

RV Pelagia Cruise Report

Cruises 64PE217 & 64PE230

Project:

Bacterioplankton cell death: competition between flagellate grazing and viral lysis and the role of bacterioplankton cell wall-derived dissolved organic matter in the ocean

(BADE)

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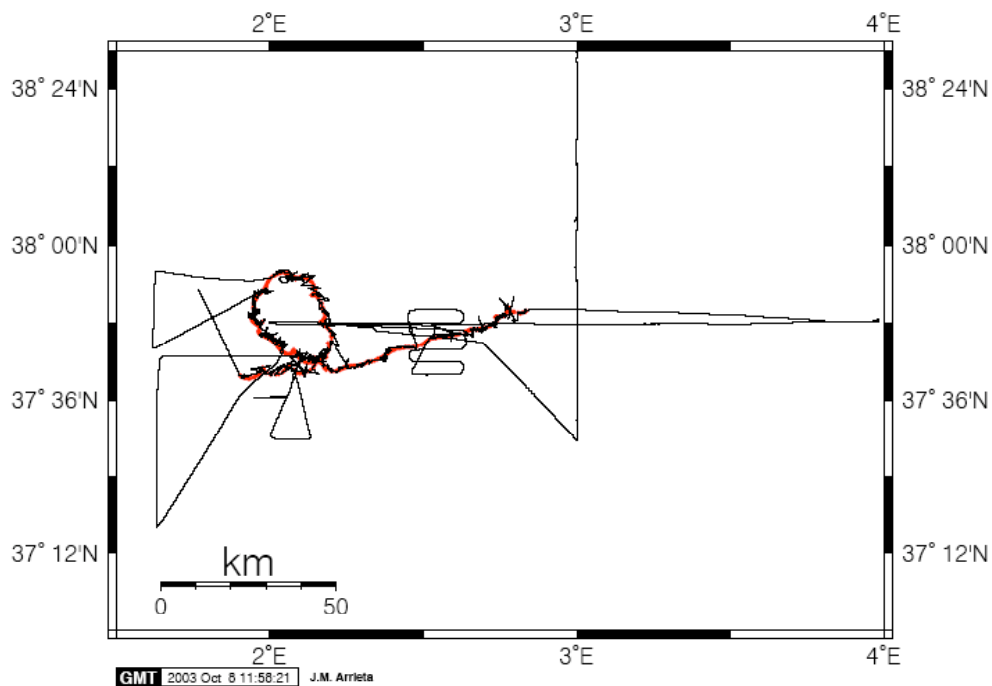
Scientific background

The world's ocean is inhabited by ca. 1.2×10^{29} prokaryotes producing ca. 9.3×10^{29} cells per year, or in terms of carbon, about 10-20 Gt C y^{-1} . Thus global oceanic prokaryotic production amounts to about 50% of global oceanic primary production (20-30 Gt C y^{-1}). From this comparison, it becomes clear that the fate of this bacterial production is of crucial importance for the oceanic carbon cycle. It is now recognized that besides flagellate grazing viral lysis is the main mechanism causing mortality of prokaryotes. On average, both mechanisms seem to be equally important for prokaryotic cell death, however, their relative contribution is highly variable over time and space. The partitioning of mortality between grazing and viral lysis is not trivial to resolve, but has considerable implications for the flow of energy and matter through the microbial food web.

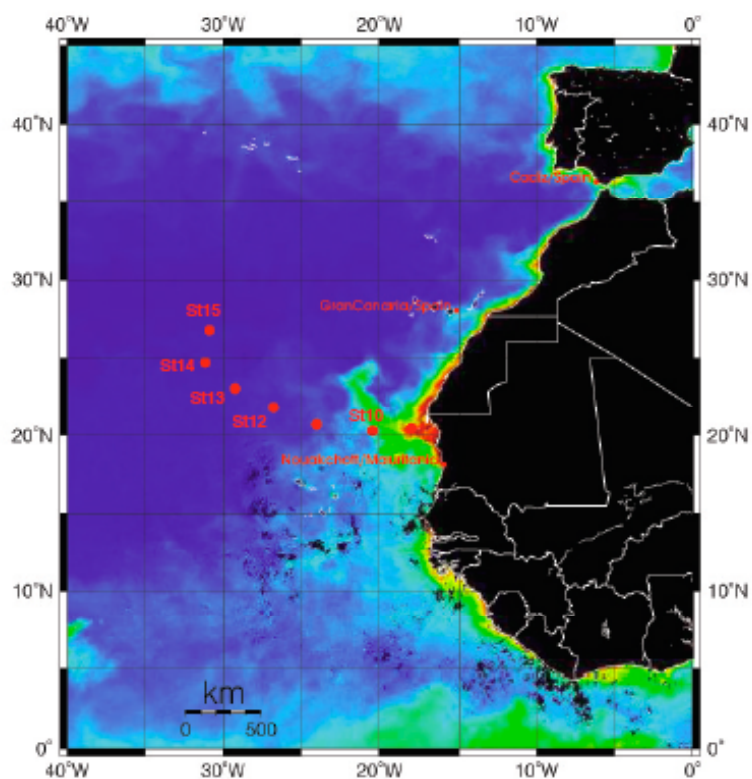
During the 2 cruises in the frame of the BADE project we addressed the question of viral control of bacterial production, as a biotic factor of bacterial cell death and abiotic factors such as ultraviolet radiation in two different systems. During BADE-1 we focused on a stable microbial community as it occurs in anticyclonic eddies. In the Algerian basin of the western Mediterranean Sea, these eddies are stable over periods of months providing ideal conditions to study viral control of bacterioplankton under near steady conditions. In contrast, during BADE-2, focus was put on changing prokaryotic abundance and variable community composition by following a transect from the Mauritanian upwelling region into the subtropical North Atlantic gyre.

Surface water samples were collected and a number of microbial parameters determined over diel cycles as outlined in more detail below. Particular emphasis was also paid to the biota of the sea-surface microlayer and to the exchange of carbon dioxide and its diel variability as mediated by the biota and by abiotic factors across the sea surface microlayer and the atmosphere.

Thus, the ultimate goal of the 2 BADE cruises was to determine the microbial activity over diel cycles under two contrasting conditions, a stable eddy system and along a trophic gradient. The underlying hypothesis was that solar radiation under otherwise stable eddy conditions generate disturbance in the microbial community large enough to induce changes in the phylogenetic composition of the prokaryotic community. Under changing trophic conditions we hypothesized that changes in surface water productivity of phytoplankton is directly linked to phylogenetic changes in the prokaryotic community composition and that related to that, to the role of viruses in controlling bacterial abundance and production.



BADE-1 cruise track in the Algerian basin of the western Mediterranean Sea. A floating drifting buoy was deployed in the center of the anticyclonic eddy and followed over a period of more than two weeks.



BADE-2 cruise. Stations occupied in the North Atlantic from the Mauritanian upwelling into the subtropical North Atlantic gyre.

Dates and list of scientific crew on BADE-1

Dates:

19 Sep 2003: emarking R/V *Pelagia* at Palma de Mallorca

10 Oct 2003: debarking at Valencia

List of scientific crew:

Name	Affiliation	function on cruise
Gerhard J. Herndl	NIOZ	chief scientist,
Susana Agusti	CSIC-Mallorca	picophytoplankton viability
Patricia Alonso	CSIC- Mallorca	picophytoplankton viability
Txetxu Arrieta	NIOZ	capillary electrophoresis, T-RFLP, ectoenzymes
Veronica Parada	NIOZ	viral production, lysogeny,
Eva Sintes	NIOZ	ultrafiltration, DOM fingerprinting, CTC, PI
Eva Alou	CSIC- Mallorca	picophytoplankton viability
Thomas Reinthaler	NIOZ	respiration, oxygen
Markus Weinbauer	LOV, Villefranche	viral ecology
Osana Bonilla-Findji	LOV, Villefranche	viral ecology
Francesca Malfatti	SIO-UCSD, San Diego	bacterial ecology
Jan Hegeman	NIOZ	inorganic nutrients, DON, DOP
Martin Laan	NIOZ	electronics, CTD operator,
Santiago Gonzalez	NIOZ	undulating CTD, DOC, POC

Dates and list of scientific crew on BADE-2

Dates:

16 Sep 2004: embarking R/V *Pelagia* at Cadiz

13 Oct 2004: debarking R/V *Pelagia* at Las Palmas, Gran Canaria

List of scientific crew:

Name	Affiliation	function on cruise
Gerhard J. Herndl	NIOZ	chief scientist, prokaryotic production
Patricia Alonso	CSIC-Mallorca	phytoplankton
Moira Llabrés	CSIC-Mallorca	phytoplankton
Txetxu Arrieta	NIOZ	capillary electrophoresis, T-RFLP, ectoenzymes
Veronica Parada	NIOZ	viral production, lysogeny,
Eva Sintes	NIOZ	MICRO-FISH, DOM fingerprinting
Ines Wilhartitz	NIOZ	viruses
Thomas Reinthaler	NIOZ	respiration, oxygen
Herman Boekel	NIOZ	High pressure sampler testing
Maria Calleja	NIOZ	CO ₂ , O ₂ , DOC,
Elena Stoica	NIOZ	archaea, MICRO-FISH
Anssi Vähätalo	NIOZ	DOC, UV experiments
Arjan Smit	NIOZ	radioactivity officer, prokaryotic production
Karel Baker	NIOZ	inorganic nutrients, DON, DOP
Jan Derksen	NIOZ	CTD operator data management

Field work of the individual participants during BADE-1

CTD work

The NOEX sampler bottles were cleaned with bleach at the beginning of the cruise, as well as halfway. Water samples were taken for biological analysis as well as for the calibration of the salinity (conductivity) and oxygen sensors. The calibration samples were processed on board. The salinity was determined by means of a Guildline 8400B salinometer in a temperature controlled laboratory container. The oxygen concentration was determined by means of a spectro-photometric method.

The data have been processed preliminarily with a vertical resolution of 1 dbar. For all water samples the CTD readings have been processed too. After the cruise these data will be corrected for the final calibration of the sensors. A preliminary analysis suggests that a linear correction of the manufacturers calibration will yield an accuracy of the pressure, temperature and salinity measured by the CTD (standard deviation) of respectively 0.7 dbar, 0.001°C and 0,001. The newly acquired SBE 43 oxygen sensor behaved quite reproducible. A calibration to an accuracy well within 2 µmol/kg by means of a linear algorithm appeared to be possible. Final calibrations of the CTD sensors will be determined after the cruise.

Configuration of the CTD sensors

Type	serial nr
SBE temperature sensor	1197
SBE conductivity sensor	2142
SBE pressure sensor	53978
Chelsea fluorometer	88/725/026
SBE 43 oxygen sensor	0234
Irradiance sensor (PAR)	4410
Seapoint turbidity meter	1737

Total and Inorganic Nitrogen and Phosphorus analyses

Jan van Ooijen, Karel Bakker, Evaline van Weerle (Dept. of Marine Chemistry and Geology, Royal Netherlands Institute for Sea Research – NIOZ)

A Traacs 800 autoanalyzer was used for spectrophotometric determination of the different nutrients using the classic methods. All the samples were taken immediately and stored frozen until analyses back in the laboratory.

Phosphate:

Ortho-phosphate is measured by formation of a blue reduced molybdophosphate-complex at pH 0.9-1.1 at a wavelength of 880nm. Potassium antimonyltartrate is used as a catalyst and Ascorbic acid as the reductant.

Described by Murphy and Riley, 1962

Ammonium:

Ammonium is measured as the indo-phenolblue-complex, using phenol and sodium hypochlorite at a pH of 10.5 using citrate as a complexant for calcium and magnesium at this pH. The resulting color is measured at 630nm.

Described by Koroleff, 1969, and optimized by Helder and de Vries, 1979

Nitrate and Nitrite:

Diazotation of nitrite with Sulfonylamide and N-(1-Naphtyl)-Ethylene Diamonium Dichloride to form a reddish-purple dye measured at 550nm. Nitrate is separately first reduced in a copperized Cd-coil using Imidazole as a Buffer and is then measured as Nitrite

Described by Grasshoff, 1983

Dissolved silica:

Measured as a blue reduced silicon-molybdenum-complex at 880nm. Ascorbic acid is used as reductant and oxalic acid is used to prevent interference of phosphate.

Described by Strickland and Parson, 1972

Total N and Total P:

Destructions were carried out by G. Kramer in a pressure-cooker using Teflon bombs and a buffered persulfate reagent.

Total N is measured as nitrate and nitrite being the oxidation products after destruction for N using the method as mentioned above.

Total P is measured after diluting the destructed samples three times with a mixture of ascorbic acid added to the seawater used as the blank for the autoanalyser.

Sample handling:

All samples were filled into high-density 125ml polyethylene sample bottles after rinsing three times with sample water.

Calibration and Standards:

Calibration curves were daily produced by diluting stock standards in plastic calibration-flasks. Nutrient depleted aged surface ocean water was used to dilute the standards to determine the calibration-lines and as water for the baseline of the autoanalyzer. As a daily check of the calibration, a lab-made cocktail-standard containing all nutrients was measured in every run. This cocktail was diluted 100-fold in the same ocean water, as a reference standard.

For TN and TP, a mixture of 10 organic compounds was treated in the same way as the samples as a recovery check of the method.

Performances:

Reproducibility of 10 replicates from one bottle within a run:

	mean μM	SD	cv. % of level	or	% off full scale
SiO ₂	14.99	0.11	0.7		0.25
PO ₄	0.875	0.004	0.5		0.15
NH ₄	0.84	0.03	3.4		0.7
NO ₃	14.07	0.03	0.18		0.06

For the CTD station 2 all 22 NOEX bottles were closed at the same depth and analyzed. The resulting statistics of this analysis are:

	mean μM	SD	cv. % of level	or	% off full scale
PO ₄	0.817	0.006	0.7		0.25
NH ₄	0.09	0.01	14		3
NO ₃	12.55	0.03	0.23		0.07

DOC sampling

Santiago Gonzales (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)

Water samples for DOC were taken directly from the NOEX bottles into combusted glass ampoules acidified with conc. phosphoric acid, sealed and stored at -20°C . DOC analyses are done back in the lab on a Shimadzu TOC-5000 analyzer.

Effects of viral lysis and predation on bacterial mortality, production and diversity.

Osana Bonilla-Findji and Markus Weinbauer (LOV, Villefranche sur mer, France)

It is well known that marine bacterioplankton play a key role in the remineralization of organic matter and thus in biogeochemical cycles. Two main mechanisms are responsible for the “top down” control of bacterial populations: 1) grazing by protists (mainly heterotrophic nanoflagellates) and 2) viral lysis. Both processes can account for an equal amount of bacterial mortality. Some studies have suggested competition between grazers and viruses for prey. Moreover preliminary evidence indicates that grazing may increase viral infection, suggesting some synergy between grazing-induced and viral-induced mortality. Also, viruses may influence bacterial diversity by keeping in check winners of resource competition. For offshore systems, viral effects on bacterioplankton remain poorly studied.

In order to compare the effects of viral lysis versus grazing on bacterial mortality, production and diversity 8 experiments were carried out with in situ communities from an eddy in the western Mediterranean Sea. Samples were collected from 20 m and the depth of the chlorophyll maximum (DCM, ca. 50 m) to create 4 treatments: bacteria without predators, bacteria with viruses (+V), bacteria with flagellates (+F), and bacteria with viruses and flagellates (V+F).

Two parallel approaches were used to address the same issue:

1) Concentrate approach.

These experiments were performed four times: two with samples from 20 m depth and two with samples from DCM layer. Between 80 and 100 liters of seawater were sequentially size-fractionated in order to obtain: the flagellate+bacteria concentrate (FC, between 0.2 μm and 3 μm), the bacterial concentrate (BC, between 0.2 μm and 0.8 μm) and the viral concentrate (VC, from 100 kDa to 0.2 μm).

The different treatments were obtained by diluting the concentrates with virus free water in order to reach ca. the in situ bacterial abundances. Heat-inactivated viruses were added to all fractions containing no viruses.

Treatments were made in triplicate in 2L polycarbonate bottles and incubated in the dark at in situ temperature for 60 to 70 hours. To study the changes in bacterial dynamics, samples were taken during the incubation to determine: bacterial and viral abundances (BA, VA), flagellates abundance (FA), bacterial production, bacterial diversity, burst size, and viral diversity (end of the experiments).

2) Dilution approach.

These experiments were also performed 4 times as previously described. In this case, 2L of seawater were gently filtered through 3 μm and 0.8 μm filters to obtain a “flagellates+bacteria fraction” and a “bacterial fraction” respectively. Then, this two fractions were diluted 10-fold with either 0.2 μm filtered seawater (with viruses) or virus-free water to obtain the following 4 treatments: Bacteria without predators

(bacteria fraction + virus free water), bacteria + viruses (bacteria fraction + 0.2 μm filtered water); bacteria + flagellates (flagellate fraction + virus-free water), bacteria + viruses + flagellates (flagellate fraction + 0.2 μm filtered water)

Treatments were done in triplicate 1 L bottles and samples for the same parameters as for the “concentrate approach” were collected during the incubation.

Determination of in situ Viral Production & Lysogeny

Osana Bonilla, Veronica Parada & Markus Weinbauer (LOV Villefranche sur mer and Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)

We know that approximately 20 to 50% of the bacterial mortality in the ocean can be caused by viral lysis, on the same order of magnitude of that caused by protistan grazing. Viral infection of bacteria can be lytic, causing destruction of the host cell, or lysogenic, in which the viral genome is instead maintained as a prophage within its host. The prophage genes remain dormant until an induction agent results in activation of prophage and initiation of the lytic cycle.

To understand the diel changes in viral production and lysogenic events, we sampled once each 4 hours at two depths (DCM and 20 m) during 4 days in a western Mediterranean eddy system. We used the dilution approach that let us determine the increase of viruses over time which arise from already infected bacterial cells by avoiding new infection. Briefly, 1 L of natural seawater from each depth was processed via tangential flow ultrafiltration to get a bacterial concentrate of 20ml that was added to 50 ml viruses free water at bacteria abundances close to in situ abundances; then one set of triplicates was amended with mitomycin C (1 $\mu\text{g/ml}$).

They were incubated in darkness at in situ temperature during 36 hours, subsampling for virus and bacterial abundance was done at three hours intervals. The subsamples were stored at -80°C to be counted later at flow cytometer.

Also aliquots from the bacterial concentrate were taken to determine burst size and the frequency of visibly infected bacteria via TEM.

Bacterial and Viral Diversity

Veronica Parada & Markus Weinbauer (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ and LOV, Villefranche sur mer, France)

To evaluate the viral diversity in situ and newly produced after 24 or 36 hours, we collected 100L of seawater from 20m and DCM depth that were processed through Millipore Pelicon tangential flow filtration to get a bacterial concentrate of approx. 600 ml that was equally distributed in 4 bottles of 20 L containing viruses-free water. Subsamples to check viral production were taken once every 6 hours.

One L of natural seawater was filtered through 0.22 μm at the onset of the experiment to obtain bacterial richness in situ; the filter was frozen at -80C to be subjected to T-RFLP analysis later. After the incubation time, we repeated the process to account for changes on bacterial richness.

Twenty L of natural seawater were processed through a 100KDa tangential flow filtration to get a viral concentrate of ca. 300 ml which will be later analyzed by PFGE

to investigate the viral diversity in situ. To check the viral diversity newly produced, the same procedure was run after the incubation time.

Microlayer Experiment

Veronica Parada & Markus Weinbauer (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ and LOV-Villefranche sur mer, France)

Samples of 300ml from the surface microlayer and 30 cm below the sea surface were taken in the early morning, after late noon and in the evening during 4 days. Measurements were performed to determine bacteriophage production and lysogeny following the same method described below.

Phytoplankton viability

Susana Agustí, Patricia Alonso, Eva Alou, IMEDEA (Instituto Mediterráneo de Estudios Avanzados), CSIC -Univ.I.B., Miquel marques 21, Esporles. Balearic Islands, Spain

Our goal during BADE-1 was to study the variability of phytoplankton cell death and evaluate its importance for the carbon flow in the oligotrophic waters of the Mediterranean Sea, confined in an anticyclonic eddy. We focused in the dynamics of picocyanobacteria abundance and cell death since this group dominates phytoplankton biomass and production in oligotrophic waters.

The study included:

Picocyanobacteria abundance and the proportion of living and dying cells throughout the water column. Their temporal variability including daily variation.

The abundance of picocyanobacteria and eukaryotic cells was quantified by flow cytometry analysis in fresh samples on board. Samples fixed in glutaraldehyde will be counted by flow cytometry at the IMEDEA lab. The proportion of living cells was quantified in fresh samples by using the cell digestion assay (Agustí and Sánchez 2002), a membrane permeability test that removes dead cells from the sample, allowing a clear identification of living cells. After the incubation with the enzymes, the living cyanobacteria cells were quantified by flow cytometry.

Picocyanobacteria growth dynamics based on cell division analysis to examine the influence of the cell division cycle on the daily variability of picocyanobacteria cell death. Samples from different depths during the 4 diel cycles made during the cruise were fixed with glutaraldehyde and frozen in liquid nitrogen for the analysis at the IMEDEA Lab for picocyanobacteria cell division by analysis of DNA. Also samples from surface, from the continuous running water system were taken every 2h by an automatic device, fixed and frozen in liquid nitrogen for the analysis of cyanobacteria cell division cycle.

Phytoplankton lysis rates and daily variability of dissolved esterase activity. Samples from 8-11 depths were taken for the analysis of dissolved esterase activity by a spectrofluorometric method. The dissolved esterase activity found in seawater will be used to calculate phytoplankton lysis rates.

The abundance and proportion of living/dying cells of other groups present in the phytoplankton community at the surface and the DCM.

Between 1 to 3 L from the surface and the DCM were concentrated by using a Millipore chamber, to a final volume of 40 ml. Replicated 10 ml were incubated with DNase and trypsin for the digestion of death cells, and replicate 10 ml were incubated without enzymes as a blank. After the incubation, the samples were filtered on 0.8 μm Nuclepore filters, fixed with glutaraldehyde and frozen for the observation and counting under epifluorescence microscopic at the IMEDEA lab.

Primary production rates and the production of dissolved organic carbon by phytoplankton.

Primary production was quantified by ^{14}C -bicarbonate assimilation, by in situ incubations of water from 5 depths between the surface and the DCM.

The temporal variability of phytoplankton carbon excretion, including immediate excretion.

To analyze the importance of phytoplankton cell death in the release of DOC by phytoplankton.

To analyze the abundance and proportion of dead picocyanobacteria cells in the upper layers (micro-layer and 20 cm layer) of the Mediterranean Sea.

To evaluate the importance of UV radiation as a factor of phytoplankton cell death, by analyzing:

- 1) the relation between underwater UVB -UVA extinction coefficients and the distribution of death cells
- 2) experimentally test the importance of UV radiation on the cell death of *Synechococcus* and *Prochlorococcus*
- 3) to evaluate the importance of UV radiation in phytoplankton carbon excretion.

Preliminary results.

The vertical distribution of phytoplankton cell abundance analyzed by flow cytometry indicated that *Synechococcus* sp. was more abundant in surface waters (5-30 m). *Prochlorococcus* sp. was, however, more abundant in deeper layers of the photic zone. The maximum abundance of eukaryotic phytoplankton was found at the depth of the DCM (around 50m). *Prochlorococcus* sp. abundance in surface waters, however, increased with time, dominating phytoplankton abundance in the surface waters by the end of the cruise.

Diel dynamics of bacterial communities and dissolved organic matter

Eva Sintes, Txetxu Arrieta and Gerhard Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)

Aquatic bacteria are recognized as playing an important role in the global carbon cycle and they are also recognized as the main consumers of dissolved organic matter. Thus a better knowledge of the relationships of the organic matter composition with the dynamics of the bacterial communities, and also with the microorganism cellular characteristics is necessary. To do this, we studied different aspects of viability and activity of the bacterial populations jointly with the characterization of the organic matter composition.

The parameters studied were:

Bacterial abundance: assessed by flow cytometry after Syto 13 staining. We also distinguished between low and high DNA bacteria depending on their green fluorescence patterns

Bacterial viability: assessed by using two different nucleic acid stains that enter the cells with damaged membrane: propidium iodide and sytox green (Molecular Probes).

Metabolically active bacteria: They were determined as those reducing CTC to its fluorescent form due to the electron transport activity and detected by flow cytometry.

Bacterial production: It was measured via [³H]-leucine incorporation into bacterial protein

Microfish: Bacterial cells that are able to uptake [³H]-leucine will be identified using different probes

Dissolved organic matter: Large (220 l) or small (0.5 l) volumes (depending on the expected concentrations) of 0.2 µm filtered water were concentrated by ultrafiltration and will be used to assess the protein and carbohydrates fingerprinting.

These factors were studied during four diel cycles following a drifting buoy developed in the center of an open sea Mediterranean eddy at a maximum of six different depths, and during four diel cycles carried out with samples from the surface microlayer and the layer 30 cm depth.

Diel Cycle of Bacterial Growth Efficiencies in a Mediterranean Eddy System

Thomas Reinthaler and Gerhard Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)

Bacteria are acknowledged to play an important role in the remineralization of dissolved organic matter (DOM). Bacterial growth efficiencies – calculated by $BGE = BP / (BP + BR)$ – serve as proxy to estimate the amount of DOM taken up and remineralized by the bacterial compartment. Generally for the whole water column a BGE of 30% is applied to model biotic organic carbon fluxes. Variations in bacterial growth efficiencies especially of open ocean waters are rarely studied. This severely hampers the calculation of carbon fluxes and carbon budgets.

During 4 diel cycles we measured bacterial respiration in the deep chlorophyll maximum layer (DCM), at 20m and in surface waters at around 5m depth. We also measured respiration and production in 4 surface micro layer (SML) diel cycles.

Methods

The sample water was filtered over 0.8µm polycarbonate filters and transferred to dekanter flasks which were incubated to reach in situ temperature and shaken vigorously to saturate the oxygen content of the water.

Subsequently bacterial oxygen demand (BOD) bottles (nominal volume of 120 cm³) were filled, t₀ bottles were stopped immediately by adding the Winkler reagents. Both t₀ and t₁ bottles were incubated submersed in temperature-controlled water baths in the dark. T₁ BOD bottles were stopped between 12 and 24 hours. All time points were done in triplicates.

We used a spectrophotometric approach based on the classical Winkler method for oxygen measurements. Measurements were done on a HITACHI U-3010 Spectrophotometer with a flow through cuvette. T₀ and t₁ bottles were measured in the same run.

From the 0.8µm filtrate samples were also drawn for bacterial abundance counts and bacterial secondary production measurements.

Diel variations in bacterial ectoenzymes and their corresponding substrates.

J.M. Arrieta E. Sintes and G.J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research –NIOZ)

Bacteria are the only significant consumers of the dissolved organic matter (DOM) pool in the oceans. However, most of the bio-reactive DOM in the oceans is bigger than 1,000 dalton and must be hydrolyzed into smaller molecules before it can be taken up by bacteria. Therefore bacterial hydrolysis of high molecular weight DOM is considered to be the rate-limiting step in bacterial cycling of DOM in the oceans. Our main goal was to characterize the diel variations in both the ectoenzyme activities and the concentrations of the corresponding substrates.

Methods

In order to achieve this goal we measured the following parameters:

Bulk ectoenzyme activities of leucine aminopeptidase, α - and β -glucosidase and phosphatase, representing the potential hydrolysis rates of proteins, carbohydrates and phosphate esters respectively. These measurements were performed by means of methyl-umbellyferyl substrates.

Diversity of ectoenzymes by capillary electrophoresis zymography as described previously. Aliquots of the enzyme extracts were exposed to UV radiation in order to characterize the potential differences in the sensitivity of ectoenzymes to UV-induced damage.

Large sample volumes have been taken in order to obtain high-quality DOM concentrates by ultrafiltration. These concentrates will be used to produce a fingerprint of the most abundant classes of carbohydrates and proteins available as potential substrates for the above mentioned ectoenzymes.

Relation between POM enrichment and bacterial community composition

Francesca Malfatti (SIO-UCSD, San Diego, California, USA)

Particles are important elements in the carbon cycle in the water column. Particles might have different origin and therefore might have developed different resident bacterial community. One can consider particles as important and unique site for biological processes. The goal of the work was to study the colonization processes and the bacterial dynamics occurring on particles in a stable oligotrophic water mass. For this purpose agar based aggregates were used as a proxy for natural particles.

Material and Methods

Agar-based beads were used enriched with different POM (casein/chitin, DNA, starch) and added to seawater from 5 m depth in triplicate to 50 ml of seawater.

Incubations were performed on deck over 24 h.

Measurements: bacterial abundance, bacterial community composition, bacterial production, enzymatic activity

Prokaryotic production measurements

Gerhard J. Herndl & Jan Hegeman (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

Prokaryotic production was measured via [3H]-leucine (20nM final conc., SA 151 and 160 Ci mmol⁻¹) and/or [3H]-thymidine (10nM final conc.) incorporation into bacterial protein and DNA, respectively. All the samples (10-40 ml) were done in duplicate with one formaldehyde-killed (3% final conc.) blank. Incubation temperature was close to in situ temperature ($\pm 1^\circ\text{C}$) and incubation period varied between 4 and 8 h, depending on the expected general activity. The filters were rinsed twice with ice-cold 5% trichloroacetic acid and transferred to scintillation vials which were stored frozen until the radioactivity was assessed in the radioisotope lab of the NIOZ.

On selected samples, prokaryotic production was also measured on the 0.6 μm filtered fraction for water column in order to allow direct comparison with the prokaryotic respiration of this fraction. Fractionation over 0.6 μm filters was done to separate free prokaryotic plankton from larger protists.

Determining prokaryotic ectoenzymatic activity

Jesus Maria Arrieta (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

Bulk bacterial ectoenzyme activities (aminopeptidase, α - and β -glucosidase, phosphatase) were measured by means of methylumbelliferyl-substrates (Hoppe 1983) in triplicate 5 ml subsamples containing the corresponding substrate at 100 $\mu\text{mol L}^{-1}$ final concentration. The increase in fluorescence ($\lambda_{\text{ex}}=360 \text{ nm}$, $\lambda_{\text{em}}=445 \text{ nm}$) was monitored during incubations at in situ temperature every 2 h for up to 8 h until a significant increase was measured. When no significant fluorescence increase was measured after 8 h, the ectoenzyme activity was considered to be 0.

Concentration of bacterial biomass by tangential flow filtration for zymography and DNA fingerprinting. Large seawater samples (150-200 L) were filtered through 0.8 μm -pore-size polycarbonate filters (142 mm diameter, Millipore) to exclude most of the eukaryotic organisms. To minimize clogging, the filter was replaced every 25 L. Bacteria in the filtrate were concentrated to a final volume of about 0.5-L using a Pellicon (Millipore) tangential flow filtration system equipped with a 0.2 μm pore-size filter cartridge (Durapore, Millipore). Bacteria in the retentate of the Pellicon system were further concentrated by centrifugation (20,000 $\times g$; 30 min; 4 $^\circ\text{C}$). The resulting pellet was washed 3 times with 0.2 μm -filtered seawater and split into aliquots for subsequent analysis (16S rDNA fingerprinting, zymography).

16S rDNA fingerprinting of the prokaryotic community using T-RFLP, and cloning and sequencing

Jesus Maria Arrieta (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis of bacterial communities is performed using the methods previously used in our lab (Moeseneder et al, 1999). Briefly, DNA is extracted from an aliquot of the bacterial concentrate and subsequently amplified by PCR using the bacteria-specific forward primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the universal reverse primer 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). The forward primer (27F) is 5'-labeled with 5-carboxy-fluorescein and the reverse primer (1492R) with 6-carboxy-4',5'-dichloro-

2',7'-dimethoxyfluorescein. All primers are synthesized by Interactiva (Ulm, Germany). With this technique two labeled fragments can be obtained from each PCR product after the restriction digest, readily distinguishable by their fluorescence emission wavelength using an ABI Prism 310 capillary sequencer (Moeseneder et al. 2001). Two different restriction enzymes *HhaI* and *MspI* are used independently on each sample to generate the restriction patterns. Only those peaks with a peak area > 1% of the total peak area of the electropherograms were counted. Once phylotypes of interest are identified they are cloned and sequenced, so that oligonucleotide probes can be designed to be used for the CARD-FISH assay in future studies on the deep ocean.

Field work of individual participants during BADE-2 (only work is described in this chapter which has not already been mentioned under BADE-1 activities; all the activities described under BADE-1 activities were also performed on BADE-2)

Carbon dioxide and oxygen flux across the air-sea interface

Maria Calleja (IMEDEA (Instituto Mediterráneo de Estudios Avanzados), CSIC - Univ.I.B., Miquel marques 21, Esporles. Balearic Islands, Spain

The main objectives were

the determination of air-sea gas (CO₂ and O₂) fluxes and partial pressures along a vertical gradient in the water column. The study of the effects of surface organic films in the air-sea gas transfer coefficient are an important point I addressed during this cruise.

the characterization of organic matter from surface sea layer and its comparison with the one from 5m depth.

MEASUREMENT OF GAS FLUXES IN THE OCEAN-ATMOSPHERE INTERFACE.

The potential role of surface water layer surfactants in modulating air-sea gas exchange has been examined in the open ocean. The area of study ranged from the highly productive zone of North-East African coast to the less biological active and more oceanic Subtropical Gyre zone.

Direct gas flux measurements were done using the chamber technique, and the relationship between gas transfer velocity and naturally occurring surfactants was evaluated at different wind speeds. Evidences that surfactants have suppressing effects on gas exchange in the air-sea interface were found.

Thus, to confirm that hypothesis and have more consistent results we performed additional measurements during BADE-2. We compared the CO₂ partial pressure from surface layer water with the one at 5m depths to evaluate the possible error done when calculating the flux using pCO₂ data from 5m depths instead of a more superficial data.

TOC sampling focused on the surface layer, not on the microlayer. The surface layer is operationally described as the top centimetre of the water-column. Thus, surface water was sampled from the zodiac with an acid cleaned syringe connected to an acid cleaned silicone tube with a floating piece allowing the collection of the first 2cm depth. Surface layer TOC for characterization will be also sampled.

CO₂ and O₂ air-sea flux; a gas analyzer was used and also a floating exchange chamber deployed on the sea surface. The chamber has to remain floating during the measurement. Analyzer and chamber were connected. Once connected, the measurement depends on the CO₂ partial pressure difference between air and surface ocean; the larger the difference, the less time is required. Normally in oligotrophic waters the pCO₂ gradient in between air-sea interface is small so measurements over a longer period are required ranging from 15 to 40 min.

pCO₂ and pO₂ vertical profile in the first 5m depths: Comparison of the partial pressures of CO₂ and O₂ in the surface layer and 5m depth (the depth from where water was taken to measure pCO₂ was used afterwards to calculate air-sea fluxes). To do that a tube with a data-logger connected to a peristaltic pump was used to pump seawater from the required depth. A TOC sample was also taken from 5m-depth water for characterization.

VERTICAL PROFILES OF CO₂ AND O₂ PARTIAL PRESSURES IN THE WHOLE WATER COLUMN

On a previous cruise we had good success measuring CO₂ and O₂ partial pressures from different depths using our gas analyzer through measurements directly of Niskin bottles collected from the Rosette. This technique was developed following just in several stations so I very much appreciated the opportunity to conduct these measurements on board of RV *Pelagia* and get some more data.

To do so, I connected the analyzer, through a gas exchangeable column to the Niskin bottles and run about 6 L of water through the exchange column. Moreover, water sample to **control alkalinity** from each depth was also taken.

CO₂ AND O₂ SURFACE WATER (5m) AND ATMOSPHERE PARTIAL PRESSURES MEASUREMENT CONTINUOUSLY ALONG THE WHOLE TRANSECT.

Gas flux air-sea exchange can be calculated indirectly from a parameterized gas exchange coefficient and the gas partial pressures in water and atmosphere. It was done by connecting the two gas analyzers, one to the water surface entrance, and the other one to the air away from the fume contamination.

To do the flux calculations some meteorological (wind speed, irradiation, water and air temperature) and navigating data (position) were needed.

Enumeration of picoplankton, Crenarchaeota and Euryarchaeota

Eva Sintes (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

Water masses were identified based on their salinity-temperature characteristics. Water from the distinct water masses was collected with NOEX-bottles mounted on a CTD frame. Samples were taken for enumeration of total picoplankton, Bacteria and Archaea and fixed instantly with formaldehyde (2% final conc.). Heterotrophic picoplankton are enumerated after DAPI staining, Bacteria and Archaea by catalysed reporter deposition fluorescence in situ hybridisation (CARD-FISH) under the epifluorescence microscope. For enumeration of Bacteria the oligonucleotide probe Eub338 are used, for Crenarchaeota Cren537 (5'-TGACCACTTGAGGTGCTG-3'), and for Euryarchaeota Eury806 (5'-CACAGCGTTTACACCTAG-3'). All the probes were tested for their specificity prior to the study. Cell walls are permeabilized for Eub338 with lysozyme (Sigma; 10 mg ml⁻¹ in 0.05 M EDTA, 0.1 Tris-HCl [pH 8]) or with proteinase-K for Eury806 and Cren537 ([1844 U mg⁻¹, 10.9 mg mL⁻¹, Sigma]; 0.2 µl ml⁻¹ in 0.05 EDTA, 0.1 Tris-HCl [pH 8]) at 37°C for 1 h. Probe working solution (50 ng µl⁻¹) are added at a final concentration of 2.5 ng µl⁻¹. Hybridisation is done at 35°C for 8-12 h.

Microautoradiography combined with CARD-FISH (MICRO-CARD-FISH).

Eva Sintes & Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

To 30-40 ml samples [³H]-leucine (SA 157 Ci mmol⁻¹, Amersham) at a final concentration of 20 nM or [¹⁴C]-bicarbonate (100 µCi, Amersham) was added and incubated in the dark at in situ temperature for 8-10 h or 60-72 h, respectively. Controls were fixed with 2% paraformaldehyde final concentration. Incubations were

terminated by adding paraformaldehyde (2% final concentration) and storing the samples in the dark at 4°C for 12-18 h. The autoradiographic development is conducted in the home lab by transferring previously hybridized filter sections onto slides coated with photographic emulsion (type NTB-2, melted at 43°C for 1h). Subsequently, the slides are placed in a dark-box with a drying agent and exposed at 4°C for 36-48 h. The slides are developed and fixed using Kodak specifications (Dektol developer [1:1 dilution with Milli-Q water] for 2 min, a rinse with Milli-Q water for 10 s, in fixer for 5 min followed by a Milli-Q water rinse for 2 min). Cells are counter-stained with a DAPI-mix (5.5 parts of Citifluor, 1 part Vectashield and 0.5 parts of PBS amended with DAPI at a final concentration of 1 $\mu\text{g ml}^{-1}$). The silver grains in the autoradiographic emulsion are detected by switching to the transmission mode of the microscope. More than 700 DAPI-stained cells are regularly counted per sample.

Photoprocesses of DOM related to upwelling

Anssi Vähätalo and Gerhard J. Herndl (Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research – NIOZ)

The DOM upwelling from deep ocean must be biologically recalcitrant, because the labile components of DOM must have been biologically decomposed during the long (>100 years) residence time of DOM in the deep ocean. A portion of the DOM in the deep ocean is likely photochemically reactive, since the previous exposure of deep-DOM to the photolytic solar UV-radiation is minimal or even non-existing. For example, the organic matter generated in the deep chlorophyll maximum that sinks to deep-ocean (e.g., fecal pellets of zooplankton) and dissolves later does not expose to solar UV-radiation until it reaches upwelling area. Although many components of the organic matter of phytoplankton are biodegradable, some parts such as pigments may resist biological decomposition for thousands of years (at least in anaerobic sediments). These biologically recalcitrant components such as pigments or decomposition products associated with chromophores are sensitive to photochemical decomposition in surface waters.

During upwelling, the DOM from deep ocean exposes to solar radiation. When the upwelled DOM reaches the surface (depths <50 m), solar radiation starts to decompose the photodegradable part of upwelled DOM. During this photochemical decomposition, photolabile DOM will be mineralized (to CO_2 , NH_4^+ , NO_2^- , Fe^{2+} , PO_4^-) and transformed into biologically labile DOM. In the case of nitrogen, photochemical reactions may convert inorganic nitrogen into DON. The photochemically produced labile DOM contributes to bacterial productivity. The photochemically produced nutrients (NH_4^+ , Fe^{2+} , PO_4^-) or incorporation of DIN to DON contribute to primary productivity. If photochemistry generates DON from upwelled inorganic N and DOM, the photochemically generated DON may not be directly available to phytoplankton. Further, photochemical processing of this DON may again release inorganic N a bit aside of the upwelling region. The photoproduction of PO_4^- is related to photoreduction of Fe in Fe^{3+} -P-CDOM complexes. The concentration of these complexes may be low in the upwelling water, and therefore the photochemical production of PO_4^- has likely low importance?

The photoproducted nutrients can be routed to food web via three pathways (1) direct photomineralization of DOM into nutrients followed by direct uptake of the nutrients by phytoplankton (2) bacterial mineralization of photoproducted labile DOM into nutrients followed by direct uptake by phytoplankton, and (3) bacterial uptake of photoproducted labile DOM into bacterial biomass followed by consumption of bacterial biomass (microbial loop, mixotrophy).

How much photodecomposition of upwelling DOM contributes to the productivity?

The magnitude depends on

- 1) The proportion of photodegradable DOM in deep ocean (<20%??)
- 2) Hydrology (i.e., how the DOM from deep ocean circulates to the surface mixing layer)
- 3) Photochemical reactivity of DOM (characterized by apparent quantum yields)
- 4) Magnitude and attenuation of solar radiation

In principle, it is possible to measure or estimate the above four parameters, which affect photochemical decomposition of deep-DOM and its contribution to the productivity of upwelling areas.

Photochemistry of C, N, P, Iron – what is important?

We estimated that photochemical processes of deep-DOM have the largest impact on carbon cycling and respiration of bacteria. Solar radiation will convert the upwelling biologically recalcitrant but partially photoreactive deep-DOC into CO₂ and biologically labile DOM. The photoproduction of biologically labile DOC can be expected to contribute to bacterial production and especially to respiration. Photoproduction of bioavailable DOC from deep-DOM must be an important process since upwelling provides a constant supply of deep-DOM and solar radiation is abundant in the study region.

Simultaneously with the decomposition of deep-DOM, solar radiation likely generates high molecular weight complex organic molecules (DOM or even particles) from the mixture of new-DOM and deep-DOM. This process reduces the bioavailability of newly produced DOM at the site of upwelling. Because photochemically produced complexes are biological recalcitrant, surface currents transport them away from the upwelling area. During this transport, the organic matter exposes to light and will be slowly photochemically decomposed again into bioavailable form. Thus, the role of photochemical reactions does not limit to the region of upwelling. Photochemical generation of biologically recalcitrant DOM at the site of upwelling facilitates the transport of DOM (by temporarily reducing the bioavailability of new-DOM) away from the region of upwelling.

As discussed above, the photochemical release of PO₄⁻ from DOP is likely low? Photochemical processes are likely important in the conversion of upwelling (colloidal and complexed) Fe³⁺ into Fe²⁺. The relative importance of this photochemical process may be damped down by the aeolian transport of Fe-containing dust from Sahara to the study region? As upwelling water already contains a large concentration of inorganic nitrogen and photoreactive DOM, it may be possible that incorporation of DIN into DON dominates the photochemical transformations related to dissolved nitrogen? This process has probably low relative

importance at the upwelling site itself, where the concentration of bioavailable inorganic nitrogen is high. As the currents transport photogenerated DON away from the upwelling area, it may become an important source of nitrogen in the regions off the upwelling region.

Measurements performed

Sterile filtered water (<0.2-um) was exposed to solar radiation together with dark controls. The rate of photoprocess is the difference between irradiated water and dark control. Bacterial response was measured in the experiments by exposing the bacterial-size fraction (e.g., 0.7-um filtrate) to solar radiation or kept in darkness. These experiments were carried out overnight, so that bactrioplankton were able to recover from UV-stress. Since the dose of absorbed radiation during the exposures was monitored, it was possible to calculate the apparent quantum yield spectrum for the photoreaction.

Presentations at international meetings resulting from work done during the BADE cruises thus far:

Abstract for oral presentation at the Ocean Sciences Meeting at Hawaii (20-24 Feb 2006)

Bacteria in the sea-surface microlayer: response to solar radiation and DOM utilization

Eva Sintes & Gerhard J. Herndl, Dept. of Biological Oceanography, Royal Netherlands Institute for Sea Research

The sea surface microlayer (SML) plays an important role in the exchange of gases and material across the air-sea interface and is inhabited by specific organisms, the neuston. Bacteria along with some basic physicochemical parameters were studied in the SML and the underlying water layer (ULW) along a trophic gradient from the Mauritanian upwelling area to the subtropical Atlantic gyre system. Additionally, their differential response to solar radiation exposure and different DOM composition was tested.

Inorganic and organic nutrients were enriched in the SML as compared to the ULW in contrast to bacterial abundance and activity. From these results and from the T-RFLP patterns obtained, we conclude that the bacterioneuston reflect the composition of the underlying bacterioplankton in the open ocean. On the other hand, MICRO-CARD-FISH revealed slight differences in bacterioneuston community composition as compared to the ULW, with Gamma-proteobacteria dominating the bacterial community composition and activity in the SML, and the Alpha-proteobacteria dominating the ULW community and activity.

In incubation experiments, SML-derived DOM supported high activity of both SML- and ULW-bacteria. SML-bacteria generally showed similar abundance and activity under exposure to solar radiation and in the dark. Our results indicate that the SML- and the ULW-bacterial communities are capable of utilizing available DOM equally well, but SML-bacteria seem better adapted to exposure to solar radiation. In accordance to this, the per cell activity quantified by MICRO-CARD-FISH indicated interspecific differences in the sensitivity towards UV exposure as well as pronounced diel cycles in the leucine incorporation per cell.

Abstract for oral presentation at the Symposium of Aquatic Microbial Ecology (SAME-9), Helsinki Finland (20-24 August 2005)

Dynamics of bacteria in the sea surface microlayer in the open ocean

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Dept. of Biological Oceanography, Royal Netherlands Institute of Sea Research, The Netherlands

The sea surface microlayer (SML) plays an important role in the exchange of gases and material across the air-sea interface and is inhabited by specific organisms, the neuston. Information on the SML of the open sea and under different trophic conditions is generally scarce particularly on bacterioneuston. Bacteria along with some basic physicochemical parameters were studied in the SML and the underlying water layer (UWL) along a trophic gradient from the Mauritanian upwelling area to the subtropical Atlantic gyre system and in an anticyclonic eddy in the western Mediterranean Sea.

Inorganic and organic nutrients were enriched in the SML as compared to the UWL. Bacterial abundance and the percentage of high nucleic acid content (HNA) cells also decreased towards the oligotrophic regions. Bacterioplankton production was higher in the upwelling area and decreased towards the gyre system while bacterioneuston productions exhibited an opposite trend increasing towards oligotrophic conditions. The percentage of highly active cells (CTC+ cells) was higher in bacterioplankton than in neuston and not related to the trophic state. Diel cycles in bacterioneuston activity, determined by CTC and MICRO-CARD-FISH, were observed both in the Mediterranean Sea and along the trophic gradient in the subtropical Atlantic with usually decreasing percentages of active cells around noon. From these results and from the T-RFLP patterns obtained, we conclude that the bacterioneuston are more independent from the overall trophic conditions than bacterioplankton.

Abstract for poster presentation at the American Society of Limnology & Oceanography meeting at Santiago de Compostela, Spain, June 2005

Virioplankton dynamics in the sea-surface microlayer in an anticyclonic eddy of the Northwestern Mediterranean Sea

Parada, V., E. Sintes, M.G. Weinbauer, G.J. Herndl

The sea-surface microlayer (SML) represents the interface between the atmosphere and ocean and is subjected to high doses of solar radiation. When studying neuston, viruses are commonly not included despite the commonly accepted view that viruses play an important role in the microbial food web. We followed a drifting buoy deployed in the center of the eddy for more than 2 weeks and investigated the dynamics in virio- and bacterioneuston abundance and viral and bacterial production. The bacterio- and virioneuston abundance in the SML was similar to those of the underlying water (ULW). Viral production rates ranged from 0 to 14.1×10^5 viruses $\text{ml}^{-1} \text{h}^{-1}$ in the SML and from 2×10^5 to 34×10^5 viruses $\text{ml}^{-1} \text{h}^{-1}$ in the UWL, thus was significantly higher in ULW than in the SML. These rates did not significantly vary on a diel basis. Lysogeny plays only a minor role in bacterial-virus interactions in both layers. We interpret the low abundance of lysogenic viruses by the constant induction of lysogens due to the high dose of ultraviolet radiation.

Abstract for poster presentation at the American Society of Limnology & Oceanography meeting at Santiago de Compostela, Spain, June 2005

Bacterial activity in the air-sea microlayer of the subtropical Atlantic: interaction between solar radiation, DOM and substrate utilization.

Eva Sintes, Gerhard J. Herndl

The adaptation of natural bacterioplankton consortia to utilize DOM of different origin and the impact of exposure to solar radiation was studied in the sea surface microlayer (SML) and the underlying water layer (UWL) in the subtropical Atlantic. Exposure of DOM to solar radiation resulted in higher activity of UWL bacteria utilizing solar radiation-exposed DOM than in the corresponding dark treatment while no differences between the activity after light treatment and DOM kept in the dark were found for the SML DOM, indicative for a lower bioreactivity of the DOM from the UWL than from SML. SML bacteria were found to be better adapted to high solar radiation than UWL bacteria. Generally, SML DOM supported high activity levels of both SML and UWL bacteria, indicating no particular adaptation of the SML bacteria

to SML DOM. Our results indicate that the bacterial communities from the SML and UWL are capable of utilizing available DOM equally well, but the SML bacteria seem better adapted to UV exposure. MICRO-CARD-FISH analyses on the response of specific groups indicate interspecific differences, however, in the sensitivity towards solar radiation.

Abstract for poster presentation at the American Society of Limnology & Oceanography meeting at Santiago de Compostela, Spain, June 2005

Evidence for an important role of autochthonous and atmospheric inputs of volatile organic carbon in the subtropical Atlantic Ocean

Calleja, M. Ll., Dachs, J., Duarte, C. M., Agustí, S., Herndl, G. J.

Atmospheric deposition is increasingly recognized to be an important vector of nutrients, carbon and pollutants to aquatic ecosystems. The ocean has been identified as the dominant sink for gas-phase anthropogenic contaminants, and biogenic organic compounds derived from land vegetation could meet the same fate, as reported for specific compounds. Indeed, recent studies have reported high total air-sea exchange of gaseous organic carbon (GOC) in the NE Subtropical Atlantic, suggesting that the ocean is a potential sink of allochthonous atmospherically deposited organic carbon. Conversely, phytoplankton may release low molecular weight exchangeable dissolved organic carbon (EDOC) that can be volatilized to the atmosphere. Here we demonstrate a metabolic role for volatile dissolved organic carbon in the ocean by providing evidence that (1) gaseous organic carbon transported from the atmosphere to the ocean is a labile fraction of the total organic carbon pool, hence utilizable by bacteria, and of (2) significant autochthonous net production and consumption of exchangeable organic carbon by phytoplankton inferred from the examination of vertical profiles and diel cycles of EDOC concentration in the subtropical Atlantic ocean.

Abstract for poster presentation at the 37th International Liege Colloquium on Ocean Dynamics, Liege, Belgium, May 2005

Evidence for an important role of surface water layer processes on air-water gas transfer in the Subtropical Atlantic Ocean.

Calleja M.Ll., Duarte C.M., Prairie Y., Agustí S., and Herndl, G.J.

Surface water layer characteristics are increasingly recognized as key determinants of air-sea exchanges of materials, such as CO₂ exchange. The CO₂ flux between water and atmosphere is inferred from the pCO₂ air-water gradient, and a gas transfer velocity (k), typically parameterized as a function of wind speed. The lack of a unique relationship between transfer velocity and wind speed suggests that processes other than wind also contribute significantly to regulate gas exchange. A comparison of organic carbon concentrations and CO₂ partial pressure at 5 m depth with those within the top 2 cm of the surface layer of the NE Subtropical Atlantic Ocean provides evidence that (1) organic carbon becomes enriched in the top 2cm layer of the sea surface, with a decline in the gas transfer velocity, standardized for wind conditions, with increasing organic carbon concentrations, and (2) the CO₂ partial pressure is also higher within the top 2cm layer of the sea surface than at 3-5 m depth, suggesting an underestimation of the pCO₂ by programs deriving this from water pumped from several meters of depth.

Abstract for poster presentation at the European Geophysical Union meeting at Vienna, Austria, April 2005

Bacterioneuston vs. bacterioplankton dynamics along a trophic gradient

E. Sintes, G.J. Herndl

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The seasurface microlayer (SML) plays an important role in the exchange of gases and material across the air-sea interface and is inhabited by specific organisms, the neuston. This environment has been mainly studied in coastal areas and in lakes. Information on the SML of the open sea and under different trophic conditions is generally scarce particularly on bacterioneuston. Bacteria along with some basic physicochemical parameters were studied in the SML and the underlying water layer (UWL) along a trophic gradient from the Mauritanian upwelling area to the subtropical Atlantic gyre system, and in an anticyclonic eddy in the western Mediterranean Sea.

Inorganic (phosphate, ammonium, nitrate and nitrite) and organic (dissolved organic nitrogen, DON, and phosphorus, DOP) nutrients were enriched in the SML as compared to the UWL. A distinct pattern of decreasing concentrations along the trophic gradient was observed for nitrite, phosphate and DOP. Concentrations of ammonium and DON were remarkably constant in the UWL, and more variable in the SML.

Bacterial abundance and the percentage of high nucleic acid content (HNA) cells also decreased towards the oligotrophic regions. Bacterioplankton production was higher in the upwelling area and decreased towards the gyre system. In contrast, bacterioneuston production increased towards oligotrophic conditions, approaching an enrichment factor (SML/UWL) of 1. These dynamics together with the decreasing DOP concentrations leads to an apparent paradox, with higher bacterioneuston production at lower DOP concentrations in contrast to the bacterioplankton production. The dynamics of bacterial production recorded in the Mediterranean eddy resembled that from the stations close to the subtropical Atlantic gyre.

The percentage of highly active cells (CTC+ cells) was higher in bacterioplankton than in neuston and not related to the trophic state. Diel cycles in bacterioneuston activity (% of CTC+ and HNA cells) were observed both in the Mediterranean Sea and along the trophic gradient in the subtropical Atlantic with usually decreasing percentages of active cells around noon. From our results we conclude that the trophic conditions have a greater impact on the bacterioplankton than on bacterioneuston.

Manuscripts are currently in preparation or submitted by several participants of the two BADE cruises.

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