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**Hydroxylamine
(NH₂OH) in the Baltic
Sea**

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Hydroxylamine (NH₂OH) in the Baltic Sea

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Abstract

The vertical distribution of dissolved hydroxylamine (NH_2OH) was measured for the first time at 10 stations in the western, southern and central Baltic Sea during a cruise in February 2004. The distribution of dissolved NH_2OH was complex due to the interplay of in-situ production in the shallow western and southern Baltic Sea and the hydrographical setting in the central Baltic Sea caused by the major North Sea water inflow event in January 2003. We conclude that nitrification might be the major source of NH_2OH , whereas anaerobic ammonium oxidation (anammox) appeared to be negligible. We suggest that a “fresh” nitrifying system, in which the NH_4^+ -oxidation rates exceeded the NO_2^- -oxidation rates, favoured the build-up of NH_2OH .

1. Introduction

Hydroxylamine (NH_2OH) is an intermediate in two important microbial processes of the nitrogen cycle: It is formed during nitrification ($\text{NH}_4^+ \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$) (e.g. Arp and Stein, 2003) as well as during anaerobic ammonium oxidation (anammox: $\text{NO}_2^- \rightarrow \text{NH}_2\text{OH} + \text{NH}_4^+ \rightarrow \text{N}_2$) (Jetten, 2001). Furthermore, NH_2OH is a precursor of nitrous oxide (N_2O) as a side product during nitrification (e.g. Arp and Stein, 2003). Oceanic nitrification is a major formation pathway of dissolved N_2O in the ocean (Bange and Andreae, 1999). Since oceanic N_2O emissions are of significant importance for the Earth’s climate (Montzka et al., 2003; Prather et al., 2001), deciphering the distribution of potential precursors such as NH_2OH is important to understand the formation pathways of N_2O . However, there are only a few published measurements of dissolved NH_2OH in oceanic environments such as the Yaquina River estuary in Oregon (Butler et al., 1987), a coastal lagoon in California (Butler et al., 1988) and the continental shelf off Oregon (Von Breyman et al., 1982). Results from an early laboratory study and the Yaquina Bay suggested that nitrification might be the principal formation process of NH_2OH (Butler et al., 1988; Rajendran and Venugopalan, 1976).

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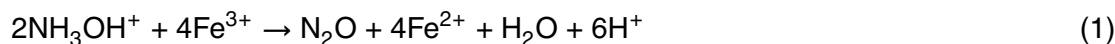
The Baltic Sea consists of a series of basins (Arkona, Bornholm, western and eastern Gotland Basins; see Fig. 1) with restricted horizontal water exchange. The fresh-water supply from the rivers and the discontinuously inflowing saline North Sea water result in a surface layer with lower salinity, and more saline deep and bottom water masses. The permanent halocline between these layers restricts the vertical exchange and is the reason for the development of stagnant deep waters accompanied by oxygen depletion and anoxia with accumulation of hydrogen sulphide. A major inflow of North Sea water, terminating the ongoing stagnation period in parts of the central Baltic Sea basins, occurred in January 2003 (Feistel et al., 2003; Nausch et al., 2003).

Here we present our measurements of dissolved NH₂OH from 10 stations in the western, southern and central Baltic Sea. The cruise took place on board the German research vessel Gauss (expedition no. 11/04/01) from 11 February to 22 February 2004 as part of the Cooperative Monitoring in the Baltic Sea Environment (COMBINE) program of the Baltic Marine Environment Protection Commission (the so-called Helsinki Commission, HELCOM, see <http://www.helcom.fi>). The locations of the stations are shown in Fig. 1.

2. Methods

2.1. Principle

NH₂OH was determined with an adaptation of the method first described by Von Breyermann et al. (1982) and later modified by Butler and Gordon (1986). The method is based on the oxidation of NH₂OH by Fe(III) to N₂O (Bengtsson et al., 2002):



The final concentration of NH₂OH, [NH₂OH], was computed as

$$[\text{NH}_2\text{OH}] = 2 * r^{-1} * ([\text{N}_2\text{O}] - [\text{N}_2\text{O}]^0), \quad (2)$$

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where r stands for the recovery factor (for the determination of r see section below), $[\text{N}_2\text{O}]$ is the total concentration of dissolved N_2O in the sample after the oxidation of NH_2OH and $[\text{N}_2\text{O}]^\circ$ is the background concentration of dissolved N_2O in the sample. In the original method, Butler and Gordon (1986) used a purge-and-trap system to extract the N_2O from the sample. Here we used a headspace equilibration technique, which is routinely applied to measure oceanic N_2O depth profiles (Bange et al., 2001). Thus, N_2O concentrations, $[\text{N}_2\text{O}]$ in nmol L^{-1} , were calculated as:

$$[\text{N}_2\text{O}] = (\beta x' P V_{wp} + \frac{x' P}{RT} V_{hs}) / V_{wp}, \quad (3)$$

where x' is the measured dry mole fraction of N_2O (in ppb) in the headspace of the sample, P is the pressure in the headspace (estimated to be constant at $1.01325 \times 10^5 \text{ J m}^{-3}$), V_{hs} is the volume of the headspace, T is the equilibration temperature (in K), R is the gas constant ($8.31441 \text{ J K}^{-1} \text{ mol}^{-1}$), β is the Bunsen solubility (in $\text{nmol L}^{-1} \text{ atm}^{-1}$) as a function of T and the salinity of the sample (Weiss and Price, 1980), and V_{wp} is the volume of the water phase.

2.2. Sample treatment and analysis

All chemicals used were analytical reagent grade supplied by Merck KGaA, Darmstadt, Germany. We always used deionised water (Milli-Q). Triplicate water samples for the determination of $[\text{N}_2\text{O}]^\circ$ and $[\text{N}_2\text{O}]$ from various depths were taken from a 5 L-bottle rosette, equipped with a CTD-sensor. The samples for $[\text{N}_2\text{O}]^\circ$ (24 mL sample volume) were immediately poisoned with 0.5 mL 1.7 mM aqueous HgCl_2 solution. The samples for $[\text{N}_2\text{O}]$ (118 mL sample volume) were acidified with 1 mL glacial acetic acid (final pH was 3). Then 1 mL of a 25 mM aqueous ferric ammonium sulphate (FAS, $\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) solution was added. After 3 h (which is the required time to complete the NH_2OH oxidation reaction, see Butler and Gordon, 1986), the samples were poisoned with 0.5 mL 1.7 mM aqueous HgCl_2 solution. All samples were stored in the dark at 5°C until measurement in our laboratory in Kiel. In order to create the headspace,

10 mL of sample were replaced with pure Helium (>99.999%, Messer Griesheim, Germany). Then the samples were allowed to equilibrate at least for 2 hours at room temperature (temperature was recorded). After equilibration, a subsample of 9 mL was drawn from the headspace with a gastight syringe. The subsample was injected through a phosphorus pentoxide (Sicapent® by Merck KGaA, Darmstadt, Germany) drying column to purge a stainless steel 2 mL sample loop connected to a gas stream selecting valve. After switching the valve, the sample was injected onto the separation column (prewashed, packed molsieve 5A column, 1.83 m length, Alltech GmbH, Unterhaching, Germany) with a argon/methane (95/5) mixture (ECD quality by Messer Griesheim, Germany) as carrier gas (flow 20 mL min⁻¹). The separation was performed isothermally at 190°C. N₂O was detected with an electron capture detector (ECD, Fisons model 800). The ECD was run in the constant current mode at 320°C with a pulse width of 1 μs, a reference current of 1 nA and a pulse voltage of 25 V. Signal processing and chromatogram integration was done with Chromstar (SCPA, Bremen, Germany) software. For the calibration procedure we used certified standard gas mixtures with 100±2 ppb and 318±6 ppb N₂O in synthetic air (DEUSTE Steininger GmbH, Mühlhausen, Germany). The check of the ECD response curve (by dilution of pure N₂O with He) revealed a linear response for N₂O in the range from 76 ppb to 11,320 ppb thus no correction for non-linearity was applied for this range. For N₂O mole fractions <76 ppb we applied the correction for non-linearity as described in Bange et al. (2001). Repeated measurements of the standard gas mixtures resulted in a mean relative error of 2.4%. The relative errors of the mean [NH₂OH] were in the range from 5 to 15%. The detection limit for [NH₂OH] was estimated to be approximately 2 nmol L⁻¹.

2.3. Determination of the recovery factor

The recovery factor *r* (see Eq. 2) was defined as the ratio of the measured NH₂OH to the theoretical NH₂OH. In order to determine the recovery factor, a series of NH₂OH standard solutions were prepared as follows: A NH₂OH stock solution was prepared by dissolution of 347.7 mg of dry NH₂OH HCl in 1 L water which was previously acidified

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with 1 mL 1N HCl. The stock solution was stored in the dark at 5°C. 1 L of Baltic Sea water (from the Kiel Fjord) was filtered and acidified with 1 mL 1N HCl. Then 1 mL of the stock solution was added. This solution was diluted with Baltic Sea water to prepare a $5 \mu\text{mol L}^{-1}$ NH_2OH solution. In a further dilution step, standard solutions with concentrations of 2, 5, 10, 30 and 50 nmol L^{-1} NH_2OH were prepared. The solution was acidified with 3 mL glacial acetic acid to a final pH of 3. After adding 1 mL of the FAS solution (see Sample treatment and analysis) the samples were allowed to react for 16 h in order to complete the NH_2OH oxidation to N_2O . Finally, N_2O was analysed as described above. The combined natural background of N_2O and NH_2OH concentrations of the used Baltic Sea water was also determined. The procedure was repeated two times. The results are shown in Fig. 2. The resulting mean recovery factor was 0.31. For the computation of $[\text{NH}_2\text{OH}]$ we used 0.31 for the recovery factor r in Eq. (2). Our recovery factor is at the lower end of the values reported by Butler and Gordon (1986), which ranged from 0.40 (Big Lagoon, California) to 0.83 (coast off Oregon). It appears that r strongly varies with the water matrices used (Butler and Gordon, 1986). We may speculate that dissolved trace metals and/or other dissolved constituents of the Baltic Sea water reduce the yield of the NH_2OH oxidation.

2.4. Nutrients

Concentrations of the dissolved inorganic nitrogen compounds nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and oxygen (O_2) were determined using standard methods (Grasshoff et al., 1983).

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3. Results and Discussion

3.1. Water column distribution

NH₂OH concentrations were in the range from 2 nmol L⁻¹ up to 179 nmol L⁻¹ (at station #22). Generally, NH₂OH concentrations were higher at the shallow, well-mixed stations (the mean for stations #360, #22 and #133 was 108 nmol L⁻¹) than in the mixed layer (ML, defined as temperature difference of <0.5°C between surface and depth) of the central Baltic Sea (26±12 nmol L⁻¹, n=7). Our measurements are comparable with the NH₂OH concentrations found in the Yaquina River (Oregon) and Big Lagoon (California) which were in the range from 0 to 362 nmol L⁻¹ and 0 to 175 nmol L⁻¹, respectively (Butler et al., 1987; Butler et al., 1988).

The vertical distributions of NH₂OH in the water column were highly variable and partly showed complex structures (Figs. 3a–d). Enhanced NH₂OH concentrations at stations #22 and #360 were associated with high salinities indicating a significant contribution of North Sea water, however, a clear relationship was not detectable. Obviously there was no correlation of NH₂OH with N₂O or O₂ suggesting that (i) a direct coupling of N₂O formation via NH₂OH was negligible and (ii) NH₂OH formation was not directly influenced by O₂. Our results are partly in contrast to the results of Butler et al. (1987; 1988) who reported a reasonable correlation of NH₂OH with N₂O. However, Butler et al. (1987) stated that the observed correlation does not necessarily prove N₂O formation via NH₂OH.

In the anoxic deep waters of the western Gotland Basin (stations #284 and #245), NH₂OH concentrations rapidly decreased close to 0 nmol L⁻¹ when O₂ was exhausted (Fig. 3d). The effect of the recent North Sea water inflow event is illustrated by the distribution of NH₂OH at station #286 (Fig. 3c) in the northern Gotland Basin. Below the mixed layer (>50 m) the dramatic decrease of NH₂OH and N₂O at 80 m is associated with the steep gradient of the O₂ concentrations (down to 7.9 μmol L⁻¹ at 90 m). Below 90 m the oxygen concentrations were enhanced due to the presence of O₂-rich North

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Sea water which ventilated the previously anoxic deep waters except for a narrow (now suboxic) depth range between 80–100 m. Therefore, the extremely low NH_2OH and N_2O concentrations at 80 m most probably were the residual signal of the “old” anoxic condition before the inflow event which is still found at station #284 (Fig. 3d).

5 3.2. Formation

NH_2OH concentrations at the shallow, well-mixed stations in proximity to the coast (especially stations #22 and #133) were associated with high NO_3^- concentrations (Fig. 4a). NH_2OH in the ML was well correlated with NO_2^- (Fig. 4b). High NH_2OH in conjunction with enhanced NO_2^- concentrations were found at stations #360, #22 and #133. The data from station #113 seem to support the correlation, however, in this case the enhanced NH_2OH and NO_2^- concentrations were found below the ML (Figs. 3a and 4b). The high NH_2OH concentrations in the western and southern Baltic Sea (i.e. stations #360, #22, #133) might result from two effects: (i) input via rivers or North Sea water and (ii) in-situ formation via nitrification.

15 Seike et al. (2004) observed NH_2OH concentrations in the range from 21 up to 3614 nmol L^{-1} in the Hii and Iu Rivers in Japan indicating indeed that rivers may serve a potential source of high NH_2OH concentrations. Since we did not find any indications for an input of high NH_2OH by riverine sources (we found no correlation with salinity) we conclude that NH_2OH input by rivers at the time of our measurements was negligible. NH_2OH measurements in the North Sea are not known, thus, the question whether NH_2OH might be brought into the Baltic Sea by North Sea waters remains speculative.

Most probably, NH_2OH was formed in-situ during nitrification at the shallow stations in the western and southern Baltic Sea. At a first glance this seems surprising because:

1. Both steps of the nitrification process are light inhibited (Olson, 1981; Ward, 1985). However, Horrigan and Springer (1990) found that “photoinhibition of NH_4^+ oxidation may be less important in estuarine environments than in oceanic ones”. Since the Baltic Sea is an estuarine system, light inhibition of the nitrification is

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most likely of minor importance.

2. Most microbial process rates show a pronounced seasonality, which follows the annual temperature cycle (ML temperatures during our study were in the range from 1.2 to 3.2°C). According to Ward (2000), however, nitrifying bacteria can easily adapt to low temperatures, thus we might expect nitrification even at the low temperatures during our study. This is in agreement with the results of Enoksson (1986) who found a significant seasonality of the nitrification rates in the ML of the western Gotland Basin (stations #284 and #245) indicating that the rates in November were considerably higher than in June. Additionally, during the time of our cruise, nitrifiers were not outcompeted by phytoplankton since the phytoplankton bloom, competing for NH_4^+ , usually starts a month later (in March).

Thus, we conclude that NH_2OH in the western and southern Baltic Sea (stations #360, #22, #133) was most likely formed by nitrification. This is in agreement with the results of Butler et al. (1987) who found a correlation between NH_4^+ oxidation rates and NH_2OH concentrations for the Yaquina River system.

Since high NH_2OH concentrations were associated with enhanced NO_2^- concentrations we may speculate that nitrification during our study was incomplete. Possibly the rates of the second nitrification step (i.e. NO_2^- oxidation to NO_3^-) were lower than the rates of the NH_4^+ oxidation (typical for a “fresh” nitrifying system, Philips et al. (2002)) or the second step was inhibited by the rather high concentrations of NH_2OH (Castignetti and Gunner, 1982).

Anammox, as an alternative formation process for NH_2OH , should only occur at the oxic/anoxic interface in the western Gotland Basin. Comparable conditions favouring anammox were found in the Black Sea and Golfo Dulce, Costa Rica (Dalsgaard et al., 2003; Kuypers et al., 2003). However, the formation of NH_2OH by anammox in the Baltic Sea could not be verified. At the oxic/anoxic interface in the western Gotland Basin (see e.g. station #284, Fig. 4d) no distinct depletion or enhancement was observed.

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4. Conclusions

The water column distribution of NH_2OH was measured for the first time in the Baltic Sea enlarging considerably the existing data set of oceanic NH_2OH measurements. The distribution of dissolved NH_2OH in Baltic Sea in February 2004 was complex due to the interplay of in-situ production in the shallow western and southern Baltic Sea and the hydrographical setting in the central Baltic Sea caused by the major North Sea water inflow event in January 2003. We found no correlation of NH_2OH with N_2O or O_2 suggesting (i) that a direct coupling of N_2O formation via NH_2OH was negligible and (ii) that NH_2OH formation was not influenced by O_2 . Nitrification appeared to be the major NH_2OH source, whereas NH_2OH formation via anammox was not detectable. We suggest that a “fresh” nitrifying system, in which the NH_4^+ -oxidation rates exceeded the NO_2^- -oxidation rates, favoured the build-up of NH_2OH .

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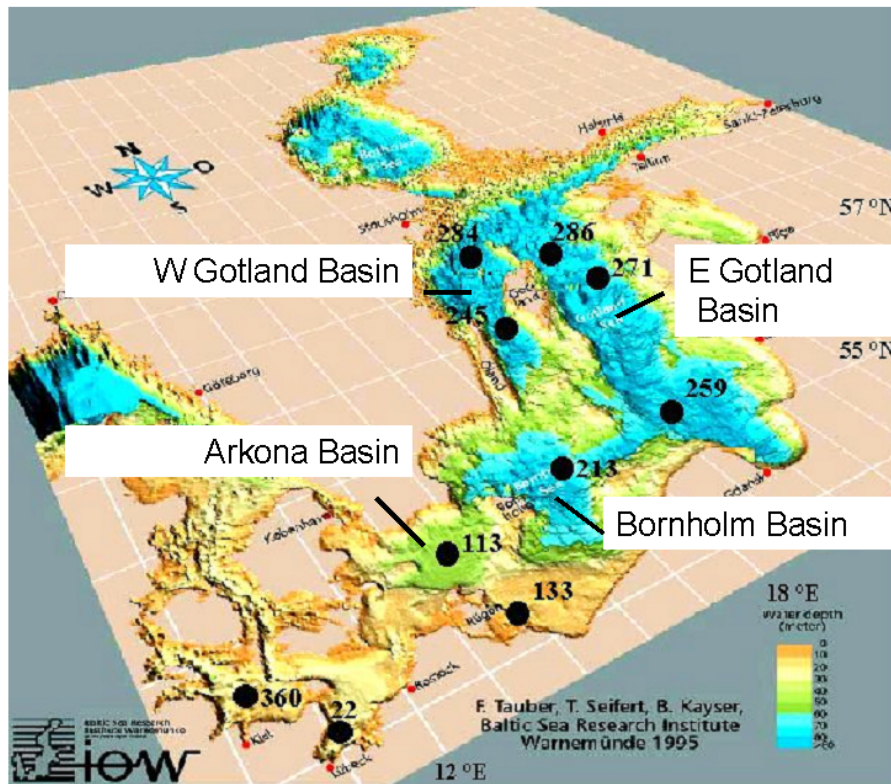


Fig. 1. Map of the Baltic Sea. Sampling stations are indicated.

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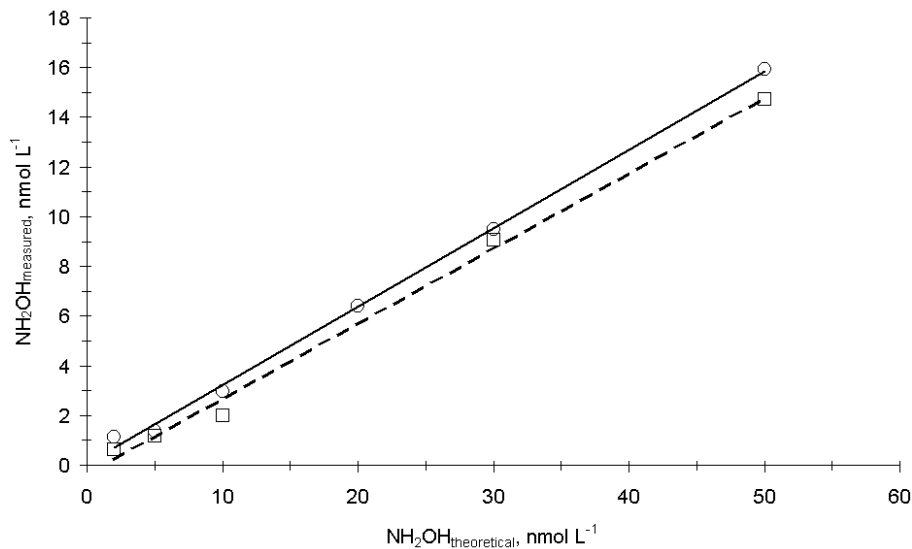


Fig. 2. Determination of the recovery factor based on two independent experiments. Data points represent mean of triplicate samples. (solid line: $y=0.32x+0.054$, $r^2=0.998$; dashed line: $y=0.30x-0.37$, $r^2=0.995$)

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Hydroxylamine
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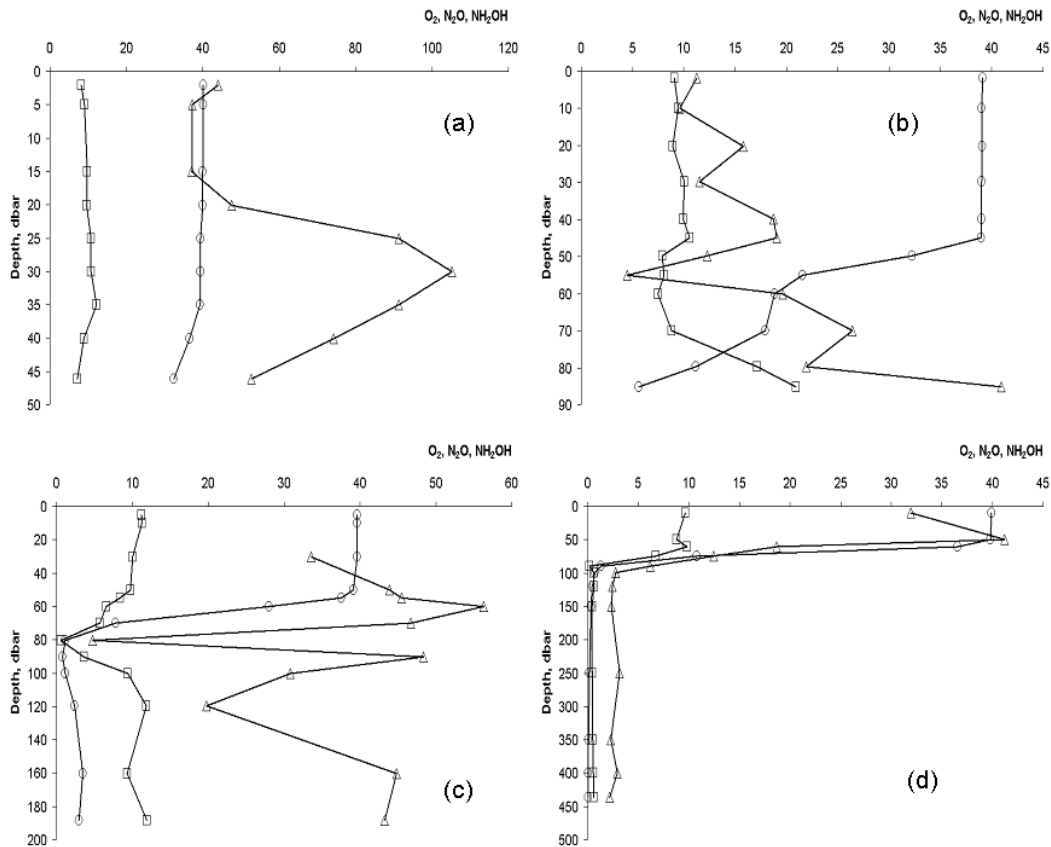


Fig. 3. NH₂OH (in nmol L⁻¹, triangles), N₂O (in nmol L⁻¹, squares) and O₂ (in μmol L⁻¹/10, circles) in the central Baltic Sea: **(a)** station #113 Arkona Basin, **(b)** station #213 Bornholm Deep, **(c)** station #286 Farö Deep, and **(d)** station #284 Landsort Deep.

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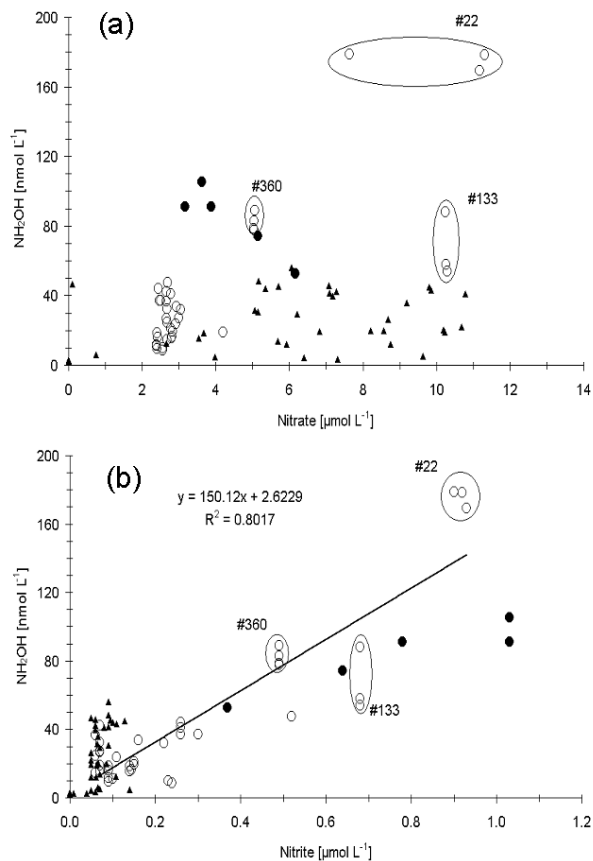


Fig. 4. (a) NH₂OH vs. NO₃⁻ and (b) NH₂OH vs. NO₂⁻. Open circles stand for data from the mixed layer, filled triangles stand for data from below the mixed layer and Filled circles indicate data below the mixed layer from station #113. Data from stations #360, #22, and #133 are marked.

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