

## Impact of sampling techniques on the concentration of ammonia and sulfide in pore water of marine sediments

by

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### Abstract

Three ex situ pore water sampling procedures (I – rhizon samplers, II – centrifugation of sediment subsamples collected from different sediment depths without core sectioning, III – core sectioning and centrifugation of sediment sections) were compared to indicate factors that may affect concentrations of pore water constituents (ammonia and sulfides). The methods were selected and modified in such a way as to determine how the concentrations are affected by different factors related to sampling procedures, e.g. contact with atmospheric air, filtration and sediment core disturbance. They were tested on nine sediment cores collected at one site in the southern Baltic Sea. The concentration of ammonia in pore water from centrifuged sediment sections was significantly higher compared to pore water extracted by rhizons – probably due to the impact of changing pH. The factor with the greatest impact on the  $H_2S/HS^-$  concentration in the analyzed pore water was the contact with atmospheric air and/or the extrusion of sediments from a core liner. Rhizons proved to be the best option for sampling pore waters analyzed for  $H_2S/HS^-$  and  $NH_4^+/NH_3$ . In the case of  $H_2S/HS^-$  we noticed the smallest loss of the analyzed constituents. For ammonia, the centrifugation of the whole sediment sections was likely to cause interferences in the indophenol blue method.

**Key words:** pore water, sediments, rhizon samplers, sediment centrifugation, ammonia, sulfide

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## Introduction

The pore water of marine sediments may account for 30 to 99% of a sediment sample, depending on the sediment core depth, grain size and the amount of organic matter (Mudroch & Azcue 1995). It contains a wide range of chemical substances that shape the environmental conditions in bottom sediments (Salomons et al. 1987; Lerat et al. 1990; Calvert & Pedersen 1993; Graca et al. 2006). Results of pore water analysis provide valuable information on the processes in bottom areas of different water bodies. The application of appropriate analytical methods is a key factor that determines the obtained concentration values. The sampling method may significantly affect the concentrations of pore water constituents (US EPA 2001a).

Sediment pore water sampling procedures can be divided into two major groups: *in situ* and *ex situ* methods. Pore waters can be collected directly at the bottom of a water body by using suction filtration devices, pore water equilibrators (peepers) or different dialysis samplers. However, in the studies of marine sediments, pore waters are more often obtained from sediment cores in ship-based laboratories or on land by core squeezers (low-pressure squeezers for surface sediments or high-pressure squeezers for deep drilled cores), centrifugation or filtration-type methods (e.g. rhizon samplers). The selection of the most suitable pore water sampling method is determined by different factors associated with the type of research, e.g. location and water depth of sampling sites, the type of sediment, pore water constituents to be measured, money available for research etc. Moreover, each sampling procedure may affect the quality of pore water samples and, as a consequence, the results of pore water analysis may not reflect the actual environmental conditions in bottom sediments of the investigated water bodies.

Low-pressure squeezers used to be one of the most often applied pore water extraction techniques (Lange et al. 1992; Manheim 1974). In the 1980s, the 1990s and the early 21st century, apart from low-pressure squeezing, the centrifugation method was also very common, used in most studies on marine sediments when analyzing the pore water (Conley et al. 1997; Karlson et al. 2005; Łukawska-Matuszewska et al. 2009). Sampling of pore waters from anoxic sediments requires special equipment, e.g. an anaerobic glovebox, to limit or even avoid contact with atmospheric air (Egger et al. 2016; Schulz 2000). Thus, in the last 5–8 years of research on sediments of continental shelf seas, rhizon samplers have become an increasingly popular method of pore water

extraction (Dickens et al. 2007; Bolałek 2010; Shotbolt 2010) and have been used in numerous studies (e.g. Jilbert et al. 2011; Müller et al. 2011; Thang et al. 2012; Wu et al. 2015; Gao et al. 2017; Naik et al. 2017; Ballas et al. 2018). Results of sediment pore water analyses are often compared between different studies. The pore water sampling techniques are not always taken into account when discussing the compared results. Moreover, their exact effect on specific pore water constituents has not been sufficiently investigated. Some researchers (e.g. Song et al. 2003; Shotbolt 2010) state that rhizon samplers have numerous advantages over other, usually “destructive” techniques for pore water sampling from marine sediments (e.g. centrifugation or pressure filtration), while other authors point to possible artifacts (e.g. degassing of CO<sub>2</sub> – Schrum et al. 2012; ammonia sorption – Ibánhez et al. 2014, or problems with resolution when taking large amounts of pore water) introduced by this relatively novel method. Although rhizon samplers are becoming increasingly popular in the research on marine sediments, the differences between parameters measured in pore waters obtained through rhizons and other methods (e.g. comparison of rhizons vs centrifugation), and whether degassing or sorption is a significant artifact introduced during rhizon procedures are still not clear.

The objective of the present study was to compare the three methods of pore water sampling, based on the results of ammonia and sulfide concentrations, and to show how different factors associated with each method, e.g. contact with atmospheric air, filtration, centrifugation and sediment core disturbance, may affect the concentrations in the extracted pore water. We decided to choose ammonia, because it may be adsorbed when using filtration-based methods (Ibánhez et al. 2014), and sulfide – because part of this anion occurs in marine sediments, depending on pH, also in the form of gaseous hydrogen sulfide.

## Materials and methods

In order to determine how sampling procedures affect the concentration of compounds dissolved in pore waters, nine sediment cores were collected at the site (EXP-1) located in the southern Baltic Sea (Gulf of Gdańsk), at a water depth of 85 m (accumulation bottom, below the permanent halocline). The cores with a length of about 40 cm were obtained in March 2010, during a cruise onboard ORP *Heweliusz*, using a Niemistö-type corer (inner diameter 7.5 cm). Visual observations of the cores indicated that there were no microbiological mats in the collected sediment, the

cores were undisturbed and the overlying water was clear. Sediments in the area of the sampling station contained fine-grained silt-size particles (Mojski 1995; supported by visual observations). General information on the sampling station is provided in Table 1. All the collected cores had an upper 5-cm layer of light olive brown color and a deeper sediment layer of black color. They were randomly divided into three groups: three cores in each group and, subsequently, pore waters were sampled with a different method for each group. The three methods used for pore water sampling were selected in such a way as to investigate how the concentrations of pore water constituents may be affected by different factors related to sampling procedures, e.g. contact with atmospheric air, filtration, core sectioning and disturbance as well as centrifugation (Table 2).

**Table 1**

## General information on the sampling station

Station ID	EXP-1
Location	54°37'N; 18°59'E
Water depth	85 m
Bottom water salinity	10.4 PSU
Bottom water temperature	5.9°C
Type of sediment	silt
C <sub>org</sub> content in layer 0–1 cm	7–8 % wt. *

\* – based on Szczepańska &amp; Uścińowicz (1994)

**Description of methods for pore water extraction**

**Method I** – rhizon CSS samplers inserted directly into sediment cores through 4-mm holes in capped Plexiglas liners, at 5-cm intervals, and connected to 3-ml syringes. The pore water extraction lasted for about 20 min. We collected about 2.5–2.8 ml of pore water. The following sediment depths were sampled: 5, 10, 15, 20, 25, 30 cm bsf (below sea floor; core No. 1, 2, 3) and additionally 35 cm bsf (core No. 1 and 2). The sample of 5 cm bsf from core No. 1 for ammonia determination was rejected due to contamination.

The resolution applied in this method was rather low compared to typical studies of short cores from the coastal marine environment (usually 0.5–2 cm). However, our objective was to obtain results in order to compare the extraction methods, while the exact layer-to-layer variations in parameters were a secondary issue. Rhizon samplers have been widely used for pore fluid sampling from soils for over 20 years (Knight et al. 1998; Sigfusson et al. 2006). They basically consist of a hydrophilic porous polymer tube (pore diameter of 0.15 µm) and PVC tubing. They can be connected either to a syringe or to a vacuum test tube; pore water is obtained from a sediment core by vacuum-driven suction (detailed characteristics of rhizon samplers can be found in e.g. Seeberg-Elverfeldt et al. 2005). This method eliminates contact with atmospheric air, ensures very little core disturbance during sampling and gives filtrated samples that are almost free of bacteria. The range of sediment dewatering for a pore water volume of ~3 ml is max 0.5 cm; cylindrical area of  $r = 0.5$  cm around a rhizon, close to the sediment edge (Seeberg-Elverfeldt et al. 2005). Therefore, sampling at a very high resolution, e.g. 0.5 cm, is impossible using rhizons. The sorption should be reduced as the samplers are made of inert polymer, which is expected to have no ion-exchange properties.

**Method II** – centrifugation of approx. 9 ml of a sediment subsample (20 min, 3500 rpm rot/min, in 10 ml tubes) directly from capped Plexiglas core liners, collected within 30 min after the core collection, using syringes with the luer tip removed (diameter of approx. 10 mm) through predrilled 11-mm holes, at 5-cm intervals. The range of the sediment layer from which each subsample was collected was 1.1 cm. Pore waters for sulfides and ammonia were sampled at 5 cm resolution: for sulfides – from sediment depths of 5, 10, 15, 20, 25 cm bsf (core No. 4); 7, 12, 17, 22, 27 cm bsf (core No. 5) and 4, 9, 14, 19 cm bsf (core No. 6); and for ammonia – at 5, 10, 15 cm bsf (cores No. 4); 7, 12, 17 cm bsf (core No. 5); and 4, 9, 14 cm bsf (core No. 6). Contact with atmospheric air was limited to a minimum when transferring a sample to centrifuge

**Table 2**

## Summary of factors associated with the applied pore water extraction methods

Factor	Method I (rhizon samplers)	Method II (centrifugation of sediment subsamples collected using syringes)	Method III (centrifugation of sediment sections pushed out of a core liner)
centrifugation	–	+	+
contact with atmospheric air	–	+/-	+
core disturbance	–	–	+
filtration	+	–	–

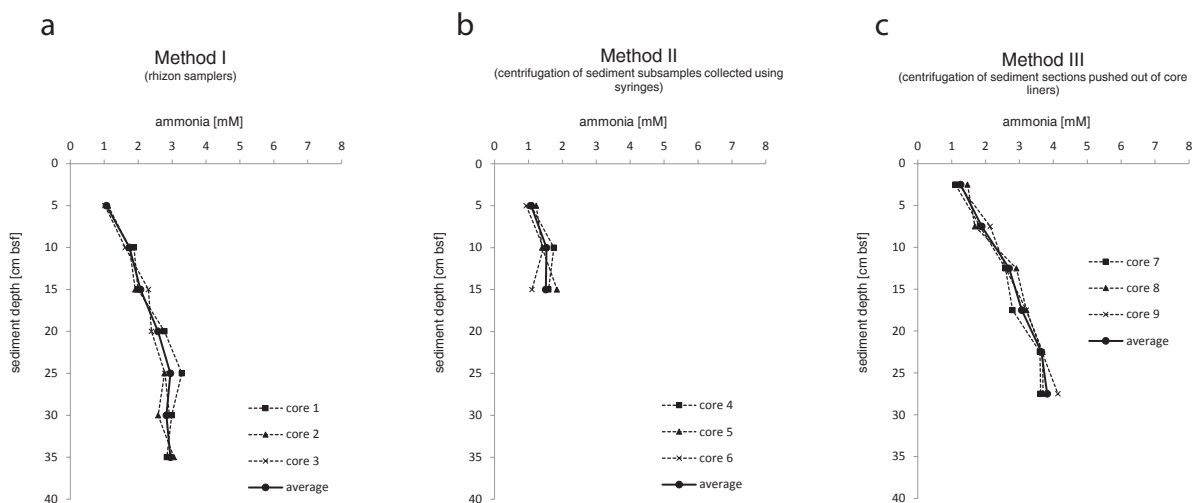
tubes. Different depths of sediment sampling resulted from not equal length of the cores in liners, which had been pre-drilled every 5 cm. In the case of ammonia, sediment sampling from only three depths of each core in this method was due to accidental disturbance of deeper parts of the cores during sampling for sulfides and the subsequent decision to reject samples from these layers for further analysis. The concentrations of sulfides and ammonia in cores No. 5 and 6 were linearly interpolated to obtain values for 5, 10, 15 etc. cm bsf. This is not a typical sampling procedure. However, it can be used to extract pore waters for the analysis of oxygen-reactive compounds, especially from long cores that are usually not sectioned in a traditional way. In this study, it was used for comparison as it eliminates the effect of sample filtration (we used turbidity correction instead; the absorbance value of a sample without reagents was subtracted from the measured absorbance value for that sample with reagents; Rainwater & Thatcher 1960), core disturbance during sampling (sectioning), and additionally it ensures limited contact with atmospheric air.

**Method III** – centrifugation (20 min, 3500 rpm, in 40 ml tubes) of 5-cm sediment sections pushed out of core liners. Sediment sectioning was carried out within 1 h after core retrieval. Vertical sediment edges of each section were discarded. Concentrations of parameters measured in each 5-cm section were assigned to the middle depth of the section, i.e. the 0–5 cm sediment

layer – 2.5 cm bsf; the 5–10 cm sediment layer – 7.5 cm bsf; the 10–15 cm sediment layer – 12.5 cm bsf, etc. Samples were not filtered after centrifugation, however, the correction for turbidity of pore water was applied. Some studies do not recommend filtering of samples for parameters related to gas content (e.g. Rainwater & Thatcher 1960) or toxicity (e.g. Ankley et al. 1994). The procedure of Method III is usually carried out under anaerobic atmosphere to avoid reactions that change redox potential of anoxic sediments. In our case, samples were exposed to atmospheric air during sectioning and transferring into centrifuge tubes as one of our objectives was to determine the effect of oxidation. Sediment cores in this method are usually disturbed during the extrusion from core liners, especially when they contain gaseous compounds (e.g. methane that migrates up the cores after decompression). The results obtained with this method are mean values for pore waters of each sediment section and are not the values for a specific sediment depth, as in the case of the first and second method. The thickness of the sections was selected as 5 cm to obtain averaged profiles of the examined parameters with the same number of subsamples as in Method I and II.

### Chemical analysis

For the purpose of this study, pore water samples for sulfides and ammonia were analyzed spectrophotometrically, immediately after the extraction (within



**Figure 1**

Profiles of ammonia concentration obtained for three methods of pore water sampling (a – rhizon samplers; b – centrifugation of sediment subsamples collected using syringes; c – centrifugation of sediment sections pushed out of core liners). In the case of Method II, concentration values for cores 5 and 6 were interpolated, and in the case of Method III, concentrations for each sediment layer are given for a sediment depth from the middle of a layer.

max 2 h after core retrieval), in a ship-based laboratory. The samples were stored in the dark at 5°C for a short period between the extraction of pore water from the cores and the analysis. Subsamples of 2 ml were analyzed for sulfides (sum of  $H_2S$ ,  $HS^-$  and  $S^{2-}$ ), using the methylene blue method described by Fonselius et al. (1999). The limit of detection for sulfides was 1  $\mu M$  and RSD < 3% (based on calibration curves). In the case of ammonia (sum of  $NH_4^+$  and  $NH_3$ ), subsamples of 0.5 ml were diluted with milli-Q water and analyzed according to the standard indophenol blue method used in marine chemistry (Grasshoff et al. 1999). The limit of detection for ammonia was 2  $\mu M$  and RSD < 3% (based on calibration curves). The statistical analysis (Statistica v.10 software) was carried out to compare the obtained results.

## Results

### Ammonia

Ammonia concentrations in pore waters from different sediment depths, obtained using Method I (rhizon samplers) were generally similar for all three cores (Fig. 1a). The concentrations ranged from 1.88 to 3.29 mM, from 1.11 to 3.05 mM and from 1.02 to 2.92 mM for cores 1, 2 and 3, respectively. The mean RSD value for this method was 8.05%.

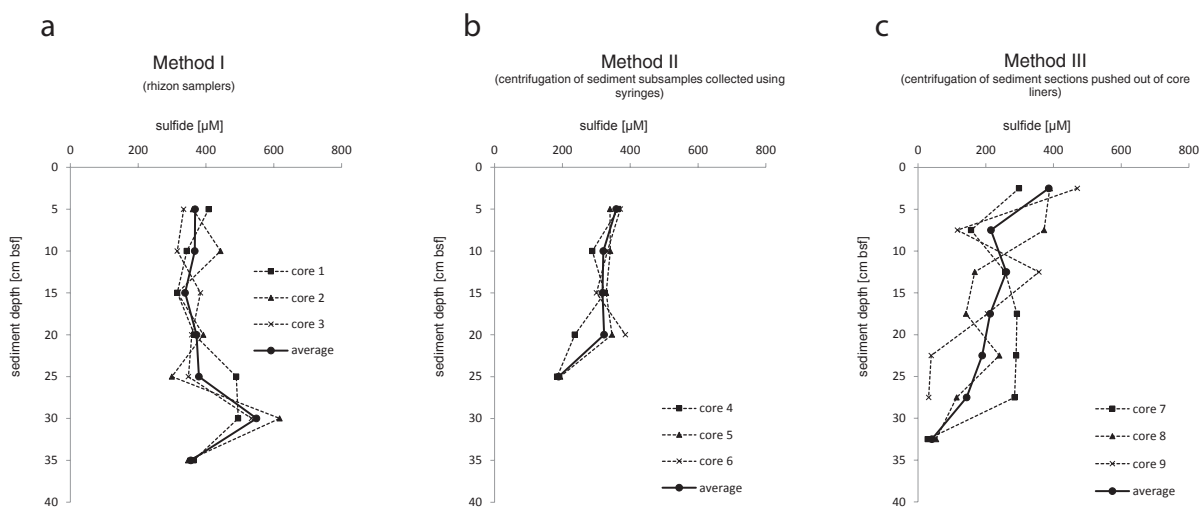
In Method II (centrifugation of subsamples obtained with luer tip removed syringes), the

obtained concentrations were within the following ranges: 1.07–1.75, 1.23–1.84, 0.93–1.44, for cores 4, 5, 6, respectively. The least similar ammonia values were measured in pore waters collected from the depth of 15 cm bsf – the difference between cores 5 and 6 was almost twofold, while the most similar values were those from the levels of 5 and 10 cm bsf (Fig. 1b). The mean RSD for this method (depths 5, 10, 15 cm bsf) was 17.02%.

The highest values of ammonia were obtained for pore water profiles of sediments sampled using Method III (core sectioning + centrifugation; Fig. 1a,b,c). They did not differ significantly between the cores (7, 8 and 9; Fig. 1c). The concentration ranges for each core were as follows: 1.11–3.62 mM (core 7), 1.47–3.69 mM (core 8) and 1.21–4.13 mM (core 9; Fig. 1c). Again, differences between the average concentration profile for this method and specific values in each core were generally < 10% (mean RSD for this method = 8.23%)

### Sulfide

Sulfide concentrations in pore water samples extracted using the first method (rhizon samplers) ranged from 315 to 495  $\mu M$ , from 299 to 618  $\mu M$  and from 315 to 535  $\mu M$  for cores 1, 2 and 3, respectively (Fig. 2a). The difference between the values for specific sediment depths of the three cores, expressed as the mean RSD, was 12.23%. The largest values were obtained for sediments at depths of 10 cm (RSD



**Figure 2**

Profiles of sulfide concentration obtained by three methods of pore water sampling (a – rhizon samplers; b – centrifugation of sediment subsamples collected using syringes; c – centrifugation of sediment sections pushed out of core liners). In the case of Method II, concentration values for cores 5 and 6 were interpolated, and in the case of Method III, concentrations for each sediment layer are given for a sediment depth from the middle of a layer.

= 18.24%) and 25 cm bsf (RSD = 26.04%), and the smallest ones for 20 cm bsf (RSD = 4.80%) and 35 cm bsf (RSD = 3.27%).

The concentrations of sulfide in pore waters of each core sampled using Method II (centrifugation of sediment subsamples collected using cut syringes) ranged from 185 to 365  $\mu\text{M}$ , from 194 to 346  $\mu\text{M}$  and from 301 to 386  $\mu\text{M}$  for cores 4 and 5, 6, respectively (Fig. 2b). The profiles of sulfide concentration obtained with this procedure were the most similar among all three tested methods. The difference between the average profile and the values in individual cores, expressed as the mean RSD, was 9.07%. At a depth of 25 cm bsf, however, the average sulfide concentration for this method was almost two times lower than for the first method, i.e. the mean value of 189  $\mu\text{M}$  for Method II and 379  $\mu\text{M}$  for Method I.

The third method (core sectioning + centrifugation of sediment sections) produced the least repeatable sulfide concentration values. The concentration ranges for the cores were as follows: 29–298  $\mu\text{M}$  (core 7), 53–389  $\mu\text{M}$  (core 8) and 31–470  $\mu\text{M}$  (core 9; Fig. 2c). The shapes of the sulfide profiles in the sediment column were completely different for each core sampled using this method (mean RSD value of 51.59%). For most layers, a particular sulfide value was sometimes over two times higher than the value for the same level but a different core, e.g. sulfide concentration in the layer of 5–10 cm bsf of cores 8 (373  $\mu\text{M}$ ) and 9 (117  $\mu\text{M}$ ). Moreover, the lowest sulfide values obtained with this method were measured in pore waters of the bottom layer of each core (Fig. 2c), i.e. 29  $\mu\text{M}$  (30–35 cm bsf, core 7), 53  $\mu\text{M}$  (30–35 cm bsf, core 8), 31  $\mu\text{M}$  (25–30 cm bsf, core 9).

## Discussion

The pore water of marine sediments in coastal areas is a complex matrix (Chapman et al. 2002) as it contains different organic and inorganic compounds, sometimes unidentified, which undergo reactions that may be difficult to predict (US EPA 2001b), e.g. microbiologically-mediated dissolved organic matter transformations. Therefore, operations that are part of the pore water extraction procedures (e.g. filtration, centrifugation, core disturbance and contact with atmospheric air etc.) can generate new compounds that, for example, may interfere with chemical reactions specific to the determination of particular parameters (Rainwater & Thatcher 1960). Knowledge of the basic sediment fluid composition may not be sufficient when discussing factors affecting the potential reactions that may change the

pore water composition after its extraction. When centrifuging thick layers of sediment (> 5 cm), the potential for secondary reactions (e.g. oxidation, complexation, sorption etc.) is even greater due to mixing of sediment layers (within one thick section) that may have different redox potential values. Thus, considering the extraction methods applied in this study, we keep in mind the fact that some results (Methods I and II) do not reflect the real shape of concentration profiles (for ammonia and sulfide) in sediments, but only a shape based on values from particular levels of a sediment core (sampling resolution for Method I – 5 cm, while the real resolution of pore water extraction is unknown; resolution of 5 cm for Method II). When sampling by rhizons (Method I), especially for small amounts of water, a sample is extracted from the edge of a core (Seeberg-Elverfeldt et al. 2005), while in the case of the method based on centrifugation of sediment (Method III), core edges are discarded. Moreover, the centrifugation of whole sediment sections (Method III) yields an averaged value for each sediment section (5 cm in our case). In the present study, this may also have affected, to some extent, the final ammonia and sulfide profiles obtained for each method and led to differences in the concentrations for particular depths/sections.

Moreover, the concentrations in which chemical constituents of seawater occur in pore waters can be orders of magnitude greater (e.g. ammonia, phosphate, silicate or DIC) than those in the water column (Rainwater & Thatcher 1960; Schulz 2000; Eby 2016). Most of the methods for seawater analysis were designed to cover concentrations in seawater (e.g. methods included in Grasshoff et al. 1999) and they were subsequently adopted for pore water measurements (Falcão & Vale 1998; Baric et al. 2002; Łukawska-Matuszewska 2016). However, sometimes the methods have not been sufficiently tested on such complex samples as pore waters of coastal marine sediments.

The trends in the concentrations of sulfide and ammonia in pore waters extracted by three different methods are described separately in the following subsections as the proportion of their different forms (gaseous vs ionic) is different with pH typical of pore waters in the Gulf of Gdańsk. Thus, their measured concentrations may be affected by different factors.

### Comparison of sampling methods for ammonia

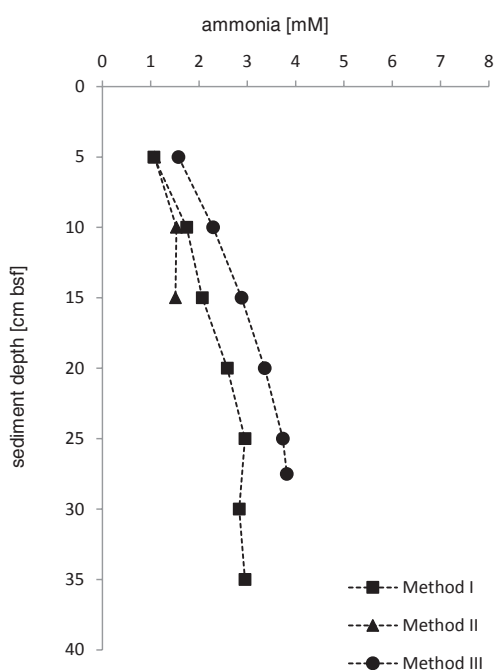
Based on the statistical analysis, it was found that a method used for pore water sampling had a statistically significant impact (Kruskal–Wallis test,  $p = 0.001$ ) on the concentration of ammonia in the

sediment pore water. The shape of the average ammonia profile was quite similar for each sampling method (Fig. 3). This was particularly clear for the cores in Method I and III. The concentration values for ammonia measured in pore water obtained by sediment sectioning and centrifugation (Method III) were on average 35% higher than the concentrations in samples from cores collected by Method I (rhizon samplers) and Method II (centrifugation of subsamples obtained with cut syringes). Based on the ammonia profiles (Fig. 1) and considering the factors related to each method, we observed that core sectioning, disturbance and contact with atmospheric air (all corresponding to Method III) led to the highest concentrations compared to factors related to the two other methods.

At pH values of pore waters from the Gulf of Gdańsk (6.44–8.04; Brodecka-Goluch & Łukawska-Matuszewska 2018; Brodecka 2013; Łukawska-Matuszewska & Kielczewska 2016), most of the ammonia content (> 97%) in pore waters is present in the form of the ammonium ion (Emerson et al. 1975) and therefore, the potential escape of gaseous

$\text{NH}_3$  while sectioning a core (Method III) is negligible for the results of the analysis. However, in the case of core disturbance occurring during sectioning, the loss of about one third of hydrogen sulfide was reported (see Results and Discussion subsections related to sulfide). Assuming that with the typical pH range of pore water samples (on average ~7), about 50% of sulfide was present in the form of hydrogen sulfide (e.g. Holmer & Hasler-Sheetal 2014), the loss of 30% of  $\text{H}_2\text{S}$  led to a pH increase of about 0.5–2, depending on the exact in situ pH values of pore waters (from a range of 6.44–8.04 – typical for pore water in the sediments from the Gulf of Gdańsk). We assume that as a result of  $\text{H}_2\text{S}$  loss and/or oxidation, pH of pore waters in Method III was changed (Chapman et al. 2002) in relation to samples in Method I and II. According to the literature, pH of samples may affect the results of ammonia determination by the indophenol blue method (Tzollas et al. 2010; Crompton 2006 and references therein). The mechanism of reactions that occur during ammonia analysis using the indophenol blue method is complicated (Aminot et al. 1997) and it largely depends on pH. The formation of monochloramine (reaction intermediate) requires a pH of 8–11.5 and the obtained indophenol is fully oxidized at pH of 10.8 (Grasshoff et al. 1999). In our study, as we previously assumed, the initial pH and buffer capacity of samples subjected to ammonia determination was different in Method I and II vs Method III. This factor may have been responsible for the differences in the ammonia profiles obtained by Method III.

Some authors (e.g. Zadorojny et al. 1973) observed the interference of amino acids in the indophenol method. Although amino acids concentrations in seawater are relatively low (0.5  $\mu\text{M}$ ; Grasshoff et al. 1999), the amounts found in the sediments are considerably greater. It has been found that the concentrations of amino acids in pore water of surface sediments are one or two orders of magnitude higher than those in the overlying water (Mintrop & Duinker 1994 and references therein). Moreover, these organic compounds can be adsorbed on clay minerals present in the sediments (Friebele et al. 1981). Despite the fact that amino acids were not analyzed in pore water samples in the present study, we speculate that to some extent they may have interfered with the ammonia measurements. They may have caused differences between the results obtained for immediately filtered and extracted pore water (Method I, rhizon samplers) and pore water collected after core sectioning and centrifugation of the whole sediment sections (Method III), where there was a greater possibility of amino acids adsorption on sediment particles (clay minerals). It has been



**Figure 3**

Profiles of the average ammonium concentration for three methods of pore water sampling (Method I – rhizon samplers, Method II – centrifugation of sediment subsamples, Method III – centrifugation of sediment sections). In the case of Method III, concentrations were averaged to obtain values for depths of 5, 10, 15 cm etc.

observed that amino acids can serve as positive (e.g. L-lysine-HCl, L-threonine) or negative (e.g. L-cysteine, L-glutamic acid or L-phenylalanine) artifacts in the ammonia determination by the indophenol blue method. Accordingly, they can increase the final result of ammonia concentration by ~3% or decrease by 5–13% (Zadorojny et al. 1973). However, previous studies tested the effect of amino acids for ammonia concentrations of about one order of magnitude lower than in pore waters.

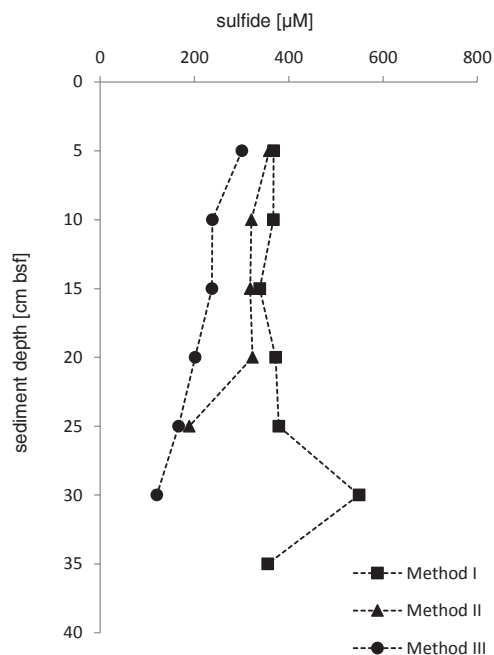
The next factor that cannot be ignored in the discussion of pore water extraction methods is filtration as it may change the concentration of an analyte in pore water (Ankley et al. 1994). Despite the fact that Song et al. (2003) and Schrum et al. (2012) successfully applied rhizon samplers (as a method based on filtration) for ammonia measurements in pore waters from lake and marine sediments and obtained accurate results, similar to those obtained by other methods applied for comparison, Ibánhez & Rocha (2014) identified and described the  $\text{NH}_4^+$  adsorption on PES membranes used in rhizons. They found that the highest potential for this adsorption occurs at low temperature and low salinity, and with a small volume of filtered water (< 2 ml). For example, at 5°C in a ~350  $\mu\text{M}$   $\text{NH}_4^+$  solution the adsorption is about 28  $\text{nM cm}^{-2}$ . According to Ibánhez & Rocha (2014), adsorption capacity of PES membranes is almost two times higher at < 5°C than at 20°C. On the other hand, the impact of salinity is associated with ion competition for adsorption sites. At  $\text{NH}_4^+$  concentrations < 400  $\mu\text{M}$ , the effect of adsorption was observed for salinity values up to 10 PSU (Ibánhez & Rocha 2014). The authors also suggested pH as a factor affecting the  $\text{NH}_4^+$  sorption capacity of membranes (as it controls the equilibrium between  $\text{NH}_4^+$  and  $\text{NH}_3$ ), however, this was not further researched by Ibánhez & Rocha (2014). In the present study, one of the objectives was to check whether rhizon samplers provide lower ammonia concentrations due to filtration (pore size of 0.15  $\mu\text{m}$ ) and subsequent adsorption. The results obtained in our study did not provide a clear answer as the concentrations in Method I (rhizon samplers) were lower than in Method III (core sectioning + centrifugation, no filtration), but similar to the concentrations in Method II (centrifugation of sediment subsamples without core sectioning, no filtration). These results would rather indicate a factor related to Method III, which contributed to the higher concentration, e.g. interferences in the indophenol blue method caused by changes in pH due to hydrogen sulfide escape/oxidation. In general, the case of ammonia adsorption on PES membranes used in rhizons needs further research in order to

obtain clear results, especially with regard to physical and chemical conditions that potentially promote this process in brackish environments.

Based on the obtained results, we believe that the differences in pH, and partly also the effect of amino acids, were the main causes of higher ammonia concentrations obtained by Method III compared to Methods I and II. However, the possibility that sample filtration (rhizons in Method I) led to the adsorption of ammonia cannot be completely excluded.

### Comparison of sampling methods for sulfide

The statistical analysis conducted for sulfide results confirmed that the method of pore water sampling had a statistically significant impact on sulfide concentrations (Kruskal–Wallis test,  $p = 0.0001$ ). The difference in the concentrations was evident not only in the case of average profiles for each method (Fig. 4), but also in the case of profiles for cores sampled using the same method (Fig. 2a,b,c). This was probably caused by the gaseous nature of the examined component. The highest sulfide concentrations were measured in cores sampled by



**Figure 4**

Profiles of average sulfide concentration for three methods of pore water sampling (Method I – rhizon samplers, Method II – centrifugation of sediment subsamples, Method III – centrifugation of sediment sections). In the case of Method III, concentrations were averaged to obtain values for depths of 5, 10, 15 cm etc.



rhizons (Method I) and the lowest ones in sediments that were pushed out, cut and centrifuged (Method III; Fig. 4). The lower concentrations of sulfide obtained using Method II and III can be explained by its upward migration and escape (in Method III during core sectioning), followed by oxidation (during exposure of sediment sections to open air in Method III; very limited exposure in Method II). The highest rates of escape and oxidation were determined in the core that was sectioned as the last one (core No. 9) compared to core No. 7 that was cut first, about 1 h before core No. 9 (average concentration of sulfide = 230  $\mu\text{M}$  for core No. 7 vs 203  $\mu\text{M}$  for core No. 9). In addition, the concentration of sulfide in the topmost sediment layer was much higher (470  $\mu\text{M}$ ) in the last core (No. 9) compared to core No. 7 (298  $\mu\text{M}$ ), which also confirms the existence of the upward migration.

Taking into account the range of pH values of pore waters in the Gulf of Gdańsk mentioned in the previous subsection and based on the relationships between sulfide speciation and pH (e.g. Holmer & Hasler-Sheetal 2014), it can be assumed that about 30–80% of sulfide in the measured samples was present in the form of dissolved, non-volatile  $\text{HS}^-$ , and the rest in the form of volatile  $\text{H}_2\text{S}$ . Thus, during our experiment we tried to minimize the time between the core retrieval and sectioning to avoid any additional artifacts. Nevertheless, the unavoidable process of the core decompression, the subsequent sectioning (sediment disturbance) and at least partial exposure of individual sediment sections to atmospheric air in Method III resulted in the greatest hydrogen sulfide loss and oxidation compared to the two other methods (Schulz 2000; Cline & Richards 1969). Part of the volatile  $\text{H}_2\text{S}$  escaped from the cores and another part was oxidized to sulfates (Chapman et al. 2002). In addition, centrifugation of sediments in Method II and III led to further  $\text{H}_2\text{S}$  loss from pore water to the headspace of centrifugation tubes (Henry's law). Based on the differences between (1) Method I and II, and (2) Method II and III, we calculated the effect of centrifugation and core disturbance with subsequent oxidation, respectively. The loss of  $\text{H}_2\text{S}$  as a result of core disturbance/sectioning and sulfide oxidation (loss of ~20% of sulfide) was greater than that resulting from centrifugation (loss of ~10% of sulfide; Fig. 4). In contrast, the loss of hydrogen sulfide in Method I was minimal as rhizon samplers ensured no contact with air during extraction. Finally, the difference between Method I and Method III with regard to sulfides was on average about 30%.

Based on the applied methods, we were not able to verify whether the filtration process (Method I) led to any adsorption of sulfides on the PES membrane.

However, even if any adsorption occurred, it was probably negligible compared to the negative effect of core sectioning (core disturbance and oxidation) or centrifugation on sulfide concentrations.

## Conclusions

It is common knowledge that sampling procedures may affect concentrations of different constituents of sediment pore water. The comparison of three pore water sampling methods (rhizon samplers, centrifugation of sediment subsamples from different core levels, centrifugation of sediment sections) for the analysis of ammonia revealed that core sectioning and centrifugation with no filtration (Method III) yielded about 35% higher concentrations than rhizons (Method I) or centrifugation of sediment subsamples, also with no filtration but additionally without core cutting, disturbance and oxidation (Method II). Centrifugation of whole sediment sections probably caused interferences in the indophenol blue method. On the other hand, the highest sulfide concentrations were obtained when rhizon samplers (Method I) were applied, while the lowest ones when using centrifugation of sediment sections in aerobic conditions (Method III). This was due to hydrogen sulfide migration, oxidation and loss during core sectioning and sediment centrifugation. The loss that occurred as a consequence of core disturbance/sectioning and oxidation (20% loss) was greater than that resulting from centrifugation (10% loss). In addition, a 1 h delay in core sectioning led to 10% loss in sulfides.

Rhizon samplers seem to be a good option for pore water analysis of volatile compounds, where core disturbance may introduce some artifacts. Moreover, they ensure quick application, no contact with atmospheric air, provide water that is filtered and free of most bacteria. This study has not proven any adsorption on the PES membrane in rhizons, however, due to unclear results we cannot reject this possibility either. The issue has to be further investigated.

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