

Supplement of Biogeosciences, 16, 1–16, 2019
<https://doi.org/10.5194/bg-16-1-2019-supplement>
© Author(s) 2019. This work is distributed under
the Creative Commons Attribution 4.0 License.



Supplement of

Controls on zooplankton methane production in the central Baltic Sea

Beate Stawiarski et al.

Correspondence to: Oliver Schmale (oliver.schmale@io-warnemuende.de)

The copyright of individual parts of the supplement might differ from the CC BY 4.0 License.

S1. Hydrographical and chemical characteristics of the water column

A SBE 911 plus CTD system (Seabird Electronics, USA) was used on the cruise to measure the temperature, salinity, chlorophyll *a*, and oxygen concentration along vertical depth profiles. The system consisted of an SBE 9 plus CTD unit with redundant sensor packs and an SBE 11 plus deck unit. The CTD unit was equipped with twin SBE 3plus temperature sensors, twin SBE 43 oxygen sensors, and a fluorescence sensor for chlorophyll *a* (FLNTURTD, Wetlabs, USA), all connected to SBE 5T submersible pumps. The SBE Seasoft V2 software package was used for data analysis.

Subsamples for measurements of dissolved methane (CH₄) concentrations and for its stable carbon isotopes ($\delta^{13}\text{C CH}_4$) were obtained from 10 L free-flow bottles (Hydrobios, Germany) attached to a rosette water sampler. Samples for methane analysis were filled into 250 ml glass flasks, spiked with 100 μl of saturated mercury chloride solution to stop biological activity and stored at 10°C until further analysis. The methane concentrations were measured with a purge and trap system connected to a gas chromatograph as described by Jakobs et al. (2014) after the cruise. For $\delta^{13}\text{C CH}_4$ analysis, 600 ml of water were sampled into 1100 ml pre-evacuated glass bottles and the gas was extracted within 3 hours upon sampling using a partial vacuum extraction line (Keir et al., 2009). A 10 ml aliquot of the gas was kept for the $\delta^{13}\text{C CH}_4$ analysis. The $\delta^{13}\text{C CH}_4$ values were determined using a modified continuous flow Isotope Ratio Mass Spectrometer IRMS (Thermo Fisher MAT253), coupled to the Gas Chromatograph (GC, Agilent 6890) by a GC-C/TC III interface (Thermo Fisher, modified after Schloemer et al., 2016). The modifications involved both a valve box with three different sampling loops, and a direct injection port. The system was equipped with a 1 m 1/16" packed column (ID 1 mm, Porapak Q mesh range 80/100), which was cooled down for 2 minutes in liquid nitrogen prior to analysis. For analysis, 7 ml of the subsamples were manually injected into the helium carrier gas stream. The water samples were stripped and cryo-focussed at a flow rate of $\sim 8 \text{ ml min}^{-1}$ for 5 minutes. The methane was separated from the other gas species by slowly thawing the trap at ambient temperature ($\sim 20^\circ\text{C}$) for 2 minutes, and a two-step GC controlled temperature program (start temperature 30°C, hold for 4 minutes, heating up to 160°C with a rate of $30^\circ\text{C min}^{-1}$, hold for 12 minutes) at a constant carrier gas flow of 1.8 ml min^{-1} . Finally, methane was combusted to carbon dioxide (CO₂) at 980°C and measured by IRMS. Daily stability tests of the system were performed using methane in air (8 ml injection volume) and the $\delta^{13}\text{C CH}_4$ values were found to have a precision of $\pm 0.5 \text{ ‰}$ (2 SD, n=12).

S2. Methane stripping-oxidation line

To measure the $^{14}\text{CH}_4$ produced by grazing on ^{14}C -labelled phytoplankton in the field, we used a sea-going methane stripping-oxidation line. The incubation bottles were connected to the line using stainless steel needles, which were attached to Perfluoroalkoxy (PFA Special Tubing, Swagelok, PFA-T2-030-100) tubings. The water samples were stripped for 10 min using a helium stream ($\sim 30 \text{ ml min}^{-1}$) and the gas stream was dried in a Nafion moisture exchanger (Ansyco, Type MD-050-

24S-2). Any $^{14}\text{CO}_2$, which was produced by respiration of phyto- or zooplankton, was removed by passing through a glass tube (150 mm x 10 mm, 5 mm inner diameter) filled 2:1 with Ascarite II (Sigma-Aldrich, ~20-30 mesh) and magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$, ELTRA, 90200). The magnesium perchlorate was added to remove any remaining water in the gas stream. The volatile hydrocarbons were focused on a trap filled with HayeSep D (CS-Chromatographie Service GmbH, 60/80 mesh; trap size: length 600 mm, diameter 1/8"). This trap was placed in an ethanol bath, which was cooled down to -120°C with liquid nitrogen (Fig. 2, position 1). It was subsequently transferred into a heated water bath (90°C , Fig. 2, position 2) to release the concentrated hydrocarbons. To finally separate the methane from the other hydrocarbons, the gas mixture was carried through an aluminum oxide column (ALLTECH, Activated Alumina F-1 60/80, 3M x 1/8" x 0.085"), fitted in a gas chromatograph (GC, Shimadzu GC2014; temperature program: start temperature 45°C , hold for 4 minutes, heating up to 200°C with a rate of $25^\circ\text{C min}^{-1}$, hold for 1 minute). The stream was redirected after 6 minutes, using a 6-port 2-position valve (VICI Instruments), so that only the methane stayed downstream in the system, while other hydrocarbons were released through the vent. The $^{14}\text{CH}_4$ was then carried through a Copper(II) oxide combustion line at 850°C using synthetic air (12 ml min^{-1}), where it was oxidized to $^{14}\text{CO}_2$. The $^{14}\text{CO}_2$ was trapped in two 10 ml scintillation vials, each filled with 3 ml of a mixture of phenylethylamine and ethylene glycol monomethyl ether (1:7 v/v) (Jakobs et al., 2013). 3 ml Ultima Gold MV (Perkin Elmer) scintillation cocktail were added and the samples were stored in the dark for 3 hours before the activity was measured by liquid scintillation counting (Perkin Elmer; Tri-Carb 2800TR). A quench curve was provided by Perkin Elmer for the corresponding scintillation cocktail.

For system calibration before the cruise, two standard injection points were built into the system. Injection point 1 was placed right after the steel needles, which connect the line with the incubation bottles and injection point 2 was placed at the entrance of the combustion line. A non-radiolabeled calibration standard consisting of CH_4 , C_2H_6 , C_3H_8 , $n\text{-C}_4\text{H}_{10}$, $i\text{-C}_4\text{H}_{10}$ (10 ppm, Linde) was injected into injection point 1 to determine the runtime and flow rate needed to recover all methane from the HayeSep D trap after heating, but also to avoid other hydrocarbons to pass the 6-port valve and enter the combustion line. For these tests the combustion line was replaced temporarily and the hydrocarbons were quantified with a flame ionization detector (FID). To determine the efficiency of the combustion line, a $^{14}\text{CH}_4$ calibration standard (stock: $1.06 \mu\text{Ci ml}^{-1}$) was injected into injection point 2 and the recovery of $^{14}\text{CO}_2$ was quantified via liquid scintillation counting. During the field campaign, the latter standard was injected daily into injection point 1 to ensure the recovery rate was consistent and stable over time.

The stripping efficiency of the system was determined with $86.8 \pm 3.7 \%$ by stripping air equilibrated deionized water samples and subsequent GC-FID quantification of methane. This value was used for correction of the methane production within the experiments. The blanks of the method were measured daily ($109 \pm 46 \text{ dpm}$), to ensure stable performance during the cruise. The dpm obtained within the methane production experiments with zooplankton grazing on *Rhodomonas* sp. were 3.7-8.1 times higher, and on cyanobacteria were 0.9-1.2 times higher than the blanks.

References

- Jakobs, G., Rehder, G., Jost, G., Kießlich, K., Labrenz, M., and Schmale, O.: Comparative studies of pelagic microbial methane oxidation within the redox zones of the Gotland Deep and Landsort Deep (central Baltic Sea), *Biogeosciences*, 10, 7863-7875, 2013.
- 5 Jakobs, G., Holtermann, P., Berndmeyer, C., Rehder, G., Blumenberg, M., Jost, G., Nausch, G., and Schmale, O.: Seasonal and spatial methane dynamics in the water column of the central Baltic Sea (Gotland Sea), *Cont. Shelf Res.*, 91, 12-25, 2014.
- Keir, R., Schmale, O., Seifert, R., and Sültenfuß, J.: Isotope fractionation and mixing in methane plumes from the Logatchev hydrothermal field, *Geochem. Geophys. Geosy.*, 10, Q05005, 2009.
- 10 Schloemer, S., Elbracht, J., Blumenberg, M., Illing, C.J.: Distribution and origin of dissolved methane, ethane and propane in shallow groundwater of Lower Saxony, Germany, *Appl. Geochem.*, 67, 118-132, 2016.