Cruise report: 64PE547 NEREIDES

Embarking: 31 July, Reykjavik (Iceland)
Disembarking: 29 Aug, Azores, Ponta Delgada

Participants:

1) Gerhard J. Herndl (UNIVIE, Chief Scientist)
2) Thomas Reinthaler (UNIVIE, Deputy Chief Scientist)
3) Chie Amano (UNIVIE)
4) Maria Papadatou (UNIVIE)
5) Kristin Bergauer (GEOMAR Kiel)
6) Theres Koch (UNIVIE)
7) Kangii Guo UNIVIE)
8) Marilena Heitger (UNIVIE)
9) Gregory Lang (Old Dominion Univ. Virginia)
10) Maria Pinto
11) Tinkara Tinta (Marine Biology Station Piran, Slovenia)
12) Manuela Pia Felsbacher (UNIVIE)
13) Karel Baker (NIOZ
14) Leo Wuis (NIOZ)

Nutrient analysis

Karel Bakker, nutrient laboratory, Department OCS, NIOZ, Netherlands.

Summary

The availability of nutrients, temperature and light play a crucial role for the production of oceanic phytoplankton. At all stations from a CTD-Rosette sampler and a few experiments, samples were collected for shipboard macronutrient determination. The macronutrient measurements were made simultaneously on four channels for Phosphate Ammonium, Nitrite and Nitrate, using a Seal QuAAtro 39 gas-segmented Continuous Flow Analyser (CFA). In total 220 samples were measured on board during the expedition.

Equipment and Methods

The colourimetric methods used

Phosphate (PO_4^{3-})

Soluble reactive ortho-phosphate is measured by formation of a blue reduced Molybdenum-phosphate-complex at pH 0.8. Potassium Antimony tartrate used as the catalyst and ascorbic acid as the reducing agent. The absorbency is measured at 880nm.

Described by J.Murphy and J.Riley, 1962. Analytica Chem.Acta

Ammonium (NH_4^+) reacts with phenol and sodium hypochlorite at pH 10.5 to form an indo-phenol blue complex. Citrate is used as a buffer and complexing reagent to prevent precipitation of calcium and magnesium at this high pH. The blue colour is measured at 630nm (Koroleff, 1969 and optimized by W. Helder and R. de Vries, 1979).

Nitrate plus Nitrite (NO_3^-+NO_2^-) is mixed with an Imidazole buffer at pH 8 and reduced by a copperised cadmium column to Nitrite. The Nitrite is diazotized with sulphanyl-amide and naphtyl-ethylene-diamine to a pink coloured complex and measured at 520nm. Nitrate is calculated by subtracting the Nitrite value of the Nitrite channel from the ‘NO3+NO2’ value. Described by Grasshof, 1983. (Seawater Methods practical handbook Weinheimverlag).

Nitrite ($NO2^-$)

Diazotation of nitrite with sulphanilamide and N-(1-naphtyl)-ethylene di-ammonium dichloride to form a pink dye measured at 520nm.

Sample handling

The samples from the CTD-rosette sampler were collected in 60ml high-density polyethylene syringes with a three-way valve mounted, and connected directly with a tygon tubing without any air contact to the Niskin bottles. After sampling, subsampling in 6ml HDPE “pony-vials” with a volume of 6ml as sample cups fitting into the auto-sampler pony-vials was done all covered with stretched parafilm to avoid evaporation and direct influx of NH_3. Samples were left for 1 to 2 hours in the dark at lab temperature to get the same temperature as the calibration standards used. The CTD samples were analysed typically within 3 hours after sampling. All tubes were pre-rinsed three times with sample before being filled. A total of 220 samples for four parameters were analysed including a test CTD station from the start on, 57 from the experiments and 163 samples collected with the CTD.

Calibration and Standards

A sampler-rate of 60 samples per hour was used (sample to wash ratio 3).
Every day Calibration standards were freshly prepared in Low Nutrient Sea Water (LNSW), diluted from separate stock standards of different nutrients. LNSW was also used as baseline-water for the analysis to wash out in-between the samples. Each run of the system had a correlation coefficient of at least 0.9999 for 10 calibration points using a linear chemistry for PO_4, NH_4 ,NO_2 and NO_3 + NO_2. Samples were measured from the lowest to the highest concentration in order to keep carry-over in the flow system as small as possible, i.e. from surface to deeper waters. Concentrations were reported in 'µmol per liter' (µM/L) at an average lab temperature of 23°C. During the expedition in each run, a freshly diluted mixed internal nutrient standard (cocktail2008), containing phosphate and nitrate, was diluted 100 times in LNSW and measured. The cocktail sample was used to monitor, independently of the standards, the performance of the system.

Stock standards

Nutrient primary stock standards were prepared at the home- lab by weighing nutrient salts p.a. in de-ionised water. All standards are kept in a so-called 100% humidity box at lab temperature to prevent any concentration change by evaporation, and to be on working temperature ready for diluting with pipettes.

PO_4^{3-}: by weighing Potassium dihydrogen phosphate in a calibrated volumetric PP flask to make 1mM PO_4 stock solution.
NH_4^+: by weighing in Ammonium Chloride in a calibrated volumetric PP flask to make a 1mM $NH4^+$ solution.
NO_3^- : by weighing Potassium nitrate in a calibrated volumetric PP flask set to make a 10mM NO_3^- stock solution.
NO_2^- : by weighing Sodium nitrite in a calibrated volumetric PP flask set to make a 0.5mM NO_2^- stock solution.

The calibration, or working standards, were prepared fresh daily by diluting the separate stock standards, using three electronic pipettes, into four 100ml PP volumetric flasks (pre-calibrated at the NIOZ) filled-up to the mark with diluted LNSW. The background values of the used LNSW were measured before the voyage started and added up to the standard values to compute the calibration target-values. The LNSW is compared with a Certified Reference Material (CRM) lot CE with assigned very low values, close to zero for all four channels.
Note; for the Ammonium background concentration of the LNSW a fixed value of 0.05uM NH_4 was used, for the other parameters; 0.005uM for PO_4 and 0.025uM for NO_3+NO_2 and 0.010uM for NO_2.

The cocktail standard is a stock mixture of Silicate, Nitrate, and Phosphate preserved with addition of 1ml saturated $HgCl_2$ per litre adjusted with a few pallets of NaOH on pH and proofed already to be stable since 2008.

Quality Control

Quality Control
Our standards have already been proven by inter-calibration exercises from ICES and Quasimeme, and since 2006 by joining the Inter Comparison exercises organised by MRI/JAMSTEC in Japan.

Our cocktail standard was measured every run for two nutrients, PO_4 and NO_3 during this expedition to gain some internal lab consistency the used Cocktail standard is monitored now since 2008, showing in-between runs reproducibility of 1.0 % for PO_4 and 0.5 % for NO_3
To obtain international comparable results, CRM's from KANSO produced by The General Environmental Technos Co., Ltd. Japan were analysed in the runs.

Statistics

Cocktail 2008 standard in-between runs data:

	average µM/L	S.D. µM/L	RSD.(%)	n
(100x dilution):				
PO_4	2.268	0.011	*0.50*	19 triplicates
NO_3	34.95	0.20	*0.58*	19 triplicates

Method Detection Limits (**MDL**) calculated (EPA norm), as 2.82 x S.D. of a 2% standard (*from the full range*) spiked LNSW sample (**n=10**).

	MDL µM	*full range µM*	SD dev. µM
PO_4	0.004	*2.50*	0.0016 µM
NH_4	0.010	*2.00*	0.0046 µM
NO_3+NO_2	0.01	*40.5*	0.0036 µM *
NO_2	0.002	*0.50*	0.0007 µM

* Note: For some surface samples NO_3 was below detection limit, so any value below 0.01uM in the data should be read as below detection limit.
Same count for PO4 analysis, every reading below 0.005 should be stated as below detection limit.

Precision in a single run: four concentration levels with Relative Standard Deviation (RSD).

	Level I μM	SD dev. μM	RSD. %	**Level II** μM	SD dev. μM	RSD. %
PO_4	0.50	0.0004	0.09	1.000	0.0023	0.24
NH_4	0.45	0.0091	1.90	0.85	0.0041	0.48
NO_3+NO_2	8.15	0.011	0.13	16.25	0.022	0.13
NO_2	0.11	0.0007	0.65	0.210	0.0000	0.00

	Level III μM	SD dev. μM	RSD. %	**Level IV** μM	SD dev. μM	RSD. %
PO_4	1.75	0.0018	0.10	2.50	0.0026	0.10
NH_4	1.55	0.0029	0.19	2.05	0.0045	0.22
NO_3+NO_2	25.36	0.062	0.20	40.5	0.097	0.24
NO_2	0.36	0.0005	0.15	0.510	0.0008	0.16

Accuracy

Accuracy is obtained by analysing Certified Reference Material (CRM) under perfect conditions (stable ship) measured at **23.0 °C** containing values for PO_4, and NO_3.

CRMs produced by KANSO lot-**CE**, **BU**, **CI** and **CH** were used.

CRM lot CE is used to verify the background concentrations of the nutrients in the LNSW.

CRM lot **BU**, n=3:

Anal:	μM	StD dev. *μM*	RSD.(%)	conv. *uM at 23°C*	**assigned *uM***	*Recovery %*
PO_4	0.354	0.001	0.16	0.346	***0.345***	*100.1*
NO_3	4.07	0.01	0.26	3.976	***3.937***	*101.0*

CRM **CI**, n=3:

Anal:	μM	StD dev. *μM*	RSD.(%)	conv. *uM 23°C*	**assigned *uM***	*Recovery %*
PO_4	0.966	0.002	0.16	0.943	***0.948***	*99.5*
NO_3	14.16	0.01	0.07	13.82	***13.77***	*100.4*

CRM **CH**, n=3:

Anal:	µM	StD dev. *µM*	RSD.(%)	conv. *uM 23°C*	**assigned *uM***	*Recovery %*
PO_4	1.200	0.002	0.17	1.172	***1.170***	*100.0*
NO_3	17.31	0.02	0.14	16.89	***16.94***	*99.7*

Evaluation issues encountered during the expedition

Some irregular peaks were detected in the NH_4 channel at a lower level, because occasionally irregular segmentation bubbles were formed due to a variety in length of the so called Inter Sample air-bubble (IS-bubble) from sampler to manifold. The sampler needle in use has to travel over different distances on an XY-platform in time so generating tiny differences in the length of the IS-bubble whereas the heating bath in the NH4 channel expanding the bubble is the cause of this variety. Because I did not have enough glass pieces to optimise the NH4-channel this problem stayed during the analysis showing a bit less performance in the calculated RSDs at the lowest levels.

References

Grasshoff, K. et al, Methods of seawater analysis. Verlag Chemie GmbH, Weinheim, 1983, 419 pp

Koroleff, 1969 and optimized by W. Helder and R. de Vries, 1979. An automatic phenol-hypochlorite method for the determination of ammonia in sea- and brackish waters. Neth. J. Sea Research 13(1): 154-160.

Murphy, J. & Riley, J.P., A modified single solution method for the determination of phosphate in natural waters. Analytica chim. Acta,1962, 27, p31-36

Microbial activity: Prokaryotic production and respiration

T. Reinthaler

Department of Functional and Evolutionary Ecology, University of Vienna,
Djerassiplatz1, 1030 Vienna, Austria

Objective

About 75% of the ocean is deeper than 200 m, however, most concepts on the interaction between the physical and chemical environment and the biota are derived from the relatively thin ocean surface layer. Moreover, the link between prokaryotic activity and biogeochemistry in the dark ocean is not firmly established despite recent studies that highlight the role of Bacteria and Archaea in the cycling of organic and inorganic matter in the dark ocean ((Herndl & Reinthaler 2013; Reinthaler et al. 2006b). Despite the major insights gained from studies on microbial activity in the surface ocean, knowledge on the microbial processing of organic matter and nutrients in the dark ocean is still in its infancy due to the lack of data. For this reason, the IPCC called attention to the fact that it is not possible to parameterize prokaryotic activity for an enhanced understanding of the global ocean carbon cycle (Intergovernmental Panel on Climate Change, 2001) and an interdisciplinary workshop of experts on integrating biogeochemistry and ecosystems in a changing ocean emphasized to study the interactions of the physics, chemistry and biology on an interdisciplinary basis (IMBER IMBIZO http://www.imber.info/IMBIZO1.html). Thus, the objectives here are to establish a biogeography of microbial diversity and activity in the meso- and bathypelagic waters of the Atlantic.

Work at sea

Prokaryotic heterotrophic production

Samples for prokaryotic heterotrophic production were taken from Niskin bottles at depth ranging from 100 m to 100 m above bottom (see Figure 1). Triplicate life and 2 control samples were incubated in 50 mL glass tubes. Microbial heterotrophic production was measured by incubating 20– 40 mL of seawater with 5 nM [^{3}H]-leucine (final concentration, specific activity 100 Ci mmol^{-1}, ARC) in the dark at in situ temperature (±1°C) for 1 to 24 h. Incubation times and volumes were chosen according to the sampling depth and based on previous experience. Duplicate formaldehyde-killed blanks were treated in the same way as the samples. Incubations were terminated by adding trichloroacetic acid (5% final concentration) to the samples. Samples and blanks were filtered through 0.2-µm polycarbonate filters (Whatman Nuclepore, 25 mm filter diameter) supported by cellulose acetate filters (Millipore HA, 0.45-µm pore size). Subsequently, the filters were rinsed with 5% ice-cold trichloroacetic acid and with Milli-Q. Filters were dried, 8 ml of scintillation cocktail (FilterCount, Canberra-Packard) added, and after about 18 h counted on board in a liquid scintillation counter (Perkin Elmer Tricarb 4810).

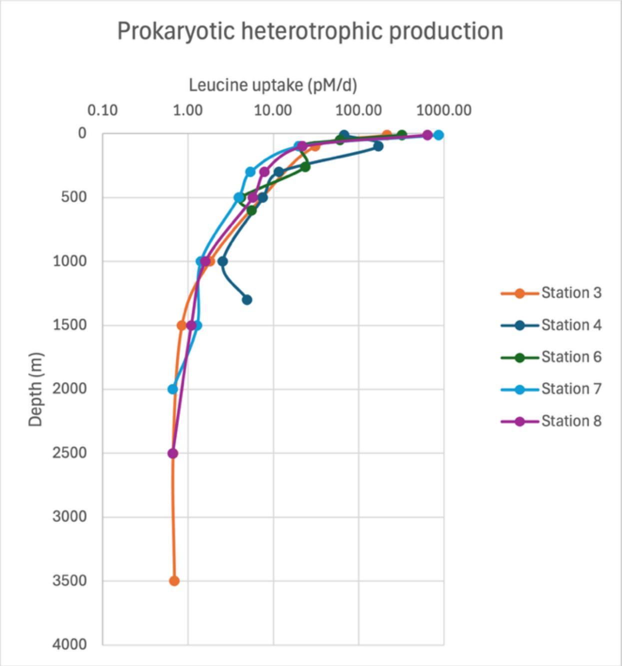

Figure 1. Exemplary profiles of prokaryotic heterotrophic production in the water column of the North Atlantic north of 60°N.

Microautoradiography and fluorescent in situ hybridization (MAR-FISH)

Samples for fluorescence in situ hybridization combined with microautoradiography were incubated in plastic tubes (Greiner Bio-One). To volumes between 20 mL and 80 mL (depending on the depth) [^{3}H]-leucine was added (5 nM final concentration), similar to the activity measurements. After 5 to 24 h the samples were fixed with 2% filtered formaldehyde and incubated for up to 18 h at 4°C to fix the cells. Subsequently the samples were filtered onto 0.2-µm polycarbonate filters (25 mm diameter, Millipore GTTP) and rinsed with Milli-Q. The filters were placed into 2 mL microfuge tubes and dried. Finally, the tubes with the filters were frozen at -80°C until analysis in the home laboratory.

Prokaryotic respiration

Around 5L seawater from 100 m, 500 and 1000 m depth were sampled into acid-rinsed polycarbonate carboys and temperature adjusted for a few hours in an incubator. Subsequently the seawater was siphoned into calibrated borosilicate glass BOD bottles with a nominal volume of 120 cm^3 using silicon tubing. For the determination of the initial O_2 concentration (t0), samples were fixed immediately with Winkler reagents and incubated together with the live samples in water baths in the dark at in situ temperature between 24–96 h when the incubations were terminated (t1). Quinduplicate bottles were used for the determination of initial and final O_2 concentrations. Oxygen concentrations of the t0 and t1 bottles were measured spectrophotometrically in a single run according to Reinthaler et al. (2006a). Measurements were done in a temperature-controlled laboratory container (set at 20°C) on a TRAACS autoanalyzer. To avoid loss of volatile iodine, the bottles were covered with parafilm and the lower end of the sampling tube was placed close to the bottom of the BOD bottle. To prevent a photochemical change in color of iodine because of ambient light, the bottles were covered with dark tubes during the analysis. The amount of total iodine was determined at a wavelength of 466 nm. The spectrophotometer was calibrated using standard additions of potassium iodate (J.T. Baker ACS grade KIO_3) to BOD bottles filled with seawater and adding Winkler chemicals in reverse order (see Figure 2).

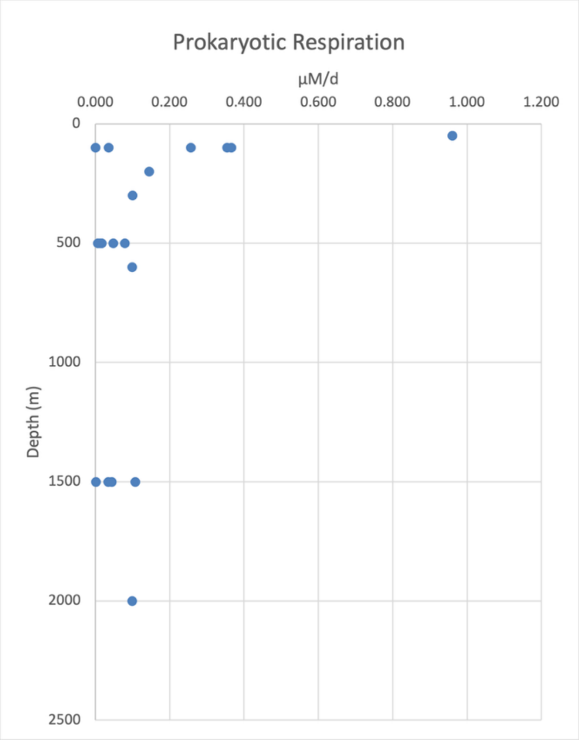

Figure 2. Preliminary results of prokaryotic respiration with depth along the transect.

High volume sampling with in situ pumps

Thomas Reinthaler

At selected stations in situ pumps (McLane Research Laboratories, USA) were used to filter seawater onto 3.0 µm and 0.2 µm polycarbonate filters at depth between 100 m and 4000 m (**Table 1**). Handling of the pumps was performed according to the detail protocols in the sample and sample-handling manual for GEOTRACES Cruises v2014. At station 3, 4, 6, 8 ,15, 16 and 18 the 3.0 µm filters were used for particle experiments. The remaining filters were shock frozen in liquid nitrogen for later metagenomic analyses.

Table 1. Station and depth of deployed in situ pumps. The pumping time and the pumped seawater volume is also indicated.

Station	Cast	Date	Depth (m)	ISP#	Time (min)	Vol (L)
1	3	08/02/25	100	ISP01	180	98
1	3	08/02/25	1000	ISP02	180	154
3	6	08/06/25	500	ISP01	240	133
3	6	08/06/25	1500	ISP02	240	162
4	3	08/07/25	300	ISP01	180	78
4	3	08/07/25	500	ISP02	180	121
6	2	08/09/25	100	ISP01	180	91
6	2	08/09/25	500	ISP02	180	159
7	3	08/10/25	200	ISP01	120	86
7	3	08/10/25	500	ISP02	120	111
8	4	08/12/25	100	ISP01	180	111
8	4	08/12/25	1500	ISP02	180	208
8	5	08/13/25	500	ISP01	300	242
8	5	08/13/25	2400	ISP02	300	failed
9	3	08/14/25	50	ISP01	120	75
9	3	08/14/25	500	ISP02	120	151
12	2	08/17/25	100	ISP01	240	174
12	2	08/17/25	500	ISP02	240	233
15	2	08/21/25	1000	ISP01	180	182
15	2	08/21/25	1800	ISP02	180	210
16	3	08/24/25	500	ISP01	180	199
16	3	08/24/25	1000	ISP02	180	257

References

Intergovernmental Panel on Climate Change, 2001. Climate Change 2001: Synthesis Report; Summary for Policymakers. p. 34.

Herndl GJ, Reinthaler T. 2013. Microbial control of the dark end of the biological pump. *Nature Geoscience*. 6(9):718–24

Reinthaler T, Bakker K, Manuels R, van Ooijen J, Herndl GJ. 2006a. Fully automated spectrophotometric approach to determine oxygen concentrations in seawater via continuous-flow analysis. *Limnology and Oceanography Methods*. 4:358–66

Reinthaler T, van Aken HM, Veth C, Aristegui J, Robinson C, et al. 2006b. Prokaryotic respiration and production in the meso- and bathypelagic realm of the eastern and western North Atlantic basin. *Limnology and Oceanography*. 51(3):1262–73

TOC/DOC, POC and amino acid sampling

Theres Koch

TOC/DOC samples were collected at stations 1 to 19 (except station 12). Samples were taken in duplicates from 6 to 7 depths (depending on the station and cast). Precombusted 40 mL glass vials were used for sampling. Water was filled directly from the Niskin bottle into the vial to avoid any carbon contamination. For acidification 100µl of 6M HCl were added to 20 mL of sample with a piston pipette and a filter tip. Samples were stored at 4°C.

POC samples were collected at stations 1 to 19 (except station 12). Samples were taken from 6 to 7 depths (depending on the station and cast). For each depth a volume of min. 5 L was taken from the respective Niskin bottle (10L carboys were used). Silicone tubes were hung directly into the carboy. Each tube was connected to a water jet pump with a 25mm filter holder in-between. POC was collected on precombusted glass fibre filters (GF/F Whatman 25mm). Filters were folded (sample-side inwards) and packed into precombusted pieces of aluminium foil. Samples were stored at -20°C.

Amino acid samples were collected at stations 1 to 19 (except station 12). Samples were taken in duplicates from 6 to 7 depths (depending on the station and cast). Precombusted 4 mL brown glass vials were used for sampling. Water was filled directly from the Niskin bottle into 100 mL sample bottles. From these, 5 mL were taken using a 5 mL syringe. For each glass vial, 2 mL of filtered sample (0.22 µm syringe filter, PES) was collected. Samples were stored at -20°C.

B-vitamin metabolic products, In situ pumps and Underwater Vision Profiler

Kristin Bergauer

The primary objective of our research at GEOMAR is to elucidate the cycling of vitamin B1 (thiamine) in marine systems, with a particular focus on exchange processes and microbial interactions. To constrain the B1 cycle, we measured concentrations of vitamin B1 and related congeners in both the particulate and dissolved pools. We hypothesize that a supply-and-demand relationship exists between B1 producers and auxotrophs, whereby different B1-type compounds act as a molecular currency exchanged among diverse microbial groups. During cruise PE547, vertical seawater profiles were collected along the transect to quantify particulate (cellular quotas of microbes and zooplankton) and dissolved B1 and precursor concentrations, as well as to estimate vertical export into the ocean interior. At six depths, 1000 mL of seawater were filtered through 0.2 µm Sterivex filters and collected into amber HDPE bottles. Filters were stored at –80 °C and HDPE samples at –20 °C until further processing.

In-situ McLane pumps

Particles for molecular characterization were collected with *in situ* McLane pumps in the epi- and mesopelagic water column, targeting organic particles across three biologically relevant size fractions (5 µm, 0.8 µm, and 0.2 µm). Pumping durations ranged from 120 to 240 minutes, filtering between 200 and 600 liters of seawater. The filters were immediately flash-frozen in liquid nitrogen and stored at –80 °C. Subsequent molecular analyses will involve DNA and protein extraction to investigate microbial endoproteomes, to assess the activity of key vitamin B1 producers and consumers.

The Underwater Vision Profiler (UVP)

We utilized a scientific particle counter with a camera that was designed to be deployed on autonomous and cabled platforms, such as a CTD. During deployment, which is feasible to a depth of 6000 m, the UVP6 images a volume of approximately 1 L at a frequency between 6 and 20 Hz. Each image is segmented in real time, and the particle size measurements are stored automatically. The UVP permits high-resolution in situ recordings of particle and zooplankton abundance in addition to derived rates (e.g., carbon export).

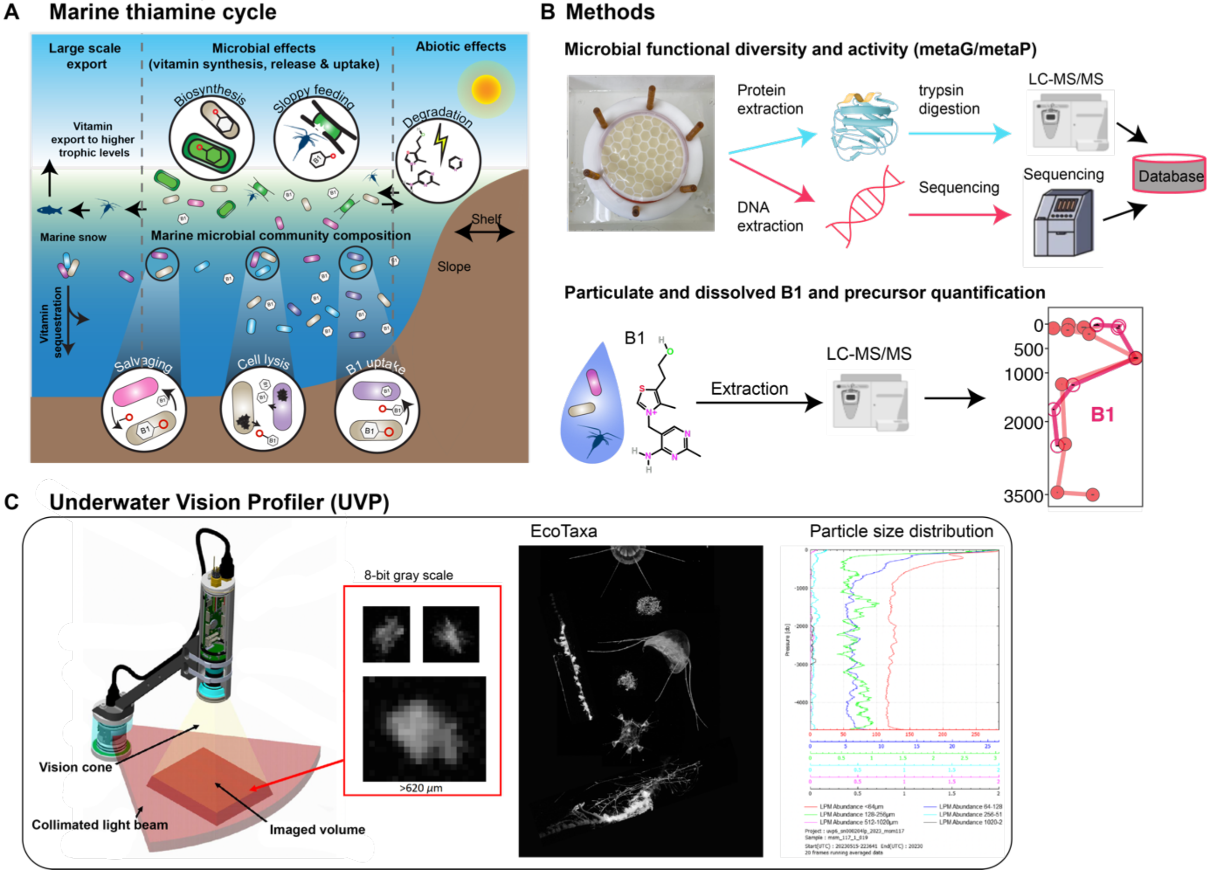

Figure 1. Sampling strategy during PE547 NEREIDES cruise. **A)** Conceptual sketch of B1 cycling in the pelagic ocean, illustrating abiotic factors that can affect the stability of B1 (e.g., solar radiation). Microbial processes illustrating biosynthesis and salvaging, as well as release and uptake of vitamins. Potential routes of large-scale export are depicted in the left section, including transfer up the marine food web. **B)** Molecular analyses that will be used to characterize microbial function and diversity, as well as quantify target metabolites. **C)** The Underwater Vision Profiler (UVP6) deployed during field operations. The instrument simultaneously records particle abundance, size spectra, and vertical distribution, providing detailed profiles from the surface to mesopelagic depths. Data generated by the UVP support the study of particle flux, plankton community structure, and carbon export processes in marine ecosystems.

Collecting and processing samples for CARD-FISH

Tinkara Tinta

Samples were collected at 19 stations along vertical profile (6-7 depths) for CARD Fluorescence in situ hybridization. Immediately after collecting specific volume (depending on depth from 80-5 mL) samples were fixed with 0.2 µm pre-filtered 37% formaldehyde to reach 2% final concentration and kept at 4°C until further processed. Afterwards (within 1-24 h after fixation) samples were filtered onto 0.2 µm white polycarbonate filters (25 diameter) with vacuum pump and stored in vials at -20°C. Table 1: List of CARD-FISH samples

Jellyfish-derived organic matter enrichment experiments

Tinkara Tinta

Sampling

Jellyfish-derived organic matter (hereinafter jelly-OM) enrichment experiments were conducted aboard RV Pelagia to better understand microbial decay rates of this type of particulate organic matter source in the ocean. Seawater for experiments was sampled at S3, S8, S13 and S15 stations from 100m and 2000-3000 m depth; in total 5 enrichment experiments were conducted.

Experimental design

Microbial production experiments

Immediately after sampling with CTD seawater samples were aliquoted into 2L Nalgen experimental bottles. For each experiment there were three biological replicates with no jelly-OM addition, which served as control, and three biological replicates enriched with jelly-OM to reach final concentration of 20 mg of jelly-OM per liter. Experimental bottles were mixed thoroughly and subsampled at 0h for microbial abundance, production (bulk using 3H-Leu incorporation method and at single-cell level using HPG method) and chemistry (total organic carbon and nitrogen and inorganic nutrients). Experimental bottles were then incubation for 3-4 days (depending on depth) at *in situ* temperature in the dark and subsampled once at intermediate time for microbial abundance and at the end of incubation for microbial abundance, production (bulk using 3H-Leu incorporation method and at single cell level using HPG method), respiration (at single cell level using Redox Sensor Green method) and chemistry (total organic carbon and nitrogen and inorganic nutrients). At the end all remaining volume from each experimental bottle was collected for microbial DNA extraction.

Microbial respiration experiment

In parallel, smaller incubations (~ 100 mL) were set up in Winkler bottles with oxygen optodes to follow microbial respiration with/without jelly-OM addition. Winkler bottles were incubated in the dark at *in situ* temperature and oxygen concentration was measured daily with PreSense Instrument Fibor 4 for 3-4 days depending on depth.

Microbial abundance

Samples for bacterial abundance were fixed with 0.2 µm pre-filtered 37% formaldehyde to reach 2% final concentration and stored at 4 °C until further processed (within 1-24h). Afterwards samples were filtered onto 0.2 µm polycarbonate filters (25 diameter) and stored at -20 °C

Microbial production (bulk)
Microbial production was measured using radiolabeled leucine (3H-Leu, Perkin Elmer, 100 Ci/mmol, 10nM final concentration at 100m depth and 5nM final concentration at 2000-3000m depth) incorporation into newly synthesized proteins. 1mL subsamples (3 live and 2 killed controls) collected at 0h and at the end of the experiment were incubated for 4-24h (depending on depth) and fixed with 100% trichloroacetic acid to reach 5% final concentration. Samples were processed with microcentrifugation protocol (centrifuged at 15.000xg for 10 min, aspired, 5% TCA wash, centrifuged at 15.000xg for 10 min, aspired, 70% EtOH wash, , centrifuged at 15.000xg for 10 min, aspired and mixed with UltimaGold Scintillation cocktail) and measured using TriCarb liquid scintillation counter aboard RV Pelagia.

Microbial production at single cell level (HPG-method)
20 mL subsamples collected at 0h and at the end of the experiment were incubated with the methionine analog L-homopropargylglycine (HPG) reagent at 20nM final concentration for 4-8 h (depending on depth) at in situ temperature in the dark. Microbial growth was stopped by fixing samples with fixed with 0.2 µm pre-filtered 37% formaldehyde to reach 2% final concentration and stored at 4 °C until further processed (within 1-24h). Afterwards samples were filtered onto 0.2 µm polycarbonate filters (25 diameter) and stored at -20 °C.

Microbial respiration at single cell level (RSG-method)
20 mL subsamples collected at the end of the experiment were incubated with the Redox sensor reagent at 20nM final concentration for 4-8 h (depending on depth) at in situ temperature in the dark. Microbial growth was stopped by fixing samples with fixed with Glycerol-TE buffer and samples were immediately afterwards filtered onto 0.2 µm polycarbonate filters (25 diameter) and stored at -80 °C.
Microbial biomass for DNA extraction
To collect microbial biomass for further DNA extraction at the end of incubation samples were collected onto 0.2 µm polycarbonate filters (47 diameter) and stored at -80 °C.

Chemistry
Subsamples for total organic carbon concentration were collected at 0h and at the end of each experiment from each experimental bottle into DOC-vials and acidified with 6N HCl and stored at 4°C. Samples for inorganic nutrients were filtered through GF/F (Whatman) filters and analyzed immediately afterwards with QuAAtro Instrument aboard RV Pelagia.

Effect of hydrostatic pressure on prokaryotic heterotrophic production and respiration

Chie Amano, Thomas Reinthaler, Marilena Heitger (University of Vienna)
Tinkara Tinta (National Institute of Biology, Marine Biology Station Piran)

Introduction

The deep ocean is a major carbon reservoir where microbial remineralization plays an important role in biogeochemical cycling, however knowledge of microbial processing of organic matter is still limited due to scarce observational data compared to the surface ocean (Aristegui et al. 2009; Herndl and Reinthaler 2013). Recent studies have shown the effects of high hydrostatic pressure on prokaryotic productivity in the deep ocean (Tamburini et al. 2013; Amano et al. 2022), indicating potential alternations in their production and respiratory rates, which in turn influence our estimates of carbon cycling in the global ocean. During this cruise, we aim to study the effect of pressure on the prokaryotic production and respiration rates using the in situ microbial incubator (ISMI) and identify active microbial taxa utilizing the organic matter at the in situ depths.

Methods

The ISMI is a device that allows autonomous collection and incubation of seawater at depth, injection of substrate, and fixation of the samples after a preprogrammed incubation time. To determine prokaryotic heterotrophic production and respiration under in situ hydrostatic pressure conditions, ^{3}H-leucine (Hartman Analytics, ART0470; final conc. 5 nM) and RedoxSensor Green (RSG; Thermo Fisher Scientific; final conc. 1 μM) were employed, respectively. In addition to the bulk seawater incubation, one extra incubation bottle was served as an amendment bottle of jellyfish powder (final conc. 20 mg/L) to see the potential of fresh organic matter degradation by microbes under in situ hydrostatic pressure conditions.

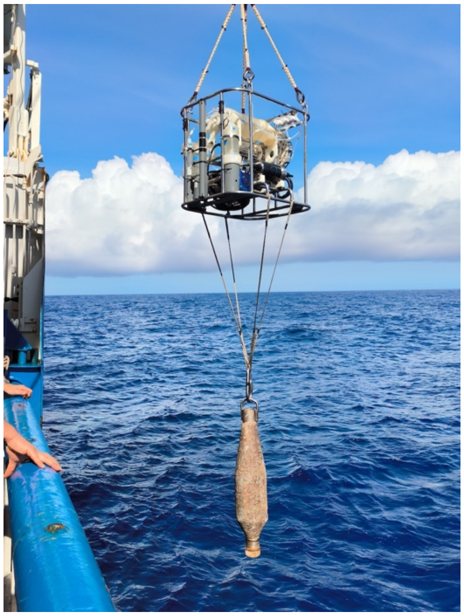

Fig. 1. Deployment of the ISMI during NEREIDES cruise.

Formaldehyde (final conc. 2%) and Glycerol-TE (final conc. 5% for Glycerol and 1x for TE) were added into the fixation bottles for 3H-leucine and RSG incubations, respectively. All the bottles in contact with the samples were acid-washed (0.2 N HCl) and rinsed with MQ water or 0.2 μm filtered seawater or 5 kDa filtrate collected from the same water mass of the ISMI deployment at the previous station. Prior to deployment of the ISMI, sampling schedule was programed with the software (N-Com communicator ver. 3.01, Nichiyu Giken Co Ltd). After recovering the ISMI on board, the samples were immediately collected into Griner tubes. Subsequently, leucine incorporation rate was determined with liquid scintillation counter (PerkinElmer, Tri-Carb 4810 TR) using filtration method (Kirchman 2001), and samples for MICRO-CARD-FISH were also taken to determine the single cell activity (Sintes and Herndl 2006). For RSG samples, incubated seawater was flash-frozen in liquid nitrogen. These samples will be kept at -80°C until further analysis at the home lab according the pervious study (Munson-McGee et al. 2022). To compare with decompressed samples, seawater samples were also taken from Niskin bottles at the same station as the ISMI deployment. These samples were incubated under atmospheric pressure conditions at in-situ ± 1°C in the temperature regulated refrigerator for the same duration as the ISMI incubation.

Preliminary results

The ISMI was deployed in total 8 times during the cruise at depths ranging from 1500 to 3000 m (Table 1). Although the full sampling sequence between the incubation and fixation bottles was not completed at the first station, overall the ISMI worked well at the following stations. Leucine incorporation rates in the samples incubated at in situ were lower than the those incubated under atmospheric pressure conditions, showing the same trend as the previous studies (Amano et al. 2022). While addition of jellyfish organic matter didn't alter the leucine incorporation rate at the station 3, a slight increase compared to the non-amended bulk seawater samples was found at the following stations (stations 8 and 12/13). Further analysis will be done at the home lab.

Table 1. Location and depths where the ISMI was deployed

Deployment/ Control ID	**ISMI/ ATM**	**Date**	**St**	**Depth (m)**	**Substrate**	**Duration of incubation (h)**
1	ISMI	04/08/2025	3	2000	Leu	11
	ATM	05/08/2025	3	2500	Leu	11
2	ISMI	10/08/2025	7	2000	RSG	2
	ATM	10/08/2025	7	2000	RSG	2
3	ISMI	13/08/2025	8	2500	Leu	6
	ATM	12/08/2025	8	2500	Leu	7
4	ISMI	14/08/2025	9	1500	RSG	2
	ATM	14/08/2025	9	1500	RSG	2
5	ISMI	18/08/2025	12	3000	Leu	6
	ATM	19/08/2025	13	3000	Leu	6
6	ISMI	21/08/2025	15	2000	RSG	2
	ATM	21/08/2025	15	2000	RSG	2
7	ISMI	24/08/2025	16	2500	RSG	2
	ATM	24/08/2025	16	2500	RSG	2

References

Amano, C., Z. Zhao, E. Sintes, T. Reinthaler, J. Stefanschitz, M. Kisadur, M. Utsumi, and G. J. Herndl. 2022. Limited carbon cycling due to high-pressure effects on the deep-sea microbiome. Nat Geosci. doi:10.1038/s41561-022-01081-3

Aristegui, J., J. M. Gasol, C. M. Duarte, and G. J. Herndl. 2009. Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54: 1501–1529. doi:10.4319/lo.2009.54.5.1501

Herndl, G., and T. Reinthaler. 2013. Microbial control of the dark end of the biological pump. 6: 718-724. doi:10.1038/NGEO1921

Kirchman, D. 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments, p. 227-237. In J. H. Paul [ed.], Methods in microbiology. Academic Press.

Munson-McGee, J. H., M. R. Lindsay, E. Sintes, J. M. Brown, T. D'Angelo, J. Brown, L. C. Lubelczyk, P. Tomko, D. Emerson, B. N. Orcutt, N. J. Poulton, G. J. Herndl, and R. Stepanauskas. 2022. Decoupling of respiration rates and abundance in marine prokaryoplankton. Nature. doi:10.1038/s41586-022-05505-3

Sintes, E., and G. J. Herndl. 2006. Quantifying substrate uptake by individual cells of marine bacterioplankton by catalyzed reporter deposition fluorescence in situ hybridization combined with micro autoradiography. Appl Environ Microbiol 72: 7022–7028. doi:10.1128/Aem.00763-06

Tamburini, C., M. Boutrif, M. Garel, R. R. Colwell, and J. W. Deming. 2013. Prokaryotic responses to hydrostatic pressure in the ocean – a review. Environ. Microbiol. 15: 1262–1274. doi:10.1111/1462-2920.12084

Functional role of microbial communities

Kangli Guo

Research Objective

The primary aim of this project is to elucidate the functional roles of microbial communities, encompassing both prokaryotes and eukaryotes, in the upper ocean. This objective builds on our recent publication in *Nature Communications*, where we demonstrated that organic matter degradation by oceanic fungi differs markedly between polar and non-polar waters [1]. We conducted study across a broad latitudinal gradient from subtropical to polar oceans, provided novel insights into the ecological roles of marine fungi and their adaptive responses to environmental variability. However, its scope was restricted to fungal communities at the surface and deep chlorophyll maximum (DCM) layers. In the present study, we extend this framework by investigating the composition and functional potential of both prokaryotic and eukaryotic assemblages, as well as their interactions, across different depths of the water column. Special emphasis will be given to their contributions to organic matter degradation under depth-dependent nutrient and environmental gradients.

Research Method

Samples were collected at each station, between 35 and 150 liters of seawater from 50 m, 100/200 m, and 500 m depths were sequentially filtered using either a tripod system or a McLane in situ pump (Table 1). Filtration was performed through polycarbonate filters (Millipore) of two size fractions: >3 μm, representing particle-attached (PA) communities, and 0.2 μm, representing free-living (FL) communities. In total, samples were obtained from 14 stations. Filters were immediately flash-frozen in liquid nitrogen and stored at –80 °C until further processing. Subsequent molecular analyses will include DNA, RNA, and protein extraction to investigate microbial community composition, functional potential, and gene expression profiles.

Reference

1. Guo K, Zhao Z, Breyer E, Baltar F. Organic matter degradation by oceanic fungi differs between polar and non-polar waters. *Nature Communications* **16**, 7589 (2025).

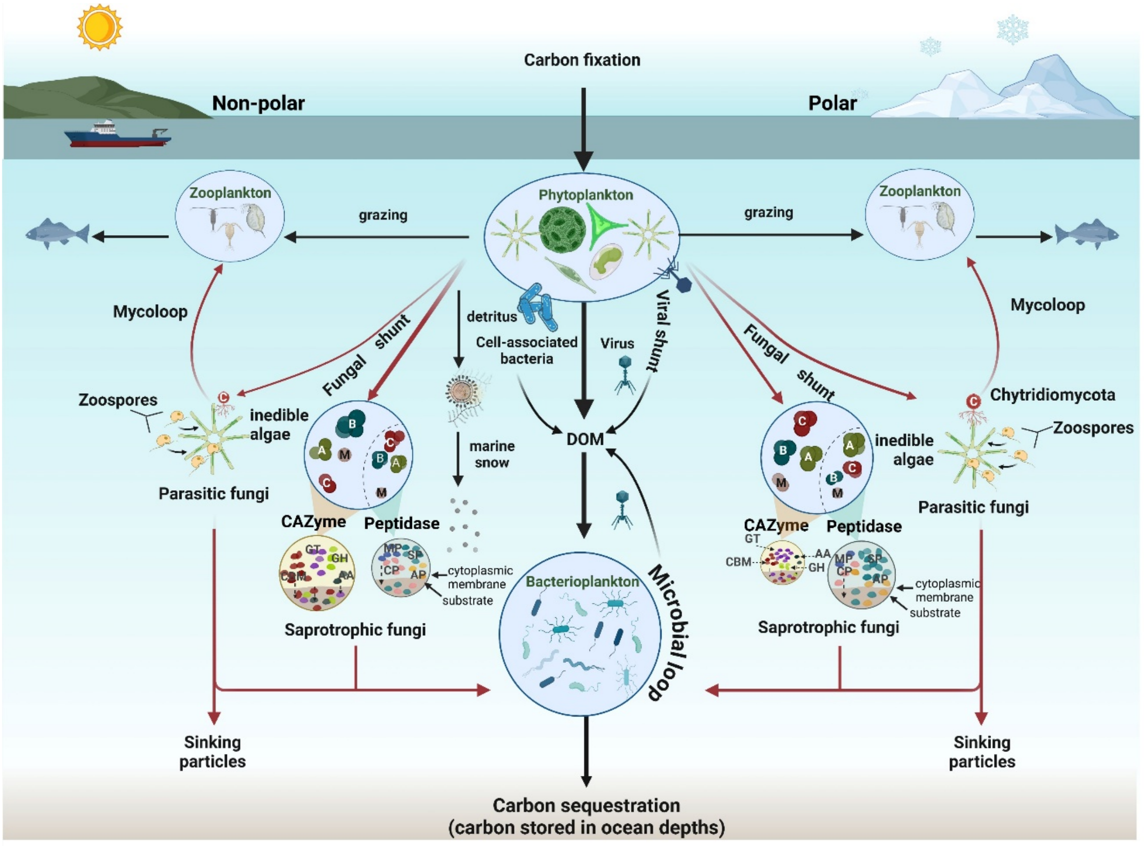

Fig. 1 Schematic overview of differences in functional diversity and taxonomic affiliation of carbohydrate and protein utilization by oceanic fungi in the surface and deep chlorophyll maximum (DCM) water layers between polar and non-polar oceans. The widespread and diverse presence of fungal functional transcripts in the open ocean highlights that their substantial influence on marine ecosystem dynamics has been previously underestimated. Our study revealed a significant association between the taxonomic affiliation of specific peptidase and CAZyme groups probably linked to the availability of carbohydrate and protein substrates characteristic of polar and non-polar waters.

Table 1. Sample station and volume of water.

station	depth(m)	size (μm)	volume (L)	date	Niskin/In-situ pump (ISP)
ST1	50	0.2 & 3	75	2025.08.02	Niskin
ST1	100	0.2 & 3	100	2025.08.02	ISP
ST1	500	0.2 & 3	100	2025.08.02	Niskin
ST1	1000	0.2 & 3	150	2025.08.02	ISP
ST2	50	0.2 & 3	100	2025.08.03	Niskin
ST2	200	0.2 & 3	100	2025.08.03	Niskin
ST3	10	0.2 & 3	75	2025.08.04-06	Niskin
ST3	100	0.2 & 3	100	2025.08.04-06	Niskin
ST3	500	0.2 & 3	150	2025.08.04-06	Niskin
ST3	1000	0.2 & 3	150	2025.08.04-06	Niskin
ST3	2500	0.2 & 3	168	2025.08.04-06	Niskin
ST4	50	0.2 & 3	125	2025.08.07	Niskin
ST4	100	0.2 & 3	100	2025.08.07	Niskin
ST4	500	0.2 & 3	100	2025.08.07	Niskin
ST5	No sampling for Kangli			2025.08.08	
ST6	50	0.2 & 3	75	2025.08.09	Niskin
ST6	100	0.2 & 3	75	2025.08.09	Niskin
ST6	500	0.2 & 3	125	2025.08.09	Niskin
ST7	50	0.2 & 3	100	2025.08.10	Niskin
ST7	200	0.2 & 3	86	2025.08.10	ISP
ST7	500	0.2 & 3	111	2025.08.10	ISP
ST8	50	0.2 & 3	75	2025.8.12	Niskin
ST8	200	0.2 & 3	100	2025.8.12	Niskin

ST8	500	0.2 & 3	75	2025.8.12	Niskin
ST9	50	0.2 & 3	100	2025.8.14	ISP
ST9	500	0.2 & 3	100	2025.8.14	ISP
ST10	No sample for Kangli			2025.8.15	
ST11	50	0.2 & 3	75	2025.8.17	Niskin
ST11	100	0.2 & 3	100	2025.8.17	Niskin
ST11	500	0.2 & 3	100	2025.8.17	Niskin
ST12	No sample for Kangli; CTD winch connection failure.			2025.8.18	
ST13	50	0.2 & 3	48	2025.8.19	Niskin
ST14	50	0.2 & 3	35	2025.8.20	Niskin
ST14	100	0.2 & 3	50	2025.8.20	Niskin
ST14	500	0.2 & 3	60	2025.8.20	Niskin
ST15	No sample for Kangli; CTD winch connection failure.			2025.8.21	
ST16	50	0.2 & 3	48	2025.8.24	Niskin
ST16	100	0.2 & 3	72	2025.8.24	Niskin
ST16	500	0.2 & 3	96	2025.8.24	Niskin
ST17	50	0.2 & 3	48	2025.8.25	Niskin
ST17	100	0.2 & 3	72	2025.8.25	Niskin
ST17	500	0.2 & 3	96	2025.8.25	Niskin
ST18	50	0.2 & 3	48	2025.8.26	Niskin
ST18	100	0.2 & 3	72	2025.8.26	Niskin
ST18	500	0.2 & 3	96	2025.8.26	Niskin
ST19	50	0.2 & 3	48	2025.8.27	Niskin
ST19	500	0.2 & 3	96	2025.8.27	Niskin

Oxic methane production in the North Atlantic

Manuela Felsberger

Objective

Biogenic methane is microbially produced in anoxic sediments and typically rapidly consumed by methanotrophic microorganisms, largely limiting emissions to the atmosphere. However, reported methane supersaturations in oxygen-rich layers of aquatic ecosystems contradict the long-held view that methane production is confined to anoxic environments and have thus been referred to as the methane paradox. Due to its proximity to the atmosphere, oxic methane production (OMP) in surface waters might constitute a particularly important source of methane, as it might escape the aquatic “microbial methane filter”. Despite this, we lack a comprehensive understanding of the involved processes and microorganisms. As part of the ERC METHANIAQ project of Ass.-Prof. Barbara Bayer (University of Vienna), I aim to answer these knowledge gaps in the scope of my PhD thesis by identifying and quantifying sources of OMP in surface waters of aquatic ecosystems with different trophic states. Additionally, metagenomic analysis is used to unravel the potential microbial drivers of OMP.

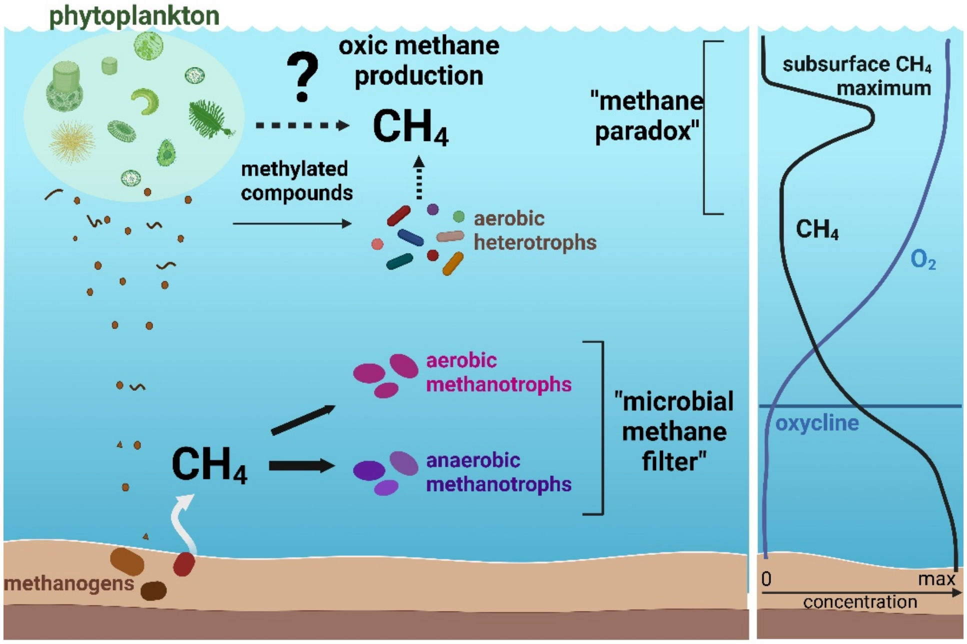

Figure 1 Schematic illustration of the influence of phytoplankton on methane cycling in aquatic ecosystems. Created in https://BioRender.com, adapted from Barbara Bayer (unpublished).

Work at sea

Stable isotope tracer experiments were performed at 16 out of the 19 stations (*Table 1*) to measure $^{13}CH_4$ production rates from isotopically labelled potential methane precursor molecules (^{13}C-methylphosphonate, ^{13}C-monomethylamine) and directly from primary production (^{13}C-bicarbonate). For this, water from discrete surface depths targeting high phytoplankton biomass (chosen according to Chl-a profile) was sampled from the Niskin bottles headspace-free in 250 mL borosilicate serum flasks, before they were closed with butylrubber stoppers and crimped. Subsequently, 250 µL of the respective ^{13}C-tracer were added to a final concentration of 1 µM (13C-MPn, 13C-MMA) or 250 µM (13C-bicarbonate), respectively, using a gastight syringe (Hamilton, 250 µL) and a displacement syringe filled with sample water. Triplicate replicates were incubated for 0-48h in on-deck incubators pumped with surface water under near *in situ* temperature and light conditions. Blue filters (LEE Filter #117 Steel Blue and/or #172 Lagoon Blue) were used to create different light levels depending on the sample water depth. At three timepoints (0h, 24h and 48h) the respective incubations were terminated by adding 300 µL $ZnCl_2$-solution (50% w/v). Until further analysis, all preserved samples were stored in the dark at 4 °C until CH_4 concentrations and $\delta^{13}C$-CH_4 were measured using the headspace equilibration technique combined with cavity ring-down spectroscopy (CRDS) on a Picarro gas analyser (G2201-i). Briefly, 50 mL headspace was created using compressed air while 50 mL of sample water were taken out with a syringe. After that, the serum bottle was vigorously shaken for 1 minute to allow equilibration of CH_4 between the aqueous and gas phase. Following equilibration, 30 mL headspace was sampled and diluted 1:1 with compressed air. The resulting 60 mL gas sample was then injected into the Picarro gas analyser (G2201-i) to quantify CH_4 concentrations. Instead of measuring on board, 25 mL gas samples of the incubations from the last two stations were transferred into pre-evacuated 12 mL exetainers for further analysis in the home laboratory.

For each incubation water depth, 2 L of water was sampled into polycarbonate bottles to analyse the microbial community (16S rRNA sequencing, metagenomics). Each sample was pre-filtered through a 180 µm nylon mesh before it was sequentially filtered using a peristaltic pump (Masterflex L/S with EasyLoad II pump heads). Cells were consecutively collected on 25 mm polycarbonate membrane filters (Isopore) with 3 µm pore size and PVDF membrane filters (Durapore) with 0.22 µm pore size by stacking two polypropylene filter holders (Swinnex 25). Filters were transferred into 2 mL gasketed bead beating tubes and immediately frozen at -20°C before being stored at -80°C.

At select stations additional incubations were performed to quantify potential CH_4 production from sinking particles by adding a resuspended particle solution from 3 µm in situ pump filters to 0.22 µm filtered seawater of the respective depth. At one station the particle resuspension was obtained from a sediment trap that has been deployed at for approximately 2.5 days. All particle incubations were performed in duplicates and incubated in the dark at the in situ temperature. Incubations were stopped after 0 h, 24 h and 48 h by addition of 300 µL $ZnCl_2$-solution (50% w/v) and further analysed as described above.

Table 1 ^{13}C-stable isotope incubation experiments performed at select stations

Station	*Depth (m)*	*^{13}C-Tracer*	*Additional Treatments/Tests*
1	10	^{13}C-MPn, ^{13}C-MMA, ^{13}C-HCO_3	
2	10	^{13}C-MPn	P_i addition, Atrazine addition, dark treatment
3	10	^{13}C-MPn	< 3µm size fraction
	500	Particle resuspension (> 3 µm) from in situ pump + ^{13}C-MPn	
5	50	^{13}C-MPn	
6	500	Particle resuspension (> 3 µm) from in situ pump + ^{13}C-MPn	
8	10	^{13}C-HCO_3	
	30	^{13}C-MMA, ^{13}C-MPn, ^{13}C-HCO_3	
	50	^{13}C-MMA	

	500	Particle resuspension from sediment trap + ^{13}C-MPn	
9	10	^{13}C-MMA	
	45	^{13}C-MMA, ^{13}C-MPn	
10	35	^{13}C-HCO_3	dark treatment
11	35	^{13}C-MMA	
13	50	^{13}C-HCO_3	Atrazine addition
14	50	^{13}C-extract from E. huxleyi culture	
15	80	^{13}C-MPn	
16	50	^{13}C-MPn	P_i addition
17	50	^{13}C-MPn	DMSP addition
18	50	^{13}C-MPn	dark treatment
19	50	^{13}C-MPn	

Bulk microbial vs. particle associated microbial activity

Marilena Heitger

Sampling strategy for bulk and particle-associated parameters

We collected samples at 19 stations between 68°N and 33°N to measure various parameters. At every station, seawater was collected using a CTD rosette equipped with 24 bottles. The whole water column from 10m to the deepest water masses (between 300 and 3500m) was covered with 6 to 9 depths per station. Because one of the aims of the NEREIDES project is to investigate the role of different particle types in the marine carbon cycle, we furthermore collected particulate organic matter using in situ pumps. The pumps were equipped with a 0.2 and 3 µm filters, which were frozen upon recovery for later molecular analyses. At selected stations and depths, only half of the 3 µm filter was frozen. From the remaining half, particles were washed off using a syringe filled with 0.2 µm filtered seawater and fitted with a sterile needle into an acid-washed bottle, and resuspended in 0.5 L of 0.2 µm filtered seawater collected from the same depth. This particle resuspension was used to measure the carbon content and amino acids (see Theres Koch`s cruise report), as well as bacterial production and respiration (see Thomas Reinthaler`s cruise report), abundance, single-cell respiration, and enzyme activity, as described below.

Prokaryotic abundance

Samples collected with Niskin bottles, as well as from the particle resuspension, were fixed with 0.5 % glutaraldehyde and frozen in liquid nitrogen for later flow cytometric analyses of prokaryotic and viral abundance.

Single-cell respiration

We used the fluorescent dye redox sensor green (RSG) to measure single-cell respiration. 1 µL of RSG was added to 1 mL samples collected with the Niskin bottles and the particle resuspension. After 2 hours of incubation at in situ temperature, glycerol (5 % final concentration) and TE-buffer (1X final concentration) were added before flash freezing the samples. By this, microbial activity stops, and the respiring cells can later be counted using a flow cytometer. Furthermore, I left samples unamended, added glycerol and TE-buffer, and froze them. These samples can be used as controls for counting the RSG-stained cells or used for single-cell sequencing.

To assess the influence of pressure on microbial respiration, I incubated selected samples in pressure chambers that mimic in situ pressure conditions. Briefly, the samples are filled into 2 mL glass vials without headspace, closed with a crimp cap with a flexible septum, and then placed inside the pressure chamber. The chamber was filled with deionized water, closed, and then placed in the housing, where pressure is applied using a hand pump. The pressure was increased to in situ pressure (20 to 25 MPa).

Extracellular enzyme activity measurements

To assess the enzymatic activity of microbes in the water column and associated with particles, I used seawater from selected depths as well as the particle resuspension for fluorogenic enzyme assays. The activity of alkaline phosphatase, β-glucosidase, and leucine aminopeptidase was estimated by adding fluorogenic substrates to triplicate samples in 96-well plates and measuring fluorescence using a FLUOstar Omega plate reader. The fluorescence was tracked over time and converted into substrate concentration using a calibration curve derived from MUF and MCA standards. The hydrolysis rates were then calculated by the increase of hydrolysed substrate over time and compared between bulk (i.e., Niskin bottle) and particle-associated samples.

High-pressure incubation of samples amended with jellyfish-derived organic matter

At three stations, samples were collected from Niskin bottles between 2000 and 3000m depth and amended with jellyfish-derived organic matter (see Tinkara Tinta`s cruise report). To mimic in situ conditions, subsamples of 100 mL were incubated in the pressure chambers at in situ pressure (20 to 30 MPa). After 96 hours, the samples were filtered onto 0.2 µm filters to later track the microbial community involved in jelly-OM degradation.

Table with samples and applied methods

Station	**PA**	**RSG**	**Enzyme assays**	**ISP particle resuspension**	**RSG high pressure**	**Jelly-OM high pressure**
1	6 depths	6 depths	3 depths			
2	6 depths	6 depths	-			
3	6 depths	6 depths	1 depth, bulk + PA	500m		3000m
4	6 depths	6 depths	1 depth, bulk + PA	300m		
5	6 depths	6 depths				

6	6 depths	6 depths	1 depth, bulk + PA	515m		
7	7 depths	6 depths			2000m	
8	9 depths	6 depths	2 depths, bulk + PA	500m, 1500m		3000m
9	8 depths	6 depths	3 depths, bulk			
10	7 depths	6 depths				
11	8 depths	6 depths	4 depths			
12	6 depths	6 depths				
13	9 depths	6 depths	4 depths			3000m
14	7 depths	6 depths				
15	7 depths	6 depths	1 depth, bulk + PA	1000m		
16	7 depths	6 depths	1 depth, bulk + PA	1000m	2500m	
17	7 depths	6 depths				
18	7 depths	6 depths	1 depth, bulk + PA	500m		
19	7 depths	6 depths				

Oxygen measurements

Theres Koch, Karel Bakker

Summary

At all stations Oxygen concentrations are observed with a Seabird 43 sensor. Depending on phytoplankton production at the sea surface or bacterial respiration at depth levels below the productive zone, maxima and minima are observed in the oxygen profiles from the CTD. At alternating stations from the CTD-Rosette sampler, some 3 to 4 samples per cast were collected for calibration purposes of the oxygen sensor. For calculating bacterial respiration, samples from in-situ temperature incubation-experiments, were collected for shipboard oxygen analysis based on Winkler (1888). The oxygen measurements were made using a Technicon TrAAcs 800 Continuous Flow Analyser (CFA) measuring the absorbance of the formed Iodine-tri-iodide closest to the isobestic point (466nm) at 460nm. In total approximately 400 samples were measured on board during the expedition, from which 43 from the CTD-

Rosette and some 380 from incubation experiments. All results were reported as concentrations in micro mole per litre (µmol/L) at 20C.

Equipment and Methods
The colourimetric oxygen measurement based on Winkler

Oxygen samples are taken using Winkler 120ml BOD bottles as the first sampling of the CTD-Rosette, with registration of its temperature on deck.
The bottles volume is three times overflowed with seawater before the Winkler reagents are added and the stopper placed and well mixed, then placed below water in a box and thoroughly mixed again after some 15 minutes. Next day after at least 24 hours the bottles were opened 1ml of a 10M H2SO4 is added and a magnetic stir-bar, covered with stretched Parafilm® against evaporation placed on the NIOZ made auto-sampler and its Absorbance measured with a connected TrAAcs 800 at 460nm in a 10mm flow cell, a LED was used as the light source in combination with a narrow band interference filter . Calibration was done using dilution of a stock KIO_3 solution with a pipette into known volumes of some Winkler bottles (used as calibration flaks) containing seawater plus all reagents in the reverse order. Each measurement run had a correlation coefficient of 1.0000 using 5 calibration points. The spectrophotometer of the TRAACS has the advantage to set optical a range of e.g. 180 to 330uM as Oxygen over the full detector range, resulting in a resolution of 0.03uM in reading-units Oxygen with a minimum of carry-over in the flow system.

Analytical Methods used
Precision at the applied range for analyses of the respiration bottles were often better than 0.04uM O2.

To obtain accuracy for the Oxygen values, a deep CTD sample of 6 litre from station 11 cast 01-#13, deep water 2000m (with salinity of 34.9) was equilibrated under atmospheric conditions (19.2C and stable barometric pressure of 1013hPa during two days) and after five days sampled following the Winkler procedure (see results below.

	Analysis uM	*THEORETICAL SATURATION* 100%SAT 19.2°C SAL 34.9 1013HPa uM	***RECOVERY* %**
SAT	234.74	*234.25*	**100.2**
SAT	234.83	*234.25*	**100.2**
SAT	234.78	*234.25*	**100.2**
SAT	234.66	*234.25*	**100.2**
SAT	234.58	*234.25*	**100.1**
SAT	234.87	*234.25*	**100.3**
AVERAGE	**234.75**		**100.2**
STDEV	0.11		
R.S.D %	*0.05*		*0.05*

Measurement results of six saturated samples with an average of **234.75uM** show a good overall agreement with the theoretical calculated value of ***234.25***uM for a 100% saturated sample with a salinity of 34.9 at 19.2°C.

Literature

Thomas Reinthaler, Karel Bakker, Rinus Manuels, Jan van Ooijen,

and Gerhard J. Herndl,

Fully automated spectrophotometric approach to determine oxygen concentrations in seawater via continuous-flow analysis, Limnol. Oceanogr.: Methods 4, 2006, p358–366.

ATP and POC measurement

Gregory Lang

In Situ Sampling:
Samples were collected from a range of depths, with higher resolution sampling conducted in the upper 500m of the water column due to larger variations in near surface waters. Beyond 500m a lower resolution sampling was conducted. Particulate organic carbon samples were taken as in one liter containers, and filtered through muffled GF/F filters. ATP samples were collected using 300 mL BOD bottles to allow for quick sampling, and were filtered through standard GF/F filters. These filters were then extracted into 1 mL of PBAC for 30 minutes prior to freezing. Both POC and ATP samples were stored at -80 °C for the remainder of the cruise. In situ samples were collected from every station.

Incubations:
Water samples collected from 10 m depths and mesopelagic depths were incubated for 96 hours, with samples taken at the start of the incubation, after 48 hours, and again at the end. Prior to beginning the incubation period, water samples were amended with 12 mL of 13C labeled *Thalassiosira weissflogii* culture to simulate organic carbon flux as well as provide a better tracer for remineralization. During the incubation period, water samples were incubated within 2 degrees of in situ temperatures, and in the dark to avoid photosynthesis. For the samples which were incubated in the on deck surface water incubator, the containers were wrapped in aluminum foil to prevent light exposure. At each of the sampling time points, two ATP samples of 5 mL each were taken, two bacterial abundance samples of 5 mL each were taken, and one POC sample of 50 mL was collected. ATP and POC samples were collected in the same manner as the in situ samples, while bacterial abundance samples were stabilized with formaldehyde and allowed to rest overnight before being filtered through 0.2 um black filters for epifluorescent microscopy. All samples were stored at -80 °C for the remainder of the

cruise. In total ten incubations (stations 1, 3, 4, 6, 8, 9, 10, 11, 15, and 16) were collected and sampled.

On shore, ATP samples were analyzed using Cell Titer Glo which reacts with ATP to create an autofluorescent signal, which was measured using a scintillation counter. Autofluorescence values were then comparerd to known standards to estimate microbial biomass. Bacterial abundance was estimated using epifluorscent microscopy and direct counting of a fraction of the filters to estimate total abundance. Finally, carbon remineralization was measured using mass spectrometry to estimate remaining carbon at each time point, and applying first order decay kinetics to estimate rates.

Metaproteomic and metagenomic sampling

Maria Papadatou

Samples from different water depths, ranging from the lower mesopelagic zone (600m) to the bathypelagic zone (4200m), were collected at 14 stations in the North Atlantic Ocean. At each selected depth, more than 200L of seawater were processed to obtain sufficient material for downstream analysis. Large volumes of seawater were required due to the extremely low biomass and protein concentrations characteristics of deep, oligotrophic waters.

Water was collected using either (1) 25-liter Niskin bottles or (2) 12-liter NIOZ bottles mounted on a CTD rosette. For large volume sampling (200-300 L) at a given depth, CTD-Niskin bottles were retrieved on individual casts. Sampling depths were chosen based on water masses of interest and varied between stations (St1 – 1000m, St3 – 2000m, St4 – 1200m & 800m, St7 – 2000m & 1000m, St8 – 2500m & 1000m, St9 – 3000m & 1500m, St10 – 3200m, St11 – 3500m & 1500m, St13 – 4200m, St15 – 600m, St16 – 2500m, St17 – 3000 & 800, St18 – 1500, St19 – 2000 & 1000).

Once the CTD was on deck, carboys were rinsed three times with water from the Niskin bottles before being filled and transferred to fill up the filtration tank. Seawater was sequentially filtered through 3 µm filter (142mm diameter) for retaining large particle-attached microbes, and 0.2 µm filter (293mm diameter) for capturing smaller free-living microbial cells. Filters were folded, placed into 15 ml or 50 ml Falcon tubes, respectively, snap frozen, and stored in -80°C until further analysis. These fractions provide material for endoproteomics (proteins inside microbial cells) and metagenomics. The <0.2µm filtrate was further concentrated to a final volume of 30-50ml using a 5KDa molecular weight cutoff, low–protein-binding regenerated cellulose filter in two steps. First, the filtrate was concentrated using the Pellicon 2/Pellicon cassette system for tangential flow filtration (TFF), followed by concentration using a Sartorius Vivaflow 200 Crossflow Cassette. The resulting retentate was snap frozen and stored at -80°C until further analyses. The 5KDa fraction represents the dissolved proteins and small molecules and provides material for exoprotemics (proteins secreted into the surrounding environment).

03	St_4	07.08.25	CTD – 800m	CTD – 1200m *	
04	St_7	10.08.25	CTD – 1000m	CTD – 2000m *	
05	St_8	12.08.25	CTD – 1000m	CTD – 2500m *	
05	St_9	14.08.25	CTD – 1500m	CTD – 3000m *	
07	St_10	15.08.25	CTD – 3200m		
08	St_11	17.08.25		CTD – 1500m *	CTD – 3500m
09	St_13	19.08.25		CTD – 4200m *	
10	St_15	21.08.25	CTD – 600m*		

11	St_16	24.08.25		CTD – 2500m *	
12	St_17	25.08.25	3000	800	
13	St_18	26.08.25		CTD – 1500 *	
13	St_19	27.08.25	CTD – 1000m		CTD – 2000m