eawag_04450

RECENT STUDIES ON SOURCES AND SINKS OF METHANE IN THE BLACK SEA

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Abstract This study focuses on the influence of gas seepage on methane sources and sinks, aerobic and anaerobic oxidation of methane and the mediating microbial organisms in the Black Sea. We present data from two cruises that took place in 2001 and 2003. Seven stations (two from the shelf, four from the upper and lower slope, and one from the central basin) were compared with respect to methane concentration and isotope signature. The stations differed in methane concentration depending on the location on the slope. A strong change in the concentration and isotopic composition of methane was observed below the oxic/anoxic interface, coinciding with increased levels of archaeal biomarkers (archaeol and sn-2-hydroxy-archaeol). Concentration and isotopic composition of methane in the water column and sediments indicate that sediments from the shelf, slope, and deep basin are only minor sources of methane. The main methane sources are seeps located on the shelf and upper slope, but also in the deep basin. The comparison of two shelf stations with and without methane seepage showed a difference in methane concentrations, isotopic composition and oxidation rates, but the presence of similar methanotrophic microbial assemblages. Also two deep stations at a seep and outside of a seep area were compared, but here methane concentrations and oxidation rates were not different from each other. Anaerobic methane oxidizers (ANME-1 and ANME-2 group) were observed at both stations with slightly higher cell counts at the seep station.

Keywords: Black Sea, methane concentration, methane isotopic composition, methane oxidation, methane seeps, methanotrophs

L. N. Neretin (ed.), Past and Present Water Column Anoxia, 419–441.
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1. INTRODUCTION

Methane is an important greenhouse gas which has increased from a level of 850 ppb before industrialization to 1.7 ppm today, further increasing with approximately 1 % per year [6, 34]. Although methane concentration in the atmosphere is small compared to CO_2 (360 ppm), its impact as a greenhouse gas in the atmosphere is about 24 times higher [15]. Main methane sources of the earth today are of human origin. The largest natural sources are wetlands and termite guts. A large proportion of the methane flux to the atmosphere comes from anthropogenic sources that are either energy related (i.e. mining and gas drilling) or agricultural (i.e. ruminants, rice agriculture and biomass burning beside landfills) [6]. Despite considerable sources of methane in the seafloor, the ocean generally contributes only a small amount of ~5-20 Tg methane per year (<2%) to the atmosphere [6] due to microbial aerobic and anaerobic oxidation processes in sediments and water column [35, 36]. Recent research has focused a lot on the role of gas hydrates as the largest reservoir of methane on earth that has been overlooked before 20-30 years ago [25]. Methane clathrates are now found at almost all continental margins with a suitable temperature-pressure field. [26] including the north-western part of the Black Sea [5, 16].

The Black Sea has a surface area of 423,000 km^2 and a maximal depth of 2212 m, and represents the world largest anoxic basin [39]. After a freshwater period during the last glacial, the Black Sea turned into a brackish basin when the Bosporus established a full connection to the Mediterranean about 7150 yrs ago [13, 40]. Due to large freshwater inflow by rivers the surface water now has a salinity of 17.5–18.5 ‰, whereas the deep water salinity is 22.3 ‰ [31]. Anoxia developed 7500 yrs ago due to the stable stratification of the Black Sea waters [20]. The aerobic surface waters are separated from anoxic deep waters by a chemocline at 100-200 m water depth depending on the geographical location [45]. On the shelf and upper continental slope where large rivers like the Danube enter the Black Sea the chemocline may even reach down to 300 m [45]. The water column contains substantial but varying amounts of methane, which has been attributed to methanogenesis in the water column [17] and sediments [37], as well as to the release of methane from gas reservoirs such as methane hydrates [16]. Whereas methane concentrations in the oxic surface waters are in the nanomolar range, methane concentrations in the anoxic deep water are much higher, sometimes exceeding 10 μM. The inventory of methane in the Black Sea adds up to 96 Tg methane [37, 44]. It is still not clear which role the deposited sediments play in the methane turnover in the Black Sea, i.e. whether they function as a methane source or sink. Reeburgh et al. [37] have suggested that sediments on the slope emit methane into the water column whereas basin sediments serve as a methane sink.

Another important question is the identity, distribution and activity of microbial organisms responsible for anaerobic and aerobic methane oxidation in the Black Sea water column. Biomarker and compound specific stable isotope investigations on particulate material collected from the anoxic water column showed that archaea may be involved in methane oxidation [43, 49]. Methane oxidation rates are around 10^{-3} nM d^{-1} in the upper water layer and increase to a few nanomoles per day below 100 m [37]. However, beside these organic geochemical investigations there are no published molecular biological reports so far that really identified those organisms.

Relatively recent findings especially in the north-western part of the Black Sea are methane seeps [2, 18]. At these locations methane enters the water column in the aerobic as well as in the anaerobic water zone and tremendously influences the methane inventory of the Black Sea.

In order to better understand methane turnover in the Black Sea we measured methane concentrations in the sediments at two shallow sites on the NW and SW slope and at a deep site in the central basin. Additionally, we investigated several biogeochemical parameters related to methane in the water column at shallow and deep methane seeps and non-seep sites. Measurements were performed in sediments as well as in the water column to investigate further the sink/source behavior of the seafloor. Methane stable carbon isotopic composition was determined for further insight into the oxidation/formation patterns of methane. Additional biomarker and molecular investigations of particulate water column material were performed to reveal which organisms are involved in the methane oxidation. The data presented are partly preliminary results from ongoing research that will be further evaluated in the future.

2. METHODS AND MATERIALS

2.1 Sampling

During cruise M51-4 in December 2001 with the German research vessel *R/V Meteor* water column profiles and sediment cores were sampled at the following stations: 7605 (42 30,71'N, 30 14,69'E) at 2130 m water depth in the central basin, 7617 (43 38,04'N, 30 02,54'E) at 1560 m water depth from the NW slope, and 7623 (41 44,77'N, 31 10,28'E) at 876 m water depth from the SW slope (Fig. 1). Additionally, samples were recovered during the CRIMEA cruise with *R/V Professor Vodyanitskiy* in 2003. Presence of gas seepage was identified hydroacoustically by an echosounder system sensitive to gas bubbles onboard. Water samples were taken at two shallow sites, namely a gas plume site above a cold seep (CTD-038: 44° 50'N, 31° 59'E, 92 m water depth) and a near by reference site without seepage (CTD-055: 44° 51'N, 32° 01'E, 76 m). Also two deep stations were sampled, a gas plume site (CTD-072: 44° 17'N, 35° 02'E, 1985 m) and a reference site without seepage to the west

(CTD-064: 44° 14'N, 32° 30'E, 1658 m, Fig. 1). Water samples were taken with a rosette system equipped with 10 l Niskin bottles. To sample the gas plume at the seep stations, the vessel was either anchored (shallow water) or the rosette was fired while drifting over the plume site, after careful mapping of the plume dimensions using side scan sonar (deep stations). Oxygen profiles were recorded using a CTD system calibrated by Winkler titration.

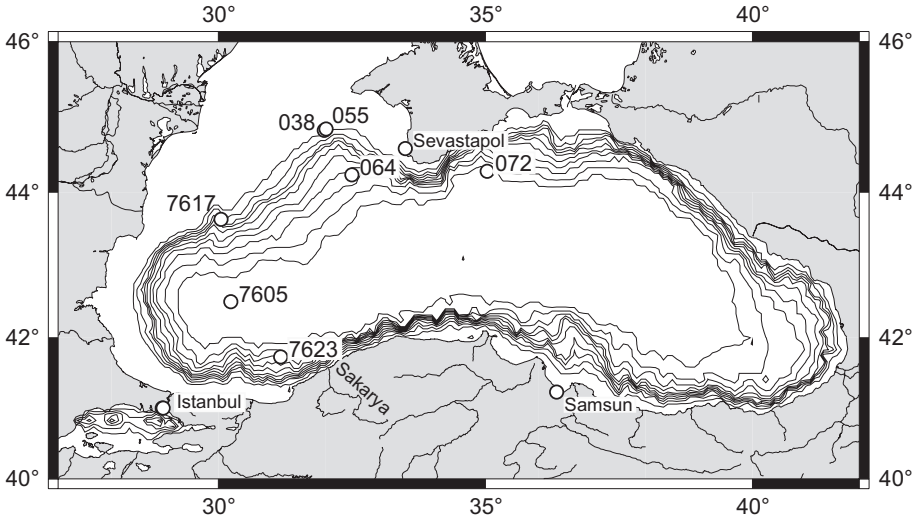

Figure 1. Map of the Black Sea showing sampling stations. Stations 7605 (central basin), 7617 (north-western slope), and 7623 (south-western slope) were sampled during the Meteor cruise in 2001 and Stations 038 (seep site on the north-western shelf), 055 (reference site on the north-western shelf), 064 (reference site on the lower slope southwest of Crimea), and 072 (seep site on the lower slope south of Crimea) were sampled during the CRIMEA cruise in 2003.

2.2 Methane Concentration and Isotopic Composition

For water sampling, 120 ml serum bottles were filled from the Niskin bottle directly after retrieval of the rosette. The water samples were poisoned with NaOH pellets, crimped immediately with a butyl-rubber stopper, and kept in the dark at 4 to 8 degrees. In the laboratory, a 20 ml helium headspace was introduced and equilibration between both phases was achieved. For sediment sampling a 5-mL cut-off plastic syringe was inserted through small holes in the core liner and the samples were placed in 50-mL serum vials containing 6 mL of 2.5 % NaOH solution. These vials were crimp sealed with butyl-rubber stoppers, shaken, and allowed to sit at room temperature for some hours before the measurement. Quantification of methane was accomplished by injecting 1 to 5 mL of headspace from the serum vials into a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector. Injector

temperature was 200°C and the detector was at 225°C. The column, 6' x 1/8" stainless steel packed with Poropak Q (80/ 100 mesh), was maintained at 40°C. The carrier gas was N_2 flowing at 25 mL min^{-1}, and the retention time for CH_4 was about 0.7 min. Peak areas were quantified with an HP 3396 Series II electronic integrator. A known amount of standard gas (Scotty, Supelco) was injected in quadruplicate and served for quantification. Analytical precision was ± 5%.

For CH_4 analysis aboard *R/V Professor Vodyanitskiy*, a modification of the vacuum degassing method described by Lammers and Suess [27] was used [38]. 1600 ml of water were injected into pre-evacuated 2200 ml glass bottles leading to quantitative degassing. The gas phase was subsequently recompressed to atmospheric pressure and the CH_4 concentration of the extracted gas was determined by gas chromatography. A Shimadzu GC14A gas chromatograph equipped with a flame ionization detector was used in connection with a Shimadzu CR6A Integrator. Nitrogen was used as carrier gas, and separation was performed using a 4 m 1/8' SS column packed with Porapack Q (50/80 mesh) run isothermally at 50°C.

The carbon isotopic composition of dissolved methane ($\delta^{13}C_{CH4}$) was determined by a method described earlier [41]. Precision of the method was ± 1 ‰.

2.3 CH_4 Oxidation Rates

Water for measuring microbial methane oxidation was filled in triplicates in 20 ml crimp-seal bottles and capped gas-tight. From each triplicate, one sample was killed with 50 μl concentrated formaldehyde solution which functioned as a blank. Aliquots of 50 μl tritiated methane (^{3}H-CH_4) were added to the bottles and incubated in the dark at ambient temperatures imitating natural conditions. Immediately after the incubation an aliquot of the water was mixed with scintillation cocktail (Ultima Gold, Packard) and measured to determine the actual amount of tracer added to the sample. After the samples stood uncovered overnight they were bubbled for 20 min with nitrogen to eliminate all unreacted tritiated methane. An aliquot of the bubbled water was mixed with the scintillation cocktail and measured again. Measurements were performed by means of a scintillation counter (1600CA Tri-Carb, Packard). Turnover rates (k value, d^{-1}) were calculated from the ratio of tracer remaining in the water to total tracer added.

2.4 Lipid Analysis

Particulate organic matter for lipid analyses was collected from specific water depths by filtration of large volumes (up to 1,000 l) of water through 142 mm diameter glass fiber filters (GFF; nominal pore size 0.7μm, precombusted at

370° C) with in situ pumps. The GFF were extracted for 24 h in a Soxhlet apparatus to obtain the total lipid extracts. Aliquots of the total extracts were saponified after addition of an internal standard and separated into fatty acid and neutral lipid fractions. The fatty acid fractions were methylated (BF_3-MeOH, Sigma) and the neutral fractions were derivatized (BSTFA, Sigma) and analyzed by gas chromatography and gas chromatography–mass spectrometry for the quantification and identification of lipids, respectively.

2.5 Fluorescence in Situ Hybridization (FISH) and Cell Counts

Bacterial abundance was determined by epifluorescence microscopy (Zeiss Axioscope 2, 1,000 magnification) of DAPI (4',6-diamidino-2-phenylindole)-stained cells. Bacterial cells were fixed by the addition of concentrated formaldehyde solution (5 % final concentration) for 15 min at room temperature and thereafter recovered by gentle vacuum filtration (20 and 50 ml for each sample) on to polycarbonate filters with a pore size of 0.2 μm (GTPB, Millipore). After washing with PBS and water, the filters were transferred into sterile PP petri dishes, sealed and stored frozen at –20 °C for FISH. The protocol of Pernthaler et al. [33] was used for the hybridization procedure. The following oligonucleotide probes (MWG, Germany) were used to describe the microbial communities: Arch915 for members of the domain *Archaea*; Eel MS 932 (ANME-2 group); ANME-1, distantly related to *Methanosarcinales* [4]; and MG84/705 and MA450, describing methanotroph groups I and II [9], respectively. Probes were labeled with the indocarbocyanine fluorescent dye CY3 and fluorescein (MWG, Germany).

3. RESULTS

3.1 Oxygen Profiles

The water column of the sampled stations clearly showed a chemocline separating the water column in an oxic and anoxic zone. Figure 4 shows the oxygen profiles of the investigated stations. At the NW station 7617 and at the central station 7605 oxygen concentrations reached up to 220 μM, the SW station 7623 had surface water oxygen concentrations below 67 μM. These low concentrations may be caused by the Sakarya river inflow and its particulate organic matter load at the sampling site. The central station 7605 showed relatively stable values of 220 μM at the surface down to 55 m and then a very fast decrease to values around zero below 100 m. Oxygen depletion was also found below approximately 100 m at the SW station and below 140 m at the NW station. Here, the transition between the oxic and the anoxic layer was not as abrupt as observed at the other two stations. Stations 064 and 072 on the

lower Crimean slope had their oxic-anoxic interfaces at approximately 120 m and 180 m, respectively, whereas the shelf stations 038 and 055 were situated fully in the oxic part of the water column. It has been previously observed that the chemocline is deeper at the slope compared to the central basin (e.g. [45] and references therein).

3.2 Sediment Methane Concentrations

Short sediment cores of up to 40 cm length were retrieved at stations 7617 and 7623 on the upper slope and at station 7605 in the central basin. Methane concentration profiles of all three cores looked relatively similar with concentrations around 10 and 12 μM at the surface and decreasing values towards the core bottom with concentrations between 4 and 8 μM (Fig. 2). The profile shape with higher concentrations at the surface and lower concentrations at the bottom indicates a methane flux from the top to the bottom of the core.

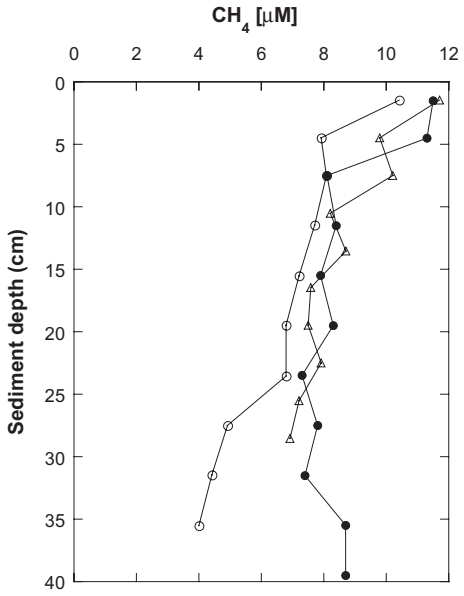

Figure 2. Methane concentrations in three sediment cores recovered from the NW slope (7617, open triangles), SW slope (7623, open circles), and the central basin (7605, full circles). The linear decrease of methane from top to bottom of the cores indicates a diffusive flux of methane from the water column into the sediments.

3.3 Water Column Methane Concentrations

All stations except the seep station 038 showed methane concentrations of 8 to 50 nM with increasing depth in the oxic water column. At the seep station 038,

methane reached 10 times higher values of up to 550 nM above the seep (Fig. 3). Below the chemocline at most stations methane values increased rapidly to approximately 10 μM at about 500 m, and remained stable around 10.5 to 11.3 μM from 500 to 2100 m water depth. Exceptions were the upper slope stations in the North-West (7617) where methane concentrations increased from 0.4 to 2.7 μM from 150 to 295 m, and in the South-West (7623), where methane concentrations increased from 0.5 at 150 m to 4.5 μM at 340 m (Fig. 4). The water columns sampled at the lower slope position (064 and 072) showed very similar concentration profiles with maximum values of 12.5 μM (Fig. 5).

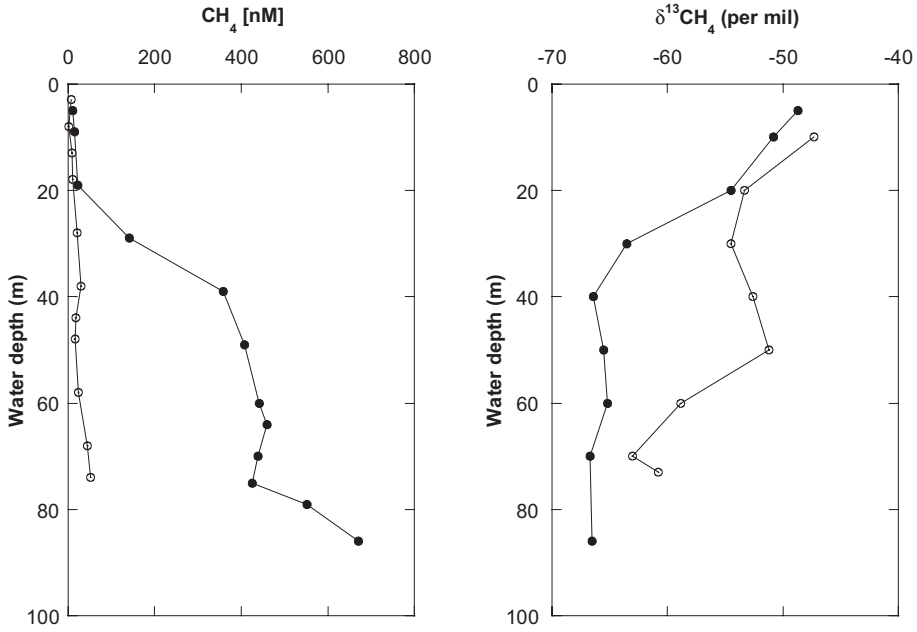

Figure 3. Water column methane concentrations (nM) at the seep 038 (full circles) and reference site 055 (open circles) located on the north-western shelf of the Black Sea. Carbon isotopic composition ($\delta^{13}C_{CH4}$ vs. VPDB) of the dissolved methane from the water column above the seep site (038, full squares) and reference site (055, open squares).

3.4 Isotopic Composition of Water Column Methane

The stable carbon isotopic composition of methane was measured at the north-western station (7617), the central station (7605), at the lower slope station (064), and at the two shelf stations (038, seep and 055, reference, Fig. 4,5,3). At the central station (7605, Fig. 4) $\delta^{13}C_{CH4}$ values in the anoxic deep water varied around - 54 ‰ and the shallowest sample taken at 30 m water depth had a $\delta^{13}C_{CH4}$ value of -42 ‰. A striking change in isotope fractionation

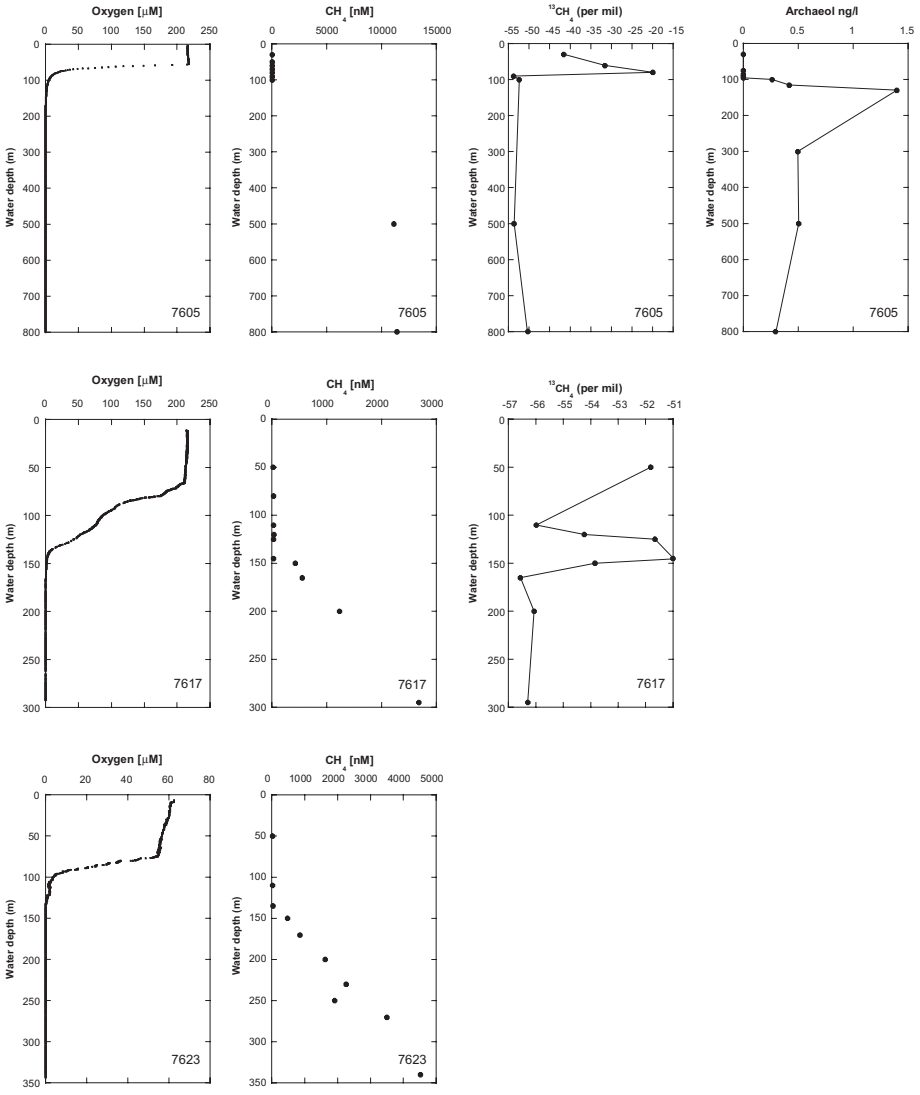

Figure 4. Oxygen (μM) and methane concentrations (nM) of stations 7605, 7617, and 7623, the carbon isotopic composition of methane ($\delta^{13}C_{CH4}$ vs. VPDB) of stations 7605, 7617 and the depth distribution of Archaeol (ng/l) extracted from particulate material collected from the water column at station 7605. Note the difference in the depth of the chemocline between stations and the change in isotopic composition of the methane due to oxidation.

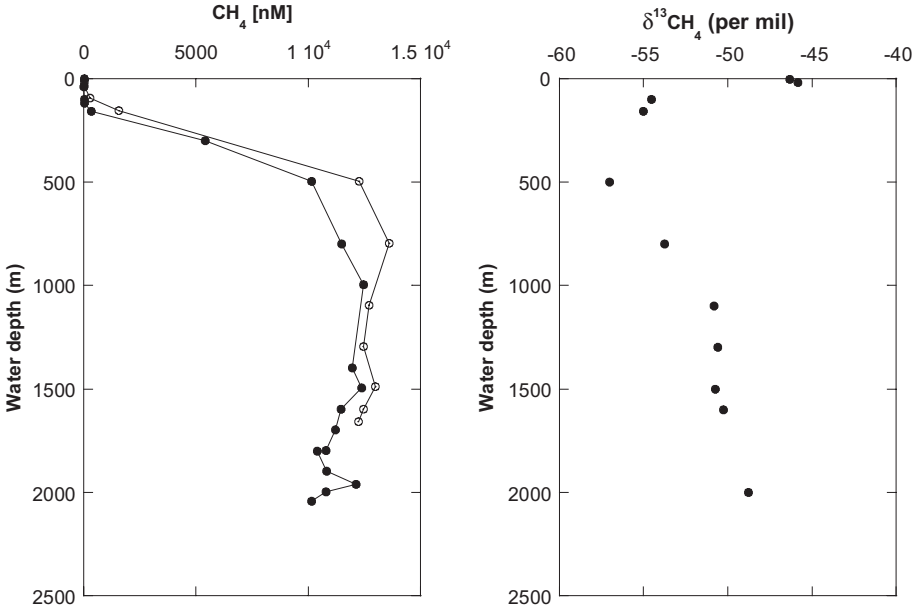

Figure 5. Depth profile of methane concentrations of reference site 064 (light circles) and a composite profile of seep sites 068 and 075 that were sampled very close to station 072 (dark circles). Carbon isotopic composition of the dissolved methane from the water column above the reference site 064. The depletion in the isotopic values from approximately 800 to 500 m water depth indicates an additional source of methane from the upper to middle slope.

occurred just below the chemocline at 100 m water depth, Here, $\delta^{13}C_{CH4}$ increased to -20 ‰. At the north-western station (7617, Fig. 4) the samples from 300 to 165 m had $\delta^{13}C_{CH4}$ values around – 56 ‰ followed by a significant increase to -51‰. The top sample at 50 m water depth had $\delta^{13}C_{CH4}$ values of -52 ‰. The stable carbon isotopic composition of methane at site 064 (Fig. 5) varied between -49 and -51 ‰ VPDB. Above 800 m $\delta^{13}C_{CH4}$ values decreased to a minimum of -58 ‰ VPDB at 500 m and subsequently increased again above 160 m to $\delta^{13}C_{CH4}$ values of -46 ‰ VPDB near the surface. The isotopic composition of the dissolved methane in the oxic water column near the seafloor at the two shelf stations (Fig. 3) differed with a $\delta^{13}C_{CH4}$ value of -67 ‰ VPDB at the bottom of the seep site (038) from the $\delta^{13}C_{CH4}$ value of -58 ‰ VPDB at the bottom of the reference site (055). A constant increase in $\delta^{13}C_{CH4}$ values from -67 ‰ VPDB to -49 ‰ VPDB from the bottom to the surface could be noted at the seep station (038).

3.5 Lipid Biomarkers for Methane Oxidizers in the Water Column

Lipids indicative for methane oxidizing bacteria and/or archaea were investigated at the two upper slope sites (7617, 7623), and at the central site (7605). Two glycerol-ethers namely archaeol and sn-2-hydroxyarchaeol, indicative of methanogenic or methanotrophic archaea, depending on the isotope signature [14, 23], were detected in the water column. Both compounds were only present in the anaerobic zone of the Black Sea and totally absent in the aerobic water layer. Concentrations in the anaerobic layer could be determined only for archaeol. They were found directly below the chemocline at 100 m in low abundances of 0.3 to 1.5 ng/l (Fig. 4). sn-2-Hydroxyarchaeol could be detected only as a trace compound in the samples at 130 m from the central station (7605) and from 170 m at the SW station (7623) where it occurred with ~0.1 ng/l. These concentrations are very low compared to those that are found in sediments off Oregon where up to 8 μg/g sediment of both compounds have been measured [4]. Due to the low abundance it was not possible to measure isotope signatures, hence it cannot be concluded whether the biomarkers are of methanogenic or methanotrophic origin.

3.6 Methane Oxidation Rates

Methane oxidation rates using tritium labeled methane were measured at the two shallow stations 038 (seep) and 055 (reference) and at the deep slope stations 064 (reference) and 072 (seep). There was on average a 30 times higher oxidation rate at the seep site (0.02 to 1.6 nM d^{-1}) compared to the shallow reference site (0.001 to 0.05 nM d^{-1}). In contrast, at the deep sites no significant difference was observed between reference and seep sites with the anoxic water column values of 0.03 to 3.1 nM d^{-1}.

3.7 Fluorescence in Situ Hybridization of Methanotrophic Microorganisms

To identify the methanotrophic community that is responsible for anaerobic methane oxidation in the anoxic Black Sea water column, filtered samples from the lower slope stations 064 (reference) and 072 (seep) were investigated by FISH (a method using specific fluorescently labeled gene probes which allows the detection of microorganisms under the microscope). Using 16S rRNA-targeted oligonucleotide probes specific to both groups, it was possible to detect ANME-1 and ANME 2 group cells, usually found in sediments, in the water column of the Black Sea. Cell counts of filters from the water column above the methane seep site revealed ANME-1 and ANME-2 cells in concentrations of up to 4 % of all DAPI stained cells (Table 1, [8]). Interestingly, cells counts

declined to below 1.2 % above 800 m, the depths where the bubble flare could not any longer be detected in the water column. At the reference station the ANME-1 and ANME-2 cells were lower than at the seep site and represented only around 1 to 2 % of total cells.

Table 1. Occurrence of ANME-1 and ANME-2 cells at the reference (064) and seep site (72) on the lower slope. Higher abundances of ANME-1 cells were measured at 1500 and 2000 m where a bubble plume could be observed with hydroacoustical means (echosounder) up to 1200 m in the water column (+ >2%, - <1.2%).

Station 064			*Station 072*		
Depth (m)	% ANME-1	% ANME-2	Depth (m)	% ANME-1	% ANME-2
160	+/-	+/-	500	-	-
500	+	-	1200	+	-
800	+/-	-	1500	+	+
1600	+/-	-	2000	+	+/-

The identification of aerobic methanotrophic bacterial cells using FISH showed at both shallow stations (038 and 055) a share of 0.1 to 4.5 % of methanotrophs type I cells of total DAPI stained cells. Methanotrophs type II were only detected with 2 % of total DAPI stained cell counts at one depth (038, 83 m) very close to the sediment. We have tested our FISH probes with pure cultures of methanotrophic bacteria of type I and II to ensure our cell detection/counting. Differences in cell numbers between our work and the data provided by Gal'chenko et al. [12] (see discussion) might have their origin in the different methods (immunofluorescence versus FISH), or in natural changes of the microbial population.

4. DISCUSSION

The following discussion compares the results of the two recent expeditions to what is known about the Black Sea methane budgets and fluxes. A closer look is taken at the interaction between the Black Sea water column and the atmosphere, the methane sink and source relationships in the sediments and water column, and at the identity of microorganisms that are responsible for methane oxidation in the oxic and anoxic part of the Black Sea water column. We especially focus on the significance of recently discovered methane seeps that have not or only marginally been considered in former publications [5, 16, 28, 42].

4.1 The Oxic Water Column as a Source of Methane to the Atmosphere

Methane concentrations in the non-seep oxic water column (055) decrease from 50 nM at around 76 m to concentrations of <10 nM at the sea surface. At the seep site (038) methane concentrations are 10 times higher above the seafloor in 80 m with values of up to 550 nM (Fig. 3). However, due to aerobic consumption of methane, surface water methane concentrations (uppermost 10 m) at the seep site are only 1.6 times higher than at the non-seep site with values of up to 16 nM. Oxidation rates at the seep site (up to 1.6 nM d^{-1}) were on average approximately 30 times higher relative to the reference station (0.001 to 0.05 nM d^{-1}). The relative turnover was 97 % at the seep and 87 % at the reference site. The oxidation of methane could also be traced using the stable carbon isotopic composition of the methane at the seep station. Here, a constant increase in the $\delta^{13}C_{CH4}$ values at the seep station from -67 ‰ VPDB (bottom) to -49 ‰ VPDB (5 m below water surface) over the entire water column clearly shows the preferably usage of light ^{12}C methane by the aerobic methanotrophs (Fig. 3).

The percentage of aerobic methanotrophs from the total cell number determined by DAPI varied between 0.1 to 4.5 % at both sites. Intriguingly, only methanotrophic bacteria of type I were detected with the exception of one sample from 100 m water depth at the seep site, where type II methanotrophs represented 2 % of total cell counts. This is at odds with earlier studies reporting that methanotrophs of type I and II are equally abundant in the water column [12]. Furthermore, Gal'chenko and coworkers found methanotrophs of type I and II representing up to 10 % of the total cell counts in the water column, whereas our findings indicate that they represent less than 5 % of the total cell counts (see results for possible explanation).

Comparing surface water methane concentrations with the methane concentration expected assuming atmospheric equilibrium [51], we find that the surface water at the seep and reference sites is 3 to 5 times supersaturated with respect to methane and therefore both stations act as a source for atmospheric methane. This is in agreement with other investigations that have measured methane fluxes from the Black Sea water column to the atmosphere [1, 42]. The latter authors found an air-sea methane flux above a shallow seep area of 0.96–2.32 nmol m^{-2} s^{-1} that is 3 times higher than calculated for the surrounding shelf (0.32–0.77 nmol m^{-2} s^{-1}) and 5 times higher than assessed for open Black Sea waters (water depth >200 m, 0.19–0.47 nmol m^{-2} s^{-1}). Hence, we can conclude that the gas seeps of the upper slope and shelf, where methane emanates in to the oxic water column, contribute substantially to the methane emission. The total number of active seeps at the upper slope and shelf is

still unknown, hence the emission of methane from the water column to the atmosphere related to gas seepage cannot be constrained at this point.

4.2 Variations in Methane Concentration in the Water Column Over Time

The key publication that deals with methane in the Black Sea arose from the 1988 *R/V Knorr* expedition [37]. Reeburgh et al. [37] showed one methane profile from the central basin with low concentrations (< 10 nM) in the oxic zone above 100 m, increasing concentrations from 100 to 550 m, and very stable concentrations around 11 μM down to 2200 m. Additionally, Reeburgh et al. [37] showed methane concentration data from the deep anoxic waters measured by Scranton [44] that were above 12 μM and speculated that this might hint to a methane decrease in the time between the two studies, i.e., 1975 and 1988. However, our measurements from 2001 and new measurements during the CRIMEA cruises in 2003 and 2004 are all between 10.5 and 13.1 μM and therefore close to Reeburgh's et al. [37] data. Hence, we conclude that any increase or decrease in this range could be reflecting regional variability, and that methane concentrations appear to be relatively stable over the past 30 years.

4.3 Sources of Methane to the Water Column

There are three potential sources for methane in the Black Sea: (1) methane is released from the sediments to the water column, (2) methane is produced in the water column or (3) methane seeps emit methane from deeper reservoirs to the water column.

(1) Methane contribution from the sediments to the water column: Depending on the location in the Black Sea the organic matter burial rates are very different. In front of rivers, high amounts of marine and terrestrial organic material are delivered to the sediments and degraded under anoxic conditions by iron, manganese, and sulfate reduction, eventually leading to methanogenesis. On the other hand, towards the central part of the Black Sea terrestrial contribution is limited and organic matter input depends on export of phytoplankton biomass to the seafloor [46]. Lander investigations by Friedl et al. [10] and Friedrich et al. [11] showed that no or only negligible amounts of methane were formed during degradation of organic material in sediments underlying oxic and anoxic bottom waters of the north-western Black Sea shelf. Additionally, Jørgensen et al. [21] could show on a sediment transect located on the north-western shelf and including sediments from water depths from 100 to 1200 m that, although methane is produced deeper in the sediments, no methane reaches the sediment surface and escapes to the water column due to the anaerobic oxidation of methane at the sulfate/methane transition zone. This is also

obvious from sediment methane concentration profiles presented by Sorokin [45]. Sediment methane concentration profiles from our investigation (Fig. 2) show high concentrations at the surface and lower concentrations at the bottom of the cores indicating that sediments from the slope and the basin are a sink for water column methane rather than a source. Dissolved water column methane diffuses into the sediments and is consumed by methanotrophic organisms. In contrast to the methane concentration profiles measured in this study (low μM range), Reeburgh et al. [37] measured concentrations in the mM range and suggested a high flux of methane from the shelf sediments to the water column. However, this is at odd with our findings and the results by Jørgensen et al. [21], Friedl et al. [10], and Friedrich et al. [11]. One explanation for the very high methane concentrations of the sediment core from Reeburgh et al. [37] may be that sediments were recovered from a seep system, an assumption made earlier also by Jørgensen et al. [21].

Most likely, sediments are only a source of methane where the gas is transported by advective processes such as fluid flow and ebullition of free gas. A high number of gas seeps that have been found close to the Crimea peninsula [16, 29] and meanwhile all around the shelf of the Black Sea (results by EU projects CRIMEA; METROL, ASSEMBLAGE) support this hypothesis.

(2) Whether methane is formed in the deep anoxic water column by methanogenesis is highly debated. Ivanov et al. [19] suggested that methane is formed in the order of 63 x 10^{10} mole per year during the process of organic matter degradation in the water column. Reeburgh et al. [37] argued that methane formation in the water column should be negligible, because sulfate reducers outcompete methanogenic bacteria for fermentation products at the presence of sufficient sulfate. Results from Konovalov et al. ([24], and manuscript in preparation) show that the profiles of ammonium and sulfide are in agreement with what would be expected when both constituents were solely derived from organic matter degradation by sulfate reduction, and that sulfate reduction would balance the export flux from surface water. This means that there is only very little place for methanogenesis in the water column and presumably not in the amount proposed by Ivanov et al. [19]. If methanogenesis is a significant process in the water column, this should show up in the isotope signature, as well as in the presence of specific biomarker lipids. We have found only minute amounts of archaeal biomarkers indicative of methanogenic archaea at site 7605. Here, they coincide with a substantial increase in the δ^{13}C of methane, pointing to a zone of anaerobic methanotrophy rather than methanogenesis. We, therefore, conclude that methanogenesis is not a significant process in the water column compared to methane oxidation.

(3) Several hundred seeps emitting methane to the water column were discovered during the last years especially on the NW shelf and south of Crimea ([2] [18] and CRIMEA Cruise Reports 2003, 2004). These seeps are so com-

mon that for instance only during the two cruises linked to the CRIMEA project more than 1000 new seeps where discovered (CRIMEA Cruise Report 2004). Gas seepage is not only found on the shelf, but occurs also on the upper and lower slope [5]. Indications for methane fluxes from seeps at the upper to middle slope could be seen in the $\delta^{13}C_{CH4}$ profile of station 064 (Fig. 5). A decrease in $\delta^{13}C_{CH4}$ values in the water column at 800 to 500 m water depth shows that methane escaping from seeps located at the deeper shelf and slope leaves an imprint on the $\delta^{13}C_{CH4}$ depth profile. This is supported by higher methane concentrations in water depths around 600 m, where the methane profile clearly deviates from other biogeochemical parameters such as NH_4 and H_2S, hence indicating an additional methane source (Konovalov, unpubl. model results). Sorokin [45] showed that the stable carbon isotope composition of methane seeping out of the Black Sea bottom is ~-58 ‰ VPDB and that the age of the methane as determined by ^{14}C dating lies between 3.500 to 5000 years BP. The $\delta^{13}C_{CH4}$ values at station 064 at around 500 m of ~ – 58 ‰ VPDB are actually very close to the values measured for methane escaping the seeps on the shelf and slope indicating a methane source from seepage. The age of the methane further confirms the argument that the methane is not formed by recent methanogenesis in the uppermost sediments, but is delivered from older Black Sea sediment deposits.

Comparing reference site (064) and seep site (072) on the lower slope, methane concentrations below 500 m water depth at both sites were more or less similar with 10-12.5 μM. First it seemed surprising that the methane plume at the seep site which was traced by acoustical means (echosounding) from 2000 m water depth up to 800 m water depth was not reflected in the methane concentration. Most likely, the huge background concentration of 12 μM methane in the deep water masks the signature of the plume. Accordingly, a plume concentration of around 500 nM as detected at the shallow seep (038) would not be resolved at a background of 12 μM.

One method to determine the methane input from seeps into the water is the distribution of noble gases in the water column. The concentrations of dissolved atmospheric noble gases in lake and ocean water correspond closely to the equilibrium concentrations determined by the surface water temperature and salinity that prevailed during gas exchange with the atmosphere [7, 22]. Noble gases are chemically inert, and therefore any observed deviations from the initial equilibrium concentrations can be used for modeling the purely physical processes. The release of gas bubbles into the water column stimulates a secondary gas exchange between the ascending gas phase and the surrounding water by gas stripping and dissolution and therefore affects the local noble gas concentrations [50].

Neon concentrations in the deep water were approximately constant with depth for each of the two profiles, but the mean Neon concentration determined

in the plume was 3.2 % lower than that determined for the reference profile (Fig. 6). This clearly proofs that a gas exchange takes place between the rising bubbles and the surrounding water; i.e., that the gas plume strips dissolved Neon from the water into the rising gas bubbles. It is, however, important to note that the observed Neon depletion in the water column is an integrated signal over the time the seep was and is active including horizontal and vertical mixing of the deep water of the Black Sea.

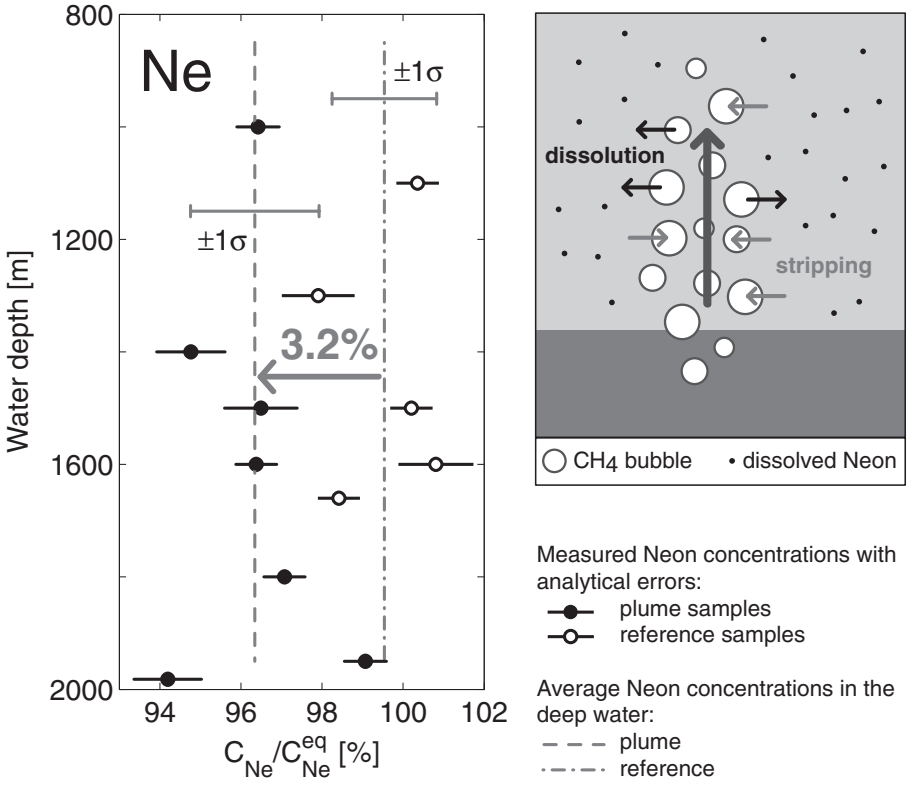

Figure 6. Atmospheric Ne concentrations (normalized to atmospheric equilibrium concentrations) in the deep water of the Black Sea (left hand panel).Water samples from the reference site (open circles) showed on average 3.2 % higher Ne concentrations than water samples of the flare from the seep site (closed circles). On the right hand panel a conceptual model shows how the gas bubbles strip the dissolved Ne from the surrounding water into the bubbles.

In conclusion we propose that a significant fraction of the methane that is found today in the water column of the Black Sea is derived from gas seeps that are mainly distributed on the shallow shelf and slope but can also be found in the deep parts of the Black Sea.

4.4 Methane Sinks

The main sinks in the methane budget of the Black Sea are, as pointed out earlier by Reeburgh et al. [37], central basin sediments, the oxidation of methane in the oxic and anoxic water column (AOM), and evasion to the atmosphere. One of the big questions as to sinks of methane in the Black Sea is the identity of methane oxidizers in the anaerobic part of the water column. It has been shown earlier that members of the order *Methanosarcinales* together with sulfate reducing bacteria are involved in the anaerobic oxidation of methane in the Black Sea [3, 30, 47]. At active gas seeps of the Black Sea, methanotrophic mats were found containing high amounts of strongly depleted archaeol and sn-2-hydroxyarchaeol. Interestingly we found archaeol and sn-2-hydroxyarchaeol in the water column of the Black Sea, coinciding with a strong fractionation against ^{13}C methane and high methane oxidation rates, indicating that methane is oxidized by organisms related to methanogenic archaea just below the chemocline. Unfortunately, due to the low concentration of these compounds in the water column it was not possible to measure their carbon isotopic composition, which would be needed for an ultimate proof that these organisms are involved in AOM. Schouten et al. [43] and Wakeham et al. [49] showed that ^{13}C depleted archaeal derived biphytanes occur in the anoxic water column of the Black Sea providing evidence that methane consuming archaea are present. Isotopically depleted phytane ($\delta^{13}C$ = -51 ‰) released after chemical (HI) treatment from an archaeal lipid precursor (e.g. archaeol, hydroxy-archaeol) has been described in the anoxic water column of the Black Sea (Wakeham et al. 2004). This $\delta^{13}C$ value is, however, rather heavy compared to values measured in sedimentary lipids extracted from venting sites (around –100 ‰, [4]) and the possibility remains that these compounds originate from methanogenic archaea.

Recently, Vetriani and co-workers [48] provided evidence based on 16S rRNA sequences and T-RFLP in one Black Sea water sample at 305 m that archaea phylogenetically related to the ANME-2 cluster are present. Using 16S rRNA-targeted oligonucleotide probes specific to both groups, we were able to detect ANME-1 and ANME 2-related cells in the water column of the Black Sea. Cell counts of samples from the water column above the methane seep (072) revealed ANME-1 and ANME-2 cells in concentrations of up to 4 % of all DAPI stained cells. Above the reference site (064) only up to 2 % ANME-1 and ANME-2 were detected. We believe that these organisms are at least partly responsible for anaerobic methane oxidation in the water column of the Black Sea but it is likely that there are other groups yet to be discovered. Incubation experiments revealed no difference in methane oxidation rates between the reference and seep site. This may be due to the fact that actual

methane concentrations at the two sites as shown above are not significantly different and cell counts were in the same range.

Interestingly, in the sediments of most methane seeps ANME-1 and ANME-2 archaea occurred in a consortium with sulfate reducers of the δ-proteobacteria groups *Desulfosarcina/Desulfococcus* [4, 30, 32]. However, no cell consortia in the water column could be observed and the methane oxidizers occurred rather as single cells. Investigations by 16S rDNA based methods are ongoing to further resolve the question which organisms are involved in the anaerobic oxidation of methane in the Black Sea water column.

From the carbon isotopic composition of the methane it is obvious that the chemocline is especially interesting when looking for methane sinks. At the central station (7605) and at the north-western station (7617) a strong isotopic enrichment of the methane was measured just below the chemocline (Fig. 4) providing strong evidence for enhanced microbial activity, i.e., methane oxidation. From tracer experiments by Reeburgh et al. [37] and own results we know that in the Black Sea water column rates of anaerobic oxidation of methane are 100 times higher than aerobic oxidation rates. However, since the strongest isotopic fractionation occurs where oxygen is already present, although in small amounts, we cannot exclude that aerobic methanotrophs contribute to the methane consumption. Higher sampling resolution at the chemocline is necessary to support this conclusion.

5. CONCLUSIONS

We have evaluated the different methane sources to the Black Sea water column and conclude that a significantly fraction of the methane derives from methane seeps located mainly on the shelf but also on the upper and lower slope. Significant microbial methane oxidation was indicated by increasing $\delta^{13}C_{CH4}$ values especially at the chemocline, but also in the oxic water column. Methanotrophic bacteria of type I are mainly responsible for aerobic methane oxidation in the oxic water column. Methane oxidation rates above the methane seep in the shallow water are approximately 30 times higher compared to a reference station. Methane concentrations and methane oxidation rates from a deep seep and a deep reference station were similar, but an additional input of methane via seepage was indicated by neon depletion in the water column due to gas ebullition. The carbon isotopic composition of methane in the anoxic water column indicates a methane source deriving from the deeper shelf/slope. Anaerobic methane oxidizers (ANME-1 and ANME-2 group) were detected over the deep seep and above the reference station. Detailed investigations are ongoing to better resolve the communities involved in anaerobic methane oxidation. To further understand the methane budget in the Black Sea it is important to determine more specifically methane oxidation rates especially

in the chemocline a boundary playing a crucial role. Additionally, rates of methanogenesis in the water column should be constrained to evaluate more precisely the sources of methane.

Acknowledgements

We like to thank Bo Barker Jørgensen from the MPI in Bremen who made it possible that Edith Durisch-Kaiser could join the research cruise with *R/V Meteor* to the Black Sea in 2001. We are especially indebted to Antje Boetius who has helped and supported us during the last years. Gabi Klockgether is thanked for her excellent analytical assistance. We especially acknowledge Marc De Batist and his enthusiasm with which he is leading the CRIMEA project. We thank all the people from the CRIMEA EU project and the captain and crew from *R/V Meteor* and R/V *Professor Vodyanitskiy* who helped us with sampling during the cruises. Funding came from the EU Project CRIMEA (EVK-2-CT-2002-00162), BBW grant (No.02.0247), the Max Planck Society and EAWAG.

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