

**Submarine Methane Seepage in the Paleo Dnepr
Area and Sorokin Trough and its Influence on the
Black Sea Methane Budget**

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrechts-Universität

zu Kiel

vorgelegt von

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Kiel 2006

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Tag der mündlichen Prüfung: ...07. Dezember 2006.....

Zum Druck genehmigt: ...Kiel, den...20. Dezember 2006.....

Der Dekan

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit selbständig und ohne unerlaubte Hilfen erstellt habe. Ferner habe ich weder diese noch eine ähnliche Arbeit an einer anderen Abteilung oder Hochschule im Rahmen eines Prüfungsverfahrens vorgelegt, veröffentlicht oder zur Veröffentlichung vorgelegt.

Oliver Schmale

*So eine Arbeit wird eigentlich nie fertig,
man muss sie für fertig erklären,
wenn man nach Zeit und Umständen
das mögliche getan hat.*

Johann Wolfgang von Goethe,
Italienische Reise, 1787

Preface

This thesis consists of a general introduction (Chapter I), five stand-alone papers (Chapter II to IV and Appendix I and II), and an overall summary and conclusion (Chapter V). Each of the Chapters II to IV as well as the Appendix I and II contain a separate introduction, description of methods, presentation of data and discussion as well as a separate reference list. Chapter III has already been published in *Geophysical Research Letters*. Chapter II has been submitted to *Marine Chemistry*, and Chapter IV will be submitted to *Global Biogeochemical Cycles*. Appendix I and II are co-author papers that are already published in *Geochemistry*, *Geophysics*, and *Geosystems*, and in the *Nato Science Series: Earth and Environmental Sciences*, respectively.

The titles and authors of the papers and manuscripts are briefly listed below:

Chapter II **Water column investigations on high-intensity seeps in the Paleo Dnepr area (NW-Black Sea) and their regional impact on the methane cycle and hydrography**

Authors O. Schmale, S. E. Beaubien, G. Rehder, J. Greinert, and S. Lombardi

Status submitted to *Marine Chemistry*

Chapter III **Methane emission from high-intensity marine gas seeps in the Black Sea into the atmosphere**

Authors O. Schmale, J. Greinert, and G. Rehder

Status published in *Geophysical Research Letters*

Chapter IV **The transient response of the Black Sea methane budget to massive short-term submarine inputs of methane**

Authors O. Schmale, M. Haeckel, G. Rehder, C. Holzner, and R. Keir

Status to be submitted to *Global Biogeochemical Cycles*

Appendix I Methanotrophic microbial communities associated with bubble plumes above gas seeps in the Black Sea

Authors C.J. Schubert, E. Durisch-Kaiser, C.P. Holzner, L. Klauser, B. Wehrli, O. Schmale, J. Greinert, D.F. McGinnis, M. De Batist, and R. Kipfer

Status published in *Geochemistry, Geophysics and Geosystems*

Appendix II Recent studies on sources and sinks of methane in the Black Sea

Authors C.J. Schubert, E. Durisch-Kaiser, L. Klauser, F. Vazquez, B. Wehrli, C.P. Holzner, R. Kipfer, O. Schmale, J. Greinert, M.M.M. Kuypers

Status published in the *Nato Science Series: Earth and Environmental Sciences*

I contributed to these papers as follows:

Chapter II, III, and IV: work at sea, acquisition and processing of all methane and CTD data, interpretation of data and calculation of methane emissions at the sea surface, the modelling of the Black Sea methane budget, graphical presentation of data, writing, and preparation of manuscripts.

Appendix I and II: work at sea, acquisition and processing of methane concentration data.

Kurzfassung

Methan ist eines der Treibhausgase, die die Strahlenbilanz und damit das Klima auf unserer Erde wesentlich beeinflussen. Geologische Methanquellen, insbesondere submarine, sind unter diesem Aspekt von großer Bedeutung, da sie wesentliche Mengen an Methan in die Atmosphäre emittieren. Die Abschätzung der globalen geologischen Quellstärke ist bis heute allerdings schwierig, da die Verbreitung dieser methanfreisetzenden Areale und die hier stattfindenden Prozesse, die auf den Methankreislauf einwirken, noch nicht ausreichend bekannt sind.

Ein Gebiet, das durch zahlreiche submarine Methanquellen gekennzeichnet ist, stellt das Schwarze Meer dar. Methanaustritte in Form methanhaltiger Fluide oder Gasblasen sind im Schwarzen Meer in allen Tiefenbereichen, vom Schelf bis in das Abyssal, dokumentiert. Dieses Randmeer weist eine einzigartige hydrographische Struktur auf, die sich aus dem Zustrom von Süßwasser über Flüsse und salzhaltigem Mittelmeerwasser über den Bosphorus ergibt. Die von ihrer Dichte sehr verschiedenen Wassermassen führen zu einer ausgeprägten Stratifizierung der Wassersäule, was sich in einer stark limitierten vertikalen Durchmischung niederschlägt. Die ausgeprägte mikrobielle Produktion organischen Materials im Oberflächenwasser führt zu einer intensiven Sauerstoffzehrung in der oberen Wassersäule, die durch den reduzierten Sauerstofftransport in tiefer gelegene Wasserkörper nicht ausgeglichen werden kann. Der ausgeprägte Redoxgradient hat einen bedeutenden Einfluss auf die biogeochemischen Stoffkreisläufe in der Wassersäule und führt zu sauerstofffreien (anoxischen) Bedingungen ab einer Tiefe von ca. 100 m. Diese besonderen Umstände macht das Schwarze Meer zum größten auf der Erde vorkommenden anoxischen marinen Becken.

Kapitel 2 und 3 dieser Arbeit (sowie die beiden Artikel im Anhang) gehen der Frage nach, welchen Einfluss Methanemissionen in verschiedenen Wassertiefen auf den Methankreislauf der Wassersäule haben und welche Bedeutung diese submarinen Emissionen als Quelle atmosphärischen Methans einnehmen. Hierzu wurde die Umgebung von Methanquellen entlang des nordwestlichen Schelfs und Kontinentalhangs (Paleo Dnepr Gebiet) sowie des nordöstlichen Tiefseebereichs (Sorokin Trog) in den Jahren 2003 und 2004 untersucht. Der betrachtete Tiefenbereich erstreckt sich von 60 bis 2100 m Wassertiefe und umfasst somit das gesamte biogeochemische Spektrum der Wassersäule. Detailliert dokumentiert wurden in dieser Arbeit die Methankonzentrationen der Wassersäule und der angrenzenden Atmosphärenschicht sowie deren stabile Kohlenstoffisotopensignaturen. Die physikalische

Beschreibung der Wasserkörper als auch der am Methankreislauf beteiligten Mikroorganismen und deren Umsatzraten begleiten die Untersuchungen. Durch großräumige Beprobungen wird die räumlich Ausdehnung der Einflussbereiche dieser Quellen ermittelt. Die auf dieser Datengrundlage aufbauende Modellierung der Methanflüsse an der Ozean-Atmosphären Grenzschicht gibt Aufschluss darüber, inwieweit die in unterschiedlichen Wassertiefen vorliegenden Methanquellen die marine Methanemission beeinflussen. Zudem ermöglicht die Erkundung dieser Gebiete in zwei aufeinanderfolgenden Jahren einen Einblick in die zeitliche Variabilität der Quellstärke dieser submarinen Methanaustritte.

In Kapitel 4 werden die eigenen in dieser Arbeit gewonnenen Ergebnisse zusammen mit den vorhandenen Literaturdaten zur Erstellung eines Methanbudgets eingesetzt, das die wesentlichen Quellen und Senken des Schwarzen Meeres quantitativ erfasst. Ein Transport-Reaktions Modell untersucht die Methanfreisetzung in den einzelnen Wassertiefen und identifiziert bedeutende Tiefen. In wieweit massive Methanfreisetzungen, wie z.B. von Schlammvulkanausbrüchen oder submarine Hangrutschungen das Methanbudget des Schwarzen Meeres und die damit verbundene Methanemission an der Ozeanoberfläche beeinflussen können, wird über verschiedene Modellszenarien ermittelt.

Die vorliegende Arbeit kann den komplexen Methankreislauf des Schwarzen Meeres und die daran geknüpften Prozesse nicht allumfassend behandeln. Vielmehr liefert sie einen Einblick, diskutiert relevante Bereiche und weist auf noch offene Fragen hin. Dass der Methankreislauf des Schwarzen Meeres weiterhin von großem wissenschaftlichen Interesse ist, zeigt die in den letzten Jahren stark zugenommene Forschungsaktivität auf diesem Feld.

Abstract

Methane is a greenhouse gas that exerts a significant influence on the radiation budget and thereby the earth's climate. Geological sources of methane, especially submarine, are particularly relevant in this regard because they emit significant quantities of methane into the atmosphere. However, it is difficult to estimate the magnitude of the global geological source because the extent of these methane-releasing areas and the processes, which influence the methane cycle, are not yet well understood.

The Black Sea is a region characterized by numerous submarine methane sources. Methane emissions in the form of methane-containing fluids or gas bubbles have been documented here at all water depths, from the shelf down to the abyss. This marginal sea features a unique hydrographic structure resulting from the inflow of freshwater from rivers and the salty waters of the Mediterranean Sea over the Bosphorus. These inputs have very different densities, and this leads to a pronounced stratification of the water column that limits vertical mixing. High biological production in the surface waters leads to an intense consumption of oxygen in the upper water column, which can not be balanced by downward ventilation of oxygenated surface waters. The pronounced redox-gradient has in turn an important influence on the biochemical cycles in the water column and leads to anoxic conditions at depths below approximately 100 m. These particular conditions make the Black Sea the largest anoxic marine basin on earth.

Chapters 2 and 3 of this work (as well as the articles in the appendix) explore the questions of what influence methane emissions have at different water depths on the methane cycle of the water column and what significance these submarine emissions have as sources of atmospheric methane. To this end, the zones of methane sources along the northwest shelf and continental slope (Paleo Dnepr Area) as well as in the northeastern deep sea region (Sorokin Trough) of the Black Sea were explored throughout 2003 and 2004. The observed depths span from between 60 and 2100 m and encompass the entire biogeochemical spectrum of the water column. The methane concentrations of the water column and the overlaying atmosphere were carefully recorded, as were the carbon isotope signatures of this methane. The physical description of the water column, the microorganisms involved in the methane cycle and their methane consumption rates were additionally documented. The area affected by the emission of methane from submarine sources was ascertained through extensive sampling campaigns. The dataset was used to model the methane flux at the sea/air interface and to evaluate the

influence of methane sources, present in different water depth on the marine methane emission. In addition, the exploration of this area throughout two consecutive years provides insight into the seasonal variability of the source strength of these submarine emissions.

Chapter 4 serves to combine the results of this investigation with those present in the literature for the purpose of constructing a methane budget that quantifies the significant sources and sinks of methane in the Black Sea. A transport-reaction model investigates the release of methane at discrete water depths and identifies those of particular interest. Various model scenarios were used to determine to what extent sizable methane emission events, for example from mud volcano eruptions or submarine landslides, affect the methane budget of the Black Sea and the associated methane emissions at the ocean surface.

The following work cannot completely address the complex methane cycle of the Black Sea and its associated processes. This work serves rather to provide new insights, discuss relevant areas and point out still unanswered questions. The increase in research on methane in the Black Sea during recent years shows that its methane cycle is of great scientific interest.

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General Introduction

The greenhouse gas methane

Methane is one of the earth's most important greenhouse gases. It influences the earth's radiation budget in two ways: directly by absorption of infrared radiation and indirectly by generating or affecting the atmospheric cycle of other greenhouse gases (O₃, H₂O, CO₂) through secondary atmospheric reactions (Lelieveld and Crutzen, 1992; Lelieveld et al., 1998). On a mole-for-mole basis the greenhouse potential of methane is 23 times higher compared to carbon dioxide (IPCC, 2001). Methane accounts for 20 % of the greenhouse forcing caused by the well mixed greenhouse gases (CO₂, CH₄, N₂O and halocarbons; IPCC, 2001).

Historical records of atmospheric methane obtained from Antarctic ice cores indicate that atmospheric methane concentrations varied strongly over the last 420,000 years (Petit et al., 1999). In this period, the CH₄ mole fraction correlates well with air-temperature variations, showing major shifts during glacial-interglacial transitions (320 - 350 glacial to 650 - 770 ppbv interglacial; Petit et al., 1999). Mechanisms which probably control fluctuations of past atmospheric methane levels are: (1) variations in the global wetland extent, (2) strong dependence of the rates of microbial methane generation on temperature, (3) changing atmospheric concentration of OH radicals, and (4) decomposition of methane hydrates (Chappellaz et al., 1993). Methane hydrates are ice-like solids formed from water and CH₄ under conditions of high pressure, low temperature, and sufficient gas concentration (Kvenvolden, 1988). Stable carbon isotopic signatures of gas hydrate methane are usually characterized by very negative values ($\delta^{13}\text{C}_{\text{CH}_4} \sim -65 \text{ ‰}$; Kvenvolden, 1988). The current atmospheric methane emissions from gas hydrates appear small, with about 10 Tg yr⁻¹ (IPCC, 2001). In comparison, the amount of hydrate-bound methane is estimated at 2.1 - 3.6 × 10⁶ Tg (Milkov et al., 2003). In case of the LPTM (Last Paleocene Thermal Maximum, 55 million years ago), negative isotopic excursions in the ocean/atmosphere inorganic carbon reservoir have been interpreted to be derived from massive destabilization of marine hydrate deposits (Dickens, 1995). This $\delta^{13}\text{C}$ anomaly is associated with rapid climate and oceanographic changes that induced a dramatic warming of high-latitude surface and deep water (Kennett and Stott, 1991). The increase in bottom water temperature may have triggered the release of large amounts of methane from the seafloor into ocean and atmosphere, causing a positive feedback in the climate system (Dickens, 1995). It is assumed that rapidly increasing

temperatures of deep waters and the associated decrease of oxygen concentrations caused the extinction of many deep-sea benthic species during this period (Kennett and Stott, 1991).

The atmospheric abundance of methane has increased by about a factor of 2.5 from 650 ppbv in the pre-industrial era to 1,751 ppbv at the beginning of the 21st century (Petit et al., 1999; Dlugokencky et al., 2003). As observed in other greenhouse gases, the average atmospheric methane concentration is still increasing over time (~ 1 % per year; Lelieveld et al., 1998), although some studies show that annual loading rates are variable and that they have decreased from a range of 25 - 40 Tg y⁻¹ in the 1980s to < 20 Tg y⁻¹ during most of the 1990s (Dlugokencky et al., 2001). There is no well-defined, quantitative explanation for the observed variability. The future evolution of atmospheric methane concentrations is of particular interest because of the potential to stabilize atmospheric methane concentrations on relatively short timescales (Lelieveld and Crutzen, 1993).

The total identified sources of atmospheric methane are estimated to be 600 Tg yr⁻¹ on average (IPCC, 2001). This number is derived from the sum of known sinks and annual increase in the atmospheric budget, as the individual source estimates underlie larger uncertainties. About 37 % is derived from natural sources, and 63 % from anthropogenic emissions (Figure 1). Sinks of atmospheric methane are tropospheric reactions with OH radicals (506 Tg yr⁻¹), stratospheric removal (40 Tg yr⁻¹), and microbial methanotrophic consumption in soils (30 Tg yr⁻¹; IPCC, 2001). By far the most important component of the natural methane sources is represented by wetlands followed by geologic emission of fossil (radiocarbon-free) methane (Etiope and Milkov, 2004). The latter includes mud volcanoes (6 - 9 Tg yr⁻¹; Etiope and Milkov, 2004), microseepage in hydrocarbon-prone areas (at least 7 Tg yr⁻¹; Etiope and Klusman, 2002), geothermal sources (2.5 - 6.3 Tg yr⁻¹; Etiope and Klusman, 2002), and seepage from the seafloor, which includes gas hydrate dissolution (20 Tg yr⁻¹; Kvenvolden et al., 2001). The main problem in regional and global estimates of geologic methane emission is the uncertainty in the actual area affected by active gas seepage. However, the recent

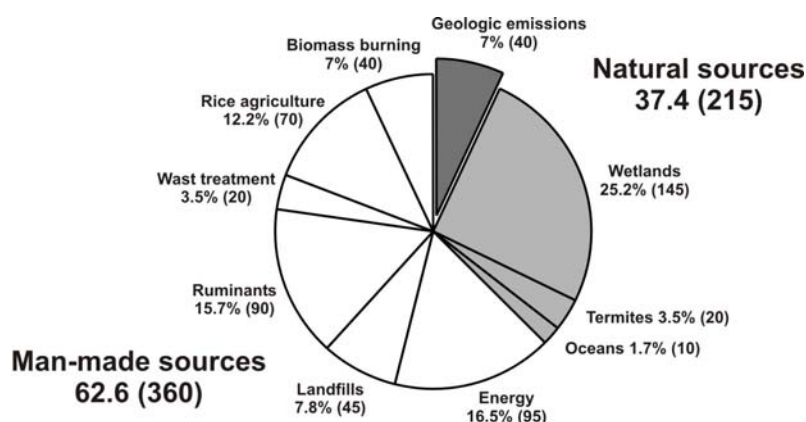


Figure 1: Sources of atmospheric methane (average fluxes in Tg CH₄ yr⁻¹ in brackets; after Etiope and Milkov (2004)).

literature illustrates that these geological features occur worldwide. They have been observed onshore and in shallow water on the continental shelves, on continental slopes and on abyssal plains (Fleischer et al., 2001; Judd, 2003; Figure 2).

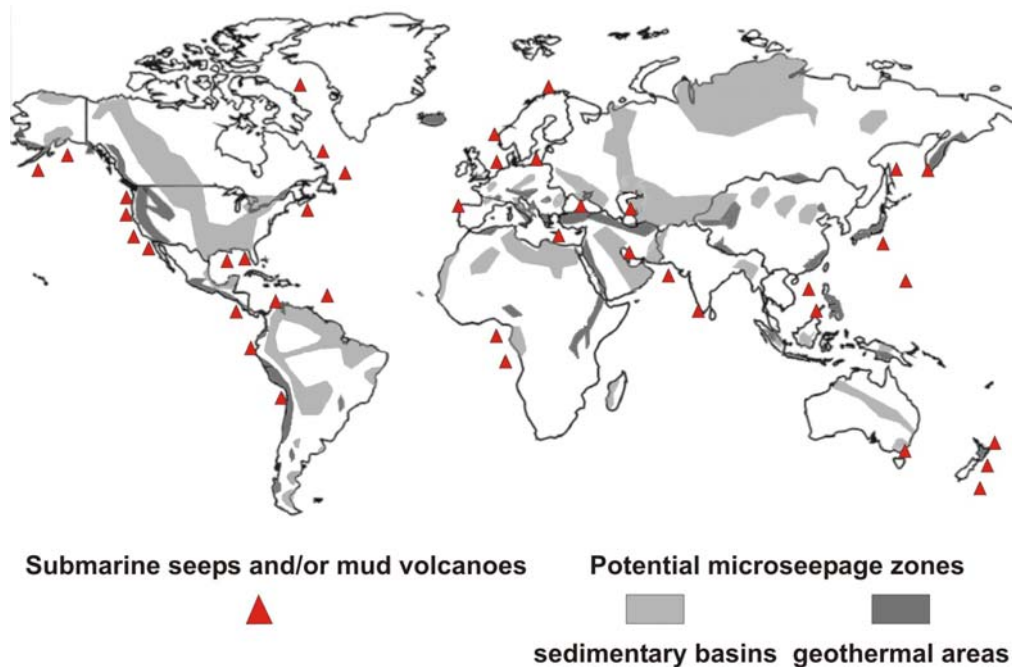


Figure 2: Geographic distribution of geologic methane emission sites (Etiope and Klusman, 2002).

Even though the continental shelf regions comprise only 7.5 % of the ocean's surface area these shallow submarine sources are of particular interest for submarine methane emission into the atmosphere (Hornafius et al., 1999). These areas are characterized by high microbial productivity and high export and burial rates due to the shallow water depth (Suess, 1980), which leads to the deposition of organic-rich sediments, resulting in an increase in microbial methane generation and possible seepage. The dissolved methane fraction is efficiently oxidized by microbes already within the sediment or later in the water column (Boetius et al., 2000; Rehder et al., 1999). However, methane transported within gas bubbles can bypass the microbial consumption, and the potential greenhouse gas can ascend into surface waters and may even reach the water-atmosphere interface. The effectiveness of gas transport by bubbles into these shallow layers largely depends on the bubble size, the existence of gas hydrate rims on the bubble surfaces, buoyancy induced by the uprising bubbles, and the methane saturation of the surrounding water (Leifer and Patro, 2002; Rehder et al., 2002). Gas transfer to the atmosphere by bubbles is most likely to occur in shallow waters where the distance to the sea surface is short and bubble dissolution or gas exchange with the surrounding water is limited (Leifer and Patro, 2002).

Physiography and Geology of the Black Sea

Physiography

The Black Sea is a nearly landlocked basin situated between Turkey, Georgia, Russia, Ukraine, Romania, and Bulgaria (Figure 3). It is connected to the Marmara Sea via the Strait of Bosphoros and the Sea of Azov through the Kerch' Strait. The Black Sea is the world's largest anoxic basin, covering an area of 423,000 km² and having a volume of around 534,000 km³ and a maximum depth of 2,206 m (Degens and Ross, 1974).

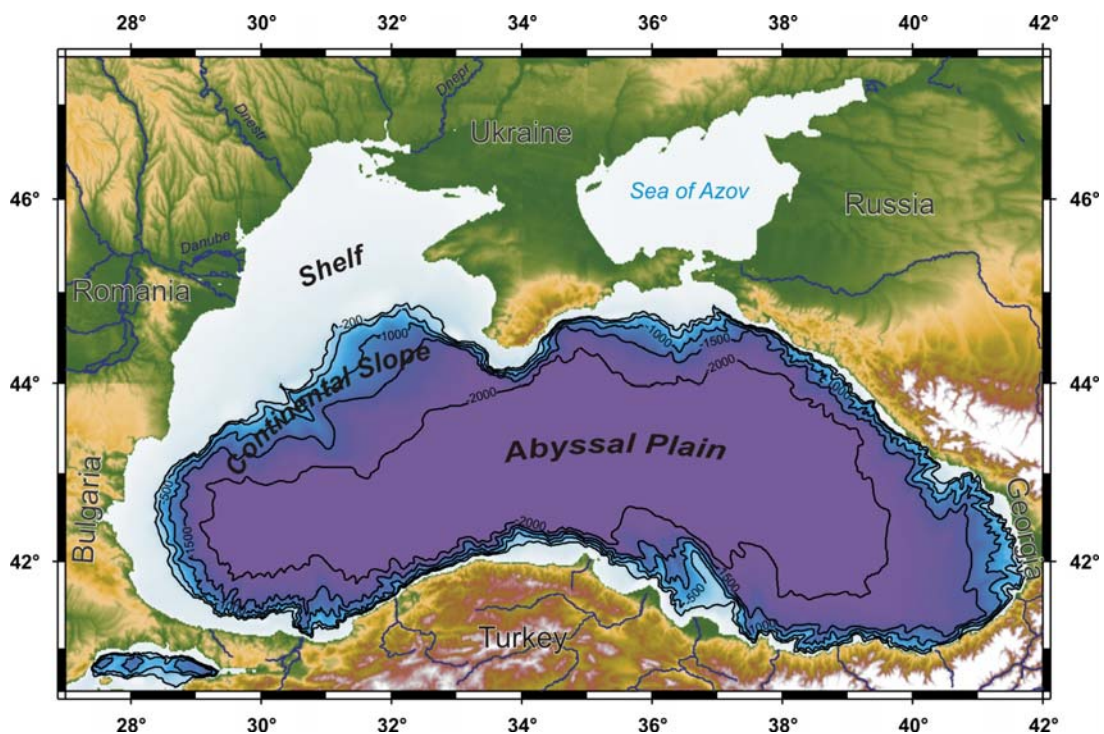


Figure 3: Physiographic map of the Black Sea region.

The Black Sea can be subdivided into three physiographic provinces: the shelf, the continental slope, and the abyssal plain (Sorokin, 2002).

The shelf, covering 28 % of the total Black Sea area, extends from the modern shore line to the edge of the steep continental slope and is generally limited by the 100 m isobath; only offshore the Crimea Peninsula and the Sea of Azov does the shelf zone extend down to 140 - 160 m water depth. The shelf edge at around 100 m water depth actually represents the

margins of the ancient Black Sea basin during the Pleistocene-Holocene regression. During that sea level lowstand the shelf area was dry and crossed by rivers flowing into the Black Sea that created delta systems and large deep-sea fan complexes.

The continental slope constitutes almost 30 percent of the total area and is divided into two subtypes according to its inclination. The first subtype is represented by a steep slope along the mountainous coast with margins situated close to the shore (e.g. coasts of the Caucasus, SE Crimea, and Anatolia). These areas are characterized by an inclination between 6 and 30° down to 1,500 - 1,900 m depth. Slopes of this type are highly dissected by canyons, which were cut by rivers during numerous sea level low stands during Pleistocene-Holocene regressions. The second subtype consists of a relatively smooth slope with inclinations between 1 and 6° and is characteristic for the western part of the Black Sea.

The spatial extension of the abyssal plain is delineated by the 2,000 m isobath and occupies 42 percent of the total Black Sea. The smooth plain, which represents the accumulation area of the Sea, is divided by a fault system along the Mid-Black Sea / Andrusov Ridge which separates the western and eastern basins. The bottom relief of the eastern basin is smoother and deeper than in the west, which is due to the influence of numerous orogenic events on the surface structures of the western basin (e.g. late phase of alpine folding processes).

Geology

The Black Sea basin is believed to have been formed via back-arc spreading developed along the northern active margin of the Paleo-Tethys, which was subducted northward during Triassic to Miocene times. Subsidence resulted in the formation of two deep sub-basins (Figure 4): (i) the western Black Sea basin, which is underlain by oceanic to sub-oceanic crust and a sedimentary cover of up to 19 km in thickness, and (ii) the eastern Black Sea basin, which is underlain by continental crust and a sedimentary cover of up to 12 km in thickness (Tugolesov et al., 1985). These basins are separated by the prominent Andrusov Ridge that is formed from continental crust and overlain by 5 - 6 km of sediments. Since the Eocene, the Black Sea region has changed to a dominantly compressional environment (Nishishin et al., 2003). The Black Sea is surrounded by Late Cainozoic mountain belts (the Great Caucasus, Pontides, Southern Crimea, and Balkanides) that are characterized by a continental crust thickness of around 40 - 50 km.

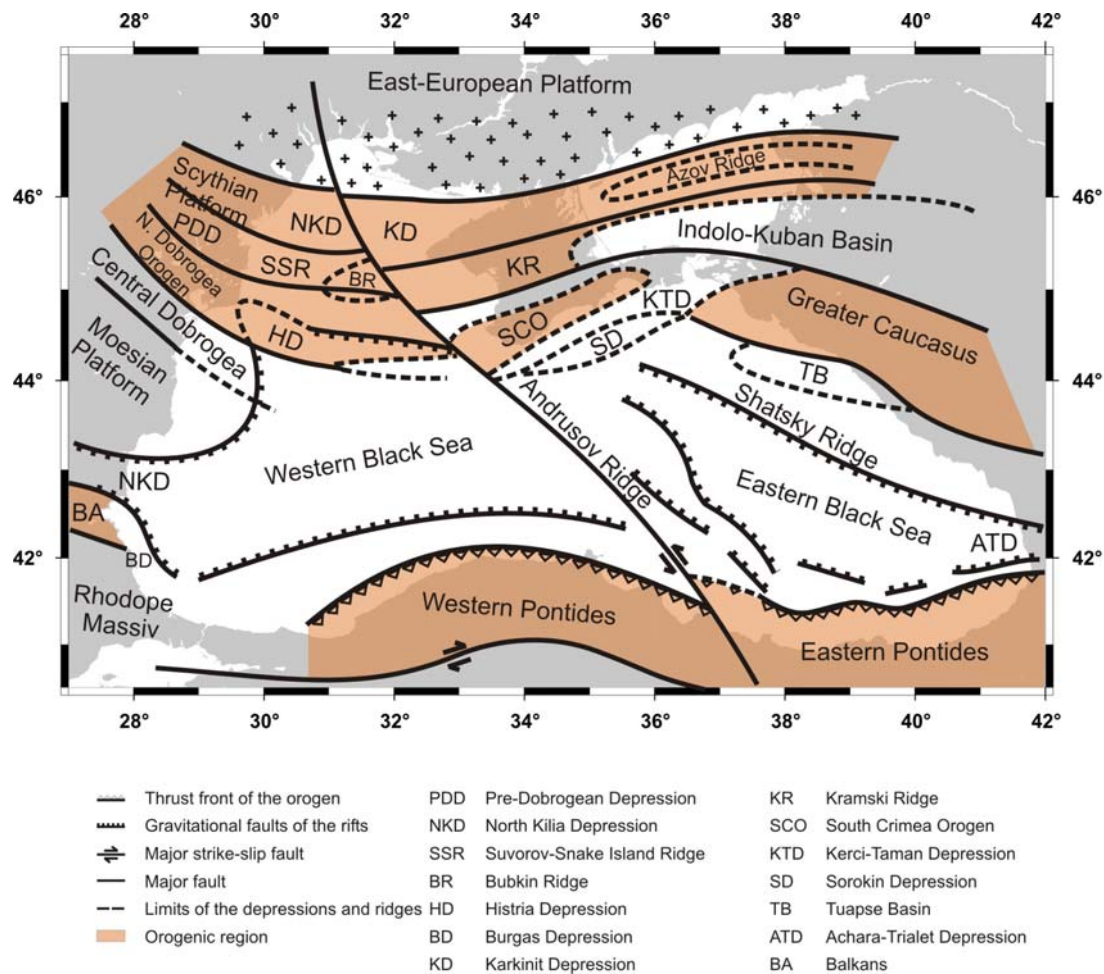


Figure 4: Tectonic map of the Black Sea region (after Dinu et al., 2002).

The opening of the Black Sea started in the western basin in the Late Barremian (Early Cretaceous), resulting in the enormous accumulation of Cretaceous to Holocene sedimentary deposits in the western and eastern basins (Görür, 1988; Banks and Robinson 1997). These units are well stratified and nearly undisturbed, although many diapiric structures are known in the north-eastern part of the eastern sub-basin as well as southeast and southwest of the Crimea peninsula (Tugolesov et al., 1985; Lüdmann et al., 2004)). The Cretaceous unit is between 5 to 6 km thick in the western basin, around 3 to 4 km thick in the eastern part, and is characterized by carbonate and evaporate deposits (Robinson et al., 1996). The overlying Palaeocene-Eocene units consist of terrigenous and carbonate rocks ranging from 5 km thickness in the western basin to 3 km thickness in the eastern Black Sea basin. The Oligocene-Lower Miocene (Maikopian Formation) clay-rich sedimentary deposits have a maximum thickness of 5 km in the western basin and 4 km in the eastern; these organic-rich deposits accumulated during intense tectonic subsidence of the bottom, coinciding with the

alpine orogenic period. Middle- and Upper Miocene units show a terrigenous composition with a thickness varying from hundreds of meters (Shatsky Ridge) to 3 km (Guriy foredeep). The Pliocene to Quaternary units have a thickness of 2 - 3.5 km in the inner parts of the basin and consist mostly of clays.

The most recent sedimentological history of the Black Sea is influenced by the opening of the Strait of Bosphorus and the resulting influx of Mediterranean water after the glacial eustatic rise of the sea level at the beginning of the Holocene. Although the exact timing and span of flooding is still highly debated, there is a general agreement on a large and rapid inflow after the last glacial maximum (LGM) some 8,000 years before present (Ryan et al., 1997; Aksu et al., 2002; Major et al., 2002; Ryan et al., 2004). The saline Mediterranean waters changed the Black Sea from a fresh water lake to a semi-marine sea. The density difference between the fresh waters from river runoff and the saline waters induced a strong density stratification which still hampers the vertical exchange between surface and deep waters. The connection with the Mediterranean led to a displacement of the nutrient-rich deep basin water into the photic zone, which stimulated the marine productivity and therefore the sedimentation of organic-rich deposits. Additional organic matter was added by the extinction of a rich freshwater benthic fauna caused by the rise of salinity and the subsequent development of a chemocline with anoxic deep waters. This episode is marked by an abrupt change from lacustrine (Unit III) to marine sediment facies (Unit II; Degens and Ross, 1974). The sediments of Unit III mainly consist of terrigenous silt with a low percentage of organic carbon (< 1 weight percent). In contrast, Unit II facies is characterized by sapropels having more than 14 weight percent organic carbon. It is believed that these deposits commenced some 7,000 years ago (Hay and Arthur, 1991). This phase ended 4,000 years ago in the shallow waters and 1,600 years ago in the deep waters (Calvert and Fontugne, 1987). The rising salinity led to the invasion of the marine coccolithophorid species *Emiliana Huxley* which marks the development of fully marine conditions and thus the boundary between Unit II and Unit I (Jones, 1990). The sediment facies of Unit I is a result of three major European glacial periods, the Elsterian, Saalian and Weichselian. The terrigenous sediments are mostly muds with interbedded silt and sand layers (Degens et al., 1986). Deposits outside regions with intense terrigenous sedimentation show alternating, finely laminated white and dark layers, which are mainly composed of coccoliths and the organic depositions produced by diatoms, respectively (Bukry, 1974).

Seeps and mud volcanoes

Gas seeps and areas of intense fluid discharge, where methane and other geochemical compounds are liberated into the water column, are widely distributed in the coastal areas, on the shelf, upper continental slope, and deep waters of the Black Sea (Figure 5). The present work is part of the European Integrated Project “CRIMEA” which aims to quantify the transfer of methane from the sea floor through the water column and into the atmosphere at shallow seeps in the Paleo Dnepr Area and deep mud volcanoes (MV) in the Sorokin Trough (Figure 5).

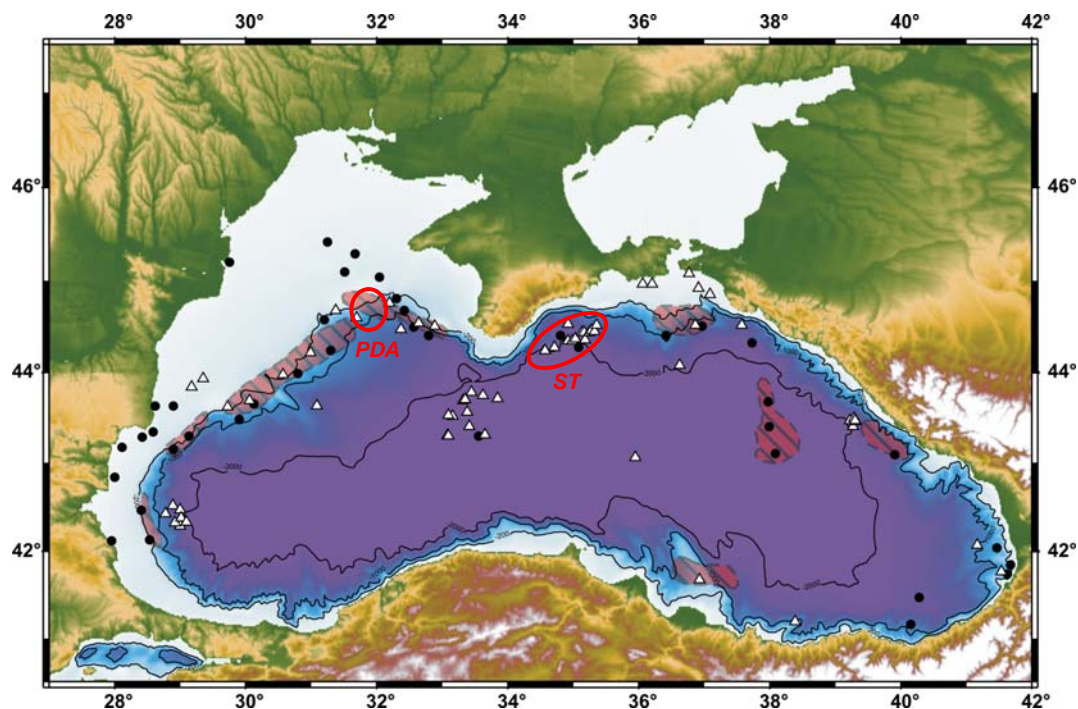


Figure 5: Map of gas and fluid discharge in the Black Sea. Triangles and dots show, respectively, locations of submarine mud volcanoes and areas of intense fluid discharge. Red areas represent regions of gas seepage and seabed pockmarks. MV locations were taken from Kruglyakova et al. (2004). Positions for fluid discharge, gas seepage and pockmarks derive from Vassilev et al. (2002). CRIMEA study areas are indicated with red circles (Paleo Dnepr Area = *PDA*; Sorokin Trough = *ST*).

Paleo Dnepr Area

The first descriptions of gas seeps on the Ukraine shelf were provided by Polikarpov et al. (1989), followed by numerous other studies which examined these features across the unique setting from shallow oxic waters, through the transitional zone, to deep anoxic waters (Egorov et al., 1998; Michaelis et al., 2002; Polikarpov et al., 1992). In the anoxic environment some

of these seeps sites are characterized by carbonate build-ups formed by microbial mats involved in anaerobic oxidation of methane (Michaelis et al., 2002). Detailed hydro-acoustic studies conducted during the 2003 and 2004 CRIMEA expeditions detected a total of 2,778 new bubble flares in the Paleo Dnepr Area (Naudts et al., 2006; Figure 6). The area of abundant gas seepage in the Paleo Dnepr Area seems to be restricted to a maximum water depth of 725 m (Naudts et al., 2006), which almost coincides with the phase boundary for pure methane hydrate at the *in situ* temperature and salinity conditions (Dickens and Quinby-Hunt, 1997). This suggests that, where gas hydrates are stable, they play the role of buffer for upward migration of methane gas and thus prevent seepage of methane bubbles into the water column (Naudts et al., 2006). Hydro-acoustic data, combined with sea-floor and subsurface seismic data gathered during the CRIMEA cruises, show that seeps are located where gas fronts intersect the sediment surface, predominantly within morphological and sub-surface features (Naudts et al., 2006). For example, shelf seeps occur primarily in association with elongated depressions or pockmarks while slope seeps are found in connection with the crests of sedimentary ridges or at submarine landslides. Sediment cores taken in the Paleo Dnepr Area during expedition P317/3 of R/V Poseidon show that the tops of the ridges (where most of the seeps occur) are composed of sand, while those taken in off-seep areas (like ridge flanks) contain clay or fine-grained sediments. This setting is in accordance with a general tendency of fluid and gas seeps to occur at bathymetric highs because of buoyancy-driven transport and the erosion of fine-grained sediments which inhibit gas and fluid flow (Hovland, 1990; Hovland, 1992).

Although the mechanisms and processes which control seep locations appear to be well understood, the source of the seeping methane and its migration pathways through the sediments are still under debate. Various local stratigraphic intervals are capable of producing methane, including organic-rich Holocene sediments, Quaternary peats and sapropels, Tertiary clays, Cretaceous coals, and highly organically enriched Devonian and Lower Carboniferous sediments (e.g. Dimitrov, 2002a). Microbial methane generation in the organic-rich shallow shelf and slope sediments of the Black Sea has been described by various authors (e.g. Amouroux et al., 2002; Ivanov et al., 2002; Reeburgh et al., 1991). The carbon isotopic values ($\delta^{13}\text{C}$) and $\text{C}_1/\text{C}_2+\text{C}_3$ hydrocarbon ratios of seep methane clearly demonstrate its biogenic origin (e.g. Michaelis et al., 2002; Schmale et al., 2006). Measurements of the natural radiocarbon content of methane ($^{14}\text{C}_{\text{CH}_4}$) on gas samples taken at Seep Area 2 show that most of the methane derives from radiocarbon-free sources (Kessler et al., 2006), supporting the theory that methane released at these two sites represents a mixture

of methane generated in organic-rich deltaic sediments deposited during various sea level low stands along the Dnepr river fan, and radiocarbon-free methane migrating upward, probably from late Eocene source rocks (Robinson et al., 1996; Kessler et al., 2006).

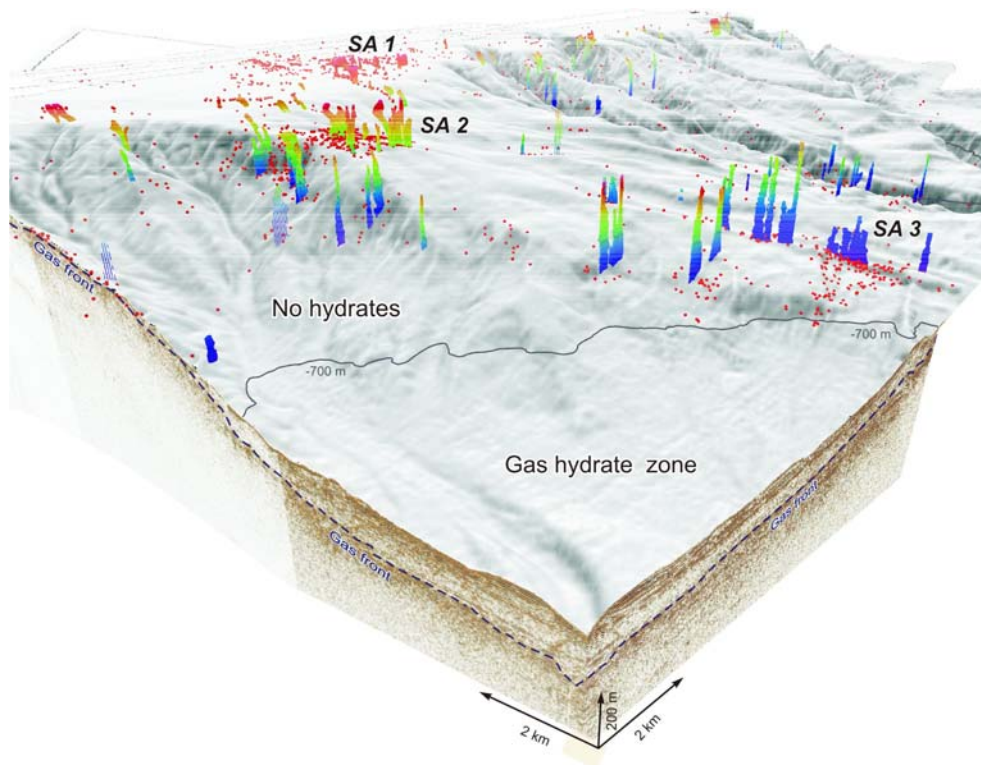


Figure 6: Oblique view of the bathymetry of the Paleo Dnepr Area (see Fig. 5 for location). Red dots are seep locations, some seeps are shown as 3D flares. GHSZ is delineated by the -700 m contour line. The two crossing seismic profiles clearly show that the gas front, i.e. the top of the free gas in the subsurface, outcrops to the surface outside the GHSZ, where gas bubbles were detected in the water column (Figure from Naudts et al., 2006). Seep Area (SA) 1 (~ 90 m water depth), Seep Area 2 (~ 220 m water depth), and Seep Area 3 (~ 600 m water depth) are indicated.

In contrast to the shallow seep sites, gas hydrates may play an important role as a source for water column methane around Seep Area 3 located in 600 m, as the shallowest water depth for methane gas hydrate occurrence in the Black Sea is about 700 m under present conditions (Vassilev and Dimitrov, 2002). The existence of a more or less continuous BSR west of the Dnepr Canyon, in a depth range of 700 to 1,350 m, has been interpreted by Lüdmann et al. (2004) as a gas hydrate layer. This layer is overlain by an approximately 100-m-thick zone of low amplitude reflection which might be related to gas hydrate cementation

(Lüdmann et al., 2004). Modeling the influence of a near-bottom temperature increase on the Black Sea gas hydrate stability zone (GHSZ), Poort et al. (2005) show that temperature changes, caused by the inflow of warm Mediterranean water after postglacial Black Sea flooding, will result in a 15 - 60 % smaller gas hydrate reservoir. This large reservoir shrinkage will be delayed by hundreds of thousands of years due to the transient nature of the heat wave propagation, so that present hydrate dissociation is only expected to occur in shelf regions at the hydrate phase boundary where the GHSZ is thinnest. Intense gas seepage in 600 m water depth (Seep Area 3) is linked to large sediment slumps which could have been caused by gas hydrate dissociation and subsequent sediment de-stabilization (Naudts et al., 2006). Compared to the biogenic methane released at the shallow seep sites (Seep Areas 1 and 2), stable carbon isotope values ($\delta^{13}\text{C}_{\text{CH}_4}$) of dissolved methane at this site are generally ~10 ‰ heavier suggesting a partial contribution of deeper thermogenic methane at this site (Schmale et al., 2006). Mud diapirs were found in the southern and southeastern part of the Paleo Dnepr Area (Lüdmann et al., 2004), which may provide migration pathways for methane from deep strata. In addition, high ^3He values indicative of input from deep-derived sources have been reported (C. Holzner, EAWAG; CRIMEA data and personal communication).

Sorokin Trough

In recent years the structure, genesis, and activity of Sorokin Trough submarine MVs, located in water depths between 1,700 and 2,100 m, have been studied during several scientific cruises (e.g. Bohrmann et al., 2003; Bouriak and Akiimetjanov, 1998; Greinert et al., 2006; Ivanov et al., 1998; for location see Figure 5).

The occurrence of MVs in the Sorokin Trough is related to the N-S orientated compressive regime generated by the northwestward motion of the buried Tetyaev and Shatskii rises (Ivanov et al., 1998; Figure 4). These structural highs act as rigid buttresses against which clays of the Maikopian formation (Oligocene-Lower Miocene) are deformed, become over-pressured and rise diapirically (Woodside et al., 1997). As a result, the sedimentary successions in the trough are penetrated by numerous diapiric ridges which have roots several kilometers deep and are bathymetrically expressed as SE-NE trending highs (Krastel et al., 2003; Bohrmann and Schenck, 2002). Several MVs are located on the culmination of these diapiric ridges, suggesting a genetic link between diapirism and mud volcanism (Ivanov et al., 1998; Bohrmann et al., 2003; Krastel et al., 2003).

It is suggested that the compressional tectonic regime in this area causes the extrusion of mud and advection of fluids (sediment dewatering) at the MVs. One of the most prominent and apparently active MV in this area is the well investigated Dvurechenskiy (*DMV*) at 2,060 m water depth (Figure 7). The sediments extruded from the *DMV* consist of clay-rich deposits most probably from the Maikopian Formation (Aloisi et al., 2004). Sediment cores reveal a disperse distribution of small gas hydrate crystals in the mud (Bohrmann et al., 2003). Optical seafloor mapping across the *DMV* shows several pieces of evidence for recent mud and fluid flow activity (e.g. occurrence of microbial mats on the sediment surface; Bohrmann et al., 2003). In addition, CTD records on top of the *DMV* show positive bottom water temperature anomalies pointing to the advective transport of warm fluids from greater depth (Bohrmann et al., 2003). This is supported by sediment temperatures of up to 16.5 °C in close contact with the ambient bottom water of 9 °C, indicating the presence of warm mud in the upper meter below the surface of the *DMV* (Bohrmann et al., 2003). *In situ* temperature probes show steep gradients, thus indicating a high fluid and/or mud flux within *DMV*. Anomalously low temperatures measured in sediment cores after core retrieval has been interpreted as a gas hydrate proxy caused by the endothermic dissociation of hydrate crystals. The formation of gas hydrates in the shallow subsurface is indirectly confirmed by negative chloride anomalies in pore water profiles (Aloisi et al., 2004). The shape of geochemical pore water profiles in sediments of the *DMV* implies an outflow of fluid and/or mud which seem to originate in the subsurface down to ~ 3 km depth (Bohrmann et al., 2003; Aloisi et al., 2004). Transport-reaction models indicate that the *DMV* is currently in a relatively quiet stage and is expelling fluids at low seepage rates (Aloisi et al., 2004; Wallmann et al., 2006).

Apart from the discussed fluid or mud-related transport processes from MVs, recent hydro-acoustic investigations above the *DMV*, the neighboring Vodianitskiy (*VMV*) and a Nameless Seep Site (*NSS*) could prove the liberation of free gas from the sediment into the overlain water column (Greinert et al., 2006; Figure 7). During this study numerous surveys were carried out between 2002 and 2004, where gas bubble flares could be detected above these MVs. These long-time studies point to temporal changes in gas seepage activity, as indicated by the occasional absence of seep-related bubbles in the water column or varying flare highs. A maximum bubble rising height of 1,300 m was determined above the *VMV*, which represents one of the highest reported thus far (Greinert et al., 2006).

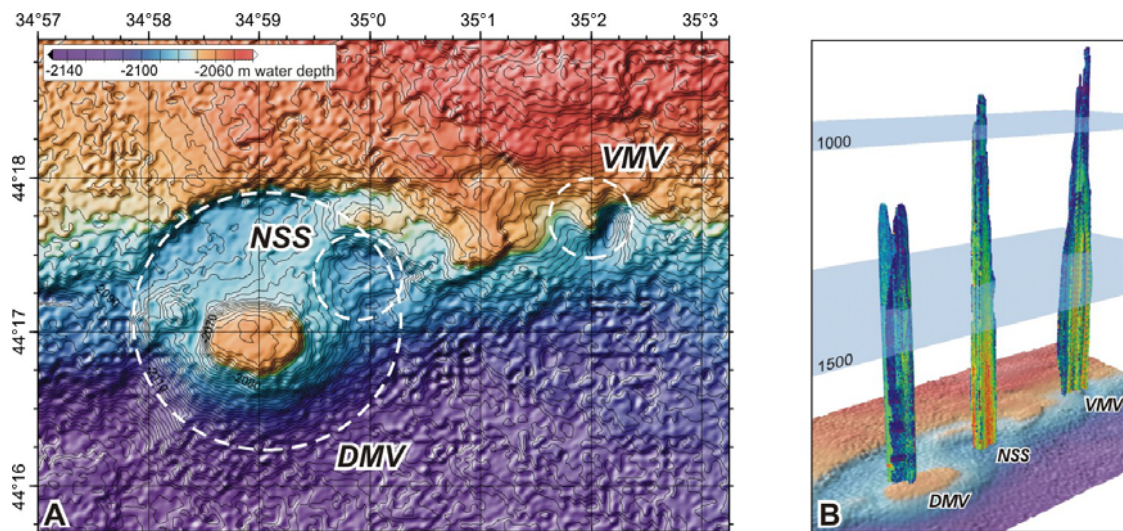


Figure 7: (A) Bathymetric map of the studied MV area, SE of the Crimea Peninsula (Ukraine). The locations of the *DMV*, *NSS* and *VMV* are indicated. (B) Example of a 3D view of the registered flares above MVs (from Greinert et al., 2006).

As already discussed, the Maikopian deposits most probably represent the source formation for sediments extruded at the MVs in the Sorokin Trough. This sedimentary sequence of over 5 km thickness is overlain by at least 1 - 3 km of Middle-Miocene to Quaternary deposits (Tugolesov et al., 1985). The sediments of the Maikopian formation are highly enriched with organic matter and thus, they have a considerable potential to generate hydrocarbons (Dimitrov, 2002b). Gas obtained from a mud breccia cored in the crater of the *DMV* is enriched in saturated high-molecular hydrocarbons ($C_2 - C_4$) as well as relatively heavy stable carbon isotope ratios ($\delta^{13}C$) of ethane. This gas signature implies a deep/thermogenic origin for the gas and thus supports the assumption that MVs in the Sorokin Trough are “rooted” in the Maikopian Formation (Blinova et al., 2003). In contrast, gas hydrate crystals from the same sediment cores mainly consist of methane with a strongly negative $\delta^{13}C$ and δD ratios and a low $C_2 - C_3$ content (Blinova et al., 2003). The results show that the methane is mainly formed by microbial CO_2 reduction. Blinova et al. (2003) suggests that this signature is caused by the generation of so called “secondary” biogenic methane, resulting from the biodegradation of hydrocarbons accumulated along mud volcano channels and deep faults below the *DMV*. This biogenic methane shows the same molecular and isotopic signatures as shallow “primary” biogenic methane.

Black Sea Oceanography

Physical Characteristics

The Black Sea represents a nearly-enclosed basin with a large riverine runoff and limited interaction with the Sea of Marmara through the Bosphorus Strait (Figure 3). Half of Europe and parts of Asia are drained by the various rivers that flow into the Black Sea, including the Danube, Dnestr and Dnepr, all of which flow into the northwestern shelf region. The excess of river runoff and precipitation over evaporation results in a positive water balance. Özsoy et al. (1997) estimate the water fluxes to be $\sim 300 \text{ km}^3 \text{ yr}^{-1}$ for precipitation, $\sim 350 \text{ km}^3 \text{ yr}^{-1}$ for runoff water, and $\sim 350 \text{ km}^3 \text{ yr}^{-1}$ for evaporation from the sea surface. The outflow via the Bosphorus of $612 \text{ km}^3 \text{ yr}^{-1}$ results in a return flow of $312 \text{ km}^3 \text{ yr}^{-1}$ (Ünlüata et al., 1989).

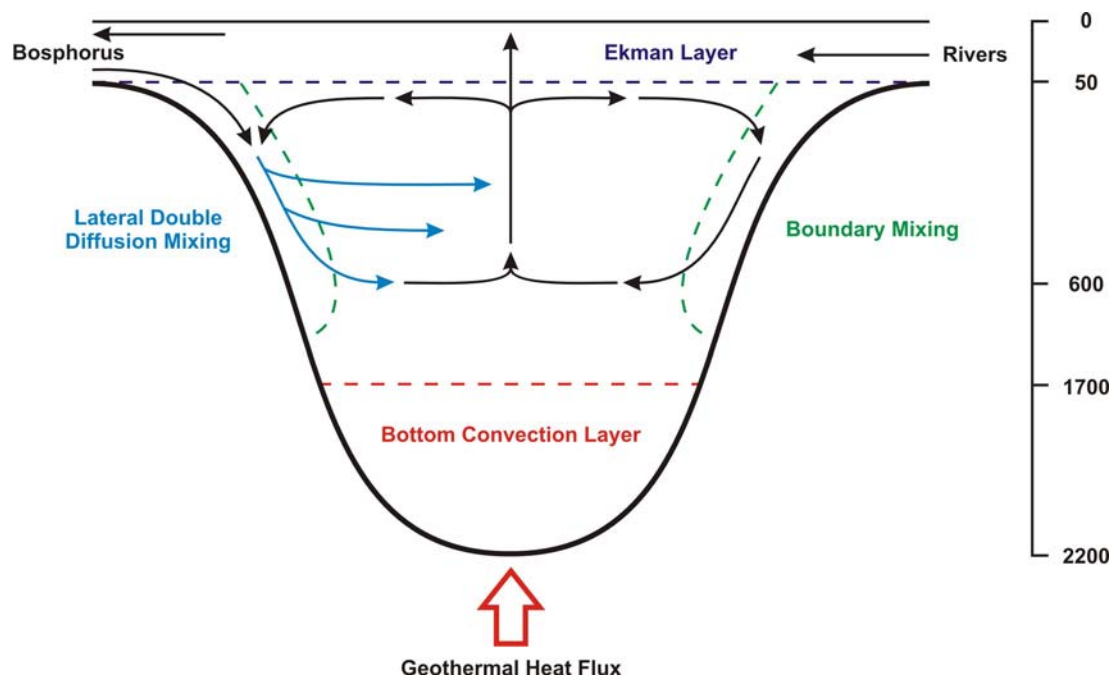


Figure 8: Vertical mixing processes in the Black Sea basin. The approximate depth range of these processes in meter is displayed on the left bar (after Özsoy et al., 1993).

Low-saline river waters and the inflow of saltier waters from the Mediterranean leads to the development of the unique hydrographical structure of the Black Sea, defined by the depth distribution of salt and temperature. The surface mixed layer is indicated by relatively low salinity values, ranging from 17.5 to 18.5 depending on seasonal changes in river runoff

(Murray et al., 1991). The surface temperature also shows seasonal variations due to solar heating and wind forcing. A temperature minimum called the cold intermediate layer (CIL) is present throughout the basin, at a water depth of approximately 50 m in the centre and 100 m in the periphery (Oguz et al., 1991). Below the CIL is the permanent halocline (50 - 200 m) which separates the surface water from the deep water. The isopycnal surfaces above 300 m are dome-shaped due to the gyre-like circulation, resulting in specific parameter values occurring at shallower depths in the centre of the Black Sea and deeper on the edges. In the deep water the vertical salinity and potential temperature gradients are small, increasing to maximum values of about 22.33 and 8.904 °C, respectively, at the bottom (Murray, 1991).

The two-layered hydrological structure of the Black Sea strongly limits the depth of efficient vertical mixing between the deep and surface waters, which is driven by turbulent exchange (or turbulent diffusion), thermal diffusion (thermal convection), vertical components of currents, internal waves, and lateral double diffusion (Özsoy et al., 1993; Sorokin, 2002; Figure 8). Seasonal wind mixing and thermal winter convection is the key mixing process which stimulates exchange within the upper 100 - 150 m. Short-period internal waves and turbulent diffusion drive the exchange processes between 200 - 500 m water depth. Lateral double diffusion mixing caused by the intrusion of Mediterranean waters in the form of multiple layers penetrating towards the basin interior takes place in the depth interval of 100 - 600 m. Boundary mixing driven by shear forces and turbulence along the continental slope can create vertical ventilation processes similar to this intrusion mechanism. This depth interval is also influenced by the Ekman pumping generated by wind stress distribution in the closed domain of the Black Sea; this drives a system of vertical circulation in the centre of cyclonic gyres which are compensated by boundary layer flows in peripheral coastal zones having anticyclonic circulation (for surface circulation pattern see Figure 9). The key mixing process below 1,700 m water depth is thermal convection, driven by geothermal heat fluxes, which has led to a homogenized bottom layer of around 400 to 500 m thickness (the largest observed in the world oceans). The strongly differing ventilation of the surface and deep waters leads to smaller mean residence times of the intermediate waters, compared to the more uniformly-aged deep waters. However, in the literature the estimates of these times vary greatly from a few decades to thousands of years, depending on the age indicators used (e.g. Sorokin, 2002; Östlund, 1974; Top et al., 1990; Murray et al., 1991).

Circulation Characteristics

The Black Sea is characterized by a cyclonic, basin-wide current system called the Main Rim Current (MRC), which flows around the entire basin along the varying continental slope and margin topography (Sorokin, 2002; Figure 9). The cyclonic character of the Black Sea MRC results from the cyclonic nature of the wind field and influences the water masses down to a water depth of 500 - 700 m. The interior of the sea is characterized by separate cyclonic cells and a series of anticyclonic eddies located between the coast and the meandering MRC zone. The Black Sea surface circulation is strongest in the upper layer, with current speeds typically larger than 50 cm s^{-1} . Below this depth, current velocities decrease to about $10 - 20 \text{ cm s}^{-1}$ within the 200 - 500 m depth range, and to $2 - 5 \text{ cm s}^{-1}$ between 500 to 1,000 m (Sorokin, 2002; Oguz and Besiktepe, 1999). In general the current pattern down to 1,000 m follows the cyclonic or anticyclonic orientation of the MRC and the peripheral coastal eddies (Sorokin, 2002). The exact knowledge of the flow field below $\sim 1,000 \text{ m}$ depth is limited and unreliable, but hydrographic investigations indicate a significant structural variability (Oguz et al., 1993; Sorokin, 2002). Based on a recent literature review, Sorokin (2002) states that the currents are mainly cyclonic, except on the NE continental slope and adjacent abyssal plain, where they are anticyclonic.

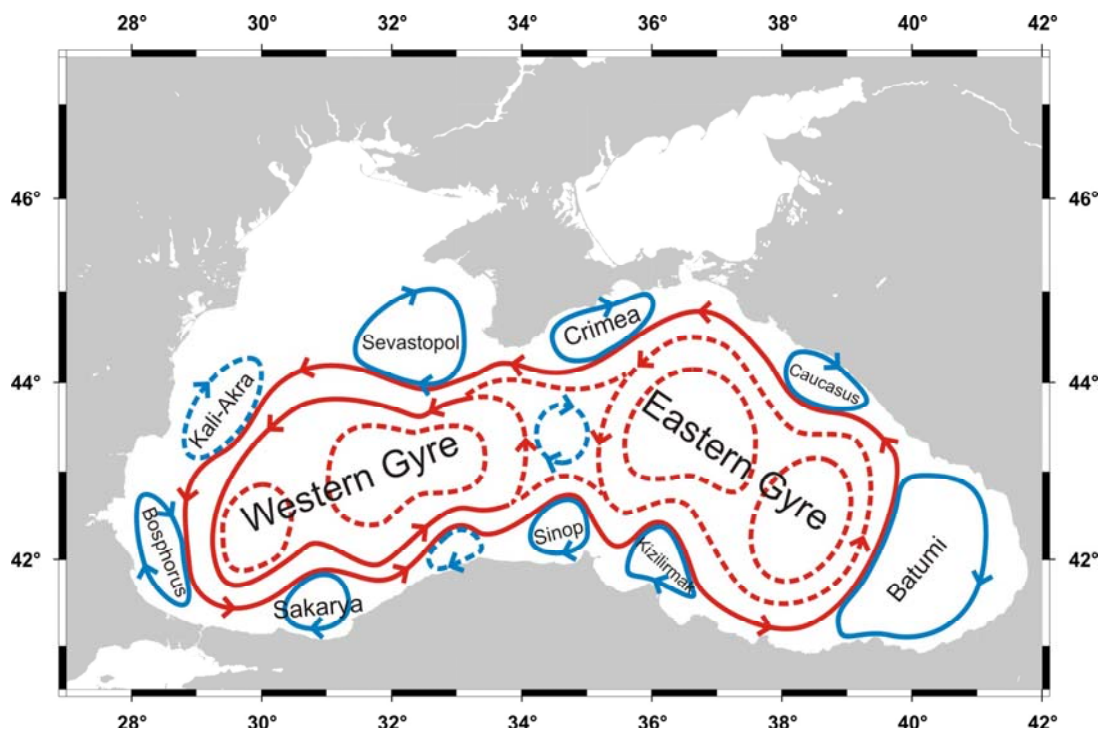


Figure 9: Circulation in the upper layer of the Black Sea. Solid line indicate quasi-permanent flows, while dashed lines correspond to current features of general circulation (Oguz et al., 1993).

Biogeochemical Characteristics

The lack of sufficient downward ventilation of oxygenated surface waters to counter organic matter fluxes from the highly productive surface waters into the deep waters has resulted in the present anoxic conditions below the pycnocline and made the Black Sea the world's largest anoxic basin. Only the upper 100 to 150 m of the total 2,200 m water depth contains oxygen (13 % of the sea volume). Based on different biogeochemical parameters, the Black Sea water column can be subdivided into four distinct layers (Oguz et al., 2000). The boundaries between these layers can be defined by means of specific isopycnal surfaces (σ_t values) to eliminate regional depth variations caused by the dome-like shape of the chemocline or by temporally-limited influences of internal waves (Saydam et al., 1993; Figure 10).

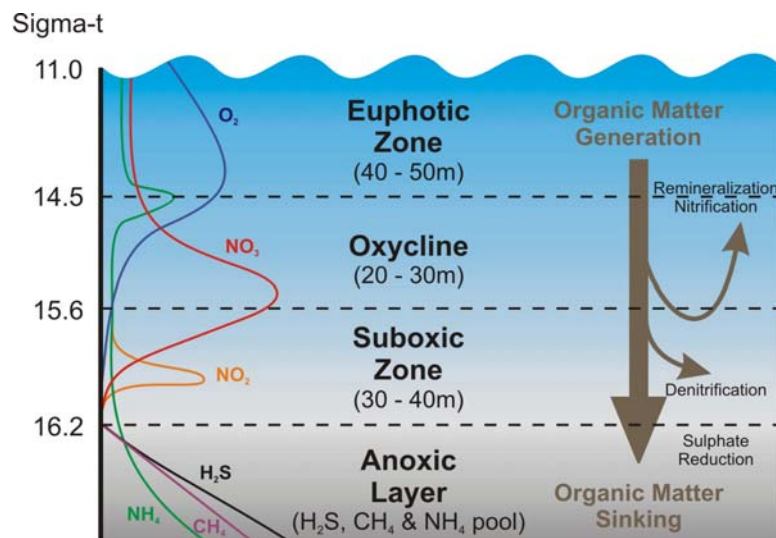


Figure 10: Main features of the vertical biogeochemical structure of the upper Black Sea water column. Qualitative distribution of oxygen (O_2), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), hydrogen sulfide (H_2S), and methane (CH_4) are presented on the left. Vertical variations are given in terms of density (Oguz et al., 2000).

(a) The “euphotic zone” (minimum light level of 1 %) has a maximum thickness of 50 m. It is characterized by high oxygen concentrations on the order of $300 \mu M$, active plankton activity (e.g. primary production and zooplankton grazing), and intense remineralization of sinking particles. (b) The underlying 20 - 30 m are called the “oxycline” and are represented by steep chemical gradients, where active degradation of particulate organic matter causes intense oxygen consumption and efficient nitrogen cycling. At the base of this layer the oxygen concentration is reduced to about $10 \mu M$ whereas the nitrate concentration increases to around $6 - 9 \mu M$. (c) Biodegradation of organic matter via denitrification takes place in the subsequent “suboxic layer” (thickness of about 20 - 40 m) which results in a rapid decrease of nitrate concentrations to zero. (d) This zone is followed by the deep “anoxic layer” where

sulfate is used to decompose organic matter and hydrogen sulphide is produced as a by-product. This deep layer is also characterized by methane concentrations which are 1,000 times higher than observed in the Black Sea surface waters (i.e. up to 11 μM in deep waters). The Black Sea methane inventory has been assessed to be ~ 96 Tg (Reeburgh et al., 1991). Thus, the Black Sea represents the largest marine water reservoir of dissolved methane. The source of water column methane, if liberated from sediments (i.e. microbial production in shallow sediment depth and / or emission from deep thermogenic sources through seeps and MVs) or *in situ* biogenic methane generation in the water column, is still part of scientific debate. The water column methane input is mainly balanced by anaerobic microbial oxidation of methane in the anoxic deep water (Reeburgh et al., 1991 and Chapter IV of this thesis).

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Water column investigations on high-intensity seeps in the Paleo Dnepr area (NW-Black Sea) and their regional impact on the methane cycle and hydrography

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Abstract

Methane concentrations and carbon stable isotopic ratios ($\delta^{13}\text{C}_{\text{CH}_4}$), oxygen concentrations and hydrographic parameters (CTD) were investigated in the water column of the north-western Black Sea (Ukrainian shelf and slope) during two cruises in the summer of 2003 and 2004. Water stations were sampled along a transect which crosses three bubble releasing seep sites at water depths of 90, 220, and 600 m. Gas bubbles emitted from the two shallow sites show high methane percentages (>80 %) having a $\delta^{13}\text{C}_{\text{CH}_4}$ ratio of around -65 ‰, clearly pointing to a biogenic origin. In both years, dissolved methane concentrations on the continental shelf are significantly higher in the area of active seeps (up to 1.6 μM near the seafloor) compared to unaffected regions (ranging between 5 and 90 nM). The input of seep methane with its light carbon isotopic signature can be traced across the entire shelf region, with $\delta^{13}\text{C}_{\text{CH}_4}$ values typically below -57.5 ‰. Intense aerobic microbial oxidation of dissolved methane in the vicinity of the 90 m site is indicated by a constant enrichment of the heavier

isotope ($\delta^{13}\text{C}_{\text{CH}_4}$ values up to -40 ‰) together with decreasing CH_4 concentrations towards the sea surface. Decreased oxygen concentrations in the region of highly active seep areas in 90 and 220 m deep waters indicate a direct seepage effect on the oxygen maximum layer (OML, between 10 and 60 m water depth) through aerobic microbial methane oxidation, gas exchange between gas bubbles and the surrounding water (stripping) and/or upwelling of oxygen depleted waters. The water column methane distribution in the deep section of the transect shows elevated concentrations above an active seep cluster in roughly 600 m water depth (up to 15.5 μM). This seep area is located close to the stability boundary of pure methane hydrates at *in situ* temperature and salinity conditions and may point to local clathrate dissolution related to stability changes. Comparison of results from the two years shows higher concentrations in 2003, implying that the methane source strength at the 600 m deep site may be variable in time. These results, together with an apparent change in the sigma-theta / methane relationship, show that elevated seep activity may have a transient influence on water column methane distribution up to the oxic/anoxic interface. Above this interface values decrease rapidly and a strong shift to heavier carbon isotope ratios ($\delta^{13}\text{C}_{\text{CH}_4} = -50$ ‰) is observed, indicating increased microbial consumption of methane within the chemocline. In contrast to shelf waters, where bubble releasing seeps represent the dominant atmospheric methane source, the relevant source in the open waters is microbial methane generation in subsurface microenvironments. This *in situ* production is indicated by a methane concentration peak at 40-50 m water depth and a corresponding shift to relatively light $\delta^{13}\text{C}_{\text{CH}_4}$ values (of around -60 ‰) compared with the under- and overlying water (around -50 ‰ and -53 ‰, respectively).

Keywords: Methane, Gas Seepages, Marginal Seas, Anoxic Basins, Carbon Isotope Ratio, Oxygen Depletion

Regional index terms: Black Sea, Dnepr paleo-delta

Introduction

The radiative properties of methane and its involvement in atmospheric reactions, which directly and indirectly moderate global climate, are well known (IPCC, 2001). As observed for other greenhouse gases, the average atmospheric methane concentration is increasing over time (~1 % per year; Lelieveld et al., 1998), although some studies show that annual loading rates are variable and that they have decreased from a range of 25-40 Tg y⁻¹ in the 1980s to <20 Tg y⁻¹ during most of the 1990s (Dlugokencky et al., 2001). This type of variability must be better understood, both in terms of anthropogenic sources as well as natural production / consumption rates, in order to provide accurate input data for forward climate modeling.

Although oceans cover 71 % of the planet surface, they are believed to contribute only about 2 % of the total flux of methane to the atmosphere (i.e. 11-18 Tg y⁻¹; Bange et al., 1994), as sediment-produced CH₄ is filtered via efficient microbial oxidation processes in the sediments and overlying water column. Hornafius et al. (1999) estimated that as much as 18-48 Tg CH₄ y⁻¹ is released into the water column by the world's continental shelf areas that have a high potential for methane seepage from the seafloor. Shelf regions comprise only 7.5 % of the ocean's surface area, but seem to be of great importance for oceanic methane emission to the atmosphere (Bange et al., 1994).

The Black Sea represents the world's largest semi-closed water reservoir of dissolved methane, but stable stratification and efficient oxidation mechanisms hamper a significant loss towards the atmosphere (Reeburgh et al., 1991; Schmale et al., 2005). This permanently stratified marine system was formed 7,200 years ago via flooding of the originally lacustrine basin by saline waters from the Mediterranean Sea. It consists of an anoxic saline base (fed by input from the Bosphorus Strait) which is overlain at a depth of approximately 150 m by fresh water fed from various fluvial systems, including the Danube, Dnepr and Dnyestr Rivers to the north. At present the primary sources of CH₄ to the Black Sea water column include seeps in water depths up to 725 m (Naudts et al., 2006) and mud volcanoes in deep waters between 800 and 2,000 m (Kruglyakova et al., 2004). As this paper addresses research conducted along the paleo-Dnepr shelf and slope area to a depth of about 750 m, the latter type of source is not addressed here.

The first descriptions of gas seeps in the Ukrainian waters were provided by Polikarpov et al. (1989), followed by numerous other studies which examined these features across the unique setting from shallow oxic waters, through the transitional zone, to deep anoxic waters (Egorov et al., 1998; Michaelis et al., 2002; Polikarpov et al., 1992). Detailed hydro-acoustic studies conducted during the 2003 and 2004 CRIMEA expeditions detected a total of 2,778

new gas plumes in the Paleo Dnepr Area (Naudts et al., 2006). These data, combined with sea-floor and subsurface seismic data gathered during the same cruises, show that seeps are located where gas fronts intersect the sediment surface, predominantly within morphological and sub-surface features (Naudts et al., 2006). For example, shelf seeps occur primarily in association with elongated depressions or pockmarks while slope seeps are found in connection with the crests of sedimentary ridges or at submarine landslides. Sediment cores taken in the same general study area during expedition P317/3 of R/V Poseidon show that the tops of the ridges (where most of the seeps occur) are composed of sand, while those taken in off-seep areas (like ridge flanks) contain clay or fine-grained sediments. This setting is in accordance with a general tendency of fluid and gas seeps to occur at bathymetric highs because of buoyancy-driven transport and the erosion of fine grained sediments which inhibit gas and fluid flow (Hovland, 1990; Hovland, 1992).

The area of abundant gas seepage in the survey area seems to be restricted to a maximum water depth of 725 m (Naudts et al., 2006), which almost coincides with the phase boundary for pure methane hydrate at the *in situ* temperature and salinity conditions (Dickens and Quinby-Hunt, 1997). The existence of a more or less continuous BSR west of the Dnepr Canyon, in a depth range of 700 to 1,350 m, has been interpreted by Lüdmann et al. (2004) as a gas hydrate layer with an average thickness of 100 ± 5 m. However, previous sedimentary and seismic studies (Lüdmann et al., 2004; and references therein) indicate that gas hydrates in the NW-Black Sea are spatially restricted to the proximal Dnepr Fan due to its high organic matter content.

Although the mechanisms and processes which control seep locations now appear to be well understood, the source of the seeping methane and its migration pathways through the sediments are still under debate. Various local stratigraphic intervals are capable of producing methane, including organic-rich Holocene sediments, Quaternary peats and sapropels, Tertiary clays, Cretaceous coals, and highly organically enriched Devonian and Lower Carboniferous sediments (e.g. Dimitrov, 2002). Microbial methane generation in the shallow shelf and slope sediments of the Black Sea has been described by various authors (e.g. Amouroux et al., 2002; Ivanov et al., 2002; Reeburgh et al., 1991), with the maximum values of methane generation in the NW shelf region occurring in organic rich deposits from the Danube river (Ivanov et al., 2002). The carbon isotopic values of methane ($\delta^{13}\text{C}$), which range from -62.4 to -68.2 [‰ vs. VPDB], clearly demonstrate its biogenic origin (Michaelis et al., 2002).

Mud diapirs were found in the southern and southeastern part of the Paleo Dnepr Area (Lüdmann et al., 2004), which may provide migration pathways of methane from deeper strata. Water samples taken in the vicinity of this subsurface structure yield high ^3He values and may also indicate input from deep-derived sources (C. Holzner, personal communication, unpublished CRIMEA data).

Despite the fact that highly significant volumes of methane are released from the Black Sea sediments, only a fraction is eventually transferred to the atmosphere due to the complex methane cycling in the water column (e.g. Ivanov et al., 2002; McGinnis et al., 2006; Reeburgh et al., 1991; Schmale et al., 2005). A simple methane budget for the Black Sea developed by Reeburgh et al. (1991) suggests that microbial methane generation in the shelf and slope sediments is the major methane source, which is balanced by anaerobic oxidation of methane in the anoxic water column (4.7 Tg yr^{-1}). According to this budget only 1.5 % of methane produced by the Black Sea sediments is emitted into the atmosphere.

This investigation is part of a European Integrated Project (“CRIMEA”) which aims to quantify the transfer of methane from the sea floor, through the water column and into the atmosphere at shallow, high-intensity bubble releasing gas seeps and deep, methane-emitting mud volcanoes.

This contribution is focused on the influence of different seep sites (located on the shelf and continental slope along the paleo Dnepr delta area) on the overlying water column. Water stations were sampled along a transect crossing three highly-active seep sites situated in different water depths (90, 220 and 600 m) and geochemical environments (oxic, transition zone, and anoxic). The dataset obtained during two cruises in the summer 2003 and 2004 will be used to discuss the spatial and temporal variability of the investigated hydrographical (T, S and density), and chemical parameters (dissolved oxygen, methane concentrations, and the $\delta^{13}\text{C}_{\text{CH}_4}$ distribution), as well as the source and fate of methane throughout the different redox zones in the water column.

Study areas

Data were gathered during RV *Professor Vodyanitsky* cruises PV58 and PV60 from May 15th to July 1st, 2003 and May 25th to June 23rd, 2004 as part of the EU Project CRIMEA (<http://www.crimea-info.org/>). The main areas of investigation were the paleo delta area of the Dnepr river in the NW Black Sea and the Sorokin Trough to the south of the Crimea peninsula (Figure 1). The present article only discusses data collected within the Paleo Dnepr

area. Our investigations focus on sub-marine high-intensity methane seeps which continuously release free gas (mainly methane) from the seabed into the water column. Numerous gas seeps have been detected within this survey area. Three seep sites located in different water depths were investigated in detail: Seep Area 1 in about 90 m water depth, Seep Area 2 in 220 m water depth, and Seep Area 3 in about 600 m water depth (Figure 1). The main database presented here consists of a hydrographic and hydrochemical section crossing all three seep areas.

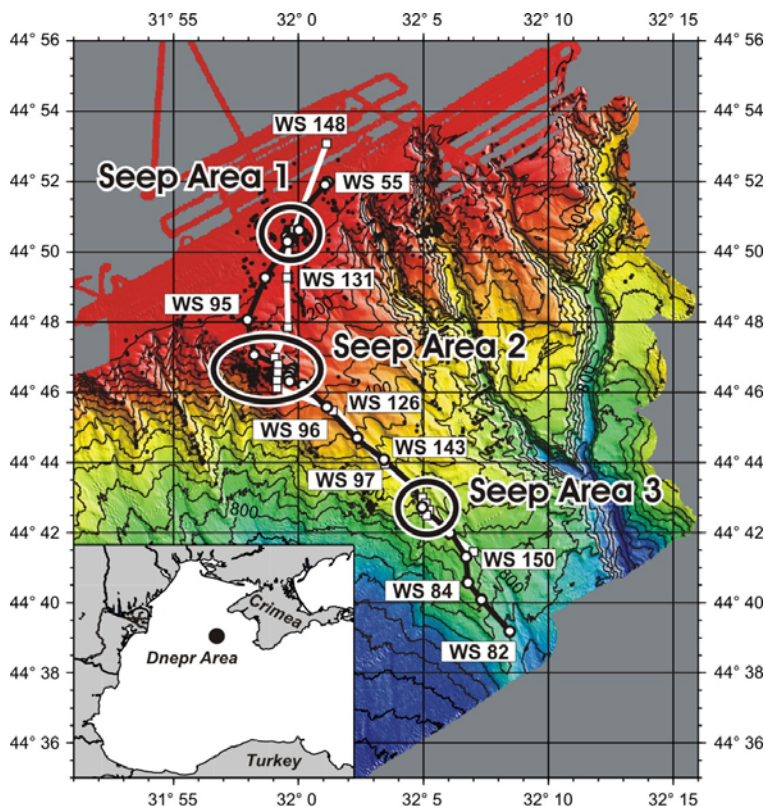


Figure 1: Bathymetric map of the Dnepr paleo-delta area showing the studied seep areas. Black dots indicate active seep positions, lines represent the two transects surveyed in 2003 and 2004. Stations are marked with circles (2003) and squares (2004), respectively; numbers indicate selected stations. The inset shows the location of the Dnepr paleo-delta study area.

Methods

Water column physical parameters were measured using a Seabird 911-plus CTD mounted on a rosette system. The pumped CTD was also equipped with a Beckmann oxygen sensor. The oxygen sensor was calibrated by collecting representative water samples and analyzing them for dissolved oxygen by Winkler titration (Grasshoff et al., 1997).

Water samples were obtained from a rosette system equipped with twelve 12 L bottles (HYDRO BIOS) and were generally analyzed within 3 hours after sampling. Dissolved gas samples were extracted using a modified version (Rehder et al., 1999) of the vacuum degassing method originally described by Lammers and Suess (1994). A total of 1,600 ml of

water were transferred directly from the Niskin bottle into pre-evacuated 2,200 ml glass bottles, which leads to almost quantitative degassing of methane. The gas phase was subsequently recompressed to atmospheric pressure and the CH₄ concentration of the extracted gas was determined by gas chromatography onboard the ship. Different gas chromatographs were used for the methane analyses conducted during the two cruises. In 2003, a Shimadzu GC14A gas chromatograph equipped with a flame ionization detector was used in connection with a Shimadzu CR6A Integrator. Nitrogen was used as the carrier gas and separation was performed using a Porapak Q packed column (4 m 1/8" SS, 50/80 mesh) run isothermally at 50 °C. In 2004, a Varian 3800 gas chromatograph was used, which was computer controlled with the Varian software package Star (version 5) and equipped with a flame ionization and a thermal conductivity detector. Hydrogen from a hydrogen generator (OPGU-1500A, STEC Inc.) was used as the carrier gas, a solenoid-actuated sample loop of 100 µl was used to inject the sample on column, and separation was performed via a 75 m-long, wide-bore Molesieve5A capillary column and a 25 m-long wide-bore PoraplotQ capillary column placed in series but separated by a bypass valve.

In addition to the analysis of extracted CH₄ concentrations on board the ship, gas subsamples were also collected for stable isotope analyses of methane carbon ($\delta^{13}\text{C}_{\text{CH}_4}$) at an IFM-GEOMAR laboratory (GC-IRMS; Finigan MAT 253). These subsamples were collected in pre-evacuated crimp-top glass vials sealed with a butyl rubber septum. 2 mL of supersaturated salt solution (degassed Millipore water) was added to each vial and the sample placed upside down to protect it from contamination by atmospheric gases during storage. Stable carbon isotope analysis involved removal of water and carbon dioxide on a NaOH/Ascarite trap, double cryofocussing at -100 °C (Ethanol/nitrogen) on Hayesep D and Poraplot S columns, methane separation on a 30 m, 0.32 I.D. Poraplot Q capillary column at 50 °C, combustion to CO₂ using a Ni catalyst run at 1150 °C, removal of combustion water using a Nafion trap supplemented by a small P₂O₅ filled inlay, refocusing of the CH₄-derived CO₂, and injection into the MAT 253 using a continuous flow technique. The $\delta^{13}\text{C}_{\text{CH}_4}$ data is expressed vs. VPDB (Vienna Peedee belemnite) standard.

Results

Water- and CTD-stations were taken along a transect which covers the shelf, shelf edge and continental slope of the Black Sea from 80 to 860 m water depth (Figure 1). The total dataset from this study consists of 26 water stations and 208 associated water samples in 2003

and 21 water stations and 245 associated water samples in 2004. All water samples were analysed for dissolved methane concentrations while 121 sub-samples in 2003 and 97 sub-samples in 2004 were analysed for the stable carbon isotopic composition of methane.

Hydrographic Data

Although CTD measurements were collected during both field seasons only the denser 2003 dataset will be shown here because both years show similar trends.

The Black Sea is a stratified marine system with fresh surface water separated from deep saline water by a sharp permanent halocline at around 100 to 150 m water depth (Figure 2a). The salinity of the sub-halocline waters is almost uniform.

The temperature distribution in Figure 2b and 3 illustrates the typical summer mixed layer, which occurs here within the upper 15 m ($T \sim 20\text{ }^{\circ}\text{C}$). The Cold Intermediate Layer (CIL, $T < 8\text{ }^{\circ}\text{C}$), which is formed during wintertime in the north-western shelf area and on top of the thermohaline domes of the cyclonic flow in the interior (Özsoy and Ünlüata, 1997), is located between 40 and 100 m depth.

The oxygen data illustrates the two layered water body, with oxic conditions in the upper 150 m and anoxic conditions below (Figure 2c). Within the oxygenated zone a concentration maximum (oxygen maximum layer, OML) occurs between 10 and 60 m water depth (conc. $\text{O}_2 > 300\text{ }\mu\text{M}$).

Specific isopycnal surfaces (i.e. sigma theta, σ_{θ} , Figure 2d) can be used to subdivide the water column based on the dominant biological and geochemical processes. The boundaries between the euphotic zone-oxycline, oxycline-suboxic layer and suboxic-anoxic layers are determined by the specific isopycnal surfaces of 14.5, 15.6 and 16.2 σ_{θ} , respectively (Saydam et al., 1993).

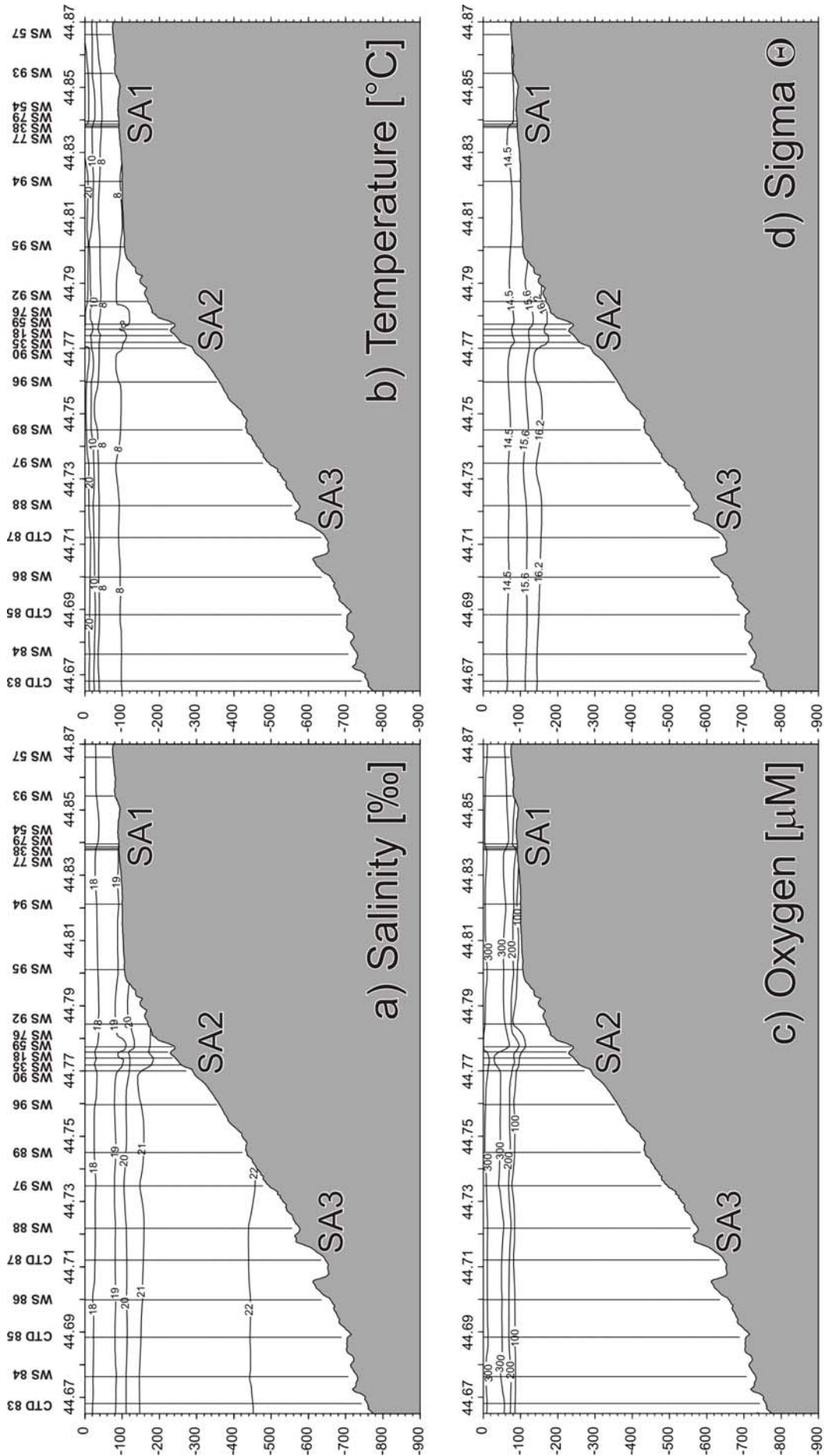


Figure 2: Contoured cross-section of (a) salinity, (b) temperature, (c) oxygen, and (d) sigma theta values along the 2003 transect along the dominant seep areas (SA). Oxygen contour lines below 100 μM are not shown because of limited sensitivity of the sensor. Vertical lines represent the position and depth of CTD casts while the numbers indicate station names.

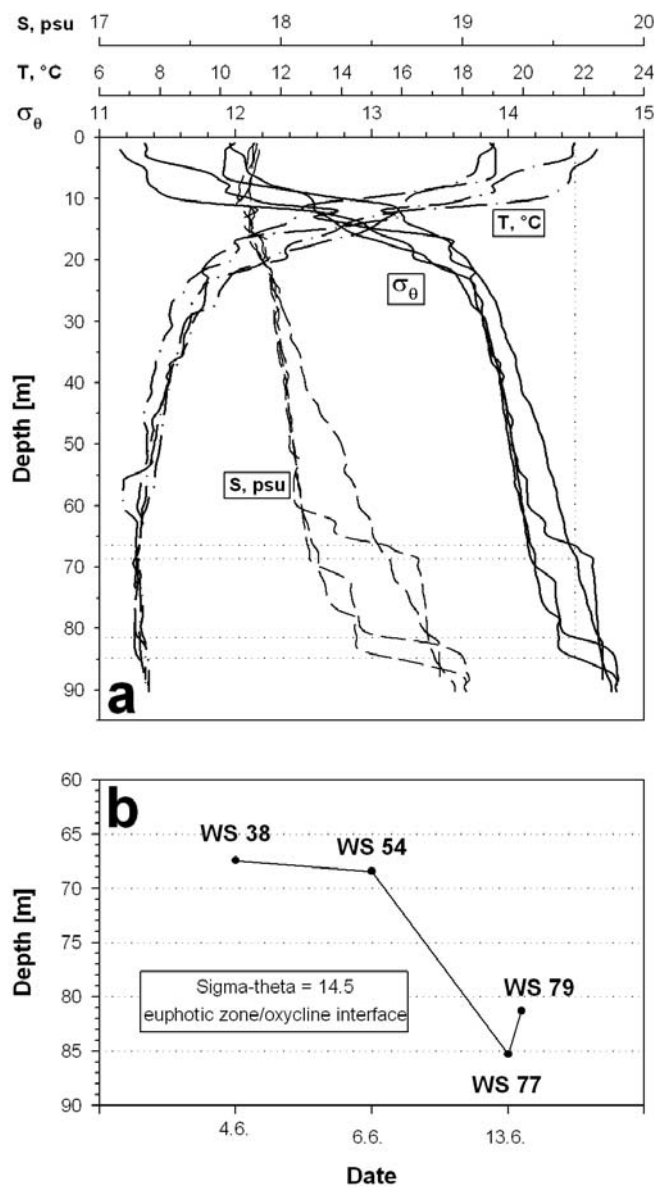


Figure 3: Physical characteristics in the water column above Seep Area 1: a) distribution of temperature, salinity, and sigma theta versus water depth; b) depth of sigma theta 14.5 (interface between euphotic zone und oxycline) versus date of sampling. Numbers indicate station names (for positions, see Figs 1 and 2).

Examination of the hydrographic parameters for all 2003 sections show that the depth of the various isolines did not remain constant over the timeframe of the expedition, with the highest variability observed above Seep Area 2. Figures 3, 4 and 5 illustrate the temporal variability observed above Seep Area 1, Seep Area 2 and in CTD-profiles situated on the continental slope.

Figure 4: Distribution of the isopycnal surfaces 14.5, 15.6, and 16.2, indicating the interfaces between the euphotic zone and oxycline, the oxycline and suboxic zone, and the suboxic and anoxic zones, respectively (Saydam et al., 1993). a) above Seep Area 2. b) off-seep stations on the continental slope. Numbers indicate station names (for positions, see Figs 1 and 2).

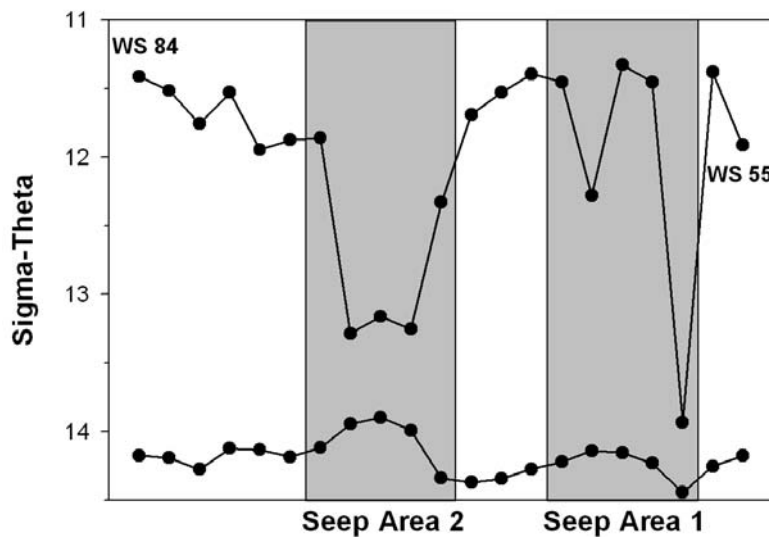
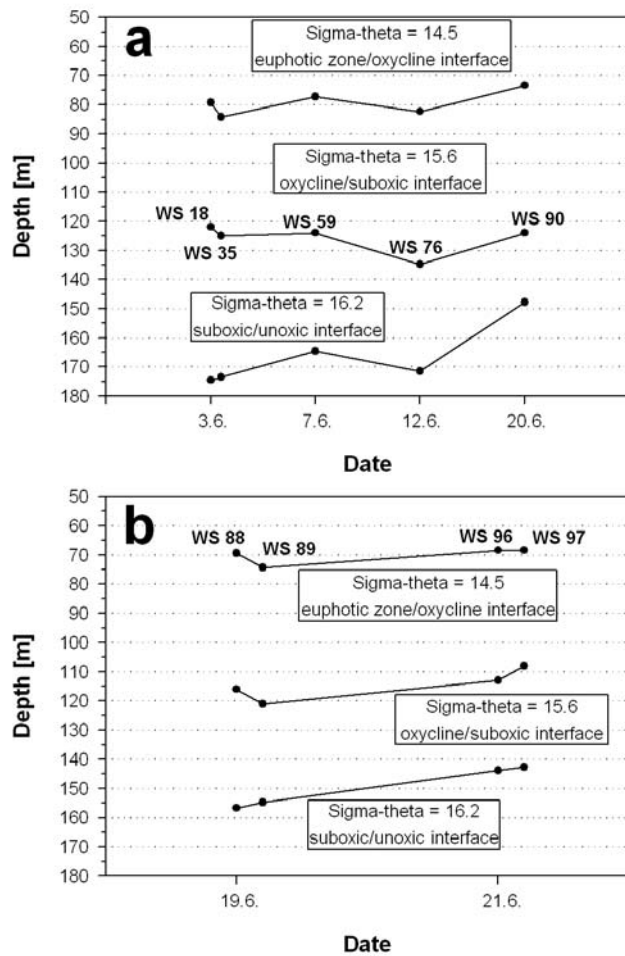


Figure 5: Upper and lower boundaries of the oxygen maximum layer (OML) across the 2003 transect, plotting the two sigma theta values for each profile where dissolved oxygen equals 300 μ M. Numbers indicate station names. Distance between stations (x-axis) is not in scale.

Although the main hydrographic characteristics are very similar in both years, different oceanographic, environmental and climatic conditions did result in cooler surface waters, a thicker euphotic zone, and many parameter contours occurring in deeper water in 2004 as compared to 2003. In part, these differences above the pycnocline may be explained by the cooler and windier conditions during the second field season, as well as by different current regimes or internal waves. As expected, values below the oxycline are essentially the same for both years.

Water column methane concentrations

Methane concentrations vary considerably along the north-south section (Figure 6a and 6c). In general, methane concentrations in the oxic part of the water body are three to four orders of magnitude lower compared to the deep anoxic waters. The border between these two water masses is situated in a water depth of around 100 to 150 m, depending on the depth of sigma theta 16.2, which marks the boundary between the suboxic layer (SOL) and anoxic zone. Both datasets from 2003 and 2004 show this general methane distribution pattern.

Methane profiles from stations located on the continental slope and away from any seep influence show concentrations ranging from around 5 nM in near-surface waters to around 12 μM in bottom waters. Water samples obtained in the region of Seep Area 3 (in a water depth of around 600 m) show the highest methane concentrations, with up to 15.5 μM in 2003 and 13.5 μM in 2004.

The methane profiles at the shelf edge, in the region of Seep Area 2, is still dominated by the gradient from oxic to anoxic waters, though at the maximum depth the concentrations are not as high as in deeper waters. Concentrations in this area range between 4 nM in near surface water samples to 6 μM (2003) or 3.5 μM (2004) in bottom water samples.

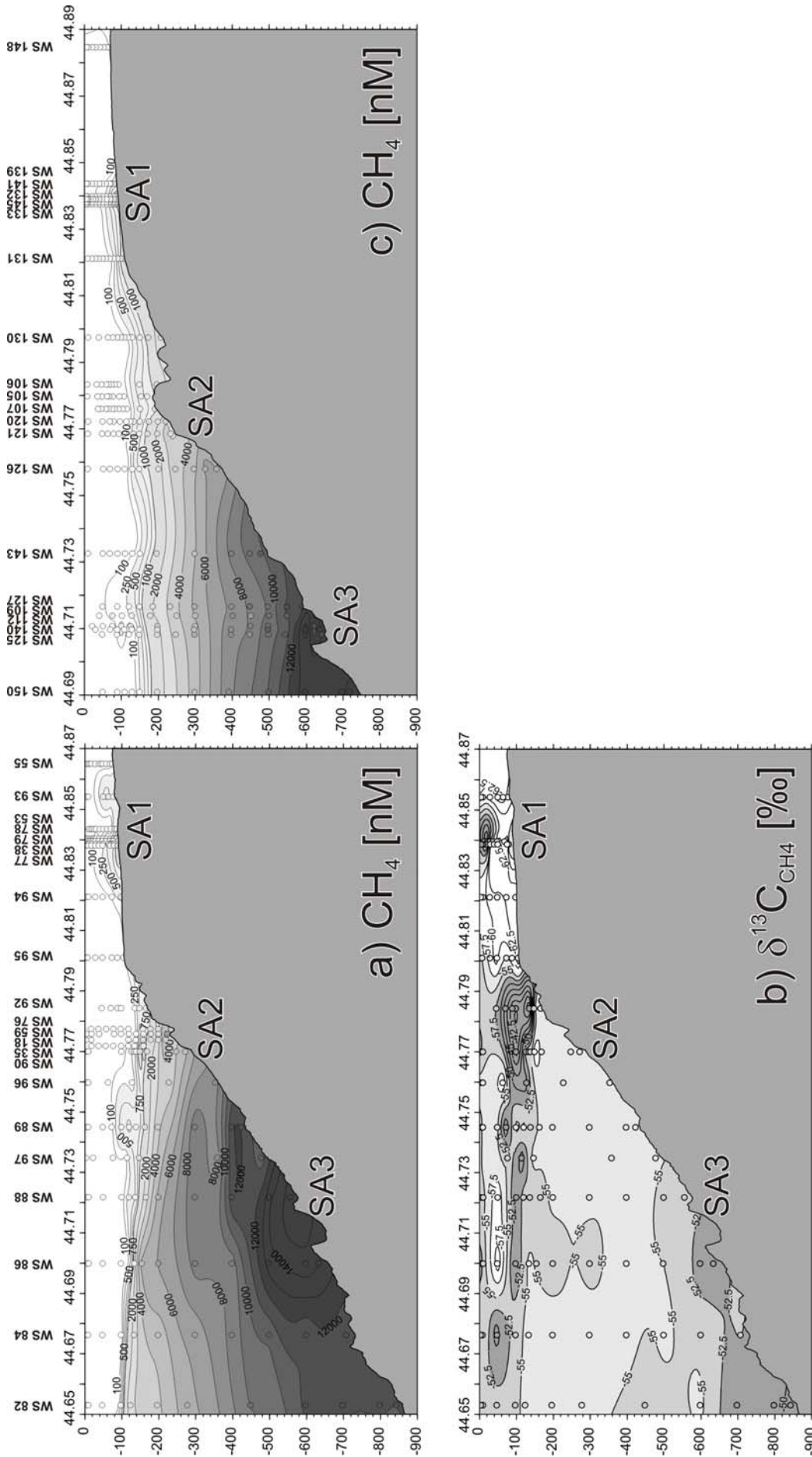


Figure 6: (a) Methane concentration and (b) carbon isotopic ratios of methane from 2003 and (c) methane concentration from 2004. The locations of the dominant seep areas (SA) and individual stations are indicated.

The typical methane distribution in the shelf region away from seep influence is, compared to the previous areas, relatively homogeneous, with concentrations increasing from around 5 nM in near-surface waters to values up to 90 nM in bottom water samples (WS 95, 55, and 148). A small sub-surface methane maximum occurs in many profiles, as illustrated by the ~40 nM peak found at 38 and 47 m water depth in profiles WS 55 and 95, respectively (Figure 7).

In contrast, the concentration profiles at Seep Area 1 differ significantly from the surrounding background shelf region, as values range from around 6 nM at the sea surface to 1.6 μ M near the seafloor for both years (Figures 7 and 8, stations affected by Seep Area 1, e.g. WS 79 and 145). Similar to the stations not affected by seepage (WS 55 and 95), water stations WS 77, 78, and 132 above Seep Area 1 show a distinct subsurface methane maximum of around 200 nM between 30 and 40 m water depth (Figure 7 and 8). However, it should be noted that this maximum is around five times higher than the one observed in the background shelf region.

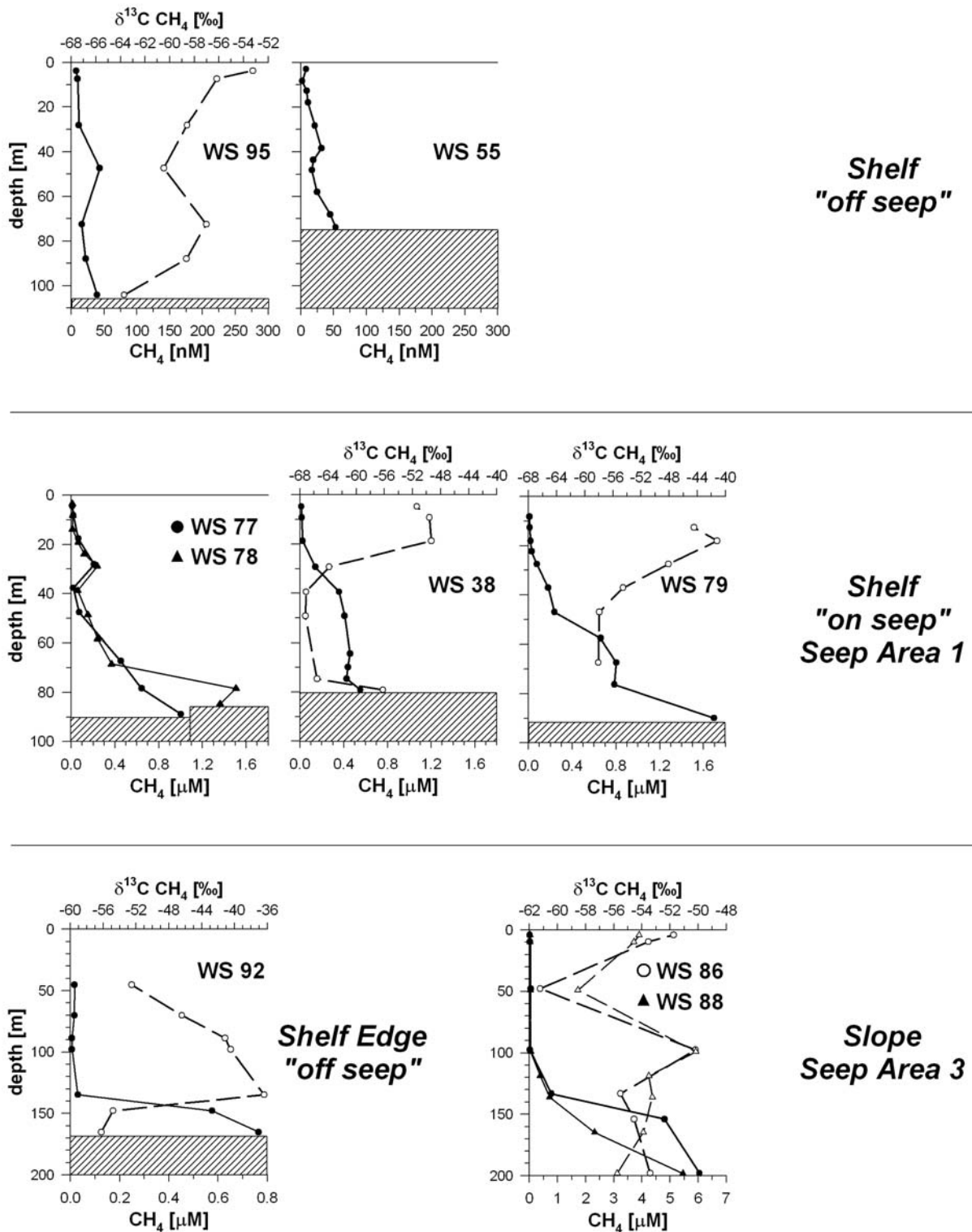


Figure 7: Distribution of methane concentration (dots) and stable carbon isotopic ratios (circles) of methane in the upper part of the water column from selected stations sampled in 2003 versus water depth.

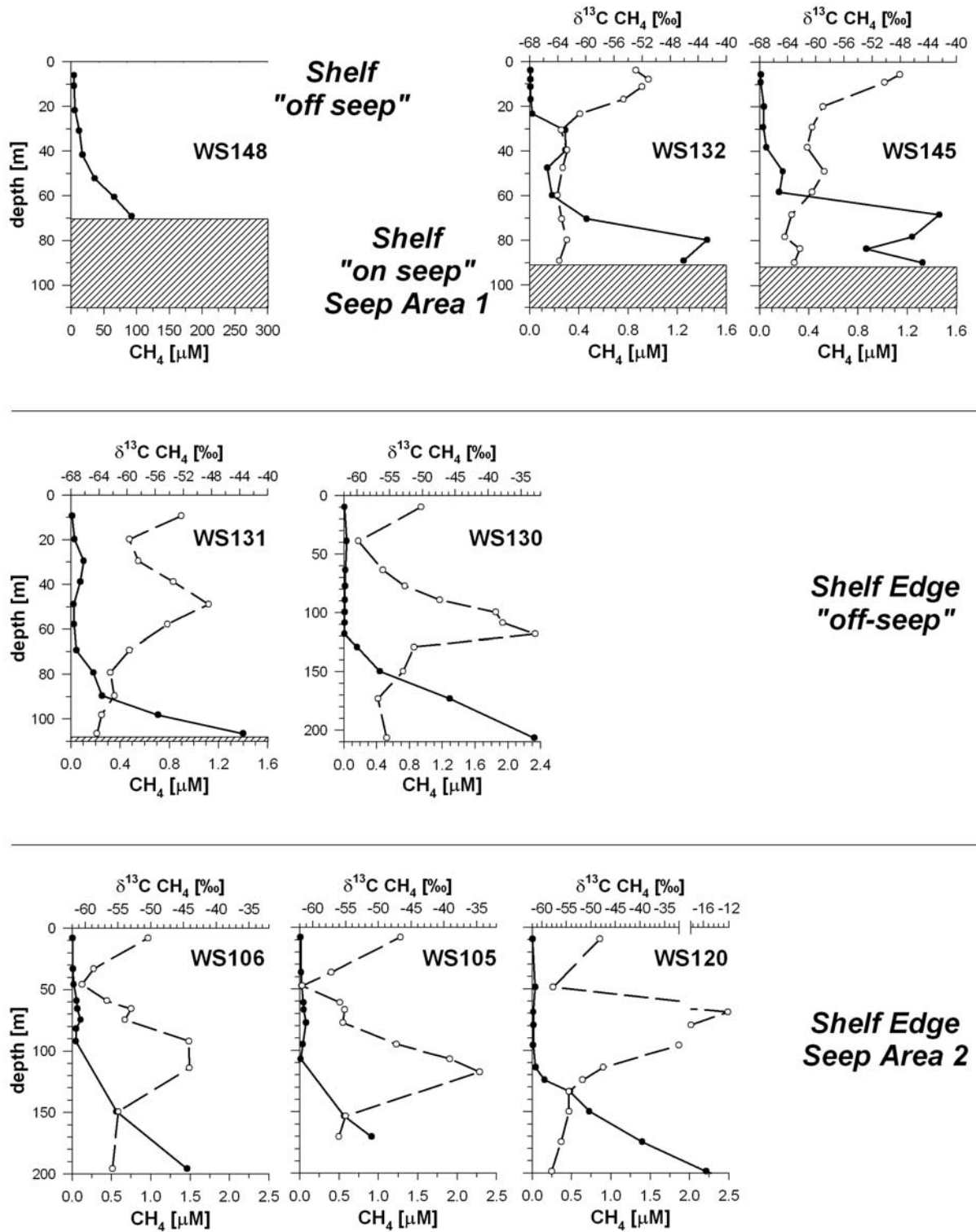


Figure 8: Distribution of methane concentration (dots) and stable carbon isotopic ratios (circles) of methane in the upper part of the water column from selected stations sampled in 2004 versus water depth.

Stable carbon isotopes of water column methane

The 2003 stable carbon isotope measurements of methane are contoured in Figure 6. Contouring was not possible for the 2004 data due to the more limited number of analysed profiles. However, the individual profiles of both years do show similar trends for the same sites. Surface waters in contact with the atmosphere have carbon isotope values from -51 to -62 ‰ over most of the shelf and average around -53 ‰ over most of the slope; analysis of an atmospheric sample taken on the slope shows a carbon isotope ratio of -46 ‰. Values throughout the oxic zone of the transect vary greatly (Figure 6b), with the lightest values occurring in the shallow waters of the continental shelf (typically < -57.5 ‰) and generally heavier values above the continental slope (-61 to -40 ‰). Below the oxycline the ratios trend towards heavier values with increasing depth, reaching a maximum value of around -49 ‰ in the deepest bottom waters.

Seep Area 1 on the continental shelf is different from the highly negative values of the surrounding area, with heavier values up to -40 ‰ being observed principally in the near-surface waters of this site (Figure 7, station WS 79). Shelf water station WS 95, taken in an off seep-region, shows a negative peak of -61 ‰ at around 40 m depth; which corresponds to the subsurface methane maximum (Figure 7).

The highest isotope values were observed above the shelf edge, near Seep Area 2. At this site values as high as -12 ‰ are observed at a water depth of approximately 70 m, while surface water values are around -48 ‰ and bottom water samples are about -59 ‰ (WS 120, and neighbouring stations (e.g. WS 105, 106, and 92); Figures 6, 7, and 8).

Gas composition and stable carbon isotopes of free gas samples

Direct bubble sampling from the seep sites was not possible during CRIMEA cruises PV58 and PV60 due to the lack of appropriate equipment. Collaboration with the METROL project (Poseidon cruise P317/3) allowed for the *in situ* collection of bubbles from seeps at the two shallow sites (90 and 220 m) with the submersible “JAGO”. These samples have been analysed in the laboratory for both their chemical and isotopic composition, as summarised in Table 1.

In regards to the other gases present, CO₂ values are extremely low, while O₂+Ar and N₂ values are surprisingly high. An examination of the N₂/(O₂+Ar) ratio in the final column gives values of 1.53, 1.62 and 2.44, which are much lower than that found in air (3.57). This indicates that the observed values are not likely due to contamination during analysis, but rather may indicate stripping of either the pore water or the water column prior to the capture

of the bubbles. This is supported by the observation that the sample collected highest above the sediments (Jago854, at 60 m above the sediments), also has the highest N₂ content, the highest N₂/(O₂+Ar) ratio (approaching that dissolved in the bottom waters at that site, i.e. 2.44 vs. 2.5), and the lowest CH₄ content.

Table 1: Gas composition and stable carbon isotopes of free gas samples.

Sample	Seep Area	CO ₂ [%]	O ₂ + Ar [%]	N ₂ [%]	CH ₄ [%]	Ethane [ppm]	Propane [ppm]	δ ¹³ C _{CH4} [‰]	C1/C2+C3	N ₂ / (O ₂ + Ar)
Jago8	2	0.002	7.0	11.4	80.6	5.8	0.6	-67	125402	1.62
Jago10	1	0.004	6.6	10.1	81.7	8.2	1.0	-63	89110	1.53
Jago854	2	0.004	7.5	18.4	64.7	2.9	0.3	-66	203719	2.44
air			21.89	78.08						3.57
bottom water			27.7	68.0						2.50

Discussion

Temporal and spatial variability of physical parameters (CTD)

Water column CTD data show that the hydrographic parameters have temporal variations along the entire transect (Figure 2). The highest variability was observed above Seep Areas 1 and 2, and small variations can also be seen in off-seep regions (Figure 2). Figure 3a shows CTD profiles (salinity, temperature and sigma-theta) taken within a 10 day period above Seep Area 1. The depth of sigma-theta (σ_{θ}) 14.5 (i.e. the euphotic zone – oxycline interface), varies between 67 and 85 m during this time interval (Figure 3b). The depths of isopycnal surfaces 14.5, 15.6 and 16.2 above Seep Area 2 also show strong variations over a timeframe of 18 days (Figure 4a). The suboxic/anoxic interface at sigma-theta 16.2 occurs between 148 and 175 m water depth, while sigma-theta 14.5 and 15.6 follow a similar trend but with smaller amplitudes. Comparable long term observations by Luth et al. (1998) of the isopycnal surfaces σ_{θ} 15.4 and 16.2 in the Paleo Dneper Area show maximum amplitudes of 8 and 34 m, respectively. Similar time series CTD-profiles were also conducted by Gulin and Stokozov (2005) near Seep Area 2. These authors defined the spatial and temporal distribution of sigma-theta 16.2, determining an amplitude of 17 m within an 8 day period and estimating a fluctuation period of approximately five days. A direct relation between seep activity and the

observed spatial / temporal variations is unlikely. Though uprising gas bubbles can cause turbulence in the water column, a direct link is contradicted by similar results from CTD-profiles gathered on the continental slope away from any seep influence (Figure 4b). These profiles were taken within a period of 3 days and show an amplitude of sigma-theta 16.2 of 14 m, comparable with shifts observed in seep regions. Internal waves or changes in the current system are a more reasonable explanation for the observed vertical shifts (Kempe et al., 1990). For example, the basin-scale cyclonic boundary current induces mesoscale anticyclonic eddies that are highly variable in time and space. These current features can control the water column stratification down to several hundred meters depth (Luth et al., 1999), and thus can drastically influence biogeochemical processes in the water column (Luth et al., 1998). The nearest anticyclonic gyre which could influence the study area is the so called Sevastopol eddy (Oguz et al., 1992).

Source of methane

The role of shelf and slope sediments in the Black Sea methane cycle and its importance as a methane source is still under debate in the scientific literature. Sediment cores taken on the Bulgarian shelf in 170 m water depth by Reeburgh et al. (1991) show increasing methane concentrations with depth, while another core collected on the abyssal plain in 2,212 m water depth shows an inverse distribution. Based on these results the authors conclude that microbial methanogenesis in the shelf sediments is the only important Black Sea methane source and that it supplies the entire Black Sea water column. Microbial investigations conducted by Ivanov et al. (2002) on the northwestern shelf show that methane formation takes place in shallow sediments and exhibits seasonal dynamics with higher formation rates in summer than in spring. Despite the high methane oxidation rates in the sediments these authors conclude that a considerable part of the methane produced in the subsurface is released into the water column. In contrast to these findings, lander observations by Friedl et al. (1998) along the Black Sea shelf and shelf edge show that no dissolved methane reaches the sediment surface. A sediment transect from the shelf to the deep sea taken by Jørgensen et al. (2001) indicates that dissolved methane is oxidized within the methane/sulfate transition zone and that shelf production of methane in off-seep sediments is of no relevance for the water column methane budget. Apart from confined seep areas in the shelf region these authors believe that only coastal sediments, where sulfate is rapidly oxidized near the sediment surface due to high organic deposition rates, may contribute methane to the water

column due to the potential that the oxidation capacity of the sulfate zone may be too small to trap the entire methane flux (Jørgensen et al., 2001). Jørgensen and co-workers reason that Reeburgh et al. (1991) probably hit an active methane-seep-influenced area to explain these contrasting results.

Water column stations sampled during this study in the shelf region, away from the dominant seep areas, may support the assumption that at least some off-seep shelf sediments represent a source of dissolved methane for Black Sea bottom waters (Figure 7 and 8; WS 95, 55 and 148). The methane concentration profiles at these stations show a smooth but constant increase with increasing water depth and thus may indicate a gentle release of dissolved methane from the sediment into the water column. One interpretation is that this methane transport into the bottom water is controlled by diffusion and may occur where the gas front comes close to the sediment surface. As proposed by Jørgensen et al. (2001) the sulfate zone in these spatial domains may be too small to oxidize the entire methane flux from below. However, out of four cores collected in the 100 m seep area during this project, only the one actually located within a bubbling seep showed elevated concentrations near the sediment-water interface (data not shown), which argues against the shelf sediments being a widespread source of methane to the water column. Another explanation for the observed concentration trend may be that dissolved methane from nearby seep sites may simply be “smeared” laterally via currents, resulting in generally higher concentrations near the seafloor.

In contrast to these low-concentration profiles (nanomolar range) observed in the background shelf areas, methane values in the micromolar range are observed above active seep regions (Figure 6-8; e.g. WS 77, 78, 79, 132, and 145). These highly active seep sites are characterized by the direct transport of methane from a subsurface reservoir into the water column via gas bubbles. This transport mechanism is clearly much more efficient compared to diffusion because of the rapid upward migration of the free gas phase and its bypassing of microbially-mediated methane oxidation processes in the sediments.

The source and depth in which methane is generated in the Paleo Dnepr Area is unknown, but bubble gas samples collected for this project at the sediment surface using the submersible Jago (Poseidon cruise P317/3 - 2004) have $\delta^{13}\text{C}_{\text{CH}_4}$ values of -63 ‰ and -67 ‰ (and $\text{C}_1/\text{C}_2+\text{C}_3$ hydrocarbons ratios of 89,000 and 203,000; Table 1) at Seep Areas 1 and 2, respectively, indicating a biogenic origin (Whiticar, 1999). Measurements of the natural radiocarbon content of methane ($^{14}\text{C}_{\text{CH}_4}$) on gas samples taken at Seep Area 2 show that most of the methane derives from radiocarbon-free sources ($5.0_2 \pm 0.4$ pMC, 24 ka ^{14}C BP; Kessler et al., 2006). We thus assume that methane released at these two sites represents a mixture of

methane generated in organic-rich deltaic sediments deposited during various sea level low stands along the Dnepr river fan and upward migrating radiocarbon-free methane from deeper strata.

In contrast; gas hydrates may play an important role as a source for water column methane around Seep Area 3 located in 600 m water depth, as the phase boundary for methane gas hydrates in the Black Sea is at a water depth of 670-700 m under present conditions (Vassilev and Dimitrov, 2002). Modeling the influence of a near-bottom temperature increase on the Black Sea gas hydrate stability zone (GHSZ), Poort et al. (2005) show that temperature changes, caused by the inflow of warm Mediterranean water after the postglacial Black Sea flooding, will result in a 15-60 % smaller gas hydrate reservoir. This large reservoir shrinkage will be delayed by hundreds of thousands of years due to the transient nature of the heat wave propagation, so that present hydrate dissociation is only expected to occur in shelf regions at the hydrate phase boundary where the GHSZ is thinnest. Intense gas seepage at Seep Area 3 is linked to large sediment slumps; which could have been caused by gas hydrate dissolution and subsequent sediment de-stabilization (Naudts et al., 2006). Compared to the biogenic methane released at the shallow seep sites (Seep Areas 1 and 2), stable carbon isotope values ($\delta^{13}\text{C}_{\text{CH}_4}$) of dissolved methane at this site are generally ~ 10 ‰ heavier (> -52.5 ‰). In addition high ^3He values indicative of input from deep-derived sources were reported by C. Holzner (CRIMEA data; personal communication). These findings suggest a partial contribution of deeper thermogenic methane at this site, although the heavier $\delta^{13}\text{C}_{\text{CH}_4}$ values can also result from microbial enrichment processes, as discussed below (see section 5.3).

Apart from sediment-derived methane, microbial methane generation in the Black Sea water column may act as an additional source. Microbial investigations by Ivanov et al. (2003) and Galchenko et al. (2004) point to methane formation in anaerobic waters. Reeburgh et al. (1991) reasons that methane formation in euxinic waters should be negligible because of the significant amounts of sulfate present, which favors sulfate reduction instead of methanogenesis. Nevertheless, the work of Winfrey and Ward (1983) and Mitterer et al. (2001) on intertidal and marine sediments show that methylamine stimulates methanogenesis in the presence of high concentrations of sulfate. Thus, microbial methanogenesis in Black Sea waters may at least be possible by the use of a non-competitive substrate, in other words one that is used by methanogenic bacteria but refused by sulfate-reducing bacteria. Micro-niches, such as the oxygen-deficient interior of floating particles, may represent an additional habitat where methane production could take place in euxinic waters, a process already known from oxic marine environments (Karl and Tilbrook, 1994). This possibility should be

even more important in the anoxic deep waters of the Black Sea, as oxygen concentration at the outer rim of the particle is already zero and sulfate diminished.

Methane generation in oxic aqueous environments is known from various oceanic regions (e.g. Owens et al., 1991; and references therein), with the typical methane distribution showing a “subsurface maximum” below the mixed layer. Similar trends can be seen in the methane concentration profiles obtained from off-seep shelf stations WS 95, 55, 130, and 131, which have a relatively good resolution in the upper 100 m of the water column (Figure 7 and 8; physical parameters of nearby water stations are given in Figure 3). It has been hypothesized that methane is generated *in situ* in zooplankton guts and the oxygen-deficient interior of particles (e.g. faecal pellets; Karl and Tilbrook, 1994). This *in situ* microbial generation results in an enrichment of the light carbon isotope ($^{12}\text{C}_{\text{CH}_4}$) at this water depth, as indicated by the stable carbon isotope profiles of stations WS 95, 130, and 131 (Figure 7 and 8). A similar isotopic tendency can be seen in slope and shelf edge stations WS 86, 88, 105, 106, and 120 (Figure 7 and 8). Even if some of these stations have a much lower resolution in the supposed area of subsurface methane generation, the isotopic trend clearly shows a steep gradient from heavy isotope values close to the chemocline ($\delta^{13}\text{C}_{\text{CH}_4} \sim -49 \text{ ‰}$) to relatively light values below the mixed layer ($\delta^{13}\text{C}_{\text{CH}_4} \sim -58 \text{ ‰}$). Within the mixed layer there is an increasing influence of atmospheric methane (measured values of -46 ‰), which causes enrichment of the heavier carbon isotopes near the sea surface. Even if the process of subsurface *in situ* methane generation is a relatively minor component in the total Black Sea methane budget, it seems to play a key role in the flux of this gas from surface waters to the atmosphere (Schmale et al., 2005).

The discussion above shows that there are diverse methane sources which can maintain the high CH_4 concentrations observed in the deep Black Sea waters, although the absolute contribution of each mechanism to the Black Sea methane budget is still not clearly understood. However, recent investigations appear to agree that the slope and plain sediments represent methane sinks rather than sources (see discussion below), and that shelf sediments do not show a general source potential. In this region, elevated advective and / or diffusive methane seepage is only expected in spatially-limited areas with high seep activity or near-surface gas fronts. If these assumptions are correct, the remaining potential sources for the large deep methane reservoir of the deeper part of the Black Sea are gas seeps, which transport large amounts of methane into the water column via bubbles, or water column methane production in the presence of sulfate.

Fate of methane

A positive $\delta^{13}\text{C}_{\text{CH}_4}$ shift occurs towards the sediments in waters deeper than 500 m (Figure 6; WS 82, 84, 86, 88) and in the deep Black Sea basin (Kessler et al., 2006) that may be the result of biogenic or abiotic processes in the deep water column or even in the sediments themselves. For example, sediment cores taken in the Black Sea abyssal plain by Reeburgh et al. (1991) show a convex downwards distribution of methane, with surface concentrations lower than those observed in the adjacent anoxic bottom water and decreasing values with increasing sediment depth. This concentration trend implies sediment oxidation of methane transferred from the water column (i.e. a sink). Microbial methane consumption in the sediments favors the lighter carbon isotope, resulting in a depletion of $^{12}\text{C}_{\text{CH}_4}$ in the pore water. The subsequent isotopic concentration gradient between bottom and interstitial waters, and the higher diffusion velocities of ^{12}C compared to ^{13}C , may cause a preferential diffusion of the light carbon isotope ($^{12}\text{C}_{\text{CH}_4}$) into the sediment and an enrichment of the heavier isotope in the residual methane pool in the adjacent bottom water. This microbially-induced process is likely to be spatially limited and would not influence the entire water column. An alternative explanation of this trend could, instead, be related to an increase in oxidation rates from below the chemocline to a depth of around 500 m due to growing substrate concentrations (Durisch-Kaiser et al., 2005; Reeburgh et al., 1991). This effect could result in decreasing turnover times of CH_4 with increasing water depth, and therefore to a faster depletion of the light carbon species ($^{12}\text{C}_{\text{CH}_4}$) in deep Black Sea water layers. Apart from these microbially-mediated processes the hypothesized addition of ^{13}C enriched (i.e. isotopically heavy) thermogenic methane liberated at the 600 m site (see section 5.2) could also cause / contribute to the observed trend.

Microbial investigations of anaerobic methane oxidation rates show that this process is responsible for intense methane consumption throughout the entire anoxic zone and that it represents the major methane sink in the Black Sea water column (Reeburgh et al., 1991; Schubert et al., 2006b). Biomarker investigations of particulate material gathered from the anoxic part of the Black Sea water column show that Archaea, probably in consortium with sulfate reducing bacteria, are responsible for this process (Schouten et al., 2001; Schubert et al., 2006a; Wakeham et al., 2003).

Methane oxidation rates in the aerobic Black Sea waters are calculated to be 10^2 times lower than those in the anoxic part (Reeburgh et al., 1991). Water samples taken during the CRIMEA PV58 cruise in 2003 and analysed for the stable carbon isotopic composition of dissolved methane ($\delta^{13}\text{C}$) point to pronounced microbial methane oxidation within the

chemocline (Schubert et al., 2006b). The strong density gradient across this structure limits upward flux from the euxinic methane pool, and thus this reduced methane supply coupled with microbial oxidation causes ^{13}C enrichment. The 2003 methane profiles gathered in the slope region (Figure 6 and 7; e.g. WS 88, 86) reflect the same isotopic trend observed by Schubert et al. (2006b). These stations show $\delta^{13}\text{C}$ values of around -55 ‰ below the chemocline and an abrupt increase within this transition zone up to values of -49 ‰.

Methane oxidation by aerobic methanotrophs in methane-seep-influenced waters is known from many regions (De Angelis et al., 1999; Suess et al., 1999; Valentine et al., 2001). Valentine et al. (2001) measured the highest oxidation rates in methane plumes overlying decomposing gas hydrates in the Eel River Basin ($5.2 \text{ mmol CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$, depth integrated), resulting in very short turnover times of CH_4 (~1.5 yr) compared to unaffected areas ($0.14 \text{ mmol CH}_4 \text{ m}^{-2} \text{ yr}^{-1}$; turnover times of decades) or similar turnover times reported for the methane-poor open ocean (Rehder et al., 1999). These results indicate that turnover times of CH_4 are inversely correlated with the amount of substrate available for microbial methane oxidation. Schubert et al. (2006b) investigated methane oxidation rates by aerobic methanotrophs at seep sites situated in the Paleo Dnepr Area during the CRIMEA PV58 cruise in 2003. Their results support the investigations conducted by Valentine et al. (2001), with methane oxidation rates approximately 30 times higher above Seep Area 1 relative to the reference station. Further evidence for the intense consumption of methane in plume regions is given in the stable carbon isotope distribution of the present dataset. Isotopically light methane is injected into the water column from seep sites, with values of -63 ‰ and -66 ‰ being observed in pure gas samples taken at the discharge zone at Seep Areas 1 and 2, respectively. The high methane oxidation rates in the dissolved CH_4 plume above Seep Area 1 result in constantly increasing $\delta^{13}\text{C}_{\text{CH}_4}$ ratios within this water body (Figure 6, 7 and 8; WS 38, 79, 132, and 145).

Interestingly, the heaviest $\delta^{13}\text{C}_{\text{CH}_4}$ values are observed on the shelf edge in the region of Seep Area 2 (up to -12 ‰ at WS 120, and similar trends at WS 92, 106, and 105; Figure 6, 7, and 8) where the oxycline and the sediment/water interface intersect. Although there is no clear indication of increased dissolved methane concentrations immediately above the bubble release points (in contrast to the 100 m site) due to the higher background values found below the oxycline, constant liberation of methane from this seep site may enhance microbial methane oxidation in the plume and yield the observed $\delta^{13}\text{C}_{\text{CH}_4}$ peak. An additional driving force for methane oxidation activity in this region could be related to increased solid-phase exchange between the sediments and the water column, such as re-suspension via high

shearing forces on the shelf edge. These shearing forces may also produce boundary currents which can result in an enhanced double eddy diffusion process fostering oxidation within the oxycline by a downward transport of oxygen and an upward transport of methane. Also; bubbles can accumulate and transport sediment, nutrients, and surface active bacteria within the plume (Leifer and Judd, 2002).

Additional evidence for intense aerobic bacterial methanotrophy on the shelf edge is provided by variations in the oxygen maximum layer (OML) in the region of Seep Area 2 and, to a lesser extent, Seep Area 1 (Figure 2 and 5). Normally the OML (conc. $O_2 > 300 \mu\text{M}$) occurs from 10 to 60 m water depth (Figure 2), however above Seep Area 2 it is far more variable. The noted thickness variation in the $O_2 > 300 \mu\text{M}$ layer is not likely to result from internal waves or currents, which would lead to fluctuations of the depth level of the layer rather than its thickness (see section 5.1). Rather this distribution could be the result of enhanced aerobic methane oxidation activity above these seep sites (causing oxygen depletion and a thinning of the OML), oxygen stripping via rising bubbles (Leifer and Patro, 2002; McGinnis et al., 2006) or the upwelling of oxygen impoverished deeper waters (Leifer and Judd, 2002).

The previously discussed microbially mediated oxidation of methane shows that this process represents a very efficient methane sink in oceanic environments. However this process is restricted to the dissolved phase, and thus a free gas phase (i.e. bubbles) can bypass this process and has the potential to transport methane from the sea floor to near-surface waters or even to the atmosphere. The release of gas bubbles from the sediment surface and their transport through the water column is a common feature in our study area (Egorov et al., 1998). McGinnis et al. (2006) conducted model simulations of a 5 mm diameter bubble, similar in size to the acoustically measured, arithmetic mean value of 4.1 (Artemov, 2006), released in the shallow Seep Area 1 (90 m). These results suggest that nitrogen and oxygen concentrations in the bubble approach atmospheric levels at the sea surface, whereas methane is nearly completely dissolved on its way through the water column. Most of it is rapidly dissolved in the lower water column, giving rise to the highest methane concentrations near the seafloor (Figure 6, 7 and 8; e.g. WS 79 and 145). The model shows that the bubble size has to be somewhat larger to directly transport methane to the atmosphere, and even then an individual bubble only contains a small fraction of the original methane released at the sea floor.

Apart from the described bubble transport, upwelling of deep water through a two-phase plume could also transfer dissolved gases to shallow water depths. High methane

concentrations in the migrating water together with the additional dissolution of the associated bubbles could result in elevated methane concentrations higher up in the water column where the plume disperses (Leifer and Judd, 2002). Leifer and Judd (2002) showed the existence of this layer well below the thermocline. This is coherent with results from Seep Area 1, as WS 77, 78, and 132 show high methane concentrations close to the thermocline at around 30 m water depth (see Figure 7 and 8 for CH₄ profiles, Figure 3 for CTD data). This subsurface methane maximum is around five times higher than the one observed in background shelf regions, and thus can not only be explained by the process of *in situ* microbial methane generation at this depth (as described above in section 5.2). From this shallow water depth vertical turbulent diffusion may transport dissolved methane to the sea surface and into the atmosphere.

However, the model calculations by McGinnis et al. (2006) clearly indicate that a significant transfer of methane into the surface waters by bubble dissolution is only likely in rather shallow regions (< 100 m), assuming that plume dynamics of large gas release rates can be neglected (Leifer et al., 2004). These results are consistent with equilibrator investigations carried out in the summer of 2003 and 2004 in the same study area (Schmale et al., 2005), and unpublished data from the CRIMEA cruise PV60 in 2004). The equilibrator dataset illustrates that spots of high methane concentrations are present in the region of Seep Area 1 (90 m). These spots are clearly separated from their surrounding and can be interpreted as a direct result of the underlying seeps. These results are confirmed by the present water column investigations, which show that only plumes in shallow waters (< 100 m water depth) reach the sea surface layer and can act as a potential atmospheric methane source (Figure 6).

Temporal variability of methane

Although the methane datasets from 2003 and 2004 show similar methane distribution patterns, two main differences can be seen in the region of Seep Area 3 (Figure 6). As outlined above this site is unique because it is located near the edge of the hydrate stability zone, its depth corresponds to the point where dissolved methane concentrations begin to become constant, and helium as well as seismic data imply the existence of a deep fault.

Methane concentrations begin to increase at the base of the oxycline (i.e. within the SOL) due to the balance between the upward diffusive transport and *in situ* microbial oxidation of methane. Due to upwelling in the centre of the Black Sea basin and corresponding downwelling on its borders, the depth of the oxycline-SOL boundary does not occur at a fixed depth, but rather it ranges from about 90 to 120 m depth. It has been found by numerous

authors, however, that this interface corresponds to the same density layer throughout the entire Black Sea, occurring at a sigma-theta value (σ_θ) of 15.6 (Saydam et al., 1993). In fact, it is generally accepted that the Black Sea is essentially at steady state (e.g. Kessler et al., 2006), and that the onset of CH₄ increase below σ_θ 15.6 should be spatially and temporally reproducible.

In contrast to this widely accepted concept, a comparison of the 2003 and 2004 datasets from the 600 m site appear to show a temporal variation that resulted in a change in the relationship between dissolved methane and the oxycline-SOL interface. Figure 9 presents methane results obtained from 8 water column profiles conducted at the 600 m site in both 2003 (dashed lines) and 2004 (solid lines), plotted against sigma-theta above (9a) and off (9b) the seep site. These plots, together with Figure 9c, show increasing methane concentrations below $\sigma_\theta = 15.6$ in 2004 as expected, but elevated methane concentrations apparently higher up in the water column in 2003. In addition, the 2003 profiles conducted over the seep area are consistently higher in concentration throughout the water column, whereas those conducted in the off-seep area are higher in the middle interval but essentially the same in the deeper water (see also Figure 9d). A possible explanation for these observations is that at some time in 2003 the area experienced an increase in seep activity. The rapid release of large amounts of methane gas would have resulted in a short term increase in the dissolved phase, with the situation returning to steady state by the 2004 sampling period. The observation that dissolved methane concentrations begin to increase near the oxycline-SOL boundary is important, as it implies that the system can in fact be “overloaded” and that it requires time to re-establish equilibrium, possibly through an increase in the local methanotrophic community and dilution of the methane signal. This indicates that - should a very large release of CH₄ occur - it may be possible that this gas will travel much higher in the water column, potentially to the atmosphere. However, the anomalous methane values measured in 2003 were observed primarily within the SOL, with values measured in the oxycline being normal except for one slightly elevated sample in an off-seep profile (Figure 9b and c, Station 82). Thus, the 2003 dataset indicates that despite elevated concentrations in the anoxic waters, possibly due to increased bubble input from the 600 m seep site, transport of elevated methane concentrations did not occur within the oxycline or overlying oxic waters.

Finally the observation that off-seep sites show increased concentrations only within two samples immediately below the oxycline (Figure 9b), within the SOL, may indicate that bubbles rose quickly above the seep, dissolved significant amounts of methane below the oxycline-SOL interface, and that this dissolved methane was then transported laterally via

currents. An alternative explanation is that the typically lower anaerobic oxidation rates and concentrations just below the SOL, compared to that in the deeper waters, may lead to anomalous dissolved methane concentrations remaining for longer periods of times at this depth. In the deeper waters, instead, greater consumption and higher background values may dissipate the signal much faster.

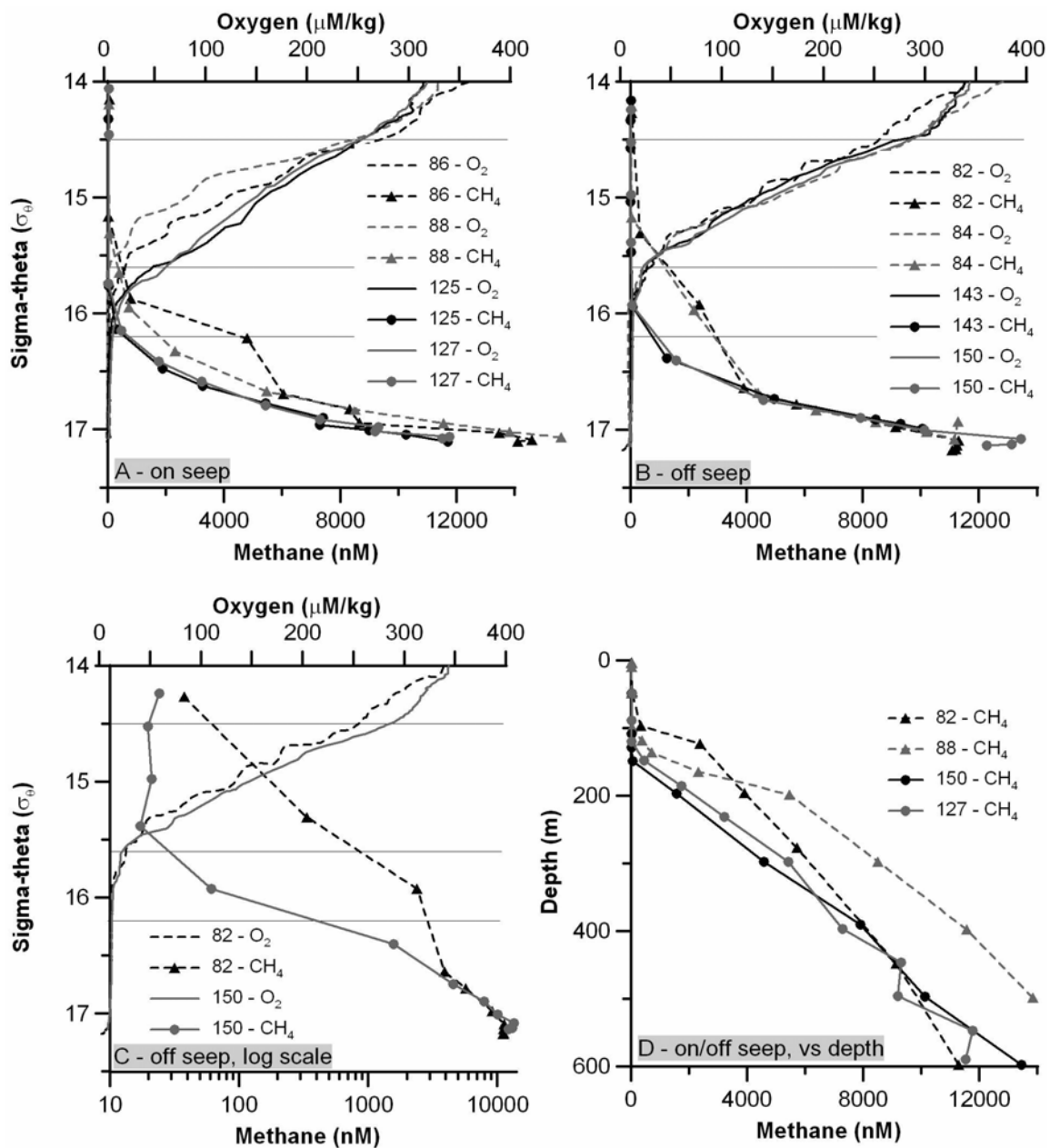


Figure 9: Methane distributions at the 600 m site, comparing results from 2003 (dashed lines) and 2004 (solid lines). A) shows on-seep results while B) shows off-seep. C) plots off-seep methane results on a log scale to show the increase of CH₄ in 2004 at the oxycline-SOL interface (sigma 15.5) while D) plots methane versus depth.

Conclusions

The present work combines dissolved methane concentrations and methane carbon stable isotopic ratios with CTD data along a water column transect in the NW Black Sea area sampled in the summers of 2003 and 2004. Work was conducted to better understand the mechanisms of methane transfer from three known seep areas located along the transect in different water depths and thus, with different stratification and redox conditions of the overlying water column. The shallowest, at 90 m water depth, is in completely oxic water, while the other two are both within the anoxic layer. Bubble flares are observed to reach the water surface at the 90 m site, cross the oxycline at the 250 m site and are restricted to the anoxic layer at the 600 m site. The transect crossing these three areas of gas seepage allows for a comparison of the oxidation processes operating in the different environments.

Shallow seeps (<100 m) were found to have a direct influence on the surface methane concentration, with significantly higher concentrations found in the seep area compared to the surrounding shelf. Despite this fact, however, methane liberated from these shallow sites is efficiently oxidized within the oxygen-rich water column, resulting in only moderately elevated surface water concentrations and relatively small methane emissions at the sea surface. In contrast, methane liberated from the deeper seep sites on the continental slope does not influence the surface water methane concentration or locally enhance the methane flux into the atmosphere because of the highly efficient microbial oxidation processes in the anoxic water column and within the oxycline. The methane flux over wide areas of the Black Sea appears to be controlled by upper water column *in situ* production rather than diffusive flux from the deep anoxic methane pool.

But this well-balanced system will not necessarily remain constant in the future. Continued eutrophication of the Black Sea may cause noticeable oxygen depletion in the shelf regions. This man-made process may reduce the microbial methane oxidation capacity in the water column above shallow seep sites, leading to higher methane fluxes from the sea surface into the atmosphere.

Significant inter-annual differences in the methane inventory across the shelf-slope section were observed. Year-on-year variations in the methane distributions imply a greater input of methane in deep water and a temporary modification of the methane distribution in the water column. Results point towards a decoupling of the well-documented methane-sigma theta relationship, with anomalously high methane concentrations observed within the sub-oxic layer (and possibly the lower oxycline). This observation indicates an apparent “overloading” of the steady-state system of anaerobic methane oxidation within the anoxic zone. This result

seems to indicate that methane could rise higher in the water column given a greater input of methane from, for example, increased hydrate dissolution induced by changes in water column temperature or sea level changes. Although it is difficult to predict if such an increased release could result in methane from deep water seeps reaching the atmosphere, results from the other sites indicate that the subsequent barriers of the oxycline and the oxic layer should be sufficient to limit this process.

Based on these results it appears critical that changes in the complex hydrographical and hydrochemical system of the Black Sea should be closely monitored to evaluate their influence on greenhouse gas emissions from seeps into the atmosphere and their impact on regional and global climate. As our data suggest, the methane budget of the Black Sea does not appear to be in steady state locally on short timescales, and the main mechanism for generation of the large deep methane pool of the Black Sea is still not clear. The understanding of the dynamics of the methane cycle in the Black Sea thus requires repeated measurement campaigns both in seep-influenced as well as non-seep areas, including new techniques to estimate the flux from gas seeps as well as production rates within the sediments and the water column. The region could serve as a model to understand the behavior of the marine gas hydrate reservoirs in a globally warming world.

Acknowledgments

We thank the crew of the RV *Professor Vodyanitsky* for their excellent collaboration during the cruises in 2003 and 2004. Thanks to Yuriy Artemov from IBSS for supplying all the seep locations and information on seep activity in 2003 vs. 2004. We thank the METROL project (EC project EVK3-2002-00080) for the opportunity to participate in the Poseidon cruise P317/3 and JAGO dives. This study received financial support through the CRIMEA project (EC project EVK2-CT-2002-00162).

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Methane emission from high-intensity marine gas seeps in the Black Sea into the atmosphere

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Abstract

Submarine high-intensity methane seeps have been surveyed in the Sorokin Trough and Paleo Dnepr Area in the Black Sea from May to June, 2003 to estimate the sea-air methane flux. The Sorokin Trough mud volcano area in around 2,080 m water depth shows no direct effects on the methane concentration in the surface water and the atmosphere (average methane saturation ratios (SR) of 143 %). The average sea-air methane flux can be determined as 0.2-0.57 nmol m⁻² s⁻¹, using two different sea-air gas exchange models; mean wind speed were extraordinary low throughout the cruise (1.16 m s⁻¹). The investigations in the Paleo Dnepr Area (60 to 800m water depth) reflects a more diverse pattern. Spots of high methane concentrations in the surface water have been recorded above a seep location in around 90 m water depth (SR up to 294 %). The air-sea methane flux above this seep site (0.96-2.32 nmol m⁻² s⁻¹) 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).

Introduction

The Black Sea is the world largest anoxic basin with a unique water column stratification caused by the influx of saline water from the Mediterranean Sea and freshwater from rivers (mainly Danube, Dnepr and Dnyestr). This induces a strong stratification of the water column with a permanent pycnocline at around 150 m water depth. The limited exchange between these separated water masses leads to anaerobic conditions in the lower zone which is highly enriched in geochemically reduced species, such as CH_4 , H_2S and NH_4 . The anoxic water column of the Black Sea represents the world largest semi-closed water reservoir of dissolved methane. Methane itself is of particular interest because of its rising atmospheric concentrations and its contribution to global warming (Lilieveld and Crutzen, 1998).

Numerous scientific studies lead to a complex figure of the Black Sea methane cycle (e.g. Ivanov et al., 1989; Reeburgh et al., 1991). Reeburgh et al. compiled a simple methane budget for the Black Sea. They suggest that microbial methane generated in the shelf and slope sediments is the major methane source, which is balanced by anaerobic oxidation of methane in the anoxic water column (4.7 Tg yr^{-1}). Less important for the methane budget are the microbial oxidation of methane in the oxic water column ($0.3 \cdot 10^{-3} \text{ Tg yr}^{-1}$) and the anaerobic oxidation in abyssal sediments ($0.4 \cdot 10^{-3} \text{ Tg yr}^{-1}$), the outflow through the Bosphorus ($0.03 \cdot 10^{-3} \text{ Tg yr}^{-1}$) and the export into the atmosphere (0.07 Tg yr^{-1}). Thus, according to Reeburgh et al. only 1.5 % of methane produced in the Black Sea is emitted into the atmosphere. The water column oxidation rate given by Reeburgh et al. (1991) suggests a short turnover time of methane of about 20 years. The general water column methane concentration below 500 m water depth is fairly homogeneous (around $11 \mu\text{M}$; Reeburgh et al. (1991) and unpublished data of the CRIMEA project).

Except the work by Reeburgh et al. (1991), little information about the role of the Black Sea as a source of the atmospheric methane has been presented. Dimitrov (2002) suggests that about 0.36 to $1.6 \text{ Tg yr}^{-1} \text{ CH}_4$ is transported into the atmosphere from the Black Sea continental shelf. His calculations are based on hydroacoustic quantifications of gas seepages, determination of seabed flux rates, and the survival time of uprising methane bubbles in the water column. In contrast, Amouroux et al. (2002) infer a shelf emission between 0.019 and 0.032 Tg yr^{-1} and a total emission from the Black Sea surface water of 0.106 to 0.189 Tg yr^{-1} , based on surface water methane measurements similar to the approach used in our study. These different estimates clearly show that additional studies are needed to understand the Black Sea methane cycle and its atmospheric source strength.

Here, we will discuss the contribution of different seep locations (Paleo Dnepr Area and Sorokin Trough) and evaluate their role as a methane source to the atmosphere. We will present methane concentrations of the surface water and atmosphere and calculate the flux of methane based on the sea-air gas exchange models of Liss and Merlivat (1986) and Wanninkhof (1992).

Study areas

Our research cruise CRIMEA 2003 with RV *Professor Vodyanitsky* took place from May 15th to July 1st, 2003 and was focused on the investigation of sub-marine high-intensity methane seeps which continuously release high amounts of free gas (mainly methane) from the seabed into the water column (<http://www.crimea-info.org/>). These gas bubble releasing seep sites are widely distributed on the shelf and at the shelf edge of the Black Sea, but a few have been reported in deep water as well (Egorov et al., 1998; Dimitrov, 2002).

Main study areas of the CRIMEA project are the paleo delta area of the Dnepr river and the Sorokin Trough south of the Crimea peninsula (Figure 1a). Detailed hydroacoustic studies in the Paleo Dnepr Area show that active seeps are distributed along the entire shelf and shelf edge, down to a water depth of 725 m, which represents almost exactly the phase boundary for pure methane hydrate at the ambient temperature and salinity conditions (Dickens and Quintby-Hunt, 1997). Three high-intensive sites have been investigated in detail: Seep Area 1 in about 90 m water depth, Seep Area 2 in 220 m water depth, and Seep Area 3 in about 600 m water depth). In the Sorokin Trough some mud volcanoes also show hydroacoustic anomalies (flares), which provides evidence for the release of free gas even in 2,080 m water depth (Dvurechenskii mud volcano). Flare imaging indicates that the gas rises more than 1,000 m (unpublished data of the CRIMEA project). These notable bubble emissions have only been found periodically, and thus seem to occur just intermittently.

Methods

The methane concentration of the surface water and the overlying air was continuously measured during the entire cruise using a fully automated, semi-continuous seawater-air equilibrator system based on gas chromatography. Technical description and physical principles of this equilibrator are described in detail elsewhere (Rehder and Suess, 2001). The

average precision of the system is better than 2 % (Rehder et al., 2002). For surface water analyses, water was pumped at high flow rate from 3m water depth through a tubing system, which was exclusively installed for this purpose to avoid contaminations from the ship seawater system. The sea surface temperature and conductivity were recorded at the same time using an online CTD (ME, TRAPPENKAMP) and a memory CTD (MCTD, Falmouth Scientific Instruments) which were mounted near the seawater inlet. For a later temperature correction between surface water and equilibrator (for details, see Rehder and Suess, 2001), the water temperature inside the equilibration vessel was measured continuously (Thermometer P650, Dossmann). The air samples were sucked in at the front of the ship 10m above the sea surface. Meteorological data, such as wind speed (taken at 10m height), temperature, wind direction, air pressure and relative humidity, were provided by an automated weather station (WS 2200). The calculations we used to determine the concentrations of dissolved methane in the sea surface layer, the SR and the methane flux from the sea surface into the atmosphere are described in detail by Rehder and Suess (2001). The sea-air methane flux was calculated using the trilinear relationship established by Liss and Merlivat (1986; hereafter referred to as LM-86) and the quadratic relationship from Wanninkhof (1992; for long-term averaged wind velocities, hereafter referred to as W-92). The models given in LM-86 and W-92 were adapted to the mean wind speed of the entire cruise calculated on the base of the daily average. We revised the dataset from the measurements taken while the ship was on station to exclude all data potentially affected by ship-related contaminations. In addition we filtered all measurements with high CO₂ values that occurred when the ship was running downwind. The final data points were gridded and visualized with GMT (Generic Mapping Tool, <http://gmt.soest.hawaii.edu/>). The grids were used to calculate the saturation ratio and the methane flux between the water and the atmosphere.

Active gas bubble releasing seeps were continuously mapped during hydroacoustic studies using a SIMRAD EK-500 echo sounder (Egorov et al., 1998; seep locations are shown in figure 1a]. It has to be considered that the displayed flare positions in figure 1a does not represent the complete image of seeps located in the Paleo Dnepr Area. Previous surveys in the Paleo Dnepr Area lead to a more complex dataset (Yuriy Artemov, personal communication, 2003) which was used to select target areas for more intense investigations. The scientific examinations during the cruise were focused on Seep Area 1 and 2 because of the noticeable seep density. These focused examinations leads to a higher data density from hydracoustic surveys for these sites compared to other areas.

Results and discussion

The spatial distribution of the concentrations of methane in the surface layer and the methane mole fraction in the overlying atmosphere of the Paleo Dnepr Area are given in Figure 1b and 1c. The results of the methane flux calculations are illustrated in Figure 1d. The CH₄ fluxes after LM-86 and W-92 were calculated on the base of the average wind speed of 1.16 m s⁻¹. The average sea surface temperatures were 19.51 °C and 19.36 °C and the average salinities were 17.88 ‰ and 17.79 ‰ for the Paleo Dnepr Area and the Sorokin Trough, respectively.

Table 1 shows the mean flux density from different surface water types and areas of the Black Sea investigated during the CRIMEA cruise in 2003. For comparison values of already published data from the Black Sea are shown (Amouroux et al., 2002; Reeburgh et al., 1991).

Table 1. Methane flux density from different surface water types of the Black Sea and values of already published data.

Water type	Area	Flux density [nmol m ⁻² s ⁻¹]		Reference
		LM-86	W-92	
open waters	Sorokin Trough	0.20	0.57	Schmale et al. (2005)
water depth > 200m	Dnepr Area	0.19	0.47	Schmale et al. (2005)
	NW Black Sea	0.34	0.58	Amouroux et al. (2002)
	Central Black Sea		0.31	Reeburgh et al. (1991)
shelf waters	Dnepr Area	0.32	0.77	Schmale et al. (2005)
water depth < 200m	NW Black Sea	0.37	0.61	Amouroux et al (2002)

The surface water methane distribution displayed in figure 1b shows a strong dependency between the individual water depth of different seep sites and their influence on the surface water methane concentration. Shelf seeps (Seep Area 1) in around 90 m water depth directly affect the methane distribution of the surface water as indicated by the coincidence of seep positions and highest methane concentrations. This direct correlation is also supported by water column investigations above Seep Area 1 during the same cruise. A plume indicated by

high concentrations of methane can be traced from the sea floor to near surface waters (data not shown). Increased surface water methane concentrations away from any seep influence could result from microbial methane generation in shelf sediments (Ivanov et al., 2002). Seeps in water depths greater than approx. 150 m (Seep Area 2, 3 and mud volcanoes in the Sorokin Trough) do not show any significant imprint on the surface water methane content. Surface concentrations above these deeper seep sites are fairly homogeneous and similar to areas where no high-intensity seeps exist. The most obvious reason for this is the dissimilarity and thickness of the water layer above the seeps.

Gas will be continuously dissolved during the ascent of the bubble at the gas/water interface. The rise velocity of approximately 15 m/min (Leifer et al., 2000) implies that the lifetime of bubbles is short enough to exclude oxidative consumption as a process influencing bubble behavior and bubble-mediated gas transport. However, anaerobic methane oxidation is responsible for intense methane consumption throughout the entire anoxic zone (Reeburgh et al., 1991). The short turnover time for methane in the order of 20 years (Reeburgh et al., 1991) and the uniform water age of 1,000 yr between 300 and 1,700 m water depth (Östlund, 1974) implies that the dissolved methane fraction below the pycnocline will be oxidized rather than transported to the sea surface. Another boundary for methane migrating upward is the oxic/anoxic interface, where consumption rates can be extraordinarily high, and the microbial oxidation of methane in the oxic water (Ivanov et al., 2002; Reeburgh et al., 1991). Thus, only shallow seeps which release methane close to the sea surface can bypass methane oxidation and have a direct influence on the local methane emission to the atmosphere.

Compared to the surface water methane concentrations, atmospheric methane was more or less uniform during the cruise period reaching an average value of 1.86 ± 0.03 ppmv (Figure 1c). These values are slightly higher than the results reported for the same timeframe by U.S. NOAA global sampling networks (1.78 to 1.83 ppmv) from the monitoring site at the Azores, which we choose because it is almost the same latitude and far from anthropogenic methane sources (<http://www.cmdl.noaa.gov/ccgg/iadv/>). In any case, methane escaping from the sea surface into the air will be diluted rapidly in the atmosphere and influences are difficult to trace by atmospheric methane measurements. The source strength of Seep Area 1 is not high enough to be responsible for increased atmospheric concentrations as observed in the southern part of the Paleo Dnepr Area. A reasonable explanation for this phenomenon is an impact of anthropogenic sources located on land. Nevertheless, our data show that the Black Sea shelf in particular but the open water also are a significant source of atmospheric methane due to its constant oversaturation with respect to the atmosphere.

The SR ranges from 98 to 294 % in the Paleo Dnepr Area and shows an average value of 143 % in the Sorokin Trough. The methane distribution and SR in surface waters above the Sorokin Trough is very homogeneous (data are not shown). At Seep Area 1 in the Dnepr Area, the surface water reaches SR of 294 % whereas the surrounding homogeneous water shows an average ratio of 148 %. Our data suggest that the SR of the open water is comparable between the two study areas and possibly reflect a rather homogeneous pattern over the entire Black Sea. However, our SRs of the Paleo Dnepr Area are relatively low compared with data published by Amouroux et al. (2002) who observed SRs for the NW Black Sea ranging from 173 % to 10,500 %. The authors describe average values of 5,340 % for the Danube river plume, 567 % for the shelf area and 401 % for the open water. Samples taken by Amouroux et al. (2002) in the vicinity of the Paleo Dnepr Area show an average SRs of around 300 % which indicates that they sampled above seep influenced areas comparable with Seep Area 1. Unfortunately they do not give any indications where samples exactly where taken or if they sampled above active seep areas.

The sea-air methane flux calculations (Figure 1d) show that methane emission from Seep Area 1 ($0.96\text{-}2.32 \text{ nmol m}^{-2} \text{ s}^{-1}$) is 3 times higher than from the surrounding shelf ($0.32\text{-}0.77 \text{ nmol m}^{-2} \text{ s}^{-1}$) and 5 times higher than from the open water ($0.19\text{-}0.47 \text{ nmol m}^{-2} \text{ s}^{-1}$). The average sea-air methane flux determined for the Sorokin Trough was $0.2\text{-}0.57 \text{ nmol m}^{-2} \text{ s}^{-1}$. It has to be emphasized that the average wind velocity were exceptionally low during the entire cruise.

We have to consider that our dataset quantifies only the flux caused by methane dissolved in surface waters. Free gas which reaches the surface layer can not be detected by the equilibrator system. This is of crucial interest for Seep Area 1 where bubbles have been visually observed at the sea surface. Thus, the gas flux into the atmosphere at this seep site could be underestimated. On locations where remaining gas bubbles reach the surface, our approach can only yield a lower limit of the direct methane flux caused by the seep.

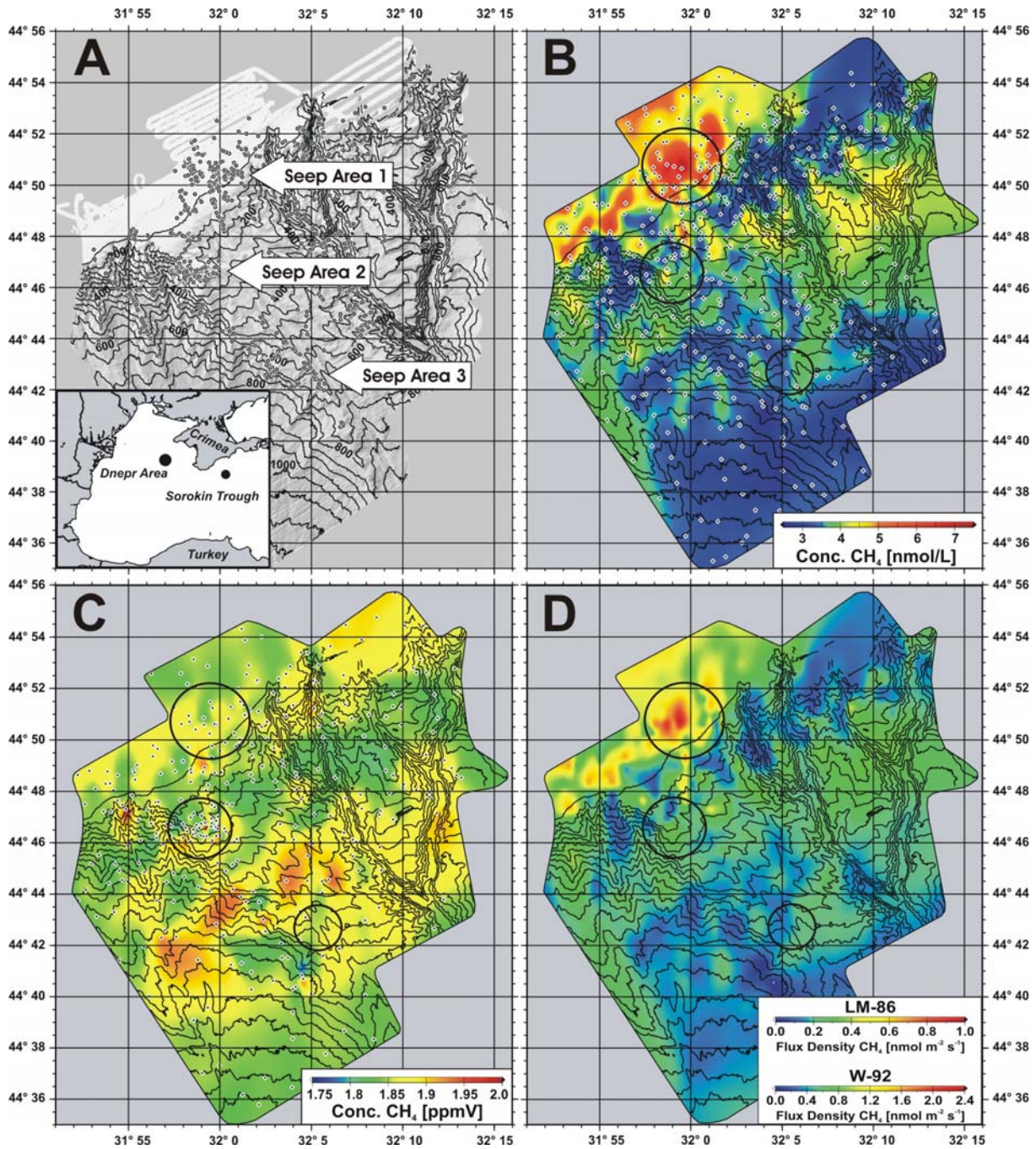


Figure 1: (A) The two CRIMEA study areas: the shallow-water Dnepr paleo delta and the deep-water Sorokin Trough mud volcano region. Bathymetric map of the Dnepr paleo delta and the three main seep areas; black dots represent active bubble seeps recorded during the cruise. (B) Dissolved methane concentration in the surface water and (C) the overlying atmosphere and (D) the results of the methane flux density after Liss and Merlivat (1986; LM-86) and Wanninkhof (1992; W-92) in the Paleo Dnepr Area. Black diamonds in figure B and C represent locations of individual water and air measurements, respectively. The black open circles emphasize the Seep Areas 1, 2 and 3.

Conclusions

Recent publications describe a direct contribution of submarine gas seeps to the global atmospheric methane budget (Milkov et al., 2003; Kopf, 2003). This hypothesis could not be supported by our results, at least not for the entire Black Sea region. Our findings suggest that only shallow seeps, in water depth shallower than 100 m, affect the surface water methane concentration and the direct local emission into the atmosphere. High intensity seep sites below this boundary show no regional influence on the surface concentration.

Another interesting finding is that the methane oversaturation in the open Black Sea surface waters does not differ considerably from that found in other highly productive areas (Cynar et al., 1993; Bange et al., 1994; Rehder et al. 2002), despite of the large methane reservoir in the underlying anoxic water masses. The different redox regimes in connection with the hydrographic structure in the Black Sea provide an effective mechanism to hamper evasion from this reservoir into the atmosphere. Thus, gas bubble transport, providing a rapid pathway through the water column and mostly unaffected by oxidative consumption, might be a major contribution to the methane flux to the atmosphere from the sea floor.

Acknowledgments

We thank the crew of RV *Professor Vodyanitsky* for their excellent collaboration and the pleasant atmosphere during the entire cruise, and numerous scientists for their helping hands. Great thank to Yuriy Artemov from IBSS for supplying all the seep locations. This study received financial support through the CRIMEA project (EC project EVK-2-CT-2002-00162). This is publication 1 of the research project CRIMEA.

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The transient response of the Black Sea methane budget to massive short-term submarine inputs of methane.

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Abstract

A steady-state box model was developed to establish a revised methane budget of the Black Sea and to estimate the methane input into the water column from seeps and mud volcanoes (MV) at various water depths. Our model results suggest a total input of methane of 67.4 Tg yr⁻¹ of CH₄ and an average methane residence time between 0.3 and 6.4 yr. The model predicts that the input of methane is largest in water depths between 100 and 600 m (82 % of the total input), suggesting that the dissociation of methane gas hydrates in water depths equivalent to their upper stability limit may represent a major source of methane into the water column. In addition to the CH₄ depth distribution, we also analysed the stable carbon isotope signature of methane. The model results point towards an increasing influence of a thermogenic methane source with increasing water depth. Finally, we discuss the effects of massive short-term methane inputs (e.g. through deep water MV eruptions or submarine landslides in intermediate water depth) on the Black Sea methane budget and the resulting methane emission into the atmosphere. This non-steady-state model predicts that these inputs will be effectively buffered by intense microbial methane consumption and that the upward flux of methane is strongly hampered by the pronounced density stratification of the Black Sea water column. For instance, an assumed eruption of 1,000 submarine MVs (equivalent to 179 Tg CH₄ d⁻¹) in water depths below 1,000 m (where most of the Black Sea MVs occur) shows that the influence on the sea/air methane flux is negligible and increases the air-sea CH₄ flux by only 0.01 %.

Introduction

For almost 30 years the Black Sea methane cycle has been in the focus of international studies (Amouroux et al., 2002; Hunt, 1974; Ivanov et al., 2002; Reeburgh et al., 1991; Schmale et al., 2005). The Black Sea water column stratification plays a key role in this complex cycle. Its structure is strongly influenced by the inflow of highly saline water via the Bosphorus and freshwater from rivers (mainly Danube, Dnepr and Dnestr), resulting in a permanent pycnocline located in water depths between 100 and 150 m. The lack of sufficient downward ventilation of oxygenated surface waters to counter organic matter fluxes from the highly productive surface waters into the deep waters has resulted in the present anoxic conditions below the pycnocline and made the Black Sea the world's largest anoxic basin with CH₄ concentrations of up to 11 μM.

Recent hydroacoustic investigations have shown that active seep sites, where gas bubbles (consisting mainly of methane) are released into the water column, are widely distributed along the coast, the shelf, shelf edge, and upper slope of the Black Sea (Egorov et al., 1998; Dimitrov, 2002a; Naudts et al., 2006; Schmale et al., 2005; Figure 1). Methane is also liberated from submarine MVs (Figure 1). Up to today, 65 MVs have been discovered in the Black Sea. They are located on the Kerch-Taman shelf, the slope of Bulgaria, Ukraine, Russia, Georgia, and Turkey, as well as in the central part of Black Sea (Kruglyakova et al., 2002). The contribution of seeps and MVs to the total Black Sea methane budget, however, is poorly constrained and needs further investigation.

The first Black Sea methane budget by Reeburgh et al. (1991) determined the total methane inventory to be 6×10^{12} mol (96 Tg). Thus, the Black Sea represents the largest marine water reservoir of dissolved methane. According to Reeburgh et al. (1991), the major methane source are shelf and slope sediments, which are balanced by anaerobic oxidation of methane in the anoxic deep water (4.7 Tg yr^{-1}). The second most important sink is the methane evasion at the sea/air interface with 0.07 Tg yr^{-1} . Less important methane sinks are the microbial oxidation of methane in the oxygenated surface waters, anaerobic oxidation of methane in abyssal sediments, and the outflow via the Bosphorus, together accounting for $7 \times 10^{-4} \text{ Tg yr}^{-1}$. The total oxidation rate of $2.9 \times 10^{11} \text{ mol yr}^{-1}$ (4.6 Tg yr^{-1}) suggests a residence time of methane of about 20 years.

Reeburgh's Black Sea methane budget was recently modified by Kessler et al. (2006) who determined the flow of methane from seeps and dissociating gas hydrates into intermediate and deep waters (water depth >150 m) to be $2.3 - 3.5 \times 10^{11} \text{ mol yr}^{-1}$ ($3.6 - 5.65 \text{ Tg yr}^{-1}$). Their budget calculations include homogeneous methane oxidation rates in the anoxic water column

and a liberation of diagenetically produced methane from sediments. However, there are some inconsistencies and contradictions with other studies that will be discussed here.

We calculate a methane budget of the Black Sea with inhomogeneous methane oxidation rates in the anoxic water column and compare our results to Kessler's methane budget. In addition, a steady-state model is developed to analyse the stable carbon isotope signature of methane in the water column. Finally, we discuss how the Black Sea methane cycle would react to massive methane liberations (e.g. caused by MV eruptions or submarine landslides), i.e. how this would effect the methane water concentrations and the resulting fluxes at the air/sea interface.

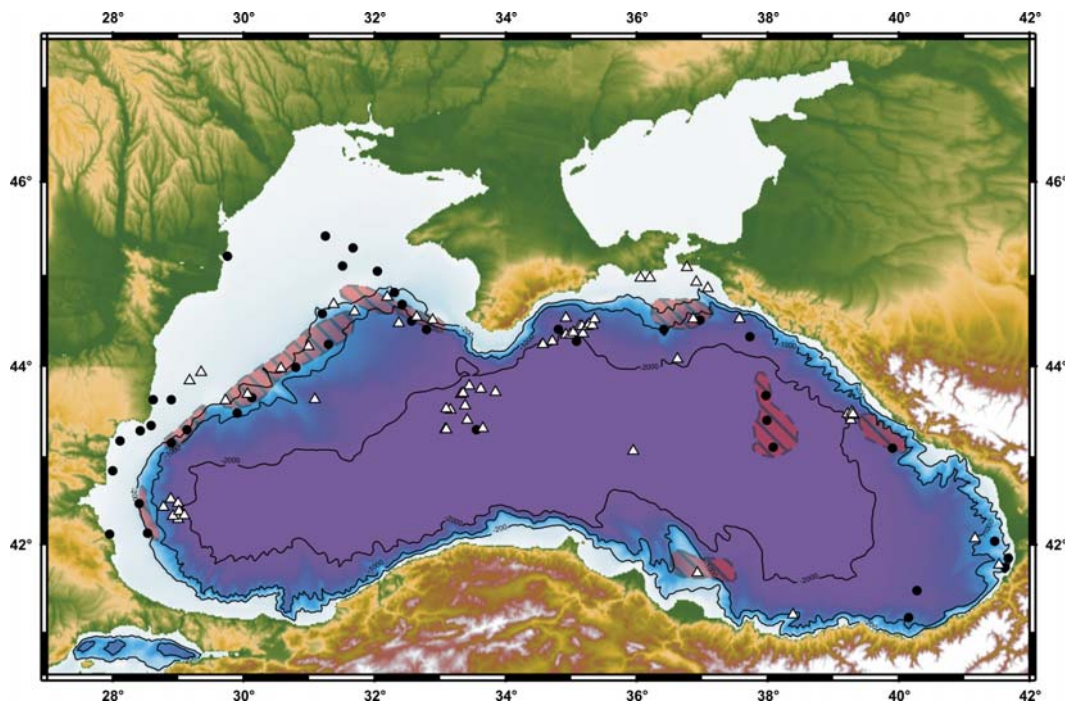


Figure 1: Map of gas and fluid discharge in the Black Sea. Triangles and dots represent locations of submarine mud volcanoes and areas of intense fluid discharge, respectively. Red areas represent regions of gas seepage and seabed pockmarks. Map is based on a data compilation from Kruglyakova et al. (2004) and Vassilev and Dimitrov (2002).

Model description

Two different model approaches were used: (*Model A*) A steady-state box model was developed to describe the recent magnitudes and $\delta^{13}\text{C}$ signatures of methane inputs into the Black Sea water column; (*Model B*) a non-steady-state box model was used to explore the effects of a methane injection into different water depth (e.g. by deep water MV eruptions and landslides in intermediate water depth) on the methane water column distribution and the methane flux between surface water and atmosphere.

Both box models consist of five well-mixed boxes with identical geometries (Figure 2). This model structure was chosen to consider the recent methane distribution in the water column and its stable isotopic signatures ($\delta^{13}\text{C}_{\text{CH}_4}$). Box 3 was created to model an injection of methane in the depth of the gas hydrate stability boundary. Box volumes (V) and areas (A) were calculated based on the GEBCO one minute global bathymetric grid (<http://www.ngdc.noaa.gov/mgg/gebco/>). Both models exchange methane with the atmosphere and include the oxic and anoxic parts of the Black Sea water column with an oxic/anoxic interface located 100 m below the sea surface. The shelf and coastal waters (<100 m water depth) are not included in our model because the distribution and intensity of methane sources (e.g. river plumes and shallow seep areas) and sinks (e.g. water column methane oxidation and evasion to the atmosphere) are very complex. At the present time, these are poorly constrained in these regions. Methane emitting areas in these shallow areas show only regional influences on the water column methane distribution and are not affecting most of the Black Sea open waters (Schmale et al. 2006).

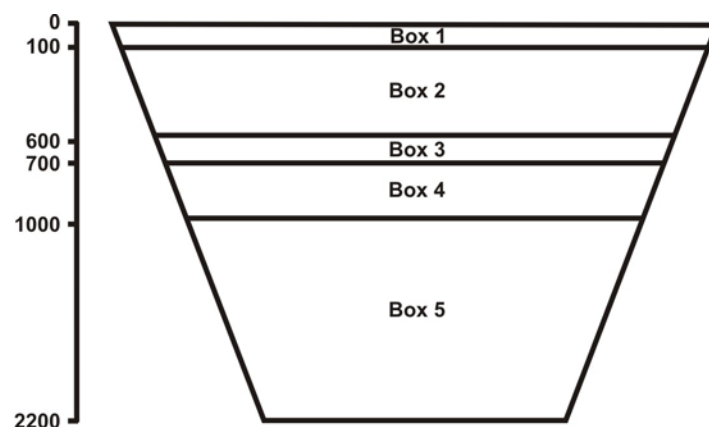


Figure 2: Black Sea model structure and depth range for each box in meters below seafloor (left bar).

Water fluxes into and out of the Black Sea follow the work of Özsoy et al. (1997) but ignore the minor effects of evaporation, rain, and river inflow on the Black Sea open ocean

methane budget. Our model considers a Bosphorus influx of $312 \text{ km}^3 \text{ yr}^{-1}$ (Özsoy and Ünlüata, 1997) and a similar outflux of $312 \text{ km}^3 \text{ yr}^{-1}$, according to our simplifications (ignoring the excess $300 \text{ km}^3 \text{ yr}^{-1}$ from the imbalance between river runoff and precipitation relative to evaporation (Özsoy and Ünlüata, 1997; Ünlüata et al., 1989). The influx of Bosphorus water is mainly restricted to a water depth between 100 and 600 m, which is balanced by upwelling that is represented by an advective transport from box 2 into box 1 (Özsoy et al., 1993; Özsoy and Ünlüata, 1997).

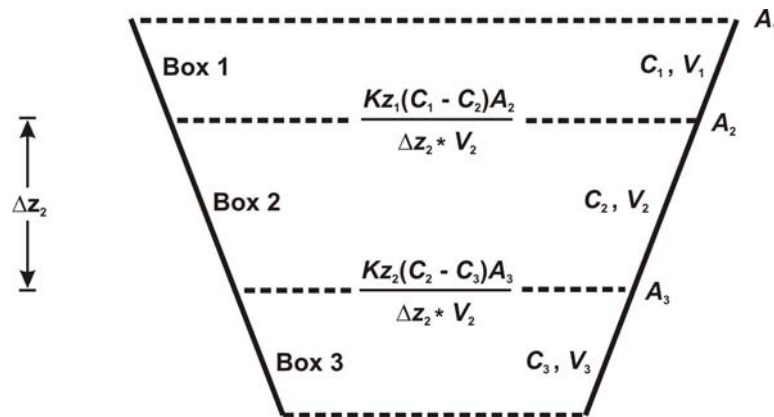


Figure 3: Notations and equations for the vertical transfer of methane by eddy diffusion. The equations are formed for box 2. Kz_1 and Kz_2 represent the Kz values at the top and bottom of box 2. A , V , C , and Δz are the area, volume, CH_4 concentration, and box thickness, respectively.

In addition to advection the model includes the vertical transfer of methane by eddy diffusion (i.e. Kz ; Figure 3). In the nearly stagnant Black Sea deep waters (600 - 2,200 m, between boxes 2 - 5) the transport of methane is restricted to eddy diffusion. Eddy diffusion coefficients (Kz) used in this work result from Thorpe scale analysis (Thorpe, 1977) and a noble gas mixing model (D. McGinnes and C. Holzner, personal communication; Figure 4).

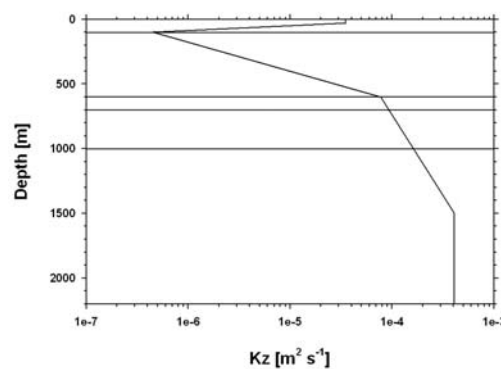


Figure 4: Vertical transfer velocity (Kz) profile for the Black Sea water column (C. Holzner, pers. com.). Note the logarithmic scale for Kz . Box boundaries are represented by horizontal lines.

The surface water box 1 is also connected to the atmosphere. The methane flux at the sea surface (F_{CH_4}) is calculated based on the sea-air gas exchange model of Wanninkhof (1992) for long-term wind averages.

$$F_{CH_4} = kw(C_A - C_1)A_1 \quad (1)$$

where kw represents the gas transfer velocity at the sea surface, C_1 the dissolved methane concentration in box 1, C_A the theoretical equilibrium concentration between surface waters and the ambient atmosphere, and A_1 the surface area of box 1. Methane solubility in seawater was calculated following Wiesenburg and Guinasso (1979). Averaged values for salinity, temperature, wind speed, and atmospheric methane concentration were taken from the literature (for parameter values see Table 1).

Methane oxidation rates were calculated based on the dataset published by Reeburgh et al. (1991; Figure 5). They have been shown to be linearly dependent on the dissolved methane concentration in the ambient water (Ward et al., 1987). We applied a first order rate law assuming that electron acceptors (SO_4^{2-} , O_2) are not limiting the microbial methane oxidation to derive depth-specific kinetic constants.

$$R_{ox} = k [CH_4] \quad \Rightarrow \quad k = \frac{R_{ox}}{[CH_4]} \quad (2)$$

where k is the kinetic constant for methane oxidation, R_{ox} the methane oxidation rate, and $[CH_4]$ the dissolved methane concentration (R_{ox} and $[CH_4]$ were taken from Reeburgh et al., 1991; Figure 5). This work contains a high-quality dataset with the densest sampling interval available for the central Black Sea. The elevated methane oxidation rates in the depth range from 100-600 m in connection with decreasing CH_4 -concentrations from the uniform deeper waters to the chemocline lead to higher values of k within this depth interval (Box 2).

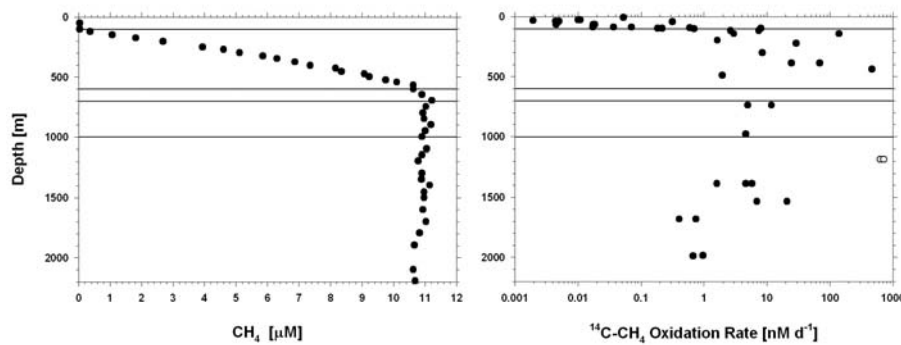


Figure 5: Dissolved methane concentration in the water column (left). Water column methane oxidation rates measured with ^{14}C labelled methane (right). Open circles represent data points which were not used in this work. All data from Reeburgh et al. (1991).

Table 1: Parameter values, notations, and references.

Parameter/Symbol/Unit	Value					Reference
	Box 1	Box 2	Box 3	Box 4	Box 5	
Volume V , km ³	32226	151045	28704	83248	249401	GEBCO grid
Area A , km ²	322267	322267	289350	284887	269058	GEBCO grid
Bosphorus inflow u_{in} , km ³	-	312	-	-	-	<i>Öszozy et al. (1997)</i>
Upwelling u_{up} , km ³		312	-	-	-	
Bosphorus outflow u_{out} , km ³	312	-	-	-	-	
Bosphorus CH ₄ concentration C_{in} , nM	-	6	-	-	-	CH ₄ concentration in box 1 C_1
Bosphorus $\delta^{13}C_{CH_4}$ signature, ppt	-	-54.1	-	-	-	$\delta^{13}C_{CH_4}$ signature in box 1
Box thickness Δz , km	0.1	0.5	0.1	0.3	1.5	
Eddy diffusion coefficient k_z ^a , km ² yr ⁻¹	1.4×10^{-5}	2.4×10^{-3}	3.6×10^{-3}	7.1×10^{-3}		<i>Holzner et al. (2006)</i>
Kinetic const. aerobic CH ₄ oxidation k_{OM} , yr ⁻¹	41.33	-	-	-	-	<i>Reeburgh et al. (1991)</i>
Kinetic const. anaerobic CH ₄ oxidation k_{AOM} , yr ⁻¹	-	3.76	0.28 ^b	0.24	0.15	<i>Reeburgh et al. (1991)</i>
Wind speed u , m s ⁻¹	4.8	-	-	-	-	<i>Sorokin (2002)</i>
Surface water temperature t , °C	15	-	-	-	-	<i>Sorokin (2002)</i>
Surface water salinity S , g kg ⁻¹	17.9	-	-	-	-	<i>Sorokin (2002)</i>
Atmospheric methane concentration C_A , ppmv	1.86	-	-	-	-	<i>Schmale et al. (2005)</i>
Atmospheric $\delta^{13}C_{CH_4}$ signature, ‰	-47	-	-	-	-	<i>Schmale et al. (2006)</i>

^a k_z values between box boundaries; ^b interpolated between box 2 and 4

The transport-reaction equations for each box were used to model the input ($R_{in\ i}$) of methane and the resulting methane distribution in the Black Sea water column are given in Table 2.

Table 2: Differential equations for each box.

Box	Differential equations
1	$\frac{\partial C_1}{\partial t} = \frac{k_w(C_A - C_1)A_1}{V_1} - k_{OM} * C_1 + \frac{u_{up} * C_2}{V_1} - \frac{u_{out} * C_1}{V_1} - \frac{Kz_1(C_1 - C_2)A_2}{\Delta z_1 * V_1} + R_{in1}$
2	$\frac{\partial C_2}{\partial t} = \frac{Kz_1(C_1 - C_2)A_2}{\Delta z_2 * V_2} - k_{AOM2} * C_2 + \frac{u_{in} * C_{in}}{V_2} - \frac{u_{up} * C_2}{V_2} - \frac{Kz_2(C_2 - C_3)A_3}{\Delta z_2 * V_2} + R_{in2}$
3	$\frac{\partial C_3}{\partial t} = \frac{Kz_2(C_2 - C_3)A_3}{\Delta z_3 * V_3} - k_{AOM3} * C_3 - \frac{Kz_3(C_3 - C_4)A_4}{\Delta z_3 * V_3} + R_{in3}$
4	$\frac{\partial C_4}{\partial t} = \frac{Kz_3(C_3 - C_4)A_4}{\Delta z_4 * V_4} - k_{AOM4} * C_4 - \frac{Kz_4(C_4 - C_5)A_5}{\Delta z_4 * V_4} + R_{in4}$
5	$\frac{\partial C_5}{\partial t} = \frac{Kz_4(C_4 - C_5)A_5}{\Delta z_5 * V_5} - k_{AOM5} * C_5 + R_{in5}$

In order to model the stable carbon isotope distribution of methane ($\delta^{13}\text{C}_{\text{CH}_4}$) in the water column, we first modify the differential equations in Table 2 to calculate the $^{13}\text{CH}_4$ concentration in each box. This modification consists of (a) specifying the sources, $^{13}\text{R}_{\text{in}}$, and the atmospheric concentration of $^{13}\text{CH}_4$, and (b) taking into account the slightly slower oxidation of $^{13}\text{CH}_4$ relative to $^{12}\text{CH}_4$. The input of $^{13}\text{CH}_4$ is determined by multiplying the total methane input, R_{in} , by the $^{13}\text{C}/^{12}\text{C}$ ratio, r . (This is approximately correct because 99 % of the total methane is in the form of $^{12}\text{CH}_4$). The isotope ratio is expressed relative to the Vienna Belemnite (VPDB) standard in the δ -notation as:

$$\delta^{13}\text{C}_{\text{CH}_4} = \frac{r}{r_{\text{std}}} - 1 \quad (3)$$

where r_{std} represent the $[^{13}\text{C}]/[^{12}\text{C}]$ ratio of the VPDB standard ($r_{\text{std}} = 0.0112372$). Thus,

$$^{13}\text{R}_{\text{in}} = r_{\text{std}}(1 + \delta^{13}\text{C}_{\text{in}}) * \text{R}_{\text{in}} \quad (4)$$

and similarly for the concentration of atmospheric $^{13}\text{CH}_4$, $^{13}\text{C}_A$, in Table 2,

$$^{13}\text{C}_A = r_{\text{std}}(1 + \delta^{13}\text{C}_A) * \text{C}_A \quad (5)$$

where $\delta^{13}\text{C}_A$ is the $\delta^{13}\text{C}$ of atmospheric methane (-47 ‰).

We have also to consider that the kinetic constant for microbial oxidation of $^{13}\text{CH}_4$ (Eq. 2) is slightly lower than for $^{12}\text{CH}_4$ (Whiticar, 1999). The kinetic constant for the oxidation of $^{13}\text{CH}_4$ (k^{13}) is given by:

$$k^{13} = \frac{k^{12}}{\alpha} \quad (6)$$

where α is the kinetic isotope fractionation factor and k^{12} the rate constant for $^{12}\text{C}_{\text{CH}_4}$ in equation 2. Thus k^{13} from equation 6 is substituted for k values in Table 2.

Kinetic isotope fractionation factors (α) reported for bacterial methane oxidation vary over a broad range (between 1.003 and 1.037 in oxygenated milieus and between 1.002 and 1.014 in anaerobic environments; Seifert et al., 2006). For our final isotope model, we use an α for CH₄ consumption of 1.02 for the aerobic water column (box 1), which represents the mean value in the literature (Seifert et al., 2006). For the anaerobic water column (Boxes 2 to 5) we used 1.009 derived from microbial investigations carried out on Black Sea sediments (Treude et al., 2005; for details see Chapter 3: Results and Discussion).

Using the modified set of equations in Table 2, the ¹³CH₄ concentrations, [¹³C]_i, for each box, *i*, are then solved for. With this result and the previously obtained concentrations of total methane, the model distribution of δ¹³C is obtained from

$$\delta^{13}C_i = \frac{{}^{13}C_i/C_i}{r_{std}} - 1 \quad (7)$$

where ¹³C_i and C_i represent the concentrations of ¹³CH₄ and ¹²CH₄ obtained in each box, *i*.

Results and Discussion

Model A: Input of methane to the Black Sea water column and its stable isotopic source signature

Model A1) Input of methane to the Black Sea water column

A steady-state box model was applied to quantify a depth-dependent input of methane to the Black Sea water column. The recent Black Sea methane distribution (Figure 6) shows methane concentrations in a nanomolar range (average of 5 nM) in water depth between 0 and 100 m. In intermediate water depths of 100 to 600 m the methane concentration is linearly increasing. The deep waters (600 - 2,200 m) are characterized by uniform methane concentrations of around 11 μM.

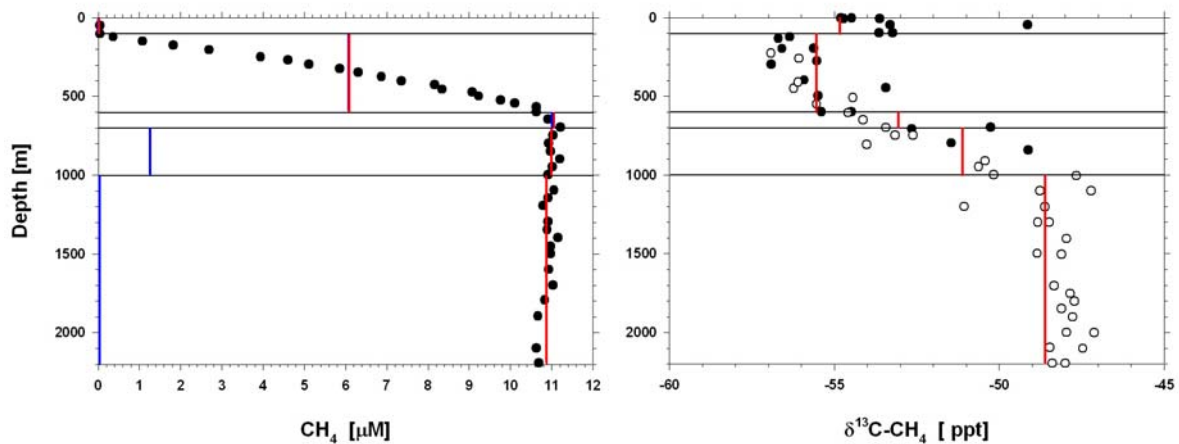


Figure 6: Dissolved methane concentrations (left) and stable carbon isotope data ($\delta^{13}\text{C}_{\text{CH}_4}$, right). Methane concentrations were taken from Reeburgh et al. (1991). Isotope values are derived from Schmale et al. (2006, black dots) and Kessler et al. (2006, open circles). Box boundaries are represented by horizontal lines. Red lines represent the model results (from *Model A1a* and *A2a*) for each box, the blue lines in the left panel are the results without inputs of methane in boxes 3, 4, and 5.

The methane inputs in each box of our model (*Model A1a*) were varied until the modelled methane concentrations agreed with the averaged measurements published by Reeburgh et al. (1991; see Figure 6 and Table 3a). Using our non steady-state model initiated with no CH_4 in the Black Sea water column shows that a simulation runtime of approximately 50 years is necessary to reach steady-state concentrations.

The modelled results in Table 3a indicate that most CH_4 is entering the Black Sea waters between 100 - 600 m water depth. The lower boundary of this depth range (represented by box 2) is close to the phase boundary of gas hydrates (670 - 700 m; (Vassilev and Dimitrov, 2002). Poort et al. (2005) have recently modelled the regional response of the gas hydrate stability zone to the Black Sea post glacial flooding and the resulting bottom water temperature increase. It predicts that at present a widespread dissociation of gas hydrates is expected to occur at the minimum water depth for hydrate stability. The model results imply that gas hydrate dissociation at the Black Sea continental slopes may take place and serve as an important methane source in intermediate waters. However, only a few active seep sites, which influence the methane concentration in the surrounding water column, have been discovered in this specific depth range so far (Schmale et al., 2005). Hydroacoustic seep detection along the slope of the NW Black Sea indicates that the minimum depth of gas

hydrate stability is not characterized by a higher-than-average number of seep sites (Naudts et al., 2006). To test the hypothesis that the emission of methane from shelf and upper slope seeps in water depth between 100 and 700 m (represented by box 2 and 3) is the only source of methane in intermediate and deep waters, we set the inputs in boxes 4 to 5 to zero. To balance the missing methane flux from box 4 into box 3 and to reach the average concentration in box 3 of about 11 μM , we set the methane input into box 3 to 3.6 Tg yr^{-1} . The model predicts that an input of methane only into box 2 and 3 is not sufficient to maintain the deep-water methane budget (see blue lines in Figure 6).

Table 3a: Model results from *Model A1a* and *A2a* (based on inhomogeneous methane oxidation rates in the anoxic water column): methane input, its isotopic input signatures ($\delta^{13}\text{C}_{\text{CH}_4}$), and the resulting concentrations, and isotopic signatures ($\delta^{13}\text{C}_{\text{CH}_4}$) of water column methane compared with measured values of Reeburgh et al. (1991), Kessler et al. (2006), and Schmale et al. (2006).

Box	CH ₄ input	CH ₄ input	$\delta^{13}\text{C}_{\text{CH}_4}$ input	modelled		measured (mean)	
	[mol km ⁻³ yr ⁻¹] $\times 10^6$	[Tg yr ⁻¹]	signature [‰]	CH ₄ [nM]	$\delta^{13}\text{C}_{\text{CH}_4}$ [‰] ^a	CH ₄ [nM]	$\delta^{13}\text{C}_{\text{CH}_4}$ [‰]
1	2	0.1	-78	6	-54.1	5	-54.0
2	22.9	55.3	-64	6099	-55.6	6097	-55.8
3	4.3	2.0	-60	11052	-53.1	11061	-53.1
4	2.7	3.5	-60	11006	-51.1	11007	-51.6
5	1.6	6.5	-57	10871	-48.6	10886	-48.2

^a modelled with $\alpha_{\text{MO}} = 1.02$ and $\alpha_{\text{AOM}} = 1.009$

Table 3b: Model results from *Model A1b* and *A2b* (based on homogenous methane oxidation rates in the anoxic water column): methane input, its isotopic input signatures ($\delta^{13}\text{C}_{\text{CH}_4}$), and the resulting concentrations, and isotopic signatures ($\delta^{13}\text{C}_{\text{CH}_4}$) of water column methane compared with measured values of Reeburgh et al. (1991), Kessler et al. (2006), and Schmale et al. (2006).

Box	CH ₄ input	CH ₄ input	$\delta^{13}\text{C}_{\text{CH}_4}$ input	modelled		measured (mean)	
	[mol km ⁻³ yr ⁻¹] $\times 10^5$	[Tg yr ⁻¹]	signature [‰]	CH ₄ [nM]	$\delta^{13}\text{C}_{\text{CH}_4}$ [‰] ^a	CH ₄ [nM]	$\delta^{13}\text{C}_{\text{CH}_4}$ [‰]
1	2	0.1	-78	6	-54.5	5	-54.0
2	5.7	1.4	-64	6042	-54.8	6097	-55.8
3	19	0.9	-60	11063	-53.2	11061	-53.1
4	6.8	0.9	-60	11063	-50.8	11007	-51.6
5	6.5	2.6	-57	10851	-48.7	10886	-48.2

^a modelled with $\alpha_{\text{MO}} = 1.02$ and $\alpha_{\text{AOM}} = 1.009$

The basin-wide CH₄ input calculated from our *Model A1a* is more than one order of magnitude higher than the one recently published by Kessler et al. (2006; Table 4, i.e. 67.4 Tg yr⁻¹ instead of 3.6 to 5.65 Tg yr⁻¹).

Table 4: Black Sea methane budget based on *Model A1a*.

			Tg CH ₄ yr ⁻¹
Sinks			
Emission across the sea/air interface			0.3
Water column oxidation	Box No.	Rate [μM yr ⁻¹]	
aerobic	1	0.21	0.1
anaerobic	2	22.91	55.4
	3	3.06	1.4
	4	2.61	3.5
	5	1.68	6.7
Outflow via Bosphorus ^a			5.9 × 10 ⁻⁵
			67.4
Sources			
	Box No.	Rate [μM yr ⁻¹]	
	1	0.20	0.1
	2	22.90	55.3
	3	4.33	2.0
	4	2.65	3.5
	5	1.63	6.5
			67.4

^a Bosphorus outflow of 612 km³ yr⁻¹ after Öszoy et al. (1997)

This deviation can only be explained by the different oxidation rates used in the two model runs. *Model A1a* considers the varying methane oxidation rates in the anoxic water column reported by Reeburgh et al. (1991; see Figure 5 with average oxidation rates ranging between 22.91 μM yr⁻¹ and 1.68 μM yr⁻¹ in box 2 and 5, respectively). These oxidation rates are based on ¹⁴C-labelled methane incubation experiments. In an additional incubation test, Reeburgh et al. (1991) determined oxidation rates using ³H-labelled methane. This results in lower and more homogenous oxidation rates in the deep waters. A compilation of the two datasets leads to an average methane oxidation rate of 0.6 μM yr⁻¹ for AOM in water depths below 100 m. This rate is used in Reeburgh's Black Sea methane budget calculations (Reeburgh et al.,

1991), and is adapted in the model of Kessler et al. (2006). In *Model A1b*, we used the lower methane oxidation rates in the deep waters as suggested by Reeburgh et al. (1991). These calculations result in a total methane input of 5.9 Tg yr^{-1} , which is in good agreement with the values published by Kessler et al. (2006), i.e. 3.6 to 5.65 Tg yr^{-1} (Table 3b).

Reeburgh et al. (1991) do not favour one of the two datasets. Both methods (^{14}C - or ^3H -labelled methane) have advantages and disadvantages as discussed by these authors, but the use of mean oxidation rates masks the inhomogeneities in the water column below 100 m, that are indicated by the ^{14}C -labelled methane incubation experiments. Recently, several studies have validated these variations using AOM rate measurements and biomarkers indicative for AOM performing microbes (Wakeham et al., 2003, Durisch-Kaiser et al., 2005; Schubert et al, 2006).

From the output of *Model A1a* we also calculated the methane inventory and residence times of each box (Table 5). The Black Sea contains a total of 78 Tg of methane, with the largest inventory below a water depth of 1,000 m, due to the dominant water volume in box 5. The methane residence time (CH_4 inventory / CH_4 total oxidation rate) varies between 0.26 and 6.4 years in the anoxic waters. The short residence time in box 2 is caused by the high methane oxidation rate, which is one order of magnitude higher than in the other boxes. In contrast, Reeburgh et al. (1991) estimated the methane residence time to be 20 yr, based on a total oxidation rate of $4.6 \text{ Tg CH}_4 \text{ yr}^{-1}$ for the deep Black Sea waters below 100 m water depth.

However, the correct knowledge of deep water methane oxidation rates is weak and further studies are needed to prove the existing datasets.

Table 5: Methane inventory, total oxidation rate, and residence time.

Box	CH₄ inventory [Tg]	CH₄ total oxidation rate [Tg yr⁻¹]	CH₄ residence Time [yr]
1	0.003	0.1	0.03
2	14.6	55.4	0.26
3	5.8	1.4	4.1
4	15	3.5	4.3
5	43	6.7	6.4
	78	67.1	

The only source of sulfate, major electron acceptor for the oxidation of methane in the anoxic waters in the Black Sea, is the inflow of Mediterranean water through the Bosphorus. To prove if the inflow of SO_4^{2-} can balance methane oxidation in the anoxic waters of the Black Sea (Table 5; $67 \text{ Tg yr}^{-1} = 4.19 \times 10^{12} \text{ mol yr}^{-1}$), we calculated the total amount of sulfate delivered by the Bosphorus. Considering SO_4^{2-} concentrations of 30.5 mM in the Mediterranean inflow (salinity ~ 38) and a water inflow of $312 \text{ km}^3 \text{ yr}^{-1}$, the total sulfate flow is $9.5 \times 10^{12} \text{ mol yr}^{-1}$. Hence, the total input of sulfate alone can account for the SO_4^{2-} amount needed for the AOM reaction in the deep waters of the Black Sea.

Due to the large predicted inputs of methane and the high methane oxidation rates, the model is relatively insensitive to changes in the water column eddy diffusion or water balance. For example increasing the eddy diffusion coefficient by one order of magnitude changes the predicted methane concentrations by 7.4 % in the anoxic boxes (2 - 5). Ignoring the water in- and out-fluxes via the Bosphorus reduces the final result in box 2 by only 0.05 %. However, the missing upwelling leads to a surface water methane concentration which is 20.5 % lower in contrast to calculation including the advective transport between box 2 and box 1.

In contrast to previously published Black Sea methane models, our model also describes the methane cycle in the upper 100 m of the Black Sea water column. The model predicts that a diffusive and advective transport is not sufficient to maintain the average surface water methane concentration in box 1 (model output of 1.8 nM in *Model A1a* and *A1b* instead of 5 nM reported by Reeburgh et al., 1991). An additional open ocean methane source in these shallow water depths is given by microbial subsurface methane generation. This process may take place in zooplankton guts and the oxygen-deficient interior of particles (e.g. fecal pellets; Karl and Tilbrook, 1994). Subsurface methane maxima together with light $^{13}\text{CH}_4$ anomalies have been observed in Black Sea surface waters by Schmale et al. (2006) and indicate that this methane source is present in the oxygenated water column. The limited exchange between box 1 and 2 by eddy diffusion and upwelling shows that subsurface generation is crucial for the methane flux at the sea surface in Black Sea open waters.

Model A2) Stable isotopic source signature

In addition, we developed a steady-state isotope model to discuss the sources of methane in the Black Sea water column (microbial vs. thermogenic). The combined stable isotope data from Schmale et al. (2006) and Kessler et al. (2006) show a nearly linear trend towards heavier values between 100 and 1,000 m water depth (Figure 6). Below this depth $\delta^{13}\text{C}_{\text{CH}_4}$ becomes uniform averaging -48.2 ‰.

We varied the methane isotope input signatures in each box of *Model A2a* until the modelled $\delta^{13}\text{C}_{\text{CH}_4}$ value of water column methane agreed with the averaged measurements summarized in Figure 6 and Table 3a. To prove if lower methane oxidation rates in the anoxic water column as suggested by Reeburgh et al (1991; see discussion in *Model A1*) influence the modelled stable carbon isotope signature of water column methane, we developed *Model A2b*. The results show that the use of these lower oxidation rates are only slightly affecting the modelled $\delta^{13}\text{C}_{\text{CH}_4}$ signature in the water column (Table 3b).

The isotope model provides further evidence for methane generation in Black Sea surface waters, as discussed in the previous section. To obtain the source signature of methane generated in the aerobic surface waters, we used the previously determined methane input predicted for box 1 (calculated in *Model A1*). The best fit to the observed $\delta^{13}\text{C}_{\text{CH}_4}$ values in the surface waters is obtained by a $\delta^{13}\text{C}_{\text{CH}_4}$ input signature of -78 ‰. This isotope signature corresponds to microbial carbonate reduction, the dominant methanogenic pathway in marine environments (Whiticar, 1999). The modelled $\delta^{13}\text{C}_{\text{CH}_4}$ value in Black Sea surface waters is -54.1 ‰ (using *Model A1a*, or -54.5 ‰ based on *Model A1b*), which comes close to measurements conducted by Schmale et al. (2006; -54.0 ‰; see Figure 6 and Table 3a and 3b). Without subsurface generation the model shows heavier stable isotope values of -40.0 ‰ (using *Model A1a*, or -35.8 ‰ based on *Model A1b*).

However, our model also shows that the predicted results are very sensitive to changes in the kinetic isotope fractionation factor α . Using minimum and maximum fractionation factors for aerobic and anaerobic methane oxidation of methane as reported by Seifert et al. (2006) leads to strongly differing $\delta^{13}\text{C}$ values of water column methane (Table 6).

Table 6: Modelled water column $\delta^{13}\text{C}_{\text{CH}_4}$ distribution using *Model A2a*, the best fit $\delta^{13}\text{C}_{\text{CH}_4}$ input signatures from our isotope *Model A2*, and different isotopic fractionation factors (α) compared with measured values of Kessler et al. (2006) and Schmale et al. (2006).

Box	$\delta^{13}\text{C}_{\text{CH}_4}$ input signature [‰]	measured (mean) $\delta^{13}\text{C}_{\text{CH}_4}$ [‰]	modelled $\delta^{13}\text{C}_{\text{CH}_4}$ [‰]		
			min. α	preferred α	max. α
			$\alpha_{\text{MO}} = 1.003$	$\alpha_{\text{MO}} = 1.02$	$\alpha_{\text{MO}} = 1.037$
			$\alpha_{\text{AOM}} = 1.002$	$\alpha_{\text{AOM}} = 1.009$	$\alpha_{\text{AOM}} = 1.014$
1	-78	-54.0	-69.2	-54.1	-39.6
2	-64	-55.8	-62.1	-55.6	-50.9
3	-60	-53.1	-58.8	-53.1	-49.0
4	-60	-51.6	-57.6	-51.1	-46.5
5	-57	-48.2	-55.2	-48.6	-43.9

The model reacts similarly sensitive to changes in the source isotope signatures. We used minimum and maximum values as given by Kruglyakova et al. (2002) for Black Sea gas hydrates and Michaelis et al. (2002) for shallow seep methane (Table 7).

Table 7: Modelled water column $\delta^{13}\text{C}_{\text{CH}_4}$ distribution using *Model A2a* and different $\delta^{13}\text{C}_{\text{CH}_4}$ input signatures.

Box	$\delta^{13}\text{C}_{\text{CH}_4}$ input signature [‰] ^a	modelled $\delta^{13}\text{C}_{\text{CH}_4}$ [‰] ^c	$\delta^{13}\text{C}_{\text{CH}_4}$ input signature [‰] ^b	modelled $\delta^{13}\text{C}_{\text{CH}_4}$ [‰] ^c
	1	-43	-24.2	-68
2	-43	-34.4	-68	-59.6
3	-43	-35.5	-68	-60.7
4	-43	-34.5	-68	-59.8
5	-43	-34.4	-68	-59.6

^a Kruglyakova et al. (2002); ^b Michaelis et al. (2002); ^c modelled with $\alpha_{\text{MO}} = 1.02$ and $\alpha_{\text{AOM}} = 1.009$

Because no isotope fractionation factor (α) for anaerobic methane oxidation in the Black Sea water column is available from the literature, we use a fractionation factor derived from sediment investigations (Treude et al., 2005) in our model *Model A2* (Table 3a and 3b). Microbial and isotopic investigations of the anaerobic consumption of methane carried out on a sediment push core taken at the NW Black Sea shelf lead to a α_{AOM} of 1.009 (Treude et al., 2005). Provided that anaerobic methane oxidation in the sediment and water column are

microbially similar, we think that it is reasonable to apply the α_{AOM} value reported by (Treude et al., 2005) to the anaerobic water column. This assumption seems to be plausible since the work of Leifer and Judd (2002) could prove the transport of particles (and thus the transport of surface active bacteria) from the seafloor to the water column by gas bubbles.

The methane isotopic source signature ($\delta^{13}C_{CH_4}$) used in our *Model A2* reflects an increasing influence of thermogenic methane with increasing water depth (Table 3a and 3b). This source distribution is supported by measurements of free gas in shallow water depths on the NW Black Sea shelf (Michaelis et al., 2002; $\delta^{13}C$ values of -62 ‰ to -68.3 ‰) and pore water samples collected in the Sorokin Trough at around 2,000 m water depth (Blinova et al., 2003; values of about -58 ‰). The MVs located in the Black Sea abyssal plane are most probably “rooted” in the Maikopian Formation which is highly enriched in organic matter and thus represents a great potential to generate thermogenic hydrocarbons (with an isotopic signature of >-60 ‰; Dimitrov, 2002b). Methane discharge from these MVs may influence the isotopic composition of methane in the deep Black Sea waters, whereas in the upper water column diagenetically generated methane in shallow sediment depths (with an isotopic signature of <-60 ‰) liberated by numerous seeps located on the shelf edge and upper slope may dominate the stable isotope distribution.

Model B: Influence of massive short-term methane injections on the Black Sea methane budget

A non-steady-state model was applied to predict the effects of massive short-term methane injections on the methane distribution in the Black Sea water column. Two scenarios are discussed: (*Model B1*) a release of methane from the seafloor in water depths below 1,000 m (box 5), the area in which most of the Black Sea MVs are located (Kruglyakova et al., 2004), and (*Model B2*) an injection of methane at the minimum water depth of gas hydrate stability where hydrate dissociation may take place (Poort et al., 2005) and submarine landslides could be expected (box 3).

The approach was to separately increase the methane input in these two water depths (represented by box 3 and 5) and to simulate the evolution of methane concentration in the Black Sea water column and the resulting flux of methane at the sea surface until a steady-state is reached.

It is difficult to designate the methane release through MV eruptions since no direct gas flux measurements are available from eruptive periods. A few estimates exist implying that gas fluxes are on the order of 10^7 to 10^{10} m³ (STP) over several days (Milkov et al., 2003), and references therein). In our model, we use a number which is based on gas flux records during strong eruptions of onshore mud volcanoes in Azerbaijan (2.5×10^8 m³ CH₄ (= 179 Gg or 1.1×10^{10} mol) per eruptive MV event; (Dadashev, 1963); a number also used by (Milkov et al., 2003) to estimate the global gas flux from eruptive MVs).

B1) Methane input in box 5

Three different model runs were performed based on different assumptions. All runs were initiated with steady-state methane concentrations calculated in our previous model (*Model A1a*) and assuming an eruptive phase of one day. Model run *B1a* considers a single MV eruption, run *B1b* is working with the assumption that all known Black Sea MVs erupt on a single day (65; taken from Kruglyakova et al., 2004), and model run *B1c* calculates on the base of a fictive number of 1,000 MV eruptions. Assuming the gas exclusively consists of methane and all gas is released within a day, a single eruption will increase the input of methane in box 5 at a rate of 4.48×10^4 mol km⁻³ d⁻¹ (0.72 Mg km⁻³ d⁻¹).

For the eruption of 1,000 MVs (model run *B1c*) the model predicts that it takes about 40 years to return to the previous steady-state methane concentrations (Figure 7). In this run the initial methane input in box 5 results in a concentration increase which is about sixfold higher than the concentration derived from our previous steady-state calculations (*Model A1a*). However, the large methane input is effectively buffered by methane consumption and pronounced water column stratification leading to a constantly decreasing influence on the methane concentrations in the overlying boxes 4 to 1. The model shows that the influence of such a major event on the sea/air methane flux is negligible and just results in an increase of less than 0.01 %. Hence, it is obvious that the smaller events represented by model run *B1a* and *B1b* (1 and 65 MV eruptions, respectively) do not show any significant effect on methane distributions and emissions into the atmosphere (Figure 7).

The modern MV activity in the Black Sea is difficult to assess but no indications for eruptive phases have been reported in the recent literature. Wallmann et al. (2006) estimated the total flux of methane from known Black Sea MVs ($n = 65$) to be 1.2×10^8 mol CH₄ yr⁻¹ (1.9 Gg CH₄ yr⁻¹) assuming that they are all in a quiescent activity stage today. His calculation is only based on an advective transport of dissolved methane (dewatering) and shows that this

contribution to the Black Sea methane budget is negligible and accounts only for less than 0.003 % of the total Black Sea methane input (based on a total input of 67.4 Tg CH₄ yr⁻¹; Table 4). This minor impact originates from the so called “benthic filter” that partly controls the transport of dissolved methane from the sediment into the bottom water. This filter efficiently consumes dissolved methane by a consortia of archaea and sulfate-reducing bacteria in shallow sediment depths (Boetius et al., 2000). During quiescent periods most of the methane is delivered by slowly ascending fluids and converted into carbon dioxide and organic matter by the microbial consortia. During eruptive stages elevated flow rates, the release of solid gas hydrates into the water column, and the ebullition of free gas can complicate the system, and large amounts of methane can bypass the “benthic filter”. In spatial limited regions these violent eruptions may create a focused two-phase plume consisting of methane gas bubbles and methane saturated ambient seawater (Leifer and Patro, 2002). These conditions reduce the bubble dissolution rates and increase the bubble live-time as well as its vertical velocity in the water column. In addition, the dissolution of methane gas bubbles has been shown to be slowed down during the travel through the gas hydrate stability zone (i.e. at depths > 700 m), which has been interpreted to be caused by a hydrate layer formed at the gas/water interface (Rehder et al., 2002, Heeschen et al., 2003). Together with a bubble induced upwelling, violent eruptions may thus have the capacity to transfer methane from deep waters into shallow water depths. However, our box model is not able to take these rapid localized transport features into account since it assumes well-mixed boxes.

We know from recent hydroacoustic investigations in the abyssal Black Sea that free gas transport via gas bubbles also occurs at mud volcanoes (CRIMEA cruise report, 2003 and 2004). These characteristics are known as gas bubble flares and can be traced from the seafloor up to 1,300 m into the overlying water column (Greinert et al., 2006), demonstrating the enhanced lifetime of methane bubbles during travel through the water column within the hydrate stability field (Rehder et al., 2002). First quantifications from the Håkon Mosby MV (HMMV) suggest that these methane gas emissions are on the order of 10⁷ mol yr⁻¹ (0.16 Gg yr⁻¹; Sauter et al., 2006). But even if we assume that the 65 known Black Sea MVs emit the same amount of free gas (together 6.5 × 10⁸ mol CH₄ yr⁻¹, i.e. 10.4 Gg CH₄ yr⁻¹), the total methane discharge via fluid flow and gas bubbles (7.7 × 10⁸ mol CH₄ yr⁻¹; i.e. 12.3 Gg CH₄ yr⁻¹) from MVs is still less than 0.02 % of the total Black Sea methane input. A fictive number of 1,000 Black Sea MVs gives a contribution of less than 6 %.

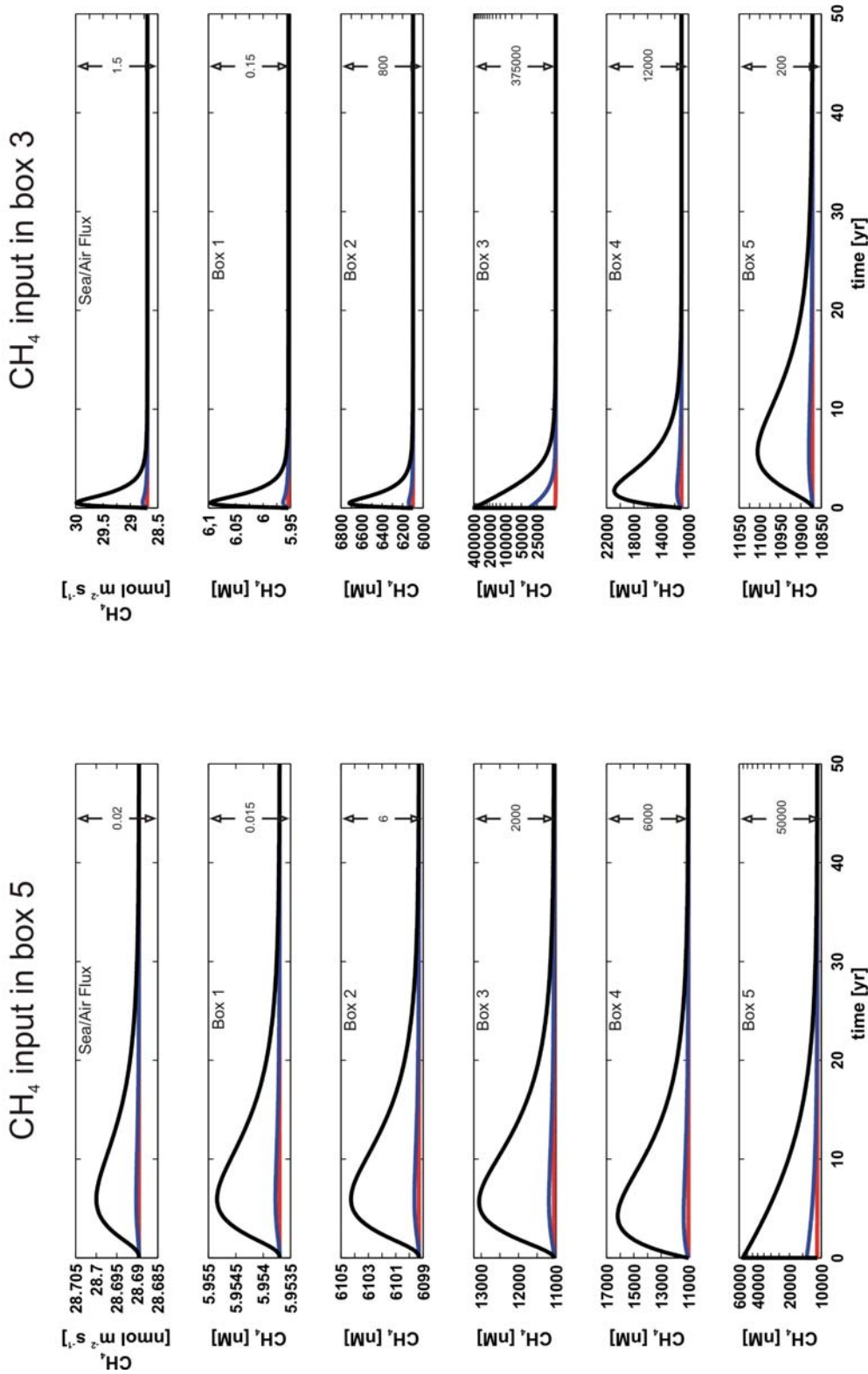


Figure 7: CH₄ distribution over time after an initial input of CH₄. Red line equals: 1 MV, blue line: 65 MV, and black line: 1,000 MV eruptions. Upper diagrams are the sea/air flux of CH₄, the diagrams below the CH₄ concentrations in the individual boxes. Note the different flux and concentration scales (log scale in event boxes 5 and 3, the range is always given on the right site of each diagram).

B2) Methane input in box 3

Our second case study focussed on the inspection of a methane release in intermediate water depths and its influence on the water column methane distribution and the subsequent atmospheric emission. Three different model runs were conducted based on the same gas inputs (pure methane) as used in our previous models for MV eruptions within box 5 (*Model B1*; Figure 7). All runs were initiated with steady-state methane concentrations calculated in *Model A1a* and assume that the methane release takes place within one day (e.g. catastrophic submarine landslide). The methane input used in model run *B2a* equals the amount of methane released during one MV eruption. Such an event will increase the input of methane in box 3 to $3.89 \times 10^5 \text{ mol km}^{-3} \text{ d}^{-1}$ ($6.2 \text{ Mg km}^{-3} \text{ d}^{-1}$).

After the maximum injection of gas (equal to 1,000 MV eruptions; model run *B2c*) the initial methane concentration in box 3 increases up to 400,000nM (about 40 times higher than the steady-state concentration calculated in *Model A1a*). The relaxation time needed to bring the system back to previous steady-state concentrations is about 35 years. Also, this study shows that methane is efficiently consumed microbially and that the exchange between individual boxes is strongly hampered, resulting in a limited transport of methane towards the sea surface. Compared to the results obtained in our previous model runs for MV eruptions in deep waters (box 5; *Model B1*), the gas input in intermediate water depths lead to an elevated upward flux of methane into boxes 3 to 1. However, the maximum gas injection in model run *B2c* increases the surface water methane concentration to 6.1 nM which is only 2.5 % larger than the steady-state surface water concentration calculated in *Model A1a*. These slightly elevated methane concentrations in box 1 increase the surface water methane emission by 4.5 %. Lower gas inputs (represented by model run *B2a* and *B2b*) do not increase the methane concentrations and surface water emissions significantly (Figure 6).

Model sensitivity studies for maximum methane inputs

To simulate the effect of low and homogenous methane oxidation rates in the anoxic Black Sea waters, we developed additional models which consider the lower oxidation rates also used in Reeburgh's methane budget (Reeburgh et al., 1991; see discussion in *Model A1*). We used the maximum methane input rates of *Model B1c* and *B2c* and calculated the water column methane distribution as well as the methane emission into the atmosphere. The methane injection into box 5 results in surface water methane concentrations of 6 nM, which is only ~1 % larger than the steady-state concentration calculated in *Model A1a*. The resulting atmospheric methane flux is only 2.4 % larger if compared to *Model A1a*. This indicates that

using Reeburgh's consumption rates does not significantly affect our predictions of a massive short-time input of methane into box 5.

In the case of a maximum input of methane into box 3, our model predicts a surface water methane concentration of 6.8 nM (14 % higher than steady-state results in *Model A1a*). This leads to surface water methane emissions, which are 28 % larger than the steady-state emission of *Model A1a*. Thus, the use of lower oxidation rates in the anoxic water column increases the methane flux at the sea surface by 23 % compared to the predictions based on higher and inhomogeneous methane consumption rates (*Model B2c*).

To test if the concentrations of SO_4^{2-} in the anoxic Black Sea waters is high enough to compensate for the high CH_4 input of the MV eruption scenario (i.e. 12×10^{13} mol of CH_4 for 1,000 MV eruptions), we calculated the total amount of sulfate below 100 m water depth. The balance shows that the total sulfate amount of about 9×10^{18} mol can easily compensate for the maximum methane injections. The inflow from the Bosphorus (0.95×10^{13} mol yr^{-1} SO_4^{2-}) is already of the same order of magnitude.

Conclusion and outlook

Previous studies on the Black Sea methane budget are based on an average AOM rate of $0.6 \mu\text{M yr}^{-1}$ in water depth below 100 m resulting in a basin-wide input of 3.6 to 5.65 Tg $\text{CH}_4 \text{ yr}^{-1}$ (Kessler et al., 2006). Our model suggests that the use of inhomogeneous AOM rates published by Reeburgh et al. (1991) significantly alters this number. In contrast to Kessler et al. (2006) our modelling studies indicate a total input of 67.4 Tg $\text{CH}_4 \text{ yr}^{-1}$. This considerable input of methane is effectively buffered by intense methane consumption and reduced vertical transport so that only a small fraction is emitted into the atmosphere ($0.3 \text{ Tg CH}_4 \text{ yr}^{-1}$). For both scenarios (homogeneous and inhomogeneous AOM rates), the observed methane concentration and stable isotopic pattern can be adequately reproduced by the model. The differences between our own methane budget and the budget published by Kessler et al. (2006) show that further studies are needed to better constrain methane oxidation rates and methane sources in the anoxic Black Sea waters.

The source of water column methane remains to our opinion still an unresolved problem in the Black Sea methane cycle. Methane emissions via quiescent dewatering and release of gas bubbles at Black Sea MVs can only account for less than 0.1 % of the total input. Our model indicates that the dissociation of gas hydrates in water depths close to the phase boundary may represent a major methane source, but until now the distribution of possible locations

are unknown. A third controversially discussed source is methanogenesis in the euxinic waters. Even if some authors support the hypothesis that in sulphate-rich environments methanogenesis is outcompeted by sulphate-reducing bacteria (Whiticar et al. 1999; Kessler et al., 2006), investigations of intertidal and marine sediments show that a non-competitive substrates such as methylamine stimulates methanogenesis in the presence of high concentrations of sulphate (Winfrey and Ward, 1983; Mitterer et al., 2001). Recent *in vitro* studies of AOM-performing Black Sea microbial mats could prove the coexistence of methanogenesis and sulphate-reduction (Seifert et al., 2006). The incubation of these mats with non-competitive substrates (methanol and trimethylamine) increases the methane production rate significantly. An additional link to water column methanogenesis is maybe given by the $\delta^{13}\text{C}_{\text{CH}_4}$ depth distribution. Our model could show that this isotopic distribution can be explained by different methane source signatures. But the stable isotope trend in the Black Sea water column is very similar to sediment depth profiles of the methane carbon isotope composition in marine environments (Whiticar et al., 1999). In these environments the shift towards heavier $\delta^{13}\text{C}_{\text{CH}_4}$ values with increasing depth is explained by substrate pool depletion and/or shift to alternative substrates for methanogenesis. Further work is necessary to prove if methanogenesis is present in the Black Sea water column, what the substrate is, and what its part in the Black Sea methane budget is.

Furthermore, our model predicts that massive short-term injections on the order of 1 to 1,000 MV eruptions will be effectively buffered in the Black Sea water column. Even if the gas is liberated in intermediate water depths methane transport to the surface and thus emission across the sea/air interface is strongly hampered by microbial methane consumption together with the hydrographic structure of the Black Sea. However, the model used in this work is based on the assumption that sufficient oxidation capacity is present in the anoxic water column to counter the massive input of methane. Future models have to consider sulphate and oxygen concentrations in the Black Sea water column and its influence on the kinetic constant for methane oxidation. The strongly reduced ventilation in the Black Sea water column (especially in deep stagnant waters) might result in local limitations of electron acceptors, so that large methane inputs may overburden the oxidation capacity. The scenario may result in reduced microbial consumption rates and elevated methane emissions into the atmosphere.

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Summary and Conclusion

High-intensity gas seeps are located on the shelf, shelf edge and upper slope of the Dnepr paleo-delta (NW Black Sea). These seeps, as well as deep water mud volcanoes in the Sorokin Trough (NE Black Sea), were investigated in the summers of 2003 and 2004 in order to understand their role in the Black Sea methane cycle and in the emission of methane at the sea surface.

Measurements of dissolved methane and its stable isotopic signature ($\delta^{13}\text{C}_{\text{CH}_4}$) show that only the seeps at 90-m depth in the Paleo Dnepr Area significantly influence the surrounding water column. Water column methane concentrations at this shallow seep site are up to two orders of magnitude higher than those measured in regions away from seep-influence. The findings of this study also suggest that only shallow seeps on the shelf (<100 m) significantly affect the surface water methane concentration, and that the sea-air methane flux above these sites is 3 times higher than that calculated for the surrounding shelf. However, methane liberated from these shallow sites is efficiently oxidized in the oxygenated water column by methanotrophic bacteria, with methane oxidation rates approximately 30 times higher than those found in the vicinity of active seeps. Further evidence for this intense consumption of methane in plume regions is given by the stable carbon isotopic distribution ($\delta^{13}\text{C}_{\text{CH}_4}$), in which the $\delta^{13}\text{C}_{\text{CH}_4}$ ratios constantly increase from the base to the top of the water column. These elevated methane oxidation rates within the plume demonstrate that this “microbial barrier” strongly decreases the upward transport of dissolved methane to the sea surface and therefore reduces the emission of this greenhouse gas at the sea surface.

Significant inter-annual differences in the methane inventory across the shelf-slope section were observed. Year-to-year variations in the methane distributions imply a greater input of methane in deep water in 2003 and a temporary modification of the methane distribution in the water column. High methane concentrations observed within the sub-oxic layer (and possibly the lower oxycline) indicate an apparent “overloading” of the steady-state system of anaerobic methane oxidation within the anoxic zone. This result indicates that methane could rise higher in the water column as a result of increased input of methane at depth. Such an increase could occur when gas hydrates dissolve in response to changes in water column temperature or sea level. Although it is difficult to predict if methane from a sudden release in deep water would reach the atmosphere, observations from the shallower sites indicate that the subsequent barriers of the oxycline and the oxic layer (microbial methane consumption and density stratification) would limit any escape to the atmosphere.

In contrast to the seep sites located in the Dnepr paleo-delta in shallow water depths, methane liberated from mud volcanoes (MVs) in the deep waters of the Sorokin Trough (about 2,000 m) does not considerably affect the methane distribution of the surrounding water column, which may be due to the very high background concentrations. Methane oxidation rates above the Vodyanitskiy MV, which was actively emitting gas bubbles during the two year field study, are similar to those observed at reference sites, although a higher abundance of archaeal cells (ANME-1 and ANME-2) have been discovered in the plume waters. It is suggested that these microbes are attached to the gas bubbles and injected from the seep-related sediment into the water column. These microbes, together with sulfate-reducing bacteria, are part of a consortium that is known to mediate the anaerobic oxidation of methane in sediments. The oxidation capacity of these microbes together with the pronounced density stratification of the Black Sea water column hampers the upward-transport of dissolved methane towards the sea surface and its liberation into the atmosphere.

The assumption that the different redox regimes, together with the hydrographic structure of the Black Sea, provide an effective mechanism that limits the transfer of methane from the deep reservoir into the atmosphere is supported by the modelling results presented in this thesis. The non-steady-state box model applied to a theoretical catastrophic methane input (through deep water MV eruptions or submarine landslides in intermediate water depths) into the Black Sea waters show that such an event would have a negligible effect on the Black Sea methane emissions at the sea surface. The model predicts that upward transport of these inputs will be effectively blocked by intense microbial methane consumption and low vertical mixing. As a result, only minimally elevated emissions of methane at the sea/air interface would be expected. Model results and the existence of a subsurface methane maxima having negative $\delta^{13}\text{C}_{\text{CH}_4}$ anomalies indicate that the methane flux over wide areas of the Black Sea appears to be mainly controlled by upper water column *in situ* production rather than diffusive flux from the deep anoxic methane pool.

Finally a steady state box model was developed in this work to quantify the depth-dependent input of methane to the Black Sea water column. The results indicate a total input of methane of $67.4 \text{ Tg CH}_4 \text{ yr}^{-1}$ and an average methane residence time ranging between 0.3 - 6.4 yr. The model predicts that the input of methane is highest in water depths between 100 and 600 m (82 % of the total input), which indicates that the dissociation of methane gas hydrates in water depths near their phase boundary may represent a major source in the water column methane budget. Modelling of the stable isotopic distribution of methane ($\delta^{13}\text{C}_{\text{CH}_4}$) implies an increasing influence of a thermogenic methane source with increasing water depth.

Appendix



Methanotrophic microbial communities associated with bubble plumes above gas seeps in the Black Sea

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Abstract

Bubbles evolving from active gas seeps can be traced by hydroacoustic imaging up to 1,000 m high in the Black Sea water column. Although methane concentrations are not distinguishable between the water column above the deep seep and reference sites, atmospheric noble gas measurements clearly show the constant input of gases (mainly methane) via seepage into the Black Sea. Archaea (ANME-1, ANME-2) and methanotrophic bacteria detected with specific 16S rRNA-targeted oligonucleotide probes are related to active gas seeps in the oxic and anoxic water column. It is suggested that methane seeps have a much greater influence on the Black Sea methane budget than previously acknowledged and that ANME-1 and ANME-2 are injected via gas bubbles from the sediment into the anoxic water column mediating methane oxidation. Our results show further that only minor amounts of methane evolving from Black Sea gas seeps reach the atmosphere due to the very effective microbial barrier. Hence, only major thermodynamically and/or tectonically triggered gas hydrate dissociation has the potential to induce rapid climate changes as suggested by the “clathrate gun hypothesis”.

Keywords: Black Sea, methane, methanotrophic organisms, ANME-1, ANME-2, Ne and He depletion, clathrate gun hypothesis

Introduction

Methane, the second most important greenhouse gas after carbon dioxide, has been held responsible for dramatic climate changes in the past (Dickens, 2003; Kennett et al., 2000). Owing mainly to anthropogenic production, atmospheric methane concentrations have doubled from 850 ppb to approximately 1,750 ppb over the last 150 years (Rasmussen and Khalil, 1984). A significant proportion of the methane emitted to the atmosphere (30 % of the total (Cicerone and Oremland, 1988) is of natural origin; however, atmospheric methane concentrations would be much higher if the huge methane pool that is stored in ocean sediments were to be released to the atmosphere. In ocean sediments, the anaerobic oxidation of methane (AOM) and the formation of gas hydrates generally hinder its release from the seafloor, since methane is almost quantitatively converted to bicarbonate (Reeburgh, 1976;

Valentine and Reeburgh, 2000). Recent observations, however, show that an unknown number of gas seeps exist at the seafloor, through which methane is emitted, thus escaping microbial transformation as streams of gas bubbles and floating hydrates. The few recent investigations that have been conducted have found neither substantial methane oxidation nor a methanotrophic community existing in the gas plumes above seeps (Damm and Budeus, 2003).

The Black Sea is the largest anoxic water body on earth and serves as a model for both modern and ancient anoxic environments. Stable stratification of the water column suppresses substantial deep-water renewal, fostering anoxia below the chemocline, which is located at 90-170 m. Methane concentrations in the deep water (below 500m) are relatively constant at a very high value (11-12 μM , i.e., $\sim 5,000$ times the atmospheric equilibrium concentration), and gradually decrease to only 10 nM in surface waters (Reeburgh et al., 1991). In the north-western Black Sea, hundreds of active gas seeps, emitting mainly methane (Blinova et al., 2003), exist along the shelf and slope of the Crimean Peninsula at water depths of between 35 and 800 m (Ivanov et al., 1989). The EU project CRIMEA has the objective to investigate the methane seeps located on the northwestern Black Sea shelf and their role as a source for methane to the atmosphere. In this paper we describe the involvement of microorganisms in the transformation of methane in the water column from the sea floor to the upper water layer with a special focus on methane seeps.

Methods

In the present study, we explored the biogeochemical dynamics at a shallow seep site ($44^{\circ} 50' \text{N}$, $31^{\circ} 59' \text{E}$, 92 m water depth) and at a deep seep site ($44^{\circ} 17' \text{N}$, $35^{\circ} 02' \text{E}$, 1985 m), together with two reference sites ($44^{\circ} 51' \text{N}$, $32^{\circ} 01' \text{E}$, 76 m and $44^{\circ} 14' \text{N}$, $32^{\circ} 30' \text{E}$, 1658 m) that were unaffected by active gas emissions. Samples were taken from the R/V *Professor Vodyanitskiy* during the CRIMEA project cruise in May/June 2003. Sampling gears involved a CTD (Seabird SBE-9) connected with a rosette of 12 water bottles each able to sample 10 L of water at a specific water depth. Water samples were taken immediately when the rosette came onboard for noble gases, methane concentration and methane isotopic composition, and fluorescence in-situ hybridization (FISH).

Methane concentration and isotopic composition

For CH₄ analysis, a vacuum degassing method was used whereby 1,600 ml of water was injected into a pre-evacuated 2,200 ml glass bottle, which leads to almost quantitative degassing (Rehder et al., 1999). The gas phase was subsequently recompressed to atmospheric pressure and the CH₄ concentration of the extracted gas was determined by gas chromatography on a Shimadzu GC14A equipped with a flame ionization detector. Nitrogen was used as the carrier gas, and separation was performed using a 4 m 1/8' SS column packed with Porapak Q (50/80 mesh) at 50 °C. The methane carbon isotopic composition was determined for the shallow stations using a *Trace Gas* linked to an *Isoprime* mass spectrometer (*GV Instruments*).

CH₄ oxidation rates

Microbial methane oxidation was measured using triplicate 20 ml crimp-seal bottles that were filled with sampled water and sealed gas-tight. From each triplicate, one sample was treated with 50 µl formaldehyde (37 %) to function as a blank. Oxygen-free aliquots of 50 µl tritiated methane (³HCH₃) with a specific activity of 20 Ci mmol⁻¹ (Biotrend, Germany) were added to the bottles and incubated in the dark at ambient water temperatures for 4 days. After incubation, samples were killed with formaldehyde (37 %), left opened overnight, and stripped with nitrogen prior to counting, to remove all non-reacted tracer. The product of methane oxidation (³HHO) was measured on a Wallac 1209 Rackbeta (Pharmacia) using a liquid scintillation cocktail (Insta-gel Plus, Perkin Elmer). Blank values were always lower than 15-50 % of the sample. Values were only approved as real microbial turnover rates if the subtraction of twice the standard deviation of all the blanks of one batch from each sample value still resulted in positive counts.

Noble gas measurements

Water samples for noble gas analysis were collected immediately when the rosette arrived on deck and stored in gas-tight copper tubes for analysis after the expedition. Noble gas measurements were performed using a specialized vacuum extraction and purification line combined with mass spectrometric analysis (Beyerle et al., 2000).

Bacterial abundance and FISH

Bacterial abundance was determined by epifluorescence microscopy (Zeiss Axioscope 2, 1,000 magnification) of DAPI (4',6-diamidino-2-phenylindole)-stained cells. For fluorescence in-situ hybridization (FISH), 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. (2002) 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* (Boetius et al., 2000); and MG84/705 and MA450, describing methanotroph groups I and II (Eller et al., 2001), respectively. Probes were labeled with the indocarbocyanine fluorescent dye CY3 and fluorescein (MWG, Germany).

Results and discussion

At the newly discovered active Vodyanitskiy mud volcano in the Sorokin Trough, at 1985 m water depth, a very prominent and constant bubble plume was found. Although the bubbles could be followed vertically by the echosounder system for more than 1,000 m, methane concentrations measured in the water column in the vicinity of the plume were not significantly different from those measured in the water column at the reference sites (Figure 1). The reason for not be able to discriminate between the two sites is most likely the already very high background values. This confirms earlier results from deep Black Sea sites indicating that elevated methane concentrations above methane seeps occur only very close to the sediments (Bohrmann et al., 2003).

To determine the methane partitioning between the bubbles and the surrounding water, we measured the distribution of noble gases in the water column. Except for helium, which can be supersaturated due to terrigenous input, the concentrations of dissolved atmospheric noble gases in lake and ocean water correspond closely to the equilibrium concentrations defined by the surface water temperature and salinity that prevailed during gas exchange with the atmosphere (Craig and Weiss, 1971; Kipfer et al., 2002). Noble gases are chemically inert, and therefore any observed deviations from the initial equilibrium concentrations are

interpretable in terms of purely physical processes. The presence of gas bubbles in the water column stimulates gas exchange between the ascending gas phase and the surrounding water by gas stripping and dissolution (see Figure 2, right-hand panel) and therefore affects the local noble gas concentrations (Clark et al., 2003; Leifer and Patro, 2002; Wüest et al., 1992).

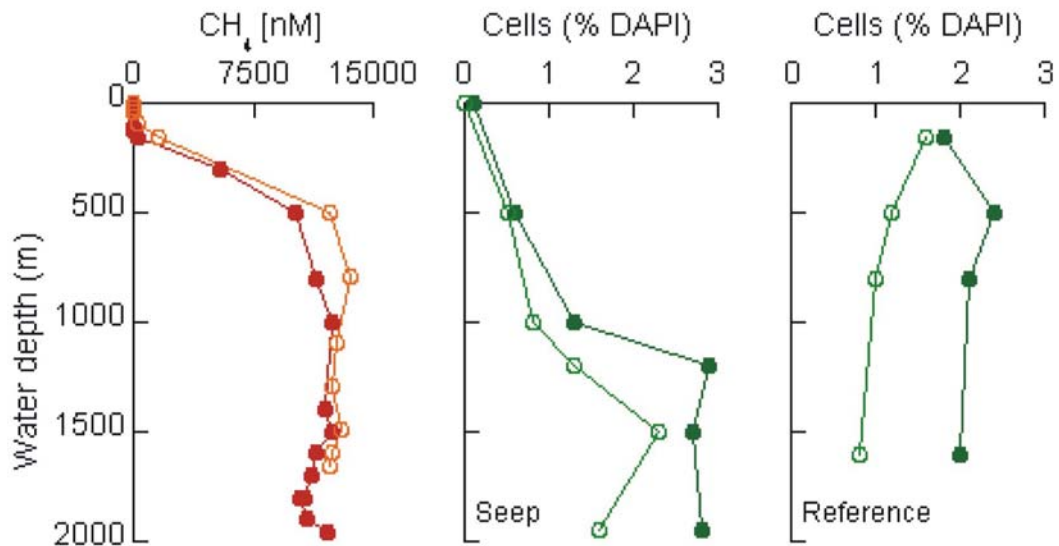


Figure 1: Methane concentrations in the water column above the deep seep site (solid red circles) and the equivalent reference site (open orange circles). The methane concentrations at these two sites were very close to each other beside a 1300 m high bubble plume influencing the water column above the seep site. The panel in the middle shows the abundance (as percent of total DAPI counts) of ANME-1 (solid dark green circles) and ANME-2 cells (open light green circles), so far known to mediate anaerobic oxidation of methane in sediments, in the water column above the seep site. Whereas both communities in the vicinity of a bubble plume are present up to 3 % of all DAPI detected cells in the water column below 1000 m with a strong decrease towards the chemocline, only 1 to 2 % of ANME-1 and ANME-2 cells are found rather homogeneously distributed over the whole anoxic water column at the reference site (right hand side panel).

Recent publications have shown high concentrations of ¹³C-depleted biphytanes and glyceryl dialkyl glyceryl tetraethers in the anoxic Black Sea water column below 700 m, which clearly indicate the involvement of archaea in the anaerobic oxidation of methane (Schouten et al., 2001; Wakeham et al., 2003). However, the actual organisms mediating the anaerobic oxidation of methane in the Black Sea water column are still unknown.

To identify the community responsible for the oxidation of methane in the gas plume we used 16S rRNA-targeted oligonucleotide probes. Using Arch 915, a general archaeal probe, a 25 % higher total archaeal cell count was found in the anoxic water column in the vicinity of the bubble plume than at the equivalent reference site. An archaeal community closely related to *Methanosarcina*, called ANME-2, together with sulfate-reducing bacteria, was found to be responsible for the anaerobic oxidation of methane in sediments from Hydrate Ridge off the coast of Oregon (Boetius et al., 2000). ANME-1 and ANME-2 cells have been detected in the sediments at seep sites in the Santa Barbara Basin and the Eel River Basin off the coast of California (Hinrichs et al., 1999; Orphan et al., 2001), whereas in a Black Sea reef structure, mainly ANME-1 was found (Michaelis et al., 2002). Methane oxidation took place at both sites with similar methane oxidation rates between 0.03 to 3.1 nM d⁻¹ and we therefore tried to identify the community involved in anaerobic methane oxidation. Using 16S rRNA-targeted oligonucleotide probes specific to both groups, we were able to detect ANME-1 and ANME-2 cells in the water column, which had previously been described only in sediments. Cell counts of filters from the water column above the methane seep site revealed ANME-1 and ANME-2 cells at concentrations of up to 3 % of all DAPI stained cells (Figure 1). At the deep reference site, cell counts detected ANME-1 and ANME-2 cells at lower concentrations of only 1 to 2 %. Additionally, the distribution of the archaeal cells over the water column was different between the reference and the seep site. Whereas at the reference station the cells are rather homogeneously distributed over the whole anoxic water column, at the seep site the archaeal abundance is higher exactly at the depth horizon where the methane plume could be observed with acoustical means, i.e. from the sea floor to approximately 1,000 m water depth (Figure 1). In general, bacteria are attached to interfaces, i.e., to solid/liquid or water/gas phase boundaries. Of these, the gas/water interface is especially important for bacteria, since enrichment of hydrophobic molecules like proteins, lipids, and carbohydrates also occurs there (Kjelleberg and Stenstrom, 1980; Norkrans and Sorensson, 1977). Our data imply that in the Black Sea water column, ANME-1 and ANME-2 cells – and presumably also other archaea attached to the gas bubbles – are released from the seep-related sediment and injected into the water column. Such interpretation is supported by the fact that the abundance of total archaeal cells, and specifically ANME-1 and ANME-2 cells, found in the water column in the vicinity of the bubble plume is higher than that found at the reference site and that the higher abundance exactly follows the height of the methane plume.

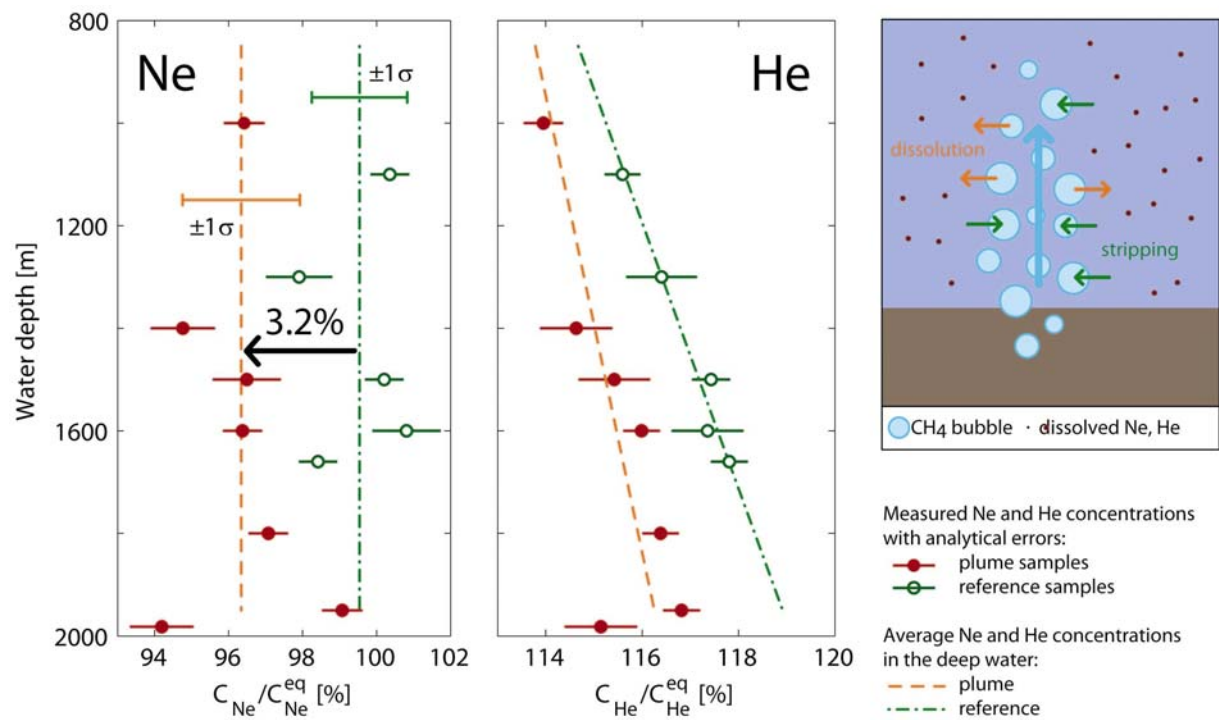


Figure 2: Neon and helium (^4He) concentrations (normalized to atmospheric equilibrium concentrations) in the deep water of the Black Sea (left-hand and centre panels). The mean of the Ne concentrations at the reference site is 3.2 % higher than that at the plume site. The difference in the noble gas concentration profiles is significant at the 1σ level (unweighted standard deviations). A similar effect was observed for He: samples from the plume are depleted relative to the reference samples ($\sim 2 \%$ on average over the given depth interval). Note that the deep water of the Black Sea contains significant amounts of terrigenic He (Top et al., 1990). The fact that He depletion is observable suggests that the deep seeps do not emit substantial amounts of terrigenic He. The right-hand panel illustrates the conceptual model of the stripping of noble gases from the surrounding water column by the ascending bubbles and concurrent bubble dissolution, leading to changes in the concentrations of the dissolved gases and in the bubble composition (Clark et al., 2003). The observed noble gas depletion reflects the bubble volume injected per volume of water. If the hydrodynamics of the seep and the physical processes controlling the secondary gas exchange were known in detail, the observed noble gas depletion in the water could be interpreted in terms of the volume of methane bubbles released by the seep. Using a simple model that assumes that solubility equilibrium is attained between the bubbles and the surrounding water (Brennwald et al., 2005) suggests that the injection of methane bubbles by the seep, if completely dissolved, corresponds approximately to the CH_4 concentration in the deep water of the Black Sea.

Although the seeps appear to currently add microorganisms to the water column, it remains an open question whether the deep bubble plumes had in the past, and still have today, the capacity to inoculate the entire anoxic water body of the Black Sea with methane-oxidizing archaea. The ^{13}C -depleted biomarkers mentioned above as having been detected in the anoxic water column of the central Black Sea (Schouten et al., 2001; Wakeham et al., 2003), an area where no seeps have yet been found, suggest that there are other methane-transforming archaea in addition to ANME-1 and ANME-2 awaiting discovery.

In contrast to the deep sites, where the additional methane influx from seeps is difficult to detect, the methane excess at the shallow seep site was very pronounced, with concentrations that were on average 10 times higher than at the reference site (Figure 3a). This excess is comparable to the situation at Coal Oil Point seep (Clark et al., 2000). Additionally, methane oxidation rates at the seep site (0.02 to 1.6 nM d^{-1}) were approximately 30 times higher than those at the reference site (0.001 to 0.05 nM d^{-1}), indicating that a methanotrophic community lives in the oxic environment affected by the bubble plume. Further evidence for enhanced microbial activity comes from the carbon isotope composition of the dissolved methane. Carbon isotope values at the shallow seep site (-66.1 ‰ VPDB) are characteristically depleted with respect to those at the reference site (-58 ‰ VPDB, Figure 3b), clearly indicating that gas seeps inject methane into the overlying water. Additionally, the decrease in methane concentration due to oxidation is associated with a carbon isotope effect that results in the remaining methane being enriched with ^{13}C (Barker and Fritz, 1981) which can be clearly seen in Figure 3b (-66.1 to -48.7 ‰ VPDB from above the sediment to the uppermost 5 m water depth). Accordingly, using oligonucleotide probes for methanotrophic bacteria types I and II (Eller et al., 2001), up to 4 % and 2.2 %, respectively, of all DAPI stained cells could be identified as methanotrophic bacteria (Figure 3c, d). Methanotrophic bacteria of type II were detected only in the deepest sample at the seep site. Type I methanotrophs were rather uniformly distributed with numbers < 0.5 % at both shallow sites with the exception of one sample. Since no higher methanotrophic bacterial numbers were detected in the water column in the vicinity of the bubble plume, higher oxidation rates must be a result of higher metabolic rates in the community above the seep site.

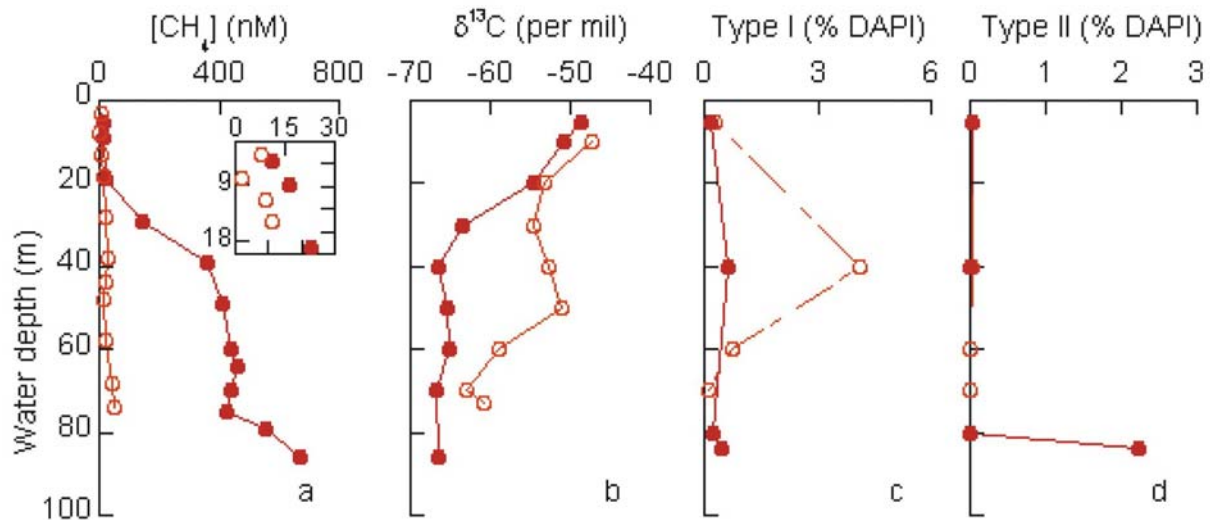


Figure 3: Methane concentrations in the water column above the shallow seep (solid circles) and reference site (open circles, a). Methane concentrations are on average 10 times higher above the seep than above the reference site. In the surface waters, methane concentrations at the seep site are on average twice as high as those at the reference site (inset). Methane carbon isotope values at the shallow seep (solid circles) and reference site (open circles, b). The lighter carbon isotopes at the seep site indicate that methane from the seep is entering the water column. The shift towards heavier carbon isotope values that is apparent with decreasing depth in the water column shows ongoing methane oxidation. Abundance of methanotrophic bacteria of type I (c) and type II (d) in the water column above the shallow seep (solid circles) and reference site (open circles) in percent of all DAPI stained cells. Almost only type I methanotrophs were detected in the water columns above the seep and reference sites in addition to one higher detection of type II methanotrophs in the deepest water sample from the seep site presumably related to sediment resuspension.

Reeburgh et al. (1991) have estimated that 2.9×10^{11} mol CH_4 are formed in the sediments of the shelf and slope of the Black Sea and that $\sim 99\%$ is oxidized anaerobically in the euxinic water column. In contrast, pore-water methane in sediment cores from the NW shelf and slope is anaerobically oxidized by sulfate and never reaches the water column (Jørgensen et al., 2001). Furthermore, flux chamber experiments showed only a negligible contribution of methane from the NW shelf sediments (Friedl et al., 1998; Friedrich et al., 2002). The methane contribution from the sediments would therefore seem to be rather small, implying that methane emanating from seeps located on the abyssal plain, shelf and slope regions must be an important term in the methane budget of the Black Sea.

Twice as much methane was detected in the uppermost 20 m of the water column at the shallow seep site than at the reference site (Figure 3a, inset). Surface waters above shallow seeps are therefore methane-enriched, and emit this greenhouse gas directly into the atmosphere. An enrichment of methane in the water column above seep stations with a subsequent release to the atmosphere has also been described recently by Schmale et al. (2005). However, higher methane oxidation rates demonstrate that microbes associated with the bubble plume are catching up with the higher methane concentrations. Our findings therefore suggest that slow dissociation of gas hydrates due to warmer bottom waters will not result in dramatic climate changes. On the other hand, the huge amount of methane released from gas hydrates as the result of a sudden tectonic event as proposed by the “clathrate gun hypothesis” (Kennett et al., 2000) will certainly exceed the oxidation capabilities of the microbial community, thus leading to an increase in atmospheric methane concentrations.

Higher mean surface water methane concentrations at the deep seep site, and hence higher rates of methane emission to the atmosphere are not observed and are also not expected due to modeling results (McGinnis et al., 2005). Although caution has to be exercised in drawing any final conclusions, we conclude that as long as the mixing dynamics of the Black Sea are strongly reduced by the prominent chemocline, methane from deep plumes will not reach the atmosphere and will therefore not result in higher atmospheric methane concentrations.

Acknowledgements

The CRIMEA expedition was carried out as part of the European Union 5th Framework project CRIMEA (Contribution of high-intensity gas seeps in the Black Sea to methane emission in the atmosphere), funded by the European Union (contract no. EVK2-CT-2002-00162) and by the Swiss Federal Office for Education and Science (contract no. 02.0247). Further financial support came from Swiss National Science Foundation grants 2100-068130 to BW and CJS, 2160-067493 to CJS, and 200020-101725, 200020-105263, and TUMSS (methane cluster of ETH) to RK. We thank the officers and crew of the R/V *Professor Vodyanitsky* and the whole CRIMEA team for their support during the cruise. Francisco Vazquez is thanked for his support in the laboratory. We explicitly thank David M. Livingstone, colleagues, and two reviewers for their insightful comments that improved our manuscript.

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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

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 (Cicerone and Oremland, 1988; Rasmussen and Kahlil, 1984). Although methane concentration in the atmosphere is small compared to CO₂ (360 ppm), its impact as a greenhouse gas in the atmosphere is about 24 times higher (IPPC, 2001). 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) . 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 (Cicerone and Oremland, 1988) due to microbial aerobic and anaerobic oxidation processes in sediments and water column (Reeburgh, 1996; Reeburgh, 2003). Recent research has focussed 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 (Kvenvolden, 1988). Methane clathrates are now found at almost all continental margins with a suitable temperature-pressure field. (Kvenvolden et al., 1993) including the north-western part of the Black Sea (Bohrmann et al., 2003; Ivanov et al., 1998).

The Black Sea has a surface area of 423,000 km² and a maximal depth of 2212 m, and represents the world largest anoxic basin (Ross et al., 1974). After a freshwater period during the last glacial, the Black Sea turned into a brackish basin when the Bosphorus established a full connection to the Mediterranean about 7150 yrs ago (Gorur et al., 2001; Ryan et al., 1997). 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 ‰ (Murray, 1991). Anoxia developed 7,500 yrs ago due to the stable stratification of the Black Sea waters (Jones, 1991). The aerobic surface waters are separated from anoxic deep waters by a chemocline at 100-200 m water depth depending on the geographical location (Sorokin, 2002). 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 (Sorokin, 2002). The water column contains substantial but varying amounts of methane, which has been attributed to methanogenesis in the water

column (Ivanon et al., 2002) and sediments (Reeburgh et al., 1991), as well as to the release of methane from gas reservoirs such as methane hydrates (Ivanov et al., 1998). 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 (Reeburgh et al., 1991; Scranton, 1977). 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. (1991) 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 (Schouton et al., 2001; Wakeham et al., 2003). 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 (Reeburgh et al., 1991). 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 (Blinova et al., 2003; Ivanov et al., 1989). 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 behaviour 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.

Methods and materials

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 2,130 m water depth in the central basin, 7617 (43° 38,04'N, 30° 02,54'E) at 1,560 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 (Figure 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, Figure 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.

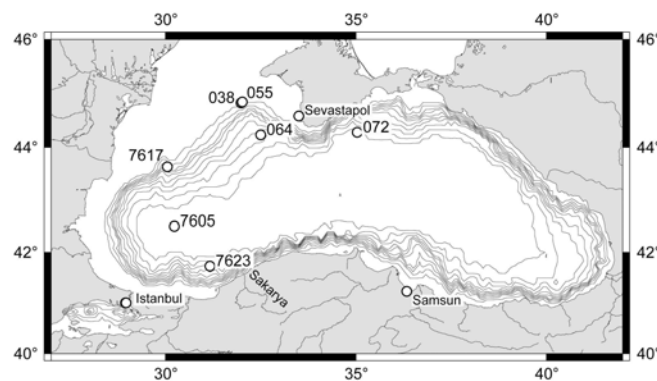


Figure 1: Map of the Black Sea showing sampling stations. Stations 7605 (central basin), 7617 (north-western slope), and 7623 (south-western slope) were sampled during the Meteor cruise in 2001 and Stations 038 (seep site on the north-western shelf), 055 (reference site on the north-western shelf), 064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

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 N₂ flowing at 25 mL min⁻¹, and the retention time for CH₄ 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 CH₄ analysis aboard *R/V Professor Vodyanitskiy*, a modification of the vacuum degassing method described by Lammers and Suess (1994) was used (Rehder et al., 1999). 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 CH₄ 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 Porapak Q (50/80 mesh) run isothermally at 50 °C.

The carbon isotopic composition of dissolved methane ($\delta^{13}\text{C}_{\text{CH}_4}$) was determined by a method described earlier (Sansone et al., 1997). Precision of the method was ± 1 ‰.

CH₄ 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 (³H-CH₃) 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.

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 fibre filters (GFF; nominal pore size 0.7 μm , 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 analysed by gas chromatography and gas chromatography–mass spectrometry for the quantification and identification of lipids, respectively.

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. (2002) (Pernthaler et al., 2002) 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* (Boetius et al., 2000); and MG84/705 and MA450, describing methanotroph groups I and II (Eller et al., 2001), respectively. Probes were labeled with the indocarbocyanine fluorescent dye CY3 and fluorescein (MWG, Germany).

Results

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. Sorokin (2002) and references therein).

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 (Figure 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.

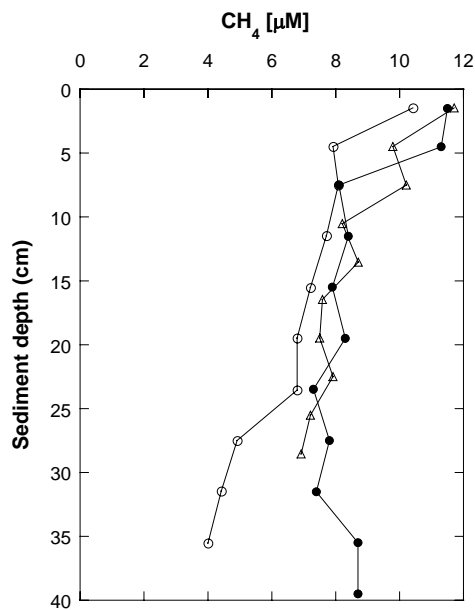


Figure 2: Methane concentrations in three sediment cores recovered from the NW slope (7617, open triangles), SW slope (7623, open circles), and the central basin (7605, full circles). The linear decrease of methane from top to bottom of the cores indicates a diffusive flux of methane from the water column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

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 (Figure 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 (Figure 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 (Figure 5).

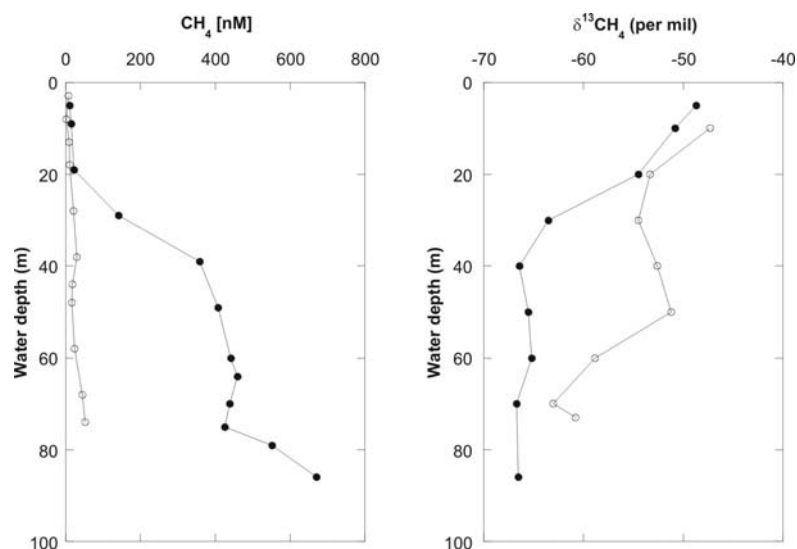


Figure 3: Water column methane concentrations (nM) at the seep 038 (full circles) and reference site 055 (open circles) located on the north-western shelf of the Black Sea. Carbon isotopic composition ($\delta^{13}\text{C}_{\text{CH}_4}$ vs. VPDB) of the dissolved methane from the water column above the seep site (038, full squares) and reference site (055, open squares).

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, Figure 2, 4, 5). At the central station (7605, Figure 4) $\delta^{13}\text{C}_{\text{CH}_4}$ values in the anoxic deep water varied around -54 ‰ and the shallowest sample taken at 30 m water depth had a $\delta^{13}\text{C}_{\text{CH}_4}$ value of -42 ‰. A striking change in isotope fractionation occurred just below the chemocline at 100 m water depth. Here, $\delta^{13}\text{C}_{\text{CH}_4}$ increased to -20 ‰. At the north-western station (7617, Figure 4) the samples from 300 to 165 m had $\delta^{13}\text{C}_{\text{CH}_4}$ values around -56 ‰ followed by a significant increase to -51 ‰. The top sample at 50 m water depth had $\delta^{13}\text{C}_{\text{CH}_4}$ values of -52 ‰. The stable carbon isotopic composition of methane at site 064 (Figure 5) varied between -49 and -51 ‰ VPDB. Above 800 m $\delta^{13}\text{C}_{\text{CH}_4}$ values decreased to a minimum of -58 ‰ VPDB at 500 m and subsequently increased again above 160 m to $\delta^{13}\text{C}_{\text{CH}_4}$ 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 (Figure 3) differed with a $\delta^{13}\text{C}_{\text{CH}_4}$ value of -67 ‰ VPDB at the bottom of the seep site (038) from the $\delta^{13}\text{C}_{\text{CH}_4}$ value of -58 ‰ VPDB at the bottom of the reference site (055). A constant increase in $\delta^{13}\text{C}_{\text{CH}_4}$ values from -67 ‰ VPDB to -49 ‰ VPDB from the bottom to the surface could be noted at the seep station (038).

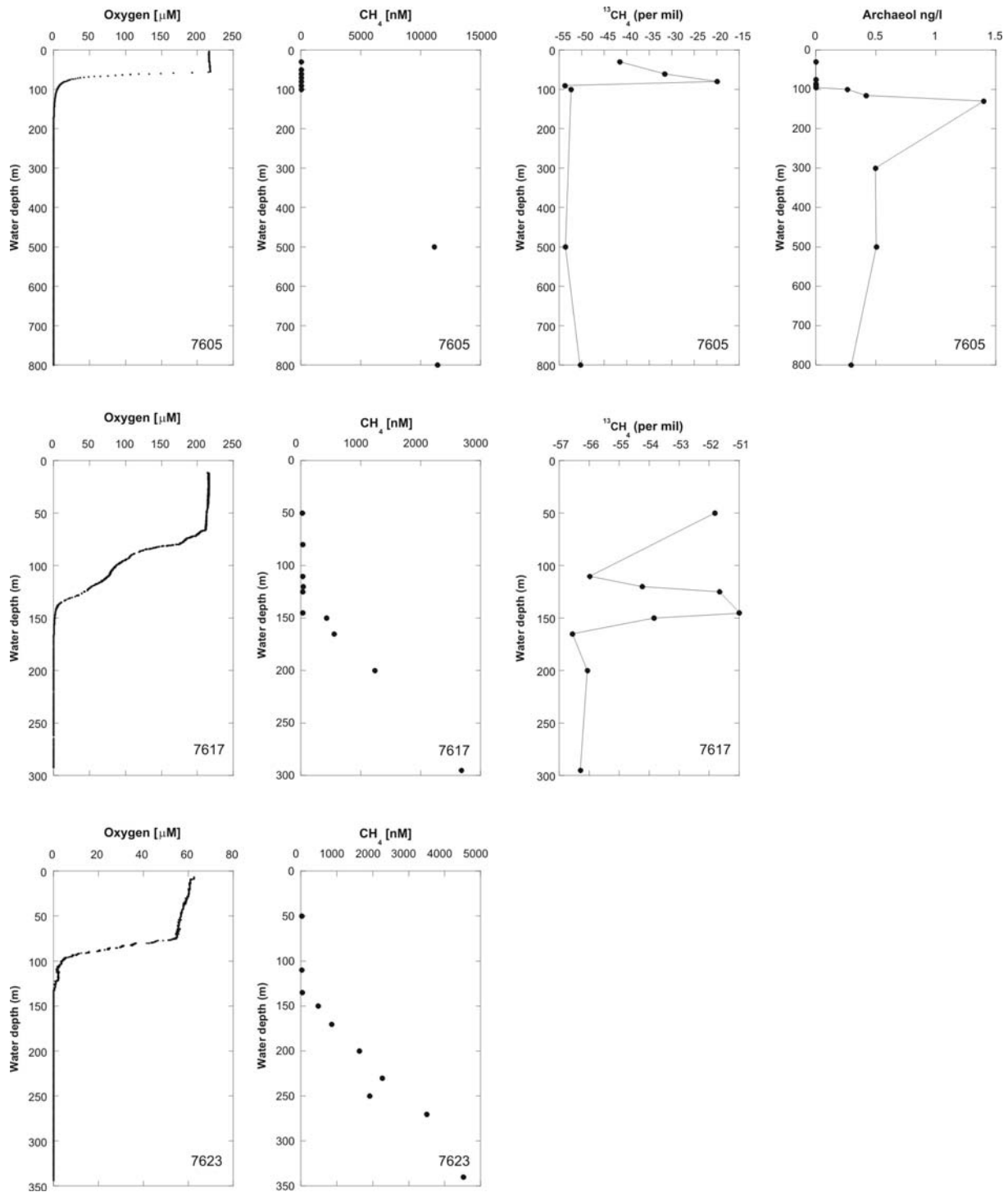


Figure 4: Oxygen (μM) and methane concentrations (nM) of stations 7605, 7617, and 7623, the carbon isotopic composition of methane ($\delta^{13}\text{C}_{\text{CH}_4}$ vs. VPDB) of stations 7605, 7617 and the depth distribution of Archaeol (ng/l) extracted from particulate material collected from the 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.

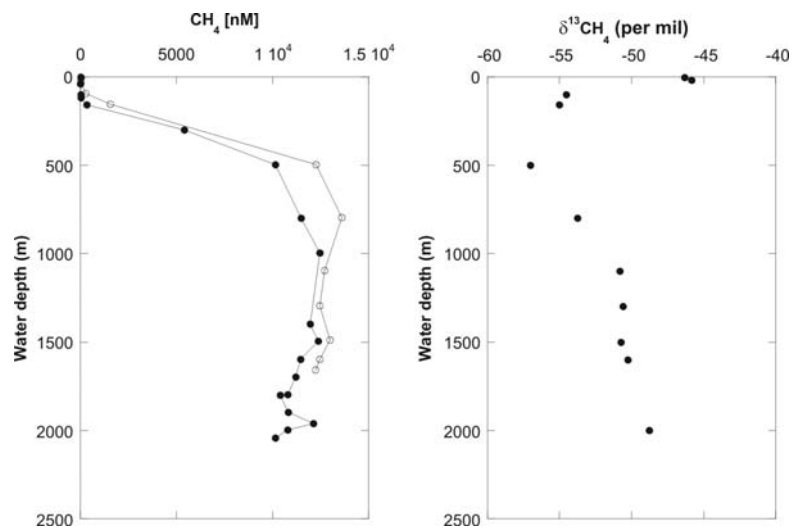


Figure 5: Depth profile of methane concentrations of reference site 064 (light circles) and a composite profile of seep sites 068 and 075 that were sampled very close to station 072 (dark circles). Carbon isotopic composition of the dissolved methane from the water column above the reference site 064. 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.

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 (Hinrichs et al., 1999; Koga et al., 1998), 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 (Figure 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 (Boetius et al., 2000). 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.

Methane oxidation rates

Methane oxidations rates using tritium labelled 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.

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 labelled 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, Durisch et al., 2005). 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.

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. (Gal'chenko et al., 1988; see discussion) might have their origin in the different methods (immunofluorescence versus FISH), or in natural changes of the microbial population.

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 1,500 and 2,000 m where a bubble plume could be observed with hydroacoustical means (echosounder) up to 1,200 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	+	+/-

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 (Bohrmann et al., 2002; Ivanov et al., 1998; Lein et al., 2005; Schmale et al., 2005).

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 (Figure 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 $\delta^{13}\text{C}_{\text{CH}_4}$ 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 (Figure 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 (Gal'chenko et al., 1988). 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 (Yamamoto et al., 1976), 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 (Amouroux et al., 2002; Schmale et al., 2005). The latter authors found an air-sea methane flux above a shallow seep area of $0.96\text{-}2.32 \text{ nmol m}^{-2} \text{ s}^{-1}$ that is 3 times higher than calculated for the surrounding shelf ($0.32\text{-}0.77 \text{ nmol m}^{-2} \text{ s}^{-1}$) and 5 times higher than assessed for open Black Sea waters (water depth $>200 \text{ m}$, $0.19\text{-}0.47 \text{ nmol m}^{-2} \text{ s}^{-1}$). Hence, we can conclude that the gas seeps of the upper slope and shelf, where methane emanates into 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.

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 (Reeburgh et al., 1991). Reeburgh et al. (1991) showed one methane profile from the central basin with low concentrations ($< 10 \text{ nM}$) in the oxic zone above 100 m, increasing concentrations from 100 to 550 m, and very stable concentrations around $11 \text{ }\mu\text{M}$ down to 2,200 m. Additionally, Reeburgh et al. (1991) showed methane concentration data from the deep anoxic waters measured by Scranton (1977) that were above $12 \text{ }\mu\text{M}$ 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 (1991) 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.

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 (Teodoru et al., 2005). Lander investigations by Friedl et al. (1998) and Friedrich et al. (2002) 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. (2001) could show on a sediment transect located on the north-western shelf and including sediments from water depths from 100 to 1,200 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 (2002). Sediment methane concentration profiles from our investigation (Figure 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. (1991) 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. (2001), Friedl et al. (1998), and Friedrich et al. (2002). One explanation for the very high methane concentrations of the sediment core from

Reeburgh et al. (1991) may be that sediments were recovered from a seep system, an assumption made earlier also by Jørgensen et al. (2001).

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 (Ivanov et al., 1998; Luth et al., 1999) 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. (2003) suggested that methane is formed in the order of 63×10^{10} mole per year during the process of organic matter degradation in the water column. Reeburgh et al. (1991) 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. (Konovalov et al. (2001), 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. (2003). 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 $\delta^{13}\text{C}$ 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 (Blinova et al. (2003), Ivanov et al. (1989), and CRIMEA Cruise Reports 2003, 2004). These seeps are so common that for instance only during the two cruises linked to the CRIMEA project more than 1,000 new seeps were discovered (CRIMEA Cruise Report 2004). Gas seepage is not only found on the shelf, but occurs also on the upper and lower slope (Bohrmann et al., 2003). Indications for methane fluxes from seeps at the upper to middle slope could be seen in the $\delta^{13}\text{C}_{\text{CH}_4}$ profile of station 064 (Figure 5). A decrease in $\delta^{13}\text{C}_{\text{CH}_4}$ 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 $\delta^{13}\text{C}_{\text{CH}_4}$ 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 (2002) 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 5,000 years BP. The $\delta^{13}\text{C}_{\text{CH}_4}$ 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 (Craig and Weiss., 1971; Kipfer et al., 2002). Noble gases are chemically inert, and therefore any observed deviations from the initial equilibrium concentrations can be used for modelling 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 (Wüest et al., 1992).

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 (Figure 6). This clearly proves 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.

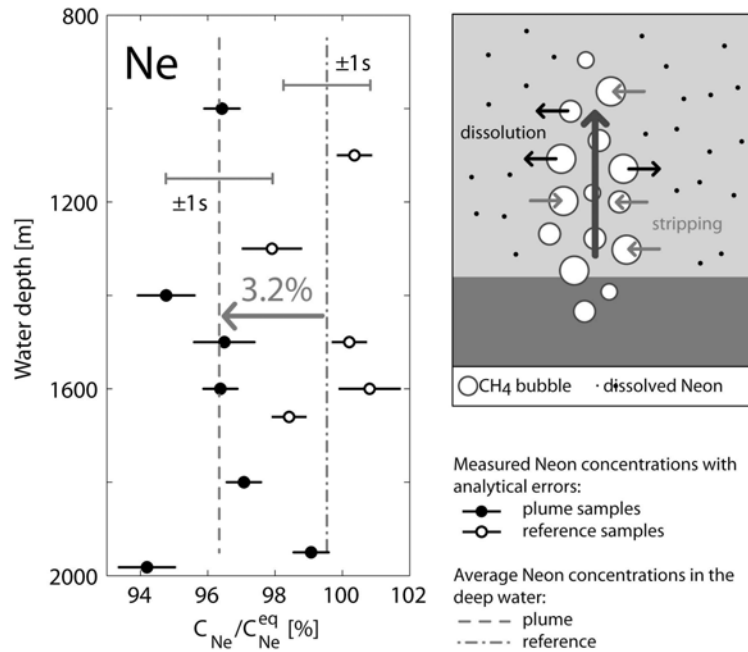


Figure 6: Atmospheric Ne concentrations (normalized to atmospheric equilibrium concentrations) in the deep water of the Black Sea (left hand panel). Water samples from the reference site (open circles) showed on average 3.2 % higher Ne concentrations than water samples of the flare from the seep site (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.

Methane sinks

The main sinks in the methane budget of the Black Sea are, as pointed out earlier by Reeburgh et al. (1991), 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 (Blumenberg et al., 2004; Michaelis et al., 2002; Lammers et al., 1994). 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. (2001) and Wakeham et al. (2003) 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 ($\delta^{13}\text{C} = -51\text{‰}$) 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 $\delta^{13}\text{C}$ value is, however, rather heavy compared to values measured in sedimentary lipids extracted from venting sites (around -100‰ , Boetius et al., 2000) and the possibility remains that these compounds originate from methanogenic archaea.

Recently, Vetriani and co-workers (2003) 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* (Boetius et al., 2000; Michaelis et al., 2002; Orphan et al., 2002). 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 (Figure 2) providing strong evidence for enhanced microbial activity, i.e., methane oxidation. From tracer experiments by Reeburgh et al. (1991) 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.

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 $\delta^{13}\text{C}_{\text{CH}_4}$ 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 their 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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Danksagung

Herrn Prof. Dr. Klaus Wallmann möchte ich für die Möglichkeit danken, dass ich diese Dissertation in der Arbeitsgruppe Marine Geosysteme am Leibnitz-Institut für Meereswissenschaften an der Universität Kiel durchführen konnte.

Mein besonders herzlicher Dank gilt Herrn Prof. Dr. Gregor Rehder, der mich in meinem täglichen Tun immer gefördert und motiviert hat und diese Arbeit durch Anregung und Diskussion sehr bereicherte. Ebenso herzlich möchte ich Stan Beaubien, Dr. Jens Greinert, Dr. Robin Keir und Dr. Matthias Haeckel danken, die mich, ob auf Forschungsfahrten oder bei der Auswertung und Diskussion gewonnener Daten, stets engagiert unterstützt haben.

Ein herzliches Dankeschön gilt allen Mitgliedern des Forschungsprojekts CRIMEA und der Besatzung des Forschungsschiffs *Professor Vodyanitskiy*, mit denen ich unvergessliche Ausfahrten auf dem Schwarzen Meer verbracht habe. Aber auch die folgenden wissenschaftlichen und privaten Treffen mit den hier gewonnen Freunden machen die Zeit meiner Promotion zu einem unvergesslichen Lebensabschnitt, an den ich mich immer wieder sehr gerne zurückerinnern werde.

Ebenso herzlich möchte ich den Mitarbeitern Martin Pieper und Michael Poser danken, die durch ihre technische Kompetenz und außerordentliche Hilfsbereitschaft einen wesentlichen Beitrag an der Gewinnung der in dieser Arbeit verwendeten Daten geleistet haben. Karen Stange danke ich für ihre engagierte und kompetente Arbeit am Massenspektrometer. Bettina Domeyer möchte ich vor allem dafür danken, dass sie mir stets bei den quälenden Formalitäten beiseite stand, die sich aus der Vorbereitung einer Forschungsfahrt ergeben.

Nicht vergessen möchte ich die Doktoranden Nikolaus Bigalke, Frank Schellig und Jens Schneider, die durch ihre Diskussionsbereitschaft (auch über wissenschaftliche Themen hinausgehend) einen nicht unbedeutenden Anteil an dieser Arbeit haben.

Mein größter Dank gilt meiner Freundin Katrin Lochner und meinem vier Monate alten Sohn Henry, der mir mehr Schlaf zubilligte als dies von befreundeten Eltern vorhergesagt wurde. Ganz herzlich möchte ich meiner Mutter und meiner Schwester für das mir entgegengebrachte Vertrauen danken, dass der von mir eingeschlagene Weg ein sinnvolles Ende findet.