see Butler and Gordon, 1986b references therein)

The turnover of NH_2OH in seawater takes 4 (artificial seawater) to 8 h (natural seawater) under oxic conditions (Kock and Bange, 2013 and their references therein). NH_2OH is a short lived intermediate (Fiaderio et al., 1967). It can help to identify areas of active nitrification in the ocean (Korth et al., 2019). On the other hand, an efficient conversion of NH_2OH during nitrification could prevent its accumulation in the water column. Under these circumstances, accumulation of NH_2OH would be an indication that the efficiency of the nitrification mechanism may be disturbed.

Korth et al. (2019) found a significant correlation between NH_2OH, N_2O and NO_3^- under oxic conditions in the ocean waters of the eastern tropical South Pacific and the equatorial Atlantic Ocean. N_2O accumulates in the water column, as it is a long lived product, whereas this is not the case for NH2OH due to its short life span. In the above areas N_2O is mainly produced by nitrification. The correlations do not provide a direct evidence that NH_2OH is a precursor of N_2O, but may indicate that conditions that favor NH_2OH accumulation may also favor N_2O production. The investigation of the conversion mechanisms of NH_2OH to N_2O by AOB and AOA could help to resolve this question. (Korth et al., 2019)

Incubation and microbiological studies could help to further investigate the role of NH_2OH during nitrification. NH_2OH is highly likely an intermediate in AOA (Vajrala et al., 2013), (Kozlowski et al., 2016). Further research is needed to clarify how NH_2OH is oxidized to NO_2^- and which side reactions can occur.

Terrestrial and reject water studies can help in further understanding the role of NH_2OH in the N cycle. (Liu et al., 2017), (Star et al., 2008), (Bikbulatova et al., 2007), (Soler-jofra et al., 2016), (Heil et al., 2015), (Zhu-barker et al., 2015), (Duan et al., 2020) etc.

1.2 Hydroxylamine measurements

The first method for the detection of low concentrations of NH_2OH sea water samples (detection limit: 0.6 nMol, confidence level 95 %) was established by Von Breymann et al. (1982) using a Gas Chromatograph with Electron Capture Detector (GC-ECD). The methods published earlier were only for high NH_2OH concentrations, or needed to be analyzed directly after sampling (see Von Breymann et al., 1982 and their references therein). Iron(III)ions (Fe^{+III}) were used for the conversion reaction of NH_2OH to N_2O (recovery: 50 %). Von Breymann et al. (1982) used the N_2O detection method (GC-ECD) published earlier (Cohen, 1977). They verified their method with seawater samples (July, 1981, 14 miles from the Oregon coast at continental shelf). Background N_2O samples and conversion samples were collected in duplicate; all samples were poisoned using mercury chloride ($HgCl_2$). Standard additions were conducted with hydroxylamine hydrochloride (NH_2OH*HCl; HACl) and quantified by titration of the reaction product Fe(II) (Rao and Rao, 1957). Different media for the HACl standards (Std.s) were tested (double distilled water, artificial seawater, open ocean surface seawater sample side, estuarine filtered and unfiltered water Yaquina Bay), they did not identify a significant difference. It was tested if ammonium ions were oxidized as well and that could be negated. The method was tested for anoxic conditions, by adding sulfide. This did not interfere with the results. (Von Breymann et al., 1982)

Butler and Gordon (1986a) further improved the earlier published NH_2OH detection method (Von Breymann et al., 1982). They found that the recovery was highly dependent on pH, as shown for natural seawater and deionized water in Figure 3. Different media for the HACl Std.s were tested: distilled water, natural seawater (surface water Pacific Gyre), salt solutions ($MgSO_4$, NaCl, artificial seawater). The method was validated with saline (coastal and offshore) and freshwater samples, the samples were acidified. Without acidification, they observed a degradation of 30 % after 3 h, room temperature (RT). (Butler and Gordon, 1986a)

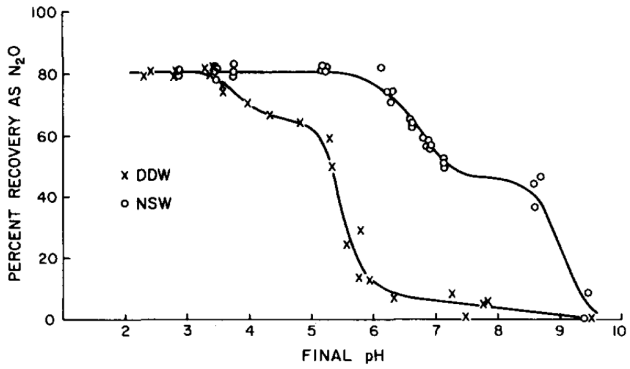

Figure 3: NH_2OH conversion to N_2O pH dependence.(Butler and Gordon, 1986a)

Recoveries decreased for water with stronger biological activity. They concluded that the pH should be adjusted between 2.8 and 3.5 pH using acetic acid, and recommended the separate addition of FAS. To the NH_2OH samples, separate conversion samples for the Std. addition (ADD) and N_2O background samples are needed. Their method reached 80 % conversion of NH_2OH to N_2O. The Figure 4 displays a potential for the mechanism of the oxidation of NH_2OH with Fe^{+III} to N_2O and HNO_2 (Butler and Gordon, 1986a).

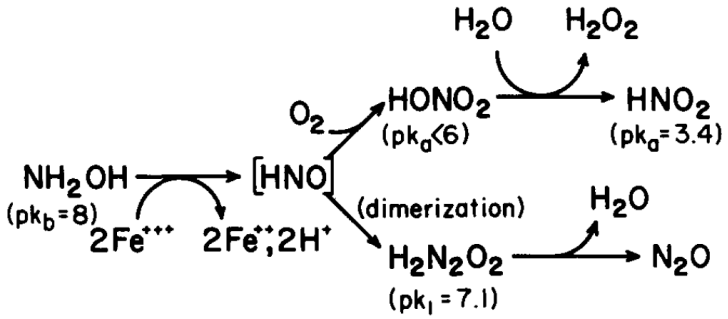

Fig. 4: Potential mechanism of NH_2OH oxidation with Fe^{+III}(Butler and Gordon, 1986a).

Bengtsson et al. (2002) further investigated the kinetics of the NH_2OH reduction/oxidation with Fe^{+III} in acetic conditions and proposed Reaction 1 for an excess of protonated hydroxylamine (NH_3OH^+) with molecular (N_2) as product and Reaction 2 for a 5 to 10 times of Fe^{+III} and N_2O as product.

$$2Fe^{+III} + 2NH_3OH^+ \longrightarrow 2Fe^{II+} + N_2 + 4H^+ + 2H_2O$$

Reaction 1: Excess $NH3OH^+$ reduction with Fe^{+III} as catalyst.(Bengtsson et al., 2002)

$$4Fe^{+III} + 2NH_3OH^+ \longrightarrow 4Fe^{II+} + N_2O + 6H^+ + H_2O$$

Reaction 2: Excess Fe^{+III} catalyzed $NH3OH^+$ oxidation.(Bengtsson et al., 2002)

Kock and Bange (2013) further improved the NH_2OH method (Butler and Gordon, 1986a) by adding sulfanilamide to remove nitrite (NO_2^-). At pH 3 (method conditions) nitrite is prevalent mainly as nitrous acid (HNO_2), which can dissociate to N_2O (Reaction 3). HNO_2 and NH_2OH can furthermore react to N_2O, with hyponitrous acid ($H_2N_2O_2$) as an intermediate (Reaction 4). They introduced the addition of the antibiotic sulfanilamide to inhibit these processes. Sulfanilamide is used as a nitrite-specific scavenger that does not interfere with the conversion reaction between NH_2OH and Fe(III).(Kock and Bange, 2013)

$$4HNO_2 \longrightarrow 2HNO_3 + N_2O + H_2O$$

Reaction 3: HNO_2 dissociation to N_2O.(Kock and Bange, 2013)

$$HNO_2 + NH_2OH \longrightarrow H_2N_2O^2 + H_2O \longrightarrow N_2O + 2H_2O$$

Reaction 4: HNO_2 reacts with NH_2OH to $H_2N_2O_2$ and dissociates to N_2O.(Kock and Bange, 2013)

1.3 Time Series Station – Boknis Eck

Continuesd sampling at the Boknis Eck (BE) Time Series Station started on 30th April 1957. It is monthly operated building one of the longest time series worldwide at least for Conductivity, Temperature and Depth (CTD) and oxygen measurements. (GEOMAR a, 2020)

Several parameters were observed: temperature (reversing thermometer: 1957 – 1975, CTD: 1979 – present), oxygen (1957 – present), salinity (refractometer: 1957 – not reported, CTD: not reported – present), phosphate (Photometer: 1957 – 7/1970, CTD: 8/1970 – present), nitrate (1979 – present), nitrite (1979 – present), ammonium (1979 – present), Chlorophyll a (photometer: 1975 – 2009, fluorometer: 2009 – present), secchi depth (1986 – now) (Lennartz et al., 2014), primary production (late 80's – late 90's), silicon dioxide (80's – present), zooplanktivores (1957 – third quarter 80's), Total Bacterial Number and Bacterial Production (late 80's – late 10's), dimethyl sulfide and dissolved inorganic carbon (2008 – present), hydroxylamine (2006 – present); trace gases (2006 – present): methane, nitrous oxide, carbon monoxide and nitrous monoxide (2019 -present) (GEOMAR b, 2020). During the time series, major gaps were from 1975 to 1979 and from 1983 to 1985 (Lennartz et al., 2014). Sensor in-situ data for salinity, pressure, temperature, Acoustic Doppler Current Profiler, oxygen, carbon dioxide and methane are linked on the Boknis Eck web page (GEOMAR b, 2020) and available from December 2016 to August 2019.

The site location is at the mouth of the Eckernförder Bay in the southwestern Baltic Sea with the coordinates: 54°31.2' N, 10°02.5'E, displayed in Figure 5. The water body is influenced through by the North Sea water inflow from Kattegat and the Great Belt. Inflows due to rivers are insignificant. With muddy sediments and a total depth of 28 m. Boknis Eck is an optimal site to investigate a coastal ecosystem under salinity changes and to study oxygen sensitive biogeochemical processes (GEOMAR c, 2020).

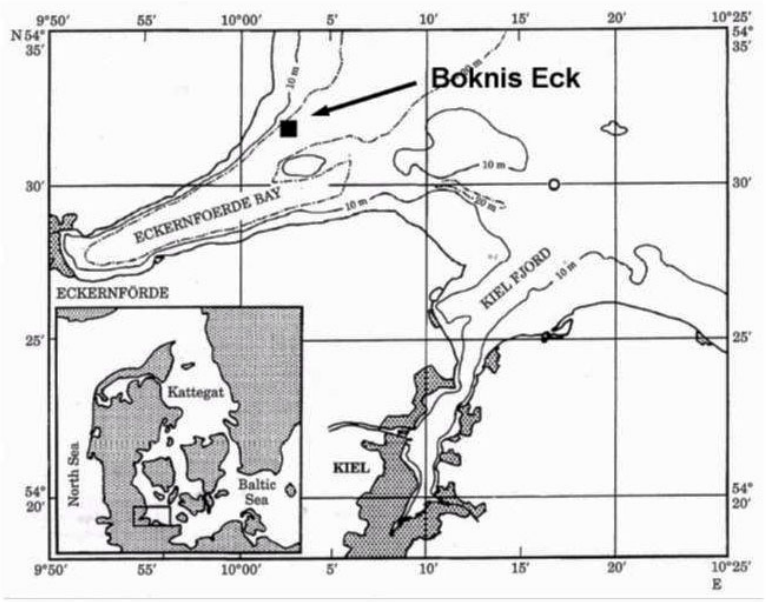

Fig. 5: Time Series Station Boknis Eck. (GEOMAR c, 2020)

Several long term trends were identified by Lennartz et al. (2014), data from the Time Series Station Boknis Eck till 2013 were interpreted. Generally, stratification of the water column is from mid-March till mid-September Decreasing oxygen concentrations could be observed during January and the summer months ranging from July (-0.8 $\mu molL^{-1}yr^{-1}$) to April (-0.5 $\mu molL^{-1}yr^{-1}$). During late summer/ autumn high oxygen depletion can occur, which can cause an anoxia of the water column. Sediments get typically anoxic during summer. A temperature increase of 0.2°C per decade could be observed; this is consistent compared to the different regions of the Baltic sea. At 10 m and 15 m thermocline can be observed starting typically from March/ April and remains until October. In March, during algal bloom, the highest chlorophyll *a* concentrations are reached. The second maxima occurs somewhere between August and December. During winter (December to February) phosphate and NO_3^- maxima' s are reached. NO_2^- and the NH_4^+ trends were not homologues for the seasons and the water column. In January, March and April, significantly decreasing NO_2^- concentrations were found. For 10 m ammonium, concentrations decrease from January to April, and for 25 m, the yearly maxima is typically obtained in May and October. (Lennartz et al., 2014)

The N cycle at Boknis Eck seems to be very dynamic when the summer stratification is broken up and the water column becomes mixed and re-oxygenated in autumn, with peak concentrations of NH_2OH and N_2O in these periods (2005). Schweiger et al. (2007) identified low NH_2OH concentrations from July to October 2005, the maxima (18.5 $nmolL^{-1}$) was reached in November (2005) and decreased concentrations from December (2005) till March (2006). N_2O and oxygen corelated linearly (July, August 2005), this changed with declining oxygen concentrations (September, October 2005) and recovered during the upwelling event in November (2005). The high NH_2OH maxima in November (2005) was associated with in-situ nitrification. (Schweiger et al., 2007) I like to mention that this Boknis Eck NH_2OH study was conducted before the implementation of the nitrite removal (Kock and Bange, 2013).

1.4 Objective

The aim of this thesis was to test the NH_2OH detection method for potential improvements. During the years of NH_2OH measurements at Boknis Eck with the standard method (Std. M.) (Kock, 2012), high variabilities in the Recovery Factor could be observed, ranging from 16 to 86 % from 2011 to 2017, while extreme Recovery Factors of -46 % and 187 % occurred. Regression coefficients ranged from 99.85 to 99.95 %, and for extreme events a minimum of 59.91 % appeared. Vajrala *et al.* (2013) reported, in their N. maritimus culture study, a quantitative conversion of NH_2OH at pH 1.4, which is in contrast to previous results by Butler and Gordon (1986a). The major task of the following master's thesis was to investigate how and whether the recoveries of NH_2OH to N_2O could be improved under different pH conditions, for the monthly measurements at the Time Series Station Boknis Eck. Sampling during the monthly cruises and analyzing these was the second task. Additionally, the Time Series Data should be assembled and good data identified. The work furthermore included a critical revision of the previous Time Series measurements to identify potentially compromised data.

2 Experimental procedure

2.1 Equipment

2.1.1 General equipment and chemicals

For the preparation of the aqueous solutions, deionized water (MQ) was used. The liquid and solid stock chemicals are listed in Table 1, except for a 2 % mercuric chloride solution, which had been already prepared for general use in the workgroup. Gases are listed in Section 2.4.

Chemical	**Molecular formula**	**Company**	**Grade**	**Batch**	**M** in g/ mol
Acetic acid	CH3COOH	J. T. Baker	99-100 % (glacial), ACS	1717301871	60.05
Hydrochloric acid	HCl	Roth	37 % (fuming), ACS	356245859	36.46
Hydrochloric acid	HCl	Merck	2 mol/ L, TitriPUR	HC077589	36.46
FAS	$(NH_4)Fe(SO_4)_2*12H_2O$	Merk	analysis, ACS	K31962976 322	482.19
HACl	$(NH_3OH)Cl$	PanReac AppliChem	99.5 %, analysis, ACS	0001288121	69.49
SA	$C_6H_8N_2O_2S$	VWR	analysis	11A070002	172.2
SA	$C_6H_8N_2O_2S$	Bernd Kraft	≥ 99 %, analysis	1404208	172.2
SA	$C_6H_8N_2O_2S$	Sigma Aldrich	≥ 99 %, analysis	SLBS4782	172.2
Sulfuric acid	H_2SO_4	Merck	95-97 %, analysis	K24833731	98.08

Table 1: Solid and liquid chemicals.

Hydroxylamine hydrochloride (HACl) had to be stored in dry air. Therefore the box was sealed with parafilm and stored in an exicator with silica gel as drying agent and Sicapent(R) to see when the silica gel had to be renewed in the drying chamber. Everything was labeled with heavy duty labels (Avery Zweckform, Oberlaindern, Germany, Silver Heavy Duty Labels 45.7 x 21.2 mm, L6009). Calculations, Tables text documents were done using Microsoft Office (Microsoft, Redmond, USA, Version: 2019), plots were either done with Excel or MATLAB (MathWorks, Natrick, USA, Version: R2018a).

The pH of the Std. MQs was verified using two different non-bleeding pH-indicator strips (Merck, Darmstadt, Germany, MQuant, Supelco, pH 0 to 2.5, HC982588; pH 2.5 to 4.5, 0C557995). For all methods, the pH of the Std. MQs and different sample types (only SA-Acid & SA-Acid + FAS) was veriefied at least once; for MQ and Boknis Eck water, a pH meter (InnoLab Chemistry, Groningen, Netherlands, pH Level 1, E163694) with an attached pH-electrode (Xylem Analytics, Weilheim, Germany, WTW SenTix 41, new 03.2017) was used. The pH meter was calibrated using the ConCal two point calibration method with a pH 3 buffer solution (Merck, pH (20°C) = 3.00±0.01, HC083129) and a pH 7 buffer solution (Merck, pH (20°C) = 7.00±0.01, HC081444).

Mass and volume determination: All masses were weighed with a fine scale (Sartorius, Goettingen, Germany, R 160 P, 0.00000 g) or (KERN & SOHN, Balingen-Frommem, Germany, ABT 220-5DNM, Min 0.001[00] g Max 220 g); to ensure maximum precision the scale had to be switched on 30 min before usage. Different volumetric/graduated pipettes were used to dispense different liquid volumes: 1 to 10 mL graduated pipette (ISOLAB Laborgeräte, Eschau, Germany, DIN AS, 1 to 10 ± 0.05 mL), 10 mL volumetric pipette (BRAND, Wertheim, Germany, BLAU BRAND, ISO 648,10 ± 0.02 mL), 20 mL volumetric pipette (BRAND, BLAU BRAND, ISO 648, 20 ± 0.03 mL), 50 mL volumetric pipette (Hirschmann Laborgeräte, Eberstadt, Germany, EM TECHCOLOR, DIN B, 50 ± 0.075 mL) and 100 mL volumetric pipette (Hirschmann, EM TECHCOLOR, ISO 648, 100 ± 0.08 mL). For the Std. MQ additional a 0.1 to 1 mL adjustable pipette (Eppendorf, Hamburg, Germany, Research, 3434651) was used. In general, several adjustable pipettes for the HACl standard solutions (Std. SOLs) were used: 1 to 10 µL (Eppendorf Reference, 2863776), 20 to 200 µL (Thermo Fisher Scientific, Waltham, USA, Electron corporation FINNPIPETTE, internal number: CH02919 4500) and 10 to 100 µL (Eppendorf Research plus, L13439B). The adjustable pipettes are allocated to the different Std. SOLs in the corresponding Section 2.2.1 (especially Table 4) and 2.3.1 (especially Table 6).

Adjustable pipettes are always specifically allocated and are excluded from the general behavior. That equipment is only completely described when first mentioned.

Bottles and vials: Clear glass sample bottles were used for the different SOLs depending on the volume 50 mL vials (Chromatographie Handel Müller, Fridolfing, Germany, R20-50fl HKL 3, 73 x 43 mm, 610069) and 100 mL vials (Chromatographie Handel Müller, R20-100fl HKL III, 95 x 52 mm, 4451178). Std. MQs were prepared in 500 mL duran glass bottles (SCHOTT, Mainz, Germany, Duran, 00416557). As sample bottles, and for the HACl Std. SOLs, 20 mL brown glass vials (Chromatographie Handel Müller, Flasche R20-20br HS, 75.5 x 23 mm, 4451254) were used, closed with butyl rubber septums (Chromatographie Handel Müller, Butylgummihohlstopfen Butyl-grau, 4451283) and crimped with aluminum caps (Chromatographie Handel Müller, Bördelkappe R20-oA gold ohne Dichtscheibe, 8 mm Loch, 772013) and labeled with heavy duty labels. In between, there were some “wrong sample vials” in the lab which had slightly different volume; this was detected afterwards. The volume of these vials was determined with MQ and the fine scale; the sample concentrations were then mathematically corrected.

Sample post-treatment material N_2O: Upon poisoning of samples, a 3 ml plastic syringe with removed plunger and a Ø 0.8 x 40 mm needle was used to enable the pressure equilibration of the samples. The 2 % $HgCl_2$-SOL was added with a 1 ml disposable plastic syringe with a Ø 0.5 x 40 mm disposable needle. Upon injection the contamination of the samples was carefully avoided. For the pressure equilibration of headspace samples, 20mL disposable syringes without plunger and 0.8x120mm needles were used.

Sample post-treatment material NH_2OH: The Acid-SA SOLs and FAS's were partly added with a gas tight repeating dispenser (HAMILTON COMPANY, Bonaduz, Switzerland, PB-600, PAT. 3161323) and an attached syringe (HAMILTON COMPANY, 2500 µL gas tight, 1000 Series) and partly with a veterinary self-filling injection syringe (Henke-Sass, Wolf, Tuttlingen, Germany, HSW Eco-Matic, 0.1 to 0.3 mL, Luer-Lock, 8300002189); a Ø 0.5 x 40 mm needle was attached to both systems. The HAMILTON repeating dispenser was filled bubble free and per captia enabled the repeated injection of 1/50 of the volume of the syringe. The chemical solutions in crimped ampules of 50 to 100mL volume were attached to the Eco-Matic, which was automatically re-filled from the ampules after injection. The injection volume could be adjusted from 0.1 to 0.3 mL. The self-refilling process of the syringe had to be carefully monitored to avoid bubbles. A precise adjustment of the Eco-Matic required the validation of the injection volume by weighing MQ injections with a fine scale. The HACl Std. SOLs were added by using 100 µL syringes (HAMILTON COMPANY, 100 µL, 1700 Series, 81008) with disposable Ø 0.5 x 40 mm needle; for each HACl Std. SOL concentration another microliter syringe and a new needle were used. Upon injection the contamination of the samples was carefully avoided. For the pressure equilibration of headspace samples, 20mL disposable syringes without plunger with 0.8x120mm needles were used.

2.1.2 Sulfanilamide comparison experiment

A comparison experiment was conducted in order to verify if the usage of different batches from different suppliers would have an influence on the NH_2OH measurements. The batches from the different suppliers are listed in Table 2. The information on the SA batch used for individual experiments is given in Table 4 (Section 2.2.1) and Table 6 (Section 2.3.1). The 50 mL glass vials for the sulfanilamide SOLs were covered with aluminum foil due to its light sensitivity. The preparation of the different SA SOLs is described in Table 2.

SA SOL			
SA Brand	**Opening Date**	**SA** / g	**DISS AcOH** /ml
Sigma Aldrich	Mar. 2017	0.088[68]	50
VWR	Mar. 2011	0.088[10]	50
Bernd Kraft	Feb. 2017	0.088[55]	50

Table 2: SA SOLs preparation.

In Table 3 the sampling plan is described; as sample medium 10.3 mL of lab air equilibrated MQ was used.

Samples in triplicates A, B, C	**Additon** in µL	**Without adjustment needle**
A1 to C3	50 $HgCl_2$	1) chemical injected 2) 2 min shaken
A4 to C6	100 VWR SA-AcOH	
A7 to C9	100 Sigma Aldrich SA-AcOH	
A10 to C12	100 Bernd Kraft SA-AcOH	

Table 3: Sample plan for SA comparison experiment.

2.1.3 Precision and accuracy of the pipettes and the Eco-Matic dispenser

2.1.3.1 Pipette uncertainty

The average uncertainty introduced from pipetting the HACl Stock SOLs to different Std. SOL solutions was determined by weighing two different pipetted volumes of MQ for each pipette. The pipetting volume was 20 µL and 40 µL (20 to 200 µL Thermo Electron corporation FINNPIPETTE, internal inventory number: CH02919 4500). Each volume was pipetted 20 times at room temperature (RT). The pipetting volume 5 µL and 10 µL (1 to 10 µL pipette Eppendorf Reference, 2863776) was pipetted 20 times each at room temperature.

Ellis (1973) identified that adjustable pipettes vary in the dispensed volume if pipette, solution and laboratory air vary in temperature. Eppendorf and Oxford pipettes in several sizes were tested (Eppendorf: 1 mL, 0.5 mL, 0.1 mL, 0.05 mL; Oxford: 0.2 mL): the pipette temperature was at 25°C and the solution (H_2O) was at 0°C and 25°C, the 1 mL pipette was tested also at 40°C. For 25°C the deviation matched with the manufacturers' specifications, for 0°C the dispensed volume was 3 to 10 % lower compared to the nominal volume, for 40°C the volume was 5% larger. The mean volume deviation was 10 times larger when the solution was at 0°C instead of 25°C. (Ellis, 1973)

The (10 to 100 µL Eppendorf Research plus L13439B) was tested twice with MQ at room temperature and MQ between 3 to 6°C, with the pipetting volume 10 µL and 80 µL and 30 repetitions for each volume at each temperature. 3 to 6°C was chosen, as this is the temperature span of the HACl Stock SOL from the fridge after a couple of minutes in the laboratory. The uncertainties were estimated using the regular standard deviation (precision) and the deviation of the arithmetic mean to the set pipette volume (accuracy). Additionally, a slightly changed standard deviation was calculated, here the mean was substituted with the set pipette volume to determine the deviation of the nominal volume.

2.1.3.2 Eco-Matic uncertainty

The Eco-Matic was tested with a 50 mL or a 100 mL MQ ampule attached and a Ø 0.5 x 40 mm needle. During the test, 100 µL of MQ were injected into a 50 mL crimped ampule and the mass of the injected volume was determined using the Kern fine scale. The syringe was permanently held in one hand with the needle pointing downwards. To test the sensitivity of the Eco-Matic for different handling, an experiment with 50 slow smooth injections and 100 mL MQ glass bottle and a second experiment with a 50 mL MQ glass bottle with 25 nearly hectic injections and 25 slow smooth injections were conducted. The precision and accuracy was calculated (see Section 2.1.3.1).

2.2 Method optimization

2.2.1 Reagent preparation

For all method optimization experiments ~0.6 g FAS were dissolved (DISS) in 50 mL MQ, using a 50 mL clear glass sampling vial, sealed with butyl rubber septum and crimped with aluminum caps. The FAS SOLs were used at maximum for 2 weeks. The 50 mL glass vial for the sulfanilamide SOLs was covered with aluminum foil due to its light sensitivity. The hydroxylamine hydrochloride (HACl) Stock solution (Stock SOL) was prepared one day before the four different standard (Std.) SOLs. Both the Stock SOL and the Std. SOLs were shaken after preparation and before usage and allowed to stand until vesicles had seeded. The Stock SOL and Std. SOLs were kept in the refrigerator at 3.3 C. A 2% $HgCl_2$ SOL (2 g $HgCl_2$ DISS in 100 mL MQ) was used as poisoning SOL. The preparation of the SA SOLs, Std. MQs, HACl Stock SOLs and HACl Std. SOLs is described in Table 4. The Table 4 is in chronological order of the

measurements, some experiments were measured before the identification of a defective Valco Valve in the gas chromatograph (GC) (further information Section 2.4.2) and pipettes were changed before method IV.

Method Medium	SA SOL		Std. MQ	Stock SOL		Std. SOL				
	SA / g (Brand)	DISS MQ / mL Acid /ml	MQ / mL Acid /ml	HACl / g	DISS in Std. MQ / mL	Std. 1	Std. 2	Std. 3	Std. 4	DIL in Std. MQ / µL
						Stock SOL / µL				
I (a) MQ***	0.186 (VWR)	13 (MQ) 87 (HCl 37%)	198.3 (MQ) 1.7 (HCl 37%)	0.0201	100	5*	10*	20*	40*	19.5
I (b) MQ***	0.173 (Sigma Aldrich)			0.0212						
Std. M MQ.***	0.179 (Sigma Aldrich)	100 (AcOH)								
II MQ*** BE 5.19; 15 m***	0.087 (VWR)	35.5 (MQ) 14.5 (H_2SO_4 95-97%)	299.199 (MQ) 0.801 (H2SO4 95-97%)	0.0210						20
Std. M. MQ***	0.087 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0201 (=BE 5.19)						
III MQ*** BE 5.19; 15 m	0.087 (Bernd Kraft)	50 (2M HCl)	297 (MQ) 3 (2M HCl)	0.0201						
Pippette and volume change for Std.'s **										
IV (x2) MQ	0.045 (VWR)	50 (AcOH)	248.5 (MQ) 1.5 (AcOH)	0.0106	100	10**	20**	40**	80**	20
IV BE8.19, 15 m			249.25 (MQ) 0.75 (AcOH)	0.0110 (=BE 8.19)						
V MQ BE 8.19; 15 m	0.086 (VWR)	37.5 (MQ) 12.5 (AcOH)	249.4 (MQ) 0.6 (AcOH)	0.0114						
VI MQ	0.089 (VWR)	43.75 (MQ) 6.25 (AcOH)	249.7 (MQ) 0.3 (AcOH)	0.0120						
(a) BE 8.19; 15 m	Same chemicals used as VI MQ. The data were lost due to software malfunction.									
VI (b) BE 8.19; 15 m	0.086 (VWR)	43.75 (MQ) 6.25 (AcOH)	249.7 (MQ) 0.3 (AcOH)	0.0107	100	10**	20**	40**	80**	20
I BE 10.19; 15 m	0.088 (VWR)	6.5 (MQ) 43.5 (HCl 37%)	198.3 (MQ) 1.7 (HCl 37%)	0.0113						
* Std. 1 & 2: 1-10 µL Eppendorf Reference (2863776),										
Std. 3 & 4: 20-200 µLThermo Electron FINNPIPETTE (internal inventory number: CH02919 4500)										
** 10 to 100 µL Eppendorf Research plus (L13439B)										
*** Theses experiments were measured before identification of the Valco Valve leakage.										

Table 4: Reagent preparation for method optimization experiments.

2.2.2 Sample preparation

The samples were prepared in laboratory air. All tests were performed in MQ and repeated with Boknis Eck water from 15 m depth (different cruises, see Table 4). Water from Boknis Eck was stored until usage, directly after the cruise, unpoisoned in bottles or canisters in the refrigerator at 3.3 °C. Before sample preparation the water allowed to stand one night in a 2 L beaker glass (Kimble Chase, Vineland, USA, KIMAX boro 3.3, 64000) covered with a paper towel in the labaratory to ensure equilibration with the lab air. The sample bottles were left overnight in the lab covered with a paper towel. The pipetting sample water volume was always 10.3 mL, equal to the volume of Boknis Eck samples with headspace. The samples were prepared using a 10 mL volumetric pipette and a 0.1 to 1 mL adjustable volume pipette (Eppendorf, Research, 3434651) with 0.3 mL as pipetting volume. I am referring to the fact that some samples may have different volumes of chemicals added due to the fact that not all samples were treated in the exact same way. The volume difference (up too 400 µL) was mathematically corrected. The sample treatment plan was like for a cruise, N_2O background samples, NH_2OH background samples and additional four different concentrations of Std. addition (Std. ADD) samples were prepared, to determine the Recovery Factor of NH_2OH to N_2O. In addition to the normal cruise samples SA-Acid control samples were prepared for the laboratory experiments to identify if N_2O production or decomposition was mediated by SA-Acid. In general, all samples were prepared in triplicates, for each method and each medium 21 samples were prepared (Table 5). The SA-Acid SOLs and FAS SOLs were added for all lab method optimization experiments using the HAMILTON repeating dispenser, except test IV, where due to the larger injection volume of 200 µL of the SA-AcOH SOL a 1 mL disposable syringe with a Ø 0.5 x 40 mm needle was used.

Samples in triplicates (A, B, C)		**Addition of chemicals**			**With** adjustment needle	**Without** adjustment needle
		1	2	3		
Code	**Purpose**	in µL				
A1 to C3	N_2O background	50 $HgCl_2$			1) all chemicals injected 2) adjustment canula removed 3) 2 min shaken	1) chemical 1 injected 2) 2 min shaken 3) chemical 2 injected 4) 2 min shaken 5) chemical 3 injected 6) 2 min shaken
A4 to C6	N_2O SA-Acid contol	100* SA-Acid				
A7 to C9	NH_2OH background	100* SA-Acid	100 FAS			
A10 to C12	HACl Std. ADD 1	100* SA-Acid	100 Std. 1	100 FAS		
A13 to C15	HACl Std. ADD 2	100* SA-Acid	100 Std. 2	100 FAS		
A16 to C18	HACl Std. ADD 3	100* SA-Acid	100 Std. 3	100 FAS		
A19 to C21	HACl Std. ADD 5	100* SA-Acid	100 Std. 4	100 FAS		
* except experiment IV, doubled SA-Acid volume (200 µL)						
I (MQ a & b, Std. M.), **II** (MQ, BE, Std. M.), **III** (MQ, BE), **IV** (MQ, BE)						
I (BE), **V** (MQ, BE), **VI** (MQ, a & b BE)						

Table 5: General experiment structure, of laboratory method optimization experiments.

2.3 Boknis Eck samples

This Section describes all experiments and Std. M. measurements which were conducted with real samples from Boknis Eck.

2.3.1 Reagent preparation

The reagent preparation for the different cruises is described in Table 6. The Table 6 is in chronological order of the measurements; some experiments were measured before the identification of a defective Valco Valve in the GC (further information Section 2.4.2) and pipettes were changed before the August cruise. The FAS, sulfanilamide SOLs, the HACl Stock SOLs and the HACl Std. SOLs were prepared, handled and stored as described in Section 2.2.1. A 2% $HgCl_2$ SOL (2 g $HgCl_2$ DISS in 100 mL MQ) was used as poisoning SOL.

Cruise Method	SA SOL		Std. MQ	Stock SOL		Std. SOL				
	SA / g	DISS / mL (in)	MQ / mL Acid /ml	HACl / g	DISS in Std. MQ / mL	Std. 1	Std. 2	Std. 3	Std. 4	DIL in Std. MQ / µL
						Stock SOL / µL				
May 19**** Std. M.	0.087 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0201	100	5*	10*	20*	40*	20
Jun. 19 Std. M.	0.091 (Bernd Kraft)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0215	100	5*	10*	20*	40*	20
Jul. 19 Std. M.	0.090 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0212	100	5*	10*	20*	40*	20
Jul. 19 Pipette Exp.						5**	10**	20**	40**	
Pippette and volume change for Std.'s * **										
Aug. 19 Std. M.	0.088 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0110	100	10	20	40	80	20
No cruise in Sept.19 due to no available ship.										
Oct. 19 Std. M.	0.085 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0107	100	10***	20***	40***	80***	20
Oct. 19 IV (x2 SA-AcOH)	0.043 (VWR)	50 (AcOH)	248.5 (MQ) 1.5 (AcOH)	0.0101						
Oct. 19 VI	0.085 (VWR)	43.75 (MQ) 6.25 (AcOH)	249.7 (MQ) 0.3 (AcOH)	0.0112						
Dec. 19 Std. M.	0.086 (VWR)	50 (AcOH)	249.25 (MQ) 0.75 (AcOH)	0.0102						
Dec. 19 IV (x2 SA-AcOH)	0.043 (VWR)	43.75 (MQ) 6.25 (AcOH)	249.7 (MQ) 0.3 (AcOH)	0.0110						
* Std. 1 & 2: 1-10 µL Eppendorf Reference (2863776) Std. 3 & 4: 20-200 µL: Thermo Electron FINNPIPETTE (internal inventrory number: CH02919 4500)										
** 1 to 10 µL Eppendorf Reference (2863776) *** 10 to 100 µL Eppendorf Research plus (L13439B)										
**** The samples of the May cruise were measured before identification of the Valco Valve leakage.										

Table 6: Reagent overview for samples from the Time Series Station Boknis Eck.

2.3.2 Sampling technique

Samples were taken on the monthly Boknis Eck cruises with the research vessel Littorina, from May 2019 to October 2019 and in December 2019 for a repetition of a method validation experiment, except for September when the cruise had to be cancelled. The samples were taken in triplicates each from 6 different depths (1 m, 5 m, 10 m, 15 m, 20 m, 25 m) using a Rosette of six 5 L Niskin bottles with an attached CTD and oxygen sensor. For each depth, N_2O background samples and NH_2OH conversion samples were taken. An additional set of 12 samples was taken from 15 m for the addition of HACl Std.s. To ensure minimum exchange of the atmosphere with the seawater, which could alter the N_2O content of the samples, the sampling for trace gases and NH_2OH was carried out before any

other samples were drawn from the Niskin bottles. No more than 2/3 of the bottle volume was emptied for the trace gas samples, otherwise the samples were taken from a fresh CTD cast. Samples were filled from the bottom using 8 mm silicon hose, left for overflowing at least one time the sample volume and closed under the running water stream, thereby avoiding any bubbles. Each sample was inspected for gas bubbles and the sampling was repeated if bubbles were detected.

2.3.3 Sample posttreatment

Samples were treated for conservation and chemical conversion at GEOMAR directly after returning from the cruise. Sample treatment comprised the poisoning of the background N_2O samples, the conversion of NH_2OH using the standard method (Std. M.) and several additional NH_2OH conversion experiments in selected months. For these experiments, an additional set of BE samples was taken from the Niskins during the BE cruise.

2.3.3.1 N_2O samples

First, a 3 mL adjustment needle was attached into the sample, with the tip remaining close to the top of the sample vial. The poisoning was done by adding 50 µL of the 2 % $HgCl_2$-SOL (2g $HgCl_2$ DISS in 100 mL MQ) via the septum. The tip of the needle from the poisoning syringe was pushed as far as possible into the sample. The $HgCl_2$ SOL sank to the bottom of the vial due to its high density, thereby ensuring that the water that is expelled via the adjustment syringe is free of $HgCl_2$. After the poising was completed the adjustment needle was removed, and when all samples were poisoned the samples were shaken for 2 min by hand.

2.3.3.2 NH_2OH samples

Samples were headspace before the addition of the chemicals for NH_2OH conversion: 10 mL helium (He, 99.9999 %, Air Liquide, Düsseldorf, Germany) was added with a gas-tight glass syringe (VICI Precision Sampling, Baton Rouge, LA, USA). The water that was replaced by the headspace and was collected in an empty 20mL syringe with .8 x 120 mm needle that was attached to the sample vial before headspace injection. This syringe was removed and the water expelled from the samples was discarded. Two different injection techniques of the chemicals (with/without adjustment syringe) were used. This causes a slight variation in the sample water volume after the addition of the chemicals; this variation was mathematically corrected. The sample treatment procedure for the NH_2OH samples of cruises is described in Table 7 and additional experiments and their sample treatment procedure is described in the following Table 8. From May till Aug. 2019 the HAMILTON repeating dispenser was used to add the SA-AcOH SOLs and the FAS SOLs. For Oct. 2019, the Eco-Matic was used to inject the SA-AcOH SOLs and the HAMILTON repeating dispenser to inject the FAS SOLs. From Dec. 19, the Eco-Matic was used to inject both the SA-AcOH SOLs and the FAS SOLs. The additional samples for the Std. ADD are used to determine the conversion of NH_2OH to N_2O, as the reaction is not quantitative and the recovery is not constant. The variations of the Recovery Factor make it indispensable to determine the conversion for each cruise, with the Recovery Factor the NH_2OH concentrations of the depth samples are calculated.

NH_2OH samples in triplicates	**Addition of chemicals**			**With** adjustment needle	**Without** adjustment needle
	1	2	3		
	in µL				
1 m	100 SA-AcOH	100 FAS		1) all chemicals injeccted 2) adjustment canula removed 3) 2 min shaken	1) chemical 1 injected 2) 2 min shaken 3) chemical 2 injected 4) 2 min shaken 5) chemical 3 injected 6) 2 min shaken
5 m	100 SA-AcOH	100 FAS			
10 m	100 SA-AcOH	100 FAS			
15 m	100 SA-AcOH	100 FAS			
15 m Std. ADD 1	100 SA-AcOH	100 Std. 1	100 FAS		
15 m Std. ADD 2	100 SA-AcOH	100 Std. 2	100 FAS		
15 m Std. ADD 3	100 SA-AcOH	100 Std. 3	100 FAS		
15 m Std. ADD 4	100 SA-AcOH	100 Std. 4	100 FAS		
20 m	100 SA-AcOH	100 FAS			
25 m	100 SA-AcOH	101 FAS			
May 19, **Jun.** 19, **Jul.** 19					
Oct. 19, **Dec.** 19					
Additionally: N_2O background samples for all depths (triplicates) + 50 µL $HgCl_2$ SOL (2 %)					

Table 7: Std. sample treatment procedure of the BE cruises.

Samples in triplicates A, B, C at 15 m depth		**Addition of chemicals**			**With** adjustment needle	**Without** adjustment needle
		1	2	3		
		in µL				
A 1 to C3	15 m NH_2OH	100* SA-Acid	100 FAS		1) all chemicals injected 2) adjustment canula removed 3) 2 min shaken	1) chemical 1 injected 2) 2 min shaken 3) chemical 2 injected 4) 2 min shaken 5) chemical 3 injected 6) 2 min shaken
A4 to C6	Std. ADD 1	100* SA-Acid	100 Std. 1	100 FAS		
A7 to C9	Std. ADD 2	100* SA-Acid	100 Std. 2	100 FAS		
A10 to C12	Std. ADD 3	100* SA-Acid	100 Std. 3	100 FAS		
A13 to C15	Std. ADD 4	100* SA-Acid	100 Std. 4	100 FAS		
* except Exo. IV, doubled SA-Acid volume (200 µL)						
Jul. 19: Pipette Exp. (A1 to C3 not needed, Std. M.) **Oct. 19:** method validation **IV** and **VI**						
Dec. 19: method validation **IV**						

Table 8: Sample structure of experiments conducted additional to Std. samples.

2.4 NH_2OH and N_2O measurements

The samples were analyzed with a gas chromatograph equipped with an electron capture detector (Agilent Technologies, Santa Clara, USA, GC-ECD, Hewlett-Packard 5890 Series II). A 6' 1/8" stainless steel column packed with a 5 Å molecular sieve (W. R. Grace & Company, Maryland, USA) was used. The oven was held at 190°C and the detector at 350°C. As carrier gas a mixture of 95.05 % argon and 4.95 % methane (AIR LIQUIDE, Paris, France,

CRYSTAL-Gemisch, ECD purity grade) was used. The flow rate was set to 36 mL min^{-1}. Samples were manually injected into a custom-made injection port that was equipped with a ~5mL glass tube filled with Sicapent(R), which was connected to a 2-Position Valco valve (VICI International, Schenkon, Switzerland) which was used for the filling and injection of a sample loop. Loop injection guarantees the injection of a constant sample volume and injection rate to the GC column.

2.4.1 Headspace equilibration method (N_2O)

Headspace addition was carried out as described in Section 2.3.3.2. During the N_2O equilibration, the 20 mL syringe that held the expelled water was kept attached to the sample vial. The sample was shaken for ~20 s using a Vortex Genie 2 shaker (Scientific Industries Inc., New York, USA, G-560E). and left to equilibrate for at least 2 h. Afterwards, a 9 mL subsample of the headspace was drawn from the headspace.

2.4.2 Calibration

In the beginning the injection port was purged with 160 mL He. To ensure that purging was successful a triplicate of He blanks (9 mL He purging syringe + 9 mL He sample syringe) was measured. If no peak was detected for the helium blanks, the calibration was started; if not, the purging procedure was repeated. The GC was calibrated each day by manual injection 9 mL of two different N_2O standard gas mixtures from Deuste Steininger GmbH (Mühlhausen, Germany, 355.8 ppb and 1044.6 ppb) using a gas-tight syringe. The standard gases were calibrated at GEOMAR against the NOAA PMEL-ARS-416396 standard. In addition to the pure standards, at least one dilution of the standard was measured. Standards

were diluted by filling the desired volume of standard into the gas tight syringes and subsequent filling of the syringe to 9 mL using a custom-made ~600 mL glass cylinder with a septum port. The gas cylinder was filled with He that was brought to atmospheric pressure prior to the dilution. All different standards were injected three times or more. The range of the standard dilutions was always chosen to exceed the range of the measured samples.For some samples with peak areas lower than the peak areas of Std., an additional Std. dilution was measured in triplicates (mostly: 3 mL Std. 355.8 ppb + 6 mL He). The precision of the standard measurements was determined on a daily basis before the start of the sample measurements. In case the standard deviation exceeded 3 %, the GC injection system was checked for leaks and the calibration was repeated until the precision was sufficient.

Some of the experiments may be compromised by the presence of a leak within the injection valve (Valco Valve) of the GC that was identified as defective on 28th of June 2019. Although the calibration measurements prior to the detection of the leak did not indicate a systematic error of the measurements, it cannot be excluded that the samples were contaminated with laboratory air or that part of the sample was lost. The Valco Valve was exchanged. I therefore chose to mark those experiments that are potentially compromised as overview in Table 4 and Table 6 and during evaluation in Section 3 and for the data Appendix III.B, III.C, III.E and III.F.

2.4.3 Sample analyses

To increase the efficiency of the GC measurements, N_2O was measured with two consecutive injections to the GC within 3 minutes. For NH_2OH, it was noticed that this kind of consecutive injection could introduce some uncertainty due to additional peaks that would overlap with the second peak. For the method optimization experiments the second peak could be related to the SA-Acid, because this second peak occurred only for samples including SA-Acid.

2.4.4 Peak identification and calculations

The peak areas were indicated by manual integration using the software ChromStar Version 6.3 (05.12.2016, Software für Chromatographie und Prozessanalytik GmbH, Weyhe-Leeste, Germany).

Since the ECD response is known to be not exactly linear, the detector response was fitted with a quadratic fit with intercept=0 (Equation 1), with peak area (PA) the calibration coefficients a and b and x_{N2O} for the mole fraction of the measured sample (in ppb). It was especially important that the peak areas of the samples was in the range of the standard peak areas.

$$PA = a{x_{N2O}}^2 + bx_{N2O} \quad (1)$$

The mole fraction of the headspace (x_{HS} in ppb) was calculated by using the pq-formula (Equation 2).

$$x_{HS} = -\frac{b}{2a} \pm \sqrt{\left(\frac{b}{2a}\right)^2 + \frac{PA}{a}} \quad (2)$$

The amount of N_2O in the headspace of the sample (n_{HS} in nmol) was determined with the ideal gas law (Equation 3), with p_{atm} for the pressure (1 atm = 101325 Pa), V_{HS} for the volume of the headspace (in m^3), R for the ideal gas constant (R = 8.3145 J mol^{-1} K^{-1}), and T_{eq} for the equilibration temperature (in K).

$$n_{HS} = \frac{x_{HS} p_{atm} V_{HS}}{1000 R T_{eq}} \quad (3)$$

The N_2O concentration in the water phase (C_w in nmol L^{-1}) was calculated as shown in Equation 4, with S for the salinity in psu; and the solubility coefficients

in mol L^{-1} atm^{-1}: A_1 (-165.8806), A_2 (222.8743), A_3 (92.0792), A_4 (-1.48425), B_1 (-0.056235), B_2 (0.031619) and B_3 (-0.0048472). (Weiss et al., 1980).

$$C_w = e^{\left(A_1+A_2\left(\frac{100}{Teq}\right)+A_3\left(\ln\left(\frac{Teq}{100}\right)\right)+A_4\left(\frac{Teq}{100}\right)^2+S\left(B_1+B_2\left(\frac{Teq}{100}\right)+B_3\left(\frac{Teq}{100}\right)^2\right)\right)} x_{HS} p_{atm} \quad (4)$$

The total N_2O concentration (C_0 in nmol L^{-1}) was calculated with Equation 5, with V_w for the volume of the water phase (in L).

$$C_0 = \frac{n_{HS}}{V_w} + C_w \quad (5)$$

2.4.5 NH_2OH calculations

The N_2O concentrations from the converted Std. ADD samples were reduced for the N_2O background, multiplied with two ($2\Delta([N_2O\ Std._n]-[N_2O\ 15\ m]^0)$), results in the NH_2OH (converted) concentrations. These were plotted against the NH_2OH concentrations calculated from the Std. SOL ADDs (100 µL $Std._n$). The slope from the linear fit is equal to the Recovery Factor (R). R represents $NH_2OH_{converted}$ per $NH_2OH_{calculated}$. To determine the NH_2OH concentration for the depth samples Equation 6 was used, $[N_2O]$ as the N_2O concentration after oxidation and $[N_2O]^0$ as the N_2O background concentration. (Gebhardt et al., 2004)

$$[NH_2OH] = \frac{2\left([N_2O]-[N_2O]^0\right)}{R} \quad (6)$$

The arithmetic mean is calculated from the sample (N_2O and NH_2OH) triplicates (SD in nmol L^{-1}, see Equation 7). F_n stands for the scalation coefficient dependent on the amount of repartition samples, for triplicates (F_3 = 1.91) and for duplicates (F_2 = 1.52). (David, 1951)

$$SD = \frac{\max(C_{0,1} \ldots C_{0,n}) - \min(C_{0,1} \ldots C_{0,n})}{F_n} \quad (7)$$

Equation 8 was used to determine the error of the NH_2OH samples. The formula is the Gaussian error propagation from Equation 6. The deviation $SD[N_2O]^0$ and $SD[N_2O]$ for the triplicates were calculated according to Equation 7. Δw is the error of the slope.

$$\Delta[NH_2OH] = \pm\sqrt{\left(\frac{2}{R} SD[N_2O]\right)^2 + (\frac{-2}{R} SD[N_2O]^0)^2 + (\left(\frac{-2}{R^2}[N_2O] + \frac{2}{R^2}[N_2O]^0\right)\Delta w)^2} \quad (8)$$

The measurement errors for the Std. addition (ADD) ($\Delta[NH_2OH_{Std.ADD_n}]$ plotted in the Recovery Factor determination were calculated with Equation 9, n stands for the different Std. ADD concentrations (1 to 4). The standard deviation (SD, calculated according to Equation 7) from the sample triplicate measurements was used and then multiplied by two as the N amount is plotted and not the N_2O amount.

$$\Delta[NH_2OH_{Std.\ ADD_n}] = \sqrt{\left(SDN_2O_{15m}{}^2 + SDStd.ADD_n{}^2\right) \times 2} \quad (9)$$

All Boknis Eck water NH_2OH samples were corrected for the dissolved inorganic carbon (DIC). Upon lowering the pH to ≤4, DIC is mobilized nearly completely from the samples in form of CO_2. (Wolf-Gladrow et al., 2007). The NH_2OH samples of all tested methods had, due to acidification, a pH below 4. As gas carbon dioxide equilibrates as well, dilutes the N_2O concentration of the headspace and increases the pressure in the sample vials. A dummy fraction was used to calculate the outgas of CO_2, using the solubility formula Equation 4 and the CO_2 solubility coefficients (Weiss et al., 1980). 99.2 % of the total CO_2 amount outgassed in the headspace. The dummy amount of CO_2 in water phase and

headspace were calculated using the ideal gas law. Proportions from the dummies (concentration independent) were used to calculate the amount and volume of CO_2 in the BE samples. Under the assumption that the DIC concentration at Boknis Eck Time Series Station had no high variability, 2000 µmol kg^{-1} (DIC) were estimated. The dilution effect was between 0.164 to 0.194 mL (20.137 to 23.797 µMol) (respective different volumes: water, chemical, vial; with and without adjustment needle). The dilution influence seems rather small (10 mL headspace: 1,64 to 1.94 %), but if the difference between $[N_2O]$ and $[N_2O]^0$ is small, this can be a critical factor to avoid negative NH_2OH concentrations. The more detailed calculation way and the exact values are presented in Section Appendix III A.

3 Results and Discussion

The error bars in Std. ADD graphs in Section 3 from Figure 6 – 23, 27 - 31 and the Appendix III D in Figure A and B reflect the uncertainty introduced by the NH_2OH and N_2O measurements in the determination of the Recovery Factor according to Equation 9 (Section 2.4.5).

3.1 Equipment

3.1.1 Adjustable pipettes

3.1.1.1 Pipette experiment at Boknis Eck

In theory, adjustable pipettes are an accurate, fast and easy way to dispense a certain volume. In the process of the method optimization experiments and Boknis Eck sampling, I chose to verify if the use of different adjustable pipettes for the Std. SOLs could explain part of the variance in the Recovery Factors from the Boknis Eck cruises. As a first test, the pipette experiment in July 2019 was conducted with samples from Boknis Eck station; see Table 6 for detailed information on SOLs and pipettes. Two sets of standard additions were prepared using the same Stock solution. It was compared if the measured Std. ADD sample concentrations, and subsequently the Recovery Factor, varies when two pipettes or one pipette was used to prepare the Std. SOLs. The Std. M. was prepared using the same pipettes as for prior Boknis Eck samples and prior method optimization experiments. After this experiment the pipettes were changed. The Pipette Experiment (Exp.) Std. SOLs were prepared using only the smaller pipette (1 – 10 µL) and therefore the higher Std.s SOLs were prepared by multiple

dispensing. The linear fit for the Std. M. is shown in Figure 6 and the linear fit for the Pipette Experiment in Figure 7.

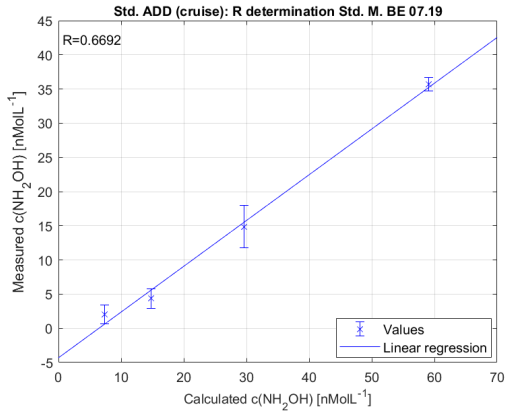

Fig. 6: BE cruise 07.19 Std. M..

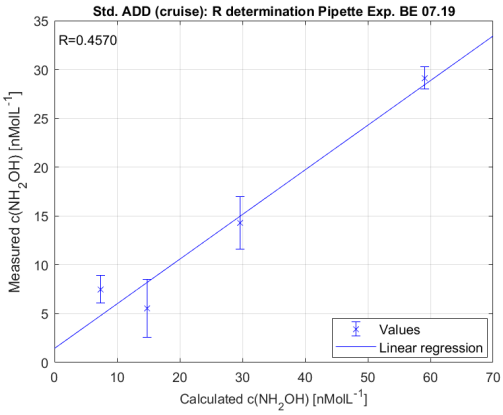

Fig. 7: BE cruise 07.19 Pipette Exp..

The conversion for the Std. M. was 66.92 % and for the Pipette Exp. 45.7 %. The experiment was a first indication that the different choice of pipettes can have an immense influence on the results. In the Pipette Exp. the error of the smaller pipette showed to add up to a big concentration difference: compared to the Std. M., the conversion rate was 21.22 % lower. This may be caused by the multiple pipetting and the accumulation of dispensing errors form the pipette. The difference in the Recovery Factors would have a large influence on the derived NH_2OH sample concentrations as presented in Table 9. The quality of the linear fit indicated by the R^2, displayed also in Table 9, was 3.91 % better for the Std. M. than for the Pipette Exp.. This experiment indicates that the concentrations of the Std. SOLs, and subsequently the conversion rates, are highly dependent on the accuracy of the used pipette, as correct volume dispensing is set as a requirement for the Recovery Factor determination. These results lead to the need to further determine the errors of the different pipettes and their influence on the results.

Cruise	Depth in m	NH_2OH	$\Delta[NH_2OH]$	R	R^2 (slope) in %
		in nmol L^{-1}		in %	/ Δw
Jul. 19	Std. M.				
	1	6.64	2.15	66.92	99.44 / 0.0356
	5	0.48	0.62		
	10	-0.46	3.52		
	15	-4.36	1.82		
	20	3.44	1.36		
	25	2.16	2.29		
	Pipette experiment				
	1	9.72	3.45	45.70	95.53 / 0.0699
	5	0.71	0.91		
	10	-0.68	5.16		
	15	-6.38	2.81		
	20	5.04	2.13		
	25	3.16	3.39		

Table 9: Pipette experiment Jul. 2019 at Boknis Eck station.

3.1.1.2 Pipette uncertainty determination

The pipette uncertainty determination experiment is described in Section 2.1.3.1. The precision and accuracy were calculated. Additionally, a slightly-changed standard deviation was calculated. The mean was substituted with the set volume reasoning; here the focus was on accuracy. The deviation from the mean is not so interesting, but the deviation towards the set pipette volume is. The set pipette volume is used to calculate the concentrations during the standard addition (Std. ADD) and in combination with the measured concentrations to determine the Recovery Factor. The results are presented in Table 10. The precision (%; room temperature, RT) of the Eppendorf Research Plus pipette was better for all volumes compared to the Eppendorf Reference and the Thermo Scientific Electron, despite for 40 µL here the Thermo Scientific Electron was more precise. This tendency was equal for the SD's (%, RT) calculated with the set volume. The accuracy (%, RT) trend of the Eppendorf reference of the Eppendorf Research Plus was not as straightforward. The pipetting with the Eppendorf Reference had a high accuracy. Nevertheless the minimas (-10% and -6 %, RT) and maximas

(8 % and 6 %, RT) deviated intensely. Therefore, this pipette was not reconsidered for further usage. The maxima for the Thermo Scientific Electron was with 8 % (20 µL, RT) was also very high. This pipette reached better accuracy, precision, SD with set V and extreme values for 40 µL than the Eppendorf Research Plus. Nevertheless, the results of the Thermo Scientific Electron did not recommend a further usage. It was chosen to double the dispensing volumes, halve the concentration of the Stock SOLs and prepare the Std. SOLs only with the Eppendorf Research Plus. Nevertheless, the Eppendorf Research Plus was not ideal in terms of some high extreme values.

Pipette			**n**	**T** (MQ) in °C	**Set V** in µL	**Standard deviation**				**Extreme V** (from Set V)				**Accuracy** mean-Set V	
						Precision Regular SD		**SD set V** (not mean)		Min		Max			
						in µL	in %	in µL	in %	in µL	Δ in %	in µL	Δ in %	in µL	in %
Product	ID	V area in µL	V1												
			V2												
			V3												
	Without wiping last drop, if n>2 smallest and biggest; not in calculation														
Eppendorf Reference	2863776	0.5 - 10	20	RT	5	0.09	4.55	0.22	4.45	4.50	-10.00	5.40	8.00	-0.03	-0.63
			20		10	0.33	3.26	0.32	3.18	9.40	-6.00	10.60	6.00	-0.01	-0.09
	V2 = 7.6, 7.9 µL (-24, -21 %)														
Eppendorf Research Plus	L13439 B	1 - 100	30	RT	10	0.20	2.01	0.21	2.09	9.53	-4.7	10.28	2.80	0.06	0.65
			10		40	0.51	1.26	0.71	1.77	39.52	-1.2	41.29	3.23	0.52	1.29
			30		80	0.37	0.46	0.71	0.88	79.5	-0.625	81.59	1.99	0.61	0.76
	V1 = 5.68, 8.29 µL (-43.2,-17.1 %); V3 = 76.41, 78.08 µL (-4.49, -2.4%)														
	L13439 B	1 - 100	30	3 - 6	10	0.12	1.22	0.15	1.47	9.70	-3.00	10.40	4.00	0.08	0.83
			30		80	0.95	1.20	1.44	1.80	75.70	-5.38	80.40	0.50	-1.09	-1.37
	Could not be observed														
Thermo Scientific Electron	CH0291 94500	20 - 200	20	RT	20	0.62	3.03	0.86	4.29	19.6	-2.00	21.6	8.00	0.61	3.03
			20		40	0.32	0.80	0.29	0.71	39.6	-1.00	40.7	1.75	0.16	0.39
	Could not be observed														

Table 10: Pipette uncertainty overview.

Ellis (1973) indicated that adjustable pipettes could have a high variation towards the nominal volume when pipette (25°C) and SOL (0°C) have a different temperature (3 % to 10 % less, see Section 2.1.3.1 for more details). The temperature difference effect was verified for the Eppendorf Research Plus for 10 µL and 80 µL. At 2 to 6°C the precision for 10°µL was better but worse for 80 µL, but the accuracy was worse for both volumes. Still, the differences between RT and 3°C to 6°C were not very high, but at 3°C to 6° the minimal extreme volume was 5.38 % too small. Also at RT the minimal extreme volume was 4.7 % too small. The extreme temperature effect as published by Ellis, 1973

could not be observed to the same extent (accuracy nominal volume 50°µL; -6.6 %, SOL 0°C and pipette 25°C).

The impact of the pipette errors on the Recovery Factor was calculated using the BE cruise Std. ADD data from August 2019 (R = 62.15 %), in contrast to the Std. procedure, where calculated Std. SOL concentration are the fix values. The opposite way was chosen: here the measured $NH_2OH_{converted}$ concentrations were seen as fix and the volume of the Stock SOL added to the different Std. SOLs was reduced or increased by the error of the corresponding pipette.

Eppendorf Research Plus: For RT calculations, the percentual errors of 10 µL (Std. 1, Std. 2) and 40 µL (Std. 3) and 80 µL (Std. 4) were used and for 0 to 6°C 10 µL (Std. 1, 2), and 80 µL (Std. 3, 4).

Eppendorf Reference and Thermo Scientific: These pipettes were used before doubling the dispensed volume and halving the Stock SOL concentrations. Nevertheless, they could be still compared to August 2019, as the percentual errors were used to calculate the impact: 5 µL (Std. 1), 10 µL (Std. 2), 20 µL (Std. 3) and 40 µL (Std. 4).

The results are presented in Table 11. Minor changes in recovery were observed when all Std. were reduced or enhanced by their respective error. The calculation with two strongest out breakers for Std. 1 & 3 towards the nominal volume that the Recovery Factor can be indeed highly influenced during random situations (5%, 0 to 6°C).

Respected Std.	Calculation Style	Eppendorf Research Plus		Eppendorf Reference & Thermo Scientific Electron
		ΔR (RT) %	ΔR (0-6°) %	ΔR (RT) %
all Std.	- Precision	0.18	0.75	0.25
two strongest	+ Accuracy two strongest	-0.65	0.99	-0.33
all Std.	- SD to Set Volume	0.33	1.19	0.29
Std. 1 & 3	- Max extreme value Std. 1 & Std. 3	0.06	-0.24	-0.03
all Std.	Most extreme outbreak (-Min + Max)	-2.22	4.99	-3.03
all Std.	+ Max.	-1.1	-0.14	-0.94

Table 11: Changes in Recovery due % errors from Table 10.

3.1.2 Eco-Matic uncertainty determination

The Eco-Matic error determination experiment is described in Section 2.1.3.2. The results of the Eco-Matic uncertainty determination are displayed in Table 12. For the SA-Acid and FAS addition, the focus was more on precision than on accuracy. The pH conditions should be the same in each sample vial, then the fact that exactly 100 µL SA-Acid is added to each vial. As FAS is added in excess, this experiment was more important for the SA-Acid addition, reasoning if the volume varies much the pH conditions would be different, and the risk would exist that the recovery of the Std. ADD would not be representative for the whole sample batch. During the method optimization experiments it was found out (see Section 3.3 and 3.4) that the recoveries react sensitive to rather small pH changes.

Sample vial size in mL	**Pressing style**	**Total** n	**Considered repetitions**	**Mean** in µL	**Accuracy**	**Precision**		**Extreme values**	
					in uL	SD in µL	SD in %	Min in µL	Max in µL
100	Slow smooth	50	1 - 25	97.63	-2.37	1.80	1.84	93.12	100.80
			26 - 50	98.90	-1.10	2.11	2.14	94.12	102.10
			1 - 50	98.27	-1.73	2.06	2.10	93.12	102.10
50	Slow smooth	25	all	101.87	1.87	2.62	2.57	92.30	104.80
	Nearly hectic	25	all	101.37	1.37	3.09	3.05	94.20	105.40

Table 12: Eco-Matic error determination results.

To eliminate the statistical influence of the higher total n repletion number with the 100 mL sample vial, the values were half splitted and the errors were calculated for the first half, second half and the total amount. Nevertheless, the influence of the doubled repetition amount is minor in this case. The precision was the best with an attached 100 mL sample vial and the slow smooth pressing style, but overall the results were similar. Noticeable is that extreme values have a bigger margin with an attached 50 mL sample vial than with the 100 mL sample vial (slow smooth, both 7.7 to 12.5 µL). The accuracy of the Eco-Matic was relatively low during the experiments, which should not largely affect the FAS conversion, but may influence the final pH from the SA-Acid additions. To

investigate whether the SA-Acid difference in the vial when an extreme case occurs makes a difference or not remained unanswered. A Boknis Eck pH buffer system experiment would be needed to answer the question if the lower or higher dispersion in the extreme cases is braced by the buffer system of the Boknis Eck water or not.

The standard deviation for the nearly hectic injection style is slightly higher (~0.5 %) than for the slow smooth injections (50 mL vial) and also the extreme value delta. As this is calculated only with the two most extreme values, this could be randomness. The statistical information value of the extreme values is not that high, and they should be seen as indication in which area the injection volumes scatter. The standard errors are not strongly dependent on the pressing style. Nevertheless, pressing fast nearly hectic is not advisable. The danger that air bubbles are overseen (~Δ=-25 %) in the self-refilling capsule is much higher if the syringe is used nearly hectically. One eye should always be kept on the self-refilling capsule, only if the capsule is air bubble-free should the injection be done.

To sum up, the Eco-Matic is a very convenient, easy and fast injection help. It is suitable if precision is more important than accuracy. The Eco-Matic is perfect if the injected agent is added in excess, or for acids lower than excess conditions if the extreme difference is braced by the pH buffer system, so that a small volume variation is tolerable.

3.2 Sulfanilamide comparison experiment

The variation from SA-Acid samples to the $HgCl_2$ samples during the method optimization experiments is shown for BE in Table 13 and for MQ water in Table 14. The standard deviation was calculated with the modified SD by (David, 1951) according to Equation 7 (Section 2.4.4). The ΔError results from the addition of the SDs from the triplicates of the $HgCl_2$ and the SA-Acid samples.

For several method optimization experiments the Δ[SA-AcOH-$HgCl_2$] was negative. This indicates that the sulfanilamide may react with N_2O, which seems rather illogical. Additionally, in several cases the error of the Δ could not completely explain the difference of the SA-Acid and the $HgCl_2$ samples. Both inconsistencies lead to the idea of the sulfanilamide comparison experiment to verify if this is caused by the fact that different sulfanilamides from the different brands were used in the beginning.

BE Tests	Sample	SA-Brand / Acid	Mean N_2O in nMol/L	SD N_2O in nMol/L	Δ[SA-AcOH - $HgCl_2$] in nMol/L	ΔError in nMol/L
II*	$HgCl_2$	VWR	28.76	0.37	-1.38	2.70
	SA-Acid	H_2SO_4	27.38	2.33		
III	$HgCl_2$	Bernd Kraft	26.23	0.86	1.26	1.44
	SA-Acid	HCl (2M)	27.49	0.59		
IV	$HgCl_2$	VWR	23.00	0.56	1.77	0.89
	SA-Acid (x2)	AcOH	24.77	0.32		
V	$HgCl_2$	VWR	26.23	0.27	-0.62	0.40
	SA-Acid	AcOH	25.61	0.13		
VI (a)	$HgCl_2$	VWR	24.48	0.17	-0.25	0.28
	SA-Acid	AcOH	24.23	0.11		
VI (b)	$HgCl_2$	VWR	26.35	0.87	-0.17	1.38
	SA-Acid	AcOH	26.18	0.51		
I	$HgCl_2$	VWR	24.36	0.70	-0.81	2.31
	SA-Acid	HCl (37%)	23.54	1.61		
*This Exp. was measured before identification of the Valco Valve leakage.						

Table 13: SA-Acid evaluation of the BE method optimization experiments.

MQ Tests	Sample	SA-Brand / Acid	Mean N_2O in nMol/L	SD N_2O in nMol/L	Δ[SA-AcOH - $HgCl_2$] in nMol/L	ΔError in nMol/L
I (a)*	$HgCl_2$	VWR	24.62	0.85	-1.62	1.73
	SA-Acid	HCl (37%)	23.01	0.87		
I (b)*	$HgCl_2$	Sigma Aldirch	25.99	1.07	1.18	3.08
	SA-Acid	HCl	27.17	2.00		
I Std. M.*	$HgCl_2$	Sigma Aldirch	25.99	1.07	0.10	3.54
	SA-Acid	AcOH	26.09	2.46		
II*	$HgCl_2$	VWR	35.23	0.32	3.36	0.68
	SA-Acid	H_2SO_4	38.59	0.36		
II Std. M.*	$HgCl_2$	VWR	35.23	0.32	-8.41	0.91
	SA-Acid	AcOH	26.82	0.59		
III*	$HgCl_2$	Bernd Kraft	29.24	0.55	1.75	0.79
	SA-Acid	HCl (2M)	30.99	0.24		
IV	$HgCl_2$	VWR	23.20	0.15	1.45	0.62
	SA-Acid (x2)	AcOH	24.65	0.47		
V	$HgCl_2$	VWR	26.27	0.55	0.17	0.94
	SA-Acid	AcOH	26.44	0.39		
VI	$HgCl_2$	VWR	26.27	0.55	-0.82	0.90
	SA-Acid	AcOH	25.46	0.35		
*These Exp.s were measured before identification of the Valco Valve leakage.						

Table 14: SA-Acid evaluation of the MQ method optimization experiments.

The SA comparison experiment was conducted after the Valco Valve exchange and is described in Section 2.1.2. Table 15 shows the core results. The mean N_2O concentrations of the MQ samples varies little. As all of the Δ[SA-AcOH-$HgCl_2$]s are within the according errors, there is no indication that the choice of the different sulfanilamides had a relevant influence. The observed deviation during the method optimization experiments shown in Table 13 and 14 could not be confirmed through the sulfanilamide comparison experiment. From the measured N_2O concentrations, the Bernd Kraft SA showed to have the smallest influence.

Sample	Mean N_2O in nMol/L	SD N_2O in nMol/L	Relative SD (%)	Δ[SA-AcOH - $HgCl_2$] in nMol/L	ΔError in nMol/L
$HgCl_2$	24.79	1.27	5.14		
VWR SA	25.06	0.39	1.54	0.28	1.66
Sigma Aldrich SA	24.32	0.02	0.06	-0.47	1.29
Bernd Kraft SA	24.78	1.41	5.69	-0.01	2.68

Table 15: Core results overview from the sulfanilamide comparison Exp.

3.3 Method optimization

3.3.1 Method optimization lab experiments

The aim of the method optimization experiments was to test whether the pH adjustments could improve the Std. M.. The Recovery Factors achieved in this thesis with the Std. M. are presented together with the associated pH and [H^+] values in Table 16. The pH was verified for each method for the Std. MQs, the MQ tests (MQ + SA-Acid + FAS) and the BE tests (BE + SA-Acid + FAS). The MQ water had a pH of 8.3 and the Boknis Eck water was in the range of pH 7.6 to 7.8. The preparation of the method optimization experiments is presented in Section 2.2. The recovery of the Std. M. varied in this thesis from 56.29 to 66.92 % and with the mean of 61.68 %, leaving out the month May 2019 with 70.26 %, as these measurements were conducted before the identification of the Valco Valve in this month.

Conditions	**R** (during thesis R over 100 % were left out) **[mean]***left out in %	**pH**	**c[H+]** in mol L^{-1}
Std. MQ Std. M.		3.1	7.94E-04
Std. M. MQ	65.71*	2.5	3.16E-03
Std. M. BE	56.29 to 66.92 (70.26*) [61.68]	2.7	2.00E-03
*Exp.s measured before identification of Valco Valve leakage			

Table 16: Std. M. core results.

3.3.1.1 Method I – Acidification with HCl

Samples were acidified with an aqueous HCl SOL (32 %).The overview of the core results achieved with method I: R, R^2, NH_2OH concentration of the

conversion samples (equivalent to depth samples) and their errors, as well as the pH conditions are presented in Table 17.

Method & medium	R in %	R^2 (linear fit) in %	NH_2OH in nMol L^{-1}	$\Delta[NH_2OH]$ in nMol L^{-1}	Condition	pH	c [H+] in mol L^{-1}
I (a) MQ*	3.96	14.46	*-65.40*	17818.04	I Std. MQ	1.3	5.01E-02
I (b) MQ*	35.01	99.86	5.94	171.26	I MQ sample	1.4	3.98E-02
I Std. M. MQ*	*112.90*	99.09	*-2.98*	2.65	Std. M. conditions see Table 16		
I BE	1.53	89.34	42.42	4562.02	I BE sample	1.2	6.31E-02
*These Exp.s were measured before indentification of the Valco Valve leakage.							

Table 17: Core results overview method I.

The Std. ADDs for method I (a), I (b) MQ, I Std. M. MQ and I BE are displayed in Figure 8, 9, 10 and 11. The recovery rates from experiment I (a) (3.96 %) and R^2 (14.46 %) were astonishingly poor. The experiment was repeated with the name I (b) MQ. For experiment I (b) new Std. MQ, SA-HCl SOL, FAS SOL, Stock SOL and Std. SOLs were prepared. To have a comparison value, the Std. M. in MQ was also tested. I Std. M. and I (b) were prepared on the same day using the Std. SOLs the method I (b) MQ.

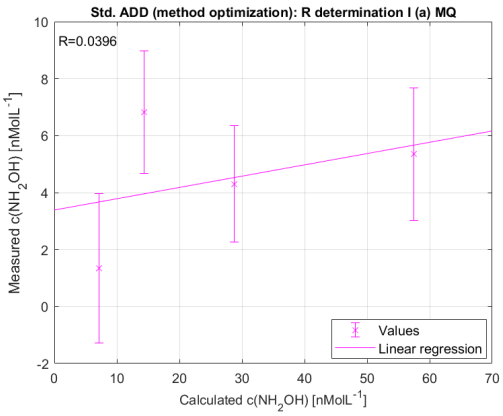

Fig.8: R determination I (a) MQ.

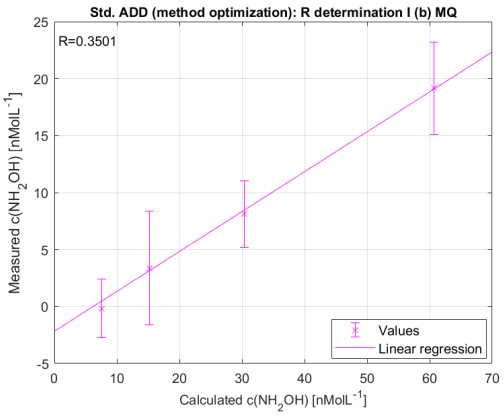

Fig. 9: R determination I (b) MQ.

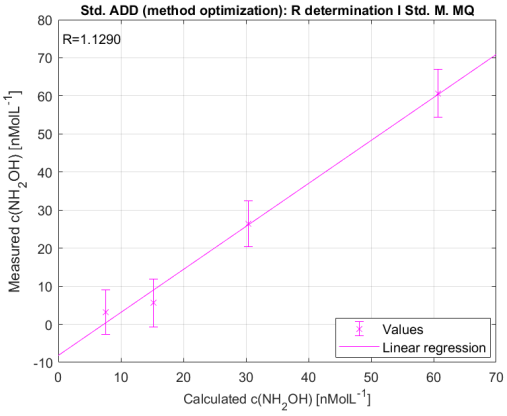

Fig. 10: R determination I Std. M. MQ.

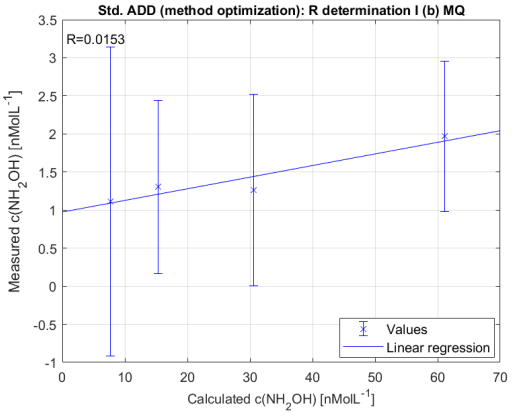

Fig. 11: R determination I BE.

I (b) still resulted in a poor recovery (35.01 %) but still better than I (a). The R^2 of I (b) MQs linear fit was with 99.86% representative. All methods I (a) MQ, I (b) MQ and I BE had extremely high $\Delta[NH_2OH]$ (see above Table 17). The experiment I Std. M. MQ resulted in a much higher conversion rate (112.9 %) and had a R^2 of 99.09 %. This would mean a >100% conversation of NH_2OH to N_2O, which is very unlikely in MQ water. Since the experiments I(a) MQ, I(b) MQ and I Std.M MQ were conducted before the detection of the Valco Valve leakage in the GC, the malfunctioning of the GC valve is a plausible explanation for these results. The defective Valco Valve has as a consequence that the results for I (a) MQ, I (b) MQ and I Std. M. MQ are questionable. The Boknis Eck water part of experiment I BE, with the water from Oct.'s 2019 cruise, was tested later with the new Valco Valve and had a recovery of 1.53 %. The R^2 of the slope was not ideal with 89.14 %. The conversion and the errors of method I (a) MQ, I (b) MQ and I BE were not acceptable at all. The conditions of method I are no alternative to the Std. M..

3.3.1.2 Method II – Acidification with H_2SO_4

Samples were acidified with an 68 % H_2SO_4 SOL. The final pH and the results from the Std. ADD achieved with this method are presented in Table 18.

Method & medium	R in %	R^2 (linear fit) in %	NH_2OH in nMol L^{-1}	$\Delta[NH_2OH]$ in nMol L^{-1}	Condition	pH	c [H+] in mol L^{-1}
II MQ*	20.93	38.74	29.08	351.71	II Std. MQ	1.4	3.98E-02
II BE*	0.08	0.01	*-4930.42*	55510535332.40	II MQ	1.5	3.16E-02
II Std. M. MQ*	65.71	91.91	*-32.28*	29.84	II BE	1.4	3.98E-02
*All Exp.s measured before identification of the Valco Valve leakage.					Std. M. see Table 16.		

Table 18: Core results overview method II.

The experiment was conducted completely before the identification of the Valco Valve leakage (see Section 2.4.2) as well as BE cruise May 2019 (same Std. SOLs II Std. M. MQ). The results from the Std. ADD for II MQ and II Std M. MQ are presented in Figure 12 and 13.

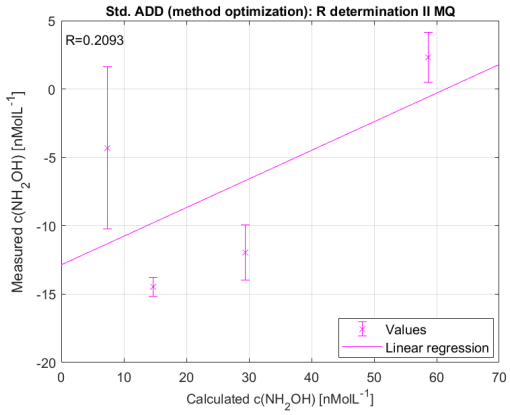

Fig. 12: R determination II MQ.

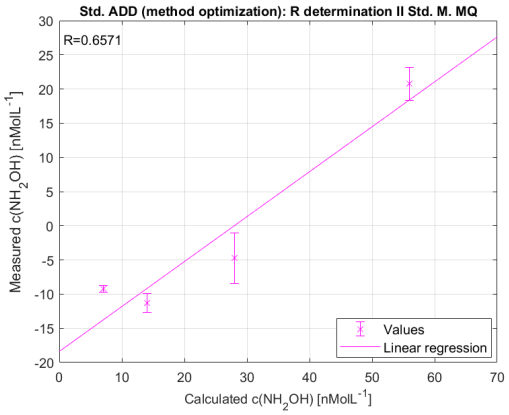

Fig. 13: R determination II Std. M. MQ.

The results from the Std. ADD in BE water are presented in Figure 14 (II BE) and 15 for the Std. M. from the May 2019 cruise. Samples from the lab experiments were prepared in lab air in contrast to the regular BE samples that were headspaced with helium. This lead to higher background N_2O values in the

laboratory experiments. The II MQ had a recovery of 20.93 % and the linear fit was not even close to be representative for these poor values (R^2=38.74 %). The conversion for II BE was nonexistent (0.08%, R^2=0.01 %). This is so insignificant that a conversion from NH_2OH to N_2O could not even be talked about, see Figure 13. The II Std. M. resulted in an expected conversion rate of 65.71 % for MQ and 70.26 % for the BE samples from May 2019. However, the strongly negative intercept of the II Std. M. indicates that the GC leak may have indeed impacted the measurements. Background N2O samples were measured on a different date than the samples from the standard additions. This may have been caused by differences in the GC calibration or by an intensification of the leak over the measurement time. Since the slope of the standard addition seems not to be affected, the use of the data for a general interpretation of the method performance seems justified.

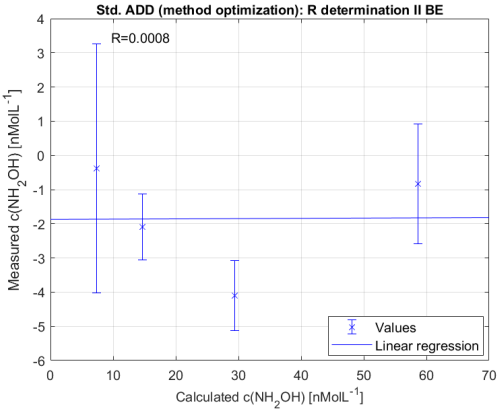

Fig. 14: R determination II BE.

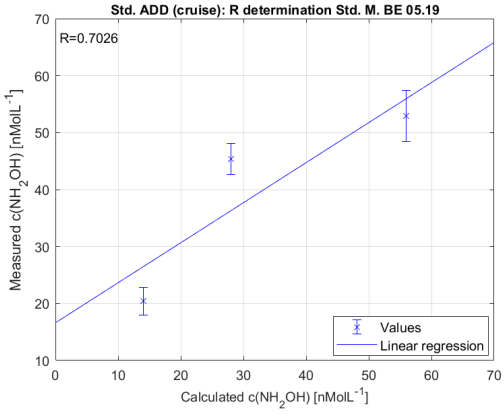

Fig. 15: Std. M. BE cruise 05.19

The NH_2OH background sample concentrations for II MQ and II BE are all in between the $\Delta[NH_2OH]$ (see Table 18). The background concentrations strongly exceeded the concentrations of the standard addition and displayed a large standard error for the II BE experiment. Also for the II Std. M. MQ the seemed compromised: the NH_2OH concentration was negative in the same range as of the $\Delta[NH_2OH]$ but the error could not explain the complete amount of the negative concentration. Method II does not provide an improvement in the Recovery Factor and is not even close to the results under Std. M. conditions. The defective

Valco Valve could have compromised the data, but also with this information the results were too poor to justify further H_2SO_4 acidification experiments even under different pH conditions. Another possible justification for the poor Recovery Factor could be that H_2SO_4 itself reacted as oxidation agent and may have compromised the conversion reaction of NH_2OH to N_2O.

3.3.1.3 Method III – Acidification with HCl

Samples were acidified with a 2 molar HCl SOL. The III MQ part of the experiment was measured before the Valco Valve exchange and the III BE part of the experiment after the Valco Valve exchange. The core results of this experiment are presented in Table 19.

Method & medium	**R** in %	**R^2** (linear fit) in %	**NH_2OH** in nMol L-1	**$\Delta[NH_2OH]$** in nMol L-1	**Condition**	**pH**	**c [H+]** in mol L-1
III MQ*	87.81	98.53	0.47	2.72	III Std. MQ	2.0	1.00E-02
III BE	61.44	98.78	*-2.92*	6.13	III MQ	2.0	1.00E-02
*The experiments measured with a defective Valco Valve.					III BE	1.8	1.58E-02

Table 19: Core results overview method III.

III MQ and III BE were prepared on the same day using the same Std. SOLs. The recoveries are presented in Figure 16 and 17. The recovery for the III MQ was with 87.81 % very promising. However, the more important experiment III BE displayed a Recovery Factor of 61.44%, which is in the range of the Std. M. recovery rates. Negative final NH_2OH concentrations obtained from the III BE experiment could be explained by small differences in the N_2O background measurements and the N_2O measurements after FAS conversion which are within the cumulative uncertainty of the N_2O determination. Despite the high recovery rate and high R^2 from the III MQ experiment, these measurements may be compromised by the leak in the GC system since the method was tested before the exchange of the Valco Valve.

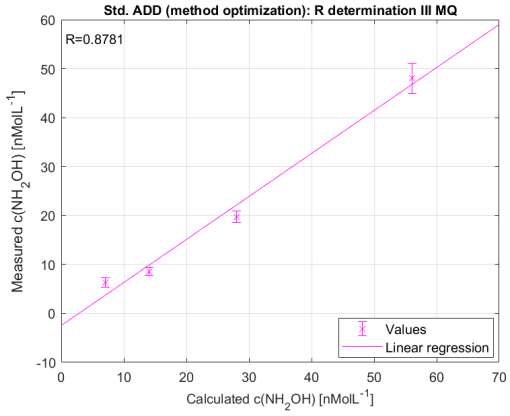

Fig. 16: R determination III MQ.

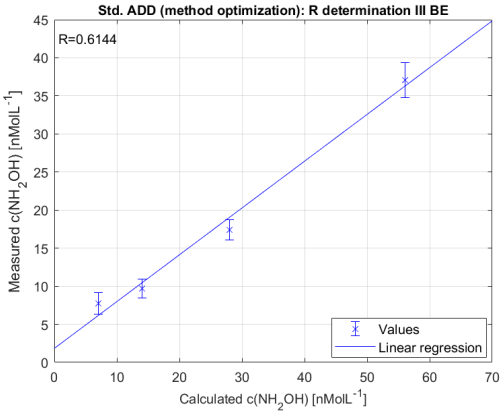

Fig. 17: R determination III BE.

Since the observed experiments with HCl (Method I and III) did not provide convincing results for an improved NH_2OH recovery, the testing of strong acids such as HCl or H_2SO_4 (Method III) was not continued. From then on, more methods were tested using different acetic acid concentrations; acetic acid was also used in the Std. M..

3.3.1.4 Method IV (x2) – Acidification with AcOH

Samples were acidified using acetic acid, like in the Std. M. the sulfanilamide (half compared to Std. M.) was DISS in glacial acetic acid but instead of 100 µL the 200 µL of acetic acid were added to the samples. The core results of method IV are presented in Table 20.

Method & medium	R in %	R^2 (linear fit) in %	NH_2OH in nMol L-1	$\Delta[NH_2OH]$ in nMol L-1	Condition	pH	c [H+] in mol L-1
IV Std. MQ					IV Std. MQ	3.1	7.94E-04
IV MQ	85.99	99.83	1.32	2.28	IV MQ	2.7	2.00E-03
IV BE	75.89	98.80	5.39	1.49	IV BE	2.5	3.16E-03

Table 20: Core results overview method IV.

The Std. ADD is shown in Figure 18 for the IV MQ and in Figure 19 for the IV BE experiment.

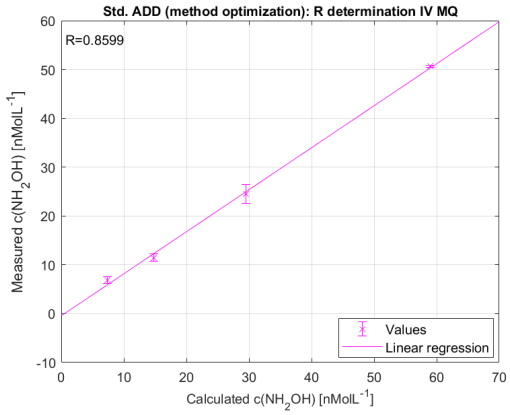

Fig. 18: R determination IV MQ.

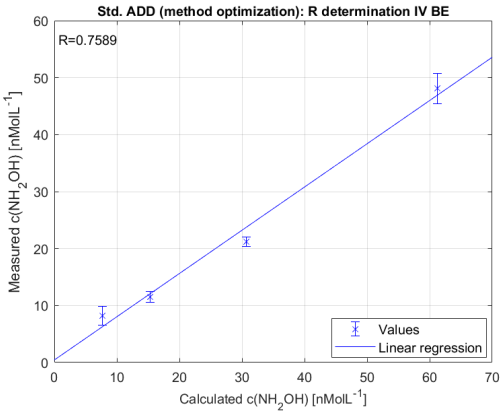

Fig. 19: R determination IV BE.

The conversion rate for the IV MQ experiment was 85.99 % and 75.89 % for the IV BE experiment. Both experiments displayed an R^2 that exceeded 99.8 % and the concentrations of the [NH_2OH] background samples resulted in matching concentrations with respectively low Δ[NH_2OH]. The Δ[NH_2O] IV MQ exceeds the [NH_2OH] IV MQ concentration, thus the it could be set to zero. The Std.'s M. BE cruise mean recovery was 61.68 % (Table 16). The method IV BE reached in the lab method optimization experiment a 14.21 % higher Recovery Factor compared to Std. M. (mean BE). Due to the promising results of the laboratory experiments, this method was further tested with samples from the Boknis Eck Time Series Station (Section 3.3.2).

3.3.1.5 Method V – Acidification with AcOH

Samples were acidified with an aqueous acetic acid SOL (25%), in contrast to the Std. M. where only glacial acetic acid is used. The core results of this method are presented in Table 21. The transfer factor determination for method V is presented in Figure 20 and 21.

Method & medium	R in %	R^2 (linear fit) in %	NH_2OH in nMol L-1	$\Delta[NH_2OH]$ in nMol L-1	Condition	pH	c [H+] in mol L-1
					V Std. MQ	2.6	2.51E-03
V MQ	67.72	99.87	0.23	2.34	V MQ	3.0	1.00E-03
V BE	70.28	99.72	*-1.10*	0.35	V BE	3.2	6.31E-04

Table 21: Core results overview method V.

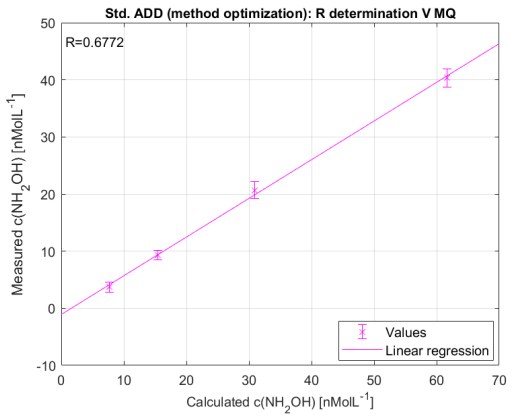

Fig. 20: R determination V MQ.

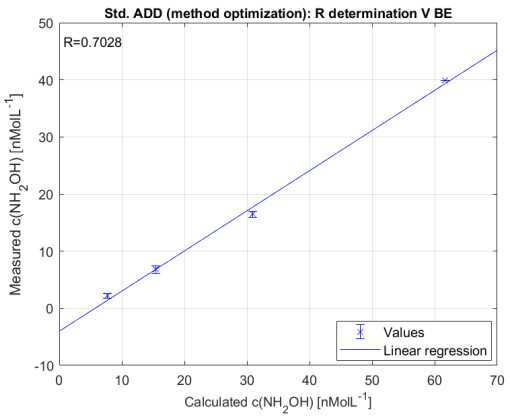

Fig. 21: R determination V BE.

The quality of the linear fit was very high and both experiments reached a R^2 >0.997. Method V reached a solid conversation rate of 67.72 % for the MQ test and 70.28 % for the BE test. Both $\Delta[NH_2OH]$ were quite good and for the V BE extremely low, that the absolute value of the negative background concentration was not completely explained (Δ-0.65 nMol L^{-1}). But this Δ could be explained due to the underestimation of the error as for Std. 4 only one sample concentration was available (data lost: software malfunction). The Recovery Factor of method V was 8.6 % higher compared to the Std. M. (mean BE cruise,

Table 16). The difference in recovery was not high enough, and this method was not tested during method validation with real samples at the Time Series Station Boknis Eck.

3.3.1.6 Method VI – Acidification with AcOH

Method VI was acidified with an aqueous acetic acid SOL (12 %), in contrast to the Std. M. where only glacial acetic acid is used. The core result overview can be found in Table 22.

Method & medium	R in %	R^2 (linear fit) in %	NH_2OH in nMol L-1	$\Delta[NH_2OH]$ in nMol L-1	Condition	pH	c [H+] in mol L-1
					VI Std. MQ	3.1	7.94E-04
VI MQ	80.62	99.93	-1.85	1.31	VI MQ	3.2	6.31E-04
VI BE	82.81	99.97	0.45	2.63	VI BE	3.4	3.98E-04

Table 22: Core results overview method VI.

During this experiment, a white brownish precipitate could be observed at the bottom of the sample vials, in both the MQ and BE experiment. The F^{+III} probably precipitated in form of iron(II)oxide-hydroxide (FeO(OH)) (Hollemann et al., 2007). Generally, if Fe^{+III} precipitated, it could be a potential risk that not enough Fe^{+III} is available for the conversion reaction of NH_2OH to N_2O. However, Fe^{+III} is added in excess, and as the recoveries of method VI are very high, this risk could be nearly certainly excluded.

The data of experiment VI (a) BE was lost due to a malfunction of the software. The experiment was repeated with the name VI (b) BE. The Std. ADD is displayed in Figure 22 for VI MQ and in Figure 23 for VI (b) BE. Method VI reached very promising results. VI MQ had a Recovery Factor of 80.62 % and the linear fit, with R^2=99.93%, equals a very good data representation by the linear fit. VI (b) BE reached a Recovery Factor of 82.81 %, even higher than in

the associated MQ test. The linear fit for VI (b) BE was also optimal and had a R^2 of 99.97 %.

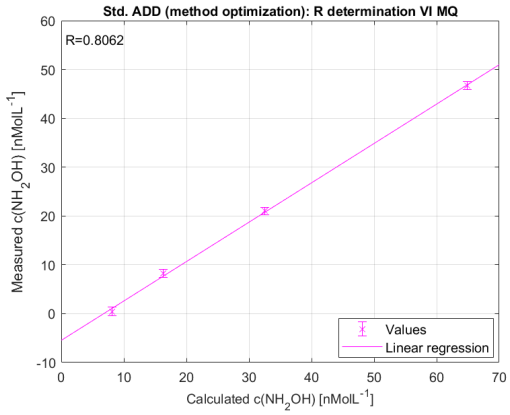

Fig. 22: R determination VI MQ.

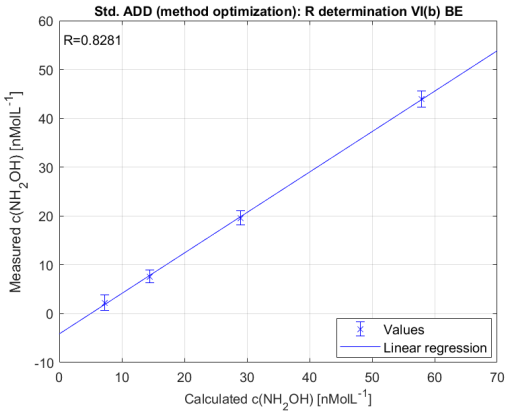

Fig. 23: R determination VI (b) BE.

Method VI reached both in the MQ and BE test higher Recovery Factors than normally achieved by the Std. M.. For VI BE they were 21.13 % higher than for the Std. M. (cruises mean, Table 16). These results were very promising in the lab method optimization experiments and could deliver a real improvement towards the Std. M.. Method VI was therefore tested with real samples from Boknis Eck Time Series Station Section 3.3.2.1.

3.3.1.7 Recovery Factor and pH

The samples' pH conditions ranged from pH 1.4 to pH 3.2 (deionized water) and from pH 1.2 to pH 3.4 (BE). The proton concentration (c[H^+]) in relationship to the Recovery Factor is shown in Figure 24 as overview for all method optimization experiments, and for methods in the pH range from 1.8 to 3.4 in Figure 25. Strong acetic conditions (32 % HCl: I BE pH 1.2, I MQ pH 1.4; 68 % H_2SO_4: II BE pH 1.4, II MQ pH 1.5) had poor recoveries from NH_2OH to N_2O. This is in contrast to the findings of Vajrala et al. (2013); they reported a quantitative

recovery at pH 1.4. Method III acidified with two molar HCl (BE pH 1.8, MQ pH 2.0), had only a high recovery for the MQ experiment, measured before the Valco Valve exchange. The BE part was measured after the exchange of the Valco Valve and the recovery was close to the recovery of the Std. M.. High Recovery Factors could be only observed in both medias (MQ and BE) when acetic acid was used (Std. M., IV, V, VI). Method IV (BE pH 2.5; MQ pH 2.7) was the only tested method where the acid addition volume was increased to 200 µL (100 % AcOH) instead of 100 µL. Method V (25 % AcOH) and VI (12 % AcOH) resulted in pH of 3.2 (V BE, V MQ pH 3.0) and 3.4 (VI BE, VI MQ 3.2 pH). All methods that were tested in higher or lower concentrations (IV, V, VI) of AcOH towards the Std. M. (BE 2.7 pH, MQ 3.1 pH) reached higher recoveries (Std. M. thesis cruise mean Table 15). The pH difference towards the different AcOH methods seems rather small, but Figure 25 shows clearly that the actual $[H^+]$ concentration strongly differs from method to method, reasoning the logarithmic scaling of the pH. If the BE $[H^+]$ concentrations are compared to the Std. M.: method IV had ~ 1.6 times higher $[H^+]$, method V had ~1/3 of the $[H^+]$ and method VI had ~1/5 of the $[H^+]$. These results implicate that the conversion reaction of NH2OH is very sensitive to small pH changes, which mean big $[H^+]$ concentration changes.

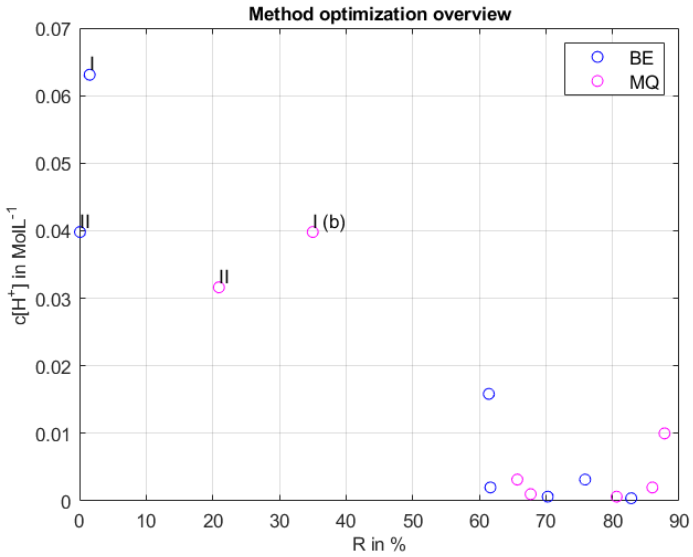

Fig. 24: c$[H^+]$ and R overview. A low pH is only reached with strong acids; all strong acid experiments did not show an improvement towards the Std. M..

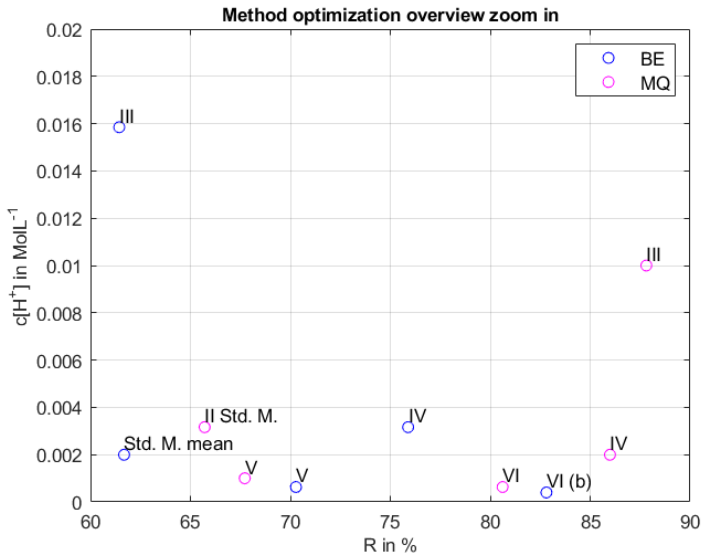

Fig. 25: c[H^+] and R zoom in for methods in the pH range from 1.8 to 3.4.

Generally, for method optimization experiments in BE water, lower proton concentrations had higher recoveries (excluding method III), but this was not consistent with the MQ tests. When only comparing the BE methods, it seems like experiments with pHs between 1.8 and 3.4 pH are interesting and reach similar recoveries (III) or better recoveries (IV, V, VI) than the Std. M. (Table 16), the recovery >100 % and May's R before the Valco Valve exchange were left out. Butler and Gordon, (1986a) advised the pH adjustment between 2.8 and 3.5 with acetic acid to reach ~80 % recovery; lower pHs in natural seawater were not tested but higher ones. They found also a significant differences in recovery between deionized water and natural seawater samples at certain pHs (see Section 1.2, especially Figure 3). For most method optimization experiments the MQ and BE Recovery Factor varied intensely, but not followed an overall trend. The nitrite removal was established later (Kock and Bange, 2013), thus it is possible that the observed recoveries (Butler and Gordon, 1986a) were biased for the natural seawater samples by side reactions with nitrite. On the one hand, method VI showed the highest recoveries of the method optimization experiments in BE water. On the other hand, the Std. M. ranged from (-46 %) 16 % to 86 % (187 %) during the time series observations (2011 – 2017), and reached, in certain months, similar recoveries as method VI (~83 %, BE method optimization Exp.). The conversion of NH_2OH to N_2O had a high dependence on pH. However,

pH is only one influence factor of many. Method validation experiments were conducted at the Time Series Station Boknis Eck, for method IV and VI in October and in December a repetition for method IV (Section 3.3.2).

Brutemark et al. (2011) evaluated a Time Series (1972 to 2009) of pH measurements from the Gulf of Finland in the Baltic Sea. They found high pH values in the summer months and the peak pH was reached in May. The lowest pH were reached during winter from January till February. The total mean was at pH 8.1 and the total span was from pH 7.4 (September 2003) to pH 9.2 (May 1993). Figure 26 shows the pHs during time series observation for the years (a) and during the different months (b). (Brutemark et al., 2011)

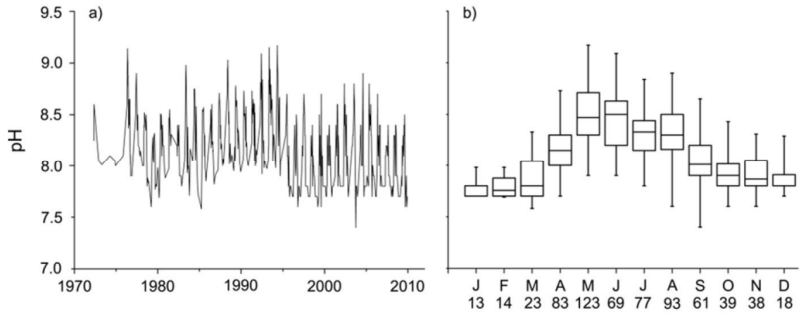

Fig. 26: a) pH from 1972 - 2009. b) pH during the months (1972 – 2009), box represents median.(Brutemark et al., 2011)

The maximal span of pH values from the Gulf of Finland was Δ1.8 pH (Brutemark et al., 2011). The pH of method VI BE was only 0.7 more alkaline than the Std. M.'s pH and had a 20.93 % higher Recovery Factor compared to the Std. M.'s thesis cruise mean (Table 15). However Boknis Eck and the Gulf of Finland are not directly comparable. The implementation of pH measurements at the Time Series Station Boknis Eck could help to clarify if changes in recovery of N_2OH to N_2O could be explained to a certain extend by pH changes in the water column.

3.3.2 Method validation at Boknis Eck

Method validation experiments were conducted in addition to the Std. M. in October and December 2019 at the Time Series Station Boknis Eck. The depth samples were measured with the Std. M.. For all method validation in addition to the Std. ADD a triplicate of NH_2OH samples was taken at 15 m.

3.3.2.1 Boknis Eck cruise Oct. 19 (Std. M., IV, VI)

The core data for October's cruise method validation experiments are presented in Table 23. In October 2019, the Boknis Eck water had a pH of 7.6. The white brownish precipitate described could be also observed at the bottom of the sample vials during the method validation experiment VI; see Section 3.3.1.6 for further details.

Cruise 10.19 Method	R in %	R^2 (linear fit) in %	15 m NH_2OH in nMol L-1	15 m $\Delta[NH_2OH]$ in nMol L-1	pH	c [H+] in mol L-1
IV	98.28	98.87	-1.55	0.87	2.4	3.98E-03
VI	84.55	99.9	-2.38	0.96	3.3	5.01E-04
Std. M.	117.90	99.15	3.62	3.27	2.7	2.00E-03
All pH values were determined with BE water from 15 m (10.19) ; pH of 7.6						

Table 23: Core data overview for the BE 10.19 cruise, including method validation Exp.s.

The Std. ADD data of all three methods reached representative R^2. Method IV was, with 98.87 % (R^2), a bit poorer than the other methods but still sufficient. The NH_2OH concentrations at 15 m for the methods IV and VI resulted to be negative and not completely explained by the $\Delta[NH_2OH]$. The NH_2OH concentration for the Std. M. at 15 m was higher than for the method IV and method VI, but if the $\Delta[NH_2OH]$ are considered the difference is small. I would like to mention that the weather on October's cruise was windy and the sea

surface was turbulent and wavy, which could have caused a bias while driving the Rossette; the depths bouncing was at about 0.5 to 1 m. To reduce this influence, all 15 m (depth of Std. ADD) samples were taken in the same cast by closing all 6 Niskin bottles at once. The Figures 27 to 29 present the Std. ADDs for the Std. M. and the method validation experiments (IV and VI).

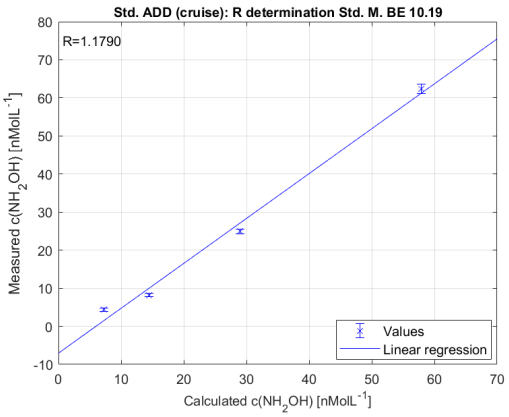

Fig. 27: R determination Std. M. BE 10.19.

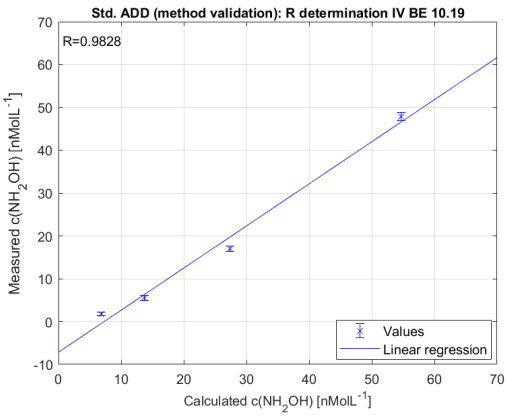

Fig. 28: R determination IV BE 10.19.

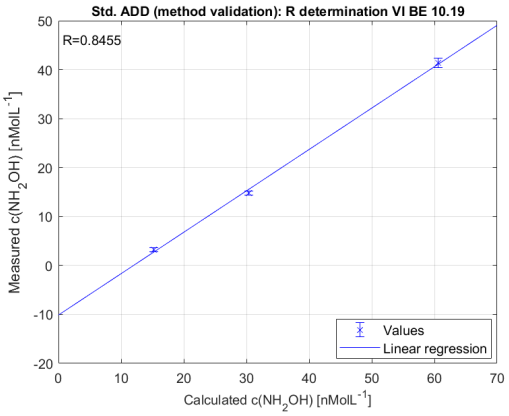

Fig. 29: R determination VI BE 10.19.

Despite the high linearity of the standard additions (R^2 of the linear fit ranging from 98.9 to 99.9 %), the results were somewhat unexpected since the Recovery Factor for the Std. M. exceeded 100% (56.22 % higher mean BE cruise Std. M., Table 16), and the Recovery Factor for Method IV was nearly 100% (high Δ 22.39 % IV BE and 12.29 % IV MQ; Table 20). In May 2016 a Recovery Factor

of 187.37 % was observed, however this was commented with bad Std.. A Recovery Factor of >100% would not be possible for a NH_2OH conversion to N_2O at a 2:1 stoichiometry. Method VI showed a Recovery Factor of 82.8 %, which was in good agreement with the previous laboratory experiments. A strongly negative intercept of the standard addition indicated a mismatch between NH_2OH and N_2O measurements. During the experiment, sample preparation and standard addition were conducted extremely carefully and all sample treatment steps were directly protocolled during the posttreatment procedure. Therefore, I would consider it unlikely that mistreatment of the samples has caused the observed results.

Instead, the reason for the surprisingly high Recovery Factors of the Std. M. and Method IV could be that other conversion reactions of NH_2OH to N_2O lead to a different stoichiometry of NH_2OH to N_2O. This would also mean that another N source was involved in the conversion reaction. It is known that NO_2^- can react with NH_2OH and form nitrous oxide (Einsle et al., 2002). The NO_2^- concentrations in October, 2019 had a high gradient from 15 m to 20 m in $\mu molL^{-1}$: 0.007 (1 m), 0.005 (5 m), 0.008 (10 m), 0.169 (15 m), 0.488 (20 m) and 0.533 (25 m). Compared to October 2018 ($\mu molL^{-1}$: 0.04 (1 m), 0.04 (5 m), 0.05 (10 m), 0.0.6 (15 m), 0.09 (20 m) and 0.012 (25 m)), the NO_2^- concentrations for 15 m and deeper were really high in October 2019. The mean NO^{2-} concentration in 2018 (too many data gaps in 2019) for 15 m was 0 .18 $\mu molL^{-1}$ and for 20 m 0.23 $\mu molL^{-1}$. The Octobers, 2019 had at 15 m average NO_2^- concentration but 20 m was more than twice as high as the average of 2018. Reasoning the high NO_2^- for 15 m to 25 m in October 2019, the turnover N cycle seems enhanced. In May 2016 (R=187.37 %), also a high gradient and NO_2^- concentrations (15 m – 25 m) could be observed ($\mu molL^{-1}$: 0.00 (1, 5 m), 0.01 (10 m) 0.51 (15 m), 0.72 (20 m), 0.82 (25 m). It could be possible that instead of FAS, it was the NO_2^- that oxidized part of the NH_2OH to N_2O, and NO_2^- contributed additional N to this reaction. However, the NO_2^- samples were taken in a different cast; due to the high depth bouncing, the actual NO_2^- concentration in NH_2OH 15 m cast may have been different. The large offset between the NH_2OH and the background N_2O samples together with the high slope of the standard additions indicate that the NH_2OH sampling from October 2019 may be compromised by additional side

reactions. Indeed, the N cycle at Boknis Eck seems to be very dynamic when the summer stratification is broken up and the water column becomes mixed in autumn, with peak concentrations of NH_2OH and N_2O in these periods (Schweiger et al., 2007). It is possible that these conditions favored the high Recovery Factors.

For the R determination of method validation method VI only the samples for the Std. ADD of Std. 2 to Std. 4 were used as the delta of Std. 1, towards the N_2O background concentration, it was negative (with Std. 1: 3.19 % lower). The results for method validation of method VI matched with the corresponding lab method optimization experiments. In the lab experiments method VI reached a conversion rate of 80.62 % at pH 3.2 in the MQ test and a conversion rate of 82.81 % in the BE test at pH 3.4 (with water from August 2019). The pH specified for the October 2019 cruise was for VI 0.1 pH more acidic (3.3 pH) than during the laboratory experiment. If the first Std. would have been left in for the calculations the recovery (VI) would have been 81.36 %, so 1.45 % lower than in the lab VI BE experiment. Without the Std. 1 (VI method validation) the recovery was slightly higher (1.74 %) than in the corresponding lab experiment.

3.3.2.2 Boknis Eck cruise Dec. 19 (Std. M., IV)

The Boknis Eck water in December 2019 had a pH of 7.6 and was in consensus with the 2019 October's cruise (Boknis Eck, 15 m depth). The core data of the method validation experiment in December 2019 are presented in Table 24.

Cruise 12.19 Method	**R** in %	**R^2** (linear fit) in %	**15 m NH_2OH** in nMol L-1	**15 m $\Delta[NH_2OH]$** in nMol L-1	**pH**	**c [H+]** in mol L-1
IV	49.67	85.28	8.62	2.71	2.5	3.16E-03
Std. M.	56.29	98.35	5.92	1.91	2.7	2.00E-03
All pH values were determined with BE water from 15 m (12.19) ; pH of 7.6						

Table 24: Core data overview for the BE 12.19 cruise, including a method validation Exp..

The NH_2OH concentrations at 15 m vary for the Std. M. and the method IV, but if the $\Delta[NH_2OH]$ are respected the concentrations of both methods overlap. The Std. ADD is shown in Figure 30 (Std. M.) and in Figure 31 (IV). The R^2 for the method IV was with 85.28 % very poor, and for the Std. M. with 98.35 % not really ideal but acceptable; Std. 3 was an outlier. The recoveries for the Std. M. (56.29 %) and the method IV (49.67 %), were significantly lower than the Recovery Factors from previous IV method optimization experiments (Table 20, R: IV BE 75.89 %, IV MQ 85.99 %) and from the previous BE samplings (Table 16: mean cruise R Std. M. 61.69 %). I would like to mention that these samples were not measured by myself and that during the measurements a lot was far from ideal. The calibrations of the GC were not conducted properly, for all N_2O and NH_2OH (Std. M.) depth samples and the 15 m NH_2OH for method IV and all Std. M. Std. ADD samples.

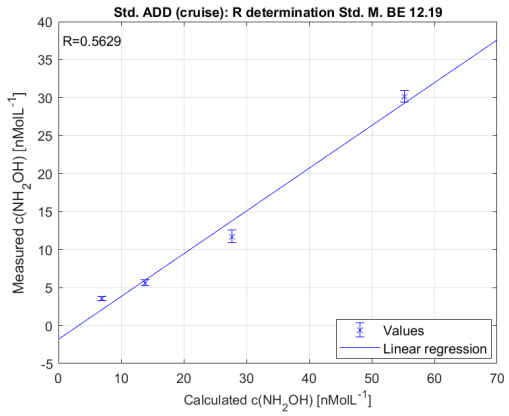

Fig. 30: R determination Std. M. BE 12.19.

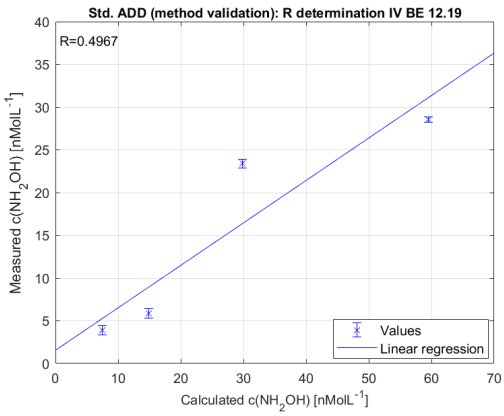

Fig. 31: R determination IV BE 12.19.

The weather during the December's cruise was calm in terms of swell and wind, but the days before were stormy which probably broke up stratification. It was also highlighted by high methane concentrations during this month. The weather conditions before the cruise had probably a high effects on the turnover of the N Cycle. For both method validation experiments in October and December, the weather conditions were not ideal and the results may be compromised to some extent. Generally, a high fluctuation of the Recovery Factors could be observed

throughout the year (Boknis Eck Time Series). For further information see Table 25 in Section 3.4.2. Seven out of ten extreme recoveries (minimum and maximum during a year) from 2013 to 2016 and in 2019 were from August to December left out 2011 and 2017 due to data gaps in this period. However, the Boknis Eck N Cycle seems to have a high dynamic when the summer stratification is broken up and the water column becomes mixed in autumn. Peak concentrations of NH_2OH and N_2O were identified in November (2005) and a fast decrease in December (2005). (Schweiger et al., 2007)

The results of December's method validation experiment (IV) were far from ideal and also highlighted by the bad results of the Std. M. during this month. To sum up the above, the issues described lead me to the conclusion that the December's cruise experiment was not representative for the performance of both the Std. M. and the tested method IV.

3.4 Time Series Station Boknis Eck

3.4.1 Data description of corrections and data gaps

Before 2011: The data collected prior to 2011 were not considered for the revision of the data. Sulfanilamide addition was introduced as a modification to the original method, and samples taken earlier than 2011 could potentially be biased from the presence of nitrite. (Kock and Bange, 2013) & (Kock, 2012)

2011 (status before thesis): The new methodology using sulfanilamide was started 2011 to remove nitrite (Kock and Bange, 2013) and (Kock, 2012). There was no Boknis Eck sampling in January, October and December. In May, the hydroxylamine measurements failed. Data from November should exist, but were not provided. The data was already corrected for the DIC. 2011 the linear regression was derived from $N_2O_{converted}$ to NH_2OH_{total} (calculated from initial weight). The slope ($m_{Std.ADD}$) was multiplied by two to derive the Recovery Factor $NH_2OH_{converted}$ per NH_2OH_{total} (Equation 10). The [NH_2OH] concentration for the depth samples was calculated according to Equation 11. (Kock, 2012) & (Kock and Bange, 2013)

$$R = 2 * m_{Std.ADD} \quad (10)$$

$$[NH_2OH] = \frac{([N2O]-[N2O]^0)}{R} \quad (11)$$

2011 (revised): The NH_2OH concentrations were underestimated by a factor of two due to a calculation error. The [NH_2OH] and Δ[NH_2OH] calculations were repeated.

The first step (Equation 10) is consistent with this thesis. The only difference is the consideration of the factor two after linear regression (Equation 10) and before linear regression (thesis). I calculated the depth sample concentrations according to Equation 6, see further description in Section 2.4.5.(Gebhardt et al., 2004)

$$[NH_2OH] = \frac{2([N_2O]-[N_2O]^0)}{R} \quad (6)$$

Comparing Equation 11 with 6, the factor of two is missing in Equation 11. The factor of two is needed as in both calculations (thesis and Equation 10) the Recovery Factor stands for $NH_2OH_{converted}$ per NH_2OH_{total} and not for $N_2O_{converted}$ to NH_2OH_{total}.

2012 (status): The samples have been measured but the data was not analyzed. Closing this gap was not a task of this master's project. Considering this, 2012 was not respected in this thesis.

2013 (status before thesis): There were no data for January. The data from February to April were not used due to different sample volume injections during the measurements, which resulted from headspace samples with severe underpressure. Underpressure was observed in samples that were stored with headspace for more than 5 months (A. Kock, p.c.). In December, the HACl initial weight was not traceable. The NH_2OH concentrations were underestimated by a factor of 2 as they were calculated equal to the data of 2011 after Kock, Annette; Bange, 2013 and Kock, 2011 without correction of DIC.

2013 (revised): The DIC was corrected for the NH_2OH samples from May to December 2013.The NH_2OH concentrations were recalculated (including the factor of 2 correction, Equation 6), using the new Recovery Factors after DIC

correction. The $\Delta[NH_2OH]$ were calculated according to Equation (8). For the December's cruise, the NH_2OH concentrations were calculated with the median recovery from May 2013 to November 2013. For some months, some depths had negative NH_2OH concentrations. The absolute values of these where reduced through the DIC correction, but still not all these were completely explained by their corresponding $\Delta[NH_2OH]$, further details are provided in the Appendix Section III. D.

2014 (status before thesis): There were no data for January 2014. In July the calibration under seeded the $[N_2O]$; these were calculated with a linear calibration. The N_2O background sample protocol was missing in August. In September there was no HACl initial weight traceable. In October to November, different HS volumes were observed. The NH2OH concentrations were underestimated by a factor of two.

2014 (revised): The NH_2OH samples were corrected for the DIC. The N_2OH concentrations were recalculated with Equation 6 and the $\Delta[NH_2OH]$ were calculated (Equation 8). For July the $[N_2O]^0$ were also calibrated linearly to increase the comparison, but these results remain questionable. The NH_2OH concentrations of July were extremely high (197 to 248 nMol L^{-1}). No median R was used to calculate the September concentrations as the ΔN_2O for 1 to 10 m were negative and the summation of SD ($SD[N_2O]^0$ and $SD[N_2O]$) values was already higher than the ΔN_2O. The $\Delta[NH_2OH]$ were for October and November were, in parts, several times higher than the NH_2OH concentrations.

2015 (status before thesis): In June there were two cruises. During one cruise, first the Std. SOLs were added, then the SA-AcOH SOL was added, followed by the FAS SOL; the samples of the second cruise was treated according to the Std. procedure. Headspace volumes were different for the NH_2OH for August and September. The 15 m NH_2OH samples were left out due to an extreme variation in the triplicate. There was no cruise in October. In December, two NH_2OH

sample batches were gathered; one was treated after the Std. procedure and for the other SA-Acid and FAS were added before headspacing. The NH_2OH concentrations were underestimated by a factor of two.

2015 (revised): The NH_2OH samples were corrected for the DIC. The N_2OH concentrations were recalculated with Equation 6 and the $\Delta[NH_2OH]$ were calculated (Equation 8). The Recovery factor of January was calculated, leaving Std. 3 out due to different headspace volume; in parts the $\Delta[NH_2OH]$ are higher than the NH_2OH concentrations.

2016 (status before thesis): There were no Data from January and February. The Recovery Factor during the May cruise was 187.37 % and was marked with bad Std. In August 2016 the N_2O Peak report was not traceable. The Std. ADD in September had a negative slope and was marked with bad Std (R^2 = 56.99 %.) There were headspace volume issues in October and November for several samples. In December Std. 1 till Std. 3 were negative, and also all depth samples. The NH_2OH concentrations were underestimated by a factor of two.

2016 (revised): The NH_2OH samples were corrected for the DIC. The NH_2OH concentrations were recalculated (Equation 6) and the $\Delta[NH_2OH]$s were calculated (Equation 8). For March, Std. 3 was excluded from the Recovery Factor determination due to a high variance of the triplicates (SD = 8.31 nMol L^{-1}).

2017 (status before thesis): Only NH_2OH data for January, March and November were provided, but in November there were no NH_2OH depth samples, the Std. ADD was tested at different depths. There was no cruise in February. The NH_2OH concentrations were underestimated by a factor of two.

2017 (status before thesis): The DIC was corrected, the NH_2OH recalculated and the $\Delta[NH_2OH]$s calculated.

2019 (thesis): The next Data were the data measured by myself, starting in May to December, despite that in September there was no cruise, and despite that this November was after the laboratory part of this thesis. December was not measured by myself but was needed for a repetition of a method validation experiment. The May cruise was measured before the identification of the Valco Valve leakage. The $\Delta[NH_2OH]$ at 15 m was in June was very high. Most of the data is discussed during this thesis; despite June and August they are presented in the Appendix III.D and III.E. All NH_2OH data are corrected for the DIC and calculated according to Equation 6 and the $\Delta[NH_2OH]$ with Equation 9. In October's cruise, a Recovery Factor of ~ 117 % could be observed.

3.4.2 NH_2OH Boknis Eck Time Series overview

The Recovery Factors during this Time Series are displayed in Table 25. The Time Series Boknis Eck data are provided in the appendix in Section III.G; please find here also additional comments for individual samples.

Std. M. Recovery Factors Time Series Station Boknis Eck in % (rounded; Prot. = Protocol)												
Year/ Month	**Jan.**	**Feb.**	**March**	**Apr.**	**May**	**June**	**July**	**Aug.**	**Sept**	**Oct.**	**Nov.**	**Dec.**
2011	None	64	64	74	None	42	54	60	None/ but c's	None	None	None
2012	No Data provided											
2013	None	None	None	None	56	82	86	70	45	68	87	No HACl data
2014	None	70	74	76	64	69	53	34	No HACl data	60	51	59
2015	61	62	67	55	56	51 60	68	63	65	None	77	72 86
2016	None	None	51	75	187**	63	67	55	Negative R	65	61	16
2017	62	None	67	No Data provided until Apr. 2019								
2019	No Data provided.				70*	61	67	62	no cruise	118	after lab part	56
* Measured before identification of Valco Valve leakage. Start measurements during this thesis.											**min.** (year)	**max.** (year)
** Comment in evaluation sheet "bad Std.".												

Table 25: Std. M. R overview (NH_2OH Time Series).

Sensor in-situ data for salinity, pressure, temperature, Acoustic Doppler Current Profiler, oxygen, carbon dioxide and methane are linked on the Boknis Eck web page (GEOMAR b, 2020) and available for December 2016 to August 2019. The NH_2OH overview plot for the time series is displayed in Figure 32. For the graph, only positive NH2OH concentrations were used; all negative concentrations were set 0, also if not fully explained by the corresponding $\Delta[NH_2OH]$s. Several months were not reconsidered for the plot, reasons are mentioned in Section 3.4.1. Several individual samples were also left out for the plot information. Which data was left out can be found in the Tables of Appendix III G.

Figure 32: NH_2OH concentrations from Time Series Station Boknis Eck.

I would like to mention that the uncertainties of the NH_2OH measurements were, in several months, higher or in the range of the NH_2OH concentrations. The tendencies of the NH_2OH concentrations at Time Series Station Boknis Eck were not straightforward. Nevertheless, NH_2OH concentrations were tendential: NH_2OH enhanced in summer (around July) and in the winter months. Generally, the NH_2OH concentrations have a high variability; peak concentrations were found in January and March 2017. Most of the months showed low or only slightly enhanced NH_2OH concentrations. The majority of NH_2OH concentrations during the time series were similar throughout the water column.

4 Conclusion

Strong acetic conditions (BE I: pH 1.2, BE II pH 1.4) led to poor or no recoveries from NH_2OH to N_2O and showed to have a high negative influence on the conversion reaction. Comparable recoveries as for the Std. M. (thesis cruise mean) were observed for method III (BE pH 1.8, 2 molar HCl). The highest recoveries were reached with different concentrations of acetic acid (IV, V, VI, Std. M.). For most of the method optimization experiments, the variation in recovery, MQ compared to BE, was significant. The highest recovery (82.81 %) was observed with the smallest tested proton concentration (pH 3.4, VI) during the method optimization experiments in BE water. These were in good agreement with the method validation experiment at Boknis Eck (VI 84.55 %). Method IV BE had the second-highest recoveries during method optimization (75.89 %). During the method validation in October and December, the Recovery Factors were significantly different (IV: Oct. 98.28 %, Dec. 56.29 %). The trend was not as clear as for method VI. The Recovery Factor of method IV observed in October would mean a quantitative conversion. However, the Std. M. had also a high variability in recovery from October's 117.9 % till December's 56.29 %. NH_2OH recoveries that exceeded 100 % were found in May 2016 and December 2019 along with high NO_2^- gradients and high NO_2^- concentrations for several depths. That might be an indicator for a stoichiometric change in the reaction and might include NO_2^- as additional N source. It was described that NO_2^- can react with NH_2OH and form nitrous oxide (Einsle et al., 2002).

The implication of pH measurements as an additional parameter at the Time Series Station Boknis Eck could help to clarify the question if changes in pH may explain some part of the Recovery Factor deviations observed during the NH_2OH Time Series. As pHs are buffered in seawater, the pH after SA-AcOH and FAS ADD in the sample vial must be determined, as well.

It would be interesting to test if the NH_2OH concentrations correlate with other parameters from the Boknis Eck Time Series (see Section 1.3). Especially temperature, salinity, oxygen, chlorophyll a, NH_4^+, NO_2^-, NO_3^-. It is well known

that oxygen concentrations have a high influence on the N_2O production via nitrification (Boontanon et al., 2000), (Goreau et al., 1980), which has NH_2OH as intermediate (see Wuchter et al., 2006 and their references). Schweiger et al. (2007), observed peak N_2O and NH_2OH concentrations and an indicative for recovered nitrification during re-oxygenation of the water column was found (November 2005, Boknis Eck).

The removal of the gaseous background, especially for oxygen, for instance by bubbling He through the samples, could be interesting to improve the last few recovery percentages. Butler and Gordon (1986 a) achieved a 4 to 6 % better recovery with removed oxygen. A great side effect would be if the gas background could be removed quantitatively. The background N_2O samples would not be needed to determine the NH_2OH concentrations. This could have the potential to reduce the $\Delta[NH_2OH]$.

On the one hand, method VI showed the highest recoveries of the method optimization experiments in BE water and reached a high recovery during October's cruise. Compared to the thesis' Std. M. cruise mean R (without R: May, Oct.), method VI might have the potential to improve the Std. M.. On the other hand, the Std. M. ranged from (-46 %) 16 % to 86 % (187 %) during the time series observations (2011 - 2017), and reached, in certain months, similar recoveries as method VI. The question, if method VI is an improvement or not, could not be answered finally. To answer that question, a comparison in performance of the methods for a longer period at Boknis Eck is needed. However, generally the conditions of the Std. M. showed the capability to reach high conversion rates. The more important issue was the fluctuation of NH_2OH conversion rates. The measured NH_2OH concentrations are highly dependent on precise, constituent and accurate SOL preparation, sample post treatment and GC analysis. Random extreme events in terms of dispensed pipetting volume during Std. SOL preparation can have a huge influence on the Recovery Factor. Therefore, I recommend implementing a second control instance during this step. There are two options. The first option is to prepare the Std. SOLs also in triplicates and use for each Std. ADD sample a different Std. SOL. Here the concentration difference in the samples accruing from not on point dispensing of

the pipettes is scattered within the Std. ADD of one Std. concentration. The impact of random extreme dispensed volumes on the Recovery Factor would be buffered through the triplicates. But option one has the potential risk that the SDs of the $\Delta[NH_2OH_{Std.ADD_n}]$ may increase, considering the potential higher concentration differences of the Std. SOL added to the Std. ADD. A second option would be to double check the dispensed pipette volume with a fine scale. The initial weights could be used to calculate the actual concentrations of the Std. SOLs more accurately. I prefer option two, as the same Std. SOL is used for the samples of the same Std. ADD, but with an enhanced accuracy in concentration calculations, and the $\Delta[NH_2OH_{Std.ADD_n}]$ might not be potentially enhanced as in option one, resulting in a more accurate determination of the Recovery Factor and therefore also a more accurate determination of NH_2OH concentrations in the depth samples.

To sum up, the Std. M. can reach good results, and it could not be certainly determined whether both method IV or VI are a real improvement towards the Std. M.. The pH was shown to influence the conversion rates. However, pH is only one influence factor of many. Accuracy during all steps might be of similar importance.

I Acknowledgement

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III Appendix

III.A DIC correction

Equation 4 (Section 2.4.4) was used to calculate the dissolved inorganic carbon (DIC) correction at typical equilibration conditions (p = 1 atm, T_{eq} = 293.15 K) and a salinity (S = 22 psu) with the solubility coefficients of CO_2 in mol L^{-1} atm^{-1} A_1 (-160.7333), A_2 (215.4152), A_3 (89.8920), A_4 (-1.47759), B_1 (0.029941), B_2 (-0.027455) and B_3 (0.0053407) (Weiss et al., 1980). A dummy mole fraction x_{HS} = 300 ppb was used to calculate the dummy water concentration (C_{Wdummy}). The headspace mole fraction was randomly chosen. It was proven that also other headspace mole fractions resulted in the same ratios. From the DIC concentration (C_{Wdummy} in µmol L^{-1}), the DIC amount in the water phase (n_{Wdummy}), the headspace ($n_{HSdummy}$) were calculated and by summation the total DIC dummy amount (n_{Tdummy}) resulted. The dummy ratios $r_{HS/Wdummy}$ and $r_{T/Wdummy}$ were calculated according to Equation I and II. The ratios are concentration independent and are constant for the particular solubility of carbon dioxide. Thus, they were used to calculate the sample carbon dioxide amounts.

$$r_{HS/Wdummy}=\frac{n_{HSdummy}}{n_{Wdummy}} \quad \text{(I)}$$

$$r_{T/Wdummy}=\frac{n_{Tdummy}}{n_{Wdummy}} \quad \text{(II)}$$

A mean DIC concentration of 2000 µmol kg^{-1}, with sampling density resulted in 2034 µmol L^{-1} was used. The density (1.017 kg L^{-1}) for the seawater from Boknis Eck was calculated under sampling conditions (S = 22 psu; T =0°C, p = 0 atm).

The total DIC amount (n_T) for the different sample water volumes was calculated. The DIC amount in the water phase (n_W) was calculated according to formula III.

$$n_W = \frac{n_T}{r_{T/Wdummy}} \quad \text{(III)}$$

The DIC amount in the headspace (n_{HS}) was calculated using formula IV. The volume of the DIC amount in the head space was calculated with the ideal gas formula 3. The DIC in the headspace dilutes the sample headspace concentration, therefore the volume of the headspace DIC was added to the 10 mL of He headspace, to correct the volume factor (V_W/V_{HS}) in the sample concentration calculations.

$$n_{HS} = n_W r_{HS/Wdummy} \quad \text{(IV)}$$

All the dummy calculations C_W, n_{Wdummy}, $n_{HSdummy}$, n_{Tdummy}, $r_{HS/Wdummy}$, $r_{T/Wdummy}$ and the real DIC concentrations n_T, n_w and n_{HS} were calculated for the different sample water volumes, chemical and vial volumes (with and without adjustment needle). Table A presents an overview about the used DIC volume corrections. In order to verify which volume correction was used for the different tests see Section 2: Table 5 (Section 2.2.2) , Table 7 (Section 2.3.3.2), Table 8 (Section2.3.3.2).

BE Sample in mL	$n_{DIC}=n_{HS}+n_W$ in µmol per sample volume (after HS)	DIC HS in mL	Sample type
9.9	20.137	0.164	Std. ADD (IV)
10	20.340	0.166	SA-Acid + FAS (IV); Std. ADD (all)
10.1	20.543	0.167	SA-Acid (IV); SA-Acid + FAS (all)
10.2	20.747	0.169	SA-Acid (all)
10.3	20.950	0.171	Std. ADD, SA-Acid+ FAS (all)
Brackest lab Exp.s: Water volume was pipetted therefore the heaspce increases here.			
11.5 (10)	23.391 (20.340)	0.191 (0.166)	Wrong vial: Std. ADD
11.6 (10.1)	23.594 (20.543)	0.192 (0.167)	Wrong vial: SA-Acid + FAS
11.7 (10.2)	23.797 (20.747)	0.194 (0.169)	Wrong vial: SA-Acid
With adjusment needle			Without adjustment needle

Table A: Overview over the DIC amount and volume in the different samples

III.B Method optimization background samples

Method optimization	$[N_2O]$ in nMol/L	$[N_2O]^0$ nMol/L	R	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	Δw	Δ$[NH_2OH]$ nMol/L
I (a) MQ*	23.33	24.62	0.0396	-65.40	2.88	0.85	0.144588	0.068149	17,818.04
I(b) MQ*	27.03	25.99	0.3501	5.94	3.06	1.07	0.998599	0.013111	171.26
I Std. M. MQ*	24.31	25.99	1.1290	-2.98	0.72	1.07	0.990947	0.076303	2.65
II MQ*	38.27	35.23	0.2093	29.08	0.53	0.32	0.387382	0.18609	351.71
II Std. M. MQ*	24.62	35.23	0.6571	-32.28	1.18	0.32	0.9191	0.137854	29.84
III MQ*	29.44	29.24	0.8781	0.47	0.86	0.55	0.985278	0.0759	2.72
IV MQ	23.77	23.20	0.8599	1.32	0.91	0.15	0.998329	0.02488	2.28
V MQ	26.35	26.27	0.6772	0.23	0.48	0.55	0.998688	0.017358	2.34
VI MQ	25.53	26.27	0.8062	-1.85	0.35	0.55	0.999258	0.015536	1.31
* measured with defective Valco Valve									

Table B: Method optimization experiments MQ.

Method optimization	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	R	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	Δw	Δ$[NH_2OH]$ nMol/L
II BE*	26.88	28.76	0.0008	-4930.42	0.50	0.37	0.000109	0.051727	55,510,535,332.40
III BE	25.33	26.23	0.6144	-2.92	0.65	0.86	0.987818	0.048248	6.13
IV BE	25.04	23.00	0.7589	5.39	0.29	0.56	0.987968	0.059222	1.49
V BE	25.85	26.23	0.7028	-1.10	0.12	0.27	0.997183	0.026414	0.35
VI (b) BE	26.54	26.35	0.8281	0.45	0.39	0.87	0.999697	0.010198	2.63
I	24.68	24.36	0.0153	42.42	0.20	0.70	0.893352	0.003727	4,562.02
* measured with defective Valco Valve									

Table C: Method optimization experiments BE.

III.C Method optimization Std. ADD

I (a) MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	23.01	23.33	0.32	0.64	2.88	0.85	4.25
7.20	23.01	23.68	0.67	1.35	1.64	0.85	2.62
14.39	23.01	26.42	3.41	6.82	1.25	0.85	2.14
28.77	23.01	25.16	2.15	4.30	1.16	0.85	2.04
57.49	23.01	25.68	2.68	5.35	1.40	0.85	2.32

Table D: Method optimization I (a) MQ, measured with a defective Valco Valve.

I (b) MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	25.99	27.03	1.04	2.08	3.06	1.07	4.58
7.60	25.99	25.91	-0.08	-0.17	1.46	1.07	2.57
15.19	25.99	27.68	1.69	3.38	3.36	1.07	4.99
30.36	25.99	30.05	4.06	8.11	1.75	1.07	2.90
60.66	25.99	35.58	9.59	19.18	2.65	1.07	4.05

Table E: Method optimization I (b) MQ, measured with a defective Valco Valve.

I Std. M. MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	25.99	24.31	-1.68	-3.36	0.72	4.13	5.93
7.60	25.99	27.57	1.58	3.16	0.19	4.13	5.85
15.19	25.99	28.79	2.80	5.59	1.48	4.13	6.21
30.36	25.99	39.19	13.19	26.39	0.95	4.13	6.00
60.66	25.99	56.34	30.34	60.69	1.47	4.13	6.21

Table F: Method optimization I Std. M. MQ, measured with a defective Valco Valve.

I BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
	24.36	24.68	0.32	0.65	0.20	0.70	1.02
7.67	24.36	24.91	0.56	1.11	1.26	0.70	2.03
15.33	24.36	25.01	0.65	1.31	0.40	0.70	1.14
30.62	24.36	24.99	0.63	1.26	0.55	0.70	1.26
61.12	24.36	25.34	0.99	1.97	0.06	0.70	0.99

Table G: Method optimization I BE.

II Std. M. MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	35.23	24.62	-10.61	-21.21	1.18	0.32	1.73
7.01	35.23	30.62	-4.60	-9.21	0.15	0.32	0.50
14.02	35.23	29.57	-5.66	-11.33	0.92	0.32	1.38
28.03	35.23	32.87	-2.35	-4.71	2.59	0.32	3.69
56.00	35.23	45.61	10.38	20.76	1.66	0.32	2.39

Table H: Method optimization II Std. M. MQ, measured with a defective Valco Valve.

II MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
	35.23	38.27	3.04	6.08	0.53	0.32	0.87
7.35	35.23	33.08	-2.15	-4.31	4.17	0.32	5.92
14.69	35.23	27.99	-7.24	-14.48	0.37	0.32	0.69
29.37	35.23	29.26	-5.97	-11.93	1.39	0.32	2.02
58.67	35.23	36.40	1.17	2.34	1.25	0.32	1.82

Table I: Method optimization II MQ, measured with a defective Valco Valve.

II BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	28.76	26.88	-1.89	-3.77	0.50	0.37	0.88
7.35	28.76	28.57	-0.19	-0.39	2.55	0.37	3.64
14.69	28.76	27.72	-1.05	-2.09	0.57	0.37	0.96
29.37	28.76	26.72	-2.05	-4.09	0.62	0.37	1.03
58.67	28.76	28.35	-0.42	-0.83	1.18	0.37	1.76

Table J: Method optimization II BE, measured with a defective Valco Valve.

III MQ							
Final Concentration of Std. in sample vial nMol/L	$[N_2O]$ nMol/L	$[N_2O]^0$ Std.ADD nMol/L	ΔN2O/nM	Δx2 nMol/L	SD $[N_2O]$ Std nMol/L	SD $[N_2O]$ 15m nMol/L	$\Delta[NH_2OH]$ Std.ADDn nMol/L
0.00	29.24	29.44	0.21	0.41	0.86	0.55	1.45
7.02	29.24	32.39	3.15	6.30	0.42	0.55	0.98
14.03	29.24	33.50	4.26	8.52	0.06	0.55	0.79
28.05	29.24	39.09	9.85	19.71	0.61	0.55	1.17
56.05	29.24	53.25	24.02	48.03	2.09	0.55	3.06

Table K: Method optimization III MQ, measured with a defective Valco Valve.

III BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	26.23	25.33	-0.90	-1.79	0.65	0.86	1.52
7.02	26.23	30.11	3.87	7.75	0.49	0.86	1.39
14.03	26.23	31.09	4.86	9.72	0.26	0.86	1.26
28.05	26.23	34.94	8.70	17.41	0.37	0.86	1.32
56.05	26.23	44.78	18.55	37.10	1.39	0.86	2.31

Table L: Method optimization III BE.

IV BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	23.00	25.04	2.05	4.09	0.29	0.56	0.90
7.68	23.00	27.10	4.10	8.20	1.03	0.56	1.66
15.35	23.00	28.77	5.77	11.54	0.38	0.56	0.96
30.68	23.00	33.59	10.60	21.19	0.06	0.56	0.80
61.23	23.00	47.04	24.04	48.08	1.81	0.56	2.68

Table M: Method optimization IV BE.

IV MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
	23.20	23.77	0.57	1.13	0.91	0.15	1.30
7.40	23.20	26.66	3.46	6.91	0.47	0.15	0.69
14.79	23.20	28.94	5.74	11.47	0.49	0.15	0.72
29.56	23.20	35.47	12.27	24.54	1.34	0.15	1.91
59.00	23.20	48.51	25.32	50.63	0.12	0.15	0.27

Table N: Method optimization IV MQ.

V MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	26.27	26.44	0.17	0.34	0.48	0.55	1.04
7.73	26.27	28.14	1.86	3.73	0.27	0.55	0.87
15.46	26.27	30.95	4.67	9.35	0.23	0.55	0.84
30.89	26.27	36.62	10.35	20.69	0.86	0.55	1.44
61.66	26.27	46.44	20.17	40.33	1.01	0.55	1.63

Table O: Method optimization V MQ.

V BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	26.23	25.85	-0.39	-0.78	0.12	0.27	0.41
7.73	26.23	27.32	1.09	2.17	0.13	0.27	0.42
15.46	26.23	29.63	3.40	6.79	0.35	0.27	0.62
30.89	26.23	34.46	8.23	16.46	0.22	0.27	0.49
61.66	26.23	46.16	19.93	39.86	only	0.27	0.00

Table P: Method optimization V BE.

VI MQ							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0.00	26.27	25.53	-0.75	-1.49	0.35	0.55	0.92
8.14	26.27	26.49	0.22	0.44	0.34	0.55	0.91
16.27	26.27	30.40	4.13	8.26	0.26	0.55	0.86
32.52	26.27	36.79	10.51	21.03	0.12	0.55	0.80
64.91	26.27	49.61	23.33	46.67	0.19	0.55	0.83

Table Q: Method optimization VI MQ.

VI (b) BE							
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	Δx2 in nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
	26.35	26.54	0.19	0.38	0.39	0.87	1.34
7.26	26.35	27.48	1.13	2.26	0.77	0.87	1.64
14.51	26.35	30.15	3.81	7.61	0.35	0.87	1.32
28.99	26.35	36.16	9.81	19.62	0.60	0.87	1.49
57.87	26.35	48.32	21.97	43.95	0.84	0.87	1.70

Table R: Method optimization VI (b) BE.

III.D Boknis Eck cruise data R plots

Here only the Recovery Factor determination plots are presented, which were examined during this thesis but are not presented in between Section 3.

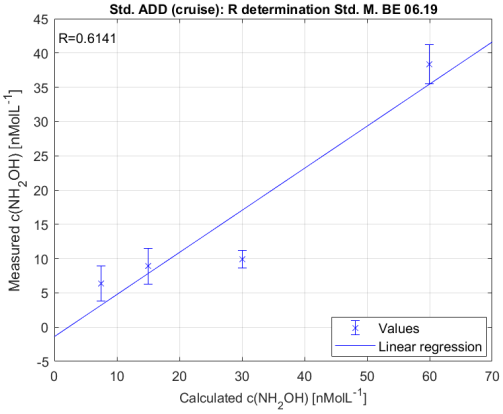

Figure A: R plot June 2019.

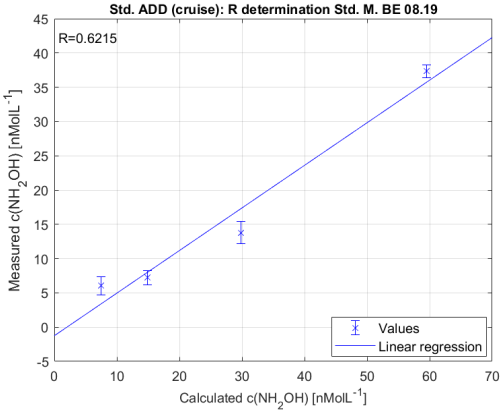

Figure B: R plot August 2019.

III.E Boknis Eck cruise data (this thesis)

The data from the Boknis Eck cruises during this master's thesis are presented in Table S. If not specified different the Std. M. was used (Kock, 2012).

Year	M	Depth m	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	$[NH_2OH]$ nMol/L	R	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	△w	△$[NH_2OH]$ nMol/L
Std. M.											
2019	5	1	12.83	6.14	19.05	0.7026	0.24	2.09	0.7788	0.3744	11.79
This month was measured with the defective Valco Valve.		5	13.26	6.24	19.98	0.7026	0.47	0.62	0.7788	0.3744	10.88
		10	14.12	6.34	22.15	0.7026	0.58	0.40	0.7788	0.3744	11.97
		15	15.34	5.72	27.38	0.7026	0.92	1.58	0.7788	0.3744	15.49
		20	16.67	Wrong vial mathematical corrected; did not match.					0.7788	0.3744	
		25	17.27	8.75	24.27	0.7026	1.81	0.83	0.7788	0.3744	14.12
Std. M.											
2019	6	1	11.64	10.23	4.61	0.6141	0.19	0.76	0.8963	0.1477	2.78
		5	13.08	10.75	7.57	0.6141	0.35	0.46	0.8963	0.1477	2.62
		10	13.16	11.77	4.55	0.6141	0.20	0.90	0.8963	0.1477	3.21
		15	18.05	12.74	17.28	0.6141	3.59	0.66	0.8963	0.1477	12.61
		20	15.32	13.57	5.69	0.6141	0.52	0.19	0.8963	0.1477	2.27
		25	13.97	12.80	3.84	0.6141	0.30	1.74	0.8963	0.1477	5.83
Std. M.											
2019	7	1	11.46	9.24	6.64	0.6692	0.71	0.04	0.9944	0.0356	2.15
		5	9.91	9.75	0.48	0.6692	0.09	0.19	0.9944	0.0356	0.62
		10	9.60	9.76	-0.46	0.6692	1.16	0.21	0.9944	0.0356	3.52
		15	8.70	10.15	-4.36	0.6692	0.01	0.60	0.9944	0.0356	1.82
		20	12.32	11.17	3.44	0.6692	0.05	0.45	0.9944	0.0356	1.36
		25	10.63	9.91	2.16	0.6692	0.29	0.71	0.9944	0.0356	2.29
Pipette Experiment											
2019	7	1	11.46	9.24	9.72	0.4570	0.71	0.04	0.9553	0.0699	3.45
		5	9.91	9.75	0.71	0.4570	0.09	0.19	0.9553	0.0699	0.91
		10	9.60	9.76	-0.68	0.4570	1.16	0.21	0.9553	0.0699	5.16
		15	8.70	10.15	-6.38	0.4570	0.01	0.60	0.9553	0.0699	2.81
		20	12.32	11.17	5.04	0.4570	0.05	0.45	0.9553	0.0699	2.13
		25	10.63	9.91	3.16	0.4570	0.29	0.71	0.9553	0.0699	3.39
Std. M.											
2019	8	1	10.69	8.82	6.01	0.6215	0.67	1.10	0.9647	0.0841	4.23
		5	12.30	8.63	11.78	0.6215	1.83	0.15	0.9647	0.0841	6.11
		10	11.04	9.73	4.21	0.6215	0.24	1.01	0.9647	0.0841	3.40
		15	9.09	7.60	4.79	0.6215	0.15	0.29	0.9647	0.0841	1.22
		20	11.32	9.52	5.80	0.6215	1.01	2.74	0.9647	0.0841	9.43
		25	9.77	7.59	7.03	0.6215	0.10	0.52	0.9647	0.0841	1.97
Std. M.											
2019	10	1	12.23	13.95	-2.93	1.1790	0.47	0.06	0.9915	0.0773	0.82
		5	12.78	14.55	-3.00	1.1790	0.66	1.07	0.9915	0.0773	2.14
		10	13.26	12.71	0.93	1.1790	0.27	0.04	0.9915	0.0773	0.47
		15	14.20	12.07	3.62	1.1790	1.91	0.21	0.9915	0.0773	3.27
		20	18.17	12.13	10.25	1.1790	5.42	1.24	0.9915	0.0773	9.45
		25	15.96	13.68	3.86	1.1790	1.32	0.46	0.9915	0.0773	2.39
Method validation IV											
2019	10	15	11.30	12.07	-1.55	0.9828	0.37	0.21	0.9887	0.0745	0.87
Method validation VI											
			11.06	12.07	-2.38	0.8455	0.35	0.21	0.9990	0.0265	0.96
Std. M.											
2019	12	15	14.33	12.67	5.92	0.5629	0.48	0.20	0.9835	0.0515	1.91
Method validation IV											
2019	12	15	14.81	12.67	8.62	0.4967	0.13	0.20	0.8528	0.1459	2.71

Table S: Boknis Eck cruise data measured during this thesis.

III.F Boknis Eck cruise data Std. ADD

May 2019 measured with defective Valco Valve, Std. 2 left out (wrong vial), mathematically corrected but c did not match						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	$\triangle N_2O$ in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	5.72	15.34	9.62	0.92	1.58	2.59
7.01	5.72	17.60	11.89	2.12	1.58	3.74
14.02	5.72	15.93	10.21	0.65	1.58	2.42
28.03	5.72	28.41	22.69	1.08	1.58	2.71
56.00	5.72	32.18	26.47	2.76	1.58	4.50

Table T: BE cruise May 2019.

June 2019; outbreaker at 15m $[N_2O]^0$, questionable, $\triangle N_2O$ negative without Std. ADD						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	$\triangle N_2O$ in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.74	18.05	5.31	3.59	0.66	5.17
7.51	12.74	15.94	3.19	1.70	0.66	2.58
15.01	12.74	17.19	4.45	1.72	0.66	2.61
30.01	12.74	17.70	4.96	0.66	0.66	1.32
59.96	12.74	31.91	19.17	1.90	0.66	2.85

Table U: BE cruise June 2019.

July 2019 Std. M., $\triangle N_2O$ 15 m negative without Std. ADD						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	$\triangle N_2O$ in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	10.15	8.70	-1.46	0.01	0.60	0.85
7.40	10.15	11.17	1.02	0.75	0.60	1.36
14.80	10.15	12.35	2.19	0.81	0.60	1.42
29.59	10.15	17.59	7.43	2.10	0.60	3.09
59.12	10.15	28.00	17.85	0.28	0.60	0.94

Table V: BE cruise July 2019 (Std. M.).

July 2019 Pipette Exp.						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	$\triangle N_2O$ in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	10.15	8.70	-1.46	0.01	0.60	0.85
7.40	10.15	13.91	3.75	0.78	0.60	1.40
14.80	10.15	12.91	2.76	2.01	0.60	2.96
29.59	10.15	17.30	7.14	1.81	0.60	2.70
59.12	10.15	24.72	14.57	0.54	0.60	1.14

Table W: BE cruise July 2019 (Pipette Exp.).

August 2019						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	7.60	9.09	1.49	0.15	0.29	0.45
7.46	7.60	10.63	3.03	0.91	0.29	1.35
14.92	7.60	11.21	3.61	0.67	0.29	1.03
29.81	7.60	14.50	6.89	1.14	0.29	1.66
59.50	7.60	26.27	18.67	0.60	0.29	0.93

Table X: BE cruise August 2019.

October 2019 Std. M.						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.07	14.20	2.14	1.91	0.21	2.71
7.26	12.07	14.25	2.18	0.24	0.21	0.45
14.51	12.07	16.17	4.10	0.20	0.21	0.40
28.99	12.07	24.53	12.46	0.39	0.21	0.62
57.87	12.07	43.27	31.20	0.86	0.21	1.25

Table Y: BE cruise October 2019 (Std. M.).

October 2019 method validation IV						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.07	11.30	-0.76	0.37	0.21	0.60
6.85	12.07	12.97	0.90	0.20	0.21	0.41
13.70	12.07	14.83	2.76	0.43	0.21	0.68
27.37	12.07	20.56	8.49	0.41	0.21	0.64
54.63	12.07	35.98	23.91	0.62	0.21	0.93

Table Z: BE cruise October (IV).

October 2019 method validation VI, Std. 1 left out Δ negative						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.07	11.06	-1.01	0.35	0.21	0.57
7.60	12.07	11.48	-0.59	0.21	0.21	0.42
15.19	12.07	13.66	1.59	0.19	0.21	0.40
30.35	12.07	19.49	7.42	0.20	0.21	0.40
60.58	12.07	32.73	20.66	0.65	0.21	0.96

Table A2: BE cruise October 2019 (VI).

December 2019 Std. M.						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.67	14.33	1.67	0.48	0.20	0.73
6.92	12.67	14.44	1.78	0.02	0.20	0.28
13.83	12.67	15.47	2.81	0.22	0.20	0.42
27.64	12.67	18.53	5.86	0.57	0.20	0.86
55.17	12.67	27.75	15.08	0.51	0.20	0.78

Table B2: BE cruise December 2019 (Std. M.)

December 2019 method validation IV						
Final concentration of Std. in sample vial nMol/L	$[N_2O]^0$ nMol/L	$[N_2O]$ nMol/L	ΔN_2O in nM/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	$\Delta[NH_2OH]$ Std. ADDn nMol/L
0	12.67	14.81	2.14	0.13	0.20	0.33
7.46	12.67	14.61	1.94	0.32	0.20	0.54
14.92	12.67	15.60	2.94	0.32	0.20	0.53
29.81	12.67	24.36	11.70	0.29	0.20	0.50
59.50	12.67	26.93	14.26	0.11	0.20	0.32

Table C2: BE cruise December 2019 (IV).

III G Core data NH_2OH - Time Series Station Boknis Eck

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]]$ nMol/L	Comments	TS overview comment Fig.
2011	2	19.2	17.4	1.79	0.64	1	5.6	0.2	0.8		3.4		
		21.1	17.9	3.13	0.64	5	9.8	2.5	0.4		9.7		
		19.8	18.5	1.35	0.64	10	4.2	0.3	0.5		2.5		
		20.1	12.6	7.53	0.64	15	23.5	1.2	0.6		6.6		
		19.4	13.2	6.21	0.64	20	19.4	1.3	1.3		9.1		
		21.1	14.1	7.03	0.64	25	22.0	1.5	0.5	no Data	7.3		
2011	3	22.2	15.2	7.03	0.64	1	22.0	0.3	0.5		4.1		
		20.1	16.9	3.26	0.64	5	10.2	0.3	0.1		2.1		
		20.7	12.5	8.16	0.64	10	25.5	0.3	0.7		4.8		
		20.3	13.8	6.48	0.64	15	20.3	0.4	0.7		4.9		
		19.7	18.4	1.29	0.64	20	4.0	0.5	0.9		4.8		
						25				no Data	0.0	no Data	
2011	4	18.8	16.3	2.48	0.74	1	6.7	0.7	0.1		2.1		
		18.7	14.2	4.48	0.74	5	12.1	0.8	0.1		2.7		
		16.5	13.3	3.16	0.74	10	8.5	0.8	0.7		4.3		
		15.3	13.8	1.49	0.74	15	4.0	0.8	0.8		4.3		
		14.5	11.2	3.33	0.74	20	9.0	1.2	0.8		5.6		
		12.3	11.6	0.70	0.74	25	1.9	0.7	0.8	no Data	4.3		
2011	6	11.2	9.1	2.16	0.42	1	10.3	0.2	0.0		1.7		
		12.7	9.4	3.24	0.42	5	15.4	0.2	1.2		7.9		
		14.7	9.9	4.86	0.42	10	23.2	0.2	0.1		2.9		
		15.9	11.5	4.34	0.42	15	20.6	0.1	0.4		3.8		
		18.8	13.9	4.96	0.42	20	23.6	0.3	0.1		3.8		
		21.6	15.3	6.37	0.42	25	30.3	0.8	0.2	no Data	7.1		
2011	7	10.7	10.6	0.07	0.54	1	0.3	0.5	0.4		3.3		
		11.7	10.8	0.88	0.54	5	3.3	0.3	0.4		2.6		
		12.9	12.9	0.02	0.54	10	0.1	0.5	0.5		3.8		
		16.8	15.2	1.63	0.54	15	6.0	0.1	0.2		1.1		
		17.3	15.4	1.88	0.54	20	7.0	0.6	0.1		2.7		
		20.3	15.9	4.32	0.54	25	16.0	0.4	0.3	no Data	3.1		
2011	8	10.0	8.5	1.47	0.60	1	4.9	0.3	0.2		2.5		
		12.2	8.6	3.68	0.60	5	12.3	0.6	0.2		4.5		
		12.3	9.3	2.96	0.60	10	9.9	1.3	0.7		8.3		
		12.4	9.7	2.74	0.60	15	9.1	0.2	0.2		2.7		
		14.9	13.9	1.00	0.60	20	3.3	0.1	0.2		1.7		
		15.5	13.9	1.61	0.60	25	5.4	0.3	0.0	no Data	1.9		
2011	9	12.4	10.0	2.41		1	7.3	0.4	0.6		3.5		
		12.9	10.0	2.87		5	8.7	0.1	0.1		0.7	R Data missing; but cs	
		13.3	9.6	3.74		10	11.3	0.3	0.2		1.8	were provided by	
		13.8	9.8	4.04		15	12.2	0.3	0.2		1.8	multiplying the old cHA	
		11.8	8.8	3.00		20	9.1	0.3	0.2		1.8	x2, the correct c could	
		11.5	7.6	3.91	no Data	25	11.8	0.4	0.4	no Data	2.7	be derived.	

Table D2: BE Time series 2011.

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]$ nMol/L	Comments	TS overview comment Fig.
2013	5	12.38	12.52	-0.14	0.5587	1	-0.50	0.46	0.14	0.9937	1.7	only 2 N2O	set 0
		12.32	12.69	-0.38	0.5587	5	-1.35	0.19	0.16	0.9937	0.9		set 0
		13.85	12.53	1.32	0.5587	10	4.73	1.15	0.29	0.9937	4.3		
		14.72	14.91	-0.19	0.5587	15	-0.67	0.22	0.40	0.9937	1.6		set 0
		15.95	15.51	0.44	0.5587	20	1.57	1.32	0.05	0.9937	4.7		
		17.09	15.69	1.40	0.5587	25	5.00	0.53	0.29	0.9937	2.2		
2013	6	13.39	11.41	1.98	0.8180	1	4.85	0.48	0.10	0.9752	1.3		
		13.55	12.42	1.13	0.8180	5	2.76	0.43	0.41	0.9752	1.5		
		13.10	12.47	0.63	0.8180	10	1.53	0.68	0.19	0.9752	1.7		
		13.65	12.82	0.84	0.8180	15	2.05	0.34	0.35	0.9752	1.2		
		19.05	16.33	2.72	0.8180	20	6.66	0.32	0.43	0.9752	1.5		
		18.98	17.29	1.69	0.8180	25	4.14	0.07	0.48	0.9752	1.3	nur 2 HA	
2013	7	15.71	10.69	5.02	0.8619	1	11.66	0.42	0.42	0.9775	1.9		
		15.66	10.92	4.74	0.8619	5	10.99	0.47	0.49	0.9775	2.0		
		22.59	14.96	7.63	0.8619	10	17.71	2.59	0.19	0.9775	6.3		
		21.13	15.40	5.72	0.8619	15	13.28	0.19	0.50	0.9775	1.9		
		20.35	14.82	5.53	0.8619	20	12.84	0.42	0.77	0.9775	2.5		
		19.95	13.85	6.10	0.8619	25	14.16	0.78	0.42	0.9775	2.6		
2013	8	9.92	9.29	0.63	0.7047	1	1.79	0.23	0.24	0.9971	1.0		
		10.01	10.29	-0.28	0.7047	5	-0.80	0.22	0.15	0.9971	0.8		set 0
		10.75	10.75	-0.01	0.7047	10	-0.02	0.05	0.14	0.9971	0.4		
		11.14	11.27	-0.13	0.7047	15	-0.36	0.28	0.15	0.9971	0.9		set 0
		12.75	11.51	1.24	0.7047	20	3.52	0.60	0.82	0.9971	2.9		
		12.52	11.76	0.76	0.7047	25	2.16	0.37	0.07	0.9971	1.1		
2013	9	11.16	9.55	1.60	0.4515	1	7.10	0.21	0.18	0.9991	1.2		
		11.25	10.38	0.87	0.4515	5	3.87	0.22	0.27	0.9991	1.6		
		10.84	9.85	1.00	0.4515	10	4.42	0.17	0.11	0.9991	0.9		
		10.93	9.79	1.14	0.4515	15	5.05	0.19	0.06	0.9991	0.9		
		10.42	9.56	0.86	0.4515	20	3.82	0.53	0.04	0.9991	2.4		
		6.63	5.31	1.32	0.4515	25	5.84	0.04	0.11	0.9991	0.5		
2013	10	11.00	10.46	0.53	0.6783	1	1.58	0.13	0.26	0.9991	0.9		
		11.06	10.38	0.69	0.6783	5	2.03	0.28	0.20	0.9991	1.0		
		10.64	10.16	0.48	0.6783	10	1.42	0.17	0.18	0.9991	0.7		
		9.75	8.59	1.16	0.6783	15	3.42	0.09	0.03	0.9991	0.3		
		9.29	8.21	1.09	0.6783	20	3.20	0.39	0.13	0.9991	1.2		
		4.21	2.71	1.50	0.6783	25	4.44	0.07	0.05	0.9991	0.3		
2013	11	12.62	10.49	2.13	0.8667	1	4.92	0.33	0.32	0.9988	1.1	Std. 1 nur 1	
		12.74	11.15	1.58	0.8667	5	3.65	0.12	0.27	0.9988	0.7		
		12.73	10.88	1.85	0.8667	10	4.27	0.25	0.20	0.9988	0.8		
		13.38	11.19	2.18	0.8667	15	5.04	0.27	0.32	0.9988	1.0		
		13.48	11.20	2.28	0.8667	20	5.27	0.16	0.25	0.9988	0.7		
		13.40	11.70	1.69	0.8667	25	3.90	0.25	0.27	0.9988	0.8		
2013	12	14.45	13.89	0.56	0.7047	1	1.59	0.31	0.31		1.3	2 N2O	Median R (5-11.2013),
		13.93	13.92	0.01	0.7047	5	0.02	0.29	0.36		1.3		
		14.43	14.48	-0.05	0.7047	10	-0.13	0.13	0.06	HACl	0.4	2 N2O	set 0
		14.50	14.26	0.24	0.7047	15	0.67	0.28	0.23	initial	1.0		11.2013),
		17.71	17.18	0.52	0.7047	20	1.49	0.24	0.11	weight	0.8	2 HA	Median Δw used
		16.87	16.59	0.29	0.7047	25	0.81	0.06	0.30	missing,	0.9	2 HA	for error

Table E2: BE Time series 2013.

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]$ nMol/L	Comments	TS overview comment Fig.
2014	2	18.68	14.28	4.40	0.6985	1	12.60	0.41	0.28	0.9998	1.4		
		18.24	15.20	3.03	0.6985	5	8.69	0.13	0.30	0.9998	0.9		
		19.38	15.81	3.57	0.6985	10	10.22	0.44	0.09	0.9998	1.3		
		19.20	15.67	3.53	0.6985	15	10.10	0.54	0.06	0.9998	1.6		
		19.83	15.75	4.08	0.6985	20	11.69	0.38	0.20	0.9998	1.2		
		18.96	15.54	3.42	0.6985	25	9.80	0.04	0.18	0.9998	0.5		
2014	3	17.36	16.17	1.19	0.7425	1	3.20	0.54	0.66	0.9950	2.3		
		17.59	18.64	-1.06	0.7425	5	-2.84	0.10	0.01	0.9950	0.3		set 0
		17.58	19.08	-1.50	0.7425	10	-4.03	0.32	0.57	0.9950	1.8	2 HA	set 0
		17.03	16.71	0.32	0.7425	15	0.87	0.45	0.30	0.9950	1.5		
		16.67	16.03	0.64	0.7425	20	1.72	0.22	0.59	0.9950	1.7	ΔStd. 1 negative, left	
		17.04	17.30	-0.25	0.7425	25	-0.69	0.53	0.32	0.9950	1.7	out	set 0
2014	4	14.38	12.07	2.30	0.7621	1	6.05	0.45	0.35	0.9829	1.6	2 HA	
		15.28	12.78	2.50	0.7621	5	6.57	0.12	0.19	0.9829	0.9		
		15.40	12.68	2.72	0.7621	10	7.14	0.07	0.01	0.9829	0.7		
		14.81	12.65	2.16	0.7621	15	5.67	0.04	0.26	0.9829	0.9	2 HA	
		15.61			0.7621	20		0.33				2 HA, N2O	left out
		15.89			0.7621	25		0.65	high deviation			2 HA	left out
2014	5	15.35	12.33	3.02	0.6443	1	9.39	0.88	1.43	0.9967	5.2		
		15.00	13.33	1.67	0.6443	5	5.19	0.32	0.31	0.9967	1.4		
		15.79	13.81	1.99	0.6443	10	6.17	0.10	0.06	0.9967	0.4		
		15.83	14.44	1.39	0.6443	15	4.33	0.08	0.25	0.9967	0.8		
		16.21	15.05	1.17	0.6443	20	3.62	0.27	0.33	0.9967	1.3		
		17.36	15.47	1.88	0.6443	25	5.85	0.15	0.10	0.9967	0.6		
2014	6	10.59	9.99	0.60	0.6894	1	1.74	0.49	0.39	0.9951	1.8		
		11.02	9.96	1.07	0.6894	5	3.10	0.36	0.48	0.9951	1.7		
		11.09	10.14	0.94	0.6894	10	2.74	0.07	0.08	0.9951	0.3		
		12.70	12.03	0.66	0.6894	15	1.93	0.29	0.17	0.9951	1.0		
		15.23	13.63	1.60	0.6894	20	4.63	1.55	0.52	0.9951	4.8		
		16.47	15.35	1.12	0.6894	25	3.24	0.14	0.22	0.9951	0.8		
2014	7	74.76	8.30	66.47	0.5365	1	247.79	22.16	0.35	0.9932	85.1	HA: Calibration not good, smaller calibration than highest HA sample peak. To increase comparison both were changed to linear fór both HA and N2O. HA Data highly questionable. Std. 1 left out, outbreaker in R plot	
		72.44	8.31	64.13	0.5365	5	239.08	9.05	0.03	0.9932	39.1		
		66.28	9.67	56.60	0.5365	10	211.02	13.29	0.26	0.9932	52.5		
		66.83	9.83	57.00	0.5365	15	212.50	9.73	0.09	0.9932	40.3		
		64.92	11.00	53.93	0.5365	20	201.05	6.71	0.16	0.9932	30.1		
		63.40	10.44	52.97	0.5365	25	197.47	9.05	0.33	0.9932	37.5		not used
2014	8	14.78		14.78	0.3411	1	86.65	1.10		0.9807	6.4		
		14.88		14.88	0.3411	5	87.25	0.13		0.9807	0.8		
		13.43		13.43	0.3411	10	78.74	0.76		0.9807	4.5		
		15.61		15.61	0.3411	15	91.55	1.03		0.9807	6.0		
		13.36		13.36	0.3411	20	78.32	0.59		0.9807	3.4		
		13.36		13.36	0.3411	25	78.34	0.91		0.9807	5.3	N2O Protocol is missing	not used
2014	9	16.67	18.11	-1.44		1		3.09	4.07			2 N2O	
		13.55	17.01	-3.46		5		0.53	4.25			2 HA; 2 N2O	
		14.50	14.97	-0.46		10		1.02	0.79			2 HA	
		15.18	14.69	0.49		15		1.17	1.02			2 HA	
		14.25	11.76	2.49		20		1.70	0.65				
		6.42	5.86	0.56	no Data	25	no Data	1.41	0.38			2 HA	not used
2014	10	20.05	15.84	4.21	0.6037	1	13.94	2.35	2.12	0.9954	10.5	delta Std. 1 bis 2 negativ	
		21.83	13.78	8.05	0.6037	5	26.67	6.09	4.22	0.9954	24.6	2 N2O, HA 1 HS differen	
		23.82	24.89	-1.07	0.6037	10	-3.55		8.62	0.9954	28.6	HA 2 HS different	
		18.66	24.84	-6.19	0.6037	15	-20.50	1.53	0.33	0.9954	5.2	2 N2O	
		18.52	27.53	-9.01	0.6037	20	-29.85	0.66	2.07	0.9954	7.2	2 N2O	
		13.52	21.91	-8.39	0.6037	25	-27.80	3.17	2.21	0.9954	12.8	2 N2O	not used
2014	11	30.87	20.44	10.44	0.5113	1	40.82	9.02	2.47	0.9656	37.4	2 HA, Δ Std. 1 negative,	
		21.46	25.20	-3.74	0.5113	5	-14.63	4.59	0.11	0.9656	18.2	x2 HS 9mL Std .1	
		26.45	25.56	0.89	0.5113	10	3.48	9.31	4.23	0.9656	40.0	2 HA, x2 HS 9mL Std. 2	
		24.71	24.53	0.18	0.5113	15	0.70	10.19	0.75	0.9656	40.0	x1 HS 9mL Std. 3	
		22.82	15.58	7.24	0.5113	20	28.32	9.99	1.82	0.9656	40.1	3x HS 9mL Std. 4	
		17.90	21.78	-3.88	0.5113	25	-15.19	0.27	4.19	0.9656	16.7	2 HA	not used
2014	12	19.80	15.58	4.22	0.5889	1	14.32	0.72	0.44	0.9969	2.9		
		19.87	16.02	3.85	0.5889	5	13.09	0.73	0.52	0.9969	3.1		
		20.72	15.57	5.15	0.5889	10	17.48	0.26	0.55	0.9969	2.2		
		19.75	15.09	4.67	0.5889	15	15.85	0.26	1.17	0.9969	4.1		
		20.89	14.61	6.28	0.5889	20	21.34	0.87	0.22	0.9969	3.2	1x Std. 2 twice as high	
		17.71	14.85	2.85	0.5889	25	9.70	0.37	0.62	0.9969	2.5	excluded	

Table F2: BE Time series 2014.

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]$ nMol/L	Comments	TS overview comment Fig.
2015	1	19.37	15.45	3.92	0.6098	1	12.86	2.91	0.55	0.9989	9.7	2 HA	
		16.66	14.47	2.20	0.6098	5	7.21	0.38	0.53	0.9989	2.2		
		19.56	14.98	4.58	0.6098	10	15.02	0.44	0.26	0.9989	1.8		
		19.52	15.32	4.20	0.6098	15	13.78	4.31	0.73	0.9989	14.4		
		21.29	15.70	5.59	0.6098	20	18.32	6.12	0.52	0.9989	20.2	Std. 3 not used 2x HS	
		20.48	14.99	5.49	0.6098	25	18.02	3.32	0.55	0.9989	11.0	12 mL, 1x11	
2015	2	17.48	14.07	3.42	0.6260	1	10.92	1.78	0.69	0.9868	6.2		
		17.36	14.86	2.50	0.6260	5	7.99	0.60	0.15	0.9868	2.1		
		17.36	15.06	2.30	0.6260	10	7.34	0.55	0.51	0.9868	2.5		
		17.97	15.13	2.84	0.6260	15	9.08	1.42	0.30	0.9868	4.7		
		18.31	15.39	2.92	0.6260	20	9.34	1.47	0.60	0.9868	5.1		
		18.56	15.68	2.88	0.6260	25	9.19	2.06	0.36	0.9868	6.7		
2015	3	17.28	13.06	4.22	0.6721	1	12.56	0.08	0.35	0.9904	1.4		
		17.13	13.28	3.84	0.6721	5	11.44	0.25	0.22	0.9904	1.3		
		17.62	15.09	2.53	0.6721	10	7.52	1.33	0.05	0.9904	4.0		
		16.89	14.48	2.41	0.6721	15	7.17	0.32	0.06	0.9904	1.1		
		16.98	13.88	3.10	0.6721	20	9.23	0.53	1.00	0.9904	3.4		
		17.20	16.25	0.95	0.6721	25	2.81	0.29	0.15	0.9904	1.0		
2015	4	13.71	13.44	0.26	0.5488	1	0.96	0.34	0.43	0.9940	2.0		
		13.88	13.63	0.25	0.5488	5	0.91	0.90	0.75	0.9940	4.3		
		13.25	13.28	-0.03	0.5488	10	-0.12	0.82	0.15	0.9940	3.0		
		14.31	14.50	-0.19	0.5488	15	-0.68	0.35	0.16	0.9940	1.4		
		15.13	15.25	-0.13	0.5488	20	-0.46	1.64	0.13	0.9940	6.0		
		19.29	18.83	0.46	0.5488	25	1.68	0.43	0.25	0.9940	1.8		
2015	5	12.89	13.30	-0.41	0.5557	1	-1.48	0.03	0.41	0.9881	1.5		
		13.03	12.50	0.53	0.5557	5	1.89	0.07	0.24	0.9881	0.9		
		13.46	13.10	0.36	0.5557	10	1.31	0.20	0.27	0.9881	1.2		
		14.00	13.26	0.74	0.5557	15	2.66	0.28	0.16	0.9881	1.2		
		15.00	15.59	-0.58	0.5557	20	-2.09	0.27	0.04	0.9881	1.0		
		16.11	16.42	-0.32	0.5557	25	-1.14	0.46	0.46	0.9881	2.3		
2015	4.06	11.45	11.94	-0.49	0.5107	1	-1.92	0.21	0.74	0.9795	3.0	Extra Exp. here first	
		11.66	12.19	-0.53	0.5107	5	-2.08	0.35	0.21	0.9795	1.6	Std. than Sa than FAS	
		12.62	12.56	0.07	0.5107	10	0.26	0.05	0.47	0.9795	1.8		
		13.01	13.17	-0.15	0.5107	15	-0.61	0.05	0.25	0.9795	1.0		
		13.32	13.60	-0.28	0.5107	20	-1.10	0.13	0.53	0.9795	2.2	Std. 4 out extreme	left out extra
		14.18	14.52	-0.34	0.5107	25	-1.35	0.10	0.10	0.9795	0.6	variation	Experiment
2015	21.06	11.31	10.51	0.80	0.6008	1	2.66	0.24	0.25	0.9851	1.2		
		11.45	10.96	0.49	0.6008	5	1.63	0.18		0.9851	0.6	nur 1 N2O	
		11.37	10.61	0.76	0.6008	10	2.54	0.20	0.34	0.9851	1.3		
		12.37	11.76	0.61	0.6008	15	2.04	0.02	0.07	0.9851	0.3		
		13.80	13.64	0.16	0.6008	20	0.54	0.05	0.28	0.9851	1.0		
		14.98	14.62	0.35	0.6008	25	1.18	0.13	0.11	0.9851	0.6		
2015	7	13.50	9.41	4.09	0.6759	1	12.12	0.20	0.30	0.9950	1.2	HS issues for all Stds.	
		13.32	9.64	3.67	0.6759	5	10.87	0.34	0.58	0.9950	2.1	and HA samples,	
		13.70	9.78	3.92	0.6759	10	11.60	0.11	0.13	0.9950	0.8	2 N2O	
		13.85	10.76	3.09	0.6759	15	9.16	0.56	0.10	0.9950	1.7	2 N2O	
		15.45	11.22	4.23	0.6759	20	12.50	0.34	0.73	0.9950	2.5	Std. 1 only x1	
		16.91	13.01	3.90	0.6759	25	11.55	0.75	0.20	0.9950	2.4	Std. 2 only x2	
2015	8	13.26	7.15	6.12	0.6333	1	19.32	0.49	0.65	0.9944	2.8	HS issues for all Stds.	
		13.45	6.63	6.82	0.6333	5	21.55	0.16	0.31	0.9944	1.6	and HA samples,	
		14.26	8.23	6.03	0.6333	10	19.04	0.32	0.27	0.9944	1.7	questionable	
		15.86	8.59	7.28	0.6333	15	22.98	0.42	0.52	0.9944	2.4		
		18.41	11.97	6.44	0.6333	20	20.33	0.78	1.69	0.9944	6.0		
		19.63	13.26	6.36	0.6333	25	20.09	0.22	0.69	0.9944	2.5	2 N2O	
2015	9	11.77	8.96	2.81	0.6498	1	8.66	0.43	0.58	0.9886	2.3		
		10.58	9.58	1.00	0.6498	5	3.09	0.51	0.39	0.9886	2.0		
		11.85	9.79	2.06	0.6498	10	6.34	0.38	0.38	0.9886	1.7		
			9.21			15	left out, strong SD and a random mean was chosen. Not						left out
		11.04	8.81	2.23	0.6498	20	6.9	0.2	0.2	0.9886	1.1	2 HA	
		11.35	8.97	2.38	0.6498	25	7.3	0.3	0.3	0.9886	1.4		
2015	10					1 5 10 15 20 25						no Data	
2015	11	13.55	11.04	2.50	0.7668	1	6.5	0.5	0.1	0.9971	1.3	2 HA	
		13.07	11.74	1.32	0.7668	5	3.5	0.5	0.5	0.9971	1.8		
		13.20	11.31	1.89	0.7668	10	4.9	0.5	0.4	0.9971	1.6	Std. 1 and 2 only x2	
		13.20	10.88	2.32	0.7668	15	6.0	0.2	0.3	0.9971	0.9		
		12.38	9.33	3.05	0.7668	20	8.0	0.4	0.7	0.9971	2.1		
		12.73	8.62	4.11	0.7668	25	10.7	0.4	0.2	0.9971	1.3	2 HA	
2015	12	13.32	7.77	5.55	0.7207	1	15.4	0.5	1.0	0.9977	3.1		
		13.13	8.63	4.49	0.7207	5	12.5	0.2	0.5	0.9977	1.5		
		12.82	8.76	4.05	0.7207	10	11.2	0.2	1.1	0.9977	3.1		
		12.74	9.98	2.76	0.7207	15	7.7	0.5	0.8	0.9977	2.7		
		12.77	9.99	2.78	0.7207	20	7.7	0.3	1.1	0.9977	3.1		
		12.51	10.53	1.98	0.7207	25	5.5	0.2	0.5	0.9977	1.5		
2015	12	13.17	7.77	5.39	0.8547	1	12.6	0.6	1.0	0.9983	2.7		
		13.42	8.63	4.78	0.8547	5	11.2	0.2	0.5	0.9983	1.3	Chem. AD 1) SA 2)Fas before HS but adjustment	
		12.73	8.76	3.97	0.8547	10	9.3	0.2	1.1	0.9983	2.6	canula in over night, possiblr	
		12.40	9.98	2.42	0.8547	15	5.7	0.3	0.8	0.9983	2.1	bias.	
		12.94	9.99	2.95	0.8547	20	6.9	0.5	1.1	0.9983	2.8	2 HA	
		12.71	10.53	2.18	0.8547	25	5.1	0.0	0.5	0.9983	1.2		not used

Table G2: BE Time series 2015.

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]]$ nMol/L	Comments	TS overview comment Fig.
2016	3	17.43	13.51	3.92	0.5147	1	15.2	0.3	0.5	0.9995	2.2		
		17.05	12.31	4.75	0.5147	5	18.5	0.4	0.3	0.9995	1.9		
		17.38	11.98	5.40	0.5147	10	21.0	0.2	1.5	0.9995	5.7	Std. 3 exluded not good	
		17.65	12.01	5.64	0.5147	15	21.9	0.2	2.5	0.9995	9.7		
		19.03	13.96	5.08	0.5147	20	19.7	0.4	0.3	0.9995	2.0	2 HA	
		18.42	12.60	5.82	0.5147	25	22.6	0.3	2.2	0.9995	8.7		
2016	4	16.29	14.61	1.68	0.7506	1	4.5	0.5	0.6	0.9905	2.2		
		17.05	14.53	2.52	0.7506	5	6.7	0.6	0.5	0.9905	2.2	2 HA	
		17.31	15.18	2.13	0.7506	10	5.7	0.4	0.1	0.9905	1.2	2 HA	
		17.01	15.27	1.74	0.7506	15	4.6	0.5	0.1	0.9905	1.4		
		17.96	16.55	1.41	0.7506	20	3.8	0.8	0.2	0.9905	2.1		
		18.80	16.75	2.05	0.7506	25	5.5	0.0	0.3	0.9905	0.9	2 HA	
2016	5	14.71	13.68	1.03	1.8737	1		0.5	0.6	0.8688			Not in Fig. but very interesting; was commented with bad Std. perhaps nitrite see Dec. 19
		15.26	13.86	1.40	1.8737	5		0.2	0.5	0.8688			
		15.86	14.37	1.49	1.8737	10		0.4	0.2	0.8688			
		16.72	15.41	1.31	1.8737	15		0.6	0.3	0.8688			
		17.56	16.14	1.42	1.8737	20		0.2	0.1	0.8688		2 HA	
		18.48	16.81	1.67	1.8737	25		0.4	0.2	0.8688			
2016	6	11.13	9.03	2.10	0.6265	1	6.7	0.3	0.1	0.9964	1.2		
		11.99	9.36	2.63	0.6265	5	8.4	0.1	0.3	0.9964	0.9		
		13.34	11.10	2.24	0.6265	10	7.2	0.1	0.3	0.9964	1.0		
		13.97	11.67	2.29	0.6265	15	7.3	0.3	0.3	0.9964	1.6		
		15.28	12.77	2.51	0.6265	20	8.0	0.3	0.4	0.9964	1.6		
		19.61	17.34	2.27	0.6265	25	7.2	0.2	0.5	0.9964	1.7		
2016	7	11.99	8.85	3.14	0.6699	1	9.4	0.3	0.2	0.9951	1.3		
		12.80	9.49	3.30	0.6699	5	9.9	0.3	0.8	0.9951	2.6		
		14.47	11.76	2.71	0.6699	10	8.1	0.2	0.5	0.9951	1.8		
		16.20	13.24	2.96	0.6699	15	8.8	0.1	0.5	0.9951	1.6		
		17.81	15.78	2.03	0.6699	20	6.1	0.8	0.5	0.9951	2.8		
		17.24	15.18	2.06	0.6699	25	6.1	0.5	0.8	0.9951	2.9		
2016	8	10.57			0.5512	1		0.1		0.9885	18.6		
		10.99			0.5512	5		0.2		0.9885	0.6		
		12.93			0.5512	10		0.4		0.9885	1.3	No N2O Data	
		14.85			0.5512	15		0.2		0.9885	0.6		
		16.55			0.5512	20		0.2		0.9885	0.9		
		13.18			0.5512	25	no Data	0.1		0.9885	0.4		left out
2016	9	10.73	9.97	0.76	-0.4631	1	-3.3	0.3	0.8	0.5991	4.2		
		10.74	10.15	0.59	-0.4631	5	-2.5	0.0	0.8	0.5991	3.8		
		10.52	10.18	0.34	-0.4631	10	-1.5	0.3	1.0	0.5991	4.7	all only 2 HA negtive	
		10.27	9.83	0.43	-0.4631	15	-1.9	0.1	0.8	0.5991	3.5	slope, wild distribution	
		10.29	9.89	0.40	-0.4631	20	-1.7	0.4	0.9	0.5991	4.3		
		2.23	2.15	0.08	-0.4631	25	-0.3	0.0	1.5	0.5991	6.4		left out
2016	10	12.54	6.12	6.43	0.6520	1	19.7	0.5	0.6	0.9995	2.4		
		13.02	5.88	7.14	0.6520	5	21.9	0.2	1.1	0.9995	3.5	HA HS issues, only 2	
		13.16	7.27	5.88	0.6520	10	18.1	0.5	1.1	0.9995	3.6	HA for 10m, 15m, 25m,	
		12.70	6.56	6.14	0.6520	15	18.8	0.2	0.3	0.9995	1.2	only 1x Std. 4 HS	
		11.39	5.24	6.15	0.6520	20	18.9	0.1	0.7	0.9995	2.2	issues	
		9.16	5.49	3.67	0.6520	25	11.3	0.2	0.5	0.9995	1.5		
2016	11	14.06	6.89	7.17	0.6118	1	23.4	0.3	0.2	0.9907	2.0		
		14.34	6.97	7.38	0.6118	5	24.1	1.9	0.2	0.9907	6.5	HA HS probleme	
		13.78	9.18	4.60	0.6118	10	15.1		1.1	0.9907	3.7	no tracable how HA c	
		14.23	6.96	7.27	0.6118	15	23.8	0.4	0.4	0.9907	2.4		
		13.20	7.11	6.09	0.6118	20	19.9	0.1	0.5	0.9907	2.3		
		9.18	6.64	2.55	0.6118	25	8.3	0.5	1.1	0.9907	3.9	2 HA, 2 N2O	left out
2016	12	4.65	6.82	-2.18	0.1600	1	-27.2	0.1	0.7	0.9950	11.4	all delta Std. 1 to 3 nega	
		4.74	5.90	-1.16	0.1600	5	-14.6	0.3	0.5	0.9950	8.4	HA depths high negative	
		4.49	6.19	-1.71	0.1600	10	-21.3	0.0	0.5	0.9950	8.3		
		4.28	6.51	-2.23	0.1600	15	-27.9	0.1	0.7	0.9950	11.9	2 HA	
		4.24	6.79	-2.55	0.1600	20	-31.9	0.1	0.3	0.9950	9.3	2 N2O	
		3.87	7.65	-3.78	0.1600	25	-47.3	0.0	0.9	0.9950	16.6	2 HA	left out

Table H2: BE Time series 2016.

Year	Month	$[N_2O]$ nMol/L	$[N_2O]^0$ nMol/L	ΔN_2O nMol/L	R	Depth m	$[NH_2OH]$ nMol/L	SD $[N_2O]$ nMol/L	SD $[N_2O]^0$ nMol/L	R^2 slope	$\Delta[NH_2OH]$ nMol/L	Comments	TS overview comment Fig.
2017	1	18.07	9.04	9.02	0.6159	1	29.3	2.6	0.2	0.9908	8.9	2 HA	
		19.95	9.18	10.77	0.6159	5	35.0	0.6	0.3	0.9908	3.1	2 HA	
		19.26	8.46	10.80	0.6159	10	35.1	0.4	0.2	0.9908	2.8		
		18.95	8.37	10.58	0.6159	15	34.4	0.5	0.6	0.9908	3.5		
		18.54	8.07	10.47	0.6159	20	34.0	0.8	0.4	0.9908	3.7		
		18.18	9.24	8.94	0.6159	25	29.0	0.7	1.1	0.9908	4.6		
2017	2					1						no cruise	
						5							
						10							
						15							
						20							
						25							
2017	3	20.89	9.54	11.35	0.6734	1	33.7	0.5	0.3	0.9941	2.6	2 HA 1m and 15m	
		20.65	10.19	10.46	0.6734	5	31.1	0.2	1.3	0.9941	4.3	high SD N2O	
		20.08	10.05	10.03	0.6734	10	29.8	0.6	0.8	0.9941	3.4	Std. 3 only two times one HS 8mL	
		19.24	11.12	8.12	0.6734	15	24.1	0.2	1.0	0.9941	3.2	If hydroxylamine decomposition is present, the actual concentration is 80%, and the conversion is increased by about 25% (Max)	
		20.67	10.88	9.79	0.6734	20	29.1	0.6	0.9	0.9941	3.5		
		21.72	13.93	7.79	0.6734	25	23.1	0.5	1.1	0.9941	3.8		

Table I2: BE Time series 2017.