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HYPOX deliverable 4.4

**Introduction and summary**

Several locations have been visited for investigating sediment signals as indicators for environmental changes in relation to oxygen depletion. Those were mainly the Black Sea, the Swiss lakes, and the Greece lagoons. Various parameters have been investigated including organic compounds (biomarkers), foraminiferal assemblages, metals (Fe, Mn, Mo). Whereas we have focused on sediments that might reach a 100-150 years back, we also included investigations on older sediments of the Cretaceous times (Dale et al. 2012).

In the Swiss lake Rotsee it was possible to follow eutrophication and subsequent oxygen depletion over the last 150 years. Several biomarkers were measured and showed the various changes controlled by sewage input over this time period. The higher sewage and nutrient input resulted in productivity increases, which predominantly led to a radiation of diatoms, primary producers and methanogens between about 1918 and 1921, but also affected all microorganism groups and macrophytes between about 1958 and 1972.

In the Swiss Lake Zurich a combination of trace metals (Fe/Mn) and long term oxygen monitoring data made it possible to reconstruct oxygen depletion in a sediment core from the deep basin. This opens new possibilities for other environments in the way that using sediment cores for reconstructing the history of oxygen content of lake bottom water with all consequences for instance for bottom dwelling organisms.

In the Greek lagoon Amvrakikos which recently was a location of several fish kills due to oxygen depletion in the water column two sediment cores were taken. They were investigated for benthic foraminifera (which are excellent marker organisms for oxygen rates in the bottom water) and also for biomarkers as indicators for bacterial communities. It could be shown that both groups react on the oxygen availability in the bottom water. Fish kills related to oxygen depletion or absence in certain years could be monitored in sediment cores using these parameters.

Our work in the Black Sea had to main regions, i.e. the Romanian shelf and the area around the Bosporus inflow.

In one paper we investigated the history of the Mediterranean inflow and its role in the ventilation of the Black Sea and discuss possible vertical fluctuations in the position of the oxic/anoxic interface in the water column during the last 7 ka. We carried out sedimentological, paleontological, and geochemical analyses and radiocarbon dating of interface sediment cores located along two depth transects ranging from -122 m to -307 m.

Another paper presents temporal, seasonal and inter-annual, variations of Dissolved Oxygen (DO) regime in the Romanian Black Sea coastal waters (Constanta area) based on data collected daily, during 1959 – 2010, from a fixed near-shore station (bottom depth of 1.5 m) and monthly/seasonally, during 1964 – 1981/1982 – 2010, from five stations (water column sampling with standard depths within 0 – 50m) located on the transect Constanta-East (50 km length, bottom depths within 16 – 54 m), respectively.
The climatic factors, which control seawater thermal regime, discharge fluctuations of the Danube River and water masses mixing, as well as biological processes, are mainly responsible for DO temporal variability in the Romanian coastal waters. Another focus lied on the investigation of the benthic fauna on the Romanian shelf area and the Bosporus inflow area. Several marker organisms and fluctuation of those over time and space have been described from our Ukrainian and Romanian colleagues.

Below we list the evolved papers either in full manuscripts or abstracts in English when the publication was in Russian.
Swiss Lakes and Amvrakikos Gulf

One manuscript is in press (in Organic Geochemistry), another paper was submitted (to Biogeosciences) and two other papers are currently in preparation. All four manuscripts are described below.

1) Impact of recent lake eutrophication on microbial community changes as revealed by high resolution lipid biomarkers in Rotsee (Switzerland)

Authors

Abstract
In this study, the effects of eutrophication on short term changes in microbial communities were investigated, using high resolution lipid biomarker and trace metal data for sediments from the eutrophic Rotsee (Switzerland). The lake has been strongly influenced by sewage input since the 1850s, being an ideal site for studying an anthropogenically altered ecosystem. Historical remediation measures had direct implications for productivity and microbial biota, leading to community composition changes and abundance shifts. The higher sewage and nutrient input resulted in productivity increases, which predominantly led to a radiation of diatoms, primary producers and methanogens between about 1918 and 1921, but also affected all microorganism groups and macrophytes between about 1958 and 1972. Bacterial biomass increased in 1933, which might have been related to the construction of a mechanical sewage treatment plant. Biomarkers also traced fossil OM or biodegraded oil contamination in the lake. *Stephanodiscus parvus*, *Cyclotella radiosa* and *Asterionella formosa* were dominant sources of specific diatom biomarkers. Since the 1850s, the cell density of methanogenic Archaea (*Methanosaeta* spp.) ranged within about 0.5-1.8 x 10⁹ cells g⁻¹ dry sediment and the average lipid content of Rotsee Archaea was about 2.2 fg iGDGTs/cell. An altered BIT index (BIT_CH) indicating changes in terrestrial organic matter supply to the lake is proposed.

*In press: Organic Geochemistry*

*DOI: 10.1016/j.orggeochem.2012.05.014*
Impact of recent lake eutrophication on microbial community changes as revealed by high resolution lipid biomarkers in Rotsee (Switzerland)


Introduction

Due to human activity such as land clearing, agriculture, forestry and urbanisation, nutrient cycling in ecosystems has been intensively altered, especially since the industrial revolution. The impact, along with climate change (Keeling et al., 2010), has profoundly altered natural biological communities in limnic, marine and terrestrial ecosystems (e.g. Vitousek et al., 1997; Smith et al., 1999). It has been estimated that human induced eutrophication has altered one third to one half of the land surface (Vitousek et al., 1997).

Today, the mechanisms of lake eutrophication are quite well understood (for an overview see Smith et al., 1999), with high nutrient loading fuelling productivity and biomass accumulation. Another factor is increased water column stratification, which leads more rapidly to O₂ depletion in the hypolimnion, which can even result in dead zones and mass mortality of species (Diaz and Rosenberg, 2008). The O₂ deficiency enhances the release of nutrients from the sediment, further increasing the cycling and bioavailability of nutrients.

However, due to the negative impact of eutrophication, water sewage treatment has had dramatic improvement over the last decades, resulting in a general increase in surface water quality (Smith et al., 1999; Matzinger et al., 2010). At the same time, efforts to remediate affected water bodies have been less successful and have not always worked out as foreseen. For example, Matzinger et al. (2010) showed that the decrease in nutrient supply to lakes did not strongly reduce O₂ consumption rate in the water column due the remineralisation of organic matter (OM) in the sediment.

Another aspect is that complex system changes from chemical, physical and biological feedback mechanisms, that rule the system ecology of lakes, may result in 'tipping points' (Scheffer, 2010). These are critical transitions indicating the fragility of an ecosystem, and resulting in dramatic changes in the abundance and composition of inhabiting organisms, either (algal) blooms or the extinction of species and the 'point of no return' (Scheffer, 2010). However, understanding more about these mechanisms is often hampered by lack of historical data - investigations have typically only started after severe cases of eutrophication, while hardly any physico-chemical and biological data are available before then (Stadelmann, 1980; Scheffer, 2010). This lack is especially the case for microbiological data, yet these biota play a central role during eutrophication. There is therefore still a need for high temporal resolution reconstruction of microbial communities to improve the understanding of...
the 'natural' state, the onset and development of eutrophication, and subsequent remediation measures.

The aim of this study was to reconstruct the eutrophication history and the response of microbial biomass, using organic and inorganic proxies, in a small eutrophic Swiss lake. The relation of biomarker concentration change to shift in microbial abundances was constrained, partly down to the species level, being supported by results from previous work on diatoms (Lotter, 1989) and methanogenic Archaea (Falz et al., 1999). Finally, we have reevaluated the effectiveness of recovery activity and the implications for microbial communities after intensive sewage input to this ecosystem.

Material and methods

Section 1

Study site and sample collection

Rotsee is a small (0.46 km²) prealpine, monomictic and eutrophic lake with a maximal depth of 16 m (Fig. 1, table 1). It formed after the retreat of the Reuss glacier after the last interglacial (Frey, 1907). Currently, it has a stable stratified water column with a strong chemocline between ca. 6 and 10 m depth and an anoxic hypolimnion during most of the year (Schubert et al., 2010). During the Holocene, the lake was mostly eutrophic and only partly mesotrophic, according to the past algal flora (Züllig, 1985).

The combination of the geographical and hydrological characteristics, together with the forested catchment, favour natural eutrophication (Bloesch, 1974), on which has been profoundly superimposed anthropogenic nutrient enrichment since the 19th century (Stadelmann, 1980). Since ca. 1850, the trophic state increased through sewage input and in 1920 the lake was classified as polytrophic (Bloesch, 1974; Stadelmann, 1980), leading to numerous blooms of Oscillatoria rubescens (today: Planktothrix rubescens, in German “Burgunderblutalge”), which turned the lake water into a red colour (Züllig, 1985). As a consequence of a canal construction from the Reuss River in the south-west corner of the lake in 1922 (Fig. 1), freshwater input increased (Kohler et al., 1984) and the water renewal time dropped from 3-4 to 0.4 yr (Lotter, 1989). However, recovery measures were unsuccessful because of continued nutrient supply from a nearby disposal site, temporal drying of the canal, and the inability of the water inflow from the canal to initiate mixing (Bachmann, 1931; Stadelmann, 1980). After the completion of construction of an interceptor sewer in 1969 and a sewage treatment plant in 1974, the lake started to recover slowly (Stadelmann, 1980).

A 56 cm long sediment core was recovered with a gravity corer in October 2009 from the centre of the lake at 16 m depth (GPS position N 47° 4.251 E 8° 18.955, WGS84). The core was sliced in continuous 1 cm intervals and frozen at -20°C until analysis.
Another 63 cm core was obtained from the same location in August 2010 for elemental analysis.

Section 2

Age model

For core dating, $^{137}$Cs and $^{210}$Pb were measured by $\gamma$ spectrometry with a high purity Ge detector (Canberra GCW-3523) using the $\gamma$ energy at 46.5 keV for $^{210}$Pb and 661.7 keV for $^{137}$Cs on freeze-dried and ground sediment. Based on the Chernobyl accident and nuclear bomb test peaks in $^{137}$Cs and a constant rate of supply (CRS) model, sedimentation (Appleby and Oldfield, 1978) and accumulation rates (Niessen et al., 1992) were calculated.

Section 3

Bulk parameters

The total carbon (TC) and total nitrogen (TN) was measured on freeze-dried samples with a Thermo Quest CE Instrument NC 2500. Total organic carbon (TOC) was measured on decalcified samples. Total inorganic carbon (TIC) was calculated as the difference between TC and TOC. The errors for TC and TOC were ±0.1 wt% and ±0.2 wt% for TN. Additional TIC measurements with a Coulomat 5011 coulometer indicated that the method led to 0.5 wt% higher TIC values, corresponding to 0.5 wt% lower TOC values. The C and N isotopic composition ($\delta^{13}$C and $\delta^{15}$N) of the OM was obtained with a GV Instruments IsoPrime isotope ratio mass spectrometry (IRMS), measured in the same run as TOC and TIC. The $\delta^{13}$C [‰ Vienna PeeDee Belemnite (VPDB)] and $\delta^{15}$N (‰ air) errors were up to ±0.3‰. The chlorin index (CI) and total chlorin concentration were determined according to Schubert et al. (2005). The analytical precision of the method is ca. 5% (Schubert et al., 2005).

Section 4

Biomarker analysis

Ca. 5 g thawed sediment was extracted (x 3) by way of ultrasonication with MeOH and dichloromethane (DCM): 1x10ml MeOH, 1x10ml MeOH:DCM (1:1, v:v), 1x10ml DCM. For quantification, a known mixture of 5α-cholestane, C$_{19}$ n-alcohol and C$_{19}$:0 fatty acid (FA) was added to the extract. Water was removed in a separation funnel with NaCl (20 ml, 5%). The extract was run over a Cu column to remove elemental S and over a column filled with Na$_2$SO$_4$ to remove traces of water. Samples were saponified (3 h, 80 °C) with 6% KOH in MeOH. Neutrals were extracted (x 3) with hexane and dried with Na$_2$SO$_4$ while the acid fraction was extracted from the aqueous phase after the addition of 6M HCl. The neutrals were divided into apolar and polar fractions via liquid chromatography over NH$_2$ columns (Hinrichs et al., 2003). The polar fraction was derivatised (1 h, at 80 °C with N,O-
bistrimethylsilyltrifluoroacetamide (BSTFA, Supelco). FAs were derivatised with 14% BF$_3$/MeOH (Sigma Aldrich) to produce the methyl esters (FAMEs). To identify multiple bond positions in FAs, an aliquot was derivatised with 2-amino-2-methylpropanol to form 2-alkenyl-4,4-dimethoxyloxazline (DMOX) derivatives (Spitzer, 1997).

The resulting fractions were measured using gas chromatography with flame ionisation detection (GC-FID, Carlo Erba HRGC 5160 Mega Series, with a 60 m VF-5 column x 0.25 mm inner diameter (i.d.) x 0.25 µm film thickness (f.t.), He carrier gas flow of 1.0 ml/min) to determine biomarker concentration. For identification purposes the samples were also measured using a gas chromatograph coupled to an electron impact mass spectrometer (GC-MS, GC8000Top Finnigan Voyager, 30 m Agilent HP-5 column x 0.32 mm i.d. x 0.25 µm f.t., He carrier gas flow of 1.0 ml/min).

Glycerol dialkyl glycerol tetraethers (GDGTs) were analysed using a fraction of the total extract, dissolved in hexane/isopropanol (1:1, v:v) and filtered with 0.45 µm PTFE filters prior to analysis via high performance liquid chromatography (HPLC) in a similar manner to that described by Hopmans et al. (2000). For quantification, a synthesized C$_{46}$ GDGT standard was added to each sample prior to analysis (Huguet et al., 2006). GDGT analysis was performed with a Thermo Surveyor LC system coupled to an LCQ Fleet ion trap mass spectrometer, as described by Bechtel et al. (2010). Isoprenoid GDGTs (isoGDGTs) were named according to (Schouten et al., 2009) and for branched GDGTs (brGDGTs) the nomenclature from Weijers et al. (2007) was used. Ethers in the polar fraction were cleaved prior to carbon isotopic analysis (Kohnen et al., 1992; Blumenberg et al., 2004). No derivatisation was necessary. For compound specific carbon isotopic analysis of the cleaved ethers, an Agilent GC 6890N with a combustion furnace using copper oxide coupled to a micromass IsoPrime mass spectrometer (Restek Rxi-5ms 60 m column x 0.32 mm i.d. x 0.25 µm FT) was used. The GC oven temperature programme was: 70 °C to 130 °C at 20 °C/min, then to 320 °C (held 20 min) at 4°C/min. He carrier gas flow was 1.0 ml/min was used.

The precision of the biomarker analysis was 10%, whereas the errors of GDGTs were within 15%. The analytical error of compound specific δ$^{13}$C values were 1–2‰.

**Section 5**

**XRF core scanning**

Relative elemental concentration was determined for the 2010 core. The core was cut lengthwise and its surface was allowed to dry at room temperature for 24 h. One half of the core was measured with the AVAATECH X-Ray Fluorescence Core Scanner at the ETH Zurich, Switzerland, at excitation energy 10 and 30 kV with a resolution of
0.3 mm for 30 s at each point (Richter et al., 2006). The unit is XRF counts (peak area). The precision of the measurement is about 2-3%, dependent on the element.

Section 6
Diatom analysis

Diatom samples were prepared in 2-4 cm intervals using standard techniques including processing with H$_2$O$_2$ (30%) and HCl (10%). Between 300 and 400 valves were counted and identified for each sample and diatom accumulation rates were calculated using the evaporation tray technique (Battarbee et al., 2002).

Section 7
Definition of the BIT$_{CH}$ index

A changed branched and isoprenoid tetraether index (BIT$_{CH}$ index) was defined as follows:

$$
BIT_{CH} = \frac{\text{brGDGTs}}{\text{isoGDGTs}} = \frac{\text{[GDGT-III]} + \text{[GDGT-II]} + \text{[GDGT-II-b]} + \text{[GDGT-II-c]} + \text{[GDGT-I]} + \text{[GDGT-I-b]} + \text{[GDGT-I-c]}}{\text{[GDGT-0]} + \text{[GDGT-1]} + \text{[GDGT-2]} + \text{[GDGT-3]} + \text{[Crenarchaeol]} + \text{[Crenarchaeol regio isomer]}}
$$

With:

a) brGDGTs:
   [GDGT-III]: m/z=1050; [GDGT-II]: m/z=1036; [GDGT-II-b]: m/z=1034; [GDGT-II-c]: m/z=1032; [GDGT-I]: m/z=1022; [GDGT-I-b]: m/z=1020; [GDGT-I-c]: m/z=1018 (Weijers et al., 2007)

b) isoGDGTs:
   [GDGT-0]: m/z=1302; [GDGT-1]: m/z=1300; [GDGT-2]: m/z=1298; [GDGT-3]: m/z=1296; [Crenarchaeol]: m/z=1292; [Crenarchaeol regio isomer]: m/z=1292 (Schouten et al., 2009)

Results and discussion

Section 1
Sedimentation rate and age model

A precise age model is a prerequisite for a high resolution biomarker and trace metal record of Rotsee sediment. Since the core was only rarely laminated and mostly uniform brown-black in colour, radionuclides, namely $^{137}$Cs and $^{210}$Pb, were used for dating (Fig. A.1; Supplementary online material). The former showed increased
activity at 9-10 cm and 18-19 cm corresponding to the Chernobyl accident in 1986 and the peak in nuclear bomb tests in 1963, respectively. The resulting sedimentation rate was calculated as 0.40 cm yr\(^{-1}\) using \(^{137}\)Cs. Using \(^{210}\)Pb dating, a very similar sedimentation rate of 0.35 cm yr\(^{-1}\) was found. Using an average sedimentation rate of 0.38 cm yr\(^{-1}\), the core contained ca. 150 yr of the lake history. The age model (Fig. A.1) could be calibrated with higher TOC concentration at 33-34 cm via the high productivity event before 1922 (Lotter, 1989). The match with the age model of Lotter (1989) supports the high precision of the age model. Hence, biomarker sampling resolution of 1 cm gives a resolution of ca. 4 yr.

Section 2

Eutrophication and sewage input history of Rotsee

TOC varied along the core, ranging between 4.3 and 6.8 wt%, with accumulation rate between 3.1 and 4.9 g cm\(^{-2}\) yr\(^{-1}\) (Fig. 2). At ca. 33-34 cm (1918-1921) and 15-18 cm (1961-1969), TOC accumulation rate maxima indicated times of higher productivity. The peak at 33-34 cm seemed to be the result of a nutrient supply induced trophic regime shift before the canal construction in 1922 (Lotter, 1989). This change became evident from reanalysis of phytoplankton samples from Bachmann (1931) in Lotter (1989), so the connection to the Reuss River was not the main driver of the higher productivity.

At the beginning of the 1960s, lake eutrophication peaked. The decreasing productivity since the end of the 1960s was a direct result of the construction of an interceptor sewer, preventing direct sewage supply to the lake from the surrounding urban area (Stadelmann, 1980). However, the Reuss River water was still rich in nutrients, but this ended in 1974 after the construction of a sewage treatment plant (Stadelmann, 1980). Between 3 and 14 cm (1972-2001), TOC accumulation remained stable and decreased only in the recent lake sediment. Two explanations are likely: Non-point source input of nutrients from agriculture, which continued to fuel productivity in the lake; or redissolution and resuspension of nutrients and OM. The latter explanation implies that, although the nutrient input to the lake was reduced, nutrient redissolution from the lake sediment still fuels productivity, similar to observations for other Swiss lakes (Matzinger et al., 2010). The continued nutrient supply explains the still conspicuous eutrophic character of the lake.

TIC increased consistently from the lower end of the core (1.4 g cm\(^{-2}\) yr\(^{-1}\)), towards ca. 33-34 cm (4.8 g cm\(^{-2}\) yr\(^{-1}\), Fig. 2) and remained almost constant until 17-18 cm (4.3-5.1 g cm\(^{-2}\) yr\(^{-1}\)). Above this point, TIC decreased to ca. 3.1 g cm\(^{-2}\) yr\(^{-1}\) at 5-6 cm, before reaching 4.2 g cm\(^{-2}\) yr\(^{-1}\) at the top of the core. The higher TIC values between ca. 17 and 34 cm (1918-1964) are in agreement with the elevated trophic state (Bloesch, 1974; Lotter, 1989). Bloesch (1974) and Lotter (1989) showed that TIC (and therefore carbonate precipitation) was related to productivity. Use of
accumulation rate prevents potential bias of the TOC as a result of dilution by way of enhanced carbonate deposition (Stein, 1991).

TN, between 0.5 to almost 1.0 wt%, coincided with the TOC profile, with higher values at ca. 17-20 cm and 29-34 cm. The much higher TOC (4.3–6.8 wt%) and TN (0.5–1.0 wt%) values (Fig. 2) vs. other Swiss lakes such as the oligotrophic Lake Brienz (0.4–1 wt% TOC, <0.1 wt% TN) and the eutrophic Lake Lugano (1.1–3.2 wt% TOC, 0.1–0.4 wt% TN) (Bechtel and Schubert, 2009) may be explained by Rotsee’s higher nutrient input, higher productivity, shallow maximum depth and anoxic hypolimnion.

The δ¹³C of the TOC was mostly below -31‰ before the beginning 1920s (Fig. 2). Then, the δ¹³C increased to -27.6 at about 33-34 cm (1918-1921), which might be in relation to the increased productivity, leading to more enriched δ¹³C values at this time. Since then, the δ¹³C remained quite constant, likely due to overall high eutrophication. However, the values decreased again since the 1960s/1970s, until reaching -32.0‰ in the surface sediment (Fig. 2), possibly in relation to a continuous recovery from eutrophication. Therefore, the carbon isotope signal also traces the trends of eutrophication and recovery during the lake history. One complication in using δ¹³C values in sediments is the so-called Suess effect. Burning δ¹³C depleted fossil fuels in conjunction with industrialisation lead to a shift in the isotopic ratio of atmospheric CO₂ which in 2004 came up to −1.7‰ compared to preindustrial values (McCarroll & Loader, 2004). Carbonates and OM deposited after the beginning of the industrialization could therefore be influenced. Hence, if a lake is in equilibrium with the atmosphere, like e.g. the Great Lakes, this has to be taken into account since it would decrease the δ¹³C of the deposited OM (Meyers, 2006). However, Lake Rotsee is not in equilibrium with the atmosphere due to the mixing into the surface water of liberated dissolved inorganic carbon derived from OM degradation. Additionally, other factors like variations in the extension of blooms and precipitation of calcareous nanoplanckton has a much stronger influence on the isotopic signature. We, hence, think that the Suess effect is of minor importance in Lake Rotsee and have not corrected the δ¹³C TOC values.

The δ¹⁵N values (Fig. 2) increased from the bottom of the core (4.0‰) to a clear maximum between 33 and 38 cm (max. 7.5‰), then decreased again upwards to the sediment surface (1.8‰). The values are within the range for soil OM (0 to +9‰), fertilizer (-4 to +4‰) and manure and septic waste (sewage, 0 to +25‰) (Heaton, 1986; Hoefs, 2009). Especially shifts of the latter potential source might strongly increase the δ¹⁵N, which might lead to large excursions in the isotopic signature. Although these different sources cannot be distinguished on the basis of δ¹⁵N values alone, the decrease in δ¹⁵N from the 1920s until 2009 may indicate reduced sewage and nutrient input. In contrast, the increasing δ¹⁵N values until the 1920s are indeed likely an indication for increasing sewage input, which is known to have taken place since the mid-19th century (Kohler et al., 1984). Nitrogen fixation and/or denitrification might have also led to the decrease in δ¹⁵N values since the 1920s.
However, nitrogen fixation is low in eutrophic systems because nitrogen is not limited (Canfield et al., 2005), so it seems to have hardly affected $\delta^{15}$N values in Rotsee. While no isotopic data of nitrate are available from the water column, the values in the sediment can trace water column nitrification-denitrification processes. Denitrification in the sediment of lakes proceeds until completion, which results in similar isotopic nitrogen signatures in the water column and the sediment, also hardly affecting the $\delta^{15}$N composition (Lehmann et al., 2003). Therefore, source changes (especially sewage supply) are the most likely reason for the observed $\delta^{15}$N signal shifts.

Other sewage input indicators are thought to be coprostanol and epicholestanol (Mermoud et al., 1985; Bull et al., 2002), which were also found in Rotsee (Fig. 2). However, coprostanol can originate from all mammals (Sherwin et al., 1993), so animal waste can be another source, which is likely due to surface runoff from nearby livestock farming north of the lake. Epicholestanol is more likely an indicator of bacterial alteration in the lake (Cordeiro et al., 2008), which might indicate higher bacterial activity during lake eutrophication, especially since the 1920s and culminating at about 1933. Sewage input started in the mid-19th century and stopped in 1974 with the construction of a treatment plant. Both markers, still observed in the surface sediment, are therefore apparently no clear sewage indicators in Rotsee.

The two C$_{27}$ isomers Ts (18$\alpha$-22,29,30-trinorneo-hopane) and Tm (17$\alpha$-22,29,30-trinor-hopane, much less abundant than Ts) and C$_{29}$ hopanoids (Seifert and Moldowan, 1978), tracers for petroleum contamination, were found with peaks at 25-29 cm (1932-1942) and 17-20 cm (1956-1964, Fig. 2). The $n$-alkane carbon preference index (CPI, defined by Bray and Evans, 1961) also showed some lower values here (1.9-3.2, Fig. A.2) although real high maturity material like fossil OM or petroleum shows typical values close to 1 (Peters, 2006) which were not observed in this part of the core.

Previous studies indicate that the presence of these hopanoids together with tricyclopolyprenanes strongly suggests a contribution from fossil OM and/or biodegraded oil (Seifert and Moldowan, 1978; Behrens et al., 1998). However, the low abundance of tricyclopolyprenanes and the CPI values (higher than 1) show that the contamination remained low. The decrease in these hopanoids at the beginning of the 1970s and the peak in the sum of odd C$_{27}$-C$_{33}$ $n$-alkanes at 13-20 cm (1956-1974) indicate that the sewage treatment plant in 1974 effectively reduced the oil supply to the lake. The saturated hydrocarbons cannot be the result of the reduction of biohopanoids by H$_2$S such the end products of that pathway remain partially unsaturated (Hebting et al., 2006).

Section 3

Terrestrial OM sources
Aquatic and terrestrial OM sources were distinguished on the basis of TOC/N values, specific terrigenous biomarkers, trace metal profiles and distributions of FAs, \( n \)-alkanes and \( n \)-alcohols.

The molar TOC/N ratio showed values between 6 and 10 (Fig. 2), indicating a predominance of aquatic OM sources (Meyers and Ishiwatari, 1993). In contrast, specific terrigenous biomarkers were detected, such as lupeol, \( \beta \)-amyrin and amyrenone (Brassell and Eglinton, 1983), but the concentrations were too low for quantification.

The maximum at \( n \)-C16:0 FA suggests predominantly autochthonous OM input to the sediment, in line with TOC/N values < 10 (Fig. 2). In contrast, the maxima in the C17, C23, C25 and C27 \( n \)-alkanes and C16, C22 and C26 \( n \)-alcohols indicate both aquatic and terrestrial sources (Meyers and Ishiwatari, 1993). The high abundance of C23 and C25 \( n \)-alkanes and C22 \( n \)-alcohol indicate a significant contribution from submerged and/or floating macrophytes (Ficken et al., 2000).

Other proxies for terrigenous input are the relative contents of Fe, K and Ti, and their covariance indicates that these three trace metals have a similar detrital source (Tribovillard et al., 2006). They show a general decrease through time until about 1922 (Fig. 3), which we interpret to be primarily the result of dilution of detrital input by an increasing input of autochthonous material resulting from eutrophication and related higher productivity. The fluxes may have remained constant over time. Ti intensity decreased to the detection limit after 1922 (Fig. 3).

These trace metals correlate well with the ratio of branched and isoprenoid glycerol dialkyl glycerol tetraethers (Fig. 3). The correlation is in line with observations that indicate that brGDGTs originate from catchment soil, e.g. Weijers et al., 2007; Bechtel et al., 2010. Even though all proxies indicate that most of the sediment material is of autochthonous, aquatic origin, the BIT index (Hopmans et al., 2004) would lead to a contrasting conclusion. Below 33 cm, the BIT index showed highest values between 0.98 and 1.0 and decreased to 0.9 at 28-29 cm. From 26 cm to the core top, the index was between 0.93-0.96. If interpreted in the classical way (Hopmans et al., 2004), one would conclude that Rotsee sediment has a primarily terrigenous source. As concluded before (Bechtel et al., 2010), however, BIT index values can be primarily ruled by the input of crenarchaeol, instead of by the input of brGDGTs. Indeed, the lower BIT value at 28-29 cm is due to a higher crenarchaeol concentration at this depth (Fig. 6). In lake settings, crenarchaeol is much less an indicator of aquatic archaeal input as it is in marine settings (Blaga et al., 2009), for which the BIT index was developed, and this may be especially the case in systems with a strong CH₄ cycle. The original BIT index purposely excluded the other isoGDGTs because their source is considered more diverse, including methanogenic and methane-oxidizing Archaea. If however, as is the case of Rotsee, a larger part of aquatic archaeal production is related to the CH₄ cycle, inclusion of the other isoGDGTs in the ratio should better reflect the relative input of allochthonous and autochthonous
OM. Indeed, two alternative BIT indices, (i) the sum of all brGDGTs over the sum of all isoGDGTs (BIT\textsubscript{CH}, definition in section 2.7) or (ii) the ratio of a single brGDGT to GDGT-0, show much higher and more reasonable sensitivity to changes in the allochthonous supply from the lake catchment (Fig. 3).

Besides dilution by autochthonous OM input as a cause for the relative changes in detrital input to the lake, this may of course also be caused by real changes in detrital input. Pfister (1999) observed that from 1828 to 1895 the frequency of extreme flood events in the Reuss River increased, but at that time it was not connected to Rotsee and therefore had no direct relation. However, precipitation in the Swiss Alps was on average 28% higher during fall compared with the period between 1901 and 1960. In the 20th century, extreme floods decreased in abundance, which could have resulted in lower erosional input from the catchment to the lake. However, such an increase in flood events since the 1970s (Schmocker-Fackel and Naef, 2010) is not apparent from the profiles of Fe, K and BIT\textsubscript{CH}, although the Ti may show such a signal (Fig. 3). We therefore conclude that these profiles most likely indicate a dilution of terrigenous detrital input by aquatic input. Profiles (Fig. 2, A.3) of the sum of long chain FAs (C\textsubscript{24}-C\textsubscript{30}), \textit{n}-alkanes (C\textsubscript{27}-C\textsubscript{33}) and \textit{n}-alcohols (C\textsubscript{24}-C\textsubscript{32}) do not show a trend comparable to the Fe, K, Ti counts and BIT\textsubscript{CH} (Fig. 3). In contrast, these compounds indicate that terrestrial input into the lake remained relatively constant.

Section 4

Input and degradation of sedimentary OM

The chlorin index (CI) is a qualitative parameter estimating OM “freshness” and degradation. The lowest value (0.55) was in surface sediment (Fig. 4), indicating relatively fresh OM (Schubert et al., 2005). However, fresh chlorophyll would show values of ca. 0.2, suggesting that chlorophylls reaching the sediment are partly degraded in the water column. With increasing sediment depth the index increased up to 0.94 (Fig. 4), close to that of inert material (1.0).

The concentration profile of total chlorins (Fig. 4) has been used to reconstruct palaeoproductivity (Schubert et al., 2005). In contrast to the TOC, there was a maximum only at 18-19 cm (16 mg/g TOC), followed by a continuous decrease with depth. Together with the strong increase in total chlorins from 6-7 cm towards the sediment surface and the rapid increase in CI values with depth, the absence of a peak at ca. 33-34 cm could be due to degradation (Fig. 4).

Impact of eutrophication on microbial community changes

Section 1

Primary producers
While C_{16} unsaturated FAs are generally related to algae and bacteria, C_{18} unsaturated FAs originate from algae, zooplankton and cyanobacteria (Volkman et al., 1980; Wakeham et al., 2007). Despite the non-specificity of these FAs, their changing abundance indicated changes in overall productivity within the lake. Because of a high correlation with the TOC and hopanoid profiles, the C_{16:1}(9) FA (double bond position at C-9) may originate from mixed sources of primary producers (peaks at 15-20 and 32-33 cm) and bacteria (peaks at 22-25 cm and 18-19 cm), whereas C_{16:2}(5,10) FA is only related to productivity (Fig. 2, A.4).

Phytol (3,7,11,15-tetramethyl-2-hexadec-2-en-1-ol), a ubiquitous marker, can originate from chlorophylls in phytoplankton, but also from land plants, bacteriochlorophylls and cyanobacterial mats (Rontani and Volkman, 2003). However, peaks in the profile traced times of higher productivity, due to the similarity to the TOC profile (Fig. 5).

Ergosterol (24-methylcholest-7-en-3β-ol, C_{29}:1Δ7, Fig. 5) has been found in fungi and yeast (Mille-Lindblom et al., 2004), but also in low amounts in algae and protozoa (Raederstorff and Rohmer, 1987). The latter source is more likely, indicated by the similarity of the depth profile to that of phytol (Fig. 5). Nonetheless, fungi could thrive on phytoplankton and/or bacterial biomass in the water column and sediment. They may play a crucial role in the cycling of nutrients and carbon, but knowledge about fungi in lakes is still limited (Grossart et al., 2010).

Certain steroids were very abundant between 1958 and 1972. They (Fig. 5 and 7), specifically stigmasterol (24-ethylcholesta-5,22E-dien-3β-ol, C_{29}:2Δ5,22E), stigmastanol (24-ethylcholestan-3β-ol, C_{29}:0), campesterol (24-methylcholesterol, 24-methylcholest-5-en-3β-ol, C_{28}:1Δ5), β-sitosterol (24-ethylcholest-7-en-3β-ol, C_{29}:1Δ7), dinosterol (4,23,24-trimethylcholest-22E-en-3β-ol) and dinostanol (4,23,24-trimethylcholestan-3β-ol) can be related to a higher productivity between 1958 and 1972 (Figs. 2, 5, 7). The absence of a peak between 1918-1922 may be due to bad preservation, because of their high relative lability vs. other lipids (Hoefs et al., 2002).

Because of the good match with higher TOC values between 1958 and 1972 (Fig. 2), the stigmasterol and stigmastanol (Fig. 5) are interpreted as being mainly derived from phytoplankton in Rotsee, although a vascular plant source cannot be excluded (Volkman, 1986).

Similarly, brassicasterol (24-methylcholesta-5,22E-dien-3β-ol, C_{28}:2Δ5,22E) and 24-methylcholesterol (Fig. 5) likely originate predominately from algae (cf. Orcutt and Patterson, 1975; Volkman, 2003) (Section 3.5.3), but a contribution from higher plants cannot be excluded (Volkman, 2003). The match with cholesterol (cholest-5-en-3β-ol, C_{27}:1Δ5), an algal/phytoplankton and zooplankton biomarker (Volkman, 1986; Volkman et al., 1998), suggests a similar origin. However, cholesterol and
brassicasterol strongly decreased within the upper 5 cm, to ca. 21% and 37%, respectively (Fig. 5). Because of the high degree of degradation, the exact source of brassicasterol remains uncertain in Rotsee. A diatom source was not evident by comparison with diatom accumulation rates obtained in this study.

β-Sitosterol (Fig. 5) is the major sterol of emersed macrophytes (Ficken et al., 2000) and is often used as an indicator for higher plant input (Volkman, 1986). A high concentration peak was observed between 14 and 20 cm. The increased biomass of emersed aquatics probably increased as a result of the higher productivity. This is supported by the increased relative abundance of mid-chain n-alcohols and C23 and C25 n-alkanes at this depth (Fig. A.3).

Section 2

Bacteria and Thaumarchaeota

Hopanoids can be used as bacterial indicators (Rohmer et al., 1984), except the hopanes discussed in Section 3.2. The peaks for C30 and C31 hopane at 28-31 cm (1926-1934) and 21-β-bishomohopan-32-ol between 25 and 29 cm (1932-1942) suggest a higher abundance of bacterial biomass and greater bacterial reworking of OM between 1926 and 1942 (Fig. 6). The higher abundance of straight and branched alkanes at 28-29 cm also indicates higher bacterial biomass at this time. Hopan-29-ol, C32-bishomohopanol and C31-homohopanol were detected, but could not be quantified due to their low abundance. The higher bacterial activity is further suggested by an Epicholestanol peak at about 1933 (Fig. 1).

After another hopanoid maximum in the 1960s, which follows the primary productivity during the eutrophication maximum, the abundance decreased, which could be a combination of biomass reduction due to reduced nutrient supply and the low degradation of their precursors in the upper sediment. The depth dependent differences when comparing different hopanoids suggest distinct unequal sources. Based on biomarker data alone, a clear source distinction within the bacteria is not possible.

At 27-28 cm (1934-1937) crenarchaeol is on average five times more abundant vs. the rest of the profile, with a trend of increasing concentration starting at about 1922 (Fig. 6). As crenarchaeol originates from Thaumarchaeota, (NH4⁺-oxidising Archaea (Pitcher et al., 2011), the high abundance of crenarchaeol suggests higher oxidation rates. It is not clear why Thaumarchaeota are only more abundant at this depth, because the trophic state was in general very high, at least between the 1920s and 1970s (Stadelmann, 1980; Kohler et al., 1984). However, the close match with bacteria between 1934 and 1937 suggests that a higher amount of NH4⁺ was supplied to the lake, which may have promoted Thaumarchaeota. The crenarchaeol maximum coincides with the construction of a mechanical sewage treatment plant in 1933 (Stadelmann, 1980), which would suggest a causal relation. However, the mechanical
water treatment should not have affected NH$_4^+$ content (Tchobanoglous et al., 2004). Either the biomass of bacteria and Thaumarchaeota might have increased due to a higher remineralisation rate of nutrients and OM during water treatment or these organisms were not removed in the treatment plant and entered the lake.

Section 3

Diatoms

A description of the diatom assemblage in Rotsee was given in Bachmann (1931) and Lotter (1989). To compare the results, diatom accumulation rates were reinvestigated in this study. In total, 71 different species were determined. The seven species Stephanodiscus parvus, Cyclotella comensis, Asterionella formosa, Fragilaria crotonensis, Fragilaria ulna var. acus, Stephanodiscus hantzschii, Cyclotella comta/radiosa represented at least 64% of all diatoms in Rotsee. Several species represent the most dominant sources for certain biomarkers. It was not, however, possible to exclude other less abundant diatoms and/or other microorganisms as sources of these biomarkers.

The pentaunsaturated C$_{20:5}(5,8,11,14,17)$ FA (Fig. 7) is assumed to originate from diatoms (Volkman et al., 1998). The high correlation of the di- and tri-unsaturated FAs C$_{18:2}(9,12)$ and C$_{18:3}(5,9,12)$ with C$_{20:5}(5,8,11,14,17)$ FA (R$^2$ 0.69 and 0.76, respectively) suggests the same origin for all three FAs (Fig. A.5). All three were sparse before 1918, most abundant between 1918 and 1924, and then continuously decreased (Fig. A.5). The increase in the upper 5 cm could be a degradation pattern, as FAs are easily decarboxylated (Hoefs et al., 2002). The correlation of these FAs with Asterionella formosa (R$^2$ 0.46 for C$_{20:5}$, 0.37 for C$_{18:3}$, 0.48 for C$_{18:2}$) and Cyclotella radios/a (R$^2$ 0.33 for C$_{20:5}$, 0.28 for C$_{18:3}$, 0.43 for C$_{18:2}$) seems to confirm them as the predominant source of these FAs. However, di- and tri-unsaturated FAs have also been referred to cyanobacteria and higher plants (Rezanka et al., 1983), which may explain the lower correlation of these diatoms with the C$_{18:2}$ and C$_{18:3}$ FAs.

The C$_{25:2}$ highly branched isoprenoid alkene (C$_{25:2}$ HBI, Fig. A.6) is also known to be derived from diatoms (Volkman et al., 1998). In freshwater settings, only the diatom Navicula sclesvincensis has been reported to contain HBIs (Belt et al., 2001), but this diatom has not been found in Rotsee. The highest correlation with C$_{25:2}$ HBI could be found for Stephanodiscus hantzschii, but is only R$^2$ 0.30, which suggests that more sources of this lipid need to be considered (Fig. A.6).

Dinosterol and dinostanol were considered to be specific for dinoflagellates (Withers, 1983), but they can also originate from diatoms (Volkman et al., 1993). Bachmann (1931) showed that dinoflagellates are rarely present in Rotsee and the deterministic C$_{22:6}$ FA of dinoflagellates (Volkman, 2003) is absent. Therefore, Dinosterol and dinostanol are interpreted as being of diatom origin. The Dinosterol/stanol profile matched the profiles of Stephanodiscus parvus, Fragilaria crotonensis, Cyclotella...
pseudostelligera and Tabellaria fenestrata together with the productivity maximum for 1958-1961 (18-19 cm; Fig. 7, A.7). However, cross plots indicate that the most dominant source seemed to be Stephanodiscus parvus ($R^2$ 0.62, Fig. 7), also the most dominant of these diatoms in the lake since the 1920s due to eutrophication. The low correlation of Fragilaria crotonensis ($R^2$ 0.30) and Tabellaria fenestrata ($R^2$ 0.28) suggest only minor sources of these lipids, while Cyclotella pseudostelligera ($R^2$ 0.40) is unlikely a significant source because of its low accumulation rate.

24-Methylcholesterol can also be derived from dinoflagellates and diatoms (Orcutt and Patterson, 1975; Volkman, 2003). Due to correlation, its main source could be Stephanodiscus parvus ($R^2$ 0.45).

Section 4

Methanogenic and methanotrophic Archaea

GDGT-0, the most dominant archaeal lipid, was used as a proxy for methanogenic Archaea. This proposed origin is supported by the fact that ca. 98% of archaeal biomass in the lake was methanogenic with 91% consisting of Methanosaeta spp. (Falz et al., 1999). More evidence came from carbon isotope analysis after ether cleavage, with high $\delta^{13}C$ values between -36‰ and -21‰ for almost all ether cleaved GDGTs in the surface sediment, which is the typical range of -35 to -22‰ of terrigenous, aquatic and also methanogenic archaeal derived lipids (Hinrichs et al., 2000), suggesting predominantly methanogenic sources. This is in contrast to much lower values, which can reach less than -100‰ in marine settings, suggesting a predominantly methanotrophic origin (Hinrichs et al., 2000). On average, GDGT-0 showed a quite constant concentration of ca. 20 µg/g TOC, interrupted by a higher concentration during the productivity maxima, with ca. 42 and 83 µg/g TOC at 17-21 cm and 33-34 cm, respectively.

Based on reported data (Falz et al., 1999), GDGT-0 was used to estimate methanogen cell density in the lake sediment. The cell counts were highest at the sediment surface and decreased continuously with depth, with ca. three times lower cell density at 10 cm vs. the sediment surface (Falz et al., 1999). Through correlation of the cores, a concentration of 4.6 x $10^8$ cells per g dry sediment of Methanosaeta spp. in the surface sediment corresponded to GDGT-0 concentration of ca. 20 µg/g TOC for most of the last 150 yr. During the productivity maxima, Methanosaeta spp. apparently increased up to at least ca. 9.2 x $10^8$ cells g$^{-1}$ dry sediment at 17-21 cm (1953-1964) and 1.8 x $10^9$ cells g$^{-1}$ dry sediment at 33-34 cm (1918-1921). For these estimates, it was assumed that the proportion of Methanosaeta spp. to all Archaea in surface sediment has remained constant at 91% for the last 150 yr.

The extent to which the supply of archaeal lipids within the sediment core compensated for degradation losses remained, however, constrained. GDGTs and archaeol are considered to be relatively recalcitrant (Pease et al., 1998). The linear
decrease in the ratio of archaeol and GDGT-0 with depth (Fig. A.8) indicates that archaeol is more rapidly degraded than GDGT-0, suggesting slow degradation of GDGT-0 in Rotsee sediment.

Based on these results, the average cell content of GDGTs was estimated on the basis of values from the literature, with 1-3 fg intact GDGTs (iGDGTs)/cell (Sinninghe Damsté et al., 2002; Wuchter, 2006; Huguet et al., 2010). IGDGTs consist of core GDGTs (cGDGTs) and polar head groups. In the sediments, polar head groups are decomposed within days to weeks (Harvey et al., 1986; Lipp et al., 2008). A cell density in the surface sediment of 4.6 x 10^8 cells g⁻¹ sediment (Falz et al., 1999) and 1-3 fg intact GDGTs, together with the correlation between iGDGTs and cGDGTs (Huguet et al., 2010) leads to cGDGT concentration between 1.6 and 4.9 µg/g sediment. This range is of the same order of magnitude as the measured concentration of the sum of isoGDGTs (2.0 µg/g sediment). In the other direction, a theoretical iGDGTs/cell value for the Rotsee sediment can be estimated, with a cell density of 4.6 x 10^8 cells g⁻¹ sediment and 2.0 µg/g sediment isoGDGTs leading to an average value of ca. 2.2 fg i-GDGTs/cell for Rotsee Archaea.

Summary and conclusions

Rotsee has been shown to be an ideal site for studying an anthropogenically altered ecosystem due to eutrophication. The multi-proxy approach, using lipid biomarkers and trace metals with a high temporal resolution of 4 years, made it possible to reconstruct short term changes in the physical, chemical and biological status. A direct impact of higher sewage and nutrient input on the increase of productivity and OM accumulation was observed, which predominantly led to a radiation of diatoms, primary producers and methanogens between about 1918 and 1921 and all microorganism groups and macrophytes between about 1958 and 1972. A higher abundance of bacteria and ammonium oxidising Archaea was likely related to the construction of a mechanical sewage treatment plant in 1933. The decrease in OM accumulation in the lake since the end of the 1960s resulted from the construction of an interceptor sewer between 1967 and 1969. Furthermore, the decrease in fossil OM or biodegraded oil related biomarkers (C_{27} T8/Tm and C_{27} hopanoids, long chain odd n-alkanes) is a clear indication for the achievement by the sewage treatment plant construction in 1974. The δ^{15}N of bulk OM, in contrast to coprostanol and epicholesterol, traced the eutrophication and recovery during the past 150 years.

Based on the correlation of trace metals (Fe, Ti, K) with branched/isoprenoid GDGTs, terrestrial input could be reconstructed. We propose an altered BIT index, BIT_{CH}, as the sum of all brGDGTs/the sum of all isoGDGTs, the ratio reflecting the balance between all aquatic Archaea, including those related to the methane cycle, and soil-derived brGDGTs better than the original BIT index.

The impact of eutrophication on microbial assemblage changes could be traced. Also based on previous microbial studies, the sources of certain biomarkers could be partly
identified down to the species level. The C_{18:2}(9,12), C_{18:3}(5,9,12) and C_{20:5}(5,8,11,14,17) FAs likely originated mainly from the diatoms *Cyclotella radiosa* and/or *Asterionella formosa*. The C_{25:2} HBI originated from mixed sources and clear source distinction between diatom species was not possible. *Stephanodiscus parvus* could be the main source of dinosterol and 24-methylcholesterol. The abundance of methanogenic Archaea and the cellular membrane lipid content (GDGTs) could be estimated.

Although microbial biomass reconstruction based on lipid biomarkers is often limited due to the non-specificity of lipids and their lability with respect to degradation, the use of high resolution multi-proxy records can improve the distinction of biomarker sources. Biomarker analysis can trace short term, eutrophication related ecosystem and microbial community changes.

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**Literature**


Figure 1. Map with Rotsee, the Reuss River and its connection with Rotsee by the Reuss-Rotsee-canal (partly below ground level) and the northwest corner of Lake Lucerne. Insert map shows the location of Rotsee within Switzerland.
Figure 2. Bulk parameters for core and profiles of coprostanol, epicholesterol, sum of C<sub>27</sub> Ts, C<sub>27</sub> Tm and C<sub>29</sub> hopanoids and sum of odd C<sub>27</sub>-C<sub>33</sub> n-alkanes, plotted vs. sediment depth and age (years AD). RRC, Reuss-Rotsee-canal; MPT, mechanical sewage treatment plant; IS, interceptor sewer; STP, sewage treatment plant (in all cases with the time of completed construction).
Figure 3. Terrestrial input to the lake, traced via the ratios of branched GDGT-III/GDGT-0 (1050/1302), sum of branched to sum of isoprenoid GDGTs (BIT$_{CH}$ index) and the XRF counts of Fe and K as running average of 9, XRF counts of Ti plotted as running average of 20 and BIT index according to Hopmans et al. (2004). All profiles are plotted vs. sediment depth and age (yr AD).
Figure 4. Chlorin index and total chlorin concentration vs. sediment depth and age (yr AD).
Figure 5. Concentration of sterols, stanols and phytol vs. sediment depth and age (yr AD).
Figure 6. Concentration of hopanoids and crenarchaeol vs. sediment depth and age (yr AD).
Figure 7. Concentration of C_{20:5}(5,8,11,14,17) FA and cell accumulation rate (acc rate) of *Cyclotella radiosa* and *Asterionella formosa* and profile of Dinosterol/stanol with accumulation rate of *Stephanodiscus parvus* vs. sediment depth and age (in yr AD).
Supplementary figures

Figure A.1. Age models of Rotsee based on $^{137}$Cs (left) and $^{210}$Pb (right) with the specific activity (Becquerel kg$^{-1}$ freeze-dried ground sediment) on x-axis vs. sediment depth.
Figure A.2. Carbon preference index (CPI; defined by Bray and Evans, 1961) of $n$-alkanes vs. sediment depth and age (yr AD).
Figure A.3. Concentration profiles of sum of even $C_{24}$-$C_{30}$ FAs and sum of even $C_{24}$-$C_{32}$ $n$-alcohols and $C_{23}$-$C_{25}$ $n$-alkanes vs. sediment depth and age (yr AD).
Figure A.4. Concentration of $C_{16:1}(9)$ FA and $C_{16:2}(5,10)$ FA vs. sediment depth and age (yr AD).
Figure A.5. Concentration of $C_{18:2}(9,12)$ FA, $C_{18:3}(5,9,12)$ FA and $C_{20:5}(5,8,11,14,17)$ FA, vs. sediment depth and age (yr AD).
Figure A.6. Concentration of $C_{25:2}$ HBI alkene with cell accumulation rate (acc rate) of *Stephanodiscus hantzschii* vs. sediment depth and age (in yr AD).
Figure A.7. Concentration dinosterol and dinosterol+dinostanol with cell accumulation rate (acc rate) of *Fragilaria crotonensis*, *Tabellaria fenestrata* and *Cyclotella pseudostelligera* vs. sediment depth and age (in years AD).
Figure A.8. Archaeol/GDGT-0 ratio vs. sediment depth and age (in yr AD). The very low ratio at 33-34 cm was removed from the data, because of bias from the high GDGT-0 concentration.
2) Environmental variations in a semi-enclosed embayment (Amvrakikos Gulf, Greece) – Reconstructions based on benthic foraminifera abundance and lipid biomarker pattern

Authors
Naeher, S., Geraga, M., Papatheodorou, G., Ferentinos, G., Kaberi, E., Schubert, C.J.

Abstract
The evolution of environmental changes during the last decades and the impact on the living biomass in the western part of Amvrakikos Gulf was investigated using abundances of benthic foraminifera and lipid biomarker concentrations. These proxies indicated that the gulf has dramatically changed due to eutrophication. Eutrophication has led to a higher productivity, a higher bacterial biomass, shifts towards opportunistic and tolerant benthic foraminifera species (e.g. B. elongata, N. turgida, T. agglutinans, A. tepida) and a lower benthic species density. Close to the Preveza Straits (connection between the gulf and the Ionian Sea), the benthic assemblages appeared to be less productive and more diversified under more oxygenated conditions. Sea grass meadows largely contributed to the OM at this sampling site. Isorenieratane, chlorobactane and lycopane together with oxygen monitoring data indicated that anoxic (and partly euxinic) conditions prevailed seasonally throughout the western part of the gulf with more severe hypoxia towards the east. Increased surface water temperatures have led to a higher stratification, which reduced oxygen resupply to bottom waters. These developments are reasons for mass mortality events and ecosystem decline observed in Amvrakikos Gulf.

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Environmental variations in a semi-enclosed embayment (Amvrakikos Gulf, Greece) – Reconstructions based on benthic foraminifera abundance and lipid biomarker pattern

Naehler, S., Geraga, M., Papatheodorou, G., Ferentinos, G., Kaberi, E., Schubert, C.J.

Introduction

Coastal development, pollution and a range of anthropogenic activities including extensive agriculture, aquaculture, urban and industrial wastes are main causes of decline and loss of coastal habitats observed over the last decades (Airoldi and Beck, 2007; Diaz and Rosenberg, 2008).

Amvrakikos Gulf, located in north-western Greece, is a semi-enclosed embayment characterized by a complex lagoonal system, extensive delta (Kapsimalis et al., 2005) and a fjord-like oceanographic regime (Ferentinos et al., 2010). The gulf is protected under the international Ramsar Convention as Wetlands of International Importance. In addition it is designated as a Special Protection Area (SPA), according to the European Union Directive 79/409/EU and it is included in the Natura2000 Network. Despite the efforts, which have been made for the protection and conservation of this unique area, the gulf is suffering from seasonal hypoxia. The dysoxic/anoxic conditions appeared during the last 20 to 30 years and have been caused by the excessive use of fertilizers, the increase in animal stocks, intensive fish farming and domestic effluents (Ferentinos et al., 2010; Kountoura and Zacharias, 2011). Recently, in February 2008 the environmental stress in the gulf reached a peak, as documented by a sudden massive mortality of fish in aquaculture rafts in the north-eastern part of the gulf (Ferentinos et al., 2010).

The purpose of the present paper is to illuminate the evolution of the environmental conditions over historic timescales in the Amvrakikos Gulf over the last 50 years through the study of proxies in the sediment. Benthic foraminifera have been proven useful in the reconstruction of palaeoenvironmental conditions since changes in abiotic and biotic parameters such as salinity, eutrophication, oxygen concentration, substrate, water depth and pollution do modify benthic assemblages (Scott et al., 1979; Jorissen, 1987; Debenay et al., 2005; Murray, 2006). Due to their short reproductive life cycles their study can detect short-term environmental changes like oxygen conditions, organic matter (OM) supply and lithology at the sea bottom (Murray, 2001).

More specific in environments where oxygen depletion in the bottom water occurs, benthic foraminifera populations and their diversity are usually reduced and the assemblages are dominated by dysoxic or seasonally by anoxic tolerant species (Sen Gupta and Machain-Castillo, 1993). The changes in benthic assemblages’
characteristics have been used to evaluate the past evolution of oxygen depletion in a wide spectrum of coastal environments (Platon et al., 2005; Filipsson and Nordberg, 2004).

Lipid biomarkers have been used as tracers for human alteration and eutrophication of water bodies (e.g. (Naeher et al., in press; Smittenberg et al., 2004). Specific indicators of severe oxygen depletion are the pigments isorenieratene, chlorobactene and okenone (or related derivatives), which have been used to trace photic zone euxinic conditions (e.g. (Brocks and Summons, 2003)). Apart from oxygen depletion, water column properties such as stability of stratification and salinity can be traced by tetrahymanol and gammacerane (Sinninghe Damsté et al., 1995; Thiel et al., 1997; Bechtel and Schubert, 2009), whereas alkenones have been useful in reconstructing surface water temperatures (Prahl and Wakeham, 1987; Müller et al., 1998).

In this study the combined approach of benthic foraminifera and lipid biomarker proxies was used to characterise environmental changes and the implications for the living biomass during the recent history in Amvrakikos Gulf.

Regional setting

Amvrakikos Gulf was formed during the Mid-Quaternary period (ca. 50-11 ka BP) (Kapsimalis et al., 2005; Anastasakis et al., 2007) and is approximately 35 km long and 6-15 km wide (Fig. 1). It is separated from the open Ionian Sea by a beach-barrier complex and is connected to the open sea through a narrow, elongated channel, the Preveza Straits, which is approximately 6 km long, 0.8 to 2.5 km wide and 20 m deep. The delta of the Arachthos and Louros Rivers and associated lagoons are located at the northern border of the gulf.

The water column is stratified during the year with a brackish surface layer and a saline bottom layer (Ferentinos et al., 2010). The surface layer is well oxygenated with concentrations ranging from 7.5 to 9 mg l⁻¹, but the dissolved oxygen content in the bottom water layer only reaches 0-2 mg l⁻¹ during the summer months in the western part and year round in the eastern part of the Gulf (Ferentinos et al., 2010 and unpublished data). Brackish water flows out through the strait with the surface layer, whereas saline water enters the Gulf through the bottom layer. Summer months temperatures and salinity in the surface water ranged between 28.3 and 29.3°C and between 32.9 and 33.7 psu, respectively. Temperatures in the bottom water ranged between 15.8 and 16.0°C, whereas the salinity was around 37.7 psu. Seasonal hypoxia has been established at the study sites since the last 2-3 decades (Kountoura and Zacharias, 2011).

Since the 1970s the gulf has been altered, mostly due to extensive agriculture, aquaculture and urban development, the construction of two dams, which control the run-off of the Arachthos River, and the establishment of oil stations along the
southern border of the gulf. Now also the surface water layer suffers occasionally from oxygen depletion (Ferentinos et al., 2010). In 2008, it seemed that high density water filled the deeper parts of the basin and caused uplifting of the anoxic layer leading to a massive fish mortality event (Ferentinos et al., 2010). Based on the testimonies of the local Fisheries Commission, fish mortality events in aquaculture rafts had also been observed in the past (at 1988, 1992 and 1998), although less intense than in 2008.

The sediment in cores Amvr15 and Amvr13 consisted of grey mud. The colour of the sediments in the top 5cm in each core appeared darker in relation to the rest of the core and was attributed to an increase in the total organic carbon (TOC, Fig. 2). At the sampling site of core Amvr15, seagrass meadows were present on the sediment surface.

Methods

Two short sediment cores were retrieved in October 2010 from Amvrakikos Gulf by a KC Kajak sediment core sampler. Core Amvr15 (42 cm long) was collected from an area close to the entrance of the gulf (38° 56’ 53’’N, 20° 48’ 31’’E) at a water depth of 32 m (Fig. 1). The 30 cm long core Amvr13 was retrieved from the inner part of the gulf (38° 59’ 15’’N, 20° 51’ 48’’E) at a water depth of 40 m (Fig. 1).

The actual sediment accumulation rates in the cores Amvr13 and Amvr15 were calculated from the vertical distribution of $^{210}$Pb, following the constant rate of supply (CRS) model of (Appleby and Oldfield, 1978). The downcore $^{210}$Pb activity was determined through the activity of its $\alpha$-emitting granddaughter $^{210}$Po, assuming secular equilibrium with $^{210}$Pb. The supported $^{210}$Pb activities, which correspond to sediment layers deposited earlier than the last 100-120 years, were calculated from the vertical profiles of $^{226}$Ra published by (Tsabaris et al., 2011) in the same area.

Benthic foraminifera were studied on 29 samples from core Amvr15 and 20 samples from core Amvr13. The mean sampling interval for faunal analyses was 1.4 cm in each core. Samples were washed over a 63 $\mu$m sieve and dried in an oven. About 200 specimens of benthic foraminifera were picked and identified from each sample; a microsplitter device was used. Each taxon was expressed as a percentage of the total benthic assemblage. An estimation of the species diversity was performed using the $H(s)$ index following the Shannon–Wiener equation (Shannon, 1948; Buzas and Gibson, 1969). The ratio of the number of benthic foraminifera per weight of dry sediment (>63 $\mu$m) was used as an index of benthic foraminifera productivity (Blackwelder et al., 1996). Hierarchical cluster analysis (R-mode) performed on 18 benthic foraminifera species and genera which were sufficiently abundant in both cores. The tree diagram was constructed using the Ward’s method based on Euclidian distance on SPSS software.
Bulk parameters were analysed and measured as described previously (Naeher et al., in press): In short, total carbon (TC), total nitrogen (TN) and total organic carbon (TOC) were determined on untreated and decalcified sediment samples, respectively, with errors of up to ±0.2 wt% by means of an elemental analyser (Carlo Erba 2500). The total inorganic carbon (TIC) was calculated from the difference between TC and TOC. The isotopic composition of OM (δ13C and δ15N) was analysed by an Isoprime mass spec connected to an elemental analyser (Carlo Erba 2500) The error was ±0.3‰ and values are reported against the international standards Vienna Pee Dee Belemnite (VPDB, carbon) and air (nitrogen). The chlorin index (CI) and total chlorin concentrations were determined according to (Schubert et al., 2005). For biomarker analysis, the same extraction and treatment produce was used as described in Naeher et al. (in press). An internal standard was added for quantification (α-Cholestane, C19 n-fatty acid, C19 n-alcohol) before extraction with MeOH/DCM. After saponification, neutrals were further separated into apolar and polar fractions over NH2 columns (Hinrichs et al., 2003). The polar fraction was derivatised with BSTFA for 1h at 80°C. FA were converted into methyl esters with 14% BF3/MeOH. FA double bond positions were determined according to (Spitzer, 1997). A sample aliquot of the polar fraction was desulfurized with Raney-Nickel catalyst (Sinninghe Damsté et al., 1988), followed by hydrogenation for 2h with PtO2 as catalyst in a solution of concentrated acetic acid and ethyl acetate (1:1, v:v). Instruments and measurement conditions are described in Naeher et al., (in press). Alkenones were quantified on an Agilent 7890A GC system, equipped with an Agilent column (30 m long x 320 µm inner diameter x 0.32 µm film thickness) and a flame ionization detector (FID). The GC oven temperature program was: 70 °C to 180 °C at 40 °C min−1, then to 320 °C at 2°C min−1 and held for 10min.

Results

Section 1

Age model

According to the CRS model, the estimated average sediment accumulation rates in core Amvr15 were 0.6 cm yr−1 and 0.8 cm yr−1 in core Amvr13. Regarding core Amvr15, the estimated rate was in agreement with the one calculated by Tsabaris et al. (2011) from the same area. From the vertical profiles of 210Pb, no significant bioturbation was observed. Based on the age models, the cores comprised sediments deposited since 1967 (Amvr15) and 1972 (Amvr13).

Section 2

Benthic foraminifera abundance

Benthic foraminifera were present throughout both cores. A total of 127 foraminiferal species were recognized in samples from core Amvr15. The number of benthic foraminifera specimen per sediment was high, except in the intervals 30-25cm, 15-
10cm, 7-6cm and 1-2cm depth (Fig. 3). The \( H(s) \) diversity index ranged between 2.6 and 3.6 and exhibited lower values from around 18cm to the top of the core. Within the upper 3cm of the core the \( H(s) \) index represented small scale oscillation with low values in the dark colour laminae. Furthermore, at around 1cm the reduction of the \( H(s) \) index was accompanied by a reduction of the benthic foraminifera population. A shift of both indices to higher values occurred in the light-coloured muddy sediments at the core top. The downcore variation of the abundances of selected taxa is shown in Figure 3. Benthic foraminifera assemblages consisted of highly diversified porcelaneous (\( Quinqueloculina \) \textit{seminulum}, \( Q. oblonga \), \( Q. laevigata \), \( Q. stelligera \), \( Q. lata \), \( Q. subpoeyana \), \( Miliolinella \) \textit{subrotunda}, \( Triloculina \) \textit{spp}) and hyaline (\( Rosalina \) \textit{globularis}, \( Discorbis \) \textit{spp.}, \( Planorbulina \) \textit{mediterranensis}, \( Cibicides \) \textit{spp}) epifauna (Murray, 2006). Infauna was represented by \textit{Bulimina aculeata}, \textit{Bolivina dilatata}, \textit{B. spathulata}, \textit{Ammonia beccarii} and \textit{A. tepida}, \textit{Nonion depressum}, \textit{Nonionella turgida} and \textit{N. bradii} (Murray, 2006). Epifauna species dominated the benthic assemblages between 42 and 20cm. The increased participation of diverse \( Quinqueloculina \) \textit{spp}, \( Rosalina \) \textit{spp}, \( Cibicides \) \textit{spp.} together with \( P. mediterranensis \) and other epifauna species may be attributed to the presence of seagrass meadows colonized locally on the coring site (Murray, 2001; Mateu-Vicens et al., 2010). Furthermore, the presence of \( Cibicides \) \textit{spp} could also be correlated to the hydrodynamic regime at the coring site since high abundances of this taxon are related to high current velocities (Szarek et al., 2006). Infaunal species showed increased abundances in the upper 20cm and \textit{B. aculeata} dominated the benthic assemblages with up to 40% of the total association. This taxon occurs “predominantly surficial” and reacts quickly to labile OM supply (Mojtahid et al., 2010).

A total of 77 foraminiferal species were recognized in samples from core Amvr13 (Fig. 4). The \( H(s) \) diversity index ranged between 1.8 and 2.7 and exhibited lower values at around 20cm and 1cm depth. The number of benthic foraminifera specimen per sediment was generally higher than in core Amvr15. Lower values were observed at around 20cm, 17cm, 7cm and 3cm (Fig. 4). Shallow and deep infaunal species dominated the benthic assemblages almost throughout the core, including high abundances of \textit{Bulimina elongata}, \textit{B. aculeata}, \textit{B. dilatata}, \textit{B. spathulata}, \textit{Hopkinsina pacifica}, \textit{A. tepida}, \textit{N. turgida} and \textit{N. bradii}. All these species have been reported as common in shelf environments, associated with high contents of OM, and being stress-tolerant taxa (Jorissen, 1987; Barmawidjaja et al., 1995; van der Zwaan, 2000; Mendes et al., 2004; Murray, 2006). The agglutinated species included mostly \textit{Textularia conica} and \textit{T. agglutinans} and the porcelaneous included Miliolids. \( Quinqueloculina \) \textit{spp} and \( Miliolinella \) \textit{spp} showed similar fluctuations and higher abundances at around 25cm, 15cm and 8cm.

Section 3

Bulk parameters and biomarkers
The TOC profile of core Amvr13 increased slightly towards the core top (1.3-2.7 wt%; Fig. 2). In core Amvr15, the TOC was higher and increased stronger towards the surface (1.4-6.1 wt%; Fig. 2). TN and δ¹⁵N values were also higher in core Amvr15 and increased in both cores towards the sediment top (Fig. 2). Whereas chlorin concentrations in core Amvr15 were constant throughout the core with 0.5 mg g⁻¹ TOC, core Amvr13 showed a strong increase in concentrations from 0.5 mg g⁻¹ TOC to a maximum concentration of 2.4 mg g⁻¹ TOC over the last 8 years (Fig. 2). The CI was slightly lower indicating fresher OM material throughout core Amvr15 compared to Amvr13 and decreased towards the core top (Fig. 2). For comparison, CI values from the Swiss lake Rotsee were added, which were similar to CI values in core Amvr15 (Fig. 2).

The atomic C/N ratio was lower in core Amvr13 than in core Amvr15 with values slightly below 10 and 13-16, respectively. In 2-3 cm (2006-2008) and 18-20 cm (1985-1988) of core Amvr13 the total nitrogen concentrations were below the detection limit, which hindered the calculation of the C/N ratios. In core Amvr15 below 20 cm (before 1977) C/N values ranged between 7 and 27 (Fig. 2). While the δ¹³C_TOC remained almost constant throughout both cores with on average about 3-4‰ higher values in core Amvr15.

The profiles of branched alkanes and isoprenoids were very similar to hopanoids in both cores (Fig. 5); all three are bacterial biomarkers (Rohmer et al., 1984; Summons et al., 2007). These lipids were relatively constant throughout core Amvr15, whereas in core Amvr13 they were most abundant in 28-29 cm (1974-1975) and in the upper 10 cm (since about 1998) and lowest in 12-14 cm (1993-1995) (Fig. 5).

Short chain n-alcohols and phytol increased quite continuously towards the surface sediment (Fig. 5). Short chain n-alcohols and phytol mainly originate from phytoplanktonic sources (Meyers and Ishiwatari, 1993; Rontani and Volkman, 2003). Dinosterol (4,23,24-trimethylcholest-22E-en-3β-ol, 4-Me30Δ22; Fig. 5) which also increased towards the surface sediment is of diatom origin in Amvrakikos Gulf, because C₂₂:₆ FA, which would hint to dinoflagellates as the source organisms is absent (Volkman, 2003; Withers, 1983). In contrast, β-sitosterol (24-ethylcholeste-7-en-3β-ol, C₂₉:₁Δ7; Fig. 5) with a similar concentration profile as dinosterol could not be assigned to a single source but has its origin in phytoplankton, higher land plants and emersed macrophytes (Volkman, 1986). The Pₐ₀ proxy is an indicator for macrophytes (Ficken et al., 2000) and ranged between 0.2 and 0.6 in both cores (Fig. 5).

Tetrahymanol is found in bacteriovoric ciliates and was often used as a stratification, stagnation and/or salinity indicator (Sinninghe Damsté et al., 1995; Bechtel and Schubert, 2009; Thiel et al., 1997). While it was relatively constant throughout core Amv15 and in the lower part of Amvr13, it increased in the most recent decade in Amvr13 (Fig. 5).
After hydrogenation, traces of isorenieratane and chlorobactane were found throughout both cores, except the lowermost sample of core Amvr13, in which chlorobactane could not be detected. Both pigments are specifically derived from phototrophic sulphur bacteria (Chlorobiaceae) and have been used as tracers for photic zone euxinia and anoxia (Brocks and Summons, 2003). Lycopane concentrations and the \( \frac{\text{lycopane} + C_{35} \text{n-alkane}}{C_{31} \text{n-alkane}} \) ratio both remained constant in core Amvr15, whereas both parameters increased with depth in core Amvr13 (Fig. 6). Although the source of lycopane is unknown, it has been used to reconstruct palaeo-redox conditions in the bottom water in marine settings (Sinninghe Damsté et al., 2003; Wakeham et al., 1993).

Alkenones were determined in both cores. These markers are specifically derived from haptophyte algae (Herbert, 2003). The UK’37 index (\( \frac{\text{Me} C_{37:2}}{\text{Me} C_{37:2} + \text{Me} C_{37:3}} \); by Prahl and Wakeham, 1987) is an alkenone based proxy, which is highly correlated with mean annual sea surface temperatures (Prahl et al., 1988; Müller et al., 1998). This index showed increasing values towards the top of both cores, between 0.61 and 0.84 in core Amvr15 and 0.63 and 0.78 in core Amvr13.

**Discussion**

**Section 1**

**Benthic foraminifera clusters**

Cluster analysis (R-mode) revealed four clusters (Fig. 8). Cluster I was composed of \( B. \) elongata, \( N. \) turgida, \( T. \) agglutinans and \( A. \) tepida (Fig. 8). In many studies \( N. \) turgida appeared as the most tolerant species to oxygen depletion and its increase in abundance is associated with enhanced OM supply (Sen Gupta and Machain-Castillo, 1993; Blackwelder et al., 1996), usually of terrestrial origin (Mojtahid et al., 2010; Goineau et al., 2011). \( A. \) tepida is considered as a species, which is tolerant to large environmental variations (Almogi-Labin et al., 1992; Debenay and Guillou, 2002), including hypoxia (Blackwelder et al., 1996) and anthropogenic pollution (Debenay et al., 2005). \( B. \) elongata is associated with food-enriched sediments related to river plumes (Guadiana River, Iberia; (Mendes et al., 2004)), closed embayment regimes (Yugoslavia; (Murray, 2001)) and fish farming products (Croatia; (Vidović et al., 2009)). \( T. \) agglutinans is an opportunistic species and exhibits a preference for food–enriched conditions and a tolerance to oxygen deficiency (Barmawidjaja et al., 1995). Furthermore, \( N. \) turgida (as \( N. \) opima) and \( T. \) agglutinans are major species correlated with a high OM content at areas under the influence of Po River in Adriatic Sea (Jorissen, 1987; Murray, 2001).

Cluster II was composed of \( H. \) pacifica, \( B. \) dilatata and \( B. \) spathulata (Fig. 8). All these species are known to participate in benthic assemblages in oxygen poor and organic rich environments (Sen Gupta and Machain-Castillo, 1993; Murray, 2006). However their low degree of opportunism (Barmawidjaja et al., 1995) in combination
of their presence in areas of a river-influenced outer shelf (Bolivina spp; (Goineau et al., 2011)) and of well oxygenated substrates (Hyams-Kaphzan et al., 2009) suggests that Cluster II should represent benthic foraminiferal associations in lower stress environments than these of Cluster I.

Cluster III was composed of Quinqueloculina spp, Miliolinella spp, other porcelaneous, Textularia conica and P. mediterranensis (Fig. 8). High abundances of Quinqueloculina spp and in general Miliolids were found in oligotrophic environments and at sufficient bottom water oxygen concentrations (Murray, 2006; Blackwelder et al., 1996; Hyams-Kaphzan et al., 2009). Furthermore, the reduction in the abundance of both porcelaneous and agglutinated groups of benthic foraminifera was used to trace palaeo-hypoxic evolution (Platon et al., 2005).

Cluster IV was composed of Cassidulina spp., Nonion spp, Cibicides spp, Rosalina spp, B. aculeata and A. beccarii (Fig. 8). As mentioned previously, species belonging to the genera of Cibicides, Rosalina, Nonion and Ammonia are known as epiphytic in the Mediterranean Sea (Murray, 2001). However, B. aculeata and A. beccarii have been reported in high abundances on the seaward part of shelves with high OM supply (Goineau et al., 2011; Debenay et al., 2005). A similar trend exhibited species belonging to the genera of Cibicides and Rosalina which have been reported in high abundances seaward on shelves under the influence of aquaculture products (Croatia; Vidović et al., 2009).

The downcore fluctuations compared to the upper core in the abundances of the benthic species of Cluster I could be used to trace high OM supply and oxygen depletion. Cluster II would represent similar environments, but under lower stress conditions. The abundances of the benthic foraminifera species of Cluster III could be used to trace environments of well oxygenated conditions and that of Cluster IV of similar environments, but probably influenced by higher marine OM input and/or seagrass development.

Section 2

Eutrophication, productivity and OM sources

The TOC increased in both cores with time (Fig. 2), indicating a higher OM supply recently. This can be explained by an increased productivity, which is supported by the increase in TN values in both cores (Fig. 2). This interpretation agrees with the higher abundance of Cluster I species in core Amvr13 and Cluster IV species in core Amvr15, which indicated a larger OM input (Fig. 9) (Sen Gupta and Machain-Castillo, 1993; Blackwelder et al., 1996; Goineau et al., 2011; Debenay et al., 2005). Also other processes like oxygen availability or degree of opportunism can affect the abundance of these species (Sen Gupta and Machain-Castillo, 1993; Murray, 2006; Barmawidjaja et al., 1995), which might explain that Cluster II species did not increase. Many biomarkers also traced an enhanced productivity with time, for
instance by the increase in short chain $n$-alcohols, phytol and dinosterol towards the uppermost sediment in both cores (Fig. 2). These markers indicated an increased abundance of primary producers (Meyers and Ishiwatari, 1993; Rontani and Volkman, 2003; Volkman, 2003). The higher productivity was a direct result of progressive eutrophication in the gulf, as suggested by increasing $\delta^{15}$N values, which have been used to reconstruct sewage supply and eutrophication in other settings (Cole et al., 2004; Wu et al., 2006). The higher chlorin concentrations in core Amvr13 within the most recent decade (Fig. 2) agrees with this explanation. In contrast, the constant chlorin levels throughout core Amvr15 indicated that productivity hardly changed at this site.

The atomic C/N ratio below 10 (Fig. 2) indicated that the OM in core Amvr13 mainly originated from algal sources, in agreement with observations in other settings (Meyers and Ishiwatari, 1993). In contrast, the higher C/N ratios in core Amvr15 suggested significant supply of plant derived OM sources (Fig. 2). The constant $\delta^{13}$C$_{TOC}$ values throughout both cores indicated that the OM source remained constant during the last decades. The $\delta^{13}$C$_{TOC}$ values in Amvr15 (average: -18.6‰; Fig. 2) are typical for marine organic matter, whereas terrigenous OM sources average at -26‰ (Sackett, 1964; Jasper and Gagosian, 1990). The lower $\delta^{13}$C$_{TOC}$ values in Amvr13 (average: -22.6‰; Fig. 2) might indicate mixed marine and terrigenous sources, but this explanation disagrees with the low C/N values (Fig. 2), which are characteristic for a predominantly marine OM origin (Meyers and Ishiwatari, 1993). Therefore, the lower $\delta^{13}$C$_{TOC}$ values in Amvr13 were more likely the result of mixing and uptake of dissolved inorganic carbon derived from OM degradation, which can lead to isotopic shifts towards depleted values in eutrophic systems (van Breugel et al., 2006). This would mean that Amvrakikos Gulf is not in equilibrium with the atmosphere. Therefore, complicating factors that can affect the $\delta^{13}$C$_{TOC}$ like the Suess effect (Meyers, 2006; McCarroll and Loader, 2004) are expected to be of minor importance in Amvrakikos Gulf.

The relatively enriched $\delta^{13}$C$_{TOC}$ values in core Amvr15 most likely resulted from additional OM input by seagrass meadows, which were present at this sampling location. In the western Mediterranean Sea it was shown that the presence of seagrass meadows led to an overprint of the OM in the sediment (Papadimitriou et al., 2005). $\delta^{13}$C$_{TOC}$ values were enriched at sites with seagrass meadows compared to sites with predominant phytoplankton derived OM sources by 4-6‰, which is well within the offset observed between cores Amvr15 and Amvr13. Furthermore, seagrass meadows can lead to 2-3‰ depleted $\delta^{15}$N values (Papadimitriou et al., 2005), which would also explain lower $\delta^{15}$N values in Amvr15.

$\beta$-sitosterol is the major sterol of emersed macrophytes (Volkman, 1986). Therefore, seagrass might be a main source of this marker at least in Amvr15 (Fig. 5). The high $P_{aq}$ index above 0.2 in both cores (Fig. 5) traced the predominance of mid chain over
long chain $n$-alkanes (Ficken et al., 2000), which indicated that macrophytes are predominant lipid sources compared to higher land plant sources.

The disagreement between constant chlorin concentrations and the increase of many lipid biomarkers in Amvr15 (for instance short chain $n$-alcohols, phytol, dinosterol, β-sitosterol; Fig. 2, 5) indicated that degradation also affected the biomarker profiles. The CI is an estimate of the OM freshness (Schubert et al., 2005). CI values obtained in the gulf sediment indicated a lower degree of degradation in the uppermost part of both cores (Fig. 2). The CI profile in core Amvr15 was almost identical with the profile obtained in the Swiss lake Rotsee (Naehler et al., in press), suggesting similar degradation rates at both settings. The CI values were higher in core Amvr13 (Fig. 2), indicating higher degradation rates at this location. These results suggest that the increase in concentration of many biomarkers (for instance short chain $n$-alcohols, phytol, dinosterol, β-sitosterol; Fig. 5) are also affected by degradation. In agreement with the constant chlorin concentration values in Amvr15 (Fig. 2), the slight biomarker concentration decrease in this core might be due to degradation. But the much higher increase in biomarker concentrations in Amvr13 (for instance short chain $n$-alcohols, phytol, dinosterol, β-sitosterol; Fig. 5) must be mainly due to a higher productivity, which is supported in the increase in chlorin concentrations in this core (Fig. 2).

**Section 3**

Impact of eutrophication on benthic foraminifera and bacteria
– Hypoxia reconstructions in the gulf

The benthic assemblages increase in population was associated with a decreasing diversity as depicted by the high negative correlation between the H(s) and the benthic productivity indices (Pearson coefficient $r=-0.71$) for the entire dataset of the cores. This is in contrast to the usual trend of microfauna which appeared less diverse and less abundant in stressful, fluctuating environments (Blackwelder et al., 1996). However, the sediments of the gulf are under the influence of fish farming and urban waste. Eutrophication can lead to an increase in benthic foraminifera density (Angel et al., 2000) in conjunction to a decrease of diversity where the opportunistic species are dominant (Debenay et al., 2005). Therefore, the summed abundances of the benthic species of Clusters I and II showed high positive correlation with the benthic productivity index (Pearson coefficient $r=0.83$) and high negative correlation with the diversity index ($r=-0.87$) for the entire dataset. The opposite trend presents the summed abundances of the benthic species of Clusters III and IV, which showed high positive correlation with the diversity index (Pearson coefficient $r=0.72$) and negative correlation with the benthic productivity index ($r=-0.74$) for the entire dataset.

The benthic foraminifera in core Amvr13 represent low diversity, but high abundance assemblages (Fig. 9). The coring site of core Amvr13 is under the influence of large OM supply and stratified water masses promoting the development of low oxygen
bottom water. This bottom-water environment is suggested by the dominance of species of Cluster I and II over those of Cluster III and IV. Furthermore, fluctuations in the abundances of the four clusters in conjunction with fluctuations in the benthic abundance and diversity index indicate fluctuating sea bottom environmental changes for the last 35 years. The development of more unfavourable benthic environments occurred at around 1985-1988, 1994-1997 and after 2000, as shown by decreases of Cluster III and increases of Cluster I (Fig. 9).

Around 1980 and since the end of the 1990s, a higher bacterial biomass was observed in the same core, as indicated by higher concentrations of branched alkanes, isoprenoids and hopanoids (Fig. 5). The profiles of these markers were especially similar to B. aculeata (Fig. 3), which is indicative of a higher supply of OM and severe oxygen depletion. These results suggest that the higher supply of OM has led to increased OM mineralisation and oxygen consumption rates. These developments might be related to aquacultures, which started around 1980. They excessively supplied nutrients and OM to the gulf, which was a main reason for eutrophication (Kountoura and Zacharias, 2011; Ferentinos et al., 2010). Furthermore, the testimonies of the fishermen often refer to a large number of fish deaths and reduction of fish populations in aquaculture rafts between 1988 and 1997.

The most stressful conditions started at around 2000, as recorded by a gradual increase in the abundance of Cluster I over Cluster III. These conditions peaked with the almost absence of Cluster III in 2008 at the time of the recently recorded seasonal hypoxic event in the gulf. However, the rapid increase of Cluster III and the H(s) index shortly after the event suggests a fast recovery of the benthic environment (Fig. 9). These conditions starting at around 2000 and peaking in 2008 were already indicated by higher concentrations of bacterial biomarkers since the mid/end 1990s (Fig. 5). These increased concentrations were in good agreement with the intensified productivity and the resulting higher OM supply to the sediment, which had the same implications as during the 1980s.

In contrast, the constant abundance of chlorins and lower concentrations of bacterial markers throughout core Amvr15 indicated less severe conditions than at site Amvr13 without significant changes in productivity and OM supply (Fig. 2, 5). Benthic assemblages at site Amvr15 appeared to be less productive and more diversified than those of core Amvr13. The dominance of Cluster III and IV species (Fig. 9) suggested lower OM supply, hence, higher sea floor oxygenation. This can be attributed to the location of site Amvr15 at the entrance of the gulf characterized by sufficient oxygen supply due to the water replenishment from the Ionian Sea (Ferentinos et al., 2010). Tziavos and Vouloumanos (1994) also reported a reduction of the benthic diversity eastwards in the surface sediments of the gulf. The observation of seagrass meadows at site Amvr15 and their absence at site Amvr13 further supports the lower impact of eutrophication (Green and Short, 2003).
More opportunistic species of Cluster IV replaced Cluster III species at site Amvr15 during times of nutrient enrichment and oxygen depletion (Fig. 9). This was especially the case between 1976 and 1980, the time interval when aquaculture development started. However, the reduction of both Clusters III and IV at 1985-1987 and 1995-1997 (Fig. 9) and a higher bacterial biomass around 1980 (Fig. 5) coincided with relative changes at site Amvr13, which suggested similar control mechanisms and time synchronicity in the bottom water oxygen regime between the two coring sites. Furthermore, similar to site Amvr13, the most severe sea floor conditions in respect to oxygen at site Amvr15, appeared in 2008 as suggested by the almost absence of Cluster III and a higher bacterial biomass around that time (Fig. 5, 9). This indicates that the effects of that hypoxic event did not only influence the benthic fauna of the inner part of the gulf, but was also spread in areas which are considered as throughout the year being well oxygenated. However the impact of hypoxia on the benthic microfauna at the bottom of the gulf entrance was less intense than that occurred in the inner part of the gulf, since species considered being sensitive to hypoxia (Cluster IV) were still highly abundant (Fig. 9) and seagrass meadows were also present at this site.

Furthermore, the more severe oxygen depletion at Amvr13 compared to Amvr15 was also indicated by the increase of the (lycopane + C_{35} n-alkane) / C_{31} n-alkane ratio with depth (Fig. 6). In contrast, the quite constant ratio in core Amvr15 (Fig. 6) suggested more oxygenated conditions.

Isorenieratane and chlorobactane were used as tracers for phototrophic sulfur bacteria (Chlorobiaceae) and phototrophic zone euxinia and anoxia (Brocks and Summons, 2003). The observation of these carotenoids throughout both cores indicated regularly occurring developments of anoxic and euxinic conditions reaching into the photic zone at both sites. The monitoring data showed that oxygen depletion has occurred seasonally during summer and fall in the western part of the gulf (Ferentinos et al., 2010; Kountoura and Zacharias, 2011). Due to the low concentrations of these pigments in the sediment of both cores only the existence of photic zone anoxia but no temporal changes could be reconstructed.

Section 4

Impact of climate on stratification and oxygen replenishment

Stratification is another key factor in the oxygen budget of the gulf water column, because oxygen is also resupplied by mixing. The relatively constant tetrahymanol concentrations in core Amvr15 (Fig. 5) suggested that stratification did not change since the 1970s. In contrast, the increase of tetrahymanol in the uppermost part of core Amvr13 (Fig. 5) clearly showed a higher stratification and stagnation, probably due to higher water temperatures and/or salinities in the gulf. To prove these relationships, sedimentary proxies were compared with monitoring data.
The UK’37 index (Me C_{37:2}/[Me C_{37:2} + Me C_{37:3}]; by Prahl and Wakeham, 1987) was used to estimate surface water temperatures in Amvrakikos Gulf. The calibration of Prahl et al. (1988) yielded values between 16.9 and 23.6°C and between 17.4 and 21.9°C in cores Amvr15 and Amvr13, respectively. For comparison, the temperature calibration by Müller et al. (1998) which is based on sediment core top samples worldwide yielded very similar results (16.5-23.4°C in core Amvr15 and 17.0-21.7°C in Amvr13; Fig. 7). The difference between estimated surface water temperatures using both calibrations was only up to 0.4°C.

The temperature estimates from both cores indicated a trend with increased surface water temperatures, which matched well with increasing air temperatures observed at the Preveza (Aktio) weather station during the last decades (Fig. 7). The UK’37 derived surface water temperatures in both cores (16.5-23.4°C, 1974-2010; Fig. 7) were within the range of monitored average annual minimum and maximum air temperatures with values between about 12 and 22°C (1970-2010), except the uppermost part of core Amvr15 with larger UK’37 temperature estimates. The annual average air temperatures ranged between 16.2 and 18.0°C (1970-2010), and were lower than estimated UK’37 temperatures (Fig. 7). Although the timing of blooms of the source organisms is unknown for Amvrakikos Gulf, the best agreement between UK’37 derived and monitored temperatures was obtained if the average temperature of March until August was used. The air temperatures ranged from 18.2 to 21.1°C (1970-2010; Fig. 7). The monitoring data showed that especially the annual minimum temperatures increased during the last decades, whereas the annual maximum temperatures hardly increased. By using only monthly average minimum temperature data, the best fit was obtained with the average minimum temperature data of June and July with a range of 16.8-21.2°C (Fig. 7). These results indicated that alkenones might have captured the lowermost surface water temperatures during June and July. In contrast, this and other calibrations showed strong correlations either with annual average temperatures or seasonal average temperatures at times of blooms (Herbert, 2003; Müller et al., 1998; Prahl et al., 1988). Therefore, a shift of the time of blooms from spring towards summer is more likely, which would also explain the large increase in estimated temperatures during the last decades.

Nonetheless, the warming trend of UK’37 derived temperatures together with the monitoring data indicated that the surface water temperatures in Amvrakikos Gulf increased during the last decades. Previous studies showed that steep temperature gradients separate surface and bottom waters (Ferentinos et al., 2010). Higher temperatures in the surface water may further increase these differences, which can explain the observation of a higher stratification during the last years.

Furthermore, also salinity controls stratification by steep salinity gradients in the water column due to the inflow of high salinity water masses through the Preveza Straits from the Ionian Sea and brackish water outflow (Ferentinos et al., 2010). This circulation pattern is similar to the Black Sea, but the reduced outflow of the Black Sea prevents the reduction of its permanent stratification (Murray et al., 2007; Ozsoy...
and Unluata, 1997), which is not the case for Amvrakikos Gulf (Ferentinos et al., 2010). However, the similar developments regarding circulation patterns, eutrophication and temperature have led to a strong decrease in oxygen concentrations, which resulted in mass mortality events and ecosystem collapse in both settings (Ferentinos et al., 2010; Kountoura and Zacharias, 2011; Lancelot et al., 2002; Mee et al., 2005).

**Conclusions**

The analysis of benthic foraminifera and lipid biomarkers revealed that Amvrakikos Gulf exhibited dramatic environmental changes due to eutrophication during the last decades. The higher productivity and OM supply to the sediment (higher concentrations of chlorins, TOC, TN and $\delta^{15}N$ values) led to a higher abundance of tolerant and opportunistic benthic species and bacteria, whereas the benthic species density decreased. Especially the increased abundance of Cluster I (B. elongata, N. turgida, T. agglutinans, A. tepida) over Cluster III species (Quinqueloculina spp, Miliolinella spp, other porcelaneous, Textularia conica, P. mediterranensis) indicated more severe OM supply and oxygen depletion in 1976, 1980, 1985-1987, 1995-1997, 2000 and 2008. Cluster III and IV species (Cluster IV: Cassidulina spp., Nonion spp., Cibicides spp, Rosalina spp, B. aculeata, A. beccarii) rapidly recovered after environmental disturbances. In core Amvr15 the benthic assemblages appeared to be less productive and more diversified with a dominance of species of Clusters III and IV under conditions of lower OM supply and higher bottom water oxygen concentrations than in core Amvr13. The presence of seagrass at site Amvr15 largely influenced the values of $\delta^{13}C_{TOC}$, C/N ratio and mid chain $n$-alkanes. Nonetheless, the presence of isorenieratane and chlorobactane in both cores traced temporarily photic zone euxinic conditions throughout the gulf. The increasing air temperatures have led to stronger stratification and hence oxygen depletion during the last decade.

**Acknowledgements**

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**Literature**


Amvrakikos gulf (Western Greece) as a part of IAEA’s campaign in the Adriatic and Ionian Seas. Radiation Protection Dosimetry, 1–14.
Figure 1. Map of Amvrakikos Gulf, Greece. The major rivers (Louros and Arachthos Rivers), the Preveza Straits (connection with the Ionian Sea) and the sampling stations of cores Amvr13 and Amvr15 are illustrated.
Figure 2. Bulk parameters of cores Amvr13 and Amvr15 plotted vs. age (yr AD), including the concentrations of total organic carbon (TOC), total nitrogen (TN), nitrogen isotopic composition ($\delta^{15}\text{N}, \%_{\text{air}}$), chlorin concentrations (mg g$^{-1}$ TOC), chlorin index (CI, including values from the Swiss lake Rotsee (Naeher et al., in press)), the atomic C/N ratio and the TOC isotopic composition ($\delta^{13}\text{C}_{\text{TOC}}, \%_{\text{VPDB}}$).
**Figure 3.** Downcore abundance variations of selected benthic foraminifera in core Amvr15 versus sediment depth together with the indices of benthic productivity (benthic foraminifera specimen/g of dry sediment) and diversity (H(s)). Grey bands in the diagrams of *Textularia spp* and *Nonionella spp* indicate the participation of *T. agglutinans* and *N. turgida*, respectively.
Figure 4. Downcore abundance variations of selected benthic foraminifera in core Amvr13 versus sediment depth, together with the indices of benthic productivity (benthic foraminifera specimen/ g of dry sediment) and diversity (H(s)). Grey bands in the diagrams of *Textularia spp* and *Nonionella spp* indicate the participation of *T. agglutinans* and *N. turgida*, respectively.
Figure 5. Concentrations (µg g⁻¹ TOC) of the sums of branched alkanes/isoprenoids, hopanoids, short chain $n$-alcohols (C₁₁-C₂₀), phytol, dinosterol, β-sitosterol, $P_{aq}$ index (by Ficken et al., 2000) and tetrahymanol plotted vs. age (yr AD).
Figure 6. Profiles of (lycopane + C_{35} n-alkane) / C_{31} n-alkane ratio vs. age (yr AD).
**Figure 7.** UK$^{37}$ index derived surface water temperatures (left) according to the correlation of Müller et al. (1998), $\text{UK'}^{37} = 0.033 \ T + 0.069$ ($R^2=0.981$) with $T =$ mean annual sea surface temperature, plotted vs. age (yr AD). For comparison, temperature data (°C) between 1970 and 2010 from the meteorological station Preveza (Aktio) were added: Annual average temperatures, average temperatures of March-August, average minimum temperatures of June-July.
Figure 8. R-mode cluster analysis for the entire dataset of the two cores.
**Figure 9.** Downcore variations of the sum abundances of the benthic foraminifera clusters (I-IV) in cores Amvr13 and Amvr15 vs. age (yr AD).
3) Maleimides in recent sediments – New insights into chlorophyll degradation and palaeoenvironmental reconstructions

Authors
Naeher S., Schaeffer, P., Adam, P., Schubert C.J.

Abstract
Maleimides (transformation products of chlorophylls and bacteriochlorophylls) were studied in recent sediments from the Swiss lake Rotsee and the Romanian Black Sea Shelf to investigate chlorophyll degradation, the role of oxygen in maleimide formation, and to identify their sources. 2-Methyl-maleimide (Me,H), 2,3-dimethyl-maleimide (Me,Me), 2-methyl-3-ethyl-maleimide (Me,Et) and traces of 2-methyl-3-isobutyl-maleimide (Me,i-Bu) occurred naturally in the sediment with a large predominance of Me,Et. A more complex distribution was obtained after chromic acid oxidation. Primary producers were the main source of Me,Et, whereas Me,H and Me,Me might originate from unknown bacteriochlorophyll precursors. 2-Methyl-3-n-propyl-maleimide (Me,n-Pr) and Me,i-Bu traced the presence of phototrophic sulfur bacteria (Chlorobiaceae), which indicated photic zone euxinic and anoxic conditions in Rotsee and throughout the Black Sea history, including the limnic phase of the Black Sea (Unit 3). The other maleimides most likely originated from unknown bacteriochlorophylls or were derived by bacterial reworking. Oxygen was crucial for maleimide formation in the water column of both settings. Novel maleimide degradation indices were proposed to estimate the degree of OM degradation. These proxies were applicable to longer timescales than e.g. the chlorin index.

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Maleimides in recent sediments – New insights into chlorophyll degradation and palaeoenvironmental reconstructions

Naeher S., Schaeffer, P., Adam, P., Schubert C.J.

Introduction

Chlorophylls and bacteriochlorophylls are the most abundant and most important pigments on earth. These structural diverse compounds are required for light absorption and are key compounds for photosynthesis (Scheer, 2006). Apart from their physiological importance, they and their transformation products (chlorins, porphyrins, maleimides) can be preserved in limnic and marine sediments and crude oils (Hodgson et al., 1968; Quirke et al., 1980; Grice et al., 1996).

These compounds can serve as important indicators for palaeoenvironmental reconstructions, such as e.g. palaeoproduction (Harris et al., 1996) and OM freshness (Schubert et al., 2010). Within the water column and in the sediment, chlorins undergo minor or major transformation processes, which continue during diagenesis and lead to the formation of porphyrins and maleimides (Villanueva and Hastings, 2000). Porphyrins, diagenetic transformation products of chlorophylls, have been intensely studied since the 1930s (Treibs, 1936; Barwise and Roberts, 1984). In contrast, maleimides (1H-pyrrole-2,5-diones), oxidation products of tetrapyrrole pigments, have been hardly examined so far. Most studies focused on the degradation of porphyrins in crude oils and ancient deposits, for instance Permian Kupferschiefer and Mid-Triassic shales (Grice et al., 1996; Grice et al., 1997), Cretaceous Boscan crude oil (Quirke et al., 1980), Cretaceous/Tertiary boundary formations (Shimoyama et al., 2001) and Neogene sediments (Kozono et al., 2001). The only study in recent deposits reported the presence of naturally occurring Me,Et in sediments from Tokyo Bay, Japan (Kozono et al., 2002). The authors explained this observation by chlorophyll oxidation in the photic zone under the presence of light and oxygen.

Me,Et and Me,Me were assigned to phytoplanktonic chlorophylls based on structural reasons (Grice et al., 1996). The origin of Me,n-Pr and Me,i-Bu are bacteriochlorophylls (BCH) c, d and e from phototropic sulphur bacteria (PSB) of the family Chlorobiaceae (Grice et al., 1996). Together with isorenieratane and chlorobactane these maleimides were used to reconstruct photic zone euxinia (Grice et al., 1996; Pancost et al., 2004). However, there is still a need for the detection and characterisation of maleimides in recent sediments to determine their partly unknown precursors and their formation processes.

The aim of this paper was to study naturally occurring maleimides in recent sediments of the Swiss lake Rotsee and the Black Sea in comparison with maleimides released after chromic acid oxidation, to investigate chlorophyll and chlorin degradation and to identify maleimide sources. The role of oxygen in maleimide formation was
evaluated. Finally, maleimide indices were proposed as new indicators for OM degradation.

**Methods**

**Section 1**

**Study sites and sample collection**

Rotsee is a small (0.46 km²), prealpine, monomictic and eutrophic Swiss lake. It has a stable stratified water column with a chemocline between ca. 6 and 10 m depth and an anoxic hypolimnion during most of the year (Schubert et al., 2010). At the maximum lake depth of 16 m, two sediment cores (54 and 58 cm long) were recovered with a gravity corer in August 2010 (GPS position N47°4.251 E8°18.955, WGS84), the same location of the cores from Naehler et al. (in press). Therefore, the age model of these cores were assumed to be identical, with a sedimentation rate of ca. 0.38 cm yr⁻¹ (Naehler et al., in press). The cores were sliced in 2 cm intervals and frozen at -20°C prior to analysis.

The Black Sea (0.46 million km², max. depth of 2220 m) is a semi-enclosed brackish sea and the largest modern anoxic basin in the world (Ozsoy and Unluata, 1997). It is connected to the Marmara Sea and the Mediterranean Sea through the Bosphorus and Dardanelles Straits. Since 9.3 ka, inflow of more saline water through the Bosphorus strait into the deep water of the Black Sea has led to a permanent stratification with the pycnocline located in 50-150 m water depth (Ozsoy and Unluata, 1997; Piper and Calvert, 2011). The Black Sea evolved from an isolated limnic-brackish lake to a restricted intermediate-salinity sea (Ross and Degens, 1974; Piper and Calvert, 2011). Since approximately 8.4 ka the abyssal part of the basin has been governed by bottom water anoxia due to permanent stratification (Piper and Calvert, 2011).

A 242 cm long sediment core was recovered with a gravity corer at the sampling station 10MA10 (N 43° 43.905 E 30° 11.962, WGS84) in a water depth of 220 m during the Mare Nigrum cruise on the Romanian Black Sea Shelf in May 2010. The core was sampled like the Rotsee cores. It showed the typical Black Sea sediment sequence (Ross and Degens, 1974; Piper and Calvert, 2011). The transition of Unit 3 to Unit 2 was at 109 cm, with a clear colour contrast and a sandy base of Unit 2. This transition could not have occurred earlier than about 6 ka BP in this shallow water depth based on estimates by Ross and Degens (1974). Unconformities at 86 cm and 97 cm indicated an incomplete sedimentation record. Peaks of the magnetic susceptibility at 86, 97 and 109 cm were the result of higher relative sand contents. Within Unit 3, the magnetic susceptibility was partly much higher due to sulphidic intercalations in the grey clay. Based on estimates of Ross and Degens (1974), the core might comprise ca. 9-10 ka.

**Section 2**
Sample preparation

2.1 Lipid biomarkers and pigments

The same lipid biomarker and pigment analysis procedure was used as reported in Naéher et al. (in press). In short, the sediment was extracted by ultrasonication with mixtures of methanol (MeOH) and dichloromethane (DCM). An internal standard was added for quantification (α-Cholestane, C19 n-fatty acid, C19 n-alcohol). After saponification, neutrals were further separated into apolar and polar fractions over NH2 columns (Hinrichs et al., 2003). The polar fraction was derivatised with BSTFA for 1h at 80°C and FA with 14% BF3/MeOH. FA double bond positions were determined according to (Spitzer, 1997). In case of Black Sea sediment extracts, an aliquot of the polar fraction was desulfurized with Raney-Nickel catalyst (Sinninghe Damsté et al., 1988), followed by hydrogenation for 2h with PtO2 as catalyst in a solution of concentrated acetic acid and ethyl acetate (1:1, v:v). Instruments and measurement conditions are described in Naéher et al. (in press).

2.2 Maleimides

For maleimide analysis the method of Grice et al. (1996) was modified. Also the same maleimide nomenclature was used. Freeze-dried and ground sediment samples from Rotsee (13-28 g) and the Black Sea (58-296 g) were three times extracted with 150-500 mL DCM/acetone (1:1, v:v), first stirring for 1 h, followed by 2x20 min ultrasonication. After each extraction step, the aliquots were centrifuged (2800 rpm, 4 min), combined and rotary evaporated. While one half of the total lipid extract remained untreated (comprising naturally occurring maleimides; free fraction (FF)), the other half was oxidised with chromic acid (comprising naturally occurring maleimides and maleimides obtained upon chromic acid oxidation of chlorophylls and related tetrapyrrole pigments in the sediment; oxidised fraction (OF)) using modifications of existing protocols (Quirke et al., 1980; Folly and Engel, 1999). For oxidation, the extract was dissolved in 1 mL trifluoroacetic acid (TFA). 50 mL of 1.7% chromic acid was added dropwise under continuous stirring for 1 h at room temperature. In a separation funnel, the solution was extracted with pure ethyl acetate (EtAc, 3x) and DCM (1x). FF and OF were separated on a silica column into three fractions, eluted with pure DCM (F1), 20% EtAc in DCM (F2) and DCM/MeOH (1:1, v:v, F3). A known aliquot of F2 was further purified by preparative thin layer chromatography (TLC), eluted with 20% EtAc in DCM on Merck silica gel plates (10x10 cm², 0.5 mm thickness, preeluted with EtAc, activated at 150°C). H,H maleimide (M=97.07 g mol⁻¹, 99%, Sigma Aldrich) was used as a retention standard (retention factor Rf=0.5), eluted in parallel with the sample. Maleimides were visualised with UV light and the band between ca. Rf=0.5 and 0.8 was recovered on a short silica column with pure EtAc. The rotary evaporated extract was dissolved in 150 µL pyridine and derivatised over night with 100 µL MTBSTFA (N-(tert-butyldimethylsilyl)-N-methyl trifluoroacetamide, M=241.3 g mol⁻¹, Pierce) to obtain TBDMS (tert-butyldimethylsilyl) derivatives. The solvent was removed under N2 at
room temperature, followed by further purification of these fractions on a silica column by elution with DCM. The purified fractions were measured on a gas chromatograph with flame ionisation detector (GC-FID) and on a gas chromatograph mass spectrometer (GC-MS). Me,H, Me,Me and Me,Et were quantified by coinjection with the MTBSTFA derivatised H,H maleimide reference (ca. 12 ng/injection). The other maleimides were quantified based on the Me,Me concentration, by the area of the m/z=75 fragment on the GC-MS. For Black Sea samples Me,n-Bu was quantified with m/z=224 of Me,i-Bu due to low concentrations. Similarly, for Me,i-Pentyl and Me,n-Pentyl the fragment m/z=238 was compared with the m/z=75 of Me,Me, so their concentrations could not be corrected for mass discrimination.

GC analysis was carried out on an Agilent Hewlett-Packard HP6890 GC-FID with an Agilent HP-5 column (30 m x 0.32 mm inner diameter (ID) x 0.25 μm film thickness (FT)) with an on-column injector and a flame ionisation detector. The GC oven temperature program started at 40°C, then heated at a rate of 10°C min⁻¹ to 100°C, followed with a rate of 4°C min⁻¹ up to 300°C after which the temperature was maintained for 30 min. A hydrogen carrier gas flow of 2.5 mL min⁻¹ was used.

GC-MS analysis was performed with a Thermo TSQ Quantum, HP-5 column (30 m x 0.25 mm ID x 0.25 μm FT), with Helium as the carrier gas (1 mL min⁻¹), equipped with a simile on-column injector (electron ionisation, 70 eV, source temperature 210°C). The m/z scan range was set between 50 and 700. The oven program started with 40°C for 10 min, then heated at a rate of 10°C min⁻¹ to 100°C, followed with a rate of 4°C min⁻¹ up to 300°C and remained isothermal for 40 min. The MS was switched on after 10 min and switched off at 50 min to prevent contamination of the filament due to too high signal intensities. Single ion monitoring (SIM) was performed on m/z=75.0, 168.1, 182.1, 196.2, 210.2, 224.2, 238.2.

**Section 3**

**Definition of Me,Me and Me,Et indices**

Novel maleimides based degradation indices were defined as follows:

Me,Me index = ([Me,Me] in the free fraction) / ([Me,Me] in the oxidised fraction)
Me,Et index = ([Me,Et] in the free fraction) / ([Me,Et] in the oxidised fraction)

Where:

[Me,Me] = concentration of 2,3-dimethyl-maleimide (µg g⁻¹ TOC)
[Me,Et] = concentration of 2-methyl-3-ethyl-maleimide (µg g⁻¹ TOC)
Results and discussion

Section 1

Me,H, Me,Me and Me,Et in the free and oxidised fraction

Me,Et was the dominant maleimide (>96%) in both fractions of Rotsee and the Black Sea (Fig. 1, 2, 3). The very large predominance in all distributions is to be expected since this compound can be formed from most of the tetrapyrrole pigments, including notably chlorophyll-a derivatives from primary producers living in the oxic part of the water column, as well as from BCH from photosynthetic sulphur bacteria (Grice et al., 1996). Naturally occurring Me,Et has only once been described before in recent sediments (Kozono et al., 2002). In Rotsee, Me,Et in the FF peaked at around 1933, whereas in the OF it was most abundant in the beginning 1920s and during the 1960s (Fig. 2). The coincidence between Me,Et in the FF and higher bacterial biomass in 1933 (Naeher et al., in press) indicated that naturally occurring Me,Et was released by bacterial degradation of chlorophylls. In contrast, the peaks of maleimides after oxidation matched well with productivity peaks during the 1920s and 1960s (Naeher et al., in press), which confirmed that Me,Et mainly originates from chlorophyll-a.

The higher Me,Et concentrations in the OF from of Black Sea Unit 1 and 2 (Fig. 3) also indicated a higher productivity, which agrees with observations of a more abundant microbial biomass compared to Unit 3 (Ross and Degens, 1974; Repeta, 1993). This interpretation is supported by higher concentrations of dinosterol, C_{22:6}, C_{25:5} and C_{25:4} FA, 24-Methyl-Cholesterol, C_{30}-C_{32} alkyl diols, C_{30} keto-ol and C_{30} highly branched isoprenoid alkenes in Unit 1 and 2, which traced more abundant dinoflagellates, diatoms and other microalgae (Volkman et al., 1992; Shanchun et al., 1994; Massé et al., 2004a; Massé et al., 2004b). However, the presence of lupeol, β-amyrrine and its degradation products together with the dominance of des-A-arbora-5(10),9(11)-dien in Unit 1 also indicated a high supply of plant OM (Hauke et al., 1992; Jaffé and Hausmann, 1995) on the Romanian Shelf, most of which is supplied by the Danube delta (Shimkus and Trimonis, 1974). Therefore, Me,Et in Romanian Shelf sediment must be derived to a large extent from plant chlorophylls. In contrast, the OM in Rotsee is predominantly of aquatic origin (Naeher et al., in press).

Me,H and Me,Me in the sediment of both settings were much less abundant than Me,Et (Fig. 2, 3). The concentrations of Me,H and Me,Me were highest at 28-30 cm (1929-1934) in the FF and OF from Rotsee (Fig. 2). In contrast, Me,H in both fractions and Me,Me in the FF were most abundant in Unit 3, whereas Me,Me concentrations were higher in the surface sediment (Fig. 3).

For the second time, Me,H was found at both sites to be the second most abundant maleimide in the OF (Fig. 2,3), as usually Me,Me being the second most common, at least in petroleum and other ancient sediments (Shimoyama et al., 2001). Based on a constant ratio of Me,Me to Me,Et, a primary production source was proposed for
Me,Me (Grice et al., 1996), but this ratio was not constant in Rotsee. This ratio ranged between 0.17 and 0.34 and between 0.14 and 0.64 in the FF and OF, respectively. Although based on structural reasons Me,Me has no related chlorophyll, it can be produced by maleimide transformation (Verne-Mismer et al., 1988). Newer studies suggested that the degradation of Me,Et to Me,Me depends on maturity (Kozono et al., 2001; Shimoyama et al., 2001), but Rotsee sediments are low maturity deposits (Naeher et al., in press), so a relationship with maturity can be excluded. In contrast, different formation pathways have been proposed for Me,H, including chlorophyll-c and pheophorbide transformation (Ellsworth, 1970; Verne-Mismer et al., 1988).

The higher concentrations of Me,H, Me,Me and Me,Et in the FF in ca. 1933 matched with a higher bacterial biomass at that time (Naeher et al., in press). Therefore, bacterial transformation rates of chlorophylls might have been higher at this time, which could explain why no bacteriochlorophyll precursors of these maleimides are known.

The concentrations of Me,H and Me,Me in the OF from the lowermost sample were slightly lower than in the FF (Fig. 2). It was expected that the concentrations of these maleimides must be equal or higher in the OF, because the OF comprises naturally occurring maleimides (found in the FF) in addition to maleimides obtained after chromic acid oxidation. Therefore, this offset was likely due to analytical uncertainties. This also means that the source chlorophylls and chlorins have been entirely degraded at this sediment depth.

Section 2

Maleimides and photic zone euxinia

Traces of Me,i-Bu was found in the FF and OF in Rotsee and the Black Sea, but Me,n-Pr was only detected in the OF at both sites (Fig. 4, 5). Me,i-Bu and Me,n-Pr concentrations were highest at 32-34 cm (1918-1924, Fig. 5). Based on structural reasons, Me,i-Bu and Me,n-Pr originate from BCH c and d (green, shallower dwelling Chlorobiaceae) and e (green-brown, deeper dwelling Chlorobiaceae) (Pfennig, 1978). Only one of the four pyrrole rings potentially contains n-Pr and i-Bu alkylations (Pfennig, 1978). Grice et al. (1996) used Me,n-Pr, Me,i-Bu and the Me,i-Bu/Me,Et ratio as tracers for Chlorobiaceae and photic zone euxinia and anoxia in the Zechstein Sea, supported by carbon isotopic data and the correlation with chlorobactane and isorenieratane. The high correlation between Me,n-Pr and Me,i-Bu in Rotsee (R²=0.95) suggested the same origin (Fig. 4), although an additional formation pathway of Me,n-Pr from chlorophyll-a was proposed (Verne-Mismer et al., 1986). This process might be responsible for the absence of any correlation between Me,n-Pr and Me,i-Bu in the Black Sea sediment.

In contrast, the principal pigments of purple PSB (Chromatiaceae) are BCH a and b, but BCH a is also present as an accessory pigment in Chlorobiaceae (Pfennig, 1978).
Based on structural reasons, chromic acid oxidation of BCH a and b can only yield maleimides with ethyl, phytyl and geranylgeranyl side chains (Pfennig, 1978). Therefore, no maleimides specific to Chromatiaceae exist, purple PSB can only be traced by the carotenoid okenone (Brocks et al., 2005).

The higher concentrations of okenone in Rotsee compared to isorenieratene (Züllig, 1985) indicated the higher abundance of Chromatiaceae compared to Chlorobiaceae, which is supported by results from microbial data (Kohler et al., 1984). Therefore, the presence of okenone and isorenieratane together with Me,i-Bu and Me,n-Pr indicated photic zone euxinic and anoxic conditions in Rotsee. Isorenieratane could not be quantified due to low concentrations, but the decrease of Me,i-Bu and Me,n-Pr since the 1920s together with continuously increasing okenone concentrations since then (Züllig, 1985) indicated a community shift towards Chromatiaceae. The reason of this shift might be the increased productivity and eutrophication (Naeher et al., in press), in line with previous observations indicating that these conditions are favoured by Chromatiaceae (Smittenberg et al., 2004).

The absence of BCH c and d and the presence of BCH e in Rotsee (Kohler et al., 1984) together with okenone only traced the occurrence of brown and purple PSB. Therefore, Me,i-Bu and Me,n-Pr must only originate from brown PSB in this lake. The BCH e maximum below the peak of BCH a (Kohler et al., 1984) agrees with the typical vertical segregation of deeper dwelling brown Chromatiaceae below shallower Chromatiaceae (Ormerod, 1983) in the chemocline of Rotsee.

The presence of Me,n-Pr and Me,i-Bu in all Black Sea units traced Chlorobiaceae in the chemocline of the Romanian Black Sea Shelf. Their detection in Unit 3 suggested at least temporary photic zone anoxia before the Bosphorus connection. In contrast, isorenieratane and chlorobactane were only found in Unit 1 and 2. Throughout the core Me,n-Pr concentrations were 3-6x higher than Me,i-Bu. In contrast, BCH e in the central Black Sea consisted of pyrrole rings with 45% 4-ethyl, 16% 4-n-propyl and 15% 4- i-butyl side chain alkylations (Repeta et al., 1989). Repeta and Simpson (1991) showed that purple and green PSB were not present, because okenone, BCH a and b and chlorobactene were absent. The predominance of the deeper dwelling brown PSB (BCH e, isorenieratene) in the Black Sea chemocline was attributed to light and sulphide limitation with the H2S chemocline at depths ≤140 m (Repeta et al., 1989; Repeta and Simpson, 1991). In contrast, CTD data during sampling of the cores from the Romanian Shelf indicated that the chemocline was located at ca. 50-70 m water depth during sampling compared to 80-100 m in the study of Repeta and Simpson (1991). The presence of chlorobactane in addition to isorenieratane and the shallower chemocline might indicate that also green PSB are present in the chemocline on the shelf. Therefore, Me,n-Pr and Me,i-Bu might originate from mixed sources of BCH c, d and e, which can be the reason for the different maleimide distribution compared to Repeta et al. (1989).
Et,Et was proposed to be of derived from BCH c, d and e based on structural reasons (Grice et al., 1996). But a Chlorobiaceae derived source is not evident due to the Et,Et concentration maximum at 28-30 cm (Fig. 5), which indicated either a bacteriochlorophyll origin or it is derived by bacterial transformation (section 3.1).

**Section 3**

**Other maleimides in the oxidised fraction of Rotsee and the Black Sea**

Chromic acid oxidation also yielded other maleimides, comprising 2-methyl-3-sec-butyl-maleimide (Me,sec-Bu), 2-methyl-3-n-butyl-maleimide (Me,n-Bu), 2-methyl-3-isopentyl-maleimide (Me,i-Pentyl), 2-methyl-3-n-pentyl-maleimide (Me,n-Pentyl) and 2-ethyl-3-n-propyl-maleimide (Et,n-Pr) with concentrations <2 µg g⁻¹ TOC in Rotsee (Fig. 5). Me,sec-Bu and Et,n-Pr could not be detected in the Black Sea. Me,n-Bu, Me, i-Pentyl, Me,n-Pentyl were not detected in the lowermost sample of Rotsee (Fig. 5). All of these maleimides were proposed to be of tetrapyrrole origin, but chlorophylls with such substituents are unknown (Grice et al., 1996). Possible porphyrin sources (Callot, 1991) can be excluded due to the low maturity of the Rotsee deposits. Porphyrin side chain alkylations by thermal cracking of kerogen as proposed for Venezuelan Boscan crude oils (Quirke et al., 1980) are unlikely due to the same reason. This also explains the absence of benzomaleimides (phthalimides) and methyl-benzomaleimides at both sites, which would be typical maleimides in kerogen-rich deposits (Kozono et al., 2001).

Bacterial reworking of chlorophylls and chlorins might also explain concentration peaks of Me,sec-Bu, Me,n-Bu, Et,n-Pr, Me,i-Pentyl and Me,n-Pentyl at 28-30 cm (1929-1934, Fig. 5). These results matched well with high hopanoid concentrations and a higher bacterial biomass at this time (Naeher et al., in press). Such specific alkylated side chains might be formed by bacterial alterations prior to tetrapyrrole decomposition of chlorophylls and chlorins, which can also explain why no precursor chlorophylls of these maleimides are known.

**Section 4**

**Chlorophyll and chlorin degradation and maleimide formation**

Due to the presence of Me,H, Me,Me, Me,Et and traces of Me,i-Bu (Fig. 2, 3), and the absence of all other maleimides in the FF (Fig. 5), questions arise regarding chlorin degradation. Maleimide formation and its timing could not be satisfactorily answered so far (e.g. Grice et al., 1996). Porphyrin degradation (Grice et al., 1996) can be excluded due to the absence of porphyrins in recent sediments (Hodgson et al., 1968). Other possible processes include photo-oxidative or enzymatically induced transformation of chlorins in the oxic parts of water columns (Brown et al., 1980; Rontani et al., 1991). However, maleimides can be also generated during early diagenesis, in lakes with overlying euxinic water columns (Magness, 2001), which could explain the occurrence of free maleimides in ancient deposits formed under
oxygen limited conditions (Grice et al., 1997). But the absence of free maleimides in Pliocene upwelling deposits (Pancost et al., 2009) implies that other maleimide formation pathways or specific prevailing environmental conditions lead to the decomposition of tetrapyrrole rings.

Maleimides can be formed by chlorophyll degradation in the photic zone under the presence of oxygen (Kozono et al., 2002). It was proposed that maleimides can be produced by sunlight irradiation of chlorophyll-a under the presence of oxygen in organic solvents (Jen and MacKinney, 1970), so measurement results are maybe biased (Grice et al., 1996; Kozono et al., 2002). But the presence of Me,H, Me,Me, Me,Et and traces of Me,i-Bu without the observation of other maleimides in the FF indicated that light cannot be the reason for the release of only specific maleimides. Therefore, the presence of oxygen might be crucial. Bacteriochlorophylls from phototrophic bacteria in the chemocline are likely preserved due to the lack of oxygen, which might explain the limitation of some maleimides to the OF. In contrast, a significant fraction of Me,H might be derived from the destruction of phaeophorbide-a (Ellsworth, 1970) and Me,Me may at least partially originate from demethylation of naturally occurring Me,Et or transformation of ethyl side chains in chlorophyll precursors (Kozono et al., 2001). At least in the Black Sea the increase of Me,H matched with decreasing Me,Et concentration, which might indicate this formation pathway. However, traces of naturally occurring Me,i-Bu indicated that maleimide formation cannot be limited to the oxygenated part of the photic zone. Therefore, also other electron acceptors might support chlorophyll destruction. This would explain why maleimides occurred naturally in settings with euxinic water columns (Magness, 2001).

**Section 5**

**Maleimide degradation indices**

In contrast to Me,n-Pr and Me,i-Bu, Me,sec-Bu increased constantly with depth ($R^2=0.93$) (Fig. 5, 6), which indicated that Me,n-Bu is not derived by PSB. This constant increase (Fig. 6) indicated that Me,sec-Bu might be a degradation product of an unknown precursor. Despite its unknown origin (Grice et al., 1996) and its slow concentration increase with depth in Rotsee ($2.7 \text{ ng} \text{ g}^{-1} \text{ TOC yr}^{-1}$; Fig. 6), Me,sec-Bu may serve as a degradation proxy. However, it was not present in the Black Sea.

The CI was used to estimate the degree of OM degradation in Rotsee (Naeher et al., in press). CI values were $>0.71$ for sediment older than about 45 years and especially high in the lowermost part of the Rotsee core (Naeher et al., in press). This indicated a rapid loss in sensitivity of this proxy and is a major drawback of this method. Therefore, novel maleimide degradation indices (Me,Me index; Me,Et index) similar to the chlorin index were defined (section 2.3) to estimate OM degradation for longer timescales.
The Me,Me index in Rotsee increased constantly from the recent sediment with 0.080 to 1.165 in 52-54 cm (1965-1970), whereas the Me,Et index increased from 0.001 to 0.155 (Fig. 6). The Me,Me index value above 1.0 in the deepest sample might be the result of measurement uncertainties (section 3.1; Fig. 6). Both indices increased constantly in the sediment (R² = 0.91 for Me,Me, R² = 0.89 for Me,Et (Fig. 6)), at rates of ca. 8.1x10⁻³ yr⁻¹ and 1.2x10⁻³ yr⁻¹, respectively. These rates indicated that the application limit of the Me,Me index with values of 1.0 were reached after ca. 124 yr, whereas extrapolation of the Me,Et would reach unity in ca. 851 yr in Rotsee sediment.

In the Black Sea sediment both indices hardly increased within Unit 1 and 2 core (Me,Me: 0.01-0.03, Me,Et: 0.017-0.022; Fig. 7). In contrast, values were much higher in Unit 3 (Me,Me: 0.78, Me,Et: 0.143; Fig. 7), likely because it consists of older and reworked material (Ross and Degens, 1974). Despite the fact that the sediment of this core is older than in the Rotsee core, the values of the Me,Me and Me,Et indices were in the same order of magnitude, which indicated that the decomposition of tetrapyrrole pigments proceeded at lower rates than in Rotsee. If it is assumed that the transition of Unit 2 and 3 occurred at 6000 yr BP based on estimates of Ross and Degens (1974) and that the maleimide sample from Unit 3 would have this age, the applicability of the Me,Me index can be extrapolated to at least 7700 yr BP, whereas the Me,Et index could be used for up to at least 42 kyr BP old sediment.

The longer applicability of the maleimide indices than the CI can be explained by the higher degradation progress of chlorophylls considered in this method. Maleimides are oxidation products, which are formed by the destruction of tetrapyrrole rings, either by natural processes or chromic acid oxidation. In contrast, the use of hydrochloric acid in CI analysis (Schubert et al., 2005) is not sufficient to decompose tetrapyrrole rings, so a lower degradation progress is considered compared to the more severe chromic acid oxidation (Ellsworth, 1970; Grice et al., 1996). Therefore, the new indices are applicable to longer timescales than the CI.

Conclusions

Maleimides were studied in recent sediments from the Swiss lake Rotsee and the Romanian Black Sea Shelf. Me,H, Me,Me, Me,Et and traces of Me,i-Bu occurred naturally in all samples. Additionally, chromic acid oxidation of sediment extracts yielded Me,n-Pr, Me,sec-Bu, Me,n-Bu, Me,i-Pentyl, Me,n-Pentyl, Et,Et and Et,n-Pr in both settings, except the absence of Me,sec-Bu and Et,n-Pr in the Black Sea. Most of the maleimides might originate from unknown bacteriochlorophyll precursors or were derived by bacterial reworking of chlorophylls and/or chlorins prior to tetrapyrrole pigment decomposition. Me,Et dominated all distributions in each sample. It predominantly originated from primary producers in Rotsee, whereas in the Black Sea also plant chlorophylls represented a large source. Me,H and Me,Me might be transformation products of chlorophylls, chlorins or Me,Et. The correlation between Me,n-Pr and Me,i-Bu suggested a common Chlorobiaceae origin. While purple and
brown PSB were observed in Rotsee, green and brown PSB could be traced on the Romanian Black Sea Shelf, indicating photic zone euxinic conditions in both settings, including the freshwater phase of Black Sea (Unit 3). In Rotsee, a community shift from Chlorobiaceae to Chromatiaceae was observed by a relative increase of okenone together with decreasing Me,n-Pr and Me,i-Bu concentrations since the 1920s due to increased productivity and eutrophication.

The presence of dissolved oxygen was crucial for maleimide formation within the water column. The constant increase of Me,sec-Bu with depth in Rotsee indicated continuous alteration and/or decomposition of an unknown precursor within the sediment. It might be used as degradation indicator, but it was absent in the Black Sea. The Me,Me and Me,Et indices were proposed as novel proxies for estimating the degree of OM degradation, which were applicable for longer timescales than the chlorin index. While the CI could hardly be used for sediment older than 45 yr, the Me,Me index could be used for up to ca. 124 yr old Rotsee sediment and the timescale of Me,Et was extrapolated to ca. 851 yr. In contrast, the Me,Me index was applicable for at least ca. 7700 yr in the Black Sea, whereas the Me,Et might be useable for at least ca. 42 kyr.

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Literature


**Figure 1.** Gas chromatogram (GC-MS) with maleimides obtained after chromic acid oxidation in the extract of the surface sediment sample in Rotsee. Insert chromatogram represents an enlargement of the chromatogram at retention times between 27 and 32 min.
Figure 2. Concentrations ("conc") of Me,H, Me,Me and Me,Et in the free ("natur.") and oxidised ("oxid") fractions in Rotsee sediment vs. age (yr AD).
Figure 3. Concentrations ("conc") of Me,H, Me,Me and Me,Et in the free ("natur.") and oxidised ("oxid.") fractions in Romanian Black Sea Shelf sediment vs. sedimentary unit.
Figure 4. Correlation between concentrations of Me,i-Bu and Me,n-Pr in Rotsee sediment with correlation coefficient (R^2) value.
Figure 5. Concentrations (“conc”) of Me,n-Pr, Me,i-Bu, Me,sec-Bu, Me,n-Bu, Me,i-Pentyl, Me,n-Propyl, Et,n-Pr, Et,Et in the oxidised fraction in Rotsee sediment vs. depth and age (yr AD).
Figure 6. Concentrations ("conc") of Me,sec-Bu and maleimide degradation indices (Me,Me index and Me,Et index) vs. age (yr AD) in Rotsee sediment with correlation coefficient ($R^2$) values.
Figure 7. Maleimide degradation indices (Me,Me index and Me,Et index) vs. sedimentary unit in Romanian Black Sea Shelf sediment.
4) **Seasonally resolved redox dynamics of manganese and quantitative bottom water oxygen reconstructions using Mn/Fe ratios in Lake Zurich, Switzerland**

*Authors*
Naeher S., Gilli, A., Hamann, Y., North, R., Schubert C.J.

*Abstract*
Redox dynamics of manganese (Mn) were studied in the sediment of Lake Zurich using precise sediment core age models, monthly long-term oxygen (O₂) monitoring data since 1936 and high resolution XRF core scanning. The age models were based on bi-annual lamination and calcite precipitation cycles (Ca XRF data). Mn was normalized with Fe to correct for terrigenous supply and dilution effects from calcite, with the latter largely dominating the Fe XRF signal. The Mn/Fe ratio was correlated with O₂ concentrations in the core from the maximum lake depth (137 m). The Mn/Fe ratio was used to quantitatively reconstruct bottom water O₂ concentrations, with a resulting average error of 1.6 mg l⁻¹ between the monitoring data and the calculated values. Bottom water O₂ concentrations (135 m) prior to the beginning of monitoring were also reconstructed (1895 to 1936). However, the general decrease of Mn XRF counts and Mn/Fe ratios up to a total peak disappearance in shallower cores indicated geochemical and sediment focusing, which can bias or even make it impossible to use Mn/Fe ratios as a quantitative palaeooxygen proxy.

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Seasonally resolved redox dynamics of manganese and quantitative bottom water oxygen reconstructions using Mn/Fe ratios in Lake Zurich, Switzerland

Naeher S., Gilli, A., Hamann, Y., North, R., Schubert C.J.

Introduction

Trace metals, such as iron (Fe) and manganese (Mn), have received much attention in the last decades due to their redox-sensitive behaviour in aquatic environments. This behaviour is strongly dependent on processes of oxidation and reduction of the coupled pairs Fe(II)/Fe(III) and Mn(II)/Mn(IV), which result either in precipitation or (re)dissolution (Davison, 1993; Calvert and Pedersen, 2007). Especially in lakes, seasonal redox changes in the hypolimnion result in the cycling of Fe and Mn (Sigg et al., 1987; Davison, 1993). Reducing conditions establish due to oxygen (O$_2$) consumption during organic matter (OM) remineralisation, which lead to a release of Fe and Mn (Nealson and Saffarini, 1994; O'Sullivan and Reynolds, 2005). After oxygenation at the chemocline due to partly or total mixing of the water column, Fe and Mn precipitates are deposited and potentially preserved in the sediment (Haworth et al., 1984; Schaller and Wehrli, 1996). Mn precipitation is furthermore catalysed by Mn oxidising bacteria, even under the absence of O$_2$ (Diem and Stumm, 1984). In fact, Mn-enrichments surrounding “Metallogenium” / Leptothrix echinata were found in Lake Zurich (Giovanoli et al., 1980; Diem, 1983).

The oxidation of Fe(II) proceeds more rapidly than Mn(II) (Haworth et al., 1984) and the involvement of numerous interdependent reactions, transformation processes and mineral phases lead to a complex pattern of redox-sensitive trace metals (Davison, 1993). However, Fe is not necessarily redox sensitive and can also have terrigenous sources, such as in the eutrophic Swiss lake Rotsee where Fe together with potassium (K) and titanium (Ti) represent detrital sources (Naeher et al., in press). Similarly, Fe is barely redox-sensitive and rather supplied by sewage treatment plant effluents, soil particles and sediment resuspension. There are two sedimentation maxima during the year, one in winter and another in summer, as shown by sediment trap data (Sigg et al., 1987; Wieland et al., 2001). A major part of the Fe in the sediment is finely dispersed as amorphous iron-oxy-hydroxide phases (only partly as goethite) or mineral phase coatings (Giovanoli et al., 1980; Sigg et al., 1987; Wieland et al., 2001).

Mn precipitates are also finely dispersed in the sediment of Lake Zurich, in the form of Mn oxides/hydroxides (MnO$_2$, Mn$_3$O$_4$, MnOOH) or with needle habitus (Giovanoli et al., 1980; Diem, 1983). Manganite ($\gamma$-MnOOH) is the predominant mineral phase in the sediments of the lake (Giovanoli et al., 1980; Diem, 1983). However, extended x-ray absorption fine structure spectroscopy (EXAFS) analysis in the eutrophic Swiss Lake Sempach showed that Mn precipitates, which are formed in the water column, are apparently of poorly crystallized H$^+$-birnessite (Friedl et al., 1997). They showed that Mn was mainly associated with authigenic particles, consisting of (Ca,Mn)CO$_3$.
and \((\text{Fe,Mn})_3(\text{PO}_4)_2\cdot8\text{H}_2\text{O}\). About 55-60% of Mn was incorporated into carbonate, whereas 40-45% entered phosphate particles.

The cycling of redox-sensitive trace metals by reductive dissolution and (re)oxidation progressively leads to geochemical focusing, which is a process of transfer and enrichment of these element in deeper waters (Schaller and Wehrli, 1996). Geochemical focusing was previously described in lower temporal resolution in similar lake systems, such as e.g. the Swiss Lake Baldegg (Schaller and Wehrli, 1996). In a lake, geochemical focusing only occurs if at some point in the year the oxygenated water column is separated due to stratification and the sediment becomes anoxic. Even without a large source of Mn through the inflow, distinct horizontal patterns of Mn in the sediment may result. The focusing seems to be triggered by horizontal and vertical mass transport as a result of turbulent mixing under specific changes in deep-water \(\text{O}_2\) abundance (Schaller and Wehrli, 1996).

Sediment focusing was observed in sediment traps in Lake Zurich (Wieland et al., 2001), and was driven predominantly by lateral downslope particle transport of resuspended and/or unconsolidated flocculated material, but also by density currents during stratification (Garrett, 1990; Wieland et al., 2001). These processes lead to higher sedimentation rates with increasing water depth, which also influences Mn and Fe precipitates (Syers et al., 1973; Engstrom and Wright, 1984).

Although the redox-sensitive behaviour of Fe and Mn is quite well understood, the effect of short-term changes in bottom water \(\text{O}_2\) concentrations on the sediment pattern of redox-sensitive trace metals still needs to be shown. Since both metals are oxidised at different rates and to a different extent, this difference can be applied in the reconstruction of short- and long-term \(\text{O}_2\) dynamics (Haworth et al., 1984). Such an analysis may also improve the understanding of short-term impacts of Fe and Mn dissolution and precipitation on their transport within lakes, potentially revealing the detailed processes behind geochemical focusing. The development of X-ray fluorescence (XRF) core scanners has enabled rapid high-resolution, semi-quantitative and non-destructive measurements of trace metals in sediment cores (Richter et al., 2006). Most importantly, until now, it was not possible to quantitatively reconstruct \(\text{O}_2\) concentrations from inorganic proxies.

The aim of this paper is to reconstruct short-term bottom water \(\text{O}_2\) concentrations as a proxy for redox dynamics reflected in the sediment contents of Fe and Mn in Lake Zurich, Switzerland. This lake was chosen as an ideal study site, because it is a well-studied system for trace metals (Giovanoli et al., 1980; Diem, 1983; Diem and Stumm, 1984; Sigg et al., 1987; Wieland et al., 2001), has a seasonally hypoxic hypolimnion and it is very sensitive to temperature changes that can induce water column mixing (Livingstone, 1997; Livingstone, 2003). Additionally, monthly \(\text{O}_2\) monitoring data for various depths exist since 1936.
The majority of the sediment is well laminated and these bi-annual layers can be precisely counted and dated. Supported by calcium (Ca) XRF data with maxima during spring/summer and minima in winter, it was possible to construct a high precision age model. Based on Mn, we show that hypolimnetic oxygenation events can be precisely traced and that the Mn/Fe ratio in the deepest core is a useful indicator to quantitatively reconstruct bottom water O$_2$ concentrations.

### Methods

#### Section 1

**Study site and sample collection**

The mesotrophic Lake Zurich has a surface area of 65 km$^2$ with a maximum depth of 137 meters (Wieland et al., 2001). The lake can behave as monomictic or dimictic depending on prevailing winter conditions (Livingstone, 2003), is orientated from the southeast to the northwest and lies at 406 m.a.s.l. The basin was formed during the last ice age (Kelts, 1978). The lake is divided into two basins by a moraine sill, forming the upper and lower lake, which are connected by a three meter deep sill (Wieland et al., 2001). Main allochthonous riverine supply from the Linth River is effectively entrapped in the upper lake, which acts as a settling basin (Wieland et al., 2001). The majority of the flow (84%) into the lower lake is over the sill connecting the two basins (Omlin et al., 2001).

Long-term monitoring of Lake Zurich began in 1936 and includes O$_2$ concentration data at 19 different water depths (typically 0.3, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 60, 80, 90, 100, 110, 120, 130 and 135 m). The lake shows simultaneous climate-driven deep water warming and cooling episodes, indicating that climate variability has a strong impact on its temperature driven stratification (Livingstone, 1997).

In November 2010, sediment cores were obtained at depths of 137 m (maximum lake depth, ZH10-15, 110 cm long), 135 m (ZH10-19, 111 cm long), and 123 m (ZH10-21, 116 cm long). The GPS positions (WGS84) of the recovered cores were N 47° 16.995'E 8° 35.624 (ZH10-15), N 47° 17.130 E 8° 35.371 (ZH10-19), and N 47° 17.062 E 8° 35.166 (ZH10-21). Cores were laminated, but in their lower part intensively intercalated by turbidites. The laminations alternated between a dark brown-black OM-rich layer and a light, brownish, carbonate-rich layer, representing summer/fall/winter and spring/summer, respectively. The three cores were correlated using the locations of varves and turbidites. The age model was based on varve counting and Ca XRF intensities (spring/summer maxima and winter minima) (Kelts and Hsü, 1978). Linear interpolation was used to connect the layers, along with a previous age model (Nipkow, 1927). The resulting age model provided a seasonal resolution of the data. The age model for ZH10-15 reaches back until 1895, for ZH10-19 until 1897 (even 1892, but not annually resolved) and for ZH10-21 until 1901. The sedimentation rates in all cores were about 0.28 cm yr$^{-1}$ on average. In ZH10-15 no
XRF data were available between 1963 and 1966 due to a hole in the core. Data between 1983 and 1988 were missing in ZH10-21 because of a turbidite that caused a deformation of this core part, whereas for ZH10-19 a continuous record was retrieved.

Section 2

XRF core scanning

For the determination of relative elemental concentrations, each core was cut lengthwise and its surface was dried for 24 hours at room temperature. The surface was carefully flattened to reduce surface roughness. One half of each core was scanned with the energy dispersive XRF Core Scanner (AVAATECH) at ETH Zurich, Switzerland. A Rhodium target X-ray tube was operated at excitation energies of 10 and 30 kV for a 30 second period and a spatial resolution of 0.3 mm. CANBERRA software was used for data processing, and results are given as XRF counts/intensities. The advantage of XRF core scanning is the rapid non-destructive high-resolution determination of elements, dependent on the measurement parameters, in the atomic mass range of aluminium (Al) to uranium (U) (Richter et al., 2006). However, XRF core scanning provides relative results as XRF counts instead of true concentrations. Furthermore, trace metal core profiles can be biased by the water content (Tjallingii et al., 2007), OM content changes (Löwemark et al., 2011) and grain size variability (Cuven et al., 2010).

Results

Section 1

Ca, Fe and Mn seasonality

The sediment core taken at deepest point of the lake (core ZH10-15, 137 m) showed a pronounced seasonal pattern in the Ca, Fe and Mn signals (Fig. 1). Sedimentary Ca maxima occurred in spring/summer, whereas Fe maxima were observed in fall/winter. Ca and Fe XRF intensities were negatively correlated (Fig. 2). Ca intensities were at least 1.5 to 38 times higher. In ZH10-15, the correlation coefficient was lowest with $R^2=0.57$ in the time interval 1980-1990 and for other decades at least $R^2=0.7$ (Fig. 2). However, if the complete dataset was used, Ca and Fe were much less well correlated ($R^2=0.32$). Fe and Ca XRF data were also negatively correlated in the other cores (ZH10-19: $R^2=0.50$ for 1900-2010; ZH10-21 $R^2=0.57$ for 1901-2010; data not shown).

In contrast to Fe, Mn showed maxima during winter/spring (Fig. 1), which is closely linked with the annual maximum deep-water O$_2$ concentrations. However, the two variables were not significantly correlated.

Section 2
Mn/Fe ratio – Calibration and reconstruction of oxygen levels

For correlation analysis of Mn and O₂ in core ZH10-15 (137 m, maximum lake depth), only annual maxima were compared in order to avoid inconsistencies due to the linearly interpolated age model, which cannot account for sedimentation rate variations during the year (Bloesch, 2007). To further improve the comparability, three-month average values of O₂ levels were used, considering the three following months with the highest oxygen concentrations, which mostly occurred during spring. Although low Mn/Fe ratios during fall/winter were not correlated with O₂ concentrations at this time, this ratio and O₂ levels exhibited a seasonal pattern (Fig. 3).

The maxima of the Mn/Fe ratio in core ZH10-15 (137 m) were correlated with maxima of the O₂ concentration data ($R^2=0.38$, $n = 71$; $p < 0.01$; 1936-2010, XRF data missing: 1963-1966) (Fig. 4). This correlation was used to calibrate the sedimentary Mn/Fe proxy to absolute bottom water O₂ concentrations.

Both Mn/Fe ratios and O₂ concentrations were consistently low with a small variance prior to 1967 (< 0.6 counts/counts and < 2 mg O₂ l⁻¹), except between 1955 and 1958 with up to 6 mg O₂ l⁻¹ and a Mn/Fe ratio of up to 1.4 (Fig. 5). After 1967, the variance increased and magnitudes were consistently higher (Fig. 5). However, between 2000 and 2010, annual maximum bottom water O₂ concentrations at 135 m were consistently below 5 mg l⁻¹, even decreasing below 3 mg l⁻¹ in 2000, 2003 and 2009 (Fig. 5).

The average error between the Mn/Fe ratio based estimates for O₂ and the actual O₂ concentrations at the same depths was about 1.6 mg l⁻¹. However, the error was > 2 mg l⁻¹ for seven values (1955, 1971, 1974, 1979, 1982, 1991, 1996), whereas one deviation was 3.0 mg l⁻¹ (1968) and the maximum error was 4.2 mg l⁻¹ (1958).

With the correlation of the Mn/Fe proxy and the monitoring data (between 1936 and 2010) winter/spring O₂ concentration maxima were reconstructed for the unmonitored period of 1895 to 1936 (Fig. 6). The resulting estimated bottom water oxygen concentrations were always below 2 mg l⁻¹, except in 1906 and 1921 when the maxima reached 3.8 mg l⁻¹ and 3.1 mg l⁻¹, respectively (Fig. 6).

However, no correlation between the Mn/Fe ratio and O₂ concentration data was observed in cores ZH10-19 and ZH-21. Despite a depth difference of only 2 m between ZH10-19 (135m) and ZH10-15 (137m), both the maximum (winter/spring) and minimum (summer/fall) Mn/Fe ratios were on average about 26% lower in ZH10-19 (Fig. 7). In the 123 m core (ZH10-21), the values were on average 45% lower than in ZH10-15 (minima 40% and maxima 50% lower, 1936-2010). If non-normalised Mn XRF intensities from the cores were compared, the peaks found in ZH10-15 only occurred at lower XRF signal intensity in 1935, 1954, 1966-1968, 1971 and 1974 in ZH10-19 (Fig. 8). In ZH10-21 no peaks were present (Fig. 8).
Discussion

Section 1

Ca, Fe and Mn seasonality

The seasonality of Ca in all cores has been governed by biogenic calcite precipitation with maxima during spring/summer. In contrast, the Fe signal (Fig. 1) might be the result of three different processes: Fe can either be (i) redox-sensitive, (ii) of detrital, terrigenous origin, and/or (iii) it is diluted by calcium in the sediment.

In the first case, Fe would have precipitated with the first traces of O₂ in the bottom water in fall/winter. This would be in agreement with monitoring data, which indicate a possible onset of O₂ resupply at that time, at least in years in which the lake was dimictic. In contrast, major O₂ resupply to the bottom water occurs during winter and/or spring, coinciding with Mn peaks during that time (Fig. 1). Furthermore, the monitoring data indicated that the lake have often only mixed partially in fall. Therefore, O₂ seems to be replenished to the deep-water mostly once a year in winter/spring. This suggests that the potential redox-sensitive behaviour of Fe can only partly explain Fe peaks in fall/winter.

However, previous studies showed that Fe can be of other origin as well (Engstrom and Wright, 1984; Davison, 1993). Sediment trap data indicated that Fe is supplied by sewage treatment plant effluents, soil particles or by sediment resuspension, with two sedimentation maxima during winter and summer (Sigg et al., 1987; Wieland et al., 2001). Their results suggested that Fe remobilisation is only minor (Sigg et al., 1987), which could mean that most of the Fe is not redox-sensitive. But still, the explanation of higher terrigenous Fe supply in summer and winter disagrees with the XRF data, which only show one Fe maximum in fall/winter.

Alternatively, dilution by other trace metals and/or other sedimentary components in the sediment is likely, because XRF core scanning is a relative determination of trace metals (Richter et al., 2006). Based on sediment trap data, CaCO₃ alone already represents about 20-80% (dry weight) of settling particles in the lake (Sigg et al., 1987). Therefore, the much more abundant calcite (compared to Fe phases) and the strong negative correlation between Ca and Fe indicates that calcite dilution affects the Fe XRF signal (Fig. 2). Fe and Ca XRF data were also negatively correlated in the shallower cores, suggesting that Fe is diluted by calcite in these cores as well.

However, the low correlation between Ca and Fe in ZH10-15 comprising all data (R²=0.32) and the high correlation of the data separated into decades (R² mostly > 0.7) suggests that the relationship between both elements was not identical throughout the lake history (Fig. 2). Especially between the 1920s and the 1960s the calcite-rich laminae were thicker with larger calcite contents compared to the previous and
following decades. It could also explain differences in slopes and offsets of Ca and Fe cross plots comprising different time intervals. Therefore, the degree of Ca dilution might have changed with time. This changing pattern is not completely understood, but might be explained by productivity changes within the lake history.

Another reason could be diagenetic alterations: During diagenesis, Fe can be potentially remobilised due to dissolution of iron-oxy-hydroxide precipitates under anoxia (Davison, 1993). However, Fe recycling from the sediment traps has been low (Sigg et al., 1987), indicating that Fe is hardly remobilised from the sediment as well. Calcite dissolution in the sediment is caused by the pH decrease due to OM mineralisation (Müller et al., 2006). The overall good negative correlation between Ca and Fe indicates that diagenesis can only have a minor impact on carbonates and iron phases in the sediment.

In contrast to Fe, the match between Mn and O2 concentration maxima during winter/spring (Fig. 1) suggests that Mn is governed by seasonal changes in the bottom water O2 content, due to changing redox conditions at the water-sediment boundary. This means that Mn can be used to trace redox conditions in the lake. However, the Mn XRF signal might be also biased by terrigenous input and calcite dilution. Therefore, Mn was normalised with Fe, which was predominantly governed by these factors. Furthermore, such a normalisation with another element can also account for grain size and porosity variability (Cuven et al., 2010; Löwemark et al., 2011), which improves the interpretability of relative XRF intensity records.

Section 2

Mn/Fe ratio – Calibration and reconstruction of oxygen levels

The high correlation of the Mn/Fe ratio with the O2 concentration data (Fig. 4) indicated that this ratio might be used as a proxy for quantitative O2 reconstructions (Fig. 5). The match of low Mn/Fe ratios with low O2 concentrations (< 2 mg l⁻¹) before 1963 (except 1955-1958) (Fig. 5) suggests that the low ratios in the deeper part of the core show a true pattern. Large diagenetic alterations such as the dissolution of Mn precipitates and loss of Mn into the upper sediment and/or water column are therefore unlikely.

The reconstruction of bottom water winter/spring maximum concentrations between 1895 and 1936 (Fig. 6) also traced low bottom water oxygen concentrations mostly below 2 mg l⁻¹, except in 1906 and 1921 with almost up to 6 mg l⁻¹ (Fig. 6). However, the large y-axis intercept of the regression function caused an offset between the reconstructed and measured O2 levels concentrations before and after 1936. Due to this offset, the calculated O2 levels seemed to be overestimated by at least 1 mg l⁻¹. Therefore, deep water oxygen levels with hypoxic conditions (< 2 mg l⁻¹) prevailed throughout most of the years between at least 1895 and 1954 (Fig. 5, 6).
The reason for the low oxygen concentrations might be eutrophication, which started with first massive blooms of *Tabellaria fenestrata* in 1896 and *Oscillatoria rubescens* in 1898 (Minder, 1938; Hasler, 1947). This might explain the O₂ decline before 1955. The construction of sewage treatment plants since 1955 has led continuously to a strong decrease in phosphate (Bossard et al., 2001), but during the 1960s, the O₂ levels decreased again. Another onset of larger oxygenation started at the end of the 1960s and the lake changed from an eutrophic to a mesotrophic state within the 1970s (Bossard et al., 2001). The non-continuous increase in O₂ concentrations since 1955 shows that also other driving forces such as for example climate need to be considered together with eutrophication. However, this is not yet completely understood. The decreasing O₂ concentration maxima since 2000 might be related to climate change, because of the lake’s tendency to reduced mixing intensities and higher hypolimnetic temperatures as a result of warmer winters in the recent years (Livingstone, 1997; Livingstone, 2003).

**Section 3**

**Processes and impacts on Mn and Fe alterations**

In some instances large deviations occurred between observed (monitoring data) and calculated (Mn/Fe ratio derived) O₂ concentrations (Fig. 5):

Persistently higher maximum O₂ concentrations in successive years seemed to result in lower Mn/Fe ratios in the following years. Oxidation of Mn in shallow waters may have effectively trapped Mn, which reduced the supply of Mn to deeper water depths. While O₂ concentrations were higher since 1955/1956, the difference between values of the monitoring data and the Mn/Fe ratio progressively increased in the subsequent years until in 1958. Similar increased differences between measured O₂ concentrations and proxy data were also observed since 1963 until 1970 and 1972 and since 1994 until 1996 and 1999. The lower than expected Mn/Fe ratios, which culminated in 1996 and 1999 apparently resulted from higher O₂ levels since 1994 (Fig. 5). These differences with lower than expected Mn/Fe ratios might be due to higher O₂ levels in subsequent years, which might have trapped Mn in shallow sediment, thereby reducing Mn supply to deeper waters. Alternatively, the higher than expected Mn/Fe ratio in 2006 might have originated from the higher dissolution of Mn precipitates or the larger supply of Mn to the deep-water the years before, which precipitated in winter/spring 2006.

Another possible explanation may be the impact of turbidites. In turbidites, sedimentary material is transported into the deep water and sediment, together with therein enclosed shallower, O₂-rich water (Loizeau and Dominik, 2000), which could also oxidise dissolved Mn. This might explain higher than expected Mn/Fe ratio in 2006. However, at the transition of other turbidites to laminated sediment, no higher Mn/Fe could be observed. Nonetheless, microturbidites, which can be too small to be visible by eye, but detected by XRF core scanning, may explain higher Mn/Fe ratios.
than expected from the monitoring data. But, additional evidence would be needed to confirm the presence of such microturbidites and their impact on the Mn/Fe ratio in core ZH10-15.

Mn and Fe distribution heterogeneities in the lake sediment might also lead to offsets. While Mn was predominantly found in sheaths of Mn-bacteria, Fe was finely dispersed throughout the lake sediment (Giovanoli et al., 1980; Diem, 1983). Difficulties with heterogeneous patterns especially arise at high spatial resolution measurements (Cuven et al., 2010; Löwemark et al., 2011). Furthermore, measurement errors can potentially cause offsets in both Mn/Fe values and O$_2$ concentrations, although at least the three-month averaging of O$_2$ reduced strong impacts of this parameter. However, the pattern of neighbouring data points did not show strong offsets concerning the Mn/Fe ratio and other trace metals, so heterogeneities and measurement errors are unlikely.

The apparently systematic signal decrease and/or progressive disappearance of Mn from the maximum lake depth towards shallower water depths (Fig. 7, 8) indicates geochemical focusing, in which redox driven changes of Mn fixation and transport progressively leads to an enrichment of redox-sensitive trace metals towards the maximum lake depth (Schaller and Wehrli, 1996). This process can explain major deviations between the Mn/Fe ratio (and Mn XRF counts) and the O$_2$ as well as progressive Mn peak decrease up to disappearance in the shallower cores. The Mn XRF signal disappeared in these cores with still intact lamination (Fig. 7, 8). However, the lamination also disappears in shallower sediment cores, likely due to bioturbation under persistently abundant oxygen in the bottom water (White and Miller, 2008).

Sediment focusing could support redox-driven transfer to deeper waters (Garrett, 1990; Wieland et al., 2001). It causes higher sedimentation rates with increasing water depth. Probably, sediment focusing can also increase Mn transfer towards deeper sediment and therefore enhancing geochemical focusing. Because of low densities and fine textures of Mn and Fe precipitates, water turbulences and mixing tend to preferentially move these particles to deeper regions of the lake, also affecting Mn and Fe (Syers et al., 1973; Engstrom and Wright, 1984).

Geochemical and sediment focusing are the most likely processes for the apparent depletion of Mn in the shallower cores and to explain partly high deviations between O$_2$ monitoring data and calculated O$_2$ concentrations based on the Mn/Fe ratio in ZH10-15.

Therefore, the application of the Mn/Fe proxy might be limited to special cases, in which oxygenated systems show pronounced seasonal redox changes throughout the year. If the whole water body is oxygenated throughout the year, Mn is trapped completely as soon as it enters the lake and remains immobilized. In contrast, Mn
remains in solution under reducing conditions and/or precipitates are dissolved (Davison, 1993; Friedl et al., 1997).

This means that if the impact of alteration processes can be assessed, the Mn/Fe ratio might be a quite robust indicator for quantitative bottom water O₂ reconstructions in similar lake settings.

Conclusions

Due to precise sediment age models, monthly long-term O₂ monitoring data and high resolution XRF core scanning, Mn redox dynamics could be studied on a seasonal resolution in the sediment of Lake Zurich, Switzerland. The age models were based on bi-annual lamination in sediment cores and yearly calcite precipitation cycles (Ca XRF data). Ca, Fe and Mn showed pronounced seasonal behaviour. Ca was governed by higher calcite precipitation during spring/summer, whereas Fe showed peaks in fall/winter and Mn in winter/spring. Fe was predominantly of terrigenous origin and not redox-sensitive. However, the high negative correlation between Ca and Fe suggested that the Fe XRF signal was governed by calcite dilution. Mn traced winter/spring oxygenation due to its redox-sensitive behaviour. The Mn/Fe ratio was correlated with bottom water O₂ concentrations. Because of this normalisation, the Mn data were corrected for terrigenous supply and dilution effects from calcite. The Mn/Fe ratio was used in core ZH10-15 (137 m, maximum lake depth) as a proxy to reconstruct bottom water O₂ concentrations. The average error was 1.6 mg l⁻¹. Based on the correlation between monitoring data and Mn/Fe ratios, O₂ concentrations could be reconstructed until 1895, which was the lower limit of the bi-annual lamination. However, it was shown that geochemical and sediment focusing can strongly bias or even prevent the utilisation of the Mn/Fe for quantitative reconstructions of O₂ in the bottom water of lakes.

Acknowledgements

This research project was funded by the European Union project “Hypox – In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas and land-locked water bodies” (EC grant 226213). Oxygen data from Lake Zurich were kindly provided by the City of Zurich Water Supply. Ulrike van Raden and Stefanie Wirth (both ETH) are thanked for field support. David Livingstone, Bernhard Wehrli and Beat Müller (both Eawag) are acknowledged for helpful discussions and suggestions.

Literature


of vertical and lateral pathways. Aquatic Sciences - Research Across Boundaries 63, 123-149.
Figure 1. Seasonality of Ca, Fe and Mn XRF intensities (x 1000) in core ZH10-15 vs. time (yr AD), example excerpt between 1980 and 1990. Grid lines indicate winter time and the beginning year is labelled.
Figure 2. Correlation between XRF counts of Ca and Fe (x 1000) in core ZH10-15 with correlation coefficients at different time intervals (1936-1980, 2000-2010).
Figure 3. Profiles of maximum (winter/spring) and minimum (summer/fall) Mn/Fe ratios in core ZH10-15 and three month average of maximum bottom water oxygen concentrations of the same time intervals in mg l$^{-1}$ (135 m, monitoring data) vs. time (yr AD). No XRF data were available between 1963 and 1966.
Figure 4. Correlation between maxima of the Mn/Fe ratio in core ZH10-15 and three month average of maximum (winter/spring) bottom water oxygen concentrations in mg l\(^{-1}\) (135 m, monitoring data). Shaded area illustrates that the Mn/Fe ratio is not applicable at very low oxygen concentrations. XRF data were missing between 1963 and 1966.
Figure 5. Profiles of maximum Mn/Fe ratios in core ZH10-15 and three month average of maximum (winter/spring) bottom water oxygen concentrations in mg l\(^{-1}\) (135 m, monitoring data) vs. time (yr AD). No XRF data were available in ZH10-15 between 1963 and 1966.
Figure 6. Reconstructed maximum (winter/spring) bottom water oxygen concentrations in mg l$^{-1}$ (135 m) between 1895 and 1936, based on maximum Mn/Fe ratios and the correlation from Fig. 3 between the Mn/Fe ratio and oxygen monitoring data (1936-2010).
Figure 7. Profiles of the Mn/Fe ratio in cores ZH10-15, ZH10-19 and ZH10-21 between 1900-2010.
Figure 8. Profiles of Mn XRF intensities (x 1000) in cores ZH10-15, ZH10-19 and ZH10-21 between 1900-2010.
Black Sea
Numerous publications have evolved from the Black Sea research mainly from HYPOX partners GeoEcoMar (Romania), IBSS (Ukraine), and ITU-EMCOL (Turkey). While publications of Romanian and Ukrainian working groups focus on hypoxia effects on faunal assemblages the work at ITU-EMCOL deals with geochemical indicators of past redox conditions.

1) Biogeography and ecology of *Trophonopsis breviata* (Jeffreys, 1882) (Gastropoda: Muricidae: Trophoninae)

*Authors*
Bondarev, I.P.

*Abstract*
Geographical spreading of *Trophonopsis breviata* viewed on the background of its natural history and ecology. Data about the species distribution in the bathymetric range and its biocenotical connections are given and analyzed. It’s stated that *Trophonopsis breviata* presents in 10 Black Sea benthic biocenosises. The species stenothermy is the determining ecological character which limits its spreading. The species ecology data evidence for its boreal roots. Ecological data confirm *Trophonopsis breviata* as separate valid species. **Keywords:** biogeography, biocoenosises, ecology, *Trophonopsis breviata*, Black Sea


*Appendix I*
2) On finding of *Archesola typhlops* (Sars, 1920), the harpacticoid new for the Black Sea, at depths greater than 100 m

**Author**
Kolesnikova, E.A.

**Abstract**

On finding of *Archesola typhlops* (Sars, 1920), the harpacticoid new for the Black Sea, at depths more than 100 m. Нахождение нового для Черного моря вида гараптиксоида *Archesola typhlops* (Sars, 1920) на глубинах более 100 м. Materials were collected with up-to-date samplers during the expedition M 72/2 MICROHAB to the northwestern and northeastern Black Sea on board the R/V Meteor (Germany). In sediments taken from the sea bottom from 24 February to 10 March 2007 at depths 120, 130, 150 and 170 m the harpacticoid *Archesola typhlops* (Sars, 1920) (Copepoda, Harpacticoida), formerly unknown in the Black Sea was found as adult females, males, and copepodites at different stages. Half a century ago in Romanian coastal sea water *Archesola typhlops pontica*, a subspecies of the genus *Esola*, was found at 69 m depth and described as new for the Black Sea (Por, 1959). The subspecies differed from the basic species in the number of setae on the exopodite of female individuals. Harpacticoids in our samples were identical to the typical species *Archesola typhlops*. Presumably the recently found *A. typhlops* inhabit hypoxic biotopes and might be a marker of this zone. Acknowledgements. This work supported by the European Union, projects HIPOX 226213 and HERMES GOCE - CT - 2005 – 511234. E. A. Kolesnikova, Ph.D. (Biol., leading researcher), Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine, Sevastopol, Crimea, Ukraine.

**Marine Ecological Journal**, Vol. IX, 1, 52., 2010, *in Russian*
3) **A new record of the *Sarsameira parva* (Boeck, 1872) and *Tachidiella minuta* G. O. Sars, 1909 (Copepoda, Harpacticoida) in the Black Sea.**

**Authors**
Kolesnikova, E.A., Sergeeva, N.G.

**Abstract**
In soft bottom sediment samples taken near the Bosphorus at the depth 85 m and 103 m were found two new species for the Black Sea Harpacticoida *Sarsameira parva* (Boeck, 1872) and *Tachidiella minuta* G. O. Sars, 1909. These species were found in the mud biotope. In the column taken sediment layers of different colors were observed: its upper oxidized layer (0 - 1 cm) are clearly distinguished from the deeper horizons, with a dark gray color with a characteristic black bedding, indicating the presence of reduced conditions in the depth of sediment. *Sarsameira parva* noted in the column of sediment from the surface to a depth of 4 cm in the whole population of this species was represented by 16 specimens (4 ♂, 9 ♀ and 3 copepodites). The greatest concentration of species recorded in the horizon of 1 - 2 cm (14 individuals). *Tachidiella minuta* was found only in the surface layer and is represented by seven specimens (2 ♂ and 5 ♀).

This work was supported by EC 7th FP project "In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land locked water bodies" (HYPOX, No.226213).


*Appendix II*
4) The lowest zoobenthos border in the Black Sea Near-Bosporus region

Authors
Sergeeva, N.G., Zaika, V.E. Bondarev, I.P.

Abstract
10 stations were carried out in the Near-Bosporus region with the depth interval of 75 – 300 m. Macrobenthos total quantity was 136 – 111 thous. indiv. m-2 at the depths of 75 – 82 m, than its number decreased sharply up to 29 – 11 thous. indiv. m-2 and remained in the limits of 11 – 4 thous. indiv. m-2 up to the depth of 250 m. Macrobenthos is represented by annelids only at the depths lower than 123 m. Polychaete Vigtorniella zaikai forms the accumulation at the depth of 250 m in the Near-Bosporus region, thought, its peaks are at the depths of 150 – 170 m, in the belt of oxygen to the hydrogen sulphide zone transition, in the northern sea half. Meiobenthos total quantity is the highest at the depth of 75 m (1861.6 thous. smpl/m2). It becomes lower, with the depth increase, forming the smaller peaks at the depths of 88 m (1011 thous. indiv. m-2), 162 m (468.5 thous. indiv. m-2) and 250 m (603.2 thous. indiv. m-2). The main share of the total quantity falls on nematode group, and harpacticoids are the following. The lowest abundance peak is at the depth of 250 m and meiobenthos quantity decreases considerably at the depth of 300 m.

Keywords: the Black Sea, Near-Bosporus region, macrobenthos, meiobenthos.


Appendix III
Sedimentary Record of Mediterranean Inflow Effect on Redox Conditions of the Istanbul Strait Outlet Area of the Black Sea.

Authors
Erdem, Z., Çağatay, M.N.

This manuscript is in preparation. Intended for submission to Quaternary Science Review
A description of the content follows:

As the world’s largest anoxic basin, the Black Sea sediments contain sensitive records of the past sea level, climatic and environmental changes, including the record of the anoxia development. It is therefore a natural laboratory for studies of anoxia evolution and biogeochemical cycling of carbon, sulphur, iron and redox sensitive trace elements. There is a consensus that the anoxia in the Black Sea has developed as a result of the restricted circulation due to sharp density stratification, weak vertical circulation and organic matter degradation (e.g., Ross and Degens, 1974; Arthur and Dean, 1998; Çağatay, 1999). However, the timing and rate of evolution of anoxia through the water column is still controversial. The onset of the Holocene anoxia and sapropel deposition has generally been assumed to be coeval in the Black Sea, although this issue is still a matter of debate. Some workers even mention that the Black Sea Holocene Sapropel Unit (Unit 2) was deposited under oxic bottom-water conditions (Calvert, 1990; Calvert and Pedersen, 1993). Another issue is the rate of development of the anoxia and the rise of the oxic/anoxic boundary in the water column.

The Istanbul Strait’s outlet area of Black Sea (ISBS), comprising the shelf and upper slope region, is a key area to investigate the above questions as well as the history of the ventilation effect of the Mediterranean water (MW), because here the MW enters the ISBS shelf through the submarine extension of the Istanbul Strait’s channel and then spreads to form a uniform 2-3 m thick saline and relatively oxygen-rich sheet over the shelf and upper slope areas (Fig 1a, b) (Özsoy and Ünlüata, 1997; Özsoy et al., 2001). This area is characterized by the Mediterranean inflow that is responsible for the ventilation and sluggish deep circulation of the anoxic Black Sea basin (Öğuz et al., 1993; Özsoy and Ünlüata, 1997) and by a submarine channel-levéé complex on
the middle and outer shelf areas that was developed by the inflow of the MW since about 7.5-8 $^{14}$C ka BP (Fig. 1b) (Flood et al., 2009; Okay et al., 2011).

Figure 1. The location and the bathymetric map of the Istanbul Strait’s outlet area of the Black Sea (ISBS). a. The location of the area with the channel-network complex formed by the Mediterranean Water (MW), b. Closer view of bottom features of the area showing the main MW-channel and its distributary channels and the location of the core transects and studied cores. Light blue area is the shelf area shallower than -110 m (Bathymetry data acquired in a NATO SACLANT Undersea Research project).

Presently, a two-way current system occurs in the Bosphorus channel, with the Black Sea water forming the upper current and the warm and saline Mediterranean Water (MW) the undercurrent (Özsoy and Ünlüata, 1997; Di Iorio et al., 1999; Özsoy et al., 2001). The oxic-anoxic boundary is located at a depth of 100-150 m with a ~30 m thick suboxic zone (Murray et al., 1989; Codispoti et al., 1991; Baştürk et al., 1994), but may have varied in the past as result of the changes in the amounts of the MW, of riverine water input and global sea level. The ISBS is therefore believed to be a sensitive recorder of the past changes in climatic and environmental conditions in the Black Sea.

Over the last several decades Black Sea has been severely affected by natural and environmental degradation mainly because of riverine input of contaminants and nutrients (Oğuz et al, 2002; 2005). There have been claims of oxic/anoxic boundary shoaling and/or thickening of anoxic zone by several tens of meters in recent years (Murray et al, 1989; Codispoti et al, 1991; Lyons, 1993; Oğuz et al, 2002; 2005).
Others indicate, however, the structure and position of the interface has been reasonably stable within the last several decades, especially when compared to existing density stratification (Tuğrul et al, 1992; Baştürk et al, 1994; Özsoy and Ünlüata, 1997).

In this paper, we investigate the history of the Mediterranean inflow and its role in the ventilation of the in the ISBS area, and discuss possible vertical fluctuations in the position of the oxic/anoxic interface in the water column during the last 7 ka. We carried out sedimentological, paleontological, and geochemical analyses and radiocarbon dating of interface sediment cores located along two depth transects ranging from -122 m to -307 m (Fig. 1b). The NE- and N-trending transects were selected on the basis of CTD casts during November 2009 Turkish R/V Arar cruise showing that the NE-trending transect was under the influence of the Mediterranean inflow, whereas the N-trending one showed no sign of such influence (Holttappel, unpublished data). Moreover, biological analysis of surface sediments from NE-trending transect revealed the presence of living benthic fauna including meibenthos and Mediterranean fauna, with decreasing water depth till the sampling depths of -300 m (Sergeeva et al., accepted). Our geochemical methods include the XRF Core Scanner analysis of the redox sensitive elements (Mn, Fe, and S) and total organic (TOC). The paleontological study involved analysis of mollusc and benthic foraminifera. The geochemical results were interpreted on the basis of the geochemical behaviour of Mn under different redox conditions (e.g., Calvert and Pedersen, 1993; Thomson et al, 1995; Crusius et al, 1996; Lepland and Stevens, 1998; Morford and Emerson, 1999; Çağatay, 1999; Yarincik, 2000; Burke and Kemp, 2002) and the principles of the Fe-S-Organic system (e.g., Berner, 1980, 1984; Berner and Raiswell, 1983; Raiswell and Berner, 1985; Raiswell et al., 1988; Canfield et al, 1992; Raiswell and Canfield, 1998; Lyons et al., 1993, 2003), using the Core scanner XRF Fe/Ti and S, and the TOC profiles of the cores.

Lithological properties and geochemical analysis of the studied cores show an abrupt change in the position of oxic-anoxic interface since the latest connection of Black Sea with world ocean system. According to the redox sensitive element Mn profiles and benthic faunal content suboxic to anoxic conditions reached between -120m and -150m depth at 6.8 ka BP over the whole upper slope area. At the eastern side of the upper continental slope area, at 150m water depth, anoxic conditions started to prevail.
at 6.8 ka BP, whereas at the western side of the area, oxic conditions continued until 5.3 ka BP, where oxygen started to be depleted in the water column below 120 m as indicated by the decreasing amount of in situ euryhaline bivalves. Furthermore, an abrupt lithological and geochemical change at -300m core on western slope area indicates that the ventilation effect of the MW continued to more than 300m depth until 5.3 ka BP. These results show that MW used the NW oriented main channel until 5.3 ka BP transporting oxygenated water to the area, but changed its course to the east after that date. On the other hand, the presence of high Mn fluctuations related to precipitation of Mn carbonate at the eastern slope area to depths of at least -300m show that MW ventilation has been active since the shoaling event of oxic-anoxic interface at 6.8 ka BP. A recent rise in the oxic-anoxic interface (250-300 a BP) is determined in a sedimentary core collected at -93m from the western side of the area. This rise is indicated by increased values of Mn-Fe-S and high TOC, corresponding to the intersection of the oxic-anoxic interface with the shelf edge area.
6) **Long-term regularities and disturbances of oxygen regime in the NW Black Sea coastal waters**

*Authors*
Luminita Lazar, M-T Gomoiu, D.Vasiliu (in prep.).

*This manuscript is in preparation. A description of the content follows:*

The paper presents temporal, seasonal and inter-annual, variations of Dissolved Oxygen (DO) regime in the Romanian Black Sea coastal waters (Constanta area) based on data collected daily, during 1959 – 2010, from a fixed near-shore station (bottom depth of 1.5 m) and monthly/seasonally, during 1964 – 1981/1982 – 2010, from five stations (water column sampling with standard depths within 0 – 50m) located on the transect Constanta-East (50 km length, bottom depths within 16 – 54 m), respectively.

The climatic factors, which control seawater thermal regime, discharge fluctuations of the Danube River and water masses mixing, as well as biological processes, are mainly responsible for DO temporal variability in the Romanian coastal waters. Seasonally, DO showed the highest concentrations in winter (maximum monthly mean of 490.9 μM in February 2007), strongly linked to low seawater temperature and intense mixing processes leading to well-oxygenated waters in the cold season. The lowest DO concentrations were measured in the warm season (minimum monthly mean of 186.4 μM in August 1998), when higher seawater thermal regime and oxygen consumption for the organic matter decomposition contribute to decreased values.

In terms of inter-annual variability, the highest winter DO concentrations (throughout the water column) were measured in cold and windy winters (maximum of 436.2 μM in 2003). Summer DO variation showed different patterns for the upper layers and bottom layers, respectively. In the surface layer, higher values (maximum of 387.2 μM in 1999) were measured in the years with larger summer discharges of the Danube River, which favor more intense photosynthetic processes due to increased nutrient stocks. In the bottom layers, DO showed lower values in the intense eutrophication period (1975 – 1988), when the seasonal cycle of primary production highlighted more pronounced summer maxima. Summers with higher seawater thermal regime showed lower DO concentrations in the bottom layers (minimum of 138.6 μM in 1986) due to stronger water stratification and increased intensities of oxidative decomposition of newly formed organic matter. The strength of these processes is responsible for the occurrence of hypoxic events in the coastal waters, but these phenomena are rather sporadic and are not a permanent feature of the Romanian coastal waters.
Meetings were results were presented

1) Holocene History of the Mediterranean Inflow and Its Influence on Formation of the Channel Network Complex and Redox Conditions in the Istanbul Strait Outlet Area of the Black Sea

Authors
Erdem, Z., Çağatay, M.N., Damcı, E., Ülgen, U.B., Holtappels, M., Lichtschlag, A.,
2) **Effect of Mediterranean Inflow on Redox Conditions of the İstanbul Strait Outlet Area of the Black Sea**

*Authors*
Erdem, Z., Çağatay, M.N.,

3) Events of hypoxia and anoxia in the Crimean coastal waters

Authors
Orekhova, N.A., Sergeevan, N.G., Gulin, M.B., Konovalov, S.K.

INQUA 501-IGCP 521 Six th Plenary Meeting and Field Trip, Rhodes, Greece, 27 September-5 October 2010

Events of hypoxia and anoxia in the Crimean coastal waters
Orekhova Natalia A.¹, Sergeeva N.G.², Gulin M.B.³, Konovalov Sergey K.⁴
¹, ⁴Marine Hydrophysical Institute, Ukrainian National Academy of Sciences, 2, Kapitanskaya Str., Sevastopol, 99011 Ukraine
², ³ Institute of Biology of the Southern Seas, Ukrainian National Academy of Sciences, 2, Nakhimova Av., Sevastopol, 99011 Ukraine
¹ naorekh-2004@mai.ru
² nserg05@mail.ru
³ s.gulin@ibss.org.ua
⁴ sergey_konovalov@yahoo.com

Keywords: oxygen deficient, the Black Sea, bottom sediments, Au-Hg-microelectrode

The number of marine environments with oxygen depletion has increased over the last decades. This is a result of many factors and human and industrial activities are widely accepted as the primary reason. The oxygen deficit becomes apparent in the most valuable coastal marine systems (estuaries, bays, harbors and etc.). In critical conditions, hypoxia is developed, when the dissolved oxygen concentration falls below 63 µM, to end up with anoxia (analytical absence of oxygen). These events result in degradation and dramatic changes in the structure of marine ecosystems: worsening of the water quality of the coastal waters and of social and economic values of the region.

Hypoxia and anoxia are consequences of eutrophication. They result from excess of organic carbon fluxes in the water column due to its higher production and discharge from the coast. Whatever is the source of excess of organic carbon, it leads to accumulation of organic carbon in the water column and bottom sediments. Organic carbon accumulation promotes more active oxygen consumption and leads to generation of reduced species: sulfides, Mn (II), Fe (II), etc. When it happens, hypoxic and anoxic conditions are formed in the bottom sediments, where hydrogen sulfide is ultimately generated. Sulfide limits and/or changes the structure of benthic communities and may support the flux of sulfide from bottom sediments to the water column. This results in anoxic conditions in the water column as well.

To study hypoxia generation and seasonal evolution, we organized on sea studies at the Crimean coast in July 2009 and are still performing observations. Three sites with organic carbon fluxes of different origin were chosen for investigation. These were:
- the internal part of the Sevastopol Bay, which is under severe anthropogenic and industrial pressure;
- the internal part of the Omega Bay, which is under anthropogenic (municipal) pressure only;
- the Tarkhankut Cape area, which is an open part of the Black Sea coast and where methane seeps are the primary source of organic carbon supporting microbiological methane oxidation and microbial biomass production.
To obtain vertical oxygen and sulfides profiles, voltammetric analysis with a solid Au-Hg microelectrode has been applied. The method allows to investigate the vertical distribution of oxygen and sulfide in pore waters of the bottom sediments and in the bottom layer of water in situ with high accuracy. The detection limits are 5 µM and 0.5 µM for oxygen and sulfide accordingly.

Hypoxia has been a permanent feature of all sediments with very few exceptions. While anoxia has been registered in the upper layer of bottom sediments in the warm period in all investigated sites, except the Omega Bay in September 2009 and the Sevastopol and Omega Bays in July 2009. The concentration of sulfide in the Sevastopol Bay don’t exceed 1µM (fig. 1a), whereas it amounts to 800 µM in the deeper layers of sediments in the Omega Bay (fig. 1b). In September, dissolved oxygen penetrated to 40 mm depth in the Omega Bay and hypoxia was not detected in the water column. In the Sevastopol Bay and near the Tarkhankut Cape, anoxia was observed in the upper layer of bottom sediments. The concentration of sulfide reached 1 µM in the Sevastopol Bay, while severe anoxia was detected near the Tarkhankut Cape, where the concentration of sulfide reached about 3 mM (fig. 2a) that is about 6-fold of the maximum concentration of sulfide in the bottom waters of the Black Sea. In June 2010, the concentration of sulfide near the Tarkhankut Cape was still high, but it did not exceed 1.5 mM and oxygen at the surface of bottom sediments was absent (fig 2b).

In the cold period of year (November of 2009, January and March of 2010), hypoxia weren’t detected at any of the monitored sites. In the Omega Bay, oxygen penetrated down to ~30 mm depth. Hypoxia was observed in the Sevastopol Bay, where sulfide was detected at the bottom surface and its concentration reached 250 µM in November 2009 (fig 3a) and ~1 µM (March 2010). In the area of the Tarkhankut Cape, hypoxia was also detected in the upper layer of sediments in November 2009, but the concentration of sulfide was about 50 µM (fig 3b).

![Fig. 1. Oxygen and sulfide in the Sevastopol (a) and Omega (b) Bays in July 2009](image-url)
The obtained data demonstrate that different sources of organic carbon (municipal in the Omega Bay, industrial in the Sevastopol Bay, and natural near the Tarkhankut Cape) may effectively support hypoxia or anoxia in different regions of the Black Sea coast. Both hypoxia and anoxia are subject of seasonal changes with the most oxygenated conditions in the cold period of year and the most severe anoxia in the warm period.

This work has been supported from the project EC 7thFP "In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies" (HYPOX, #226213).
Overview of publications by Hypox members related to Hypox topic but not funded by Hypox


Appendix:

Publications in Russian and publications by Hypox members and related to Hypox topic but not funded by Hypox
Appendix

Appendix I

Biogeography and ecology of Trophonopsis breviata (Jeffreys, 1882) (Gastropoda: Muricidae: Trophoninae).

Authors
Bondarev, I.P.

Географическое распространение Trophonopsis breviata (Jeffreys, 1882) рассмотрено на историческом и экологическом фоне. Приведены и проанализированы данные о распространении вида по глубине и его биоценотические связи. Установлено, что T. breviata встречается в составе 10 донных биоценозов Чёрного моря. Степень стабильности является определяющей экологической характеристикой, которая лимитирует распространение вида. Данные по экологии вида свидетельствуют о его бореальных корнях. Экологические особенности T. breviata подтверждают его видовую самостоятельность.

Ключевые слова: биогеография, биоценозы, экология, Trophonopsis breviata, Чёрное море

Trophonopsis breviata (Jeffreys, 1882) – один из наиболее характерных моллюсков джеметинских геологических слоёв, соответствующих последней, новочерноморской, стадии эволюции Черноморского бассейна [16], и заслуживает рассмотрения в специальном исследовании как важный элемент черноморской макрофауны, вселение и широкое развитие которого тесно связано с развитием экосистемы Чёрного моря в голоцене. Высокая степень вариабельности вида [22] в совокупности с его разнообразными биоценотическими связями представляет интерес для теории и практики экоморфологии и систематики моллюсков.

Целью данной работы является исследование T. breviata на природно-историческом фоне для создания целостного представления о биологическом виде как результате взаимодействия с факто-рами среды. Для этого прослежены биогеографические корни вида, исследованы его экологические особенности, показана экологически обусловленная внутривидовая изменчивость, проведено сравнение с близкородственными видами. Комплекс экологических и конхологических данных используется для решения таксономических и систематических вопросов, связанных с видовой самостоятельностью T. breviata и обоснованностью подвидового и инфраподвидовых названий.
Названия биологических таксонов приводятся в соответствии с современной редакцией WoRMS [30].


Сравнительно недавние исследования [23] свидетельствуют о присутствии T. breviata в Мраморном море, а фотографии, представленные на Интернет-сайте www.conchology.ru, дают информацию о наличии T. breviata в районе острова Bozcaada, расположенного у пролива Дарданеллы в Эгейском море.

Наиболее вероятной «прародиной» T. breviata является северо-западная Атлантика, откуда его предок попал в северо-восточное Средиземноморье. Представители подсемейства Trophoninae распространены преимущественно в высоких широтах, как северного, так и южного полушарий [28]. Виды трофонин, встречающиеся в более низких широтах, обычно приурочены к большим глубинам, где стабильно существует холодноводный режим; например, Trophonopsis droueti (Dautzenberg, 1889) из района Азорских о-вов, обитающий на глубинах свыше 1000 м. Проникновение Trophoninae, также как и других представителей бореальной и арктической фауны, в более низкие широты связано с охлаждением вод в ледниковый период [17]. В результате последующего таяния ледника и повышения уровня Мирового океана трофонины проникли в Средиземное море. Дальнейшее проникновение в Чёрное море средиземноморских видов, в том


Отражением бореального происхожде- ния вида является ограничение его распростра- нения в Средиземноморской биогеографиче- ской провинции наиболее холодноводной её частью – Мраморным морем и проливом Дар- данеллы. Стоит упомянуть, что ещё А. А. Ост- роумов в 1896 г. отмечал, что фауна Мраморно- го моря отличается от остального Средизем- номорья значительным удельным весом бореаль- ных (кельтских) видов [17].

Отсутствие *T. breviata* в самом Средиземном море не позволяет отнести его к группе средиземноморских эндемиков. Не является он и эндемиком Чёрного моря, хотя и распростра- нён преимущественно в черноморском бассейне. Следовательно, *T. breviata* должен быть отнесен к группе средиземноморско-бореальных видов.


Видовой статус *T. droueti* не оспарива- ется, благодаря ограниченности его ареала (Азорских о-ва) и приуроченности только к большим глубинам (более 1000 м) [26, 28].

**Экология.** *T. breviata* – небольшая хищ- ная гастропода (средний размер раковины 7 – 9 мм), которая питается, в основном, мелкими двустворчатыми моллюсками, просверливая их раковину [19]. Наши исследования показыва- ют, что жертвами *T. breviata* могут быть и мал- кие гастроподы, вплоть до представителей сво- его же вида. Пустые раковины *T. breviata* с
характерными небольшими круглыми отверстиями чаще встречаются на глубинах свыше 100 м, где количество потенциальных жертв этого хищника существенно уменьшается. Очевидно, что каннибализм вызван дефицитом объектов питания.

*T. breviata* имеет прямое развитие без пелагической стадии. Эта особенность является причиной достаточно низкой способности вида к расселению и объясняет тот факт, что он появился в Чёрном море в последнюю очередь. Линзообразные капсулы размером около 2 мм крепятся к субстрату плоским основанием [19]. Реализация базовых функций определяет биотопические и биоценотические связи. Традиционно *T. breviata* рассматривают как обитателя зоны развития фазеолиновых илов [2, 5, 19, 24], при этом его стенотопность и предпочтение илсного грунта рассматриваются как специфическую экологическую характеристику [24]. Такое определение верно лишь формально, поскольку на самом деле *T. breviata* наиболее характерен для фазеолинового пояса Чёрного моря, где развиты илсные грунты. Но это не означает, что этот вид является пелофилом, поскольку ценообразующий двусторчатый моллюск *M. phaseolina* формирует в зоне развития илов пятна различной формы и размера или даже гряды [21], которые обычно развиваются на створках уже отмерших раковин. Именно эти пятна и служат биотопом с твёрдым субстратом, необходимым для крепления яйцевых капсул, а сама фазеолин и ассоциированные с ней моллюски составляют биоценоз, создающий условия для полноценного питания *T. breviata*. Это положение действительное для всех биоценозов рыхлых грунтов, в которых обнаружено присутствие этого моллюска. Приведенные ниже данные показывают, что *T. breviata* обладает гораздо большей экологической валентностью, чем это принято считать.

Табл. 1 Донные биоценозы и глубины обнаружения в их пределах *T. breviatus*  

<table>
<thead>
<tr>
<th>Биоценоз</th>
<th>Глубины распространения биоценоза, м</th>
<th>Глубины обнаружения <em>T. breviatus</em>, м</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phillophoracea</td>
<td>20 – 60</td>
<td>40 – 60</td>
</tr>
<tr>
<td><em>Mytilus galloprovincialis</em> (Lam.)</td>
<td>20 – 60</td>
<td>40 – 60</td>
</tr>
<tr>
<td><em>Upogebia pussila</em> (Petanga) – <em>Pitar rudis</em> (Poli)</td>
<td>25 – 27</td>
<td>25 – 27</td>
</tr>
<tr>
<td><em>Gouldia minima</em> (Montagu)</td>
<td>20 – 50</td>
<td>30</td>
</tr>
<tr>
<td><em>Pitar rudis</em> (Poli)</td>
<td>25 – 28; 50 – 55</td>
<td>50 – 55</td>
</tr>
<tr>
<td><em>Cardium simile</em> Milashevich</td>
<td>40 – 52</td>
<td>40</td>
</tr>
<tr>
<td><em>Terebellides stroemi</em> Sars</td>
<td>40 – 115</td>
<td>40 – 60</td>
</tr>
<tr>
<td><em>Ophiurae</em></td>
<td>50 – 15</td>
<td>75 – 105</td>
</tr>
<tr>
<td><em>Modiolula phaseolina</em> (Philippi)</td>
<td>50 – 125</td>
<td>60 – 120</td>
</tr>
<tr>
<td><em>Pachycerianthus solitarius</em> (Rapp)</td>
<td>120 – 160</td>
<td>120 – 158</td>
</tr>
</tbody>
</table>

Примечание: Границы биоценозов и глубины обнаружения в них *T. breviatus* даны преимущественно в соответствии с [11], биоценоз филлофоры – по [9], *Terebellides stroemi* – по [18], *Pachycerianthus solitarius* – по [25; собств. данные].

Тесная связь *T. breviata* с биоценозом фазеолины подтверждается и данными радиоуглеродного анализа [20], которые показывают, что их появление в Чёрном море произошло почти синхронно с незначительным опрежением фазеолиной. *M. phaseolina* встречается в Чёрном море на глубинах от 4 до 174 м [11], однако полный жизненный цикл она способна осуществлять при температурах не выше 8°C [14], что является отражением её бореальных корней. Наибольшие плотности фазеолина создаёт на глубинах 60 – 100 м, где расположен холодный промежуточный слой (ХПС). Глубже плотность фазеолины падает, контролируясь

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набором факторов: повышением температуры воды, усилением гипоксии, увеличением крутизны склона дна, более жидкой консистенцией илов [21]. Гравитационное перемещение грунта объясняет наличие фазеолины на максимальной известной глубине [21]; этот же процесс может быть причиной присутствия T. breviata или его раковин на максимальных глубинах. Ядро биоценоза фазеолины расположено на глубине 80 – 90 м, к ним же приурочен пик численности T. breviata. Обычно плотность T. breviata в ядре фазеолинового биоценоза не превышает 10 экз. м^-2, снижаясь по мере как уменьшения, так и увеличения глубин.


В составе других биоценозов, характерных для глубин, не превышающих 50 – 55 м (табл. 1), T. breviata встречается редко и только в осенне-зимний период, начиная с ноября. Однако на глубинах 45 – 55 м, соответствующей верхней границе ХПС, T. breviata создаёт повышенные концентрации (4 – 8 экз. м²), почти такие же, как в ядре фазеолинового биоценоза.

Таким образом, можно выделить два экологических комплекса T. breviata, соответствующих зонам двух пиков численности: более мелководный комплекс верхней границы распространения, где биоценозы и биотопы более разнообразны, но преобладает биоценоз мидии, и более глубоководный, где преобладает биоценоз фазеолины.

В некоторых районах на обычных глубинах распространения фазеолины она занимаает подчинённое положение, а основу биоценоза составляют либо Polychaeta Terebellides stroemi (Sars, 1835) [18], либо Ophiurae [11]. На глубинах свыше 120 м, где доминантом биоценоза является Coelenterata Pachycerianthus solitarius (Rapp, 1829) [21], T. breviatus встречается редко. Биоценоз P. solitarius (Rapp) детально не описан, но по данным наблюдений из ПОА [4]...
и результатам бентосных исследований [25], на глубинах свыше 110 – 130 м фазеолина практически исчезает, и её биоценоз замещается на биоценоз, в котором доминирует P. solitarius. Распространение T. breviata на этих глубинах контролируется теми же факторами, которые ограничивают распространение фазеолины. Кроме того, дополнительным фактором является минимальное количество потенциальных жертв, приводящее T. breviata к каннибализму.

Биогеографические корни объясняют и экологические предпочтения вида. Представители Trophoninae в подавляющем большинстве — стенотермные организмы [25]. T. muricata — один из немногих эвритермных представителей Trophoninae, что важно учитывать для выводов о степени его родства с T. breviata.

Характерная для Чёрного моря структура вод позволяет существовать бореальной бентофауне и флоре на сравнительно небольших глубинах. Холодный промежуточный слой (ХПС), расположенный на шельфе на глубинах 45 – 150 м, формируется за счёт зимнего охлаждения поверхностных вод и имеет плотность выше, чем у последних, но ниже, чем у расположенного ниже слоя постоянных температур, обладающего более высокой солёносностью [6]. Динамические колебания верхней границы ХПС и зимнее охлаждение прибрежных вод позволяют T. breviata продвигаться на более мелкие глубины, богатые молодью митилид и другими мелкими двустворчатыми моллюсками. Невозможность преодолеть температурный барьер ограничивает распространение T. breviata на мелководе, а наличие большого количества потенциальных жертв создаёт повышенные концентрации вида у этого барьера. Экологические данные, наряду с прямыми измерениями температур, позволяют подтвердить стенотермность T. breviata, для которого жизнедеятельность в Чёрном море возможна при температурах, не превышающих 8.5°C. При температурах ниже 7.2°C T. breviata также не обнаружен, что позволяет охарактеризовать его как узкостенотермный холодолюбивый вид. Вероятно, у T. breviata не только полный жизненный цикл и, в первую очередь, гаметогенез, но и просто процесс жизнедеятельности индивида могут протекать в узком диапазоне температур. В Средиземном море таких низких температур нет; даже в Мраморном море, где температура воды в поверхностном слое зимой опускается до 8°C, летние температуры выше, чем в Чёрном море. В структуре вод Мраморного и Эгейского морей также присутствует ХПС, располагающийся глубже 40 – 50 м. Поэтому все особи T. breviata, чьи изображения приведены на сайте www.conchology.be, собраны на глубинах 80 – 85 м, что соответствует глубине максимальной численности вида в Чёрном море. В средиземноморской биогеографической провинции распространение T. breviata ограничено Мраморным морем и непосредственно прилегающим к нему северным участком Эгейского моря. Не исключено, что при более высокой по сравнению с Чёрным морем солёносности, в морях северо-восточного Средиземноморья T. breviata может существовать при несколько более высоких температурах, чем вышеуказанный максимум. Осеннезимнее охлаждение прибрежных вод Чёрного моря позволяет T. breviata проникать на более мелкие глубины — до 25 м, а, возможно, и меньше. Пустые раковины T. breviata были собраны на глубине 5 м; есть сведения об их обнаружении в выбросах на пляжах. Попадание раковин на мелководье может объясняться и турбулентным переносом штормовыми волнами, и перемещением раками-отшельниками.

Экоформы и таксономия. Несмотря на то, что ареал T. breviata достаточно ограничен, этот вид обладает столь высокой степенью конхологической варииабельности, что его крайние формы могли бы быть отнесены к разным видам. Даже экземпляры из одной пробы, особенно из комплекса верхней границы распространения, демонстрируют большие различия конхологических характеристик. При этом экземпляры T. breviata из этой зоны достигают наиболее крупных размеров, и их раковины
чаще пигментированы, даже в районах, где отсутствуют красные водоросли [22]. Причиной такого конхологического разнообразия является, очевидно, разнообразие биоценозов и объектов питания.

В начале 20 века самые плотные скопления *T. breviata* были зарегистрированы в СЭЧМ на филлофорном поле Зернова [13]. Обитающие здесь особи характеризовались интенсивной красно-коричневой окраской, что связано с мощным развитием красных водорослей. Биоценоз красных водорослей был одним из наиболее продуктивных в Чёрном море [7]. Существенное сокращение запасов филлофоры в третьей четверти 20 века вплоть до практического исчезновения было обусловлено сокращением запасов филлофоры в третьей четверти 20 века вплоть до практического исчезновения биоценоза на большей части СЭЧМ повлекло за собой существенное сокращение местной популяции *T. breviata*. Соответственно, характерная для поля Зернова форма с рельефными рёбрами и интенсивной красно-коричневой окраской встречается гораздо реже, чем в сборах, которыми располагал К. О. Милашевич [22]. Характер имеющихся сборов дал основание К. О. Милашевичу усомниться в том, что экземпляры, имеющиеся в распоряжении автора описания вида *T. breviata*, являются типичными для вида, и он описал два, по его мнению, редких вариетета, которые фактически соответствовали оригинальному описанию. Экоморфы, у которых «вместо продольных рёбер раковина снабжена многочисленными тонкими неправильными пластинками; местами замечается выраженная утолщение» — var. *striata* и с характерным «молодо-белым цветом» — var. *lactea* [13, стр. 105], фактически встречаются чаще и соответствуют тем изображениям *T. breviata*, по которым известен вид [1, 10, 26, 28]. В данном случае логика, которой руководствовался К. О. Милашевич, привела к появленю избыточных терминов.

В описании *T. breviata* Ф. Джеффрис [27] указывал в качестве его ближайшего родственника *T. muricata*, который характеризуется более скульптурированной раковиной. В монографии по истории черноморских гастро-
Выводы 1. *T. breviata* является холодолюбивым стенотермным видом с бореальными корнями. Современное распространение вида ограничено Чёрным и Мраморным морями и частью Эгейского моря, непосредственно прилежащих к проливу Дарданеллы (Чанаккале), что даёт основание отнести его к средиземно-морско-бореальным видам. 2. Глубины постоянного обитания *T. breviata* (45 – 150 м) контролируются распространением холодного промежуточного слоя и колебанием его верхней границы. Обнаружение его на меньших глубинах (до 25 м) связано с процессами осенн-зимнего охлаждения прибрежных вод и глубинах (до 25 м) связано с процессами осенн-зимнего охлаждения прибрежных вод и объясняется сезонными миграциями. Установленная нижняя граница распространения вида (158 м) обусловлена биотическими и абиотическими факторами, которые лимитируют развитие объектов питания. 3. Распределение *T. breviata* характеризуется двумя пиками численности: максимальные показатели связаны с ядром биоценоза фазеолины (80 м), второй пик приурочен к зоне максимального встречаемости молоди мидии (50 м). 4. *T. breviata* обнаружен в пределах 10 биоценозов Чёрного моря, что, несмотря на стенотермность, позволяет характеризовать его как вид с достаточно широкой экологической валентностью.

Biogeography and ecology *Trophonopsis breviata*... 


Appendix II

A new record of the *Sarsameira parva* (Boeck, 1872) and *Tachidiella minuta* G. O. Sars, 1909 (Copepoda, Harpacticoida) in the Black Sea.

*Authors*
Kolesnikova, E.A., Sergeeva, N.G.


При изучении сообществ мейобентоса района Босфорского пролива (Чёрное море) в донных осадках на глубине 82 м (41°24,02′ Е, 29°03,21′ N) найдены два новых для Чёрного моря вида гарпактикоид. Сборы донных осадков в диапазоне глубин 75 – 300 м были выполнены в рейсе НИС Arar Стамбульского университета (Турция) 9 – 21 ноября 2009 г. Отбор проб проведен мультикorerом, позволившим сохранить стратификацию колонки грунта. Для анализа вертикального распределения мейобентоса в толще донных осадков изучение колонки грунта (площадью 38.5 см²) проводили послойно с интервалом 1 см от поверхности до 7 см глубины. На одной из станций (ст. 3) зарегистрированы ранее не отмеченные для данного водоёма виды гарпактикоид Sarsameira parva (Boeck, 1872) и Tachidiella minuta G. O. Sars, 1909. Первый из них характерен для северных морей. Его местообитание – илистые и песчано-илистые грунты на глубинах 60 – 120 м. Вид морской, полигалинный, с температурным оптимумом 0 – 20 °C. Второй вид описан из северных акваторий Норвегии, Швеции и Франции. Морской полигалинный вид, обитающий в иллюстных биотопах на глубинах 8 – 70 м в интервале температур 0 – 20 °С (Lang, 1948; Lee, Huys, 1999). В Чёрном море эти виды также найдены в иллюстном биотопе. В колонке взятого грунта наблюдались слои различной окраски: его верхний окисленный слой (0 – 1 см) чётко отличался от более глубоких горизонтов, имеющих тёмно-серый цвет с характерной чёрной слоистостью, указывающей на присутствие восстановленных условий в глубине осадка. Sarsameira parva отмечен в колонке грунта от её поверхности до глубины 4 см. В целом популяция данного вида была представлена 16 особями (4♂, 9♀ и 3 копеподита). Наибольшая концентрация особей отмечена в горизонте 1 – 2 см (14 особей). Tachidiella minuta обнаружена только в поверхностном горизонте и представлена 7 особями (2♂ и 5♀).


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Appendix III

The lowest zoobenthos border in the Black Sea Near-Bosporus region

Authors
Sergeeva, N.G., Zaika, V.E. Bondarev, I.P.

НИЖНЯЯ ГРАНИЦА ЗООБЕНТОСА
В ПРИБОСФОРСКОМ РАЙОНЕ ЧЁРНОГО МОРЯ

В прибосфорском районе Чёрного моря выполнено 10 станций в интервале глубин 75 – 300 м. На глубинах 75 – 82 м общая численность бентоса составила 136 – 111 тыс. экз. м\(^{-2}\), после чего резко снижалась до уровня 29 – 11 тыс. экз. м\(^{-2}\) и оставалась в пределах 11– 4 тыс. экз. м\(^{-2}\) до глубин 250 м. Глубже 123 м макробентос представлен только аннелидами. Полихета *Vigtorniella zaikai* в прибосфорском районе образует скопление на глубине 250 м, хотя в северной половине моря её пики располагаются на глубинах 150 – 170 м, в переходной полосе от кислородной к сероводородной зоне. Общая численность мейобентоса наиболее высока на глубине 75 м (1861.6 тыс. экз. м\(^{-2}\)). С увеличением глубины она снижается, образуя меньшие пики на глубинах 88 (1011.1 тыс. экз. м\(^{-2}\)), 162 (468.5 тыс. экз. м\(^{-2}\)) и 250 м (603.2 тыс. экз. м\(^{-2}\)). Основная доля в общей численности приходится на нематод, следующей по обилию группой были гарпактикоиды. Нижний пик обилия расположен на глубине 250 м, а на 300 м численность мейобентоса значительно убывает.

Ключевые слова: Чёрное море, прибосфорский район, макробентос, мейобентос.

Вопрос о нижней границе макрофауны в Чёрном море детально обсуждался по результатам съёмок, выполненных ещё в 1925 – 1935 гг. [4, 5, 6, 8]. Тогда было показано, что глубина границы колеблется от 120 до 170 (200) м, причём наиболее заглублена она близ пролива Босфор. В работах по макрообентосу этого района, выполненных отделом экологии бентоса ИнБЮМ, обсуждались результаты съёмок 1958, 1960 и 1989 гг. [1, 3], в ходе которых суммарно было сделано 18 станций в пределах глубин 70 – 113 м. Это не позволяло рассмотреть вопрос о нижней границе бентоса, расположенной на заведомо большей глубине. Мейобентос исследовали лишь на некоторых станциях, и были приведены только его общая численность и биомасса для двух типов сообществ [3]. Из более ранних работ только одна содержала данные о составе мейобентоса прибосфорского района [9].

Поскольку многие авторы считают, что во второй половине XX века в экосистеме Чёрного моря произошли существенные изменения, важно получить новые сведения о границе распространения макро- и мейофауны по глубине, в частности, в районе влияния нижней струи босфорского течения, где уже отмечалось обеднение макробентоса в результате загрязнения [9].

Ниже изложены данные о составе и численности основных групп фауны в районе исследования и приведена новая информация о нижней границе распространения по глубине как макро-, так и мейофауны. Материалы по видовому составу обеих групп будут представлены после завершения обработки.

Материал и методы. Материалом служили сборы донных осадков 11 – 15 ноября 2009 г. с борта НИС «Арар» Стамбульского технического университета. В прибосфорском районе Чёрного моря выполнены сборы донных осадков на 10 станциях в интервале глубин 75 – 300 м (рис. 1).
При отборе проб использованы мультикорер и гравити-корер (далее именуемые кореры) с внутренним диаметром трубок 7 см (38.5 см²). Для сравнения с прежними данными по макробентосу [10] на двух станциях (75 и 94 м) пробы взяты дночерпателем с площадью захвата 0.1 м² (табл. 1).

Табл. 1 Местоположение бентосных станций, характер осадка и метод отбора проб

<table>
<thead>
<tr>
<th>№ станции</th>
<th>Глубина, м</th>
<th>Координаты</th>
<th>Характер субстрата</th>
<th>Орудие отбора</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>41°20.33′ 29°06.03′</td>
<td>Чёрный ил, верхние 10 см окисленные, глубже восстановленные</td>
<td>Ди, МК</td>
</tr>
<tr>
<td>2</td>
<td>94</td>
<td>41°24.02′ 29°03.21′</td>
<td>Из пелитовый</td>
<td>Ди, МК</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>41°24.02′ 29°03.21′</td>
<td>Из пелитовый, верхний 1 см окисленный, глубже серый с чёрными прожилками</td>
<td>МК</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>41°23.29′ 29°12.24′</td>
<td>Алевритово-пелитовый ил, со створками илом</td>
<td>ГК</td>
</tr>
<tr>
<td>5</td>
<td>103</td>
<td>41°26.86′ 29°12.95′</td>
<td>Алевритово-пелитовый ил</td>
<td>ГК</td>
</tr>
<tr>
<td>6</td>
<td>122</td>
<td>41°28.68′ 29°14.81′</td>
<td>Из чёрный</td>
<td>ГК</td>
</tr>
<tr>
<td>7</td>
<td>160</td>
<td>41°28.99′ 29°15.14′</td>
<td>Из чёрный</td>
<td>ГК</td>
</tr>
<tr>
<td>8</td>
<td>190</td>
<td>41°29.36′ 29°15.53′</td>
<td>Из чёрный с запахом (H_2S)</td>
<td>ГК</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
<td>41°29.93′ 29°16.12′</td>
<td>Из чёрный текущий с запахом (H_2S)</td>
<td>ГК</td>
</tr>
<tr>
<td>10</td>
<td>300</td>
<td>41°30.14′ 29°16.34′</td>
<td>Из чёрный текущий с запахом (H_2S)</td>
<td>ГК</td>
</tr>
</tbody>
</table>

Дн – дночерпателем, МК – мультикорер, ГК – гравити-корер УС

Кореры давали колонки осадков высотой 10 см. Для изучения глубины проникновения организмов от поверхности в толщу осадка сразу после взятия проб полученные колонки грунта делили послойно на подпробы с шагом 1 см. Каждую из них фиксировали 75° спиртом. В лаборатории ИнБЮМ пробы промывали дистилированной водой через сите 1000, 250 и 63 мкм. Чтобы отличить живых гидробионтов в
месте взятия проб, особенно моллюсков и формамицифер от пустых створок, применяли окраску бенгальским розовым. К числу живых относили только интенсивно окрашенные организмы, не имеющие никаких нарушений морфо-анатомических признаков.

Результаты и обсуждение. Новые данные, полученные с помощью пробоотборников, позволяющих сохранить стратификацию донных осадков в колонке грунта и предотвратить попадание в пробу посторонней фауны, с небольшими интервалами между станциями и в достаточно широком диапазоне глубин, позволяют описать современное распределение черноморского макро- и мейобентоса по глубине.

Макробентос. Прежде всего, обсудим данные, полученные на всех станциях с помощью кореров. В период исследования общая численность макробентоса в прибосфорском районе составила 136 – 111 тыс. экз. м\(^2\) на глубинах 75 – 82 м, после чего резко снижалась до уровня 29 – 11 тыс. экз. м\(^2\) и оставалась в пределах 11– 4 тыс. экз. м\(^2\) до глубины 250 м (рис. 2).

Рис. 2 Распределение общей численности макро- и мейобентоса по глубине в прибосфорском районе (ноябрь, 2009)
Fig. 2 Total macro- and meiobenthos quantity distribution along the depths at the Bosporus region (November, 2009).

Сравним численность пяти наиболее обильных групп макробентоса. На глубине 75 м значительно преобладали ракообразные, в основном амфиподы, (95 тыс. экз. м\(^2\)). Второй по обилию группой были аннелиды (29 тыс. экз. м\(^2\)), затем - иглокожие (офиуры) и моллюски (двустворчатые) (табл. 2; рис. 3).

<table>
<thead>
<tr>
<th>Глубина, м</th>
<th>Crustacea</th>
<th>Annelida</th>
<th>Bivalvia</th>
<th>Echinodermata</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>94640</td>
<td>28860</td>
<td>1820</td>
<td>8320</td>
</tr>
<tr>
<td>82</td>
<td>7020</td>
<td>22100</td>
<td>260</td>
<td>4940</td>
</tr>
<tr>
<td>88</td>
<td>520</td>
<td>2080</td>
<td>6760</td>
<td>780</td>
</tr>
<tr>
<td>103</td>
<td>520</td>
<td>4680</td>
<td>0</td>
<td>1560</td>
</tr>
<tr>
<td>123</td>
<td>0</td>
<td>1300</td>
<td>1560</td>
<td>260</td>
</tr>
<tr>
<td>162</td>
<td>0</td>
<td>10920</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>190</td>
<td>0</td>
<td>780</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>0</td>
<td>6760</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Табл. 2 Численность макробентоса (экз. м\(^2\)) в прибосфорском районе (по данным кореров)
Table 2 Macrobenthos (indiv.m\(^2\)) quantity in the Near-Bosporus region (according to the corers data)

Морський екологічний журнал, № 1, Т. Х. 2011
На глубине 82 м численность ракообразных снизилась в 14 раз, а офиур – вдвое. Преобладающей группой стали аннелиды, сохранявшие высокое обилие (22 тыс. экз. м\(^{-2}\)). К глубине 88 м общая численность резко упала (рис. 2, 4). Здесь наблюдался пик численности двустворчатых моллюсков, и они стали доминирующей группой. Среди ракообразных на глубинах до 88 м присутствовали амфиоподы, танайды и кумацеи, но с дальнейшим увеличением глубины в составе бентоса отмечены только амфиоподы.

При малом различии глубин сложно вычленить возможное влияние таких факторов, как глубина и свойства биотопов, на изменения обилия групп от станции к станции, но нужно иметь в виду, что ранее подчеркивалась пятисостояние поселений макробентоса в данном районе [1, 8]. Глубже 105 м не встречались морские звезды, а на 122 м в последний раз зарегистрированы офиуры. Представители губок, ракообразных (амфиопод), двустворчатых моллюсков и асcidий тоже встречались только до глубины 123 м. На большей глубине в прибосфорском районе из макробентоса зарегистрированы только аннелиды. Помимо них в мейобентосных пробах на глубине 160 м найдено две особи, а на 190 м – одна особь двустворчатых моллюсков. В пробе с глубины 250 м найден один живой брюхоногий моллюск. Эта форма не встречалась на глубинах 162 и 190 м, и данную находку можно считать случайной, во всяком случае, пока не будут получены дополнительные данные из дночерпательных сборов. На глубине 300 м не найдено ни одного представителя макробентоса, в том числе в пневдомейобентосе.

Поскольку в прибосфорском районе Чёрного моря аннелиды проникают наиболее глубоко, остановимся на их распределении. Численность аннелид распределена по глубинам очень неравномерно (рис. 4), а основной пик наблюдался на глубинах 75 – 82 м (28860 экз. м\(^{-2}\)). Отметим также пики на 162 м (10920 экз. м\(^{-2}\)) и на 250 м (6760 экз. м\(^{-2}\)). В пике на 250 м численность аннелид была в 4,2 раза меньше, чем в основном пике, но интерес вызывают два обстоятельства. В первых, пик был сформирован, по-видимому, в основном, олигохетами (4940 экз. м\(^{-2}\)), с добавлением (1820 экз. м\(^{-2}\)) полихет, в частности, Vigtorniella zaikai Kisselevа, которых гораздо больше было в мейобентосе (7020 экз. м\(^{-2}\)). В период исследования этот вид представлен в большей степени молодыми стадиями, которые, благодаря небольшим размерам, отнесены к категории мейобентоса (пневдомейобентоса). Во-вторых, в других районах Чёрного моря на такой глубине макробентос отсутствует, а V. zaikai образует пики на меньших...
глубинах. Так, в северной половине Чёрного моря пики, образованные мейобентосными полихетами *V. zaikai* и *Protodrilus* sp., наблюдались на глубинах 150 – 170 м, в переходной полосе от кислородной к сероводородной зоне [2]. В 2009 г. в прибосфорском районе скопление *V. zaikai* впервые отмечено на глубине 250 м.


Пробы, полученные в 2009 г. корером, трудно сравнивать с материалами прежних съёмок из-за различия глубин и методов сбора. Дночерпательные пробы были получены только на двух станциях, на глубинах 75 и 94 м.

Для сравнения используем выборку из 4 групп, приведенную в [10], и только одну станцию 2009 г. (75 м, наиболее богатую по численности бентоса). Рассчитаны проценты от данных 1958 г., принятых за 100 % (табл. 3).

Можно видеть, что по сравнению с 1958 г. численность аннелид и ракообразных в 2009 г. сильно упала. При этом снижение регистрировалось ещё в 1989 г. В то же время численность моллюсков сохранилась на уровне 1989 г., а обилие иглокожих даже несколько возросло. Разумеется, небольшие значения численности фауны на единственной ноябрьской станции на глубине 75 м может объясняться разными причинами, но на глубине 94 м она была на порядок ниже; относительно обильны были моллюски и иглокожие (по 16 экз.). Именно эти группы показывают, что влияние сезона не сказалось на обилии. Много вопросов возникает, если сравнивать результаты, полученные дночерпателем, с данными корера (см. табл. 2, 3), но обсуждение сравнительной уловистости разных пробоотборников увело бы нас от основной темы.
Табл. 3 Сравнение дночерпательных данных разных лет
Table 3 Comparison of the bottom-sampler data of different years

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Число станций</td>
<td>10</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Глубина, м</td>
<td>70 – 100 экз. м²</td>
<td>70 – 100 экз. м²</td>
<td>75 эхз. м²</td>
</tr>
<tr>
<td>Среднее обилие</td>
<td>% 100</td>
<td>% 100</td>
<td>% 75</td>
</tr>
<tr>
<td>Annelida</td>
<td>752</td>
<td>146</td>
<td>19.4</td>
</tr>
<tr>
<td>Crustacea</td>
<td>865</td>
<td>37</td>
<td>4.3</td>
</tr>
<tr>
<td>Mollusca</td>
<td>51</td>
<td>42</td>
<td>82.3</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>426</td>
<td>67</td>
<td>15.7</td>
</tr>
</tbody>
</table>

Мейобентос.
Общая численность мейобентоса наиболее высока на глубине 75 м (1861.6 тыс. экз. м²). С увеличением глубины она снижается, образуя меньше пики на глубинах 88 м (1011.1 тыс. экз. м²), 162 м (468.5 тыс. экз. м²) и 250 м (603.2 тыс. экз. м²) (табл. 4, рис. 2).

Табл. 4 Численность основных групп мейобентоса в прибосфорском районе в 2009 г
Table 4 The main meiobenthos groups quantity in the Near-Bosporus region in 2009

<table>
<thead>
<tr>
<th>Глубина</th>
<th>Nematoda</th>
<th>Harpacticoida</th>
<th>Foraminifera</th>
<th>Ciliophora</th>
<th>Gromida</th>
<th>Ostracoda</th>
<th>Общая численность</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>1513720</td>
<td>215800</td>
<td>21060</td>
<td>7280</td>
<td>11960</td>
<td>0</td>
<td>1861600</td>
</tr>
<tr>
<td>82</td>
<td>251840</td>
<td>173420</td>
<td>17800</td>
<td>14300</td>
<td>6720</td>
<td>0</td>
<td>502480</td>
</tr>
<tr>
<td>88</td>
<td>696540</td>
<td>173940</td>
<td>49400</td>
<td>28600</td>
<td>8840</td>
<td>4420</td>
<td>1011140</td>
</tr>
<tr>
<td>103</td>
<td>648700</td>
<td>80600</td>
<td>18200</td>
<td>44980</td>
<td>1300</td>
<td>2860</td>
<td>865280</td>
</tr>
<tr>
<td>123</td>
<td>207740</td>
<td>49920</td>
<td>10920</td>
<td>9880</td>
<td>4160</td>
<td>2080</td>
<td>306280</td>
</tr>
<tr>
<td>162</td>
<td>356720</td>
<td>72540</td>
<td>16120</td>
<td>2080</td>
<td>520</td>
<td>1300</td>
<td>468520</td>
</tr>
<tr>
<td>190</td>
<td>69160</td>
<td>780</td>
<td>2600</td>
<td>780</td>
<td>0</td>
<td>900</td>
<td>80860</td>
</tr>
<tr>
<td>250</td>
<td>491920</td>
<td>33800</td>
<td>6240</td>
<td>48620</td>
<td>1940</td>
<td>520</td>
<td>603200</td>
</tr>
<tr>
<td>300</td>
<td>2600</td>
<td>520</td>
<td>260</td>
<td>520</td>
<td>0</td>
<td>0</td>
<td>9360</td>
</tr>
</tbody>
</table>

Основная доля в общей численности приходилась на группу нематод, поэтому кривая распределения нематод повторяла по форме кривую изменений общей численности. Следующей по обилию группой были гарпактикоиды, кривая их численности относительно плавно снижалась по глубине с небольшими пиками на 162 и 250 м (см. рис. 2).

Обращает на себя внимание тот факт, что на глубине 250 м, где в других районах Чёрного моря донная фауна представлена только мейобентосом весьма низкой численности, в прибосфорском районе на данной глубине явно прослеживается повышение обилия некоторых форм. Именно здесь отмечены пики аннелид, относящихся к группам макробентоса и мейобентоса, что отчасти объясняется попаданием разных стадий одного вида (V. zaikai) в разные размерные группы. Кроме того, здесь наблюдаются пики таких групп мейобентоса, как уже упомянутые Harpacticoida, Ciliophora, Foraminifera. Небольшое повышение численности проявляется на данной глубине также у Gromia sp. и Ostracoda. Здесь же встречено большое количество науплиальных стадий рачкообразных – 22.6 тыс. экз. м² (они не вошли в численность гарпактикоид).

Таким образом, мелкая фауна на этой глубине представлена относительно обильно, образуя характерный пик. Об этом свидетельствует проба, взятая с глубины 300 м, в которой насчитывалось Ciliophora – 20 экз. (что соответствует 5200 экз. м²), Nematoda – 10 экз. (2600 экз. м²), по одному экземпляру Foraminifera, Oligochaeta (260 экз. м²) и два экземпляра Harpacticoida (520 экз. м²).

Основываясь на изложенных материалах, мы приходим к заключению, что в Морской экологический журнал, № 1, Т. X. 2011
прибосфорском районе нижней границей распространения макробентоса следует считать глубину 250 м. Отдельные представители мейобентоса, как показывают данные последних лет, проникают до максимальных глубин Чёрного моря [7]. Как видно из приведённых данных по прибосфорскому району, на глубине 300 м численность мейобентоса значительно снижается, а в его составе повышается доля таких групп, как Nematoda и Ciliophora.

Благодарности. Работа выполнена при частичной поддержке EC 7th FP project "In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies" (HYPOX, #226213).

Авторы выражают большую признательность проф. Намик Чагатай (Namik Chagatay) из Стамбульского технологического университета за предоставленную возможность принять участие в исследованиях бентоса прибосфорского района и содействие в этих работах. Авторы благодарны коллегам из отдела экологии бентоса ИНБЮМ к. н. С.А. Мазлумян за участие в отборе бентосных материалов, а также Е. И. Бабич и В. Г. Копий за техническую помощь при оформлении рукописи.


Поступила 17 мая 2010 г.
После доработки 28 августа 2010 г.

Нижняя граница зообентосу в Прибосфорском районе Чорного моря Н. Г. Сергєєва, В. Є. Заіка, І. П. Бондарів

прибосфорском районе нижней границей распространения макробентоса следует считать глубину 250 м. Отдельные представители мейобентоса, как показывают данные последних лет, проникают до максимальных глубин Чёрного моря [7]. Как видно из приведённых данных по прибосфорскому району, на глубине 300 м численность мейобентоса значительно снижается, а в его составе повышается доля таких групп, как Nematoda и Ciliophora.

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Поступила 17 мая 2010 г.
После доработки 28 августа 2010 г.

10 stations were carried out in the Near-Bosporus region with the depth interval of 75 – 300 m. Macrobenthos total quantity was 136 – 111 thous. indiv. m\(^{-2}\) at the depths of 75 – 82 m, than its number decreased sharply up to 29 – 11 thous. indiv. m\(^{-2}\) and remained in the limits of 11 – 4 thous. indiv. m\(^{-2}\) up to the depth of 250 m. Macrobenthos is represented by annelids only at the depths lower than 123 m. Polychaete *Vigorniella zaikai* forms the accumulation at the depth of 250 m in the Near-Bosporus region, thought, its peaks are at the depths of 150 – 170 m, in the belt of oxygen to the hydrogen sulphide zone transition, in the northern sea half. Meiobenthos total quantity is the highest at the depth of 75 m (1861.6 thous. smpl/m\(^2\)). It becomes lower, with the depth increase, forming the smaller peaks at the depths of 88 m (1011 thous. indiv. m\(^{-2}\)), 162 m (468.5 thous. indiv. m\(^{-2}\)) and 250 m (603.2 thous. indiv. m\(^{-2}\)).The main share of the total quantity falls on nematode group, and harpacticoids are the following. The lowest abundance peak is at the depth of 250 m and meiobenthos quantity decreases considerably at the depth of 300 m.

Key words: the Black Sea, Near-Bosporus region, macrobenthos, meiobenthos.

3АМЕТКА


Благодарности. Работа выполнена при частичной поддержке EC 7thFP project "In situ monitoring of oxygen depletion in hypoxic ecosystems of coastal and open seas, and land-locked water bodies" (HYPOX, #226213). Авторы выражают признательность к.б.н. В. А. Гринцову за предоставляемый материал из Мартыновой бухты.
Рис. 1 *Darcythompsonia fairlensis* (А – самка, Б – самец) (Чёрное море; оригинал); Fig. 1 *Darcythompsonia fairlensis* (A – female, B – male) ((the Black Sea; original)
Appendix IV

Controls on organic carbon and molybdenum accumulation in Cretaceous marine sediments from the Cenomanian–Turonian interval including Oceanic Anoxic Event 2

Authors
Dale, A.W., Stephen R. Meyers, David R. Aguilera, Sandra Arndt, Klaus Wallmann 2012: –

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Controls on organic carbon and molybdenum accumulation in Cretaceous marine sediments from the Cenomanian–Turonian interval including Oceanic Anoxic Event 2

Andrew W. Dale a,⁎, Stephen R. Meyers b, David R. Aguilera c, d, Sandra Arndt d, Klaus Wallmann a

a IFM–GEOMAR Leibniz Institute of Marine Sciences, Wischhofstrasse 1–3, 24148 Kiel, Germany
b University of Wisconsin-Madison, 1215 W. Dayton St., Madison, WI 53706, USA
c DELTARES, Princetonlaan 6, 3508 TA Utrecht, Netherlands
d Department of Earth Sciences, Utrecht University, P.O. Box 80.021, 3508TA, Utrecht, Netherlands

1. Introduction

The Cretaceous period (145.5–65.5 Ma) was characterized by warm climates and high eustatic sea level (Barron, 1983). The corresponding marine sedimentary record is punctuated by dark colored fully- or partially-laminated facies enriched in organic carbon (OC) and trace metals known as black shales (Arthur and Sageman, 1994). Within these sequences, which were deposited over 104–105 yr timescales, the δ13C values for OC and marine carbonates show a positive excursion, explained as regional to global scale perturbations in the coupled ocean–atmosphere carbon cycle (Arthur et al., 1988). Multi-proxy data suggest that these sediments were deposited under water column anoxia or even euxinia, conditions more generally described as ocean anoxic events (OAE) (Schlanger and Jenkyns, 1976). Importantly, this occurs in parallel with oxygenated bottom waters and high rates of aerobic carbon degradation in the surface sediments, implying that elevated Mo burial in ancient marine facies do not necessarily reflect euxinic or even anoxic conditions within the water column. Our findings suggest that both an increase in production and preservation lead to enrichment in organic carbon in the Western Interior Seaway. More generally, the results demonstrate that a careful consideration of the coupling between iron, carbon and oxygen cycles during the early stages of diagenesis is critical for interpreting geochemical proxies in modern and ancient settings.

⁎ Corresponding author. Tel.: +49 431 600 2291; fax: +49 431 600 2928.
E-mail address: adale@ifm-geomar.de (A.W. Dale).

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the oxic sediment layers increases (Hedges and Keil, 1995). However, Middelburg et al. (1993) caution that the perceived importance of oxygen could simply be a consequence of diagenetic maturity, whereby aerobic respiration consumes the most reactive OC fractions, leaving behind the less reactive material for the anaerobic microorganisms. Mechanistically, the relationship between OET and OC accumulation could also be attributed to changes in the rate of delivery of labile OC to sulfide-rich pore water, as hydrogen sulfide serves as control on organic matter vulcanization, ‘hydrogenation’ and preservation (e.g., Hebbing et al., 2006; Arndt et al., 2009). With regard to the production argument, higher rates of primary production and deposition of organic detritus may simply enrich the sediment in OC. However, because anoxic sediments preferentially release phosphorus back to the water column, the potential exists for a positive feedback between primary production and benthic OC enrichment (Van Cappellen and Ingall, 1994; Ingall and Jahnke, 1997). Consequently, higher levels of production and preservation are both possible drivers for OC enrichment during anoxic events; a fact that is not always obvious from the debate in the geological literature (Demaision and Moore, 1980; Pedersen and Calvert, 1990).

Key to interpreting measured data with regard to the extent to which production and preservation processes may have developed at the start of anoxic events and their ultimate control on OC accumulation is a mechanistic understanding of the coupled physical and biogeochemical processes occurring within the sediments themselves. A transition towards anoxia at the start of the anoxic event would have been met by a shift from predominantly aerobic respiration of OC (c.f. the contemporary deep ocean) towards sulfate-based anaerobic respiration (c.f. the contemporary silled basins and productive shelf settings). A thinner aerobic layer will lead to enhanced transport of labile OC to the anaerobic sulfate reduction zone where preservation is more likely (Canfield, 1994; Tyson, 2001; Meyers et al., 2005; Burdige, 2007). Preservation will be further enhanced if bulk accumulation rates also increase simultaneously. Yet, too high sedimentation rates will dilute the OC concentration to the point where OC enrichment is no longer perceptible (Tyson, 2001). Paradoxically, the sediment carbon burial efficiency (CBE=burial flux/deposition flux x 100%) in this case will be high because of the enhanced delivery flux to the sulfate reduction zone (Burdige, 2007). On the other hand, OC will be extensively degraded in sediments that accumulate very slowly under oxic bottom waters due to the long residence time of particles in the oxic layer, leading to a low CBE. These coupled processes imply that to accurately interpret sedimentary proxy records, due consideration must be given to the dynamic interplay between the major transport and biogeochemical processes in sediments (Arndt et al., 2009).

An additional and widely-considered indicator for ocean anoxia is the enrichment of trace metals in black shales (Sageman and Lyons, 2003). Molybdenum (Mo) is currently of high interest in this regard because of the different geochemical speciation it exhibits under different redox conditions. Dissolved molybdate (MoO$_4^{2-}$) behaves conservatively in oxic aquatic environments, yet undergoes quantitative sulfidation to dissolved thiomolybdate (MoS$_4^{2-}$) when hydrogen sulfide ion (HS$^-$) reaches a critical concentration threshold of ca. 50–250 μM (Helz et al., 1996; Zheng et al., 2000). Tetrathiomolybdate (MoS$_4^{2-}$) is immobilized through adsorption onto mineral phases and organic substrates and eventually becomes buried to the sediment repository (Helz et al., 1996; Tribovillard et al., 2004). Consequently, Mo concentrations in black shales (10$^{-1}$–10$^{-2}$ ppm) tend to be at least an order-of-magnitude larger than average crustal values (1–2 ppm) (Wedepol, 1991) and they also show a degree of correlation with the OC content in both contemporary (Algeo and Lyons, 2006; McMahan et al., 2006) and ancient facies (Kolonic et al., 2005). This indicates that the controls on OC and Mo accumulation rates may be related. However, the reductive dissolution of reactive iron by sulfide can dramatically modulate the concentration of free hydrogen sulfide in the porewater (Jørgensen, 1977; Raiswell and Canfield, 1996) and prevent formation of MoS$_4^{2-}$. This sulfide sink can potentially limit the utility of Mo as a paleoproxy (Meyers, 2007).

In this study, our prime objective is to quantify the processes controlling OC and Mo enrichment in marine sediments from OAE2. We employ a numerical reaction-transport model that integrates physical processes and biogeochemical reactions into a quantitative framework. As a case study, we focus on the Cretaceous Western Interior Seaway—a shallow (300 m paleo-water depth) epicontinental water body linking the Boreal Ocean to the north and the Tethys Ocean to the south in what is now the western USA. Specifically, we address the Bridge Creek Limestone Member, which brackets the C–T boundary, including OAE2 and the post-OAE2 period (94.34–93.04 Ma) (Sageman et al., 2006). Our approach constitutes a new quantitative framework for the analysis of deep-time biogeochemical perturbations, and the results are of significance for the accurate interpretation of proxy records in both ancient and modern sediments.

2. Geomological and paleoceanographic setting of the WIS

During the late C–T interval, high eustatic sea level and foreland basin subsidence led to the development of the Western Interior Seaway (WIS) in western North America (Kaufman, 1977). The WIS was a meridional seaway that connected the high-latitude Boreal Ocean to the low-latitude Tethys Sea, with the Sevier Orogenic Belt lying to the west (e.g. Kaufman, 1977). Reconstructions of water mass dynamics within the seaway, based on paleobiologic, sedimentologic, geochemical evidence and circulation modeling (e.g. Slingerland et al., 1996; Kump and Slingerland, 1999; Fisher and Arthur, 2002), indicate a dynamic interplay between distinct Boreal and Tethyan water masses during the Cenomanian and Turonian. In summary, these studies suggest that the WIS hosted a cyclonic gyre, with Boreal surface water influx from the north and Tethyan surface water influx from the south. Turbulent mixing within the seaway likely prohibited the development of a stable density stratification of the water column (Kump and Slingerland, 1999).

The present study evaluates data from the USGS #1 Portland core (Sageman et al., 1997; Meyers et al., 2005), located in the central portion of the seaway, and composed of sediments deposited at approximately 300 m paleowater depth (Sageman and Arthur, 1994). At this location, the C–T boundary interval is predominantly composed of rhythmically alternating decimeter-scale limestone and marlstone beds, which constitute the Bridge Creek Limestone Member (Greenhorn Formation) (Sageman et al., 1997). Individual beds of the Bridge Creek Limestone Member can be traced for over 1000 km (Elder et al., 1994). This basin-wide rhythmic sedimentation has been quantitatively linked to an orbital driver (Sageman et al., 1997; Meyers et al., 2001; Meyers and Sageman, 2007), yielding a high-resolution chronometer that permits detailed evaluation of geochemical fluxes through the C–T boundary interval, both during and immediately following OAE2 (Meyers et al., 2001; 2005).

A selection of the Bridge Creek data from Meyers et al. (2005) which have been smoothed with a 2 m moving average filter (Fig. 1) reveals large secular changes in OC, Fe and Mo concentrations and accumulation rates across the C–T interval. These rates are not spectacular compared to other OAE2 sequences (e.g. Kolonic et al., 2005) and are more comparable to those encountered in modern deep sea settings. However, the data are unique because the OC and Mo accumulation rates are highest post-OAE2, which suggests that ‘OAE2-like’ conditions in the WIS lagged behind the global ocean. According to Meyers et al. (2005) and Meyers (2007), the elevated fluxes of reactive oxidized iron (i.e. iron oxides–hydroxides) relative to OC during OAE2 (Fig. 1e), perhaps enhanced by an additional (hydrothermal?) iron source decoupled from the detrital flux, had a strong influence on these trends. They hypothesized that abundant reactive iron efficiently removed dissolved sulfide from sedimentary pore waters through oxidation reactions, effectively inhibiting molybdate sulfidation. This hypothesis is analyzed in detail in the present study in the context of OC and Mo accumulation.
3. A model for Cretaceous sediments of the WIS

On the basis of identifiable changes in bulk sediment and carbon accumulation rates, the data in Fig. 1 were subdivided into 15 distinct time intervals. It is our intention to use a model to simulate the OC, Fe and Mo concentrations and accumulation rates for each of these time intervals in the unconsolidated (porous) sediments as they were being deposited. This presents some complications since the sediments are now fully lithified material. We thus use geochemical data measured in the strata to extract the necessary boundary conditions and parameters required to describe the changing conditions at the sea floor over the C–T interval. In other words, 15 model simulations of the unconsolidated sediments of the Cretaceous WIS are performed, each having boundary conditions and parameters which differ from one time interval to the next. Comparison of the model output with the measured data in Fig. 1 will provide a quasi-validation that the derived model forcing functions have been adequately described. The 15 time intervals are sufficiently long (>10^4 yr) so that each of the simulations was run to steady state, that is, until there is no temporal change in the concentration–depth profiles (see Section 3.1).

The strategy outlined in this section proceeds as follows. First, we introduce the model framework for simulating coupled reaction and transport in unconsolidated (porous) marine sediments. We then describe how key model boundary conditions and parameters for each time slice were extracted from the data in Fig. 1. We end this section by describing how the model can be used in conjunction with a system analysis to extract the factors controlling Mo and OC accumulation rates in Cretaceous sediments. In what follows, the subscripts ‘cr’, ‘BCM’ and ‘co’ indicate that the corresponding parameters or boundary conditions apply to unconsolidated Cretaceous sediments, to consolidated Bridge Creek Limestone Member strata, or to contemporary marine sediments, respectively.

3.1. Model set-up

3.1.1. Modeling coupled reaction and transport

The model is designed to simulate the concentration profiles of aqueous, Cr(z) and solid species, Cs(z) in the upper 150 cm of Cretaceous sediments at a water depth characteristic of the WIS (300 m, Slingerland et al., 1996; Meyers et al., 2005). Solutes considered are oxygen (O₂), sulfate (SO₄²⁻), total hydrogen sulfide (TH₂S), molybdate (MoO₄²⁻) and ferrous iron (Fe²⁺) and solids considered are pelagic particulate organic carbon deposited on the seafloor (OC, chemically defined as CH₂O), reactive iron oxide–hydroxide (Fe(OH)₃), iron sulfide (FeS) and thiomolybdate (MoS₄²⁻). In this paper, the term reactive iron refers to the iron oxide–hydroxide fraction. The speciation of dissolved sulfide is not explicitly calculated and unless indicated TH₂S refers to ΣH₂S + HS⁻ + S²⁻. The one-dimensional mass–conservation equation (Berner, 1980; Boudreau, 1997) was used to simulate concentrations along the vertical (depth) axis, z:

$$\frac{\partial c(z)}{\partial t} = \frac{\partial}{\partial z} \left( \psi(z) \cdot \left( \frac{\partial c(z)}{\partial z} + D_{c crim}(z) \cdot \frac{\partial c(z)}{\partial z} \right) \right)$$ (1a)

$$- \frac{\partial}{\partial z} \left( \psi(z) \cdot v_{c crim}(z) \cdot c(z) \right) + \kappa_{c crim} \left[ C(z) - C_{d}(z) \right] + \Sigma \left[ \psi(z) \cdot \left( 1 - \psi(z) \right) \right]$$

(1b)

$$\frac{\partial c(z)}{\partial t} = \frac{\partial}{\partial z} \left( \left( 1 - \psi(z) \right) \cdot \left( c_{d}(z) + D_{c crim}(z) \cdot \frac{\partial c(z)}{\partial z} \right) \right)$$

$$- \frac{\partial}{\partial z} \left( \psi(z) \cdot c_{d}(z) \right) + \kappa_{c crim} \left[ C(z) - C_{d}(z) \right] + \Sigma \left( 1 - \psi(z) \right)$$

where t is time, ψ(z) is the sediment porosity and Σ is the sum of the rates of change of concentration due to biogeochemical reactions. The model parameters are D_{c crim}(z), D_{c crim}(z), ω_{c crim}(z), v_{c crim}(z) and κ_{c crim}, which represent the molecular diffusion coefficient of solutes in sediments, mixing by bioturbation, the burial velocity of solids, the burial velocity of pore water, and solute exchange by bioirrigation, respectively. Details on how these parameters are estimated are described below. Solutes and solids are modeled as molar (mol L⁻¹) and mass (weight % (wt.%)) concentrations. Model parameters and boundary conditions are listed in Table 1.

3.1.2. Sediment porosity

The porosity of muddy marine sediments typically decreases fairly rapidly in the surface layers (upper dm) due to compaction and then shows a more attenuated decrease with increasing depth. The change
in porosity with depth was thus described using an exponential function:

$$\psi(z) = \psi(0) + (\psi(0) - \psi(\infty)) \cdot \exp \left( -\frac{z}{z_{por}} \right)$$ (2)

where $\psi(0)$ is the porosity at the sediment–water interface, $\psi(\infty)$ is the porosity where the porosity gradient ($\partial \psi(z) / \partial z$) is small relative to the surface layers, and $z_{por}$ is the porosity depth attenuation length. Because the low rate of sediment accumulation in the WIS (Fig. 1b) resembles the contemporary deep ocean, we used the porosity profiles measured in the deep ocean by Archer et al. (1989) and Haackel et al. (2001) to obtain $\psi(0)$, $\psi(\infty)$ and $z_{por}$ values of 0.95, 0.80 and 0.2, respectively. The sediment thus undergoes compaction with a quadrupling of the solid volume fraction from the sediment surface to 40 cm depth. It is implicitly assumed that the sediment below the modeled depth (150 cm) continued to compact slowly until becoming the sedimentary rock present today. Although additional degrees of freedom are introduced to the model to parameterize compaction (rather than assuming depth-invariant porosity), we take this approach since a reasonable estimate for the porosity profile in surface marine sediments can be made in the absence of measured data. We anticipate that this provides more realistic modeled rates and fluxes than those obtained assuming constant porosity.

3.1.3. Bulk sediment burial velocities

Burial of solids ($\omega_c(z)$, cm ky$^{-1}$) and porewater ($v_c(z)$, cm ky$^{-1}$) in Cretaceous sediments are given by the following conservation equations assuming steady state compaction (Berner, 1980):

$$\omega_c(z) = \frac{1 - \psi(z)}{1 - \psi(\infty)} \cdot \frac{\partial \psi(\infty)}{\partial z}$$ (3)

$$v_c(z) = \frac{\psi(z) \cdot \omega_c(z)}{\psi(\infty)}$$ (4)

where $\omega_c(\infty)$ is the unknown sediment burial velocity at the sediment depth where the porosity gradient is small relative to the sediment–water interface (around 40 cm according to Eq. (2)). Calculation of $\omega_c(z)$ and $v_c(z)$ in Cretaceous sediments thus requires a knowledge of the porosity profile (given above) and $\omega_c(\infty)$.

To calculate $\omega_c(\infty)$ for each time interval we used the measured bulk sedimentation velocities, $\omega_{b,cm}$ (cm ky$^{-1}$) (Fig. 1a). Assuming that the unconsolidated sediments below 150 cm gradually

---

**Table 1**

Model parameters and boundary conditions used in the simulation of Cretaceous sediments. Values which are different for each of the 15 time intervals are indicated as ‘Variable’.

<table>
<thead>
<tr>
<th>Symbol (unit)</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>Bottom water temperature</td>
<td>7.5</td>
</tr>
<tr>
<td>S (–)</td>
<td>Bottom water salinity</td>
<td>35</td>
</tr>
<tr>
<td>$\rho$ (g cm$^{-3}$)</td>
<td>Density of solid material</td>
<td>2.5</td>
</tr>
<tr>
<td>$\psi(z)$ (–)</td>
<td>Porosity at sediment–water interface</td>
<td>0.95</td>
</tr>
<tr>
<td>$\psi(z)$ (–)</td>
<td>Porosity in compacted sediments</td>
<td>0.80</td>
</tr>
<tr>
<td>$\omega_c(z)$ (cm ky$^{-1}$)</td>
<td>Porosity attenuation length</td>
<td>5</td>
</tr>
<tr>
<td>$D_b(z)$ (cm$^2$ yr$^{-1}$)</td>
<td>Burial velocity in compacted sediments</td>
<td>Variable</td>
</tr>
<tr>
<td>$\alpha_b(z)$ (yr$^{-1}$)</td>
<td>Surface bioturbation coefficient</td>
<td>Variable</td>
</tr>
<tr>
<td>$\tau_z$ (cm)</td>
<td>Mixing depth by animals</td>
<td>Variable</td>
</tr>
<tr>
<td>$D_w(z)$ (cm$^2$ yr$^{-1}$)</td>
<td>Diffusion coefficient in seawater for $O_2$</td>
<td>402</td>
</tr>
<tr>
<td>$D_{w}(S\mathbf{O}_4^{-})$ (cm$^2$ yr$^{-1}$)</td>
<td>Diffusion coefficient in seawater for $SO_4^{2-}$</td>
<td>197</td>
</tr>
<tr>
<td>$D_w(H_2S)$ (cm$^2$ yr$^{-1}$)</td>
<td>Diffusion coefficient in seawater for $H_2S$</td>
<td>360</td>
</tr>
<tr>
<td>$D_w(Fe^{2+})$ (cm$^2$ yr$^{-1}$)</td>
<td>Diffusion coefficient in seawater for $Fe^{2+}$</td>
<td>132</td>
</tr>
<tr>
<td>$D_w(MoS_4^{2-})$ (cm$^2$ yr$^{-1}$)</td>
<td>Diffusion coefficient in seawater for $MoS_4^{2-}$</td>
<td>184</td>
</tr>
<tr>
<td>$K_{CO}$ (µM)</td>
<td>Half-saturation constant for $O_2$</td>
<td>4</td>
</tr>
<tr>
<td>$K_{Fe^{3+}}$ (µM)</td>
<td>Half-saturation constant for $Fe(OH)_{3}$</td>
<td>50</td>
</tr>
<tr>
<td>$K_{SO_4}$ (mM)</td>
<td>Half-saturation constant for $SO_4^{2-}$</td>
<td>0.5</td>
</tr>
<tr>
<td>$k_1$ (yr$^{-1}$)</td>
<td>Rate constant for $OC$ mineralization with $Fe(OH)_{3}$</td>
<td>Variable</td>
</tr>
<tr>
<td>$k_2$ (yr$^{-1}$)</td>
<td>Rate constant for $OC$ mineralization with $SO_4^{2-}$</td>
<td>Variable</td>
</tr>
<tr>
<td>$k_3$ (M$^{-1}$ yr$^{-1}$)</td>
<td>Rate constant for $Fe^{2+}$ oxidation with $O_2$</td>
<td>1.0 $\cdot 10^8$</td>
</tr>
<tr>
<td>$k_4$ (M$^{-1}$ yr$^{-1}$)</td>
<td>Rate constant for $Fe^{2+}$ oxidation with $O_2$</td>
<td>1.0 $\cdot 10^8$</td>
</tr>
<tr>
<td>$k_5$ (M$^{-1}$ yr$^{-1}$)</td>
<td>Rate constant for $Fe^{2+}$ oxidation with $O_2$</td>
<td>1.0 $\cdot 10^8$</td>
</tr>
<tr>
<td>$k_6$ (M$^{-1}$ yr$^{-1}$)</td>
<td>Rate constant for $MoS_4^{2-}$ precipitation</td>
<td>6.9 $\cdot 10^4$</td>
</tr>
<tr>
<td>$[H_2S]^*$ (µM)</td>
<td>Threshold $H_2S$ concentration for $MoS_4^{2-}$ precipitation</td>
<td>65</td>
</tr>
<tr>
<td>$[O_2]^*$ (µM)</td>
<td>Threshold concentration for hypoxia</td>
<td>0.05</td>
</tr>
<tr>
<td>$[O_2]_{cr}(0)$ (µM)</td>
<td>Bottom water $O_2$ concentration</td>
<td>Variable</td>
</tr>
<tr>
<td>$[SO_4^{2-}]_{cr}(0)$ (mM)</td>
<td>Bottom water $SO_4^{2-}$ concentration</td>
<td>10</td>
</tr>
<tr>
<td>$[Fe^{2+}]_{cr}(0)$ (µM)</td>
<td>Bottom water $Fe^{2+}$ concentration</td>
<td>0</td>
</tr>
<tr>
<td>$[H_2S]_{cr}(0)$ (µM)</td>
<td>Bottom water $H_2S$ concentration</td>
<td>0</td>
</tr>
<tr>
<td>$[MoS_4^{2-}]_{cr}(0)$ (µM)</td>
<td>Bottom water $MoS_4^{2-}$ concentration</td>
<td>0.1</td>
</tr>
<tr>
<td>$F_{OC}$ (g cm$^{-2}$ ky$^{-1}$)</td>
<td>OC flux to sea floor</td>
<td>Variable</td>
</tr>
<tr>
<td>$F_{Fe^{2+}}$ (g cm$^{-2}$ ky$^{-1}$)</td>
<td>$Fe(OH)_{3}$ flux to sea floor</td>
<td>Variable</td>
</tr>
<tr>
<td>$F_{Fe^{2+}}$ (g cm$^{-2}$ ky$^{-1}$)</td>
<td>Fe$^{2+}$ flux to sea floor</td>
<td>0</td>
</tr>
<tr>
<td>$F_{MoS_4^{2-}}$ (g cm$^{-2}$ ky$^{-1}$)</td>
<td>$MoS_4^{2-}$ flux to sea floor</td>
<td>0</td>
</tr>
</tbody>
</table>

$^4$ Irrigation of $H_2S$ and $Fe^{2+}$ are set to zero (see text).
$^5$ Diffusion coefficient for $MoSO_4^{2-}$ is taken from Zheng et al. (2000). All other diffusion coefficients are from Schulze (2000).
$^6$ The half-saturation constants are based on values given by Jourabchi et al. (2005), Berg et al. (2003) and Van Cappellen and Wang (1996). The model is insensitive to these parameters over the range of values provided in these publications.
$^a$ From Erickson and Helz (2000), corresponding to the slowest (and final) step of $MoS_4^{2-}$ sulfidation to $MoS_2^{2-}$. 

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compacted to become rock, $\phi_\omega$ was approximated from $\phi_{\text{BCM}}$ using a similar conservation equation applied to Eq. (3):

$$\phi_\omega = \phi_{\text{BCM}} \left( \frac{1 - \varphi_{\text{BCM}}}{1 - \varphi(\omega)} \right)$$ (5)

where the porosity of the sedimentary rock ($\varphi_{\text{BCM}}$) is zero and $\varphi(\omega)$ is given in Eq. (2).

3.1.4. Solute diffusion coefficients

Depth-dependent molecular diffusion coefficients for Cretaceous sediments ($D_{\alpha}(z)$, cm$^2$ yr$^{-1}$) were calculated from the molecular diffusion coefficients in seawater ($D_W$, cm$^2$ yr$^{-1}$):

$$D_{\alpha}(z) = \frac{D_W}{1 - \ln \left( \frac{\varphi(z)}{\varphi(\infty)} \right)^2}$$ (6)

Values for $D_W$ are listed in Table 1 and correspond to a bottom water temperature of 7.5 °C and a salinity of 35 (Schulz, 2000). The bottom water temperature was based on sea surface temperatures of the WIS predicted by Slingerland et al. (1996) and the decrease in temperature with depth in the modern ocean. Temperature and salinity in the model were assumed constant in time and with sediment depth.

3.1.5. Bioturbation and bioirrigation rates

Bioturbation ($Db_{\alpha}(z)$, cm$^2$ yr$^{-1}$) and bioirrigation ($\alpha_{\alpha}(z)$, yr$^{-1}$) coefficients were estimated by first considering their magnitude in modern ocean sediments. Bioturbation was calculated from the burial velocity using the empirical logarithmic expression derived by Tromp et al. (1995):

$$Db_{\alpha}(0) = 43 \phi_{OC}^{0.85}$$ (7)

where the subscript ‘co’ indicates contemporary ocean sediments and $\phi_{OC}$ has units of cm yr$^{-1}$. Although it is not explicitly stated by Tromp et al. (1995), we assumed that $\phi_{OC}$ is analogous to $\phi_\omega(\omega)$, that is, the burial velocity in compacted sediments. A similar constitutive relationship for the surface bioirrigation rate, $\alpha_{\alpha}(0)$ (yr$^{-1}$), is unavailable. We thus assumed a value of 10 yr$^{-1}$ based on modern pelagic sediments (Thullner et al., 2009):

$$\alpha_{\alpha}(0) = 10$$ (8)

The foregoing equations are applicable to normal oxic bottom waters. However, discontinuous sequences of homogenized and laminated WIS rock strata, indicated by the frequency of sediment lamination (FOL, %) (Fig. 1g), imply that the sediments throughout the OAE2 and post OAE2 period were intermittently inhabited by bioturbating organisms due to fluctuating oxygen concentrations at the benthic boundary layer. Consequently, a correction must be made to Eqs. (7) and (8) for their application to Cretaceous sediments. We employed the FOL (%) as an indicator of the intensity of faunal activity through the following relationships:

$$Db_{\alpha}(0) = \left( 1 - \frac{\text{FOL}}{100} \right) Db_{\alpha}(0)$$ (9)

$$\alpha_{\alpha}(0) = \left( 1 - \frac{\text{FOL}}{100} \right) \alpha_{\alpha}(0)$$ (10)

where $Db_{\alpha}(0)$ in Eq. (9) is calculated using Eq. (7) and the derived $\alpha_{\alpha}(0)$ (yr$^{-1}$) values are used in place of $\phi_{OC}$. The rate of bioirrigation for $H_2S$ and $Fe^{2+}$ in Cretaceous sediments was set to zero to reflect their rapid oxidation on the walls of animal burrows in surface sediments (Berg et al., 2003).

The depth dependency of bioturbation and bioirrigation was imposed using Gaussian-type functions (Boudreau, 1996):

$$Db_{\alpha}(z) = Db_{\alpha}(0) \cdot \exp \left( -\frac{z^2}{2 z_{cr}^2} \right)$$ (11)

$$\alpha_{\alpha}(z) = \alpha_{\alpha}(0) \cdot \exp \left( -\frac{z^2}{2 z_{cr}^2} \right)$$ (12)

![Fig. 2. Conceptual diagram of the coupled carbon, sulfur, iron and molybdenum cycles used in the model. Reactions r1–r9 are detailed in Table 2.](image-url)

**Table 2**

Model reaction network and rate formulations.  

<table>
<thead>
<tr>
<th>Stoichiometry*</th>
<th>Rate expression*</th>
<th>Rate units</th>
</tr>
</thead>
<tbody>
<tr>
<td>r1: CH$_4$O + O$_2$ -&gt; CO$_2$ + H$_2$O</td>
<td>$k_1\cdot[CH_4O]\cdot Fe_{o(c)}$</td>
<td>mol CH$_4O$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r2: CH$_4$O + 4Fe(III) -&gt; 7H$_2$O + 4Fe$^{2+}$ + HCO$_3^-$ + 10H$_2$O</td>
<td>$k_2\cdot[CH_4O]\cdot Fe_{o(c)}\cdot (1 - Fe_{o(c)})$</td>
<td>mol CH$_4O$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r3: CH$_2$O$+0.5\cdot SO_4^{2-}$ -&gt; H$_2$O + H$_2$S + HCO$_3^-$</td>
<td>$k_3\cdot[CH_2O]\cdot Fe_{o(c)}\cdot (1 - Fe_{o(c)})\cdot (1 - Fe_{o(c)})$</td>
<td>mol CH$_2O$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r4: Fe$^{2+}$ + 0.25 O$_2$ + 2.5H$_2$O -&gt; Fe(OH)$_3$ + 2H$^+$</td>
<td>$k_4\cdot[Fe^{2+}]$</td>
<td>mol Fe$^{2+}$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r5: Fe$^{2+}$ + H$_2$S -&gt; FeS + 2H$^+$</td>
<td>$k_5\cdot[Fe^{2+}]\cdot[TH_2S]$</td>
<td>mol TH$_2S$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r6: Fe$^{2+}$ + Fe(III) -&gt; Fe$^{3+}$</td>
<td>$k_6\cdot[Fe^{2+}]\cdot[TH_2S]$</td>
<td>mol TH$_2S$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r7: 8H$_2$S + Fe(III) + H$_2$O = 8H$_2$O + 8FeS + 4H$^+$</td>
<td>$k_7\cdot[Fe(III)]\cdot[TH_2S]$$^*\cdot f_{Fe}$</td>
<td>mol TH$_2S$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r8: Fe$^{3+}$ + 2Fe$^{2+}$ -&gt; Fe$^{2+}$ + SO$_4^{2-}$</td>
<td>$k_8\cdot[Fe^{3+}]\cdot[Fe^{2+}]$</td>
<td>mol Fe$^{3+}$ L$^{-1}$ yr$^{-1}$</td>
</tr>
<tr>
<td>r9: MoO$_4^{2-}$ + Fe$^{2+}$ + SO$_4^{2-}$ -&gt; MoS$_2$ + Fe(III) + 4H$_2$O</td>
<td>$k_9\cdot[MoO_4^{2-}]\cdot[TH_2S]$$^<em>\cdot (1 - TH_2S)$$^</em>\cdot b$</td>
<td>mol MoO$_4^{2-}$ L$^{-1}$ yr$^{-1}$</td>
</tr>
</tbody>
</table>

Conversion factor between particulate (wt%) and dissolved inorganic carbon (mol L$^{-1}$): $f_{OC} = \frac{1000}{1000 \cdot (1 - \varphi(\omega))}$

Conversion factor between particulate (wt%) and dissolved iron (mol L$^{-1}$): $f_{Fe} = \frac{1000}{1000 \cdot (1 - \varphi(\omega))}$

$|b| = 1$ if $[TH_2S] > [TH_2S]^*$, otherwise $|b| = 0$.

* Reactions are written assuming that TH$_2S$ = FeS for equation balancing.

b Kinetic limitation term: $Fe_{o(c)} = \frac{Fe_{o(c)}}{Fe_{o(c)}}$ for (j = O$_2$, Fe (=Fe(OH)$_3$), SO$_4^{2-}$).
where \( z_{cr} \) (cm) controls the depth of irrigation and particle mixing. Since hypoxia leads to a shallowing of habitat depth of animals (Middelburg and Levin, 2009), \( z_{cr} \) was calculated from the mixing depth by animals in modern sediments (\( z_{co} \)):

\[
z_{cr} = \left( 1 - \frac{FOL}{100} \right) z_{co}
\]

\( z_{co} \) was assigned a value of 5 cm based on a global database compiled by Teal et al. (2008).

### 3.2. Biogeochemical reaction network

The reaction network was designed to elucidate the major controls on OC preservation and Mo accumulation rate during the OAE and post-OAE period. For expediency, only the most important reactions were implemented and those suspected of having minor importance were excluded. A conceptual diagram of the reaction network is shown in Fig. 2 and the kinetic rate expressions are listed in Table 2.

The primary redox reactions describe the rate of degradation of OC through aerobic respiration \((r_1)\), dissimilatory iron reduction \((r_2)\) and sulfate reduction \((r_3)\). The rates are first-order in OC to reflect the general observation that OC availability is the rate-limiting step of the reaction (Berner, 1980). The rate of each pathway is determined by the concentrations of the oxidants \((\text{e.g. } O_2, \text{Fe(OH)}_3, \text{SO}_4^{2-})\) through kinetic limitation terms. These were formulated so that the reactions proceed sequentially as each electron acceptor becomes depleted with depth in the sediment. Denitrification was ignored since nitrogen cycling is not the focus of the study and only becomes a major pathway of carbon diagenesis in sediments undergoing severely hypoxic bottom waters \((O_2 < 20 \text{ µM})\) (Canfield, 1993; Bohlen et al., 2011).

Carbon mineralization coupled to manganese oxide reduction was also ignored because Mn concentrations in the WIS formation are 10 times lower than iron (Sageman and Lyons, 2003) and it is generally a minor pathway of carbon mineralization (Thullner et al., 2009). Finally, methanogenesis was not considered because it is inhibited by sulfate concentrations of \(\geq 1 \text{ mM}\); conditions which do not occur in our simulations. These omissions do not seriously affect the model result since \(> 90\%\) of benthic OC is oxidized by \(O_2\) and \(\text{SO}_4^{2-}\) (Jørgensen and Kasten, 2006).

The secondary redox reactions are centered on Fe, S, and Mo geochemistry \((r_4-r_9, \text{Table 2})\). The rate laws used to describe these processes employ encounter-limited, or bimolecular, kinetics. This approach minimizes the number of parameters to be defined and is consistent with the idea that the rate law should depend linearly on the concentrations of the reactants in reactant limited-environments (Van Cappellen and Wang, 1996). Ferrous iron \((\text{Fe}^{2+})\) liberated by dissimilatory iron reduction can be oxidized abiotically back to particulate iron oxide \((r_4)\) or precipitated as \(\text{FeS}\) using dissolved sulfide \((r_8)\). FeS can be oxidized aerobically \((r_9)\) or permanently buried. Dissolved sulfide can be oxidized biologically to sulfate using oxygen \((r_5)\). In addition to oxidizing OC, \(\text{Fe(OH)}_3\) can also be used as the oxidant for chemical sulfide oxidation \((r_7)\). Normally, this reaction is assumed to produce ferrous iron and elemental sulfur \((S^0)\) (Van Cappellen and Wang, 1996). Yet, for clarity, the net reaction is written assuming that \(\text{Fe}^{2+}\) production is coupled to \(\text{FeS}\) precipitation and that \(S^0\) disproportionate to sulfide and sulfate \((S^0 + \text{H}_2\text{O} \rightarrow 3/4 \text{H}_2\text{S} + 1/4 \text{SO}_4^{2-})\). For additional insight into the complexities of benthic Fe and S cycles, the interested reader is referred to Jørgensen and Kasten (2006).

The final geochemical sink for \(\text{H}_2\text{S}\) is through reaction with \(\text{MoO}_4^{2-}\).

In sulfidic solution, the latter undergoes sulfidization leading to the production of tetrathiomolybdate \((\text{MoS}_4^{2-})\):

\[
\text{MoO}_3\text{S}_4^{2-}(aq) + \text{H}_2\text{S}(aq) = \text{MoO}_3\text{S}_4^{2-}(aq) + \text{H}_2\text{O}(l)
\]

where \(1 \leq x \leq 4\).

The reaction proceeds in four steps that conserve \(\text{Mo(VI)}\), and the reaction rate decreases by an order-of-magnitude over each successive step (Erickson and Helz, 2000). Consequently, mixtures of thiomolybdates can accumulate in seasonally or intermittently sulfidic porewaters. Yet, due to the long time scales of the 15 intervals modeled in this study \((> 10^4 \text{ yr})\), we assume that the equilibria can be simplified as a single step reaction between molybdate and sulfide to produce \(\text{MoS}_4^{2-}\) \((r_6, \text{Table 2})\). The bimolecular rate law prescribed for this pathway is consistent with laboratory experiments which show that the sulfidization steps are first-order in the reactants (Erickson and Helz, 2000). \(\text{MoS}_4^{2-}\) is particle-reactive and can be rapidly scavenged from the pore water by metal oxhydroxides, iron sulfide or organic material (e.g. Vorlicek et al., 2004). Therefore, \(\text{MoS}_4^{2-}\) is modeled as a solid species on the assumption that it is immediately sequestered by particulate phases and undergoes no further reaction.

Experiments and theory have shown that the rate of \(\text{MoS}_4^{2-}\) scavenging is rapid when \(\text{HS}^-\) concentration reaches a critical \(\text{pH}\)-dependent threshold (Helz et al., 1996; Erickson and Helz, 2000). For a \(\text{pH}\) of 7.5 and 8.3, this threshold is equal to ca. 50 and 250 \(\mu\text{M}\) \(\text{HS}^-\) respectively, at 298 K (Helz et al., 1996). The \(\text{pH}\) of anoxic marine sediments is typically of the order 7.5. For this \(\text{pH}\) and the in situ temperature \((280 \text{ K})\) and pressure \((30 \text{ bar})\) of the WIS, \(\text{HS}^-\) accounts for around 80% of \(\text{TH}_2\text{S}\). This implies that the geochemical switch for the above reaction should occur at in situ \(\text{TH}_2\text{S}\) concentrations upwards of ca. 65 \(\mu\text{M}\). There is some field evidence to suggest that lower sulfide thresholds \((0.1 \mu\text{M})\) are required for \(\text{MoS}_4^{2-}\) scavenging onto iron minerals compared to organic material \((100 \mu\text{M})\) (Zheng et al., 2000). Since these values have yet to be confirmed by laboratory studies, we use the higher \(\text{pH}\)-dependent threshold sulfide concentration, \([\text{TH}_2\text{S}]^*\), of 65 \(\mu\text{M}\) whilst noting that it is likely to be somewhat site specific. The reaction formulation only allows \(\text{MoS}_4^{2-}\) formation if \([\text{TH}_2\text{S}]^*\) is greater or equal to \([\text{TH}_2\text{S}]^+\) \((r_9, \text{Table 2})\).

### 3.3. Boundary conditions

Boundary conditions at the top and bottom of the sediment column are required to solve Eqs. (1a) and (1b). At the top, the \(\text{SO}_4^{2-}\) concentration in seawater was fixed at 10 mM to represent the Cretaceous ocean (Horita et al., 2002) and \(\text{TH}_2\text{S}\) was set to zero since the WIS was not sulfidic (Table 1). Proposed fluctuations in \(\text{SO}_4^{2-}\) concentration throughout
the Cretaceous of between 2 and 12 mM (Wortmann and Chernyavsky, 2007; Adams et al., 2010) are not likely to have an important impact on the model results since methanogenesis will only become important if $\text{SO}_4^{2-}$ falls to $<1$ mM. Boundary conditions for the other species are described separately below. At the bottom of the simulated sediment column (150 cm depth), all species are prescribed with zero-gradient (Neumann) boundary conditions.

3.3.1. Bottom water oxygen concentration

Oxygen concentrations at the benthic boundary layer in the WIS, $[\text{O}_2]_{cr}(0)$, were estimated using a method borrowed from a study on the hypoxic St. Lawrence River estuary by Katsev et al. (2007). These workers scanned intact sediment cores from sites with different bottom water oxygen concentrations using computerized axial tomography in order to observe the effect of decreasing oxygen on bioturbation and sediment structure. They derived a non-linear empirical relationship between bioturbation and oxygen concentrations which we use as the basis for our model. Using this relationship and the equality between $\text{Db}_{cr}(0)$ and $\text{FOL}$ in Eq. (9), $[\text{O}_2]_{cr}(0)$ can be calculated as follows:

$$[\text{O}_2]_{cr}(0) = [\text{O}_2]^* - \frac{1}{\tau} \ln \left( \frac{\text{FOL}}{100 - \text{FOL}} \right)$$

(15)

where $[\text{O}_2]^*$ is the threshold oxygen concentration due to hypoxia which leads to large changes in faunal community structure. Mechanistically, $[\text{O}_2]^*$ defines the oxygen concentration at which $\text{Db}_{cr}(0)$ falls to half the value of $\text{Db}_{cr}(0)$ due to severe hypoxia (Katsev et al., 2007). It is assigned a value of 62 $\mu$M based on work by Diaz and Rosenberg (1995). The time-invariant coefficient, $\tau$, controls the steepness in decline of $[\text{O}_2]_{cr}(0)$ with increasing FOL. This parameter was assigned a value of 0.3 by Katsev et al. (2007).

The relationship between $[\text{O}_2]_{cr}(0)$ and FOL for two values of $\tau$ is shown in Fig. 3, where the shaded band indicates the region corresponding to the FOL from the WIS. The parameter $\tau$ controls the steepness of the curve whereas $[\text{O}_2]^*$ determines its vertical displacement. The range in $[\text{O}_2]_{cr}(0)$ is small (61–70 $\mu$mol L$^{-1}$) for the value of $\tau = 0.3$ and implies that fully laminated sediments occur at oxygen concentrations of ~50 $\mu$M which are well-above tolerance levels for many macrofauna and meiofauna (Levin, 2003). Furthermore, zero lamination corresponds to a bottom water oxygen concentration of 86 $\mu$M. One would expect higher baseline oxygen concentrations in a shallow oligotrophic setting such as the WIS (Bralower and Thierstein, 1984; Pratt, 1985). We thus adjusted the value of $\tau$ to 0.05 so that the bottom water oxygen concentration for non-laminated sediments reflects contemporary oxygenated bottom water values, assumed to be 200 $\mu$M, and that only anoxic conditions lead to laminated sediments. The range in $[\text{O}_2]_{cr}(0)$ using $\tau = 0.05$ then increases to 54–106 $\mu$mol L$^{-1}$ for the observed range of FOL.

3.3.2. Carbon deposition flux

Meyers et al. (2005) report values of organic carbon accumulation in the Bridge Creek Limestone Member (FC-BCM, Fig. 1c). These are used in conjunction with the carbon burial efficiency (CBE, %) to calculate the rate of organic carbon flux deposited at the sediment surface during the Cretaceous. In practical terms, the CBE is almost impossible to calculate accurately because of the long time, potentially millions of years, between deposition at the sea floor and preservation as kerogen. However, evidence suggests that the bulk of the reactive carbon is mineralized at an early stage of diagenesis, leading to asymptotic carbon concentrations close to the sea floor (Berner, 1982). Burdige (2007) collated data of CBE and the bulk sediment accumulation rate in contemporary marine sediments ($\text{FBS}_{-\text{co}}$, g cm$^{-2}$ ky$^{-1}$), redrawn in Fig. 4 with additional data from Betts and Holland (1991). Environments with normal (>30 $\mu$M) and low (<30 $\mu$M) bottom water oxygen concentrations which include euxinic environments, are distinguished.

The following logistic equation was identified as an appropriate regression to the data in Fig. 4:

$$\text{CBE} = \frac{A_1 - A_2}{1 + \frac{\text{FBS}_{-\text{co}}}{A_1}} + A_2$$

(16)

where $A_1$ and $A_2$ are the burial efficiencies at zero and infinite sedimentation accumulation rates, respectively, and $A_3$ is the center of the regression. For normal marine conditions, $A_1 = 0.5\%$, $A_2 = 75\%$ and $A_3 = 0.07$ g cm$^{-2}$ yr$^{-1}$. Sediments underlying low-oxygen environments are better represented using $A_1 = 5.0\%$, $A_2 = 75\%$, and $A_3 = 0.01$ g cm$^{-2}$ yr$^{-1}$. The CBE was calculated for Cretaceous sediments for high and low oxygen conditions by substituting the bulk accumulation rates ($\text{FBS}_{-\text{co}}$, Fig. 1b) for $\text{FBS}_{-\text{cr}}$ in Eq. (16). If the curve for low oxygen concentration in Fig. 4 corresponds to $[\text{O}_2](0) = 30$ $\mu$M, and assuming that the curve for normal oxygenated bottom waters can be assigned a value of $[\text{O}_2](0) = 200$ $\mu$M as defined above, then the end-member OC fluxes to the sea floor during the Cretaceous are:

$$\text{F}_{C-30} = 100 \times \frac{\text{F}_{C-BCM}}{\text{CBE}(\text{O}_2 = 30 \mu\text{M})}$$

(17)

$$\text{F}_{C-200} = 100 \times \frac{\text{F}_{C- BCM}}{\text{CBE}(\text{O}_2 = 200 \mu \text{M})}$$

(18)

The carbon deposition flux during the Cretaceous ($\text{F}_{C-\text{cr}}$) can then be estimated from Eqs. (17) and (18) by scaling to the previously derived $[\text{O}_2]_{cr}(0)$ values:

$$\text{F}_{C-\text{cr}} = (\text{F}_{C-200} - \text{F}_{C-30}) \times \frac{[\text{O}_2]_{cr}(0)-30}{200-30} + \text{F}_{C-30}$$

(19)

3.3.3. Iron boundary conditions

The accumulation rate of iron in Fig. 1e (Fe-BCM) distinguishes between a terrigenous or detrital iron source and an ‘excess’ iron pool during OAE2 (Meyers, 2007). A cornerstone of the hypothesis by
Meyers (2007) is that the excess iron was highly reactive, possibly hydrothermal in origin (e.g., Snow et al., 2005), and promoted sulfide buffering and low Mo accumulation rates during OAE2 in the WIS. To address this hypothesis effectively, the fluxes and reactivity of detrital and excess iron must be considered individually.

Based on Fe–C–S relationships, Dean and Arthur (1989) argued that the iron is mainly present as particulate iron sulfides. Sedimentary iron can be visualized as a continuum of mineral assemblages, each with a discrete reactivity, yet whose chemical properties are generally poorly characterized. Operationally-defined chemical extractions on natural and synthetic iron minerals have been employed to determine the reactivity of sedimentary particulate iron (Raiswell and Canfield, 1996; Poulton and Raiswell, 2002). These workers defined the following reactive fractions; (i) highly reactive (FeHR) iron as the fraction which reacts on short timescales and which includes amorphous and crystalline iron oxides, (ii) poorly reactive iron (FePR) as the fraction which reacts slowly on 10^2–10^3 yr time scales and includes reactive silicate iron, and (iii) a fraction bound within sheet silicates which is reactive on much longer timescales, and termed unreactive (FeU). A compilation of data by Poulton and Raiswell (2002) further indicates that the relative proportions of FeHR (25%), FePR (25%), and FeU (50%) are broadly uniform in non-euxinic sediments and have changed little throughout the Phanerozoic. This is in contrast with the present data where most of the particulate iron appears to be present as sulfides and was thus available for reductive dissolution (Dean and Arthur, 1989). However, it is not possible to infer from the Bridge Creek data whether FeU was absent in the deposited iron oxide or whether FePR was slowly converted to sulfide during lithification. Therefore, in line with Poulton and Raiswell (2002), we defined the flux of sulfidizable reactive iron ([FeHR + FePR]) to the Cretaceous sediments (2 g cm^{-2} ky^{-1}) as half the measured detrital iron flux in Fig. 1e plus the excess iron flux. This should be viewed as a conservative estimate given the findings of Dean and Arthur (1989). FeU comprises the remaining 50% of the detrital iron and is not considered in the model. For simplicity, the same reactivity toward dissolved sulfide was prescribed for the reactive detrital iron and excess iron with a rate constant whose value allows the iron to be reduced on the time scale of sediment burial over the modeled 150 cm column (10^4–10^5 yr). The importance of the rate of iron dissolution by sulfide on Mo accumulation is addressed through the system analysis (see below). The concentration of Fe^{2+} at the sediment–water interface and the deposition flux of FeS was set to zero due to the presence of oxic bottom waters in the WIS (Kump and Slingerland, 1999).

3.3.4. Molybdenum boundary conditions

The flux of thiomolybdate (MoS_{4}^{2-}) to the sea floor was set to zero since the WIS was not euxinic. An additional flux of detrital Mo was implicitly included by multiplying the mass accumulation rate (F_{BS-BCM}) by the Mo concentration in world average shale (1.5 ppm; Wedepohl, 1991). Detrital Mo is considered to be unreactive and is included for the sake of completion.

The flux of molybdate (MoO_{4}^{2-}) adsorbed to iron oxide particles was not considered in the model since its significance is essentially unknown. A tenuous interpretation of laboratory results presented by Goldberg et al. (2009) suggests that the adsorbed Mo concentration in oxic seawater is smaller than the detrital Mo concentration. Furthermore, several studies across a broad range of oxic and anoxic settings have reported that processes occurring at or below the sediment–water interface are mainly responsible for Mo accumulation (e.g. Crisius et al., 1996; Erickson and Helz, 2000; Zheng et al., 2000). Transport of dissolved molybdate across the sediment–water interface was thus assumed to be the only source of reactive Mo, although the processes responsible for Mo accumulation are still poorly understood.

It should be mentioned that Mo enrichment in some black shales from the proto-North Atlantic is lower in the OAE2 sections compared to those marking the onset and termination of the anoxic event (Hetzelt et al., 2009). This has been suggested as a drawdown of the global Mo inventory due to sulfidization and burial. Algeo and Lyons (2006) discuss how a similar reservoir effect could operate in modern-day silled basins such as the Black Sea. However, Meyers (2007) argued that this was a second-order effect in the WIS because the major shifts in Mo accumulation are extremely rapid relative to the Mo residence time and are associated with independent evidence for pronounced environmental changes. The concentration of dissolved MoO_{4}^{2-} in the sea water was thus set to contemporary values (100 nM) and assumed to be time-invariable across the C–T interval.

3.4. Parameterization of the biogeochemical reactions

Distinct rate constants were imposed for the degradation of OC through aerobic respiration (k_1) and anaerobic respiration by sulfate reduction (k_2). Specifically, k_1 was prescribed a higher value than k_2. We used the study by Tromp et al. (1995), based on earlier work by Toth and Lerman (1977), as an empirical basis to constrain the rate constant for aerobic respiration and dissimilatory sulfate reduction from the sediment accumulation velocity (in cm yr^{-1}):

\[ k_1 = 2.97 \cdot \omega_{cr}^{0.62} \]  
\[ k_2 = 0.283 \cdot \omega_{cr}^{1.94} \]  

No information is available on the rate constant for dissimilatory iron reduction (k_3). We assumed that this parameter value was equivalent to k_1 and tested this further with the system analysis (see below). Note that we increased the value of the empirical constant in Eq. (21) by a factor of 5 from the original value of 0.057 to 0.285 since lower values failed to generate sulfide. This may be because the anaerobic rate constants were extracted from deep anoxic layers where the material is more refractory or because the reported accumulation velocities were not considered in the model since its significance is essentially unknown.

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correspond to surface sediments where sediment burial velocities are higher. Nonetheless, the resulting $k_3$ values using Eq. (21) are within the scatter of the data presented by Tromp et al. (1995).

No similar constitutive relationships exist for the rate constants of the secondary redox reactions ($k_{4-8}$, Table 2). We attempted to overcome this problem by analyzing previous modeling studies from a range ofoxic and anoxic basins from the continental shelf to the deep sea where bimolecular rate constants have been reported (Fig. 5). The range of values over many orders-of-magnitude is quite striking. For example, the rate constant values for aerobic sulfide oxidation ($k_5$) extend over 10 orders-of-magnitude. The lower end estimates of 1.6 - 10$^5$ M$^{-1}$ yr$^{-1}$ (Van Cappellen and Wang, 1995; Wang and Van Cappellen, 1996) were based on experiments by Millero et al. (1987a). At the upper end of the scale, Boudreau (1991) constrained $k_5$ to 1.0 - 10$^{15}$ M$^{-1}$ yr$^{-1}$ using field data from Aarhus Bay sediments. This anomaly is easily explained by the role of sulfide-oxidizing microorganisms. The laboratory experiments were performed under abiological conditions whereas sulfide-oxidizing bacteria are present in abundance at the site investigated by Boudreau (1991) and efficiently catalyze the reaction between sulfide and oxygen.

Rate constants for secondary redox reactions are generally regarded as fitting parameters in models and thus integrate the effect of many environmental variables. These include (i) the role of ionic strength and pH, (ii) thermodynamic constraints on reactions imposed though the pore water chemistry, (iii) geochemical catalysts or inhibitors, and (iv) microbial community structure. The spread of reported values in Fig. 5 likely reflects a change in the relative importance of these variables among the contrasting environments. Nonetheless, more than half of the studies listed report that the rate constants were ‘constrained’ or ‘fitted’ using site-specific data, yet contain little or no relevant model-data comparison to evaluate the goodness-of-fit. It should be remembered that sediment reaction-transport models are coupled through the chemical species and that ‘fitted’ may refer to a specific aspect or output of the model. This means that a parameter value could be sourced from elsewhere in the literature, yet still be reported as ‘fitted’ if the overall model output is satisfactory. Rate constants $k_2$ to $k_9$ for the WIS were estimated only from those studies that provide some indication of the validity of the parameterizations, i.e. concentration or rate data, and were further assumed to be time-invariant.

The considerable uncertainty in the unknown rate constants and the parameters used to hindcast the boundary conditions, is not a major cause for concern. The highly coupled nature of the reaction network places constraints on the number of possible permutations of the entire set of model parameters when tested against field data (Van Cappellen and Wang, 1996). That is, large errors in a single parameters or forcing functions will become obvious in one or more of the modeled variables. Simultaneous reconstruction of OC, Mo and Fe concentrations and accumulation rates is a firm indication that the parameterization is satisfactory. The uncertainty if further quantified using the system analysis described below.

3.5. Numerical solution

The set of coupled partial differential equations for solutes and solids was transformed using the method of lines (Boudreau, 1996). The resulting set of ordinary differential equations was solved using the NDSolve algorithm in MATHEMATICA over a grid spacing increasing from sub-mm at the top of the core to sub-cm at the bottom with a total of 100 depth intervals. The model typically requires ca. 120 s to complete a single simulation.

3.6. System analysis

The reaction network is limited to 9 reactions and is thus relatively small compared to other diagenetic models (e.g. Van Cappellen and Wang, 1996). Yet, it is nonetheless a highly interconnected biogeochemical system. One is thus confronted with a large number of potential couplings between parameter values and boundary conditions that control the Mo and OC accumulation rate. A piecewise analysis of the model, that is, changing single parameter values one-by-one and observing the change in model output, is not an optimal means of accurately determining the major controls on specific processes since it cannot identify important couplings between parameters.

To disentangle the interconnectivity in the model, we carried out a system analysis based on a two-level factorial design. Factorial analysis is a statistical methodology (Box et al., 1978) that determines the response of a pre-defined system output (e.g. a reaction rate or concentration) to a change in n model ‘factors’ (e.g. parameters or boundary conditions). Each factor is assigned a high and low level, such that for n factors there are a total of 2$^n$ system responses. The ‘effect’ of all possible factor permutations is calculated from the responses using a simple algorithm. The effects can then be illustrated on a normal probability plot to visualize the factor or interactions thereof that have the largest impact on the system response (Box et al., 1978). An example of an application of factorial analysis to marine sediment dynamics is given by Dale et al. (2006, 2011).

In this study, three model responses have been identified: Mo and OC accumulation rate (or the burial rate at the lower model boundary of 150 cm) and the corresponding CBE. Two factorial analyses were performed using the factors suspected to have the largest effect on these responses. The first set of factors tested were those associated with transport and boundary conditions, that is, the environmental parameters ($F_{C_{cr}}, \alpha_{cr}(\infty), \alpha_{cr}(0), z_{cr}, [O_2]_{cr}(0)$). The 7 environmental factors require (2$^7$ =) 128 model simulations to fully test the complete array of factor combinations. The second set focuses on the reaction-specific parameters which are the biogeochemical rate constants ($k_1$ to $k_9$), requiring a further (2$^9$ =) 512 simulations. The factors which directly control Mo accumulation ($F_{Mo_{aq-cr}}, [MoO_4]^{2-}_{cr}(0), k_6$ and $[THS]^+$) were not included since the interest is on the peripheral mechanisms which are conducive to authigenic Mo accumulation in Cretaceous sediments.

To elucidate the major controlling factors on Mo and OC burial and CBE across the C–T interval, the high and low factor levels were determined from their mean values derived for the OAE2 and post-OAE2 period. The values for the rate constants $k_4$ to $k_9$ were fixed in the model and not derived from the data. Their prescribed values in the factorial analysis were determined by varying the baseline values in Table 1 by ±50%. Although this is much lower than the reported parameter values (Fig. 5), it is the same relative change calculated for the other derived parameters (see Table 3, Results). It is important to remember that the system analysis results may not be universally applicable and are only valid for the specific ranges over which the factors were varied.

4. Results

4.1. Critical examination of model forcings and comparison with modern pelagic sediments

The parameters and boundary conditions derived in Section 3 applicable to the unconsolidated Cretaceous sediments are shown in Fig. 6. Averaged values for the OAE2 and post-OAE2 periods are summarized in Table 3 and compared to those from contemporary marine settings where possible.

The derived mean sediment burial velocity, $\alpha_{cr}(\infty)$, varies over time, ranging from 3.2 to 4.4 cm yr$^{-1}$ (Fig. 6a, Table 3). These are similar to those encountered in the modern deep ocean but around 3 times lower than the OAE2 sequences of Tarfaya Basin in the proto-North Atlantic (Kolonic et al., 2005). Consequently, the bioturbation coefficients ($D_{B_{cr}}(0)$, Fig. 6b) are also similar to deep sea values since $D_{B_{cr}}(0)$ is derived from $\alpha_{cr}(\infty)$. Bioirrigation coefficients, $\alpha_{cr}(0)$, of 4 to 9 yr$^{-1}$
Table 3

Mean values of parameters and boundary conditions derived for the OAE2 and post-OAE2 periods shown graphically in Fig. 6 and the relative change between the two periods (Δ). Also listed are the rate constants k1–k8. Representative values for the contemporary deep sea, shelf and oxygen minimum zones (OMZ) are also shown where data is available. The final column defines the range of values used in the system analyses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Deep sea</th>
<th>Shell</th>
<th>OMZ</th>
<th>OAE2</th>
<th>Post-OAE2</th>
<th>Δ (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOCr (g cm⁻² yr⁻¹)</td>
<td>OC flux to sea floor</td>
<td>0.04–0.04</td>
<td>-10⁶</td>
<td>2–14</td>
<td>0.37</td>
<td>0.45</td>
<td>+23</td>
<td>0.37–0.45</td>
</tr>
<tr>
<td>FOCr (g cm⁻² yr⁻¹)</td>
<td>Fe(OH)₃ flux to sea floor</td>
<td>0.003</td>
<td>2.7</td>
<td>0.2–3.4</td>
<td>0.018</td>
<td>0.007</td>
<td>-59</td>
<td>0.007–0.018</td>
</tr>
<tr>
<td>ocR (cm yr⁻¹)</td>
<td>Sediment burial velocity</td>
<td>0.1–10</td>
<td>-10²–10³</td>
<td>50–300</td>
<td>4.4</td>
<td>3.2</td>
<td>-27</td>
<td>3.2–4.4</td>
</tr>
<tr>
<td>DBCr(0) (cm yr⁻¹)</td>
<td>Surface bioturbation coefficient</td>
<td>2–2.2</td>
<td>10²</td>
<td>-0</td>
<td>0.33</td>
<td>0.16</td>
<td>-52</td>
<td>0.16–0.33</td>
</tr>
<tr>
<td>αOCR (yr⁻¹)</td>
<td>Surface bioirrigation coefficient</td>
<td>2–20</td>
<td>&gt;10⁴</td>
<td>-0</td>
<td>7.7</td>
<td>4.8</td>
<td>-37</td>
<td>4.8–7.7</td>
</tr>
<tr>
<td>zoc (cm)</td>
<td>Mixing depth by animals</td>
<td>5</td>
<td>10</td>
<td>-0</td>
<td>3.8</td>
<td>2.4</td>
<td>-37</td>
<td>2.4–3.8</td>
</tr>
<tr>
<td>[O₂]cr(0) (μM)</td>
<td>Bottom water O₂ concentration</td>
<td>&gt;150</td>
<td>&gt;120</td>
<td>0–40</td>
<td>87</td>
<td>61</td>
<td>-30</td>
<td>61–87</td>
</tr>
</tbody>
</table>

Environmental parameters

- FOCr: OC flux to sea floor
- FOCr: Fe(OH)₃ flux to sea floor
- ocR: Sediment burial velocity
- DBCr(0): Surface bioturbation coefficient
- αOCR: Surface bioirrigation coefficient
- zoc: Mixing depth by animals
- [O₂]cr(0): Bottom water O₂ concentration

Reaction-specific parameters

- k1, k2, k3: Rate constants for OC mineralization with O₂
- k4, k5, k6, k7, k8: Rate constants for other reactions

Fig. 6: Derived parameters and boundary conditions for Cretaceous sediments corresponding to the 15 time intervals defined in Fig. 1. Height refers to relative spatial location within the Bridge Creek Limestone Member. (a) sediment burial velocity (ωcr), (b) bioturbation coefficient (DBcr(0)), (c) bioirrigation coefficient (αocR), (d) depth of faunal activity (zoc), (e) bottom water oxygen concentration ([O₂]cr(0)), (f) OC flux to the sediment surface (FOCcr), (g) total iron flux to the sediment surface and the reactive fraction used in the model (FFe-cr), (h) rate constant for aerobic degradation of OC (k1), and (i) rate constant for anaerobic degradation of OC by sulfate (k5). The gray shaded band indicates the OAE2 interval.

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bottom waters of the WIS (Kump and Slingerland, 1999). On balance, the presence of a deep hypoxic water layer across the C-T interval seems more reasonable than anoxic or sulfidic bottom waters.

The flux of OC to the sediment–water interface ($F_{OC}$, Fig. 6f), ranges from 0.37 to 0.45 g cm$^{-2}$ kyr$^{-1}$. This is comparable to those on the lower continental slope and abyss in the modern ocean, but at least a factor of 10 lower than sediments on the shelf and from oxygen minimum zones (Table 3). This is a further indication that the WIS must have been significantly oligotrophic compared to the modern ocean of comparable water depths (300 m) where one would expect to find OC fluxes of ca. 1–5 g cm$^{-2}$ kyr$^{-1}$ (Burdige, 2007; Thullner et al., 2009). Our approach further predicts that the post-OAE2 sediments received a 20% higher flux of OC relative to those in the OAE2 period. Yet, the data show that carbonate accumulation rates were lower post-OAE2 (Fig. 1d), which led Meyers et al. (2005) to argue that paleoproduction, and thus $F_{OC}$, were also lower during this interval. We return to this discrepancy in more detail in the Discussion.

The imposed reactive iron fluxes are compared with the total iron influx in Fig. 6g which includes the unreactive iron fraction. There is very little data in the literature for comparison of these fluxes. Model-constrained reactive iron fluxes for the deep MANOP sites (Dhakar and Burdige, 1996) and mass balances for Pacific pelagic clays (Glasy, 1991) are in the region of 0.003 g cm$^{-2}$ kyr$^{-1}$. These are up to a factor of 10 lower than those measured in the Bridge Creek data. On this basis, we infer that the WIS sediments received a disproportionately large flux of reactive iron compared to carbon with respect to other modern deep sea (Table 3) or ancient (e.g. Hetzel et al., 2009) ocean analogs. For comparison, iron fluxes in contemporary shelf sediments are 2–3 orders-of-magnitude higher (Table 3) since most terrigenous particulate material is trapped and buried there (Wallmann, 2010).

The rate constant for aerobic OC mineralization, $k_3$, ranges from 74 to 113 kyr$^{-1}$, which is ca. 4–5 orders-of-magnitude larger than the rate constant for anaerobic mineralization by sulfate reduction ($k_2$) which ranges from 5.5 · 10$^{-4}$ to 2.1 · 10$^{-3}$ yr$^{-1}$ (Fig. 6h,i). There is no scientific consensus on a theory that clearly explains these differences and the major theories proposed have been detailed in the Introduction. Nonetheless, the constitutive equations used by Tromp et al. (1995) to derive the rate constants are empirically based and have been shown to provide a good estimate of carbon burial efficiencies in contemporary marine sediments (Meile and Van Cappellen, 2005). Even so, the assignment of a single rate constant value at any given time conflicts with the observation that OC degrades at different rates over a wide spectrum of temporal and spatial scales (Westrich and Berner, 1984; Middelburg, 1989). For example, the inclusion of 2 or 3 different OC pools are often required to adequately simulate anaerobic OC mineralization by sulfate reduction (Dale et al., 2009). Yet, we argue that the need for additional OC pools in model simulations is less important for oligotrophic environments with low sediment burial velocities compared to eutrophic settings with high sediment burial velocities. This is because much more of the reactive carbon will be mineralized by aerobic processes in the water column and the uppermost surface layers in oligotrophic systems, leading to decreased burial rates of reactive fractions (Middelburg, 1989). In other words, the suitability of the Tromp et al. (1995) equations in describing a single bulk OC pool undergoing either aerobic or anaerobic decomposition may increase with decreasing sediment burial velocity. Given that the sediments of the WIS accumulated at rates similar to those in the contemporary deep sea, the use of a single rate constant in the model seems to be defensible.

### 4.2. Geochemical characteristics of Cretaceous sediments

The results of the 15 steady state model simulations are shown in Fig. 7 (thick lines), which compares modeled and measured accumulation rates and concentrations of OC, Fe and Mo. Model-predicted CBEs are also presented. OAE2 is characterized by relatively low OC and Mo accumulation rates and high Fe accumulation, whereas OC and Mo accumulation rates are relatively high and Fe accumulation rates are low following this global event. To put these data in perspective, the Mo accumulation rates of ca. 7–14 μg cm$^{-2}$ kyr$^{-1}$ (Fig. 7c) are much lower than the range of 50–1500 μg cm$^{-2}$ kyr$^{-1}$ reported for modern sediments (Zheng et al., 2000; McManus et al., 2006; Morford et al., 2009; Scholz et al., 2011). Overall, the model shows good agreement with the observations and is able to reproduce the major trends in the data. This provides some confidence that the reaction network and parameterizations are satisfactory. A series of causal mechanisms explaining these trends has been proposed (Meyers et al., 2005; Meyers, 2007) which we discuss later in conjunction with the results from the system analysis. Beforehand, we explore how the sediment porewater and solid phase concentration profiles may have evolved in the unconsolidated sediments.

The geochemical profiles of solids and solutes in the unconsolidated Cretaceous sediments shown in Fig. 8 are generated by running the model using the mean parameter values for the OAE2 and post-OAE2 period (Table 3). The model predicts a 20 mm penetration depth of O$_2$ into the sediment during OAE2. Thus, despite contemporaneous global anoxia, the WIS sediments retain a relatively thick upper oxic layer similar to the modern deep sea (Reimers et al., 1986). Moreover, aerobic respiration accounts for 99% of total OC mineralization over the upper

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**Fig. 7.** Comparison between measured (circles, from Fig. 1) and modeled (thin lines) data from Bridge Creek Limestone Member. Accumulation rate of (a) OC ($F_{OC}$), (b) total iron ($\text{Fe(OH)}_3 + \text{FeS}$) ($F_{Fe-BCM}$), (c) Mo ($F_{Mo-BCM}$), and concentrations of (d) OC, (e) total iron ($\text{Fe(OH)}_3 + \text{FeS}$), (f) Mo, and (g) carbon burial efficiency (CBE). The modeled concentrations and accumulation rates are calculated at the bottom of the simulated core, (b) and (e) only show the simulation of the reactive iron pool with that calculated from the raw data in Fig. 1e (see Section 3.3.3). The dashed lines in (c) and (f) indicate detrital Mo and the gray shaded band shows the OAE2 interval. The thick lines denote the uncertainty of the model (see system analysis (Fig. 9)), and correspond to the largest observed effect (boxed numbers in each panel, see text).
150 cm (Table 4). Iron and sulfate reduction account for <1% and sulfate concentration barely decreases over the model sediment column. The same tendencies have been quantified in contemporary deep sea sediments (Jahnke et al., 1982; Dhakar and Burdige, 1996; Haeckel et al., 2001), which reiterates the oligotrophic nature of the WIS over the C-T interval. Particulate (reactive) iron decreases from ca. 3 wt.% at the surface to ca. 1 wt.% at the base of the simulated sediment core due to reductive dissolution (Fig. 8g). This implies that sulfate reduction and release of sulfide to the porewater would only become significant at a greater depth than modeled here. These concentrations agree with observations in the Peru Basin (König et al., 1997) but are lower than Pacific red clays that have iron contents in excess of 5 wt.% (Glasby, 1991). The presence of reactive iron severely inhibits the rate of sulfide production by dissimilatory sulfate reduction and particulate Mo concentrations remain at the detrital background level of 1.5 ppm (Fig. 8h).

The sediments below the oxic layer are highly ferruginous with Fe2+ concentrations reaching 1 mM at 150 cm. These high levels contrast with reports of low or undetectable levels of dissolved iron in modern abyssal sediments (Froelich et al., 1979; Emerson et al., 1980; Haeckel et al., 2001), possibly because our model does not account for siderite formation or Fe2+ incorporation into clay lattices (König et al., 1997). Furthermore, the apparent equilibrium constant between adsorbed and dissolved iron is on the order of 10^3 for marine sediments (Van Cappellen and Wang, 1996), and the model did not account for this large sink of Fe2+. Thus, the true Fe2+ concentrations were likely much lower than those simulated here.

There are striking geochemical differences between OAE2 and post-OAE2 sediments. The latter are OC-enriched with concentrations (2–3 wt.%) that are without parallel in the modern deep ocean (<1 wt.%; Seiter et al., 2005). The sediments have a 10 mm thick oxic layer and aerobic respiration accounts for 98% of total OC mineralization and sulfate reduction for around 2% (Table 4). Despite this huge imbalance, the sediments below the oxic layer become sulfidic, with TH2S reaching 100 μM at 150 cm depth (Fig. 8c). Particulate iron and dissolved iron are completely consumed and FeS burial becomes a sink for sulfide (Fig. 8i). The presence of free dissolved sulfide allows Mo to accumulate once the threshold sulfide concentration of 65 μM is reached at ca. 60 cm.

These trends support the idea that the rate of Mo accumulation is tightly coupled to the availability of dissolved iron in the porewater (Zheng et al., 2000; Meyers, 2007). Importantly, our results also show that enhanced Mo accumulation can occur in parallel with

Table 4
Modeled depth-integrated reaction rates (r1 to r9) for the OAE2 and post-OAE2 periods corresponding to the species indicated in parenthesis (see Table 2 for the reaction stoichiometry). ΔTA is the change in total alkalinity (TA) per formula reaction, and the last two columns show the net depth-integrated rate TA balance calculated by multiplying ΔTA by the corresponding reaction rate (positive values indicate net alkalinity production).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>OAE2a</th>
<th>Post-OAE2a</th>
<th>ΔTA</th>
<th>ΔTA: OAE2</th>
<th>ΔTA: post-OAE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>r1 (CH2O)</td>
<td>296 (99%)</td>
<td>342 (98%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>r2 (CH2O)</td>
<td>2.9 (0.9%)</td>
<td>0.25 (&lt;1%)</td>
<td>+8</td>
<td>+23.1</td>
<td>+2.0</td>
</tr>
<tr>
<td>r3 (CH2O)</td>
<td>0.007 (&lt;1%)</td>
<td>5.9 (1.7%)</td>
<td>+1</td>
<td>+0.01</td>
<td>+5.9</td>
</tr>
<tr>
<td>r4 (Fe2+)</td>
<td>11.5</td>
<td>1.0</td>
<td>−2</td>
<td>−23.0</td>
<td>−2.1</td>
</tr>
<tr>
<td>r5 (TH2S)</td>
<td>5.8 · 10^-6</td>
<td>0.45</td>
<td>−2</td>
<td>−1.2 · 10^-5</td>
<td>−0.90</td>
</tr>
<tr>
<td>r6 (TH2S)</td>
<td>3.4 · 10^-3</td>
<td>0.89</td>
<td>−2</td>
<td>−6.7 · 10^-3</td>
<td>−1.8</td>
</tr>
<tr>
<td>r7 (TH2S)</td>
<td>1.6 · 10^-4</td>
<td>1.5</td>
<td>−2</td>
<td>3.5 · 10^-5</td>
<td>−0.33</td>
</tr>
<tr>
<td>r8 (Fe2+)</td>
<td>8.6 · 10^-4</td>
<td>0.93</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>r9 (MoO4^-)</td>
<td>0</td>
<td>1.3 · 10^-3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ΔTA: OAE2 (eq m^-2 yr^-1)
ΔTA: post-OAE2 (eq m^-2 yr^-1)

* Values in parentheses are the fraction of OC mineralization channeled through each primary redox pathway.
4.3. System analysis of Mo and OC accumulation and CBE

The results of the factorial analysis of the 7 environmental factors (\(F_{Cr}\), \(F_{Fe}\), \(\alpha_\text{cr}(\omega)\), \(D_{Cr}(0)\), \(\alpha_\text{cr}(\omega)\), \(Z_{Cr}\), \([O_2]_{cr}(0)\)) generated with the model are illustrated on normal probability plots in Fig. 9a–c. The panels show the effects of single factors or their interactions on OC accumulation (Fig. 9a), CBE (Fig. 9b) and Mo accumulation (Fig. 9c) or, stated differently, the change in these responses when the factor(s) increase from their low to high level. Data points (‘effects’) which fall on or close to the solid line are normally distributed about zero whereas those which lie away from the line cannot be explained as chance occurrences and, consequently, are regarded to have a large impact on the model response. For example, there are 3 main environmental factors which control OC accumulation (Fig. 9a): \([O_2]_{cr}(0)\), \(\alpha_\text{cr}(\omega)\) and \(F_{Cr}\). Of these, \([O_2]_{cr}(0)\) and \(F_{Cr}\) lie farthest away from the normal line which means that they exert the largest effect on OC accumulation. In addition, \([O_2]_{cr}(0)\) appears to the left of the normal line, such that an increase in \([O_2]_{cr}(0)\) will decrease OC accumulation through higher rates of aerobic mineralization, as expected. \(F_{Cr}\) lies to the right, which is simply because higher depositional fluxes increase the rate of OC accumulation. The overall ‘effect’ of increasing oxygen from the low (61 μM) to high (87 μM) concentration (Table 3) is to decrease OC accumulation by 0.014 g cm\(^{-2}\) ky\(^{-1}\). The effect of the most sensitive parameter, in this case \([O_2]_{cr}(0)\), provides an estimate of the uncertainty in the model-predicted concentrations and rates (thick lines in Fig. 7).

A similar pattern for \([O_2]_{cr}(0)\) emerges with the CBE, whereby the latter is reduced by ca. 4.5% if oxygen increases from the low to high level (Fig. 9b). The critical control of OC dynamics by oxygen over this conservative concentration range is quite remarkable, and alludes to oxygen limitation as the most important control on OC burial. As a first approximation, the OET can be quantified as the thickness of the oxic layer (L) divided by \(\omega_{cr}(\omega)\). Using \(\omega_{cr}(\omega)\) values from the OA2 and post-OAE2 periods in Table 3 and the depth of oxygen penetration in Fig. 8a, the OET is 50% greater during OA2 compared to post-OAE2.

Bioturbation and bioirrigation do not exert a dominant control on OC accumulation rate and CBE (Fig. 9a,b). There is, however, a weak interaction between \([O_2]_{cr}(0)\) and the depth of sediment mixing by animals, \(Z_{Cr}\), on CBE which is identified in Fig. 9b as the \([O_2]_{cr}(0)-Z_{Cr}\) term. The model includes a simplified formulation for faunal activity and does not account for the complexities of sediment transport and carbon cycling by animals caused by mechanical breakdown of aggregates, digestion and egestion of OC and production of microorganisms (Meysman et al., 2006). It is clear, however, that bioturbation is an ineffective mechanism of sediment oxygen uptake compared to molecular diffusion, which can be seen by comparing the in situ molecular diffusion coefficient for oxygen (ca. 400 cm\(^2\) yr\(^{-1}\)), Table 1) with the biodiffusion coefficient (ca. 0.5 cm\(^2\) yr\(^{-1}\)). Accordingly, increased \([O_2]_{cr}(0)\) and thus rates of oxygen diffusion into the sediment (Fig. 8a) mainly explain the lower CBEs during OA2.

The CBE data further show that \(\omega_{cr}(\omega)\) and \(F_{Cr}\) lie to the right hand side of the normal line, such that these factors will increase the rate of OC delivery to the sulfate reduction zone where preservation is more likely (Fig. 9b). This cause–effect relationship between carbon concentration and burial velocity has been corroborated by extrapolation of experiential data into statistical generalizations (Tyson, 2001). Yet, their individual effect is to increase CBE by roughly...
1.5% only, and so are of minor importance compared to \([O_2]_{cr}(0)\). This apparently contradicts the empirical relationship in Fig. 4, which shows that accumulation rate is the most important factor influencing preservation (Betts and Holland, 1991; Canfield, 1994). This is because the accumulation rate varies by only \(\pm 30\%\) across the C–T interval, compared to 5 orders-of-magnitude when the whole spectrum of marine sediments is considered (Fig. 4). In contrast, the perceived role of oxygen on CBE in Fig. 4 at low sediment accumulation rates is much more pronounced, as shown by the divergence of the two regression curves for high and low oxygen environments.

Of the reaction-specific factors, the rate constant for aerobic degradation \((k_1)\) mostly influences OC accumulation and CBE (Fig. 9d, e). The datum lies to the left of the normal line since higher \(k_1\) values will lead to more extensive carbon degradation. There are also weak interaction terms between \(k_1\) and the rate constant for sulfate reduction \((k_3)\). Overall, however, the effect of the rate constants on these responses is much lower than for the environmental factors.

The system analysis for Mo accumulation shows that an increase in reactive iron flux \((F_{Fe-cr})\) and \([O_2]_{cr}(0)\) will both decrease Mo burial by about 6 \(\mu g\ cm^{-2}\ ky^{-1}\), respectively, whereas an increase in reactive iron flux \((F_{Fe-cr})\) will promote Mo burial by 2–3 \(\mu g\ cm^{-2}\ ky^{-1}\) (Fig. 9c). This confirms the expectation of a prominent role for reactive iron on Mo accumulation based on previous work (Meyers et al., 2005; Meyers, 2007). The rate constants \(k_1\) and \(k_3\) invoke similar magnitudes of Mo accumulation (Fig. 9f). The effect of \(k_1\) is to decrease the Mo accumulation rate by reducing the amount of carbon buried to the sulfate reduction zone, whereas higher \(k_3\) will lead to more sulfide production and higher rates of Mo accumulation. However, because of the significant interaction terms between \(k_1\) and \(k_3\), \(F_{Fe-cr}\) and \([O_2]_{cr}(0)\) and, to a lesser extent, \(F_{Fe-cr}\) and \(F_{C-ox}\) on Mo accumulation, additional analysis is required to fully interpret the main effects of these factors. As an example, the bubble plots in Fig. 10 deconvolve the interaction terms for the environmental parameters. With regards to the \(F_{Fe-cr}–[O_2]_{cr}(0)\) interaction (Fig. 10a), it is clear that iron is much more dominant in controlling Mo accumulation than oxygen. A high reactive iron flux \((0.018 g\ cm^{-2}\ ky^{-1})\) decreases Mo burial to 2–3 \(\mu g\ cm^{-2}\ ky^{-1}\), which is essentially equal to the detrital Mo flux (Fig. 7c). Importantly, this occurs regardless of whether oxygen is present at the high or low level. High \([O_2]_{cr}(0)\) causes a reduction in Mo accumulation to 4 \(\mu g\ cm^{-2}\ ky^{-1}\) but only when the iron flux is low. The role of oxygen here is related to the lower OC accumulation rate and CBE when \([O_2]_{cr}(0)\) is high (Fig. 9a,b), which ultimately leads to lower free dissolved sulfide in the porewater. Similarly, the \(F_{Fe-cr}–F_{C-ox}\) interaction (Fig. 10b) reveals that higher OC fluxes can promote a doubling of the Mo burial rate, but only when the iron flux is low.

Overall, the system analysis reveals that there is a complex relationship between OC, \(O_2\), Fe and Mo accumulation. Changes in OC flux and bottom water oxygen concentration can potentially radically alter the Mo burial rate when iron fluxes are low. Low iron burial fluxes characterize the post-OAE2 period in the WIS and, in general, OAEs from other geographical regions (e.g. Hetzel et al., 2009).

5. Discussion

5.1. The role of iron cycling on Mo enrichment

Black shales from sites around the world are typically enriched in carbon and trace metal sulfides (Arthur et al., 1988; Arthur and Sageman, 1994; Kuypers et al., 2002; Kolonic et al., 2005; Brumsack, 2006; Hetzel et al., 2009). The supporting paradigm for this observation relies on the idea of enhanced carbon burial through increased primary productivity and/or preservation induced by the absence of oxygen in ocean bottom waters. This leads to elevated rates of sulfate reduction and sulfide accumulation in the water column and/or porewater, conditions under which particulate trace metal sulfides become stable (Calvert and Pedersen, 1993).

On the contrary, Mo accumulation rates in the WIS were lower during OAE2 and only increased following the global anoxic event (Fig. 7c). Several studies have argued that this was due to a high rate of detrital iron flux from the adjacent Sevier Orogenic Belt supplemented by a source of non-detrital, possibly hydrothermal, iron (Sageman and Lyons, 2003; Meyers et al., 2005; Meyers, 2007). Our results support the hypothesis of an effective benthic redox control Mo accumulation by iron and further suggest that reactive iron may have accumulated to several wt.% during OAE2 (Fig. 8g). At this time, rates of iron reduction \((r_2)\) and ferrous iron oxidation \((r_4)\) are elevated compared to post-OAE2 (Table 4) due to efficient re-oxidation of reduced iron within the upper bioturbated layers (Canfield et al., 1993). The abundance of iron curtails Mo accumulation by (i) allowing dissimilatory iron reducing bacteria to inhibit sulfate reducers for labile substrate, and (ii) oxidizing the little free sulfide which becomes available. This type of setting closely resembles contemporary abyssal sediments that are iron-rich and sulfide-free (Glasby, 1991; König et al., 1997). The system reaches a tipping point at the C–T transition where sulfide production surpasses the buffering capacity of iron oxide, allowing concentrations to reach the threshold for Mo sequestration. Beyond this point the rate of FeS cycling becomes much higher \((r_5, r_7, r_8)\), indicating fundamentally different pathways of iron recycling across the C–T interval. One can expect the balance between hydrogen sulfide production and depletion to be most delicate during this transitional period towards anoxia.

The importance of iron is somewhat unique to the WIS and should not be considered representative of OAEs. However, the role of oxygen and organic carbon revealed by the system analysis are a reminder that iron alone does not definitively control Mo accumulation, and this is probably true of sediments in general. For example, Mo enrichment has been shown to be sensitive to carbon flux and oxygen levels.
in modern surface sediments along the oxygen minimum zones of the Americas (McManus et al., 2006; Scholz et al., 2011). Due consideration of coupled iron, carbon and oxygen cycling is critical to interpreting paleoredox indicators such as Mo.

Adsorption of MoO$_4^{2-}$ onto iron (and manganese) oxohydroxides in oxic waters (Helz et al., 1996) and deposition on the seafloor provides an additional mechanism for Mo transfer to the sediment in addition to diffusion from sea water (Cruisius et al., 1996). Subsequent reductive dissolution of the iron oxides releases Mo to anoxic sediment porewaters, which can then be sequestered and precipitated as thiomolybdate or diffuse back to the water column. In general, this transfer pathway is small compared to the diffusive Mo flux across the sediment water interface (Cruisius et al., 1996; Erickson and Helz, 2000; Zheng et al., 2000). However, iron-associated Mo may lead to strong benthic enrichment of authigenic Mo in silled basins with weakly or seasonally anoxic bottom waters where the chemocline migrates through the sediment–water interface (Algeo and Lyons, 2006). Given the abundance of iron in the WIS, one would expect to see the Bridge Creek data punctuated with extreme Mo enrichments if the Mo pump was periodically active. However, such enrichments are absent from both the 2 meter smoothed Mo concentrations (~10 ppm, Fig. 7f) and the raw data (max. 20 ppm, data not shown). For this reason, we exclude this mechanism as a vector for Mo enrichment in the WIS.

5.2. Production versus preservation of OC across the C–T interval

Increased global burial of OC during OAE2 is usually inferred from the marked positive excursion in $\delta^{13}$COC and $\delta^{13}$CCO$_3$ followed by a return to more negative values in the post-OAE2 period as paleoproduction and OC burial decreased (Arthur et al., 1988). The Bridge Creek $\delta^{13}$COC data essentially track the trend of the global ocean (Sageman et al., 2006). Yet, the striking increase in OC concentration and accumulation rates occurring after OAE2 (Fig. 7) suggests that the extent and timing of ‘OAE2-like’ conditions in the shallow epicontinental WIS were offset from the rest of the ocean (Meyers et al., 2001; Sageman and Lyons, 2003). To help identify the contribution of productivity and preservation on OC accumulation in the WIS, Meyers et al. (2001; 2005) employed carbonate accumulation as a qualitative proxy for paleoproduction (Fig. 1d). The lower carbonate accumulation rates post-OAE2 were inferred to represent lower paleoproductivity, and the OC enrichment relative to OAE2 was thus explained as carbon preservation. In accordance with this idea, they adopted a simple preservation scaling approach, that is, dividing the carbon accumulation rate by the FOL, which predicted lower rates of paleoproduction post-OAE2 compared to OAE2. These researchers went on to argue that OC preservation was driven by (i) a decline in reactive iron flux which led to increased dissolved sulfide concentrations in the porewater, reduced bioturbation due to faunal toxicity to sulfide and a shallower sulfate reduction zone (thinner oxic layer), and (ii) a reduction in bulk sediment dilution. Equally, less OC preservation occurred during OAE2 because the decrease in sulfide levels by iron buffering led to more efficient ventilation of the sediments by burrowing animals; a hypothesis later termed the ‘sulfide buffer/phosphorus trap’ (Meyers, 2007). However, despite the obvious role of iron on accumulation (see above), our analysis does not indicate any substantial role for iron on OC accumulation (Fig. 9a). Instead, our results which are based on a more rigorous estimate of reconstructed carbon flux allude to the combined effects of paleoproduction and preservation, whereby a modest increase in paleoproduction (i.e. higher F$_{OC}$) in conjunction with preservation (i.e. lower $[O_2]_{cr}(0)$), conspired to allow OC (and Mo) to accumulate at higher rates post-OAE2 and with a higher CBE. A plot of $[O_2]_{cr}(0)$ versus F$_{OC-Cr}$ (Fig. 11) shows the inverse relationship between the two factors across the C–T interval. We reiterate that this result is by no means certain due to the inherent uncertainties in reconstructing the boundary conditions of the WIS sediments. It does, however, illustrate how simultaneous production and preservation can give rise to geochemical trends that are characteristic of OAE-like conditions.

The use of carbonate as a paleoproductivity proxy assumes that the phytoplankton composition remained relatively uniform in the central WIS over the C–T interval and that carbonate dissolution and precipitation were constant over time (Meyers et al., 2005). The model results presented here provide circumstantial evidence that the latter condition may not have been fulfilled. To explain, the diagenetic regimes between OAE2 and post-OAE2 are radically different (Fig. 8); being highly ferruginous during OAE2 and sulfidic thereafter. There is a transition from dissimilatory iron reduction and net sulfide buffering toward sulfate reduction and sulfide accumulation between the two periods. Critically, the number of protons, or alkalinity equivalents, produced and consumed by these reactions is different (ΔTA, Table 4). Stoichiometrically, much more alkalinity is produced by dissimilatory iron reduction (+8 equivalents per mole of carbon oxidized) compared to sulfate reduction (+1). Despite the small net production of alkalinity during OAE2 (+0.14 eq m$^{-2}$ yr$^{-1}$), it is very likely that carbonate minerals were in a state of disequilibrium in the surface sediments. For instance, dissimilatory iron reduction (r$_i$) produces +23.1 eq m$^{-2}$ yr$^{-1}$ of alkalinity, which will favor carbonate preservation and possibly authigenic carbonate precipitation in these layers. However, ferrous iron oxidation consumes 23 eq m$^{-2}$ yr$^{-1}$ of alkalinity, which will lead to highly undersaturated pore waters within the thin upper oxic layer (Jourabchi et al., 2005). Since the rate of carbonate dissolution depends on the mineral saturation state (e.g. Keir, 1980), which in turn depends on the porewater alkalinity and pH, this reaction creates a strong thermodynamic drive for carbonate dissolution in the surface layers.

The post-OAE2 sediments are associated with a larger net alkalinity production (2.8 eq m$^{-2}$ yr$^{-1}$ of alkalinity), which is due to burial of FeS. Again, one may expect sulfide oxidation reactions in the surface layers to cause an undersaturation with respect to carbonate minerals. Furthermore, the depth-integrated rate of aerobic OC mineralization post-OAE2 and, therefore, CO$_2$ production is...
46 mol m$^{-2}$ yr$^{-1}$ higher than during OA2E. Although this reaction does not affect total alkalinity directly, CO$_2$ will rapidly neutralize carbonate ion alkalinity (CO$_2$ + CO$_3^{2-}$ + H$_2$O $\rightarrow$ 2HCO$_3^-$) and lower the carbonate saturation state. This ‘metabolic dissolution’, to distinguish from thermodynamically-driven dissolution, is a common characteristic of surface marine sediments (Emerson and Bender, 1981; Archer et al., 1989) and could conceivably account for the lower rates of calcite accumulation post-OA2E. This cautions against the use of carbonate content as a proxy for primary production. A more sophisticated model than the one presented here would be required to test this idea more rigorously (Jourabchi et al., 2008).

In summary, the above calculations highlight the difficulties in distorting the role of production and preservation through a qualitative interpretation of carbonate accumulation rates where there are large shifts in benthic redox potential. Erba (2004) raised similar concerns for the interpretation of calcareous nanofossils in black shales. The interpretation of proxies through the application of models using highly resolved data from OA2E sequences elsewhere (e.g. Kolonic et al., 2005; Hetzel et al., 2009) shows great promise in delivering a more global picture of the significance of benthic processes during the onset of ocean anoxia.

6. Conclusions

Proxy signals recorded in marine sediments can be amplified or diminished by changes in the intensity of physical processes (e.g. burial rates, bioturbation) as well as biogeochemical reactions. Benthic cycles are highly coupled through the chemical species and can interact synergistically or antagonistically on the accumulation of key geochemical indicators. It thus becomes challenging, if not impossible, to deduce the major forcings by a qualitative analysis of the data. Reaction-transport models integrate transport processes and biogeochemistry into a quantitative framework and can be used to extricate the overarching controls on single- or multi-proxy inventories.

We applied a model to reconstruct the geochemical paleorecord preserved in strata of the Bridge Creek Limestone Member of the Western Interior Seaway and evaluate the controls on organic carbon (OC) and molybdenum (Mo) accumulation rate. Our attention focused on the Cenomanian–Turonian (C–T) boundary interval (94.34–93.04 Ma) which includes OA2E. To our knowledge, this is the first model application to reconstruct multi-proxy data recorded in fully consolidated sedimentary rock. This approach thus represents a new dimension for studying deep-time biogeochemical processes in a dynamic manner.

Important implications follow from this work. Our results clearly corroborate Meyers’ (2007) argument that the accumulation rate of particulate Mo in black shales is a poor proxy for ocean euxinia or even anoxia in ancient marine systems. We show that high rates of Mo accumulation can occur with high rates of aerobic OC degradation in the surface sediments. Furthermore, Mo sequestration can be severely restricted by the availability of reactive iron oxides which buffer porewater sulfide to levels below those required for Mo sequestration. Evidence suggests that the bottom waters became progressively more hypoxic, but not anoxic, over the C–T interval in the WIS in parallel with increasing OC flux to the seafloor. This alludes to a positive feedback between the sea floor and the photic zone, probably via phosphate, on the advance of anoxia. Future modeling work will address this hypothesis by incorporating phosphorous data into the model. The results presented simultaneously support both long-standing notions that OC enrichment in ancient sediments is driven by increased preservation and increased export production. There is no reason why these should be regarded as uncoupled, independent processes.

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References


Appendix V

Bacterial Chitin Hydrolysis in Two Lakes of Contrasting Trophic Status

Authors

Bacterial Chitin Hydrolysis in Two Lakes with Contrasting Trophic Statuses

Krista E. Köllner,a,b,c Dörte Carstens,a,c Esther Keller,b Francisco Vazquez,a Carsten J. Schubert,c Josef Zeyer,c and Helmut Bürgmanna

Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters-Research and Management, Kastanienbaum, Switzerlandb; Eawag, Department of Aquatic Ecology, Dubendorf, Switzerlandc; and Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerlandd

Chitin, which is a biopolymer of the amino sugar glucosamine (GlcN), is highly abundant in aquatic ecosystems, and its degradation is assigned a key role in the recycling of carbon and nitrogen. In order to study the significance of chitin decomposition in two temperate freshwater lakes with contrasting trophic and redox conditions, we measured the turnover rate of the chitin analog methylumbelliferyl-N,N’-diacetylchitobioside (MUF-DC) and the presence of chitinase (chiA) genes in zooplankton, water, and sediment samples. In contrast to the eutrophic and partially anoxic lake, chiA gene fragments were detectable throughout the oligotrophic water column and chiA copy numbers per ml of water were up to 15 times higher than in the eutrophic waters. For both lakes, the highest chiA abundance was found in the euphotic zone—the main habitat of zooplankton, but also the site of production of easily degradable algal chitin. The bulk of chitinase activity was measured in zooplankton samples and the sediments, where recalcitrant chitin is deposited. Both, chiA abundance and chitinase activity correlated well with organic carbon, nitrogen, and concentrations of particulate GlcN. Our findings show that chitin, although its overall contribution to the total organic carbon is small (~0.01 to 0.1%), constitutes an important microbial growth substrate in these temperate freshwater lakes, particularly where other easily degradable carbon sources are scarce.

Chitin is a homopolymer of β-1,4-linked N-acetylated glucosamine (GlcNAc). It is a structural component of the cell walls of fungi and the exoskeletons of invertebrates but is also found in protozoa (45) and algae (23, 31). Due to its wide distribution, chitin is, after cellulose, the second most abundant biopolymer on earth (35). The annual production and the steady-state amount in the biosphere are on the order of 10^{12} to 10^{14} kg (30, 47). On the basis of literature data on chitin production by arthropods, the biosphere are on the order of 10^{12} to 10^{14} kg (30, 47). On the basis of literature data on chitin production by arthropods, the total annual chitin production in aquatic environments was estimated at 2.8 × 10^{10} kg chitin year^{-1} for freshwater ecosystems and at 1.3 × 10^{12} kg chitin year^{-1} for marine ecosystems (10). The role of chitin as a significant component of the aquatic carbon and nitrogen budget was studied extensively during the 1990s, but almost exclusively in estuarine and marine environments (7, 17, 33, 34, 48). Studies on bacterial chitin degradation in lake water are rare (4, 14, 37, 42).

Not only phyto- and zooplankton and insect carcasses, but also zooplankton molting (exuviae) and excretion of fecal pellets (peritrophic membranes) contribute to the production of huge amounts of chitinous particles in the water column (61). These chitinous particles are part of the marine or lake snow, which was shown to represent a hot spot of particulate organic matter solubilization (18, 19, 52). In the ocean, chitinolytic bacteria were found to be responsible for the hydrolysis of chitin (35, 64). After adhering to the polymeric substrate, chitinolytic bacteria express a multitude of enzymes and other proteins required for its catabolism (33). The hydrolysis of the β-(1,4)-glycosidic bonds between the GlcNAc residues is accomplished by extracellular chitinases (EC 3.2.1.14) (22). The end products of chitin degradation in the chitinolytic pathway are monomers and dimers of GlcNAc, which can be catabolized in the cytoplasm to fructose-6-P, acetate, and NH_3 (3, 33).

Based on amino acid similarities, chitinases are classified into family 18 and family 19 (22). Family 19 chitinases were formerly thought to be restricted to plant origin but have since also been found in various *Streptomyces* species and other bacteria (4, 49, 57). However, the most information on bacterial diversity and distribution in diverse environments is available for family 18 A chitinases (11, 24, 25, 36, 38, 55).

In the present study, we aimed to identify the main sites of chitin hydrolysis and the significance of chitin as a bacterial substrate in two temperate freshwater lakes with contrasting trophic and redox conditions. For this purpose, we analyzed the chitinase activities and the abundances of bacterial chitinase genes (chiA) in zooplankton, water from 10 different depths, and sediment samples from oligotrophic Lake Brienz (LB) and eutrophic Lake Zug (LZ). The lakes were sampled in spring and fall 2009.

**MATERIALS AND METHODS**

**Sampling sites.** The characteristics of the study sites are listed in Table 1.

LB is an oligotrophic perialpine lake located 70 km southeast of Bern, Switzerland. The lake is fully oxic throughout the year. The catchment of the lake is drained by the two main inflows, Aare and Lütschine, which together transport an annual average of 3 × 10^8 kg suspended material into LB (16). Both, the hydrological regime and the suspended particle load of the river Aare are influenced by hydropower operations. The continuous supply of suspended glacial particles, causing reduced light penetration, together with the scarcity of nutrients has led to an unusually low phytoplankton biomass (on average, <10 g m^{-2}) (15, 21).

LZ is a eutrophic subalpine lake 30 km South of Zurich, Switzerland. The lake consists of two basins: a shallow (40- to 60-m depth) North Basin and a 200-m-deep South Basin. The South Basin is meromictic, with mix-
ing depths that do not exceed 100 m. Together with the eutrophic status of LZ, this leads to seasonally anoxic conditions at a depth of 140 to 160 m and permanent anoxia below that depth (41, 43).

**Sampling water and sediments.** LB was sampled in mid-May and mid-September 2009 in the central part of its basin at position 46°43'N, 7°58'E. LZ was sampled at the end of March and the end of October 2009 in the South Basin at position 47°6'N, 8°29'E.

Profiles of temperature, oxygen (\(\text{O}_2\)), and conductivity were taken with a conductivity-temperature-depth (CTD) profiler (Seabird SBE19; Sea-Bird Electronics, Inc., Bellevue, WA). Based on the temperature and \(\text{O}_2\) profiles determined (Fig. 1), water was sampled from 10 depths using a Niskin water sampler, poured into autoclaved 1-liter glass bottles, and transported cool and in the dark to the laboratory. We sampled LB at water depths of 5, 10, 20, 30, 40, 70, 100, 150, 200, and 240 m and LZ at 5, 10, 15, 25, 60, 80, 100, 130, 170, and 190 m.

Particulate organic matter (POM) from the same depths was sampled on 0.7-μm glass fiber filters (Whatman Inc., Florham Park, NJ) with in situ pumps (McLane Research Laboratories Inc., Falmouth, MA) until the filters were clogged.

Sediment cores were recovered from the two sampling sites using a gravity corer (32). The first 5 (spring) to 7 (fall) centimeters of each core were sliced at intervals of 1 centimeter. Subsamples of each layer were processed for microbiological and biogeochemical analysis (see below).

**Zoo- and phytoplankton communities.** Zooplankton samples were taken with a 95-μm double-closing net (8) from 0 to 100 m (2 replicates) and preserved in 2% formaldehyde. Phytoplankton was sampled with an integrated sampler according to the method of Schröder (51) from 0 to 20 m (2 replicates). Lugol-fixed phytoplankton species were counted using the technique of Utermöhl on an inverted microscope (58). Crustacean species and their developmental stages were enumerated under a binocular dissecting microscope at \(\times 10\) to \(\times 75\). Phyto- and zooplankton biomass fresh weights were calculated from the mean cell/organism dimensions of each species (9, 21). For LB, these analyses were carried out at the Laboratory for Water and Soil Protection of the Canton of Bern.

### TABLE 1 Characteristics of study sites

<table>
<thead>
<tr>
<th>Property</th>
<th>Lake Brienz</th>
<th>Lake Zug South Basin</th>
<th>Reference(s)</th>
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<tr>
<td>Position</td>
<td>46°43'N, 7°58'E</td>
<td>47°7'N, 8°29'E</td>
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<tr>
<td>Elevation (m)</td>
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<tr>
<td>Surface area (km²)</td>
<td>29.8</td>
<td>16</td>
<td>21, 43</td>
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<tr>
<td>Maximum depth (m)</td>
<td>259</td>
<td>200</td>
<td>21, 43</td>
</tr>
<tr>
<td>Volume (km³)</td>
<td>5.15</td>
<td>2.0</td>
<td>21, 43</td>
</tr>
<tr>
<td>Primary inflow</td>
<td>Aare, Lütschine Rigiaa</td>
<td>Aare</td>
<td></td>
</tr>
<tr>
<td>Primary outflow</td>
<td>Aare</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean hydraulic residence time (yr)</td>
<td>2.7</td>
<td>14</td>
<td>21, 43</td>
</tr>
<tr>
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<td>Eutrophic</td>
<td></td>
</tr>
<tr>
<td>Oxygen status</td>
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<td>Anoxic below 140–160 m</td>
<td>43</td>
</tr>
</tbody>
</table>

**FIG 1** Profiles of temperature, oxygen, and conductivity of the water columns of LB sampled in May (a) and September (b) 2009 and of LZ sampled in March (c) and October (d) 2009. The dots along the oxygen profile indicate sampled water depths.
Zooplankton chitin. The chitin biomass in LB and LZ was calculated from the zooplankton biomass and body chitin content published by Cauchie (10), which were 4.3% and 9.8% (dry weight) for lentic branchiopoda and copepoda, respectively.

Chemical analysis. The zooplankton, water, and sediment samples were subdivided for microbiological and chemical analyses.

The dissolved organic carbon (DOC) and dissolved nitrogen (DN) concentrations in lake water were determined after filtration through a 0.2-μm Supor membrane filter ( Pall Corporation, Port Washington, NY). DOC and total organic carbon (TOC) were measured by high-temperature catalytic oxidation (720°C) with a Shimadzu TOC-V CPH (Shimadzu Scientific Instruments, Kyoto, Japan). The total nitrogen (TN) content and DN concentration were determined with a Shimadzu TOC-V CPH/TNM1.

Phosphate (PO₄³⁻) concentrations were determined following filtration through 0.45-μm cellulose acetate filters (Schleicher & Schuell GmbH, Dassel, Germany). PO₄³⁻ was determined photometrically by the molybdenum blue method according to Vogler (59).

Concentrations of GlcN in zooplankton samples, in POM, and in the sediments were measured by a slightly modified method according to Zhang and Amelung (63) with a derivatization step according to Guer rant and Moss (20) and with myo-inositol (Aldrich) as an internal standard. Filters were treated with 10 ml of 6 M HCl for 10 h at 100°C, which should ensure complete hydrolysis of biopolymers of GlcN, including chitin and peptidoglycan, in which it occurs in its N-acetylated form (GlcNAc). Hydrolysis causes deacetylation, and thus, concentrations of GlcN are the sums of both forms, GlcN and GlcNAc. The gas chromatography (GC) system was equipped with a flame ionization detector (HRGC 5160; Carlo Erba Instruments, Milan, Italy), a split-splitless injector, and a VF-5 MS column (60 m, 0.25-mm inner diameter, and 0.25-μm film thickness). The injector temperature was 250°C and the temperature of the detector was 300°C. Hydrogen was used as the carrier gas, with a flow rate of 2 ml min⁻¹. The temperature profile was as follows: 120°C to 200°C at 20°C min⁻¹, 200°C to 250°C at 2°C min⁻¹, and 250°C to 270°C at 20°C min⁻¹, held for 10 min at 270°C. A GlcN standard (n-glucosamine; Sigma- Aldrich Chemie GmbH, Buchs, Switzerland) was also derivatized and used for quantification.

Chitinase activity. Chitinase activity on water, sediment, and zooplankton samples in fall 2009 was determined using the cliinit substrate analog methylumbelliferyl-N,N’-diacetylchitobioside (MUF-DC) (Sigma-Aldrich) according to a previous method (34, 37) with slight modifications. Referring to the manufacturer’s instructions, MUF-DC was dissolved in 100% dimethyl formamide (DMF) to a final concentration of 5 mM (stock solution). To the manufacturer’s instructions, MUF-DC was dissolved in 100% dimethyl formamide (DMF) to a final concentration of 5 mM (stock solution). To the manufacturer’s instructions, MUF-DC was dissolved in 100% dimethyl formamide (DMF) to a final concentration of 5 mM (stock solution). To the manufacturer’s instructions, MUF-DC was dissolved in 100% dimethyl formamide (DMF) to a final concentration of 5 mM (stock solution).

In a preliminary test, the effect of the substrate concentration was determined by incubating LZ surface waters and mixed LZ sediment samples (0 to 5 cm) with six different MUF-DC concentrations (1, 5, 10, 50, 100, and 300 μM) at 20°C and 4°C, respectively. In addition, we tested DMF for inhibitory effects on Streptomyces griseus chitinase (Sigma). DMF was found to linearly inhibit activity up to 80% at 6% DMF, the concentration in the highest MUF-DC concentration (300 μM) used in the assay. Therefore, we adjusted the concentration of DMF for all assays to 6%.

Centrifuge tubes (50 ml: Greiner Bio-One, Frickenhausen, Germany) were filled with water samples mixed with formalin to a final concentration of 0.25% to prevent microbial growth (27). The influence of 0.25% formalin on the fluorescence of released MUF and on the activity of S. griseus chitinase was tested in preliminary experiments. No significant effects were found. Formalin-fixed water samples were stored cold in the dark until assayed (within 4 h after sample collection).

Sediment samples (0.5 g) and 1.48 ml of autoclaved, 0.2-μm-filtered and formalin-treated (0.25%) lake water were well mixed and assayed within 12 h of sample collection.

Water and sediment samples were amended with aliquots of the MUF-DC stock solution and incubated at 4 and 20°C, which correspond to the minimum and maximum temperatures measured in the water columns of both lakes over a year. As in situ temperatures for different samples differ from the incubation temperatures (Fig. 1) and sediments were analyzed as slurries, our data (see Fig. 3 and 4) are potential chitinase activity rates. After incubation for 1 to 3 h, the reactions were stopped by adding aliquots of 100 and 150 μl of water and sediment-in-water suspension by adding 10 and 15 μl of ammonium glycine buffer (pH 10.5) (12), respectively. The fluorescence of free methylumbelliferone (MUF) was measured in the water samples and sediment supernatants at 360-nm excitation and 460-nm emission using a Synergy HT microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). The samples were shaken (300 rpm) between measurements.

Zooplankton was killed by 3% hydrochloric acid and washed with autoclaved and 0.2-μm-filtered lake water. Zooplankton (0.05 g) was suspended in 2 ml 0.25% formalin-treated, autoclaved, and 0.2-μm-filtered lake water. After adding an aliquot of the MUF-DC stock solution, samples were incubated and processed as described for the sediments. The sediments were measured repeatedly up to 11 h, zooplankton up to 9 h, and water up to 100 h.

Positive controls were assayed in autoclaved, 0.2-μm-filtered, and formalin-treated (0.25%) lake water and in autoclaved sediments containing aliquots of MUF-DC stock solution and S. griseus chitinase. In addition, negative controls without S. griseus chitinase were run to test for antibiotic degradation of MUF-DC.

DNA extraction. Autoclaved glass bottles (1 liter) were filled with water samples and transported on ice and in the dark to the laboratory. About 5 liters of water from each sampled depth was filtered through a 5-μm-isopore membrane filter (Millipore, Billerica, MA) and a 0.2-μm polycarbonate filter (Whatman), each 142 mm in diameter, connected in series. The filters were frozen in liquid nitrogen immediately after filtration and stored at −80°C until DNA extraction. For extraction, a filter segment (1/4) was cut into small pieces using sterile scissors and mixed with 0.2 g of glass beads (0.1 g of 106- and 0.1 g of 150- to 212-μm glass beads; Sigma-Aldrich) in a 2-ml screw-cap tube (Brand GmbH & Co KG, Wertheim, Germany). Ice-cold extraction buffer (1.4 ml) (26) was added. The cells were disrupted in a FastPrep-24 bead-beating system (MP Biomedicals, Solon, OH) by beating twice for 40 s at 4 m s⁻¹, placing the tubes on ice in between. Bead beating was followed by a freeze-thaw cycle in liquid nitrogen. The supernatant was treated with 50 μg ml⁻¹ RNase A (Sigma-Aldrich) for 30 min at 37°C and extracted with an equal volume of phenol-chloroform-isomylalcohol (25:24:1; pH 8; Sigma-Aldrich). After precipitation with 1 volume of isopropanol, the pelleted DNA was dissolved in Tris-EDTA (pH 8) buffer and stored at −80°C.

For extraction of sediment and zooplankton, 0.5-g and 0.05-g samples, respectively, were mixed with 1.4 ml ice-cold nucleic acid extraction buffer (26). The samples were frozen in liquid nitrogen and stored at −80°C until DNA extraction. After thawing, 0.25 g sterile 0.1-mm Zincoria beads (Biospec Products Inc., Bartlesville, OK) was added, and the samples were processed on a vortex adaptor (MoBio Laboratories, Inc., Carlsbad, CA) for 1 min at maximum speed. DNA extraction was performed as for the water filters.

The quality of DNA extracts was checked by agarose gel (1%) electrophoresis. Extracted DNA was quantified by fluorescence spectroscopy using the Quant-iT PicoGreen double-stranded DNA (dDNA) Assay Kit (Molecular Probes, Eugene, OR) and a Synergy HT microplate reader (Bio-Tek Instruments).

The reproducibility of the applied DNA extraction protocols was tested on quadruplicate samples of sediment slurries and on four filter segments (1/4) of a single 0.2-μm polycarbonate filter, one for each lake.

Amplification and quantification of chitinase gene fragments. We used the primer pair chi2 (GAGCGGATCGACATCGATTGG) and chiR (CGTCGACGCCGCGSCCRTA), which was reported to target chitinase family 18 group A (chiA) gene fragments from a broad range of chitinolytic bacteria (62). The performance and specificity of chiA PCR primers were tested on spring water samples (LB at 10 m, LB at 240 m, LZ at 10 m, and LZ at 190 m).

Each PCR mixture (20 μl) contained 10 μl 2× Power SYBR green PCR Master Mix (Applied Biosystems, Foster City CA), 0.4 μl of each primer.

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(10 μM; Microsynth, Balgach, Switzerland), 2 μl bovine serum albumin (BSA) (10 mg ml⁻¹; Sigma-Aldrich), and 5 μl template diluted in nuclease-free water (Qiagen GmbH, Hilden, Germany). Amplification was performed in a 7500 Fast Real-Time PCR System (Applied Biosystems) with PCR conditions consisting of an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 60 s, and extension at 72°C for 30 s, and finally by melting-curve analysis.

PCR resulted in products yielding a single sharp band of the expected size of ~430 bp in gel electrophoresis (data not shown). To evaluate the specificity of the chiA PCR primers, we constructed clone libraries from the spring water samples. PCR products of chitinase gene fragments from samples were cloned into the pGEM-T vector by using the pGEM-T Easy Vector System according to the instructions of the manufacturer (Promega). Screening of the clone libraries constructed from these samples resulted in 15 different restriction fragment length polymorphism (RFLP) types among 71 screened plasmids. We sequenced 1 to 5 clones of each RFLP type, 31 clones in total (sequencing by Microsynth AG, Balgach, Switzerland). All sequences could be assigned to chitinases, indicating high specificity of the PCR protocol (unpublished data).

In preliminary experiments, the PCR amplification was assayed in 4-fold serial dilutions of total DNA extracts in nuclease-free water (Qiagen) to determine the effects of inhibiting contaminants. For instance, according to the resulting copy numbers, we assayed lake sediments in 64-fold dilutions, as they gave comparable but slightly higher (~13%) chiA copy numbers than 16-fold-diluted templates, while results dropped off significantly for the 256-fold dilution (~40%). Four-fold dilution of zooplankton samples and 16-fold and 64-fold dilutions of water and sediment samples, respectively, were applied in the final analysis. Therefore, different amounts of genomic DNA template were used in quantitative PCR (qPCR), i.e., less than 5 ng DNA for LB samples and ~10 to 20 ng DNA for LZ samples. Additionally, the results of quadruplicate analyses of DNA extraction yields showed high standard deviations, up to 31%, for water filters. For sediment slurries, the standard deviation was 6%.

**RESULTS**

**Biogeochemistry of lake water columns.** (i) Oxygen. The temperature, O₂, and conductivity profiles for both lakes sampled in spring and fall are shown in Fig. 1. In both seasons, the water column of LB was fully oxic. For LZ, anoxic conditions were measured below 130 m (O₂ < 0.1 mg liter⁻¹), a shallower depth than reported previously (41, 43).

(ii) Organic carbon. In LZ waters, the TOC concentrations were roughly three to four times higher than in LB waters (see Fig. S1a in the supplemental material) and ranged from 1.86 to 2.54 mg C liter⁻¹ (error of measurement, 0.20 mg C liter⁻¹). The DOC values ranged between 1.80 ± 0.20 mg C liter⁻¹ (March 2009; 170 m) and 2.40 ± 0.20 mg C liter⁻¹ (October 2009; 5 m). For LB, the DOC concentrations were below or close to the detection limit of 0.50 mg C liter⁻¹ in both seasons.

(iii) Nitrogen. The TN and DN concentrations were below the detection limit of 0.50 mg N liter⁻¹, except for LZ waters in spring (data not shown).

(iv) Phosphate. The PO₄³⁻ concentrations were below 5 μg P liter⁻¹ for all sampled water depths of LB, with the exception of the 5-m and 20-m surface water depths sampled in September 2009 (see Fig. S1c in the supplemental material). For LZ the PO₄³⁻ concentrations increased with water depth. The concentrations ranged from 3.8 ± 0.5 μg P liter⁻¹ (October 2009; 15 m) to 217 ± 0.5 μg P liter⁻¹ (October 2009; 190 m).

(v) Glucosamine. For both lakes, concentrations of particulate GlcN were highest in the euphotic zone and decreased with depth (see Fig. S1d in the supplemental material). The highest value of 70.6 nmol liter⁻¹ was measured at the 5-m water depth of LZ sampled in March 2009. For LB, the GlcN concentrations were roughly 1 order of magnitude lower than for LZ.

**Biogeochemistry of sediment profiles.** For LB sediments, the TOC, TN, and GlcN contents were always lowest for the 1- to 2-cm layer, in which the sediment becomes anoxic (46), and increased again with depth (Table 2). Biogeochemical parameters in LZ sediment (Table 3) were roughly 1 order of magnitude higher than in LB sediments, with the exception of the 6- to 7-cm layer sampled in fall, which showed similar TOC, TN, and GlcN contents in both lakes.

**Zoo- and phytoplankton community compositions.** The phytoplankton communities in both lakes were composed of chrysophytes (golden algae), diatoms, cryptophytes, dinoflagellates, chlorophytes (green algae), haptophytes (only in LB in May 2009), euglenoids (only in LZ in October 2009), and cyanobacteria. The phytoplankton biomass was dominated by diatoms, except for LZ in March 2009, when the predominant organisms were cyanobacteria (60%). Diatoms accounted for 40% (May 2009) and 66% (September 2009) of the biomass in LB and for 16% (March 2009) and 71% in LZ (October 2009).

In both lakes, the predominant zooplankton species were cladocerans (Daphnia sp., Diaphanosoma brachyurum, Leptodora

---

**TABLE 2 Biogeochemistry of sediments from LB sampled in May and September 2009**

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>TOC (% dry wt)</th>
<th>TN (% dry wt)</th>
<th>GlcN (μmol g dry wt⁻¹)</th>
<th>TOC (% dry wt)</th>
<th>TN (% dry wt)</th>
<th>GlcN (μmol g dry wt⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>0.59</td>
<td>0.05</td>
<td>1.30</td>
<td>0.67</td>
<td>0.04</td>
<td>0.48</td>
</tr>
<tr>
<td>1–2</td>
<td>0.39</td>
<td>0.04</td>
<td>0.62</td>
<td>0.48</td>
<td>0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>2–3</td>
<td>0.48</td>
<td>0.04</td>
<td>0.97</td>
<td>0.70</td>
<td>0.06</td>
<td>0.97</td>
</tr>
<tr>
<td>3–4</td>
<td>0.61</td>
<td>0.06</td>
<td>0.84</td>
<td>0.65</td>
<td>0.06</td>
<td>0.73</td>
</tr>
<tr>
<td>4–5</td>
<td>0.67</td>
<td>0.06</td>
<td>0.92</td>
<td>0.70</td>
<td>0.06</td>
<td>1.04</td>
</tr>
<tr>
<td>5–6</td>
<td>0.82</td>
<td>0.08</td>
<td>0.75</td>
<td>1.23</td>
<td>0.13</td>
<td>2.58</td>
</tr>
</tbody>
</table>

**TABLE 3 Biogeochemistry of sediments from LZ sampled in March and October 2009**

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>TOC (% dry wt)</th>
<th>TN (% dry wt)</th>
<th>GlcN (μmol g dry wt⁻¹)</th>
<th>TOC (% dry wt)</th>
<th>TN (% dry wt)</th>
<th>GlcN (μmol g dry wt⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>4.72</td>
<td>0.72</td>
<td>14.2</td>
<td>3.27</td>
<td>0.44</td>
<td>14.9</td>
</tr>
<tr>
<td>1–2</td>
<td>2.85</td>
<td>0.39</td>
<td>6.56</td>
<td>3.08</td>
<td>0.38</td>
<td>13.7</td>
</tr>
<tr>
<td>2–3</td>
<td>4.99</td>
<td>0.73</td>
<td>12.3</td>
<td>4.12</td>
<td>0.49</td>
<td>19.1</td>
</tr>
<tr>
<td>3–4</td>
<td>4.94</td>
<td>0.74</td>
<td>13.3</td>
<td>2.64</td>
<td>0.33</td>
<td>7.29</td>
</tr>
<tr>
<td>4–5</td>
<td>3.60</td>
<td>0.48</td>
<td>10.5</td>
<td>2.80</td>
<td>0.39</td>
<td>5.55</td>
</tr>
<tr>
<td>5–6</td>
<td>2.11</td>
<td>0.30</td>
<td>8.23</td>
<td>1.31</td>
<td>0.17</td>
<td>4.36</td>
</tr>
</tbody>
</table>

* Error of measurement (standard deviation) of one sample: TOC, ±0.1%; TN, ±0.02%; GlcN, ±10%.
The plankton bloom is in agreement with chlorophyll counted for 72% of the total plankton biomass of 28.1 g m$^{-2}$

zooplankton biomass from LB collected in September 2009. The scarce food sources for zooplankton, like forms of diatoms, such as Asterionella formosa and Fragilaria crotonensis, also goes along with the predominance of large grazing-resistant zooplankton biomass (Diaptomidae copepods) in LB and LZ, respectively.

**Zooplankton biomass**. In March/May 2009, the zoo- and phytoplankton biomass contributed equally to the total plankton biomass in both lakes (Fig. 2). In LZ, the plankton biomass (25.9 g m$^{-2}$) was more than double that of LB (11.7 g m$^{-2}$). In October 2009, the vast majority of the plankton biomass (33.9 g m$^{-2}$) was phytoplankton (>90%). The late fall phytoplankton bloom is in agreement with chlorophyll a measurements from the same year (Environmental Agency of Canton Zug, unpublished data). The low proportion of zooplankton biomass also goes along with the predominance of large grazing-resistant forms of diatoms, such as Asterionella formosa and Fragilaria crotonensis, and the scarcity of food sources for zooplankton, like small algae and cyanobacteria (data not shown). In contrast, the zooplankton biomass from LB collected in September 2009 accounted for 72% of the total plankton biomass of 28.1 g m$^{-2}$. Judging from LB plankton-monitoring data for the year 2009 (Environmental Agency of Canton Bern, unpublished data), the zoo- and phytoplankton biomass generally were approximately equal, with the exception of January and late summer (July to September), when the zooplankton biomass was significantly greater (up to 74% of the total plankton biomass).

**Zooplankton chitin**. In March/May 2009, chitin estimated from zooplankton abundances was 134 mg m$^{-2}$ for LB and therefore more than 2 times higher than in LB (55.3 mg m$^{-2}$) (Fig. 2). In contrast, in fall, zooplankton chitin in LB (154 mg m$^{-2}$) was about 8 times higher than in LZ (18.7 mg m$^{-2}$).

**Zooplankton chitin biomass compared to TOC and GlcN concentrations**. For the spring sampling campaign, relating the chitin estimates from the zooplankton to the carbon pool, zooplankton chitin contributed 0.04% of the TOC integrated over the upper 100 m of the water column of LB (63.5 g C m$^{-2}$) and 0.03% of the TOC pool of LZ (205 g C m$^{-2}$). For the fall sampling campaign, the contribution of zooplankton chitin to the TOC was almost 30 times higher in LB (0.1270%) than in LZ (0.0045%).

In LB, the particulate GlcN concentration integrated over the 0- to 100-m water column was about 2-fold (March 2009) and 6-fold (October 2009) higher than the zooplankton chitin biomass (Fig. 2). In LB, it was as high as the zooplankton chitin biomass in May 2009, but it was only one-sixth of the zooplankton chitin biomass in September 2009. This discrepancy could be caused by the higher inorganic glacial particle load from the inflows in the second half of the year (15, 16), which caused higher turbidity in the surface waters (1). As a consequence, the primary production maximum was shifted to a lower water depth of 2.5 m compared to May 2009, when it was observed at a water depth of 5 m (D. Carstens, K.E. Köllner, H. Bürgmann, B. Wehrli, and C.J. Schubert, submitted for publication). Since the shallowest in situ pumping was performed at 5 m, the fall sampling probably missed a significant proportion or even the maximum of the zooplankton biomass in the surface waters of LB.

**Zooplankton chemistry**. The GlcN content of zooplankton sampled in fall accounted for 69.6 ± 4.5 and 94.8 ± 7.1 μmol g dry weight$^{-1}$ in LB and LZ, respectively, and was, therefore, about one-third higher in the zooplankton of LZ. The results for the TOC, TN, and GlcN contents of zooplankton samples are summarized in Table 4.

**Chitinase activity**. The effect of the substrate concentration on chitinase activity was tested in LB surface waters and mixed sediments. Chitinase gene copy no.

<table>
<thead>
<tr>
<th>Site</th>
<th>TOC (% dry wt)</th>
<th>TN (% dry wt)</th>
<th>GlcN (μmol g dry wt$^{-1}$)</th>
<th>Chitinase activity at 4°C (nmol MUF$^b$ h$^{-1}$ g dry wt$^{-1}$)</th>
<th>Chitinase activity at 20°C (nmol MUF$^b$ h$^{-1}$ g dry wt$^{-1}$)</th>
<th>Chitinase gene copy no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(μmol mg$^{-1}$ DNA$^{-1}$)</td>
<td>(μmol mg$^{-1}$ DNA$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>52.1 ± 0.7</td>
<td>8.77 ± 0.09</td>
<td>69.6 ± 4.5</td>
<td>26.0 ± 4.4</td>
<td>1600 ± 705</td>
<td>1.39 ± 0.61</td>
</tr>
<tr>
<td>LZ</td>
<td>42.4 ± 0.7</td>
<td>9.00 ± 0.24</td>
<td>94.8 ± 7.1</td>
<td>50.4 ± 9.3</td>
<td>5726 ± 578</td>
<td>13.9 ± 1.4</td>
</tr>
</tbody>
</table>

$^a$ The errors given are standard deviations of triplicate measurements of one sample.

$^b$ MUF, fluorescent methylumbelliferone released after the hydrolysis of the chitin substrate analog methylumbelliferyl-$N,N'$-diacetylchitobioside.
ment slurries. For the sediment samples, MUF-DC turnover was found to be highest (2.63 ± 0.22 nmol h \(^{-1}\) g dry sediment \(^{-1}\)) at a substrate concentration of 50 μM (Fig. 3) and dropped at 100 and 300 μM. For the water samples, the chitinase activity was below the limit of detection for all substrate concentrations, even after 4 days of incubation. For negative controls, no turnover rates could be detected, which implies that only biotic degradation of MUF-DC was detected during the incubation.

We assayed the sediments and zooplankton from both lakes sampled in fall 2009 with 50 μM MUF-DC at 4°C and 20°C. MUF-DC turnover rates at 20°C were roughly one-third higher than at 4°C for the zooplankton samples from both lakes and up to 3- and 8-fold higher for LB and LZ sediments, respectively. For reasons of simplicity, only the data for 4°C are discussed and shown in Table 4 and Fig. 4.

(i) Zooplankton. Chitinase activity on LZ zooplankton was about double that measured for LB zooplankton on a dry-weight basis (Table 4).

(ii) Sediment. Chitinase activity (at 4°C) in LB sediments ranged from 0.08 (1 to 2 cm) to 0.69 (6 to 7 cm) nmol MUF h \(^{-1}\) g dry weight \(^{-1}\) and in LZ sediments from 0.59 (5 to 6 cm) to 5.10 (0 to 1 cm) nmol MUF h \(^{-1}\) g dry weight \(^{-1}\) (Fig. 4). Comparing the two lake systems, LZ's chitinase activity per gram dry sediment was up to 40 times higher than that measured in LB sediments but decreased with depth and converged on LB values in the 5- to 6- and 6- to 7-cm layers (Fig. 4). However, normalized to the GlcN concentrations, the chitinase activities were in the same range for both lakes, with no clear depth-related trend (data not shown).

Normalized to the TOC, TN, and GlcN contents, the chitinase activities were on the same order of magnitude as the values measured for zooplankton (data not shown).

Chitinase gene copies. (i) Water. For the 0.2- to 5-μm water fractions of LZ, specific chiA fragments could be amplified only for the 5-m and 25-m water depths sampled in spring and for the 5-m to 25-m water depths sampled in fall (Fig. 5). In comparison, the chiA gene copies detected per ml of water were 2 to 15 times higher in the surface waters of LB, with the exception of the 10-m water depth of LZ in October 2009 (272 ± 17 chiA copies ml \(^{-1}\)). The maximum chiA concentration in LB waters (340 ± 7 chiA copies...
may affect comparisons between different sample types. Therefore, chiA copy numbers normalized to the amount of DNA used in the PCR are shown in Fig. 5B and 6B. For the water column of LB, chiA gene copy numbers per ng DNA exceeded the numbers detected for the surface waters (Fig. 5B). In the sediments, chiA gene copy numbers were higher for all sediment layers of LB than for the sediments of LZ (Fig. 6B).

(iii) Zooplankton. We determined the chiA gene copy numbers on zooplankton samples, considering them a main source of chitin in our lake ecosystem and therefore a hot spot of bacterial chitin hydrolysis. The number of chiA copies detected in LZ zooplankton samples was more than 3-fold higher than in LB zooplankton samples on a dry weight basis (Table 4). Normalized to the amount of DNA used in the PCR, it was 10-fold higher.

Normalized to the GlcN concentrations, the chiA copy numbers associated with zooplankton were approximately on the same order of magnitude as the chiA copies in the sediment of LZ but up to 10-fold lower than the values detected in the sediment from LB (see Fig. S3a in the supplemental material). Compared to the results for the water columns, the chiA concentrations normalized to GlcN were 100- to 1,000-fold lower in zooplankton samples from LZ and LB, respectively (see Fig. S2a in the supplemental material).

Correlations between chitinase activity, chiA abundance, and biogeochemical parameters. (i) Water. For both sampling campaigns, the chiA copy numbers detected in the water column of LB and the GlcN concentration were highly significantly correlated ($P < 0.01; n = 10$) (Table 5).

(ii) Sediment. For both lake sediments, the chiA content and the chitinase activity correlated highly significantly ($P < 0.01; n = 7$) (Table 5). For the LB core sampled in the fall, significant correlations were found between the chiA copy number and the GlcN, TOC, and TN contents. In spring, these correlations were not significant, except for the correlation between the chiA copy number and GlcN. Chitinase activity and GlcN, TOC, and TN concentrations also correlated significantly for the fall cores from both lakes, but in LZ, the correlation was not as highly significant as in LB sediments.

**DISCUSSION**

Sources of chitin and glucosamine in freshwater lakes. Chitin was previously reported to be produced in large amounts in aquatic ecosystems (10, 47). The mean chitin standing biomass from marine planktonic crustaceans (copepods, cladocerans, and decapod larvae) was calculated to be 26.3 mg m$^{-2}$ (30), which is on the same order of magnitude as the zooplankton chitin biomass we estimated for our two different lacustrine ecosystems. Our results also agree with the results from a study on chitin dynamics in a mesotrophic bog and a hypereutrophic lake, in which the chitin biomass (from crustaceans) fluctuated between 2 and 200 mg m$^{-2}$ (42). The available data thus point to similar chitin biomasses in marine and lacustrine ecosystems, with considerable local and seasonal variability.

However, zooplankton is not the only chitin source in an aquatic ecosystem. It is known that chitin is also produced by protozoa, fungi, and algae, especially by diatoms (2, 23, 45). Diatom chitin, known as chitan, can amount to a significant constituent of the cellular biomass. For the diatom *Thalassiosira fluviatilis*, for example, chitan was found to represent 31 to 38% of the total cell mass (40). In contrast, Smucker reported only 7% and

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**FIG 6** chiA concentration per mg of dry sediment (A) and per ng extracted DNA (B) of LB and LZ. The error bars represent standard deviations of triplicate measurements of one sample.
found significant differences between various diatom species (ranging from 2 to 10% [dry weight]) (54). However, he also attributed this discrepancy to chitin losses due to the extraction method that had been used. To our knowledge, chitin content estimates for diverse algal species are not available in the literature, and a calculation of phytoplankton chitin, analogous to the one provided for zooplankton chitin, is thus currently not feasible. As a rough estimate, assuming a chitin content between 5% and 30% of dry diatom biomass and a mean dry weight of 300 pg per diatom cell (49), diatom chitin would constitute 102 to 610 mg m⁻² for LB in May 2009, 177 to 1,061 mg m⁻² for LB in September 2009, 261 to 1,568 mg m⁻² for LZ in March 2009, and 503 to 3,015 mg m⁻² for LZ in October 2009. Thus, significant amounts of chitin, exceeding the zooplankton contribution, may have been produced by this source. This may also explain our observations in LZ, where the particulate GlcN pool (0 to 100 m) was indeed found to be higher than the zooplankton chitin (Fig. 2).

GlcN is not only the main constituent of the biopolymer chitin, but together with muramic acid (MurA), it also forms the disaccharide backbone of the bacterial cell wall polymer peptidoglycan. Based on measurements of MurA concentrations, Carstens et al. determined the contribution of bacterial cells to the particulate GlcN (Carstens et al., submitted). Bacterium-derived GlcN accounted for up to 26% and 34% of total GlcN in the ephotic zones of LZ and LB, respectively. Compared to the water column of LZ the proportions of bacterial GlcN were higher in the water column of LB, with a maximum of 94% at 200 m sampled in fall 2009.

Chitinolytic activity and populations in freshwater lakes. Several studies on chitinase activity in aquatic environments have been published, mainly for marine and estuarine water and sediments, which applied a wide range of substrate concentrations, ranging from 20 nM to 5 mM (6, 27, 28, 34, 44, 53, 60). This makes the determination of substrate saturation curves for the environment under study crucial. We found an optimum substrate concentration of 50 μM for the lake sediments, whereas no chitinase activity could be measured in the water at any substrate concentration. Using a comparable approach (with 20 μM MUF-GlcNAc), Martinez et al. could not detect any chitinase activity in seawater (39). In contrast, in alkaline hypersaline Mono Lake, turnover rates for 10 μM MUF-DC in water and sediment samples were 1,000-fold higher than the chitinase activities of the sediments analyzed in the present study (37). However, Mono Lake is an environment extremely rich in chitin from shrimp exuvia and carcasses. Its lack of detection in the water samples of our lake ecosystems indicates that chitinase activity was mostly associated with particles. In aquatic environments, aggregates are known as hot spots of exoenzymatic activities (18, 53). Chitinase activity, in particular, was previously reported to be mainly associated with particulate fractions, e.g., it was found associated with the >3-μm or the 2- to 100-μm particle size class (29, 50). Similarly, we found significant activity in the water only on the zooplankton samples, i.e., the >95-μm fraction. Particle-associated microbes have the advantage of benefiting directly from the soluble oligomers produced by chitin hydrolysis. However, planktonic bacteria can also profit from chitin hydrolysis products, which has been shown recently for members of planktonic freshwater Actinobacteria (5).

By chiA-specific quantitative PCR, we could confirm the presence of chitinolytic bacteria in zooplankton and sediment and in the 0.2- to 5-μm water fractions. The chiA abundance normalized to any parameter (DNA, GlcN, or TOC) that was associated with the 0.2- to 5-μm water fraction was significantly higher than that detected in the zooplankton samples, which we had assumed to contain high concentrations of chitinolytic bacteria. Access to zooplankton chitin (α-chitin) is probably hindered by cross-linked structural components, such as glycans and proteins. These have to be degraded by different microbial communities initializing the decomposition of zooplankton carcasses (17, 56, 57). However, we did not analyze the fraction of eukaryotic DNA in the DNA extracts to normalize the results to only bacterial DNA, which could change the described ratios.

The bacterial degradation of chitin is known to be highly regulated (33). Therefore, the detection of bacterial chiA gene copy numbers indicates only the presence of bacteria capable of chitin degradation and is not a direct measure of active chitin hydrolysis, i.e., chitinase activity. However, in the present study, chiA gene abundance in sediments was highly correlated with chitinase activity, and the increased abundance of the chiA gene in the water

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlated with:</th>
<th>Pearson correlation coefficient (r²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>GlcN (nmol liter⁻¹)</td>
<td>Chitinase gene copy no. (chiA copies liter⁻¹)</td>
</tr>
<tr>
<td>Sediment</td>
<td>Chitinase activity at 4°C (nmol MUF h⁻¹ g⁻¹ dry wt)</td>
<td>Chitinase gene copy no. (chiA copies mg dry wt⁻¹)</td>
</tr>
<tr>
<td></td>
<td>GlcN (nmol g dry wt⁻¹)</td>
<td>TOC (% dry wt)</td>
</tr>
<tr>
<td></td>
<td>TOC (% dry wt)</td>
<td>GlcN (nmol g dry wt⁻¹)</td>
</tr>
<tr>
<td></td>
<td>GlcN (nmol g dry wt⁻¹)</td>
<td>Chitinase activity at 4°C (nmol MUF h⁻¹ g dry wt⁻¹)</td>
</tr>
<tr>
<td></td>
<td>TOC (% dry wt)</td>
<td>TN (% dry wt)</td>
</tr>
<tr>
<td></td>
<td>TN (% dry wt)</td>
<td>0.84b</td>
</tr>
</tbody>
</table>

a P < 0.05.
b P < 0.01.
column of LB, where chitin made a greater contribution to the carbon pool, also indicates a relationship between gene abundance and chitin degradation. The higher prominence of chitin as a bacterial substrate in oligotrophic compared to eutrophic lakes has also been shown in culture-dependent analyses of chitinolytic bacteria in Polish lakes (13, 14).

In conclusion, significant correlations between chiA gene abundance, chitinase activity, and biogeochemical data evidenced the contribution of chitin to the carbon and nitrogen budget in the lake sediments, in particular for the oligotrophic system of LB (Table 5). We therefore assign chitin a role as a significant microbial growth substrate in temperate freshwater lakes, especially where other easily degradable carbon sources are scarce.

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acetylglucosamine) fibers of the diatom *Thalassiosira fluviatilis* Hustedt.


Appendix VI

Distribution of branched and isoprenoid tetraether lipids in an oligotrophic and a eutrophic Swiss lake: Insights into sources and GDGT-based proxies

Authors
Bechtel, A., Smittenberg, R.H., Bernasconi, S., and Schubert, C.J.

Full publication: Organic Geochemistry, Vol. 41, 822-832.
Distribution of branched and isoprenoid tetraether lipids in an oligotrophic and a eutrophic Swiss lake: Insights into sources and GDGT-based proxies

Achim Bechtel a,*, Rienk H. Smittenberg b, Stefano M. Bernasconi b, Carsten J. Schubert a

a EAWAG, Department of Surface Waters, Seestrasse 79, CH-6047 Kastenienbaum, Switzerland
b ETH Zürich, Geological Institute, Sonneggstrasse 5, CH-8092 Zürich, Switzerland

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A B S T R A C T

Distributions of isoprenoid (isoGDGT) and branched glycerol dialkyl glycerol tetraethers (brGDGTs) were measured in the water column and sediments of the eutrophic Lake Lugano and the oligotrophic Lake Brienz, Switzerland. Absolute concentrations of isoprenoid, i.e. archaeal GDGTs, were highest in the euphotic zone of both lakes, as well as in sediments deposited at times when lake eutrophication occurred. This indicates that GDGT concentrations may be used as indicators for primary productivity. Both lakes, including the anoxic bottom water of Lake Lugano, are characterised by GDGT distributions typical for group I Crenarchaeota with GDGT-0/crenarchaeol ratios of around 1. Comparison of the distribution of brGDGTs with isoGDGTs and other terrestrial biomarkers throughout the Lake Lugano water column, together with CBT/MBT-derived temperatures that resemble that of the lake, suggest significant in situ production. BIT index values for Lake Brienz sediments (ca. 0.4) were significantly higher than water column values (ca. 0.1), most probably because terrestrial run off events were not captured during water sampling. TEX 86-derived temperatures reflect surface water conditions to within a few degrees, while lower values obtained from deeper water layers suggest a contribution of in situ produced isoGDGTs. For both lake sediments, TEX 86-derived temperatures could be matched reasonably with mean annual lake surface water temperature variation, albeit with a larger offset for Lake Lugano. This suggests that absolute temperatures can only be reconstructed from lake sediments for which a local calibration is known.

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1. Introduction

Over recent years, a number of new proxies based on glycerol dialkyl glycerol tetraethers (GDGTs; Appendix, structures 1–V) have been proposed. Isoprenoid GDGTs (isoGDGTs) with sn-2,3 stereochemistry are produced by at least two of three main groups of Archaea, the Crenarchaeota and the Euryarchaeota (De Rosa and Gambacorta, 1988; Koga and Morii, 2006), that occur ubiquitously, ranging from open oceans (e.g. Karner et al., 2001) and lakes (e.g. Auguet and Casamayor, 2008), to soils (Leininger et al., 2006). Crenarchaeota are widespread in many lacustrine waters (Urbach et al., 2001; Keough et al., 2003) and freshwater sediments (MacGregor et al., 1997; Schleper et al., 1997). The depth distribution of aquatic Crenarchaeota in lacustrine water columns is highly variable and in many cases they are numerically dominant at greater depth (Urbach et al., 2001; Keough et al., 2003). Crenarchaeol is suggested to be a specific GDGT for group I Crenarchaeota (Sinninghe Damsté et al., 2002). Recent studies argue for a seasonal aspect to crenarchaeotal productivity as well as a predominant surface water source for it (Blaga et al., 2009).

A palaeotemperature proxy, TEX 86, based on the distribution of isoprenoid GDGTs differing in the extent of cyclic moieties, has been calibrated with a global set of core-top sediments from the world’s oceans (Schouten et al., 2002, 2007; Wuchter et al., 2005; Kim et al., 2008). The proxy is increasingly being used for marine systems. (Powers et al., 2004, 2005; Tierney et al., 2007; Blaga et al., 2009). The most recent calibration, provided by Powers et al. (2010), is based on lake sediments from globally distributed lakes. All studies indicate that TEX 86 should be applied only for lakes with sufficient production of GDGTs by aquatic Crenarchaeota relative to isoprenoid GDGTs derived from soil in the watershed or other sources like methanotrophs.

Another type of GDGTs is that with sn-1,2 stereochemistry and basic n-alkyl chain architecture, with varying numbers of methyl branches and cyclopentane moieties (Appendix, structures VI–VIII). These so-called branched GDGTs (brGDGTs) have been attributed to soil bacteria (Sinninghe Damsté et al., 2000; Hopmans
et al., 2004; Herfort et al., 2006), but their exact biological source is unknown. Weijers et al. (2007) demonstrated that the relative extent of cyclopentane moieties, expressed in the cyclisation ratio of branched tetraethers (CBT), is related to the pH of the soil, while the relative extent of methyl branches (methylation index of branched tetraethers – MBT) is positively correlated with the mean annual air temperature (MAT) and to some extent to soil pH. The relative extent of the branched vs. isoprenoid tetraethers, expressed as the BIT index (Hopmans et al., 2004), has been used as a proxy for soil organic matter (OM) (Hopmans et al., 2004; Weijers et al., 2007), although low BIT index values have been observed in sediments with a high terrigenous load (Huguet et al., 2007; Walsh et al., 2008).

Although to some extent successful, the GDGT proxies suffer from a bias in some situations. For instance, TEX$_86$ – derived temperatures can be affected by soil-derived isoGDGTs (Weijers et al., 2006; Blaga et al., 2009). The relative abundance of Euryarcheota vs. Crenarcheota or different crenarcheotal populations that have different GDGTs patterns could also influence TEX$_86$ values (Turich et al., 2007; Trommer et al., 2009; Blaga et al., 2009), or the extent to which sedimentary GDGTs reflect surface water conditions compared to deeper water layers (Menzel et al., 2006; Huguet et al., 2007). The CBT/MBT proxy may be influenced by in situ production of the brGDGTs in lakes and marine sediments (Sinninghe Damsté et al., 2009; Tierney and Russel, 2009; Peterse et al., 2009).

The aim of the present study was to assess the distribution of isoprenoid and brGDGTs in two Swiss lakes and their sediments, and their potential use as a palaeoenvironmental and palaeoclimatic proxy. The lakes were chosen because of their different trophic levels and evolution over the last 80–100 years in order to identify sources of archaeal lipids and to compare factors that affect their distribution in the water columns and the sediments. The results are discussed in relation to physical and chemical characteristics of the water columns, taking into account the results of previous studies on samples obtained during spring and autumn sampling campaigns (Bechtel and Schubert, 2009a).

2. Materials and methods

2.1. Sites and sample collection

Lake Brienz is an oligotrophic, deep perialpine lake (Fig. 1A) with surface area 29.8 km$^2$ and maximum depth 260 m. While the Lütschine, one of the two major contributing rivers, drains a partly calcareous catchment of an unaltered hydrological regime, the Aare flows predominantly from a crystalline catchment whose flow is heavily altered by several reservoirs built for hydropower generation (Sturm, 1976; Finger et al., 2007). The predominant organisms are chrysophytes (golden algae), diatoms, cryptophytes, dinoflagellates, chlorophytes (green algae) and cyanobacteria (up to 70% of total phytoplankton); cladochlors and calanoid copepods account for 75% of the zooplanktonic biomass (GBL, 2007). Primary productivity in the euphotic zone is highest during late spring (May) and is reduced during summer (June–September) due to enhanced light attenuation caused by high particle load (Finger et al., 2007). During the 1970s until 1986, mesotrophic conditions occurred as a result of anthropogenic influence. Improved waste water treatment have restored the oligotrophic conditions since then (Finger, 2006). There is no information regarding archaeal ecology.

Lake Lugano is a deep subalpine lake at the border of Switzerland and Italy (Fig. 1C). The sampling site was chosen within the northern basin, which has a volume of 4.69 km$^3$ and a maximum depth of 288 m. Because of its morphology, with steep slopes, and geographic position protected against strong winds, permanent anoxic conditions occur below 70 m (Barbieri and Polli, 1992). Eutrophication, which started in the second part of the last century, led to significantly increased primary productivity, with the highest values (>400 g C m$^{-2}$ yr$^{-1}$) in the 1980s. At present, productivity has decreased to ca. 300 g C m$^{-2}$ yr$^{-1}$ (Barbieri and Simona, 2001). Chlorophytes (green algae), cyanobacteria and diatoms, as well as cyclopooids and cladocerans, predominate in the phytoplanktonic and zooplanktonic biomass, respectively (Barbieri and Simona, 2001; Bechtel and Schubert, 2009a). The productivity is characterised by blooms of diatoms during spring and blooms of green algae and cyanobacteria during summer. High primary productivity during the June sampling campaign and significantly lower autochthonous OM production in autumn are indicated by chlorophyll contents of 6.6 mg/m$^3$ and 3.0 mg/m$^3$, respectively, in the photic zone. There is also no information on the archaeal ecology.

Depth profiles of temperature, oxygen content and turbidity were measured with a CTD profiler (Seabird, SBE-19). Water and particulate OM (POM) samples were collected in June 2007 (spring samples) and during October/November 2007 (autumn samples). For Brienz, sampling was carried out in the central part of the basin (Fig. 1B) at a 247 m station [position 641150/174850 (Swiss Grid)] SW of the village of Oberried. The site was selected on a side plain, where sediment deposition was found to be undisturbed (Anselmetti et al., 2007). Lugano samples were obtained from a 266 m station [position 719490/094630 (Swiss Grid)] south of the village of Castagnola (Fig. 1C). Particulate material was collected by in situ filtration of lake water (20–160 l) at 10, 40, 70, 100, 150 and respectively, at 200 (Brienz) and 250 m (Lugano) depth, during spring, and at 10, 40, 70, 100, 150, and 200 m during the autumn, using sampling campaigns using McLane Research filtration systems (WTS-142) and GFF filters with a nominal size of 0.7 µm, used in a double layer to reduce nominal size. The cores from both lakes were collected at the same positions as the POM samples with a gravity corer during June 2007 (Brienz) and March 2008 (Lugano) at water depths of 247 and 266 m, respectively. The cores (65 and 57 cm, respectively) were sliced in 2 cm intervals and frozen at −20 °C.

2.2. Lipid extraction and GDGT separation

Aliquots (50% of GFF filters) of the particulate material collected from different water depth were Soxhlet extracted with dichloromethane (DCM)/MeOH (9:1) mixture. Aliquots (2–6 g) of selected samples from the cores were extracted at 70 °C for 7 min with DCM/MeOH (9:1) mixture using a high performance microwave digestion unit (mls 1200 mega; Milestone). Half of the total extract from each sample was evaporated using a Zymark TurboVap II concentrator and redissolved in 2 ml hexane:DCM (9:1). These aliquots were further separated into several fractions with activated Al$_2$O$_3$ (Supelclean LC-Aluminia-6 N ml SPE Tubes). Hexane:DCM (9:1 v/v, 4 column volumes) eluted the apolar fraction. Polar fractions containing the GDGTs were eluted with DCM/MeOH (1:1 v/v, 3 column volumes). After solvent evaporation the polar fractions were redissolved in 400 µl HPLC-grade hexane/isopropanol (99:1 v/v) and were filtered through a 0.45 µm PTFE filter prior to analysis. Prior to GDGT analysis, 5 ng of a C$_{46}$ GDGT standard was added to each sample for quantification (cf. Huguet et al., 2006).

2.3. GDGT analysis

GDGT analysis was performed at the Geological Institute of the ETH Zürich using high performance liquid chromatography/atmospheric pressure chemical ionisation–mass spectrometry (HPLC/APCI–MS) with a Thermo Surveyor LC system coupled to an LCQ Fleet ion trap mass spectrometer equipped with a PAL LC
2.4. TEX$_{86}$, BIT, and CBT/MBT

BIT indices were calculated following the equation of Hopmans et al. (2004):

\[
\text{BIT} = \frac{[VI] + [VII] + [VIII]}{[VI] + [VII] + [VIII] + [V]} \tag{1}
\]

TEX$_{86}$ was calculated as follows (Schouten et al., 2002):

\[
\text{TEX}_{86} = \frac{[III] + [IV] + [V]}{[II] + [III] + [IV] + [V]} \tag{2}
\]

Temperature reconstructions were based on the linear relationship between mean annual lake surface temperature (LST) and TEX$_{86}$ values based on sediment top data from globally distributed lakes (Powers et al., 2010):

\[
\text{LST} = -14.0 + 55.2 \times \text{TEX}_{86} \tag{3}
\]

The MBT and CBT indices were calculated according to Weijers et al. (2007):

\[
\text{MBT} = \frac{[VI] + [Vlb] + [Vlc]}{[VI] + [VII] + [Vlb] + [Vlc] + [VIIb] + [VIIc] + [VIII]} \tag{4}
\]

\[
\text{CBT} = -\log_2 \frac{[Vlb] + [Vlb]}{[VI] + [VII]} \tag{5}
\]

MBT and CBT values were used to infer mean annual air temperature (MAT) and pH values of soil using the following equations of Weijers et al. (2007):

\[
\text{MBT} = 0.122 + 0.187 \times \text{CBT} + 0.020 \times \text{MAT} \tag{6}
\]

\[
\text{CBT} = 3.33 - 0.38 \times \text{pH} \tag{7}
\]

Uncertainty in GDGT concentration, caused by uncertainty in especially integration of smaller peak areas, resulted in relative errors in the range of 5% of the calculated proxy data (BIT, TEX$_{86}$, CBT, MBT). The uncertainties are in the range of the symbol sizes used in the respective diagrams. The resulting uncertainties in temperature estimates are indicated by error bars. The uncertainty in pH reconstructed from Eq. (7) is in the range of 1 pH unit (Weijers et al., 2007).

3. Results

3.1. Physical and chemical characterisation of water columns and sediments

The physical and chemical characteristics of the water columns of both lakes during spring and fall were discussed previously...
(Bechtel and Schubert, 2009a); only the most important results are outlined below. The Brienz water column is characterised by a nearly homogenous oxygen concentration of >300 \( \mu \text{mol/l} \), a thermocline between 5 and 10 m during spring and a minor temperature decrease at 35 m during fall (Fig. 2). TOC, alkalinity and major anion concentration are low (Bechtel and Schubert, 2009a), consistent with the oligotrophic conditions and high oxygen contents. Lugano is characterised by anoxic conditions (oxygen content <40 \( \mu \text{mol/l} \) during spring and <20 \( \mu \text{mol/l} \) during autumn) below 70 m (Fig. 2). A pronounced thermocline was located at 15 m in June 2007, whereas a reduced temperature decline occurred at 20 m in the fall (Fig. 2). Bulk geochemical parameters indicate high primary productivity (Bechtel and Schubert, 2009a).

3.2. Age model for cores and composition of sediments

The reported sediment ages (Figs. 3 and 4) are based on unsupported \(^{210}\text{Pb} \) activity and the age assignment of the two \(^{137}\text{Cs} \) peaks.
3.3. GDGT distributions

Both the isoGDGTs (I–V) and the brGDGTs (VI–VIII) were detected in all POM samples, dominated by GDGT-0 (I) and crenarchaeol (V). Total GDGT concentration in Brienz POM was low, with the highest values (Table 1) in the surface water, both in spring (ca. 1 ng/l) and fall (ca. 0.8 ng/l), decreasing rapidly with depth (Fig. 2). The greatest differences between spring and fall total GDGT concentration occurred at the surface. All deeper POM samples contained similar amounts of GDGTs, between 0.2 and 0.4 ng/l, with a slightly lower content in the spring samples. In fall, the surface water layers – defined by thermocline depth – extended to a deeper depth, resulting in higher GDGT content at 40 m during fall (Table 2). The absolute concentration of brGDGTs was higher in the spring surface water of Brienz, most probably reflecting high particle load (e.g. soil; Finger et al., 2007). A comparable GDGT pattern could be seen in the Lugano water column (Table 2, Fig. 2) but with values ca. 1.5 times higher than those for Brienz. Here, however, the brGDGTs did not decline with depth, like the isoGDGTs. At the chemocline (70–100 m) of Lugano the GDGT concentrations increased a little in springtime.

Brienz and Lugano sediments also contained the GDGTs I–V (Appendix, Fig. A1) and branched GDGTs (VI–VIII), including compounds with 1 or 2 cyclopentane moieties (VIb, c; VIIb, c; VIIIb, c). GDGT-0 (I) and crenarchaeol (V) were the dominant GDGTs in most samples, except in the upper part of the Brienz core, where branched GDGTs dominated. TOC-normalised GDGT concentrations (Tables 3 and 4) were higher in the Lugano sediments than in the Brienz sediments. Total archaeal lipid concentration in Brienz sediments (Fig. 3) showed a general decrease with depth from 2100 to 400 ng/g TOC, with highest values in the 14–16 cm and the 24–26 cm intervals (Fig. 3). In the Lugano sediments, highest GDGT concentrations were found close to the top of the core (4–20 cm), corresponding to the interval of maximum eutrophication, and in the lowermost sample (Fig. 4). Crenarchaeol was the dominant GDGT in all Lugano sediment samples (Table 4, Fig. 4).

3.4. TEX_{86}, BIT and CBT/MBT proxies

The BIT index of the Brienz POM samples was generally low (<0.13), with minor variation with depth during spring and fall (Fig. 5, Table 1). However, the values for the upper sediments were significantly higher (ca. 0.50, Table 3; Fig. 3). The average value for the Lugano POM samples (0.28; Table 2; Fig 5) was similar to that for Lugano sediments (0.25 ± 0.07, Table 4; Fig. 4). However, in the lake the value differed significantly between the upper oxic (ca. 0.15) and lower anoxic (ca. 0.40) part (Table 2; Fig. 5) of the water column.

**Fig. 3.** GDGT concentration profiles and evolution of trophic level (based on total P contents; Finger, 2006) within Lake Brienz (A), variation in environmental proxies from GDGT distribution (TEX_{86}, BIT, CBT, MBT) within the water column (B) and (C) the comparison of TEX_{86}-based mean LSTs (according to Powers et al., 2010) within the cores (black squares) and mean annual LST for Lake Brienz (GBL, 2009; black diamonds), as well as estimated mean annual LST variation (grey filled circles) based on regional air temperature – LST correlations. In (D) CBT/MBT-based MAT estimates (Weijers et al., 2007) are compared with the 3 year moving average from mean annual air temperature record, measured at the Bern meteorological station (Meteoswiss). Relative errors in proxy data are within the range of the symbol sizes. Uncertainties in reconstructed temperatures are indicated by error bars.

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The variations in TEX$_{86}$ and the reconstructed temperatures (Eq. (3)), within the water columns of both lakes are shown in Fig. 5. Variations in the TEX$_{86}$-derived temperatures from the upper parts of the cores were compared with records of measured lake surface temperature (LST) for the upper 10 m of both lakes (Figs. 3 and 4) (GBL, 2009; CIPAIS, 1986-2007).

Based on the CBT index, a slightly higher average pH of 7.4 (±0.4) was obtained for the Lugano sediments (Table 4) vs. the Brienz sediments (pH = 6.8 ± 0.3; Table 3). The application of the CBT/MBT proxy to the Brienz sediments resulted in highly fluctuating, but comparable, temperatures to measured air temperatures (data from MeteoSwiss, <http://www.meteoswiss.admin.ch/web/de/...>

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth (m)</th>
<th>Temp (°C)</th>
<th>Total GDGTs (ng/l)</th>
<th>GDGT-0 (ng/l)</th>
<th>Crenarchaeol (+isomer) (ng/l)</th>
<th>GDGTs 1-3 (ng/l)</th>
<th>Branched GDGTs (ng/l)</th>
<th>GDGT-0/crenarchaeol</th>
<th>BIT$^a$</th>
<th>TEX$_{86}$$^b$</th>
<th>TEX$_{86}$-derived temp.$^c$ (°C)</th>
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<tr>
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<tr>
<td>FLB 200</td>
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<td>0.37</td>
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</table>

$^a$ According to Hopmans et al. (2004).

$^b$ According to Schouten et al. (2002).

$^c$ According to Powers et al. (2010).
As revealed from records of total phosphorus content and bioproductivity within the lakes (Barbieri and Simona, 2001; Finger, 2006). The observed correlations between GDGT concentration and bioproductivity in the sedimentary records may be related to enhanced ammonia concentration in the lower parts of the water columns, as species of the group I Crenarchaeota are known to be ammonia oxidizers (Könneke et al., 2005; Leininger et al., 2006; Wuchter et al., 2006).

A high relative amount of GDGT-0 vs. crenarchaeol may indicate a contribution from methanogenic Euryarchaeota (Blaga et al., 2009; Sinninghe Damsté et al., 2009). GDGT-0/crenarchaeol values in the Brienz water column range from 1.0 to 1.6 (Table 1), so the lake appears to contain an archaeal community dominated by group I Crenarchaeota (Wucher et al., 2005). The values fall within the range observed for marine samples (Sinninghe Damsté et al., 2009) and half of the European lakes investigated by Blaga et al. (2009). The same is also true for Lake Lugano, with GDGT-0/crenarchaeol values of ca. 1 (Table 2). Similar values throughout the water column indicate that methanogenic Euryarchaeota are not very prevalent in Lugano (Blaga et al., 2009) during the periods of POM sampling. Compared to the water column, GDGT-0 occurs in lower abundance relative to crenarchaeol in the Lugano sediments (GDGT-0/crenarchaeol ratio of 0.48 ± 0.20 in the sediments and 1.04 ± 0.12 in the upper water column, as species of the group I Crenarchaeota are known to be ammonia oxidizers (Könneke et al., 2005; Leininger et al., 2006; Wuchter et al., 2006).

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in the water column). One viable explanation is that the ‘snapshot’ POM samples do not reflect the annual budget of the various GDGTs. This suggests that thearchaeal community changes through the year, producing varying amounts of the different GDGTs, depending on, for instance, nutrient availability, or other ecological drivers.

The BIT index values increase significantly from ca. 0.3 in the lower Brienz sediments to ca. 0.5 in the upper part (Table 3; Fig. 3), which is higher than the POM BIT values (0.13). It is most likely that the brGDGTs are supplied together with soil-derived OM, which occurs particularly during spring melt and other high flow (erosion) events. The ‘snapshot’ Brienz POM samples did not capture such an event, so displayed lower BIT values than the average sediment. Lateral transport of sediment with a relatively higher terrestrial signature (Sturm and Matter (1978)), including brGDGTs, may also have increased sedimentary BIT values relative to the water column (cf. Bechtel and Schubert, 2009a).

The BIT index differed significantly between the upper oxic (ca. 0.15) and lower anoxic (ca. 0.40) part of the Lugano water column (Table 2). This is primarily caused by a decreasing concentration of crenarchaeol downward, while the brGDGT concentration remained virtually constant (Fig. 2). Possibly, the two types of GDGTs are present in different types of POM, where isoGDGTs are incorporated in larger fast sinking particles, e.g. faecal pellets, while brGDGTs sit in finer grained material that settles very slowly. Recent evidence suggests, however, that brGDGTs can also be produced in sediments or the water column, and not only in soil (Peters et al., 2009; Sinninghe Damsté et al., 2005; Tierney and Russell, 2009). Indeed, the concentrations of long chain fatty acid and n-alkanol distributions for the same samples (Bechtel and Schubert, 2009a) do not show any evidence for relatively greater terrigenous OM content in the anoxic bottom water of Lugano. Furthermore, the CBT/MBT-derived temperature, based on the brGDGTs, does not match Lugano air temperatures but resembles that of the lake deep water quite well, as discussed below. These lines of evidence suggest that the virtually unchanging depth profile of brGDGTs can be best explained by a relatively large fraction of brGDGTs produced in situ in the water column.

4.2. TEX<sub>86</sub> and CBT/MBT proxies

4.2.1. Water column

The highest TEX<sub>86</sub>-based temperature values (Powers et al., 2010) were obtained from the surface water samples (10 m) of both lakes during spring and fall and were 0.0–1.2°C lower than the measured surface water temperature (Tables 1 and 2, Fig. 5). The TEX<sub>86</sub> temperatures are in excellent agreement with the observed LSTs, taking into account the calibration error of 3.6°C (Powers et al., 2010). TEX<sub>86</sub>-derived temperatures from POM samples at greater depth are relatively constant throughout the water column of both lakes during spring and fall (Table 4). TEX<sub>86</sub>-based temperatures in the deeper parts of the water columns were slightly higher than the in situ temperatures in Brienz (Fig. 5a) and much higher in Lugano (Fig. 5b). This temperature offset is consistent with findings for marine mesopelagic waters (Wuchter et al., 2005; Igalls et al., 2006). The temperatures warmer than in situ temperatures and colder than surface temperatures likely reflect a mixing via sinking of surface water lipids, that record the lake surface temperature, and GDGTs produced in situ. Furthermore, deep water isoGDGTs are usually older than surface water GDGTs and may reflect different temperature regimes, important during periods of rapidly changing temperature such as spring and fall. This might explain the lower temperatures in the spring, with GDGTs produced in winter, but for autumn higher temperatures would be expected, with GDGTs produced in summer. IsoGDGTs derived from other sources may also influence the TEX<sub>86</sub> values from the deeper parts of Lake Lugano, as indicated by the high BIT index (Table 4).

4.2.2. Sediments

In order to compare the TEX<sub>86</sub>-derived temperatures with lake surface temperatures deeper downcore, mean annual LSTs were estimated from mean annual air temperatures (MATs) of Bern...
and Lugano towns, respectively (data from MeteoSwiss, <http://www.meteoswiss.admin.ch/web/de/wetter.html>). This was done by first correlating the measured LSTs (upper 10 m) of the last 15 years in both lakes (GBL, 2009; CIPAIS, 1986–2007; Figs. 3 and 4; see Fig. 1 for sampling sites) with air temperatures (Brienz LST = 0.46 + MAT + 5.29, R² 0.71; Lugano LST = 0.84 + MAT + 2.77, R² 0.49) and then using the correlation further back in time. The climates of the towns Brienz and Bern are very comparable, but for Bern is a longer record available. Below 30 cm sediment depth, i.e. before ca. 1965, the BIT index values in Lake Brienz ranged around 0.3, and for this part of the core TEX86-based temperatures (Powers et al., 2010) are in good agreement with the measured and estimated mean annual LST (Fig. 3). In the upper part of the Brienz core, BIT values exceed 0.35 and a significant offset is observed between TEX86 temperatures and LST. It can be assumed that the reconstructed temperatures are biased through the input of soil-derived isoGDGTs, consistent with recent observations that BIT indices >0.4 will affect TEX86 temperature reconstructions for lakes (Blaga et al., 2009).

The TEX86-derived temperatures for Lake Lugano are on average 3–4 °C lower than the estimated annual LST variation (Fig. 4). Considering that most of the mismatches are equal to or less than 3.6 °C, the values still fall within the calibration error. Furthermore, both the TEX86 temperatures and the estimated LSTs show a general increase over the time interval covered by the core. The offset may partly result from Weak LST estimates based on the LST – air temperature correlation at Lake Lugano (R² 0.49). A better match could be obtained by considering only lake surface temperature data during spring (April–June), because these are on average 3 °C lower than mean annual LST.

The CBT index values for Brienz sediments translate to a pH of 6.8 (Table 3), while this alkaline lake has a pH around 8.5. This argues for primarily soil-derived brGDGTs in Lake Brienz. Indeed, most terrestrial material derives from the river Aare, which drains a crystalline, i.e. non-carbonate, catchment area (Sturm, 1976). CBT-derived pH values for Lake Lugano were 7.4 (Table 4). This is similar to the measured average pH of 7.7 in water column, taking into account the overall error in the range of 1 pH unit (Weijers et al., 2007). However, the surroundings of the lake also contain a significant amount of carbonate rocks and alkaline soils, and no conclusion can be drawn concerning a terrestrial or aquatic origin for brGDGTs, based on CBT data alone.

The CBT/MBT-reconstructed air temperatures from Lake Brienz sediments fluctuate significantly but compare reasonably with measured air temperatures at Bern (Fig. 3). In contrast, temperatures which are much too low (3–6 °C) were obtained for Lake Lugano that has a MAT of around 12 °C (Fig. 4). This mismatch suggests that at least a proportion of the branched GDGTs is derived from in situ production. The temperatures below the thermocline are around 5.5 °C (Table 2, Fig. 2), matching the CBT/MBT-derived values within error.

5. Conclusions

Based on isoGDGTs concentration, the surface water archaeal community at Lake Lugano above the chemocline appears similar to that of Lake Brienz. Comparison of the present and past trophic levels of both lakes with POM and sedimentary isoGDGT concentrations suggest that GDGT concentrations in lacustrine sediments might be used to detect periods of eutrophication.

The TEX86 index values record the lake surface temperatures (LST) reasonably when applying the Powers et al. (2010) calibration. TEX86-derived temperatures in the deeper parts of the water column of both lakes suggest mixing of exported and in situ produced GDGTs or the presence of older isoprenoid GDGTs, reflecting different temperature regimes. In Lake Brienz, reconstructed mean LSTs matched the instrumental record, until soil-derived OM input
increased in the latter half of the 20th century, evidenced by way of increased BIT indices. In Lake Lugano, the TEX\textsubscript{86}\textsuperscript{+}-based temperatures exhibit a −4 °C offset. This is at the edge of the calibration error, but can also be interpreted as a springtime temperature record, or an annual record that is influenced by GDGT production in deeper and colder water.

In Lake Brienz, the brGDGTs appear to be genuinely soil derived, but, based on their distribution through the water column and the calculated CBT/MBT proxy, the brGDGTs in Lake Lugano appear to be at least partially produced in situ.

Overall, the data appear to validate the new TEX\textsubscript{86}\textsuperscript{+} calibration for lacustrine sediments, but also show that this can only be carried out after careful examination of the sources of archaeal GDGTs. This is even more true for the sources (i.e. soil vs. lacustrine) of the brGDGTs and the use of the MBT and CBT proxies.

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Appendix

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References


Fig. A1. Isoprenoid and branched GDGT membrane lipids.
Appendix VII

A biogeochemical study of sediments from the eutrophic Lake Lugano and the oligotrophic Lake Brienz, Switzerland

Authors
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A biogeochemical study of sediments from the eutrophic Lake Lugano and the oligotrophic Lake Brienz, Switzerland

Achim Bechtel *, Carsten J. Schubert

EAWAG, Department of Surface Waters, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland

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ABSTRACT

The biomarker composition and stable isotope (C, O) ratio values of organic matter (OM) and carbonate from sediment cores of the oligotrophic Lake Brienz and the eutrophic Lake Lugano (both in Switzerland) are compared, in order to obtain information about OM sources and transformation processes. Eutrophic conditions at Lake Lugano are reflected in elevated total organic carbon (TOC) content and hydrogen index (HI) values, as well as higher lipid concentrations. Parallel down core trends in δ13C values of TOC and calcite in the Lake Lugano sediments reflect bioproductivity cycles. Variations in δ18O values of calcite are consistent with changes in mean summer temperature over the time interval covered by the core. In contrast, such a correlation does not exist for Lake Brienz and there the stable isotope composition of calcite reflects its allochthonous origin. In the sediments of both lakes, fatty acid (FA) distributions and the composition of n-alkanols and n-alkanes indicate highly variable proportions of autochthonous OM sources (algae, zooplankton, bacteria) and OM from land plants. Enhanced in situ microbial synthesis during sediment deposition in Lake Lugano is suggested by the higher TOC-normalised concentrations of branched chain FAs (C15–C17), hopanoic acids and triterpenoid alcohols (i.e. tetrahymanol, diplopterol). Variations in the concentrations of cholesterol are related to contributions from zooplankton and/or green algae, while sitosterol concentrations reflect the input of vascular plants. Periods of increased input of OM from diatoms are evidenced by high 24-methylcholesta-5,22-dien-3-β-ol (either epibrassicasterol or brassicasterol) and/or highly branched isoprenoid (HBI) alkenes concentrations. High relative concentrations of diplopterol in Lake Lugano sediments are consistent with the predominance of cyanobacteria commonly observed in eutrophic lakes. The presence of archeol and hydroxyarcheol in very low concentrations in the Lugano sediments argues for the activity of methanogens and/or anaerobic methanotrophs.

Differences in OM degradation processes are reflected in higher chlorin index values in the Brienz sediments but higher saturated vs. unsaturated n-FAs in the core from Lugano. Higher concentrations of branched chain FAs and 16:1ω7 n-FA, as well as enhanced 18:1ω7/18:1ω9 n-FA, are consistent with enhanced bacterial biomass in the Lugano water column or sediments. The preservation of phytoelm seems to be enhanced in sediments with a high relative contribution of land plant OM. Major factors affecting OM accumulation in the lakes are differences in OM sources (i.e. terrestrial OM vs. autochthonous production), extent of bacterial activity and most likely oxygen availability in the water column.

1. Introduction

Lake sediments are important archives of past environmental change by way of containing a diverse range of lipids derived from organisms in the lake and from allochthonous material derived from the catchment areas (Berglund, 1986). Therefore, changes in lipid composition reflect changes in lake and catchment biota related to changes in local and regional environment (Meyers, 2003; Müller et al., 2005; Muri and Wakeham, 2006; Pearson et al., 2007).

The use of lipids as palaeoenvironmental indicators requires knowledge of their biological precursors. The distribution of amino acids, lipids and sugars in the environment is related to sources and transformation processes of OM (Amon et al., 2001). Extensive research on the composition of lipids as biomarkers specific for single organisms, a group of organisms, plant species and/or biogeochemical processes has been performed with respect to the water column and underlying sediments (Hedges and Keil, 1995; Niggemann and Schubert, 2006; Wakeham et al., 2007). Lipid geochemistry of sediment cores provides information on biological activity fuelled by oxygenic and anoxygenic photosynthesis,
chemoautotrophy and heterotrophy (Wakeham and Beier, 1991; Wakeham et al., 2007). The presence of biological molecules can provide further details on certain biotic sources within a lake and its catchment, as well as about the preservation of OM (Schubert et al., 2006; Pearson et al., 2007).

Although lacustrine systems account for only a small percentage of the water body on Earth, their role in the global carbon cycle appears to be relevant since they accumulate carbon more efficiently than oceans (Müller et al., 2005; Cole et al., 2007). Lakes participate in biogeochemical cycling and act as carbon sinks or sources in response to environmental parameters. Environmental parameters such as OM sources, oxygen exposure time, anoxia and sedimentation rates, have been identified as important factors influencing OM accumulation (Hedges and Keil, 1995).

The aim of this paper was to identify sources of OM and to compare major factors that affect OM accumulation in the sediments of the oligotrophic Lake Brienz and the eutrophic Lake Lugano (both in Switzerland). Bulk organic geochemical parameters, carbon isotopic composition of TOC and \(d^{13}C\) and \(d^{18}O\) values of calcite were measured on samples collected from cores. The study focusses on the use/usefulness of FAs, aliphatic alcohols, sterols and aliphatic hydrocarbons in distinguishing different OM sources. Chlorins are used as a proxy for the “freshness” of OM (Schubert et al., 2005).

2. Materials and methods

2.1. Study sites and sample collection

Lake Brienz is an ultra-oligotrophic, perialpine lake with a maximum depth of 260 m (Fig. 1A). Information about its hydrological conditions, catchment and predominant planktonic communities are outlined by Bechtel and Schubert (2009). Mesotrophic conditions were established between 1960 and 1986 as a result of anthropogenic influence. Decreasing phosphorus concentrations in the lake water through the installation of waste water treatment plants resulted in the present ultra-oligotrophic conditions (Müller et al., 2007). The coring site was selected on a side plain, where sediment deposition was found to be undisturbed (Anselmetti et al., 2007), 46°44’N, 8°00’E SW of the village of Oberried (Fig. 1B). The core was collected with a gravity corer in June 2007 at a water depth of 247 m. The core (65 cm long) was sliced in 2 cm intervals and frozen at –20 °C.

Lake Lugano is a deep subalpine lake located on the border with Italy (Fig. 1C). The sampling site was chosen within the northern basin, which has a maximum depth of 288 m. Eutrophication, which started in the second part of the last century, led to a significantly increased primary productivity, with the highest values (>400 g C m\(^{-2}\) year\(^{-1}\)) in the 1980s. Nowadays, it has decreased to about 300 g C m\(^{-2}\) year\(^{-1}\). After a 40 year period of permanent anoxic conditions below 70 m depth (Barbieri and Polli, 1992) the lake became mixed during very cold and windy winters in between 2004 and 2006 (Holzner et al., 2009). However, anoxic conditions in the hypolimnion were re-established from 2006 on. A summary of the present predominant planktonic communities is provided by Bechtel and Schubert (2009). The core was collected with a gravity corer in March 2008 in the northern basin (Fig. 1C) at a 266 m deep station (46°00’N, 9°00’E), south of the village of Castagnola. The core (57 cm long) was sliced in 2 cm intervals and frozen at –20 °C.

2.2. Age control

For supported \(^{210}\)Pb and \(^{137}\)Cs activities, 1–2 g homogenised dry samples were analysed with a Ge gamma spectrometer (Canberra) using the line 46.5 keV for \(^{210}\)Pb and 661.7 keV for \(^{137}\)Cs. The precision of the method was in the range of 10–15% (relative error).
Unsupported $^{210}$Pb activities were calculated by subtracting the lowest measured activity from the supported activities.

### 2.3. Bulk organic parameters

Total carbon (C) and total nitrogen (N) concentrations of homogenised samples were measured with a CNS elemental analyser (Hekatech). The reproducibility was in the range 0.1–0.2 wt.% for C and better than 0.03 wt.% for N. Total inorganic carbon (TIC) concentrations were measured with a CO$_2$ coulometer (Coulometric Inc., 5011). The overall analytical precision was better than 0.2 wt.%. TIC content was calculated as the difference between C and TIC. From the TOC and N concentrations TOC/N values were calculated.

Pyrolysis of OM was carried out using a Delsi Rock-Eval instrument (Version RE II). The amount of hydrocarbons (mg HC/g rock) released during gradual heating in a He stream was normalised to TOC to give the hydrogen index (HI).

### 2.4. Stable isotope analysis

Carbon isotope measurements on TOC were made on homogenised samples after removal of carbonate by treatment with 6N HCL. Portions of each sample (between 1 and 10 mg) were packed into tin capsules and combusted in excess oxygen at 1050 °C using a NC 2500 elemental analyser (ThermoQuest). Residual oxygen was removed by reaction with reduced Cu at 600 °C. After passing through a H$_2$O trap (MgClO$_4$), the resulting CO$_2$ was analysed on line with a Micromass isotope ratio mass spectrometer. The $^{13}$C/$^{12}$C isotope ratio of the CO$_2$ was compared with the corresponding ratio of a reference calibrated against the Pee Dee Belemnite (PDB) standard. The reproducibility of the total procedure was in the range 0.2–0.3%.

For the decomposition of calcite for mass spectrometric analysis, portions of the samples were weighed in glass vials that were evacuated and flooded with He. Carbon and oxygen isotope measurements were performed by addition of 100% H$_2$PO$_4$ to the samples heated at 90 °C in a MultiFlow on-line system for analysis of $^{13}$C and $^{18}$O in carbonate (Micromass Ltd., UK). Analysis was carried out with a Micromass isotope ratio mass spectrometer attached to the MultiFlow system. The results are reported relative to the PDB standard for both $\delta^{13}$C and $\delta^{18}$O. The reproducibility was better than 0.2%.

### 2.5. Chlorins

Chlorin concentrations were determined using homogenised sediment, following the method of Schubert et al. (2005). In brief, aliquots (10–20 mg) were extracted ($3 \times$) with 5 ml acetone on a sonication bath. Fluorescence was measured on the extracts with a spectrophotometer (Cary, Varian) at an excitation wavelength of 428 nm and emission of 671 nm. Phaeophytin for quantification was prepared from fresh chlorophyll a oxidised with 25% HCl. The chlorin content was normalised to TOC. The analytical precision of the procedure was 5% (relative error). To determine the chlorin index (Cl) the extracts were acidified with two drops of 25% HCl and measured at the same wavelength. The Cl was calculated from the fluorescence intensity of the acidified sample divided by the fluorescence intensity of the non-acidified sample, as outlined by Bechtel and Schubert (2009). It is a tool for a rapid estimation of OM freshness.

### 2.6. Lipid extraction and fractionation

FAs and neutral lipids (e.g. alkanols and alkenols, sterols, triterpenoid alcohols, alkanes and alkenes) were analysed using a modified method from Wakeham and Beier (1991). Aliquots (2–6 g) of selected samples from the cores were extracted at 70 °C for 7 min with a dichloromethane (DCM)–MeOH mixture (9:1) using a high performance microwave digestion unit (mls 1200 mega; Milestone). Half of the total extract was saponified with a solution of 6% KOH in MeOH (80 °C, 3 h) after addition of nonadecanoic acid (19:0) and 5x-cholestanole as internal standards. Neutral lipids were extracted ($3 \times$) with 2 ml n-hexane at pH 14. Prior to analysis, the neutral fractions were derivatised with BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide, Fluka] for 1 h at 80 °C to form trimethylsilyl ethers. FAs were extracted after acidification with 6N HCl (pH 1) with n-hexane. The samples were derivatised with BF$_3$/CH$_3$OH (14%, Sigma) for 2 h at 100 °C. After addition of 1 ml nanopure water, the methyl esters were extracted ($3 \times$) with 2 ml n-hexane.

### 2.7. Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

Lipid samples were analysed using a GC system with a flame ionisation detector (HRGC 5160, Carlo Erba Instruments) equipped with a split-splitless injector and a VF-5 ms column (60 m x 0.25 mm i.d. 0.25 µm film thickness, Varian). The carrier gas was H$_2$ (2.4 ml min$^{-1}$). The oven temperature programme for the FAs was: 90–140 °C at 10 °C min$^{-1}$, to 260 °C at 4 °C min$^{-1}$ and to 320 °C (held 15 min) at 20 °C min$^{-1}$. For quantification of the neutral fractions the programme was: 90–140 °C at 10 °C min$^{-1}$ and to 300 °C (held 30 min) at 4 °C min$^{-1}$. The injector temperature was 280 °C and the detector temperature 320 °C.

Identification of FAs was carried out by comparison of retention times with standard methylated FA mixtures (FAME and BAME, Supelco). For identification of individual compounds, the neutral fractions were injected on-column (RTX-5, 30 m x 0.25 mm i.d. 0.25 µm film thickness, Varian). The carrier gas was He (1.5 ml min$^{-1}$). The oven temperature programme was identical to that for GC analysis of the neutral fractions. The mass spectrometer was operated in the EI (electron ionisation) mode over the range 54 m/z–680 m/z (0.57 s total scan time). Identification of individual compounds was based on retention time and comparison of spectra with published data.

Quantification was performed by comparison of FID response in relation to that of internal standards (19:0 n-FA for FAs and 5x-cholestanol for neutral fraction, respectively). The analytical precision of the method was 4–8%. Concentrations were normalised to TOC.

### 3. Results and discussion

#### 3.1. Age control

A sedimentation rate of 0.82 cm/year was obtained for Lake Brienz by applying an exponential fit to the unsupported $^{210}$Pb activity (Fig. 2). This calculation is only valid assuming a constant sedimentation rate and a constant rate of supply of unsupported $^{210}$Pb to the sediments (Appleby and Oldfield, 1978). A slightly lower rate of 0.76 cm/year was determined using the $^{137}$Cs distribution with the peak caused by the Chernobyl reactor accident, clearly recognised at 14–18 cm (Fig. 2). The calculation using the $^{137}$Cs distribution with the peaks caused by the Chernobyl accident (1986) and nuclear weapons testing (1963) results in a lower sedimentation rate of 0.70 cm/year. The values are in agreement with rates calculated in previous studies (Anselmetti et al., 2007). To relate the down hole depths to the corresponding ages, the sedimentation rates obtained from the $^{137}$Cs peaks are used. For the time
Fig. 2. (a) Unsupported $^{210}$Pb activity with exponential fit and derived overall sedimentation rate (SR), (b) $^{137}$Cs and (c) $^{40}$K activities, (d) TOC and TIC contents vs. age, (e) TOC/N values, (f) HI, (g) carbon and oxygen isotopic composition of calcite and (h) $\delta^{13}$C of TOC vs. age for Lake Brienz sediments.
periods before 1963, age models are based on the sedimentation rates derived from the 210Pb data (Fig. 2d and h).

Similar calculations result in a sedimentation rate of 0.55 cm/year for Lake Lugano using the unsupported 210Pb data (Fig. 3). From the 137Cs distribution, a rate of 0.64 cm/year for the time after 1986 and of 0.61 cm/year for the interval between 1963 and 1986 are obtained (Fig. 3). The results are in agreement with sedimentation rates obtained in previous studies (Dominik and Span, 1992). Corresponding down core ages are shown at the right hand sides of Fig. 3d and h.

The presence of younger material mixed within a deeper layer of sediment or of slumps transported from shallower regions (turbidities) is indicated by 210Pb activity deviating from the exponential fit. Two of these deviations recognised in the Brienz record (Fig. 2a) correspond to the time of deposition of major turbidites (Sturm and Matter, 1978; Anselmetti et al., 2007).

3.2. Bulk organic parameters

The concentrations of TOC and total N in the sediment core from Lake Brienz are very low (0.4–1 wt.% and 0.02–0.14 wt.%, respectively; Fig. 2d). In the whole core the molar TOC/N ratio varies between 5 and 20, suggesting variable terrestrial OM input and/or variation in N degradation. Enhanced TOC/N values are recognised at 40–50 cm (Fig. 2e), consistent with the inferred deposition of a major turbidite in the lake, resulting in greater input of terrestrial OM. TIC content is highly variable (between 0.6 and 2.7 wt.%; Fig. 2d) and reflects the deposition of detrital calcite (Sturm, 1976). The antibiotic variation in 40K activity and TIC suggests the presence of varying contributions of clay minerals and carbonate. HI values are highest at 20–32 cm depth (Fig. 2f), consistent with elevated phytoplankton productivity during the inferred time period in which mesotrophic conditions were established in the lake (1960–1985).

Higher concentrations of TOC and total N in the Lugano sediments (TOC between 1.1 and 3.2 wt.%, N between 0.10 and 0.35 wt.%) reflect enhanced OM production and preservation in the anoxic water column and sediments. High TOC contents are obtained at 6–17 cm, corresponding to the inferred period of maximum eutrophication (Fig. 3d). Low TOC/N values between 8 and 11 suggest only minor terrestrial OM input (Fig. 3e). TIC content is lower than in Brienz and reflects the formation of autochthonous calcite, as indicated by overall parallel trends in TOC and TIC depth profiles (Fig. 3d). An apparent positive correlation between TOC and carbonate content has been reported as supporting a link between planktonic blooms and carbonate precipitation (Imbus et al., 1992). The diagenetic variation in 40K activity and TIC suggests that the presence of varying contributions of clay minerals and carbonates is related to variation in primary productivity, with higher values during periods of enhanced productivity (Branasconi et al., 1997). In lacustrine systems, phytoplankton is often enriched in 13C during blooms, resulting in low CO2 content and high 13C values of dissolved inorganic carbon in the surface waters (Imbus et al., 1992). Calcite 13C values vary between −3.0 and 0.6% (Fig. 3g). The positive correlation (R2 = 0.78) between 13C of TOC and calcite argues for carbonate precipitation governed by planktonic blooms (Imbus et al., 1992). The autochthonous origin of the calcite is further supported by the fact that 13C values co-vary with 3 year moving average of mean summer (April–October) temperatures reported for the lake for the time interval covered by the core (Fig. 4a). The positive relationship (Fig. 4b) results from the combined effects of the temperature-dependent O isotope fractionation between calcite and water, the temperature-dependent isotopic composition of rain water and the effect of evaporation from the surface water of the lake (Mayer and Schwark, 1999; Leng and Marshall, 2004).

3.4. Chlorins

Chlorin concentrations of selected sediment samples from Brienz vary between 163 and 597 μg/g TOC, with highest values in the middle part of the core (20–32 cm) corresponding to the interval of mesotrophic conditions in the lake (Fig. 5). Chlorin Index (CI) values in the range 0.75–0.92 (Fig. 5) indicate advanced degradation of OM (Schubert et al., 2005). The lower CI values at 19–32 cm reflect slightly enhanced OM preservation during the period of mesotrophic conditions.

Higher concentrations between 323 and 833 μg/g TOC and generally lower CI values in the sediments from Lugano are attributed to eutrophic conditions (Fig. 5). Maximum chlorin contents correspond with the period of maximum eutrophication. This period is further characterised by better preservation of OM, as seen from the depth trend in CI values varying between 0.58 and 0.87 (Fig. 5).

3.5. Lipid composition

The dominant lipids are carboxylic acids, alkanols and alkenols, n-alkanes and sterols. Other groups include alkenes, hoophanoic acids and triterpenoid alcohols. Higher concentrations of lipids are obtained from Lugano sediments (between 1592 and 3390 μg/g TOC) than Brienz sediments (between 1048 and 2143 μg/g TOC), as expected for a eutrophic lake (Tables 1 and 2). Major features of the lipid composition are described below.

3.5.1. Carboxylic acids

Total FA concentrations are highest in most Lugano horizons (between 1117 and 2511 μg/g TOC) than those from Brienz (between 834 and 1939 μg/g TOC) (Fig. 6). Most samples from both lakes have n-alkanoic FA distributions from C12 to C28, with a strong even/odd preference and maximum intensity at n-C16 and n-C18, respectively, characteristic of autochthonous OM production in the lakes (Cranwell et al., 1987; Stefanova and Disnar, 2000), with
variable inputs from aquatic macrophytes and land plants (long chain saturated n-FAs; Ficken et al., 2000). To illustrate differences in the FA composition that are expected to reflect differences in OM sources and preservation, the relative contribution of different compound classes to the sedimentary OM are plotted vs. down core depth (Fig. 6).
Fig. 4. (a) Depth trend of $\delta^{18}$O of calcite in sedimentary profile of Lake Lugano and variation in mean summer (April–October) temperature measured at the meteorological station of Lugano projected to corresponding depth using age model from overall sedimentation rate, as well as corresponding 3 year moving average of mean summer temperature. (b) Cross correlation between $\delta^{18}$O of calcite and the 3 year moving average of mean summer temperature.

Fig. 5. Chlorin concentration and variation in chlorin index (CI) in sediments from Lake Brienz and Lake Lugano.
The relative concentrations of long chain saturated n-FAs indicate a high abundance of allochthonous OM in the upper part (<20 cm) of the Brienz core and low terrestrial input in the lower part (>30 cm) of the profile (Figs. 6 and 7). Because of the increased relative concentrations of long chain FAs already during the period of mesotrophic conditions (19–32 cm), the results are interpreted to reflect increased in-wash of terrestrial OM rather than reduced eutrophication. Land plant OM input to Lugano sediments varies over a narrower range, being enhanced in the middle and lower parts (>30 cm), as indicated by the relative proportions of short chain vs. long chain FAs already during the period (<20 cm) of the Brienz core and low terrestrial input in the lower part (>30 cm) of the profile, respectively.

Besides the n-C16:0 FA, C18 mono- and poly-unsaturated compounds are the most abundant alkenoic acids at Lake Brienz, with a dominance of C18:1 acids, reflecting algal, bacterial and cyanobacterial contributions (Cranwell, 1978; Volkman et al., 1980; Zegough et al., 2000). Low concentrations of 18:3 plus 18:2 n-FAs in the Brienz samples (Fig. 7) correspond to low relative abundances of cyanobacteria in the euphotic zone. However, the good positive relationship between the concentration of the 16:1o7 FA and the content of branched chain FAs in the Lugano sediments argue that 16:1o7 reflects the contribution of bacterial biomass from manganese-, iron- and sulfate-reducing bacteria and/or methanothrophs in these samples (Fig. 8), consistent with the results of previous studies (Wakeham et al., 2007; Bechtel and Schubert, 2009). The lack of correlation within the Brienz sediments, characterised by low 16:1o7 FA concentrations, suggests a complementary diatomaceous origin for this biomarker.

Unsaturated acids are present in high relative abundance in the sediments from the middle and lower parts (>30 cm) of the Brienz core (Fig. 6), suggesting enhanced OM preservation during sediment accumulation (Cranwell, 1984). Higher values of saturated n-FAs relative to mono- and poly-unsaturated n-FAs occur in the upper part (<20 cm) of the Brienz core (Table 1), corresponding out in a previous study (Rezanka et al., 1983), although a contribution from green algae cannot be excluded.

For Lugano, comparable relative abundances of C16 and C18 monounsaturated FAs are obtained. The C16 alkenoic acids, as well as 20:5 and 20:4 n-FAs, have been found in sediments characterised by the presence of diatom remnants (Volkman et al., 1998; Pearson et al., 2007). High concentrations of C16 n-FA (Fig. 7) together with high relative contents of 16:1 n-FAs in the Lugano sediments are consistent with the presence of diatoms in the photic zone. However, the good positive relationship between the concentration of the 16:1o7 FA and the content of branched chain FAs in the Lugano sediments argue that 16:1o7 reflects the contribution of bacterial biomass from manganese-, iron- and sulfate-reducing bacteria and/or methanothrophs in these samples (Fig. 8), consistent with the results of previous studies (Wakeham et al., 2007; Bechtel and Schubert, 2009). The lack of correlation within the Brienz sediments, characterised by low 16:1o7 FA concentrations, suggests a complementary diatomaceous origin for this biomarker.

Unsaturated acids are present in high relative abundance in the sediments from the middle and lower parts (>30 cm) of the Brienz core (Fig. 6), suggesting enhanced OM preservation during sediment accumulation (Cranwell, 1984). Higher values of saturated n-FAs relative to mono- and poly-unsaturated n-FAs occur in the upper part (<20 cm) of the Brienz core (Table 1), corresponding out in a previous study (Rezanka et al., 1983), although a contribution from green algae cannot be excluded.
Fig. 6. Concentration of total FAs and relative abundances of FA groups in sediments from Lake Brienz and Lake Lugano.

Fig. 7. Concentration of \( n-C_{16} \) FAs, \( n-C_{18} \) FAs, \( n-C_{24:0} \) FA and sum of \( n-C_{18:3} \) plus \( n-C_{18:2} \) FA in sediment profiles from Lake Brienz and Lake Lugano.
to the zone of high input of terrestrial OM characterised by high abundances of long chain saturated n-FAs (Fig. 6). The overall relationship between the degree of saturation and land plant input (indicated by long chain FAs) is also observed for the Lugano sediments, where even higher ratios of saturated vs. unsaturated n-FAs are obtained (Fig. 6). The results indicate that the relative concentrations of saturated FAs in the samples are governed by the contribution of long chain saturated n-FAs from land plants rather than reflecting the degradation of OM in the water columns of the lakes. Results from particulate organic matter (POM) in both lakes showed that the degree of saturation of FAs of predominantly planktonic origin could be indicative of OM degradation (Bechtel and Schubert, 2009).

The relative contribution of bacterial biomass to sedimentary OM can be estimated from the concentrations of branched chain FAs (Volkman et al., 1980; Kaneda, 1991; Niggemann and Schubert, 2006). Depth profiles of the concentrations of branched chain and polyunsaturated FAs indicate enhanced bacterial biomass and OM degradation in the Lugano sediments, especially in the upper part (6–17 cm) of the profile, corresponding to the interval of maximum eutrophication (Fig. 6). The ratio of 18:1o7 FA relative to 18:1o9 FA has also been used to trace bacterial reworking of planktonic material as 18:1o7 is a major FA of bacteria (Wakeham et al., 1997; Niggemann and Schubert, 2006). Higher values are obtained from the middle part (20–45 cm) of the Brienz core and in the upper (<20 cm) and lower middle (27–35 cm) part of the Lugano succession. The data suggest a generally higher contribution of bacterial biomass to the OM in the Lugano sediments. Similar results were obtained for the POM of both lakes (Bechtel and Schubert, 2009).

Comparable relative concentrations of hopanoic acids (<3% of total acids), dominated by 17k(H), 21l(H)-bishomohopanoic acid, are found in the sediments of both lakes. This compound has been thought to be indicative of the contributions of bacterial biomass or the reworking of primary OM, as often seen in sediments dominated by OM from soil in-wash (Grimalt et al., 1991; Meyers and Ishiwatari, 1993). The concentrations of hopanoic acids are unrelated to the variation in long chain FA concentrations in the samples from both lakes, arguing for its origin from in situ microbial synthesis.

3.5.2. Neutral lipids

The normalised concentrations of the total neutral fractions are significantly higher in the Lugano sediments (between 475 and 879 μg/g TOC) vs. the Lake Brienz succession (between 186 and 313 μg/g TOC; Fig. 9).

3.5.2.1. Alkanols and alkenols. n-Alkanols (C$_{13}$–C$_{28}$) are the predominant compounds in the neutral lipid fractions of most samples. The total concentrations range from 98 to 166 μg/g TOC for Brienz and from 234 to 460 μg/g TOC for Lugano. The C$_{23}$–C$_{30}$ n-alkanols (denominated short chain n-alkanols; Fig. 9) are indicative of algal or bacterial inputs (Albro, 1976; Weete, 1976). The presence of an odd/even predominance in the C$_{13}$–C$_{17}$ range, as well as the occurrence of branched alkanols (C$_{13}$–C$_{17}$) in low concentration, argues for in situ microbial synthesis (Cranwell, 1980). All samples are characterised by the predominance of the C$_{13}$ n-alcanol. The comparable n-alkanol distribution in the POM samples has been interpreted to reflect higher contributions of algal OM vs. microbial plus diatomaceous biomass during autumn (Bechtel and Schubert, 2009). Long chain n-alkanols (C$_{22}$–C$_{28}$) with an even predominance and maximum at C$_{25}$ or C$_{26}$ have been reported to be major components of aquatic macrophytes (Ficken et al., 2000), but they are also abundant in the waxes of higher plants (Eglinton and Hamilton, 1967). According to Lachavanne et al. (1992), the amount of macrophyte vegetation on the shores of the northern basin of Lugano is very low. This suggests a significant contribution of long chain alkanols from higher plant waxes to these sediments.

Generally, the high relative proportions of long chain n-alkanols in the upper part of the Brienz core and middle and lower part of the Lugano succession (Fig. 9) are consistent with the depth trends for the long chain n-FAs (Fig. 6) and reflect the input of allochthonous OM. The concentration of n-alkanols generally increases relative to the sum of alkanols plus sterols in sediments characterised by lower contributions of terrestrial OM (lower part of the core from Brienz; Fig. 9).

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) is the only branched alkenol present in amounts sufficient for peak integration in the samples (Fig. 9). It derives mainly from the side chain of chlorophyll a of phytoplankton and land plants (Shi et al., 2001), although it can be derived by hydrolysis of bacteriochlorophyll a of purple sulphur bacteria (Marchand and Rontani, 2003) and has been found in cyanobacterial mats (Rontani and Volkman, 2005). Our results indicate that the content is generally higher in samples characterised by an enhanced input of terrestrial OM (Fig. 10), arguing that phytol is more resistant to degradation in those sediments.

3.5.2.2. Steroidal alcohols. Sterols are present in considerable concentrations (15–44 μg/g TOC for Brienz, 38–133 μg/g TOC for Lugano), especially within the upper part (<20 cm) of the Lugano core (Figs. 9 and 11). The dominant ones in most samples are cholesterol (cholest-5-en-3β-ol) and sitosterol (24-ethylcholest-5-en-3β-ol). Sitosterol has been reported to be a typical marker of higher plant input and marsh grasses (Canuel et al., 1997). However, it may also partly be derived from algal and cyanobacterial inputs (Volkman et al., 1999; Rontani and Volkman, 2005).

The variability in sitosterol concentration in the Brienz core (Fig. 11) is attributed to the input of vascular plants. This is supported by the good positive relationship ($R^2$ 0.71) between sitosterol concentration and the content of long chain n-alkanols in the Brienz sediments (Fig. 12a). An increased in-flow of soil OM in the upper part of the Brienz profile and throughout the Lugano sediments is indicated by the data and by high C$_{30}$ sterols (sitosterol + stigmasterol) relative to the sum of sterol concentrations for both lakes (Tables 1, 2). However, deviations in the concentration of sitosterol from the overall relationship in the Lugano succession ($R^2$ 0.19, excluding the three data points from the top of the profile) may indicate additional inputs from microalgae, especially at the top of the profile (Fig. 12a).
Cholesterol has been used to indicate inputs from zooplankton grazing in the water column (Gagosian et al., 1983), although it has been reported as a predominantly algal/phytoplankton marker (Volkman et al., 1998). In a study of POM from both lakes, significantly increased cholesterol concentrations in the deeper parts of the autumn water columns, as compared to the situation during spring, have been related to enhanced OM from zooplankton grazing (Bechtel and Schubert, 2009). This interpretation was based on the accompanying increase in C$_{18}$ FAs at these water depths. However such a relationship is missing from the sediments of both lakes. Therefore, the high relative proportions of cholesterol in the middle and lower part (>26 cm) of the Brienz sediment profile (Fig. 11; Table 1) can only be linked to increased relative contributions of autochthonous OM of planktonic origin.

The C$_{28}$ sterol 24-methylcholesta-5,22-dien-3$eta$-ol (either epi-brassicasterol or brassicasterol) is considered a diatom marker (Brassell et al., 1982; Killops and Killops, 1997). This interpretation has been supported by the overall positive relationship between it and highly branched isoprenoid (HBI) alkene concentrations in the POM samples from both lakes during spring and autumn (Bechtel and Schubert, 2009). HBI alkenes are biomarkers for diatoms (Section 3.5.2.4). In contrast to these results, only a general tendency towards higher concentrations of the C$_{28}$ sterol with increasing HBI alkene concentrations ($R^2$ 0.17) are found in the Lugano sediments (Fig. 12b). No relationship between HBI alkene concentration and C$_{28}$ sterol content exists in the sediments from Brienz. Additional sources for 24-methylcholesta-5,22-dien-3$eta$-ol (e.g. green algae) have to be considered for the sediments as a result of seasonal and/or annual changes in planktonic communities. The differences for the sediments from both lakes may be explained by differences in the diatom communities. In the euphotic zone of Lake Brienz, the diatom species Stephanodiscus sp., Fragilaria ulna and Asterionella formosa predominate (GBL, 2007), whereas diatom communities at Lake Lugano are dominated by Stephanodiscus sp., Melosira islandica sp. and Fragilaria crotonensis (Pollì and Simonà, 1992; Simonà personal communication). Until now, the occurrence of HBI alkenes has been reported for only a few specific diatom phylogenetic groups (i.e. Rhiqosolenia setigera, Haslea ostrearia, Pleurosigma sp.; Sinninghe Damsté et al., 1999, 2004; Belt et al., 2000; Grossi et al., 2004) not abundant in both lakes.

Fig. 9. Concentration of total lipids in neutral fractions and relative abundances of major compound groups in sediments from both lakes.

Fig. 10. Cross correlations of TOC-normalised concentration of phytol vs. long chain n-alkanols in sediments from both lakes.
Stanols are present in lower relative abundance than sterols (stanols/sterols <0.35) and generally higher stanols/sterols values are obtained for the Lugano sediments (Tables 1, 2). Some stanols have a direct input from microalgae (Volkman, 1986), phytoplankton and zooplankton (Nishimura and Koyama, 1977). However, their occurrence is usually considered as evidence of degradation of OM during diagenesis (Gaskell and Eglinton, 1976). Hence, the higher stanols/sterols values in the Lugano sediments most probably reflect enhanced OM degradation in the lake.

3.5.2.3. Triterpenoid alcohols. Greater proportions of cell membrane rigidifiers from both eukaryotes (i.e. sterols) and prokaryotes (i.e. triterpenoids) occur in the Lugano neutral lipids than the Brienz neutral lipids, suggesting greater contributions of microbial biomass to the Lugano sedimentary OM (Fig. 13a).

Diplopterol [17β(H), 21β(H) hopan-22-ol] is the most abundant triterpenoid alcohol in the dataset (up to 5% of total neutrals), with the greatest concentration (>30 μg/g TOC) in the upper part of the Lugano succession (Fig. 11). It has been found in several eukaryotic phyla (e.g. ferns, mosses, lichens, fungi) as well as in haptophyto-producing bacteria (Bottari et al., 1972; Ourisson et al., 1979; Rohmer and Bisseret, 1994). Recently, a cyanobacterial source was proposed (Summons et al., 1999). The occurrence of diplopterol in highest abundance in the Lugano sediments deposited during the interval of maximum eutrophication (Fig. 11) is consistent with this interpretation (Barbieri and Simona, 2001). Increased nutrient supply as a result of anthropogenic activity is known to be responsible for shifts towards plankton communities dominated by cyanobacteria. (Xu and Jaffé, 2009).

Tetrahymanol (gammaceran-3β-ol) is present in most samples (up to 27 μg/g TOC) with the highest concentrations in the upper and middle part of the Lugano core (Fig. 11). It is probably indicative of primitive organisms such as ciliates, protozoa, phototrophic and green and purple sulphur bacteria (Kleeman et al., 1990; Harveys and MacManus, 1991), although it has been reported to occur in algal or microbial mats in hypersaline environments (Barbé et al., 1990). Tetrahymanol-producing protozoa can be attributed to the development of a stratified water column, because dense bacterial populations thrive at oxic–anoxic boundaries and provide abundant food for these bacteriovores organisms (Sinninghe Damsté et al., 1995). The higher contents of tetrahymanol in the Lugano sediments are consistent with these findings (Fig. 11).

3.5.2.4. n-Alkanes and alkenes. n-Alkanes with a marked odd/even predominance from n-C15 to n-C31 were found in concentrations up to 110 μg/g TOC. The bimodal distribution, with maxima at C17 and in the C27–C29 range, reflects the combination of autochthonous and allochthonous inputs (Ficken et al., 2000). The results are in agreement with the distribution patterns obtained for n-FAs and n-alkanols. Higher relative abundances of n-alkanes occur in the neutral fractions from Brienz, arguing for increased decarboxylation of FAs from wax esters and phospholipids. Decarboxylation is the favoured process for FA decomposition in oxic to suboxic environments (Welte and Waples, 1973).

Two HBI alkenes commonly attributed to diatoms (Sinninghe Damsté et al., 1999) are abundant throughout the cores from both lakes. They have been tentatively assigned as br-25:2 and br-30:2 on the basis of the mass spectra (Belt et al., 2000) and are considered as biomarkers for the contribution of diatoms to photoautotrophy in the lakes. Higher abundances are noticed in the Brienz sediments (Fig. 9), arguing for a greater contribution of diatoms to the biomass.

Cross correlation of the relative contents of HBI alkenes vs. diplopterol (Fig. 13b) in the neutral lipid fractions indicates higher relative contributions of OM from cyanobacteria and lower inputs from diatoms to the Lake Lugano OM than to the Lake Brienz OM. The results are supported by recent biomarker compositions...
3.5.2.5. Archaeol and hydroxyarchaeol. Bis-O-phytanylglycerolidether (archaeol), biosynthesised by microorganisms from the archaea domain, occurs in very low concentration, insufficient for peak integration, in the Lugano sediments. It is accompanied by sn-2-hydroxyarchaeol, another biomarker diagnostic for archaea. Both compounds have been found in halophiles, thermophiles and methanogens, as well as in methane-oxidising methanotrophs (Koga et al., 1993; Pancost et al., 2001; Wakeham et al., 2003; Schubert et al., 2006; van Dongen et al., 2006). In the case of Lugano, these archaeal lipids most probably reflect the activity of methanogens in the sediments. In contrast to methanogens, no indications have been found in the POM samples for the activity of methane oxidisers (Bechtel and Schubert, 2009). However, a contribution from anaerobic methanotrophs cannot be excluded.

4. Summary and conclusions

Differences in the trophic level and associated OM production and preservation in the sediments from Lake Brienz and Lake Lugano are reflected in enhanced TOC contents, elevated HI values, as well as higher TOC-normalised lipid concentrations in the core from the eutrophic Lake Lugano. Additionally, total concentrations of chlorins, FAs and neutral lipids are higher in the Lugano sediments than the Brienz sediments. Bioproductivity cycles within Lake Lugano are indicated by co-varying δ¹³C values of calcite and TOC.

Major sources of OM and differences in the two lakes are revealed from the lipid compositions of the sediments, dominated by carboxylic acids, alkanols, sterols, triterpenoid alcohols and hydrocarbons. The Lake Brienz sedimentary OM is characterised by a high autochthonous contribution (algae, zooplankton and bacteria) and low terrestrial input in the lower part and high abundance of allochthonous OM in the upper parts of the profile. The variability in sitosterol concentration in the Brienz core is attributed to the input of vascular plants. Contributions of diatom related OM are reflected in relative abundances of C₁₅₋₁ H-FAs, HBI alkenes and 24-methylcholesta-5,22-dien-3β-ol (either epibrassicasterol or brassicasterol). A higher relative contribution of diatoms to Lake Brienz primary production is indicated by the data.

Land plant OM input to the Lugano sediments varies over a narrower range, as indicated by relative proportions of short chain vs. long chain n-FAs. Relative concentrations of C₂₉ sterols are suggested to be additionally influenced by the contribution of OM from microalgae. The occurrence of diplopterol in highest concentrations in Lugano sediments deposited during the interval of maximum eutrophication is suggested to reflect a shift towards phytoplankton communities dominated by cyanobacteria. A high contribution of bacterial biomass is evidenced by high relative proportions of branched FAs and triterpenoid alcohols. The
predominance of 16:1o7 n-FA in the Lugano sediments reflects the activity of anaerobic bacteria (manganese-, iron- and sulfate-reducing bacteria and methanogens). High concentrations of tetrahymanol-producing protozoa are attributed to the development of a stratified water column.

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Appendix VIII

Biogeochemistry of particulate organic matter from lakes of different trophic levels in Switzerland.

Authors
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Biogeochemistry of particulate organic matter from lakes of different trophic levels in Switzerland

Achim Bechtel *, Carsten J. Schubert

EAWAG, Department of Surface Waters, Seestrasse 79, CH-6047 Kastanienbaum, Switzerland

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A B S T R A C T
Biomarker compositions of particulate organic matter (POM) from the oligotrophic Lake Brienz and the eutrophic Lake Lugano (both Switzerland) are compared, in order to obtain information about organic matter (OM) production and transformation processes in relation to water column stratification. Eutrophic conditions in Lake Lugano are reflected by enhanced alkalinity, elevated total organic carbon (TOC) and chlorin contents compared with Lake Brienz. Lower δ13C values of dissolved inorganic carbon (DIC) in Lake Lugano reflect enhanced OM respiration in the water column.

Differences in OM dynamics between both lakes, as well as seasonal variations, are evidenced by TOC-normalised concentration profiles of total fatty acids (FAs) and total neutrals. In Lake Brienz, the results reflect the relative contributions of primary productivity and refractory, allochthonous OM to POM, governed by particle load and interflows due to density stratification. The depth trends at Lake Lugano are a result of high primary productivity, water column stratification and associated particle load in the upper layers, as well as microbial degradation close to the chemocline and greater preservation under anoxic conditions. Minor differences exist with regard to the OM composition. In both lakes, FA distributions and the composition of n-alkanols indicate a predominant autochthonous OM source (algae, zooplankton, bacteria). Inputs of OM from diatoms are reflected in highly-branched isoprenoid (HBI) alkenes, 16:1 n-FAs and 24-methylcholesta-5,22-dien-3β-ol (either epibrassicasterol or brassicasterol). Differences in relative proportions of n-C16 vs. n-C18 FAs and alkanols, respectively, as well as in the percentages of C27, C28 and C29 sterols relative to the sum of sterols are related to differences in the abundances of planktonic algae, diatoms and green algae within the euphotic zone of both lakes as well as in bacterial activity and soil in-wash. High relative proportions of cholesterol in the autumn samples, most pronounced at Lake Lugano, were attributed to an increased input from zooplankton grazing in the water column.

Differences in OM degradation processes are reflected in slightly higher chlorin index values and higher relative proportions of saturated vs. unsaturated n-FAs in Lake Lugano. Higher contents of branched chain FAs, 16:1o7 n-FA, and enhanced 18:1o7/18:1o9 n-FA ratios suggest enhanced bacterial biomass in the water column of Lake Lugano close to the chemocline. Increasing proportions of saturated n-FAs and n-alkanols with increasing water depth, most distinct in the autumn for both lakes, argue for intensified bacterial activity and degradation of OM during autumn. High relative contents of sterols and low n-alkanol concentrations in POM close to the chemocline at Lake Lugano during spring are interpreted to reflect higher primary productivity in the photic zone, OM export to the deeper parts and enhanced degradation rates of more labile constituents (i.e. C11–C20 n-alkanols), as compared to Lake Brienz.

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1. Introduction

Extensive research on the molecular composition of lipids as biomarkers specific for single organisms or biogeochemical processes has been performed in ocean waters with respect to the
et al., 2005; Cole et al., 2007). Lakes participate in biogeochemical cycling and act as carbon sinks or sources in response to environmental parameters.

Lacustrine OM contains a diverse range of lipids, which can be used to track changes in lake and catchment biota (Meyers, 2003; Muri and Wakeham, 2006). The use of lipids as palaeoenvironmental indicators requires knowledge of their biological precursors. Lipid geochemistry of POM at different depths in stratified water columns provides information on biological activity fuelled by oxygenic and anoxygenic photosynthesis, chemosynthesis, and heterotrophy (Wakeham and Beier, 1991; Wakeham et al., 2007). Biological molecules characterising certain biotic sources within the lake and its catchment can be applied to provide further details on production, delivery and preservation of OM (Schubert et al., 2006; Pearson et al., 2007).

The aim of the paper was to assess sources of OM and to compare major factors that affect its degradation in the water columns of the oligotrophic Lake Brienz and the eutrophic Lake Lugano (both Switzerland). Water depth distributions of oxygen content, temperature, turbidity, TOC, alkalinity, major anion concentrations (i.e. Cl\(^{-}\), NO\(_3\), SO\(_4^{2-}\)) and carbon isotopic composition of DIC were measured on samples obtained during spring and autumn sampling campaigns. This study focusses on the significance of FAs, aliphatic alcohols, sterols and aliphatic hydrocarbons in order to reveal differences in OM sources and transformations in response to differences in trophic level and water column stratification. Chlorins are used as proxies for primary productivity and freshness of OM (Schubert et al., 2005).

2. Materials and methods

2.1. Study sites and sample collection

Lake Brienz is an ultraoligotrophic, deep perialpine lake (Fig. 1A). It has a surface area of 29.8 km\(^2\) and maximum depth of 260 m. While the Lütschine, one of the two major contributing rivers, drains a partly calcareous catchment of an unaltered hydrological regime, the Aare flows from a crystalline catchment whose flow is heavily altered by several reservoirs built for hydropower generation (Sturm, 1976; Finger et al., 2007). The predominant organisms in the water column are chrysophytes (golden algae), diatoms, cryptophytes, dinoflagellates, chlorophytes (green algae) and cyanobacteria (up to 70% of total phytoplankton) and cladocerans and calanoid copepods account for 75% of the zooplanktonic biomass (GBL, 2007). Phytoplankton communities show minor differences in relative abundance during the sampling campaigns. During autumn a lower abundance of diatoms was noticed compared to the situation in June 2007.

Fig. 1. (A) Overview of study area around Lake Brienz. Small arrows show river flow direction (modified after Finger et al., 2007). Hydropower reservoirs are situated 15 km upstream on Aare (large arrow indicates direction). (B) Lake Brienz bathymetry map (contour lines every 20 m) showing location of sampling site for in situ filtration of POM (modified after Girardclos et al., 2007). (C) Map of Lake Lugano with sampling station in Northern basin offshore from village of Castagnola (Switzerland).
permanent anoxic conditions occur below 70 m (Barbieri and Polli, 1992). Eutrophication, which started in the second part of the last century, led to significant increased primary productivity, with the highest values (>400 g C m⁻² yr⁻¹) in the 1980s. Nowadays, primary productivity has decreased to about 300 g C m⁻² yr⁻¹. Chlophytes (green algae), cyanobacteria and diatoms, as well as cyclopoids and cladocerans predominate in the phytoplanktonic and zooplanktonic biomass, respectively (Barbieri and Simona, 2001; Simona, personal communication). Seasonal variation in the phytoplankton community leads to decreasing abundances of cyanobacteria and higher relative percentages of diatoms during the autumn sampling campaign as compared to June 2007.

Profiles of temperature, depth, oxygen content and turbidity were measured with a CTD profiler at the sampling site in June and November 2007. Water and particulate matter samples were collected from the northern basin (Fig 1C) at a 266 m deep station [position 719490/984630 (Swiss Grid) S 5° of the village of Castagnola. Particulate material was collected by in situ filtration of water (20–110 l) at 10, 40, 70, 100, 150 and 250 m depth during spring and at 10, 40, 70, 100, 150, and 200 m during the autumn sampling campaign, respectively. High primary productivity during the spring sampling campaign and significantly lower autochthonous OM production during autumn are indicated by chlorophyll contents of 6.6 and 3.0 mg/m², respectively, in the photic zone of Lake Lugano (Simona, personal communication). In Lake Lugano, the allochthonous (detrital) particle load has been estimated to vary between 30 and 60% of the total accumulation in the sediments (Niessen et al., 1992), contributing only minor proportions of organic carbon in relation to autochthonous OM production within the lake. Suspected matter concentrations have been reported to be enhanced during autumn in Lake Lugano compared to those in summer (Hofmann and Dominik, 1995).

2.2. Water analysis

TOC concentration of water samples was measured using high temperature catalytic oxidation with a Shimadzu 5050A analyzer (Benner and Strom, 1993). Alkalinity was determined with an automatic titration system (716 DMS Titrino) with 0.1 N HCl to pH 4.3. For determination of chloride, nitrate and sulfate concentrations, aliquots were filtered through cellulose nitrate filters with a pore size of 0.45 μm and dried. Analysis was performed using an ion chromatograph (Dionex DX-500, Carlo Erba Instruments), a split–splitless injector and a VF-5 ms column (60 m × 0.25 mm i.d., 0.25 μm film thickness) using a GL-8000 gas chromatograph (Carlo Erba Instruments). The carrier gas was H₂ (2.4 ml min⁻¹). The oven temperature programme for analysis of FA was: 90–140 °C at 10 °C min⁻¹, to 260 °C at 4 °C min⁻¹ and to 320 °C (held 15 min) at 20 °C min⁻¹. For quantification of the neutral fractions the oven temperature programme was: 90–140 °C at 10 °C min⁻¹ and to 300 °C (held 30 min) at 4 °C min⁻¹. The injector temperature was 280 °C and the detector temperature 320 °C.

Identification of FAs was carried out by comparison of retention times with standard methylated FA mixtures FAME and BAME (Supelco). For the identification of individual compounds, the neutral fractions were injected on-column (RTX-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness) using a GL-8000 gas chromatograph (Carlo Erba Instruments) quadrupole mass spectrometer. The carrier gas was He (1.5 ml min⁻¹). The oven temperature programme was identical to that used for GC analysis of the neutral fractions. The mass spectrometer was operated in the EI (electron ionisation) mode over a range from m/z 54 to m/z 680 (0.57 s total scan time). Identification of individual compounds was based on retention time and comparison of mass spectra with published data. Quantification was performed by comparison of FID response vs. that of internal standards (19:0 n-FA for FAs and 5x-cholestane for neutral fraction, respectively). The precision of the method was 4–8%. The concentrations were normalised to the amount of water filtered.

2.3. Chlorin

Chlorin concentrations were determined on aliquots (0.04 vol% of GFF filters) of the POM following the method of Schubert et al. (2005). In brief, the aliquots of the filters were extracted (3 ×) with 5 ml acetonitrile in a sonication bath. Acetone was decanted after centrifugation for 10 min at 4 °C. Fluorescence was measured with a spectrophotometer (Cary, Varian) at an excitation wavelength of 428 nm and an emission of 671 nm. Phaeophtin for quantification was prepared from fresh chlorophyll a acidified with 25% HCl. The precision of the analytical procedure was 5%. To determine the chlorin index (CI), the extracts were acidified with two drops of 25% HCl and measured at the same wavelength. The CI was calculated from the fluorescence intensity of the acidified sample divided by the fluorescence intensity of the non-acidified sample. It represents the ratio of chlorophyll and its degradation products in a sample that could still be chemically transformed and those inert to chemical attack. The CI is a tool for a rapid estimation of OM freshness. The ratio varies between 0.2 for fresh phytoplankton and unity for samples containing only chemically inert chlorins.

2.4. Lipid extraction and fractionation

FAs and neutral lipids (e.g. alkanols and alkenols, sterols, alkanes and alkenes) were analysed using a modified method from Wakeham and Beier (1991). Aliquots (half of the GFF filters) of the particulate material collected from different water depths were Soxhlet extracted with CH₂Cl₂/MeOH (9:1). Half of the total extract was saponified with a solution of 6% KOH in MeOH (80 °C, 3 h) after addition of nonadecanoic acid (19:0), 5x-cholestan and C₃₅ n-alanine as internal standards. Neutral lipids were extracted (3 ×) with n-hexane at pH 14. Prior to analysis, the neutral fractions were derivatised with BSTFA [N,O-bis(trimethylsilyl)] trifluoroacetamide, Fluka] for 1 h at 80 °C. FAs were extracted with n-hexane after acidification with 6 N HCl (pH 1). The samples were derivatised with BF₃/MeOH (14%, Sigma) for 2 h at 100 °C. After addition of 1 ml nanopure water, the methyl esters were extracted (3 ×) with hexane.

2.5. Gas chromatography (GC)

Lipids were analysed using a GC system equipped with a flame ionisation detector (HRGC 5160, Carlo Erba Instruments), a split–splitless injector and a VF-5 ms column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Varian). The carrier gas was H₂ (2.4 ml min⁻¹). The oven temperature programme for analysis of FAs was: 90–140 °C at 10 °C min⁻¹, to 260 °C at 4 °C min⁻¹ and to 320 °C (held 15 min) at 20 °C min⁻¹. For quantification of the neutral fractions the oven temperature programme was: 90–140 °C at 10 °C min⁻¹ and to 300 °C (held 30 min) at 4 °C min⁻¹. The injector temperature was 280 °C and the detector temperature 320 °C.

Identification of FAs was carried out by comparison of retention times with standard methylated FA mixtures FAME and BAME (Supelco). For the identification of individual compounds, the neutral fractions were injected on-column (RTX-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness) using a GL-8000 gas chromatograph (Carlo Erba Instruments) quadrupole mass spectrometer. The carrier gas was He (1.5 ml min⁻¹). The oven temperature programme was identical to that used for GC analysis of the neutral fractions. The mass spectrometer was operated in the EI (electron ionisation) mode over a range from m/z 54 to m/z 680 (0.57 s total scan time). Identification of individual compounds was based on retention time and comparison of mass spectra with published data. Quantification was performed by comparison of FID response vs. that of internal standards (19:0 n-FA for FAs and 5x-cholestan for neutral fraction, respectively). The precision of the method was 4–8%. The concentrations were normalised to the amount of water filtered.

3. Results and discussion

3.1. Physical and chemical characterisation of water columns

The water column of Lake Brienz is characterised by a nearly homogeneous oxygen concentration of >300 μmol/l, a thermocline between 5 and 10 m during spring and a minor temperature decrease at 35 m during autumn (Fig 2). During spring, high turbidity in the surface water with a sharp maximum at 15–20 m is

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observed, most probably reflecting high particle load caused by
density currents (Sturm and Matter, 1978; Finger et al., 2007)
and a minor influence of maximum phytoplankton abundance, as
the sampling date is close to the annually observed time interval
of maximum primary productivity (Finger et al., 2007). No nutrient
removal (NO\textsubscript{3}/C\textsubscript{0}\textsubscript{3}) is evident from the anion concentration profiles
(Fig. 2). The autumn water column is characterised by enhanced
turbidity values from 5 m down to 90 m, probably due to clay min-
erals settling down in the water column during and after diminish-
ing density stratification (Sturm and Matter, 1978). Enhanced
turbidity below 220 m most probably reflects the existence of
high-density turbidity currents in the lake (Sturm and Matter,
1978) or of a nepheloid layer close to the bottom. Alkalinity and
major anion concentration are low (Fig. 2). The autumn water col-
umn is characterised by slightly increased nitrate and chloride con-
centrations vs. the situation during June 2007, consistent with

![Fig. 2. Physical and chemical characteristics of water columns of Lake Brienz and Lake Lugano at sampling sites in June 2007 and October/November 2007. The continuous line indicates oxic–anoxic interface in water column of Lake Lugano.](image-url)
reduced primary production in the euphotic zone (Finger et al., 2007). Low TOC content of the water column (62–174 μmol/l) and sediments (TOC < 1 wt%; Sturm and Matter, 1978) is consistent with the oligotrophic conditions and oxygen availability in the lake. The δ¹³C values of DIC ranged from −8.8 to −6.1‰ (Table 1, Fig. 2), as typically found in freshwater where DIC is not significantly influenced by organic carbon respiration (Hellings et al., 2000).

Lake Lugano is characterised by anoxic conditions (oxygen content < 40 μmol/l) during spring and <20 μmol/l during autumn) below 70 m (Fig. 2). A pronounced thermocline was located at 15 m depth in June 2007, whereas a reduced temperature decline occurred at 20 m in the autumn water column (Fig. 2). During spring, a sharp maximum in turbidity is observed at 10–15 m depth, corresponding to high primary productivity in the photic zone (Simona, personal communication). Like Lake Brienz, the zone of enhanced turbidity extended down to 40 m in November 2007, probably as a result of enhanced particle load and decreased temperature gradient and density stratification (Hofmann and Dominik, 1995). High values of turbidity in the bottom layer below 210 m resulted from the input of excavation material from a construction site in the city of Lugano. TOC content varied between 106 and 355 μmol/l (Table 2, Fig. 2), with the highest values during spring in the oxic to suboxic layers (10 and 40 m, respectively) indicating high primary productivity. Together with the enhanced preservation of OM due to anoxic conditions in the deep water, TOC content of the sediments is reported to vary between 2 and 8 wt% (Niessen et al., 1992). Eutrophic conditions are further reflected in enhanced alkalinity (>2 mEq/l) as well as enhanced chloride, nitrate and sulfate concentrations (Table 2, Fig. 2). The carbon isotopic composition of DIC is characterised by lower δ¹³C values (<−8.5‰) than Lake Brienz, with the lowest values (<−12‰) close to the oxic–anoxic interface (Fig. 2) probably as a result of greater OM respiration.

3.2. Chlorsins

Chlorin concentrations in POM from Brienz varied between 10 and 439 μg/g TOC, with the highest values in the photic zone (Table 1, Fig. 3). A lower TOC-normalised value is observed in the top layer of the autumn water samples. The depth profile in October 2007 shows a less pronounced decrease than in the spring, resulting in higher chlorin content at elevated water depth. The results could be explained by an increased export of chlorins due to intensified settling velocity of particulate matter. Chlorin index (CI) values increased from 0.2 to 0.6, indicating decreasing OM freshness (Schubert et al., 2005) towards the bottom of the lake (Fig. 3). The higher CIs during autumn at depths between 40 and 100 m argue for an intensified degradation of OM.

Eutrophic conditions in Lugano are reflected in high chlorin concentrations between 12 and 814 μg/g TOC (Table 2, Fig. 3). Comparable depth trends, with rapidly decreasing concentrations were observed in the spring and autumn. However, intensified degradation of OM at the chemocline (70 m) is indicated by elevated CIs in the autumn vs. the spring (Fig. 3).

3.3. Lipid composition

Lipid analysis enabled the identification of individual compounds in the POM. The dominant compound groups are carboxylic acids, alkanols and alkenols and sterols. Other groups include alkanes and alkenes. Lower concentrations of lipids were obtained from Brienz and values during autumn were lower than during spring for both lakes (Figs. 4–7). Major features of the lipid composition are described below. The results from the 250 m sample at Lake Lugano should be interpreted with care because of the anthropogenic input of excavation material. Identified compounds in neutral fractions and FAs comprise only for small amounts of TOC (between 0.03 and 1%; Tables 3 and 4). TOC in the water column of lakes is usually dominated by carbohydrates, amino acids, lignin, proteins, pectin, chitin, etc. (de Leeuw and Largeau, 1993). However, although lipids comprise only a small fraction of the total organic matter their source and process information are valuable and are therefore used in this study.

3.3.1. Carboxylic acids

Total FA concentrations decrease from 8.7 mg/g TOC (spring) and 3.5 mg/g TOC (autumn), respectively, at the Brienz photic zone of towards 0.2 mg/g TOC (spring) and 1.1 mg/g TOC (autumn), respectively, in the bottom waters (Table 3, Fig. 4). In Lugano, total FA concentrations vary over comparable ranges if normalised to TOC in the water column (6.8–1.0 mg/g TOC; Table 4, Fig. 4). During autumn, significantly lower FA contents are observed in the Brienz euphotic zone most probably due to low primary productivity. As a result of water column stratification and bacterial activity close to the chemocline, a more pronounced decrease of FAs in the suboxic zone (40–70 m) is observed in the water column of Lugano vs. Brienz during autumn. In contrast, the TOC-normalised concentrations remain on a comparable level in the lower layers of the water columns, being anoxic in Lugano and oxic in Brienz. During spring, comparable FA contents and depth trends were found in the POM from the upper layers of the water columns (<100 m) of both lakes. Also during this time, significantly reduced TOC-normalised concentrations of FAs were observed in the bottom layers of Brienz vs. Lugano, reflecting either decreased OM preservation or the contribution of turbidity currents enhancing OM accumulation.

Most samples have n-alkanoic FA distributions from C₁₂ to C₂₄ with a strong even/odd preference and maximum at n-C₁₆ or n-C₁₈. The distribution patterns are characteristic of predominantly autochthonous OM production (Cranwell et al., 1987; Stefanova and Disnar, 2000) and minor inputs from aquatic macrophytes and land plants (long chain saturated n-FAs; Ficken et al., 2000). To illustrate differences in the FA composition, the relative contribution of different compound classes to POM are plotted vs. water depth (Fig. 4). The individual FAs were grouped into mid-chain saturated (C₁₂–C₂₀), long chain saturated (C₂₁–C₂₈), monounsaturated, polyunsaturated and branched-chain (iso- and anteiso-) FAs. Unsaturated acids are present in high abundance in POM from the upper layers of both water columns (Fig. 4). The increase in saturated vs. mono and polyunsaturated n-FAs from the photic zone towards increasing water depth reflects degradation of OM in the water columns (Tables 3 and 4). The results indicate enhanced degradation of OM during autumn in both lakes. Increased relative proportions of monounsaturated n-FAs in the POM from bottom waters can be attributed to enhanced contributions from bacterial lipids (Fig. 4). The relative amount of bacterial OM can be described with the relative concentrations of branched FAs (Volkman et al., 1980; Kaneda, 1991; Niggemann and Schubert, 2006). Water depth profiles of branched vs. polyunsaturated FAs indicate enhanced bacterial biomass and OM degradation in the deep water of both lakes and an additional maximum close to the oxic–anoxic interface in Lugano (Figs. 4, 8a; Tables 3 and 4). C₄₉ mono and polyunsaturated compounds are the most abundant alkenoic acids, with a dominance of 18:1, reflecting algal, bacterial and cyanobacterial contributions (Cranwell, 1978; Volkman et al., 1980; Zegough et al., 2000). The ratio of 18:1o9 relative to the 18:1o9 FA has been used to trace bacterial reworking of planktonic material, as 18:1o7 is often a major bacterial FA (Wakeham et al., 1997; Niggemann and Schubert, 2006). Slightly increasing values from 0.2 to 0.6 with increasing depth in Brienz and from 0.3 to 1.0 in Lugano were obtained (Tables 3 and 4). The results are
consistent with a higher contribution of OM from bacteria in the water column of Lugano.

The TOC-normalised content of 18:3 and 18:2 acids is considered a biomarker for cyanobacteria (Rezanka et al., 1983), but high contents are also found in most green algae and higher plants. Higher concentrations of 18:3 plus 18:2 n-FAs during spring than during autumn in Lugano, despite comparable TOC-normalised concentrations of C16 and C18 n-FAs (Fig. 5), correspond with a higher contribution of cyanobacteria to autochthonous OM production during spring at Lugano (Simona, personal communication). Lower concentrations in the Brienz samples (Fig. 5) are consistent with the presence of diatoms in the euphotic zone. However, a lower relative contribution of diatoms to primary productivity was found in the euphotic zone of Lugano during spring. In the anoxic zone of its water column (70–250 m) the dominant monounsaturated C18:1-FAS was 16:1o7, which is abundant in manganese-, iron-, and sulfate-reducing bacteria and methanotrophs (Wakeham et al., 2007). The good positive relationship between the concentration of 16:1o7 FA and the content of branched chain FAs argues that 16:1o7 can be used as an indicator of bacterial biomass in the samples (Fig. 8b).

Table 2

<table>
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<tr>
<th>Sample</th>
<th>Depth (m)</th>
<th>Temp. a (°C)</th>
<th>Oxygen (μmol/l)</th>
<th>Turbidity (FTU) b</th>
<th>Water filtered (l)</th>
<th>TOC c (μmol/l)</th>
<th>Alkalinity (mEq/l)</th>
<th>Cl− (μmol/l)</th>
<th>NO3− (μmol/l)</th>
<th>SO42− (μmol/l)</th>
<th>δ13C DIC (‰, PDB)</th>
<th>Chlorins (μg/g TOC)</th>
<th>Cl− (mEq/l)</th>
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<td>69.8</td>
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<td>116.3</td>
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<td>106</td>
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<td>64.0</td>
<td>19.7</td>
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<tr>
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a Temperature.  
b Total organic carbon.  
c Chlorin index.  
d Formazin turbidity units.
Significant differences in the relative proportions of \( n-C_{16} \) vs. \( n-C_{18} \) FAs are evident from comparing the composition of POM from the spring and autumn water columns of both lakes (Fig. 9). These seasonal differences may be related to changes in the phytoplankton community (diatoms vs. other algae, cyanobacteria). However, the major differences are noticed for the deeper parts of the lakes (>40 m) and the increase in relative proportions of \( n-C_{18} \) FAs during autumn is mainly caused by increasing amounts of 18:0 n-FA. The results suggest a greater contribution of zooplankton grazing in the autumn water columns. Deviations from the data point from 200 m water depth (Fig. 9) at Lugano are most likely related to the huge input of construction site material from the city of Lugano.

3.3.2. Neutral lipids

The depth trends in normalised concentrations of the total neutral fractions are similar to the observed variations in total FA concentrations in both lakes (Tables 3 and 4; Figs. 4 and 6).
3.3.2.1. Alkanols and alkenols. n-Alkanols (C₁₃–C₂₄) are the predominant compounds in the neutral lipid fractions of most samples. Their total concentration ranges from 660 to 35 μg/g TOC in Brienz and from 443 to 46 μg/g TOC in the POM of Lugano. The C₁₃–C₂₀ n-alkanols (denominated mid-chain n-alkanols; Fig. 6) are indicative of algal or bacterial inputs (Albro, 1976; Weete, 1976). The presence of an odd/even predominance and C₁₇ being the dominant n-alkanol during spring, as well as the occurrence of branched alkanols (C₁₃–C₁₉) in low concentration, argues for in situ microbial synthesis (Cranwell, 1980). Autumn POM samples are characterised by a predominance of C₁₈ n-alkanol, probably reflecting different phytoplankton and or bacterial communities. Changing contributions of microbial plus diatomaceous biomass vs. algal OM would be consistent with the observed changes in phytoplankton composition of the spring and autumn water columns. Generally, the very low relative proportions of long chain n-alkanols (Fig. 6) are consistent with a predominant autochthonous OM origin, as deduced from the FA composition. Long chain n-alkanols (C₂₂–C₃₀) with an even predominance and maximum at C₂₂ or C₂₄ were reported to be major components of submerged and emerged aquatic macrophytes (Ficken et al., 2000), but they are also abundant in the waxes of higher plants. According to Lachav-
anne et al. (1992), the amount of macrophyte vegetation on the shores of the northern basin of Lake Lugano is very poor. Therefore, a significant contribution of higher plant waxes to the measured concentration of long chain n-alkanols in the samples is suggested.

The relative abundances of n-alkanols generally increase towards the bottom waters and the trend is more pronounced in Brienz and in the autumn than for the depth profiles in June 2007 (Fig. 6). The data can be explained by differential degradation or synthesis. In the spring water column of Lake Lugano, n-alkanol abundances relative to the total concentrations of lipids in the neutral fractions are lowest close to the oxic–anoxic interface (Fig. 6). This can be explained by high primary productivity in the photic zone and the associated export of OM into the deeper parts of the water column. Rapid changes in the microbial communities involved in various biogeochemical cycles (nitrate-, manganese-, iron- and sulfate-reduction; as well as methanogenesis) at the oxic–anoxic interface are seen to be responsible for enhanced OM degradation of the more labile constituents (such as mid-chain n-alkanols) at the chemocline.

Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is the only branched alkenol present in amounts sufficient for peak integration in samples from shallow water depth (Fig. 6). It derives from the side chain of chlorophyll a of phytoplankton (Shi et al., 2001), although it can be derived by hydrolysis of the bacteriochlorophyll a of purple sulfur bacteria (Marchand and Rontani, 2003) and has been found in cyanobacterial mats (Rontani and Volkman, 2005). Our results indicate the highest concentration in the photic zone and rapidly decreasing abundance with increasing depth for both lakes (Fig. 6), arguing that phytol can be used as a marker for phytoplanktonic OM and/or cyanobacterial activity. Consistent with this interpretation, the TOC-normalised concentrations in the euphotic zone of Lugano are higher than for Brienz. Higher relative abundances in the suboxic to anoxic zone of Lugano are attributed to enhanced preservation of phytol under anoxic conditions.

3.3.2.2. Steroidal alcohols. Sterols are present in highly variable concentration in the POM (between 786 and 8 mg g TOC). High TOC-normalised concentrations were found within the photic zone and in the spring water column of both lakes (Fig. 6). The dominant sterols in most samples from the spring water columns are sitosterol (24-ethylcholest-5-en-3β-ol) and cholesterol (cholest-5-en-3β-ol). Sitosterol has been reported to be a typical marker of higher plant input and marsh grasses (Canuel et al., 1997). However, it may also partly be derived from algal inputs (Volkman et al., 1999; Rontani and Volkman, 2005). Other important sterols include stigmastanol (24-ethylcholesta-5,22-dien-3β-ol), associated with inputs from vascular plants, green algae and other phytoplankton (Volkman, 1986; Rontani et al., 2004). The overall positive relationship between sitosterol and the TOC-normalised content of long chain saturated n-alkanols argues for a land plant origin for this biomarker (Fig. 10a). An increased inflow of soil OM during spring is indicated by the data and by high C29 sterols (sitosterol, stigmastanol) relative to the sum of sterol concentrations in both lakes (Tables 3 and 4). However, increased TOC-normalised abundances of sitosterol at and below the oxic–anoxic interface in Lugano (especially pronounced in the spring; Fig. 7) may indicate inputs from green algae, which are abundant in the photic zone of Lugano.

The C27 sterols, cholesterol and dehydrocholesterol (cholesta-5,22-dien-3β-ol), have been used to indicate inputs from zooplankton grazing (Gagosian et al., 1983), although they have been reported as predominantly algal/phytoplankton markers (Volkman et al., 1998). In autumn POM samples, cholesterol is the only sterol present in considerable concentration (Fig. 7). Its high relative proportions in the autumn samples (Tables 3 and 4), most pronounced for Lugano, may indicate increased input from zooplankton grazing. This interpretation is consistent with the observed predominance of C18:0 n-FA in the autumn water columns of both lakes below the euphotic zone. However, an algal source of cholesterol would be also consistent with the observed increase in relative contributions of Chlorophyceae in Lugano and Chrysophyceae in Brienz to primary productivity during autumn (Simona, personal communication; GBL, 2007).

The C28 sterol, 24-methylcholesta-5,22-dien-3β-ol (either epi-brassicasterol or brassicasterol), is considered a diatom marker (Brassell et al., 1982; Killops and Killops, 1997). This interpretation is supported by the overall positive relationship between TOC-normalised concentrations of 24-methylcholesta-5,22-dien-3β-ol and...
HBI alkene concentrations (Fig. 10b). This C_{28} sterol is enriched in the upper layers of both lakes, indicating the high contribution of diatoms to primary production of OM (Fig. 7). The other C_{28} sterol in the samples is 24-methylcholesterol, commonly found in green algae and higher plants (Volkman, 1986). Good positive relationships between 24-methylcholesterol and sitosterol (R^2 0.98 for Brienz, 0.82 for Lugano, excluding the data point from 10 m depth in the autumn water column) exist, arguing for a predominant land plant origin for this biomarker.

Dinosterol (4α,23,24-trimethyl-5α-cholest-22-en-3β-ol) was found in low concentration within the POM from both lakes, indicating the presence of dinoflagellates (Robinson et al., 1984; Volkman, 1986). A higher relative abundance of dinoflagellates has been found in the photic zone of Brienz during spring than during autumn (GBL, 2007). The 24-methylcholesta-5,22-dien-3β-ol vs. dinosterol concentration ratio values in the surface water samples (10 m) of Brienz (about 3.0 during spring and about 2.3 during autumn) are consistent with the differences in abundances of diatoms relative to dinoflagellates (3.1 during spring, 2.6 during autumn) in the euphotic zone of Brienz.

Stanols are present in lower relative abundance than sterols (stanols/sterols < 0.25) and generally higher values occur in the deeper layers (Tables 3 and 4). Some stanols have a direct input from microalgae (Volkman, 1986), phytoplankton and zooplankton (Nishimura and Koyama, 1977). However, their occurrence is usually considered as evidence of degradation of OM during diagenesis (Gaskell and Eglington, 1976). No significant differences in the stanols/sterols ratio of POM from both lakes could be found.

### Table 3
Dominant lipid characteristics of POM from Lake Brienz during spring and autumn.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total FAs (μg/g TOC)</th>
<th>Total neutrals</th>
<th>Sat./unsat. FAs</th>
<th>Branched-chain/unsat. FAs</th>
<th>C_{27} sterols/sterols</th>
<th>C_{29} sterols/sterols</th>
<th>C_{30} sterols/sterols</th>
<th>Stanols/sterols</th>
<th>n-Alkanols/(n-alkanols + sterols)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB 10</td>
<td>8728</td>
<td>1934</td>
<td>1.31</td>
<td>0.24/0.32</td>
<td>0.43</td>
<td>0.17</td>
<td>0.40</td>
<td>0.12</td>
<td>0.53</td>
</tr>
<tr>
<td>LB 40</td>
<td>2323</td>
<td>714</td>
<td>1.33</td>
<td>0.20/0.19</td>
<td>0.28</td>
<td>0.28</td>
<td>0.44</td>
<td>0.14</td>
<td>0.56</td>
</tr>
<tr>
<td>LB 70</td>
<td>2069</td>
<td>803</td>
<td>1.64</td>
<td>0.24/0.47</td>
<td>0.27</td>
<td>0.27</td>
<td>0.46</td>
<td>0.19</td>
<td>0.74</td>
</tr>
<tr>
<td>LB 100</td>
<td>899</td>
<td>242</td>
<td>2.47</td>
<td>0.27/1.75</td>
<td>0.36</td>
<td>0.21</td>
<td>0.42</td>
<td>0.09</td>
<td>0.81</td>
</tr>
<tr>
<td>LB 150</td>
<td>222</td>
<td>98</td>
<td>1.30</td>
<td>0.36/1.60</td>
<td>0.24</td>
<td>0.20</td>
<td>0.56</td>
<td>0.15</td>
<td>0.73</td>
</tr>
<tr>
<td>LB 200</td>
<td>330</td>
<td>57</td>
<td>1.71</td>
<td>0.34/1.77</td>
<td>0.41</td>
<td>0.16</td>
<td>0.43</td>
<td>0.20</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Autumn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLB 10</td>
<td>3544</td>
<td>563</td>
<td>1.34</td>
<td>0.25/1.00</td>
<td>0.63</td>
<td>0.18</td>
<td>0.19</td>
<td>0.06</td>
<td>0.69</td>
</tr>
<tr>
<td>FLB 40</td>
<td>2167</td>
<td>196</td>
<td>1.79</td>
<td>0.45/1.61</td>
<td>0.55</td>
<td>0.35</td>
<td>0.10</td>
<td>0.08</td>
<td>0.82</td>
</tr>
<tr>
<td>FLB 70</td>
<td>2683</td>
<td>219</td>
<td>7.72</td>
<td>0.26/1.36</td>
<td>0.78</td>
<td>0.15</td>
<td>0.07</td>
<td>0.15</td>
<td>0.95</td>
</tr>
<tr>
<td>FLB 100</td>
<td>1455</td>
<td>110</td>
<td>10.92</td>
<td>0.28/1.60</td>
<td>0.70</td>
<td>0.13</td>
<td>0.17</td>
<td>0.12</td>
<td>0.91</td>
</tr>
<tr>
<td>FLB 150</td>
<td>1321</td>
<td>131</td>
<td>4.83</td>
<td>0.60/6.00</td>
<td>0.69</td>
<td>0.07</td>
<td>0.24</td>
<td>0.18</td>
<td>0.91</td>
</tr>
<tr>
<td>FLB 200</td>
<td>1105</td>
<td>82</td>
<td>4.60</td>
<td>0.53/5.00</td>
<td>0.77</td>
<td>0.06</td>
<td>0.17</td>
<td>0.16</td>
<td>0.90</td>
</tr>
</tbody>
</table>

- Fatty acids.
- Saturated.
- Unsaturated.
- Total organic carbon.

Fig. 8. Cross correlations of relative proportions of (a) branched chain FAs vs. polyunsaturated n-FAs and (b) the concentrations of 16:1\text{\textalpha} FAs vs. the TOC-normalised contents of branched chain FAs.

Fig. 9. Correlation plot of relative abundances of C_{16} FAs vs. C_{18} n-FAs.
This bimodal distribution reflects the combination of autochthonous and allochthonous inputs (Ficken et al., 2000). Two HBI alkenes commonly attributed to diatoms (Sinninghe Damsté et al., 1999) were abundant throughout the water columns of both lakes. They have been tentatively assigned as br-25:2 and br-30:2 from the mass spectra (Belt et al., 2000). Despite the fact that there is no clear depth trend in TOC-normalised content throughout the water columns (Fig 7), these compounds are considered biomarkers for the contribution of diatoms to photoautotrophy in the lakes. The results provide good evidence for the presence of diatom cells at greater depths. Overall positive relationships ($R^2$ 0.78 for Brienz, 0.72 for Lugano) are observed between HBI alkenes concentrations and the TOC-normalised contents of 16:1 $n$-FAs, indicating that these FAs can be used to track diatomaceous biomass.

4. Summary and conclusions

OM dynamics in the eutrophic Lake Lugano and the oligotrophic Lake Brienz were evaluated from the molecular composition of lipids in the POM. Differences in the trophic level and in associated primary productivity are reflected in enhanced alkalinity, elevated total organic carbon (TOC), and high chlorin contents in the water column of the Lake Lugano. Total absolute concentrations of FAs and neutral lipids are higher in the water column of Lugano during spring and autumn as compared to Brienz. The lipid compositions of POM in both lakes are dominated by carboxylic acids, alkanols, sterols and hydrocarbons. Total FA and neutral lipid contents vary over comparable ranges if normalised to TOC.

Comparison of the normalised concentration profiles of FAs and neutral lipids from both lakes results in higher contents in the euphotic zone of Brienz during spring, whereas the contents are higher in Lugano during autumn. Degradation of FAs and neutrals is more pronounced in the water column of Brienz. In Lugano the content of FAs relative to TOC remains constant below the chemocline. The results are interpreted as reflecting the relative contributions of primary productivity and refractory, allochthonous OM to POM. Increased particle load during late spring/early summer and

Fig. 11. Sketch of hydrological conditions, particle load and phytoplankton communities during spring and autumn in (a) Lake Brienz and (b) Lake Lugano.
inflows due to density stratification in contrast to the intensified settling of fine-grained particles and associated OM towards the bottom during autumn could explain the observed concentration profiles in Brienz (Fig. 11a). The depth trends at Lugano are explained as a result of high primary productivity, water column stratification and associated particle load in the upper layers, as well as microbially induced degradation close to the chemocline and greater preservation under anoxic conditions (Fig. 11b).

FA distributions and the composition of n-alkanols indicate a predominant autochthonous OM source (algae, zooplankton, bacteria) and generally minor contributions from aquatic macrophytes and land plants to both lakes. Decreased contributions of microbial plus diatomaceous biomass vs. algal OM to primary productivity during autumn in both lakes are consistent with changes in the relative proportions of C₁₆ vs. C₁₈ n-FAs and n-alkanols. Inputs of OM from diatoms are reflected in high relative abundances of C₁₆:₁ n-FAs, HBI alkenes and 24-methylcholesta-5,22-dien-3β-ol (either epibrassicasterol or brassicasterol). The concentration profiles of 18:₃ plus 18:₂ n-FAs from both lakes are in agreement with a greater contribution of cyanobacteria to autochthonous OM production during spring at Lake Lugano. Sitosterol is attributed to vascular plant input. An increased inflow of soil OM during spring is indicated by high C₂₉ sterols (sitosterol, stigmasterol) relative to the sum of sterol concentrations in both lakes. High relative proportions of 18:₀ n-FA and cholesterol in the autumn samples are interpreted to reflect increased input from zooplankton grazing in both lakes. A summary of lipid biomarkers and their suggested attribution to sources based on the data is provided in Table 5.

Intensified OM transformation processes are indicated by elevated chlorin index and lower δ¹³C values of DIC in Lake Lugano. Water depth trends in FA distribution and relative proportions of branched chain FAs argue for enhanced bacterial reworking of OM in the Lugano water column, most pronounced above and at the chemocline. The predominance of 16:₁₀₋₁₇ FA in the anoxic zone of the water column of Lugano is attributed to the activity of manganese-, iron- and sulfatereducing bacteria and methanogens. Intensified degradation of OM during autumn in both lakes is evidenced by high contents of mid-chain saturated n-FAs in the deeper parts of the water columns. In the spring water column

Table 4
Dominant lipid characteristics of POM from Lake Lugano during spring and autumn.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total FAs (μg/g TOC)</th>
<th>Total neutrals</th>
<th>Sat/unsat. FAs</th>
<th>Branched-chain/polyunsat. FAs</th>
<th>C₂₇ sterols</th>
<th>C₂₉ sterols</th>
<th>C₃₆ sterols</th>
<th>Sterols</th>
<th>n-Alkanols/n-alkanols + sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL 10</td>
<td>6828</td>
<td>1678</td>
<td>0.50</td>
<td>0.33</td>
<td>0.43</td>
<td>0.50</td>
<td>0.26</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>LL 40</td>
<td>1450</td>
<td>488</td>
<td>1.30</td>
<td>0.46</td>
<td>1.03</td>
<td>0.50</td>
<td>0.20</td>
<td>0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>LL 70</td>
<td>1283</td>
<td>503</td>
<td>0.95</td>
<td>0.57</td>
<td>1.25</td>
<td>0.31</td>
<td>0.20</td>
<td>0.49</td>
<td>0.13</td>
</tr>
<tr>
<td>LL 100</td>
<td>1792</td>
<td>649</td>
<td>0.76</td>
<td>0.77</td>
<td>2.57</td>
<td>0.32</td>
<td>0.21</td>
<td>0.57</td>
<td>0.22</td>
</tr>
<tr>
<td>LL 150</td>
<td>1293</td>
<td>229</td>
<td>1.16</td>
<td>0.64</td>
<td>2.35</td>
<td>0.49</td>
<td>0.14</td>
<td>0.36</td>
<td>0.08</td>
</tr>
<tr>
<td>LL 250</td>
<td>2154</td>
<td>504</td>
<td>2.22</td>
<td>0.48</td>
<td>1.82</td>
<td>0.64</td>
<td>0.08</td>
<td>0.28</td>
<td>0.09</td>
</tr>
</tbody>
</table>

| Autumn |
|--------|----------------------|----------------|----------------|-----------------------------|-------------|-------------|-------------|--------|-----------------------------|
| FLL 10 | 6728                 | 789            | 0.90           | 0.37                        | 0.38        | 0.46        | 0.48        | 0.06   | 0.01                        |
| FLL 40 | 1334                 | 155            | 1.52           | 0.33                        | 0.71        | 0.67        | 0.19        | 0.14   | 0.07                        |
| FLL 70 | 1437                 | 142            | 5.96           | 0.73                        | 10.67       | 0.70        | 0.07        | 0.23   | 0.10                        |
| FLL 100| 1468                 | 154            | 5.05           | 0.72                        | 7.38        | 0.62        | 0.11        | 0.28   | 0.15                        |
| FLL 150| 978                  | 73             | 5.63           | 0.89                        | 3.86        | 0.70        | 0.12        | 0.18   | 0.13                        |
| FLL 200| 1465                 | 118            | 1.92           | 1.02                        | 10.50       | 0.63        | 0.10        | 0.27   | 0.19                        |

Notes:
- Fatty Acids.
- Unsaturated.
- Saturated.
- Total organic carbon.

---

Table 5
Summary of lipid source input interpretations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Probable source</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₀ n-alkanoic acid (FA)</td>
<td>Algae, bacteria, high productivity</td>
</tr>
<tr>
<td>C₁₃ n-alkanoic acid (FA)</td>
<td>Zooplankton, algae, cyanobacteria</td>
</tr>
<tr>
<td>C₁₆ and C₁₄ n-alkanoic acid (FA)</td>
<td>Higher plants, macrophytes</td>
</tr>
<tr>
<td>Iso- and anteiso-C₁₆ and C₁₇ FA</td>
<td>Bacteria, microbial inputs</td>
</tr>
<tr>
<td>C₁₈ mono-unsaturated acid (FA)</td>
<td>Diatoms</td>
</tr>
<tr>
<td>C₁₆ mono-unsaturated acid (FA)</td>
<td>Cyanobacteria, algae, bacteria</td>
</tr>
<tr>
<td>16:₁₀₋₁₇ n-FA</td>
<td>Anaerobic bacteria (sulfate-, manganese-, iron-reducing bacteria)</td>
</tr>
<tr>
<td>18:₁₀₋₁₇ n-FA</td>
<td>Bacteria, microbial inputs</td>
</tr>
<tr>
<td>C₁₈ bi- and tri-unsaturated acids (FA)</td>
<td>Cyanobacteria, green algae, higher plants</td>
</tr>
<tr>
<td>C₁₆ n-alkanol</td>
<td>Diatoms, bacteria</td>
</tr>
<tr>
<td>C₁₈ n-alkanol</td>
<td>Algae, zooplankton</td>
</tr>
<tr>
<td>C₂₂- C₂₉ n-alkanols</td>
<td>Higher plants, macrophytes</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>Vascular plants, algae</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>Vascular plants, phytoplankton</td>
</tr>
<tr>
<td>24-Methylcholesta-5,22-dien-3β-ol</td>
<td>Diatoms, microalgae</td>
</tr>
<tr>
<td>24-Methylcholesterol</td>
<td>Higher plants, algae</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Zooplankton, phytoplankton</td>
</tr>
<tr>
<td>Dinosterol</td>
<td>Dinoflagellates</td>
</tr>
<tr>
<td>HBI alkenes</td>
<td>Diatoms</td>
</tr>
</tbody>
</table>
of Lugano, n-alkanol abundances relative to the total concentra-
tions of sterols in the neutral fractions are lowest close to the
oxic–anoxic interface. This is explained by high primary productiv-
ity in the photic zone and the associated export of OM into the dee-
per parts of the water column. Rapid changes in the microbial
communities involved in various biogeochemical cycles (nitrates-
manganese-, iron- and sulfate-reduction, as well as methanogene-
sis) at the oxic–anoxic interface are seen to be responsible for en-
hanced OM degradation of the more labile constituents (such as
C<sub>13</sub>–C<sub>20</sub> n-alkanols) at the chemocline.

Acknowledgements

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O. Scheidegger, M. Schurter, A. Brandt, and A. Zwyssig (EAWAG
Kastanienbaum, Switzerland) for technical assistance. We are
grateful to M. Simonov and M. Veronesi from the Institute of Earth
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Appendix IX

Rapid Changes in the Ecological Conditions of the Black Sea Over the Last 3 kyr
(Turkish with an extended abstract in English)

Authors
Güngör, E., Çagatay, M.N

Full publication: ITÜ Dergisi/d Mühendislik, 4, 5, 23-33
Karadeniz’de son 3000 yıllarda ani çevresel değişimler

Emin GÜNGÖR*, M. Namık ÇAĞATAY
İTÜ Avrasya Yer Bilimleri Enstitüsü, Yer Sistem Bilimi Programı, 34469, Ayazağa, İstanbul

Özet

Bu çalışmada Batı Karadeniz havzasından çoklu karotiyer (multi-corer) sistemi ile alınmış toplam iki karotta jeokimyasal analizler uygulanarak, son 3000 yılda önemli ani oluşmuş doğal ve antropojen kökeni çevresel değişimler saptanmış ve bu değişimler 210Pb yöntemi ve yaygınlanmış 14C verileri ile tarihendirilmiştir. İki ayrı istasyonun 600 m (BS98-09) ve 1319 m (BS98-15) su derinliğinde alınan bu karotlarda 210Pb radyoizotopu kullanarak tayin edilen toplam kütte birikim hızı (MAR) sırasıyla 171.5 ve 71.3 g.m⁻².y⁻¹ dir. Sedimentasyon hızı ise BS98-09 karotunda 72 cm.ky⁻¹ ve BS98-15 karotunda ise 24 cm.ky⁻¹ olarak bulunmuştur. Son 125 yıl içerisinde BS98-15 istasyonunda alınan karotta toplam organik karbon ve karbonat nortalama MAR değerleri 1.56 ve 24.26 g.m⁻².y⁻¹ olarak bulunmaktadır, BS98-09 istasyonundan alınan karotta ise toplam organik karbon ve karbonat nortalama MAR değerleri 13.4 ve 48.1 g.m⁻².y⁻¹ olarak bulunmuştur. Sapropel bimaminin en üst kısmında ise toplam organik karbon ve karbonat nortalama MAR değerleri 3.45 ve 13.54 g.m⁻².y⁻¹ olarak hesaplanmıştır. Metal (örneğin, Ba, Cu, Pb, Zn) konsantrasyonlarında son 80 yıl içerisinde çok hızlı bir artış gözlemektedir. Baryum zenginleşmesinin her iki karotun üst kısmında, normal doğal seviyesinden 5-5.5 kat daha fazla olduğu ve bunun da Karadeniz’de artan ötrüfikasyondan kaynaklandığı sanılmaktadır. BS98-15 karotunun üst kısmında Zn, Cu ve Pb’nün zenginleşmesi normal doğal seviyelerinden sırasıyla 5, 2 ve 9 kat daha fazladır. BS98-09 karotun üst kısmında ise aynı elementler sırasıyla 3.6, 2.4 ve 2 kat daha fazladır.

Anahtar Kelimeler: Karadeniz, kütte birikim hızı, organik karbon, karbonat, metaller.
Rapid changes in the ecological conditions of the Black Sea over the last 3 kyr

Extended abstract
Geochemical studies of two cores from the western continental margin of the Western Black Sea Basin at water depths of 600 (BS98-09) and 1319 (BS98-15)m revealed naturally and anthropogenically driven environmental changes in the Black Sea over the last 3000 yr. These changes were dated using the \(^{210}\text{Pb}\) analysis of this study and published \(^{14}\text{C}\) data. The studied cores were obtained using a multi-corer during a cruise of the International Atomic Energy Agency (IAEA) in 1998.

Mass accumulation rates (MAR) based on \(^{210}\text{Pb}\) dating are 171.5 g.m\(^{-2}\).yr\(^{-1}\) for BS98-09 and 71.3 g.m\(^{-2}\).yr\(^{-1}\) for Core BS98-15, respectively. The considerably high MAR value in Core BS98-09 is in agreement with its location being closer to the Danube delta than that of Core BS98-15. The average MARs of total organic carbon (TOC) and carbonate during the last 125 yr in Core BS98-15 are 1.56 and 24.26 g.m\(^{-2}\).yr\(^{-1}\), whereas the corresponding values in Core BS98-09 are 13.41 and 48.12 g.m\(^{-2}\).yr\(^{-1}\). The MARs of TOC and carbonate in the upper part of the sapropel unit are 3.43 and 13.54 g.m\(^{-2}\).yr\(^{-1}\) in Core BS98-15. Core BS98-15 includes a Coccolith Unit and the top of the underlying Sapropel Unit, whereas Core BS98-09 contains the Coccolith Unit only. The two units in Core BS98-15 have been deposited under anoxic conditions in the last 7500 yr BP (before present). The top of the Sapropel contains the “first coccolith band”, marking the first entry of Emiliania huxleyi in the Black Sea during the Holocene. The Coccolith Unit is microlaminated and consists of alternations of white E. huxleyi and dark organic-rich clay laminae. The unit in the studied cores contains 57.3-62.7% wt total carbonate and 3.5-4.1% wt total organic carbon (TOC). High carbonate content of this unit is almost totally made up of calcitic E. huxleyi coccoliths. The Sapropel Unit is an organic-rich black mud containing 26.3-36.1% wt carbonate and 5.6-10.4 % wt TOC.

The high MAR\(_{\text{CaCO}_3}\) value of the Coccolith Unit is caused by the presence of calcitic coccoliths and coccospores of E. huxleyi. The average sedimentation rate, calculated for whole of the Coccolith Unit using the 2000 yr (corrected) \(^{14}\text{C}\) age of Coccolith/Sapropel boundary (Arthur and Dean, 1999) and assuming a linear sedimentation rate, was found to be 1.5 to 3 times lower than the sedimentation rates for the last 125 years computed from the \(^{210}\text{Pb}\) data. This indicates that the sedimentation rate has not been constant during the last 2000 years and that it increased drastically especially in the last few hundred years as a result of human impact in the form of deforestation and agricultural activities. The “first coccolith band”, dated 2000 yr BP (present before) by Arthur and Dean (1999) and 2720 BP by Jones and Gagnon (1994), marks the first appearance of E. huxleyi in Holocene. This was an important event in the oceanographic and sedimentological history of the Black Sea, causing a large carbonate flux to the seafloor. Colonization of the Black Sea surface waters by E. huxleyi at about 2000 yr BP was most probably because of the increase in the sea surface salinity to a threshold value of 11% that is needed for the survival of these organisms (Paasche, 2002).

A period of highest carbonate deposition, corresponding to high E. huxleyi production, was observed in both cores at different core depths. According to the \(^{210}\text{Pb}\) dating, these depths correspond approximately to AD 1700, with the carbonate peak widths corresponding to a period of about 100 years between AD 1750-1650. This time period can be correlated by the “Late Maunder Minimum”, the coldest phase of the “Little Ice Age”, which was characterized by glacier advances in Northern and Southern Hemispheres, average annual temperatures 1-2°C lower than the present, and an increased precipitation in most of Europe.

Metals (Ba, Cu, Pb, Zn) show sharp increases in concentrations towards the top of the cores. Barium enrichment is 5-5.5 times the background values in both cores, attesting to the increased eutrophication of the Black Sea. Zn, Cu and Pb enrichments at the top of the cores are 5, 2 and 9 times the background values in Core BS98-15 and about 3.6, 2.4 and 2 times in Core BS98-09, showing the strong industrial metal pollution of the Black Sea sediments in the last 80 years.

Keywords: Black Sea, holocene sediment, organic carbon, carbonate, metals.
Giriş
Maksimum 2250 m derinliğe sahip olan Karadeniz dünyanın en büyük oksijensiz (anoxic) denizidir (Şekil 1). Takriben 100-150 m derinliğinde oksijenli ve az tuzlu (yaklaşık %18) üst su tabakasında, daha derinde H2S içeren oksijensiz özellikle sahip alt su tabakası ile bular arasında çeşitli biyokimyasal ve redoks reaksiyonlarının olduğu yaklaşık 40-50 m kalınlığında bir geçiş zonu (suboxic) mevcuttur (Murray vd., 1989; Oğuz vd., 2002). Karadeniz’in Holosen döneminde ait sedimentleri üç birimden ibarettir (Ross ve Degens, 1974; Çağatay, 1999). Bu birimler, karotun üst kısmından başlayarak tabana doğru; 1) laminalı (ince tabakalı) Coccolithic marl Birim (Kokolit Birimi veya Birim I), 2) organik madde açısından zengin, mikro-laminalı sapropelik bir birim (Sapropel Birimi veya Birim II) ve 3) tatlı-acı su göl ortamında köklenmiş Lutite Birimidir (Birim III). Üstteki iki birim, Akdenizin tuzlu sularının Çanakkale ve İstanbul Boğazları vasıtasıyla Karadeniz’i istila ettiği sırasında depolanmıştır (Ross ve Degens, 1974; Jones ve Gagnon, 1994; Ryan vd., 1997; Arthur ve Dean, 1999; Ryan vd., 2003).


Bu çalışmada, Batı Karadeniz’in kita sahanlığından alınan (BS98-09, BS98-15) iki sediment karotunda (Şekil 1) sediment (SR) ve toplam kütle birikim hızı (MARSED), toplam organik karbon (TOC), toplam karbonat ve metal dağılımları (Ba, Cu, Pb, Zn) incelenmiştir.

Şekil 1. BS98-09 ve BS98-15 karotlarının alındıkları lokasyonlar (BS4-9 ise Buesseler ve Benitez (1994)’in inceledikleri karotun bulunduğu lokasyon)
Sediment karotlarının yaşlandırılması 210Pb yöntemi ile yapılmıştır. Buna dayanarak, Birim I’in üstü için MAR TOC ve MAR CACO3 değerleri hesaplanmıştır. Ayrıca Birim I/Birim II sınırı için yayınlanmış kalibre 14C yaşıları kullanarak Birim II’nin üstü için MAR TOC ve MAR CACO3 değerleri hesaplanmıştır. Sonuçlar ıklim değişimi ile metal kirliliği ve tarihçesi açısından yorumlanmıştır.

**Tablo 1. Karadeniz’de holosen yaşlı çökel istifinin kronolojisi**

<table>
<thead>
<tr>
<th>Stratigrafik seviye</th>
<th>Yaş (yı)</th>
<th>Metod</th>
<th>Kaynak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sapropel Birimi tabanı (Birim II/III sınırı)</td>
<td>7090±180</td>
<td>14C</td>
<td>Ross ve Degens (1974)</td>
</tr>
<tr>
<td>(Birim III)</td>
<td>6600</td>
<td>14C</td>
<td>Calvert v.d. (1987)</td>
</tr>
<tr>
<td>(Birim II/III sınırı)</td>
<td>7540±130</td>
<td>14C</td>
<td>Jones ve Gagnon (1994)</td>
</tr>
<tr>
<td>(Birim II)</td>
<td>7800</td>
<td>14C</td>
<td>Arthur ve Dean (1999)</td>
</tr>
<tr>
<td>(Birim I)</td>
<td>5083</td>
<td>Varv sayımı</td>
<td>Degens ve Stoffers (1980)</td>
</tr>
<tr>
<td>İlk Kokolit bandı</td>
<td>3450±120</td>
<td>14C</td>
<td>Ross ve Degens (1974)</td>
</tr>
<tr>
<td></td>
<td>2720±160</td>
<td>14C</td>
<td>Jones ve Gagnon (1994)</td>
</tr>
<tr>
<td></td>
<td>1633</td>
<td>Varv sayımı</td>
<td>Hay v.d. (1991a)</td>
</tr>
<tr>
<td>Son Kokolit istilası ve süreleri</td>
<td>3200±140</td>
<td>14C</td>
<td>Ross ve Degens (1974)</td>
</tr>
<tr>
<td>Kokolit Birimi (I) başlangıcı</td>
<td>1635±60</td>
<td>14C</td>
<td>Jones ve Gagnon (1994)</td>
</tr>
<tr>
<td></td>
<td>998</td>
<td>Varv sayımı</td>
<td>Degens ve Stoffers (1980)</td>
</tr>
<tr>
<td></td>
<td>1256</td>
<td>Varv sayımı</td>
<td>Hay v.d. (1991a)</td>
</tr>
</tbody>
</table>

**Malzeme ve yöntem**

BS98-09 (44°28.118’N, 31°15.178’E, 600 m derinlik) ve BS98-15 (43°29.094’N, 30°42.367’E, 1319 m derinlik) karotları, Uluslararası Atom Enerjisi Ajansı’nın (IAEA) Batı Karadeniz’den düzenlediği CRUISE98 RADEUX isimli bilimsel sefer sırasında temin edilmiştir (Sekil 1). Karotlar, MARK II 400 multicorer kullanarak sulu çamurlu üst kısmıl ile birlikte bozulmadan alınmıştır. Daha sonra 1 cm’lik dilimler halinde örnekler hazırlanmıştır.

Laboratuvara getirilen örneklerin ilk olarak yaş ağırlıklarını tahmin edilmiş ve daha sonra örnekler döndürülen ve kurutulularak kilometrelik (freeze-drier) yöntemi ile kurutulmuştur. Kurutma işlemi, örnekler sabit ağırlığa gelinceye kadar devam etmiştir. Tarihlemeye yönteminde 210Pb’dan meydana gelen 210Po radionüklidinin 5.30 Mev’deki (T1/2 : 138 gündür) metastabil nötral ve numunede olmasından dolayı (135) standard 210Po radyonüklidinin 137 odaya gelinceye kadar buharlaşımıştır. Daha sonra kurutulan ve örnekler üzerinde çözdürülen örnekler sabit konsantrasyonu sabit tutulmuş ve örnekler 0.5M HCl çözeltisi içine alınarak 8 saat boyunca polonyumlar transfer edilmiştir. Daha sonra örnekler Degens ve Stoffers (1980) yöntemi kullanılarak analiz edilmiş ve her bir örnek için 50 mg/ml askorbin asit ilave edilerek 8 saat boyunca polonyumların kaplama işlemi yapılmıştır.

Kaplama işlemi tamamlandıktan sonra, örnekler saf su ile yıkanarak, daha oteden etiketlenmiş zarflarla yerleştirilmiştir. ORTEC marka alfa spektrometre kullanılarak örneklerin analizleri yapılmıştır. Sayım süresi, %5’lik mutlak standard hatanın altında inecek şekilde ayarlanmıştır. Her bir örnek ait kiyasal verim yaklaşık %80 civardadır. Sonuçların güvenilirliğini sağlamak amacıyla IAEA’nın düzenlediği karşılştırmalı analiz çalışmasında BS-1 örneği analiz edilmiş ve elde edilen sonuçlar, referans değerler ile birlikte Tablo 2’den verilmiştir.

Ayrıca analizlerin güvenilirliğini temin etmek için IAEA-135 standardi kullanılarak doğrulama ve tekrarlanabilirlik testleri de yapılmış olup sonuçlar Tablo 3’tedir verilmektedir.
210Pb olmayan karotun alt kısımlarında sediment birikim hızı Bioturbasyonun olmadığı durumda sabit kalır. Karotun 32 cm kalınlığı, supérieur 210Pb ise karotun alt kısımlarında sediment birikim hızını belirler. BS-1 karotunun üst 32 cm'lik birikim periyoduna ait 210Pb akışları, 1N HCl ile çözülür. Ayrıca TOC analizleri için, örnekler 4M HCl ile muayene edilir ve 4M HCl ile muayene edilen örneklerin %95 güvenilir aralığı, %5 den daha küçük ve terimal ile ayırt edilebilir. Toplam karbonat analizlerinde ise, örneklerin 4M HCl ile muamele edilmesi sonucu oluşan CO₂ hacminin ölçülmesi yöntemi kullanılmıştır (Loring ve Rantala, 1992). Örnekler toplam asit karbonat toplam karbonat %ağırlıkta verilen denklemi ile hesaplanır; 

\[
\text{MAR} = \frac{\text{TOC(CaCO}_3\text{)}}{100\times\text{SRx}(1-\rho/\rho_a)} (2)
\]

Burdan \(SR = \text{sediemantasyon hızı (cm.ky}^{-1}\), \(\Phi = \text{özneşiklilik (\%)}, \rho = \text{yoğunluk, g/cm}^{3}\).

Kokolit Biriminin toplam karbonat miktarı %57.3-81.3 arasında ve C_{organic} miktarı %2.9-5.6 arasında değişmektedir. Sapropel Biriminde ise toplam karbonat %26.2-37.1 ve C_{organic} %5.6-0.4 aralıklarında değişmektedir (Şekil 2).

BS98-09 karotundaki çökelt istifisi Kokolit ve organik madde zengin kılıçlı laminaların ardalanmasından meydana gelmiştir. Bu karotun Kokolit Birimindeki toplam karbonat miktarı %23-51 ve C_{organic} miktarı %0.9-4.7 arasında değişmektedir (Şekil 2).

Maksimum karbonat miktarları BS98-15 karotunda 9.5 cm’de, BS98-09 karotunda ise 18 cm karot derinliğinde görülmektedir (Şekil 2). Bu değerler yüksek Kokolit üretiminin olduğu bir dönemde temsil etmektedir. Bu dönemin yaş ve iklimde olan ilişkisi aşağıdaki tartışılması.

**Toplam sediment, organik karbon ve karbonat kütle birikim hızları**

BS98-09 ve BS98-15 karotlarındaki fazla 210Pb değerinin derinlikle azalığı görülmüştür (Şekil 3). Denklem 1 kullanılarak, BS98-09 ve BS98-15 karotları için sedimantasyon hızı (SR) sırasıyla 72 cm.ky^{-1} ve 24 cm.ky^{-1} olarak bulunmuştur, toplam kütle birikim hızı (MAR) ise 171.5 g.m^{-2}.y^{-1} ve 71.25 g.m^{-2}.y^{-1} olarak bulunmuştur. BS98-09’daki sedimentasyon hızının BS98-15’deki sedimentasyon hızına göre yaklaşık 3 kat daha fazla olduğu görülmektedir. BS98-09’da da yüksek sedimentasyon hızı, bulunduğu yerin önemli bir sediment kaynağı olan Tuna Nehri Delta’sı’na olan yakınılığı ile ilişkilidir.

BirimI/BirimII arasındaki sınırlık yaşının 14C ile yapılan yaş tayinine göre 2000 yıl (Arthur ve Dean, 1999) ve karotun üst kısmından BirimI/BirimII sınırlarında kadar lineer bir sedimentasyon hızı olduğu kabul edilerek BS98-09 ve BS98-15 karotlarında BirimI için sedimentasyon hızı sırasıyla 25 ve 16.3 cm.ky^{-1} olarak bulunmuştur (Şekil 3, Tablo 4).

**Tablo 4. Sedimantasyon hızı (SR), organik karbon ve karbonat birikim hızları**

<table>
<thead>
<tr>
<th>Karot</th>
<th>Birim</th>
<th>TOC (a%)</th>
<th>Karbonat (a%)</th>
<th>SR (cm.ky^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS98-09 I</td>
<td>2.93</td>
<td>31.90</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>BS98-15 I</td>
<td>3.83</td>
<td>59.50</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>BS98-15 II</td>
<td>7.96</td>
<td>33.20</td>
<td>16.3</td>
<td></td>
</tr>
</tbody>
</table>


Ortalama 2000 yıl boyunca hesaplanan bu oranlar, 210Pb ile son 125 yıl için hesaplanan oranlara göre 1.5 ve 3 kez daha küçüktür. Buradan son 2000 yıl içerisinde meydana gelen sedimentasy-
Karadeniz`de son 3000 yıldaki ani çevresel değişimler

yon hızının sabit olmadığı ve muhtemelen son birkaç yüzyl içerisinde insan etkilerinden dolayı önemli bir oranda arttığı tahmin edilebilir.

Calvert vd. (1991) Batı Karadeniz`in 2087 m su derinliğinden aldıkları karotta (Şekil 1, BS4-9) 14C kullanarak yaptıkları çalışmada son 2000 yılın ortalama toplam kütle birikim değerini 38.7 g.m⁻².y⁻¹ olarak tahmin etmişlerdir. Bu'nunla beraber Buesseler ve Benitez (1994) aynı karotta (BS4-9) ²¹⁰Pb ile yaptıkları çalışmada yaklaşık son 125 yıl için ortalama toplam kütle birikim değerini 69±3 g.m⁻².y⁻¹ olarak bulmuşlardır. Kütle birikim hızlarındaki bu farklı zamanda sedimentasyon hızındaki önemli bir artış göstermektedir. Buesseler ve Benitez`in (1994) MAR değeri, bu çalışmada bulunan 71.25 g.m⁻².y⁻¹ MAR değerine benzerdir. BS98-15 karotunda son 125 yılı temsil eden karotun en üst 3 cm'inde ortalama MAR_TOC ve MAR_CaCO₃ değerleri sırasıyla 1.57 ve 24.26 g.m⁻².y⁻¹ olarak bulunurken, BS98-09 karotunda ise bunu karşılık gelen MAR_TOC ve MAR_CaCO₃ değerleri 13.41 ve 48.12 g.m⁻².y⁻¹ olarak bulunmuştur (Tablo 5).

MAR_TOC ve MAR_CaCO₃ değerleri ile MAR_TOC ve MAR_CaCO₃ değerlerinin toplam arasındaki fark silsili kırımı malzeme miktarını vermektedir. BS98-09 karotunda bu kırımı malzemesi BS98-15 karotuna göre 3.3 kez daha fazladır. Birimi/BirimII sınavının yaşı 2000 yıl olarak alındığında (Arthur ve Dean, 1999), BS98-15 karotunda Birim için ortalama sedimentasyon hızı 16.25 cm.kyr⁻¹ olarak bulunmuştur. Bu sedimentasyon hızını kullanarak, sapropel biriminin üst kısmını için ortalama MAR_TOC ve MAR_CaCO₃ değerleri ise 3.43 ve 13.54 g.m⁻².y⁻¹ olarak bulunmuştur (Tablo 5).

Tablo 5. MAR_TOC ve MAR_CaCO₃ değerleri

<table>
<thead>
<tr>
<th>Karot</th>
<th>Birim</th>
<th>MARSED</th>
<th>MAR_TOC</th>
<th>MAR_CaCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS98-0</td>
<td>I</td>
<td>171.5</td>
<td>13.4</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.86-20.4</td>
</tr>
<tr>
<td>BS98-1</td>
<td>I</td>
<td>71.3</td>
<td>1.57</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.35-1.84</td>
</tr>
<tr>
<td>BS98-1</td>
<td>II</td>
<td>-</td>
<td>3.43</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.65-4.09</td>
</tr>
</tbody>
</table>

Şekil 3. BS98-15 (sol) ve BS98-09(sağ) karotlarda fazla (Excess) ²¹⁰Pb`un grafiği

HYPOX Deliverable 4.4 version 1
Sapropel birimindeki MAR\textsubscript{TOC} değeri birimlerdeki MAR\textsubscript{TOC} değeri nazaran 2.2 kez daha fazla ve MAR\textsubscript{CaCO\textsubscript{3}} ise birimlerdeki MAR\textsubscript{CaCO\textsubscript{3}} değeri nazaran 1.8 kez daha azdır. Birimlerde yüksek MAR\textsubscript{CaCO\textsubscript{3}} değeri genellikle yaz ve sonbahar aylarında çoğalan ve sedimentte beyaz ince laminali tabakayı oluşturan E. huxleyi türündeki Kokolit’lerden kaynaklanmaktadır. Bu organizmalar Karadeniz’in ilk defa 2000-3000 yıl öncesinde ve muhtemelen Karadeniz’de üst su tuzluluğun %11’lik eğri ile çakıyan en sırfi (M.S 1300-1900) olarak isimlendirilen peri-...

Toplam karbonat değerinin %11’lik etozluluk öncesinde ve muhtemelen Karadeniz’de üst su tabakasına kaynaklanmıştı ve Bu elementin BS98-15 karotunun üst 2 cm’inde hızla yükselenler alttaki normal seviyesinden 5 kat daha fazla olduğu görülmektedir (Şekil 4). Bu yüksek derecedeki artışin \(^{210}\text{Pb}\) tarihlendirmesine göre yaklaşık son 80 yıl içersinde organik üretimden (ötrüfikasyon) kaynaklandığı tahmin edilmiştir. Sapropel Biriminin üst kısımda alüminyum ile normalize edilen Ba değerlerinin yüksek olması, sapropel oluşumu sırasında daha fazla organik madde üretiminden kaynaklanmıştır.

Karbonat değerleri ve iklim değişimi

Metal kirliliği ve tarihiçe
Baryum organik üretim önemli bir belirtecdir (Bishop, 1988; Wefer vd., 1999; Gingegele, 1999). Bu elementin BS98-15 karotunun üst 2 cm’inde hızla yükselenler alttaki normal seviyesinden 5 kat daha fazla olduğu görülmektedir (Şekil 4). Bu yüksek derecedeki artışın \(^{210}\text{Pb}\) tarihlendirmesine göre yaklaşık son 80 yıl içersinde organik üretimden (ötrüfikasyon) kaynaklandığı tahmin edilmiştir. Sapropel Biriminin üst kısımda alüminyum ile normalize edilen Ba değerlerinin yüksek olması, sapropel oluşumu sırasında daha fazla organik madde üretiminden kaynaklanmıştır.

Sonuçlar
Karadeniz’in son 3000 yıl boyunca dört önemli ani çevresel değişim olduğu söylenebilir. Bunlar:

1. Yaklaşık 2700 yıl önce Karadeniz’in yüzey suyu tuzluluğunu ~11 ppt değeri ulaşarak, E. huxleyi türü organizmaların yaşaması için uygun bir ortam haline gelmiştir. Bunun sonucu o zamana kadar çökelen Sapropel yerine Koko-}

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Karadeniz’de son 3000 yıldaki ani çevresel değişimler

Şekil 4. BS98-15 karotunda Al ile normalize edilen metallerin derinlik boyunca dağılımları (metal/Al x 10⁻⁴)

Şekil 5. BS98-09 karotunda Al ile normalize edilen metallerin derinlik boyunca dağılımları (metal/Al x 10⁻⁴)
son birkaç yüzyılda çevresel üzerinde özellikle insan aktivitelerinin neden olduğu erozyon hizndaki artıştan kaynaklanmıştır.

(3) Kokolit üretimi M.S.1700 yılı civarında 100 yıllık bir zaman dilimi boyunca artmıştır. Bu zaman dilimi, “Küçük Buzul Devri” döneminin en soğuk safhası olan “Late Maunder Minimum (AD1645-1715)” periyodunda karşılk gelmektedir. Bu soğuk dönemde Karadeniz’i etkileyen okenus–atmosfer etkilemesi göreceli olarak yüksek Kokolit üretimine neden olmuştur.

(4) Ba, Cu, Pb ve Zn gibi metallerin konsantrasyonlarında son 80 yılı içerisinde önemli artışlar meydana gelmiştir. Ba, organik üretimin artışını gösteren önemli bir veridir (Bishop, 1988; Wefer vd. 1999; Gingele, 1999). Karotlarda son 80 yıllık Ba zenginleşmesi doğal seviyesine göre yaklaşık 4-5 kat daha fazladır. Karotlar boynuzca metal değerlerinin değişimi, Karadeniz’in son 80 yıl boynuzca yoğun bir şekilde örtüfikasyon ve metal kırlılığı ile karşı karşıya kaldığını göstermektedir.

### Kaynaklar


