BBL Characteristics at the Iberian Sea transect

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Task III.1.1
Objectives: to determine and quantify the distribution, amount, composition and residence time of SPM in nepheloid layers and in clear water.

Sediment stability and characteristics of resuspended aggregates of the western European continental margin

Most studies on particle behaviour indicate that the quality and quantity of deposited organic matter is strongly influenced by decomposition processes during the flow-controlled processes of sedimentation, lateral sediment transport and resuspension (Auffret *et al.*, 1994) in addition to microbial and faunal activity at the sediment-water interface and in the surface sediments (Graf *et al.*, 1995; Thomsen *et al.*, 1995). Shields-type diagrams then provide the links between flow, bed stability/behaviour and transport mode. Cohesive sediments with biological interaction, found at mid- and lower-slope regions of continental margins, are part of a complex chain of events which are not fully understood in their relation to bed stability, flow structure, material fluxes, and ecosystem dynamics. For example, the latest studies on benthic boundary layer characteristics of continental margins reveal that major particle transport occurs as a low-concentration suspension in the lowest few decimetres above the sea floor (Thomsen and van Weering, 1998; Thomsen, 1998).
To understand and quantify these transport processes, both laboratory and field studies are required to link hydrodynamics with aggregate and bed formation. For models as well as calculations of the mass fluxes associated with this bed-flow-biology interaction, several important particle and hydrodynamic parameters need to be determined: the bottom stress (τ), expressed as friction velocity (u_*), the turbulence intensities and the mean local horizontal flow speed together with the controlling variables of sediment transport: the critical erosion stress (τ_ε), the critical deposition stress (τ_δ), and the particle settling velocity (w_s). The task was to determine at different transects and water depths of the western European continental margin the sea bed erodibility, especially when significant biological interaction was expected.

Methods

Sediments were collected during six cruises to the European continental margins between 45° and 55° N, 5° and 25° W in the months of July 1995, August 1996, May 1997, July 1997, August 1998 and January 1999 (1996-1999 as part of OMEX II-II). Cores were taken with a box-corer built by the Netherlands Institute for Sea Research (NIOZ). For the determination of the critical erosion stress τ_ε (N m^{-2}= Pa) = u_{*c}^2 x ρ (where u_{*c} is the critical friction velocity [m s^{-1}] and ρ is the fluid density [kg m^{-3}]), sub-cores of 20 cm diameter were taken from the box corers and stored under in-situ temperatures. Critical erosion stress was obtained onboard in an erosion chamber with controlled bottom stress (Gust, 1990), into which the 20-cm sub-core and *in situ* seawater (overlying water height 10 cm, Fig. 1a) were inserted.
With the selected erosion/respiration chamber it was possible to incrementally increase the spatially homogeneous bottom stress in the range $0.005 < \tau < 0.5$ N m^{-2}, of which we chose as minimum value $\tau = 0.01$ N m^{-2} and stepwise increases τ of 0.001 N m^{-2}. Each step of constant stress exposure lasted for a minimum of 10 min. Onset of sediment erosion for the disaggregated fine sediment fraction (< 100 µm diameter) was determined by a NTU turbidity meter. Data on turbidity were observed every 10 minutes and water samples for size analyses were taken, after sediment erosion had started. The size distribution and mean diameter of these samples was analysed with a Coulter Counter (Type Multisizer), and the sediment size/weight distribution was determined by the modified Atterberg method. Onset of sediment erosion for the aggregated fraction (> 100 µm diameter and named

100^{+}aggregates) was determined by a particle camera with 20-fold magnification (Thomsen *et al.*, 1996), focussed on the water layers immediately above the sediment.
Data on density and kinematic viscosity of the sea water at the different stations were derived from a CTD (Seabird SeaCat) which was deployed at each station during core collection.

Results
Sediments on the continental margin (212 to 4940-m water depth) consisted of an aggregated surface layer which on erosion at all transects (22 stations) yielded aggregates > 100 µm (called 100^{+}aggregates) at critical friction velocities [u_{*c}] of 0.45 to 1.16 cm s^{-1}. The erosion thresholds of underlying sediments typically increased from 0.68 to 2.05 cm s^{-1} from shallow to deeper sites on each transect, with upper-slope sediments being more sandy and the cohesive clay sediments found at deeper sections of the continental rise. Table 1 and Fig. 2 (threshold data of the individual transects) summarise these results. At all stations, 100^{+}aggregates of the top layer appeared as the first resuspension product. The aggregates consisted up to 75% wt of organic matter, which was mostly refractory with a carbon/nitrogen ratio exceeding 8, and the lithogenic material (25%) was embedded in the amorphous matrix of the organics. Aggregates contained remnants of faecal pellets, meiofauna organisms and shell debris of foraminifera. 32 - 71% of the bacterial mass of the BBL was particle attached and covered the organic matrix of the aggregates. Approximately 1% of the organic fraction was labile bacterial organic carbon.
The 100^{+}aggregates found at the stations of the Goban Spur, Whittard Canyon and Vigo transect showed median diameters of 224 - 344 µm. Aggregates of that size had a settling velocity of 0.05 - 0.08 cm s^{-1}, and an estimated excess density of ~ 0.026 g cm^{-3}. The value was back calculated from Stokes Law and measured settling velocities for particles with Re < 1, determined in a settling column (Thomsen, 1998).
On the continental margin, the grain size of the sediment underlying the 100^{+}aggregates significantly decreased with increasing water depth (Kendall's Tau = - 0.6, $p < 0.0001$). At the Goban Spur, Whittard Canyon and Vigo sites 100^{+}aggregates were photographed and collected in the water column at 40 cm above the bed with the BIOPROBE water sampler (Thomsen and van Weering, 1998). Median sizes of these aggregates, shown in Tab. 1 (marked by *), compare well with the size of eroded aggregates from the shipborne experiments (Rho = 0.92, p= 0.009, Spearman Rank). For all critical friction velocities determined, there was a negative correlation between particle size and erosion resistance (Kendall's Tau = - 0.75, $p < 0.0001$). The settling velocity of the particles was experimentally determined over the temperature range of 2 - 12°C and the empirical size/settling velocity relationship for BBL aggregates in the 100 - 600 µm size range is: $0.225 \times D^{0.95}$ with ws given in cm and D given in mm.
These results are accepted as a manuscript to *Deep-Sea Research* by Thomsen and Gust, G.

Task III.1.2
Objectives: to assess the spatial and temporal magnitudes and variability of benthic boundary layer dynamics
Deliverable: still under analyses from 4 cruises, the last ended in January 1999. The most important result concerning sediment chlorophyll concentrations is, that during January 1999 CPE concentrations in the upper 10 cm ranged from 0.7 to 3.7 µg-CPE cm^{-2}, with one order of magnitude higher values in the Nazaré Canyon.

Task III.1.5
Objectives: to define contrast between upwelling and non-upwelling dominated sediment transport processes.
Deliverable at month 30.

Task III.3.1
Objectives: to determine the role and importance of bio-entrainment and deposition in carbon cycling in surface sediments

Aggregate scavenging rates of benthic interface feeders at the Iberian continental margin

Experimental studies in laboratory flumes show that benthic *Foraminifera* (*Marsipella* spp.) dominating a mid-slope station (1645 m) at the Iberian continental margin can scavenge aggregates transported in the benthic boundary layer. These BBL aggregates occur in concentrations of 0.5 and 5 cm^{-3} at 0.5 cm height above the sea floor, when free stream flow velocities were in the order of 33 and 3 cm s^{-1} respectively. The estimated POC biodeposition of the foraminifer community was 0.22 - 0.67 mg m^{-2} d^{-1}, which is roughly 1 - 4% of the total carbon deposition needed to feed mid-slope benthic communities.

The benthic community at the station was dominated by tubular arenaceous *Foraminifera* with a total abundance of 647±39 m^{-2}. *Marsipella elongata* dominated the foraminifer community with a mean abundance of 583 ind. m^{-2}, followed by *Marsipella caervicornis* with 64 ind. m^{-2}. During time of investigation *Marsipella elongata* had built up a permanent cyst of sponge spicules and debris in the aperture region. The spicules were used to support a spherical shaped pseudopodial network, which served as filter apparatus. The tests stood erected in the sediments. Approximately 50 - 60% of the tube were embedded in the sediment while the remainder penetrated up to 0.4 cm into the water column. The less dominant foraminifer *Marsipella caervicornis* was branched and penetrated 0.3 - 0.4 cm into the boundary layer with 60% of the agglutinated tube being embedded in the sediment. Sponge spicules were also used to build up a hemispherical collector, but the upper part of the tubes were also covered with protoplasm and used as cylindrical filter apparatus. Both types of *Marsipella* were able to withstand flow velocities exceeding 50 cm s^{-1} an u_{100} although they were fully exposed to the turbulent logarithmic layer. POC deposition *via* aggregates was low when compared to the biodeposition rates estimated for the Goban Spur study site of the OMEX I project. Under high flow conditions, *Marsipella* deposits 0.67 mg POC m^{-2} d^{-1} total deposition. Under low flow conditions total biodeposition was 0.22 mg POC m^{-2} d^{-1}.

Determinations of the biodepositional flux of carbon from all experiments carried out so far results in a mean value of 0.005% of the horizontal carbon flux (recalculated after Thomsen *et al.*, 1995; Thomsen and Flach, 1997; Thomsen and Jähmlich, 1998; Thomsen 1999). This results in an averaged additional carbon flux of up to 4.5 g m^{-2} y^{-1} due to biodeposition under high flow conditions, but only 75 mg m^{-2} y^{-1} under low flow conditions. Thus biodeposition alone can only be the dominant feeding mechanism under high flow conditions at the upper slope region, whereas with increasing depth, interface feeding dominates. By using newly developed sampling devices, final experiments on the nutritional value of BBL aggregates will add to the understanding of the carbon source for the benthic carbon demand at greater water depth, regardless if sediment traps over -or underestimate the real vertical carbon fluxes.

These results are in final review at Limnology and Oceanography

Erosion resistance: see also **Task III.1.1**.

Table 1. Critical shear velocity measurements for different sediments at different depth collected during three cruises to the European continental margins of the Celtic Sea between 45° and 55° N, 5° and 25° W in the months of July 1995, August 1996, May 1997 and August 1997. Data in column B, which are marked with * are in-situ particle camera data measured with the BIOPROBE system.

		Underlying sediments					Aggregate layer at the sediment surface			
Date	Station	depth [m]	mean [µm size]	d_{50} [µm size]	u_{*c} [cm/s]	N m^{-2}	mean [µm size]	d_{50} [µm size]	u_{*c} [cm/s]	N m^{-2}
7/95	Goban Spur	670	90	90	0.86	0.08	325 ±120	293*		
		1028	61	54	0.98	0.10	381 ±190	322*		
		1445	20	20	1.62	0.27	226 ± 97	207*		
		3660	8	6	1.80	0.34	301 ±142	276*		
		2150	30	25	1.35	0.19	335 ±102	302		
5/96	Rockall	845	18	20	1.40	0.20	987 ±607	895	0.67	0.05
		3035	16	12	1.80	0.34	1452 ±922	1210	1.15	0.14
		2215	15	10	1.61	0.27	2085 ±970	1898	0.55	0.03
		2820	18	12	1.85	0.36	2768 ±1353	2403	0.65	0.04
		2815	25	15	1.61	0.27	2312 ±1121	2167	0.55	0.03
8/96	Whittard	212	125	115	0.68	0.05	528 ±364	413	0.95	0.09
		803	34	30	1.25	0.16	406 ±239	348	0.92	0.09
		1530	25	24	1.79	0.33	138 ± 38	125	0.98	0.10
		3711	6	5	2.05	0.44	199 ±112	146	1.16	0.14
7/97	Vigo	2275	9	8	1.73	0.31	212 ± 54	204	0.81	0.07
		1645	16	12	1.82	0.34	206 ± 52	203	1.13	0.13
		1756	11	5	1.61	0.30	352 ±124	327	1.15	0.14
		2602	5	8	1.49	0.23	298 ±147	264	0.92	0.09
8/98		879	35	20	1.05	0.11	798 ±333	721	0.45	0.02
		2570	34	15	0.80	0.07	513 ± 69	447	1.00	0.10
		3380	12	8	1.25	0.16	435 ±177	406	0.90	0.08
		4940	8	6	1.85	0.36	382 ±100	361	0.95	0.10

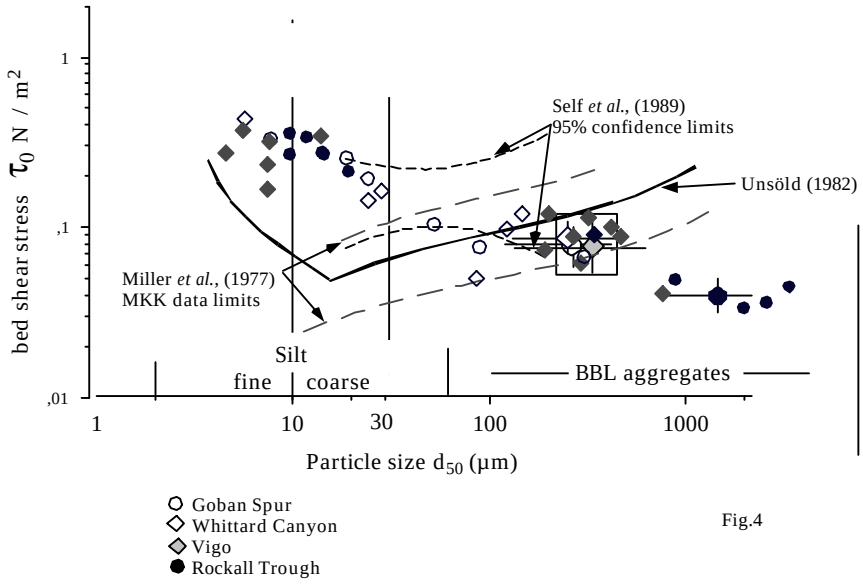

Fig.4

Figure 1. Critical bed shear stress for erosion of continental margin sediments, showing the onset of a cohesion effect at about 30 µm (grey dots). The black curve represents the Shields curve modified after Unsöld (1982). The wide-dashed lines refer to the uncertainty limits of available high quality data evaluated by Miller et al. (1977), and the narrow-dashes lines those of Self et al. (1989). Black dots represent the critical bed shear stress of sediments from the upper continental margin. Open squares and circles represent aggregates from the sediment surface at different locations. The horizontal dashed line at 1 Nm-2 demonstrates the separation between primary cohesive particles and surface aggregates.

Task III.3.4
Objectives: to investigate benthic community structure in relation to BBL dynamics
Deliverables: month 24:

Microbially mediated dynamics of organic matter in the benthic boundary layer.
(contribution from Karl-Paul Witzel and Will Ritzrau)

1. General:
During the last period of OMEX II-II, studies on microbial processes in the benthic boundary layer focused on microbial activities and the genetic structure of the microbial communities in the water column, in the benthic boundary layer and the surface sediments. Samples were available from two cruises: In Summer 1998 with RV *Pelagia PE64/121* (30.7.98 - 16.8.98) and in winter 1998/99 with RV *Meteor M43/2* (28.12.98 - 14.1.99). Due to space limitations during the *Pelagia* cruise, no rate measurements of microbial transformations of dissolved into particulate organic matter could be carried out on board of the ship. Bacterial biomass samples for analysing the composition of the microbial community were taken by Laurenz Thomsen. From this cruise, we got a total of 18 samples from surface water, the benthic boundary layer, and sediment that are currently analysed with molecular techniques.
During *M43/2*, samples were collected at 7 locations across the Iberian Margin and analysed on board ship and afterwards in the laboratory. Due to bad weather conditions, samples from the benthic boundary layer could be retrieved at one station only.

The following research was completed:

1. Measuring the uptake rates of [4,5-^{3}H] L-leucine and [*methyl*-^{3}H] thymidine into microbial biomass (*Task a*). A total of 21 samples from different depths of the water column and the BBL was incubated in time course experiments with these substrates on board ship under controlled temperature conditions. Active incorporation of ^{3}H into the particulate fraction was measured to calculate uptake rates. Additionally in laboratory experiments the microbial activity associated with two different size fractions of resuspended surface sediments were completed at selected sites on the shelf, at 2900 and 3700-m water depths.

2. A total of 25 samples was taken to analyse bacterial abundance and biomass in different depths of the water column and the BBL after DAPI staining.

3. In order to elucidate the composition of the microbial community (*Task b*), bacterial biomass was collected by filtration and frozen on board ship. In the laboratory, DNA was extracted and purified with various techniques to get PCR-amplifiable templates from a total of 65 samples taken from the BBL, the water column and the sediment at different stations (see Table 1). PCR was carried out with eubacterial primers and primer sets which are specific for autotrophic, ammonia-oxidising bacteria. The PCR-products were analysed by denaturing gradient gel electrophoresis (DGGE) to identify different populations of the microbial community.

4. In order to analyse the composition of particle associated bacterial communities (*Task c*), resuspension experiments were carried out with the ‚Gust-chamber' on board ship. Defined particle fractions that are characterised by Thomsen *et al.* were generated in these experiments from different sediment samples, and bacterial communities adhering to the different fractions were compared by the molecular techniques mentioned above.

2. Results:

1. Uptake rates of [4,5-^{3}H] L-leucine and [*methyl*-^{3}H] thymidine into microbial biomass (*Task a*)
The preliminary results of the distribution of the microbial thymidine and leucine incorporation (without quench correction) in the water column displayed the well-accepted pattern with highest rates found in the surface waters and decreasing with depth. Unfortunately, at this station samples from the benthic boundary layer were not available. However, due to differences in the bacterial

abundances, which have not been evaluated yet, cell specific activities may display a different distribution. Generally the microbial communities along the Iberian Margin appeared to be less active during winter compared to the summer communities at the Whittard Canyon.
In a first attempt, the distribution of microbial activity associated with various size fractions of resuspended surface sediments was evaluated at selected sites. Even though not statistically supported, the results suggest differences in the role of the various particle fractions of eroded material for microbial dynamics in the benthic boundary layer. On the shelf, the bacteria associated with larger particles appear to be more active than communities attached to smaller more easily erodable fractions. This appears to be supported by the idea, that on the shelf small particles are winnowed out by the dynamic hydrodynamic regime, leaving the larger fraction behind.
In contrast at greater depth, where the hydrodynamic regime is most likely not as dynamic compared to the shelf, smaller particles appear to be more important for microbial processes than the larger particles, which require larger shear velocities to be eroded. This finding has to be considered with respect to the idea that particle-associated bacteria could benefit from the hydrodynamic regime of their host particle and the absolute abundance of erodable particles. However, it has to be evaluated if this result is strongly related to the particle size distribution at the various stations. So far we were not able to relate the results of the distribution of microbial activities and community structures to the composition and distribution of particles. Furthermore, relationships between the distribution of microbial activities and the composition of the various communities will be evaluated on the basis of the results, which have been produced.

2. Composition of microbial communities in the BBL, overlaying water and sediment (*Task b*)
Based on the results from the Whittard Canyon (last OMEX report), we had expected differences in the composition of the bacterial populations - especially those associated with particles - between sediment, pelagial and BBL, parallel to characteristic patterns in the distribution of microbial activity. Microbial communities in these environments are usually very complex and heterogeneous, and thus difficult to study. As a first access, we focused our studies on autotrophic ammonia-oxidising bacteria because they seem to be ideal model organisms for our purpose: (1) they are phylogenetically and physiologically homogenous and well defined; (2) they play a key role in N-cycling by oxidising ammonia to nitrite; (3) some of them tend to adhere to particles, while others are predominantly free-living. Future studies could be extended to other physiological groups of micro-organisms as well.
The composition of the nitrifying bacterial communities was observed on the basis of band patterns after denaturing gradient gel electrophoresis of specific PCR products of 16S rDNA. Bacteria in vertical zones of the water column, the benthic boundary layer, and the sediment were much more different than within the same layer but at very distant stations. Most of the PCR products have been cloned in order to sequence the 1100 bp stretch of the 16S rDNA. Sequencing is still in progress and should reveal within the next weeks information for a detailed taxonomic and phylogenetic analysis of the bacterial community.
Especially with DNA extracted from sediment samples, we had often difficulties to amplify the target molecules, probably due to co-extraction of substances that inhibit the PCR. This may lead to an artificially lower complexity of the community. Therefore, these results are preliminary and need further verification. Further efforts are undertaken to purify the DNA extracts and to increase the efficiency of the PCR reaction.

3. Composition of particle associated bacterial communities (*Task c*)
Our hypothesis that particles of different composition and size are colonised by different microbial communities was tested using the “GUST-chamber”. By applying different shear forces to sediment surfaces, different size classes of particles were resuspended on board ship. Communities of nitrifying bacteria on the particles were analysed after filtration and extraction of DNA with the molecular techniques described above.

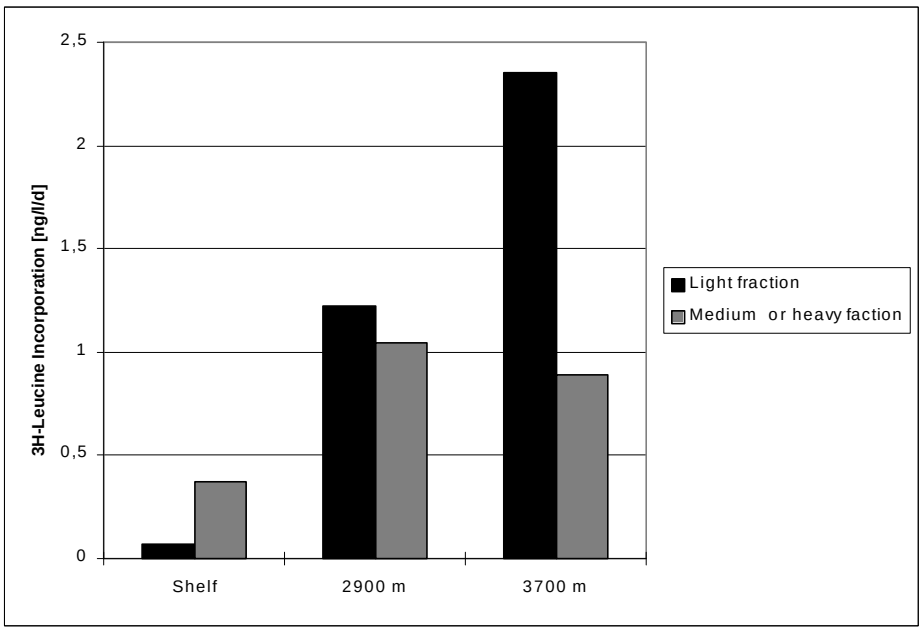

Fig. 2. Distribution of ^{3}H-Leucine Incorporation by microbial communities associated with different fractions of erodable material from the sediment water interface. The fractions ”light” and ”medium and heavy” describe the erodability of the fractions.

Figure 3 shows an example from 2 stations. Each band in the gel represents a different population of these bacteria. At the two stations in the Nazaré Canyon with 3700 and 2900-m water depths, the communities within the same fractions are very similar. However, the light and heavy fractions harbour, as was expected, clearly different populations of nitrifying bacteria. The fact that from the sediment samples only a few bands were detected, is most probably due to inhibition of the PCR or absorption of DNA to sediment particles. Further improvements of the method are necessary to circumvent this in the future. Further verification of these experimental results shall also include other groups of bacteria and reveal information on the factors that are influencing different colonisation patterns of the particles.

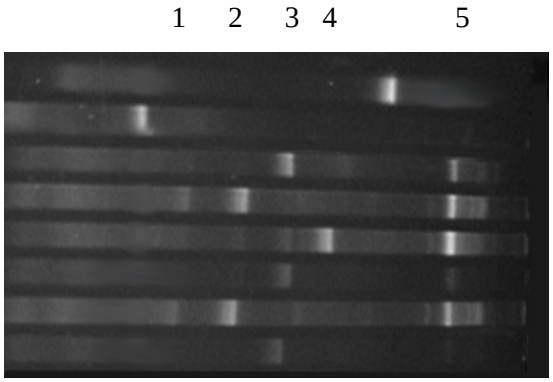

Fig. 3: Denaturing gradient gel electrophoresis of 16S rDNA amplified from ammonia-oxidising bacteria on different particle fractions generated in the GUST-chamber. As standards, two cultures (*Nitrosomonas eutropha* and *Nitrosovibrio tenuis*) and the original sediment sample are shown.

3. Outlook:

By using a specialised group of bacteria as model organisms, our results show that the bacterial communities in the different layers of water and sediment and BBL, are more different than on a horizontal transect across the Iberian Margin. This is most likely due to a better transfer in lateral than in vertical direction, and may open a possibility to roughly estimate the origin of the particulate material. Presently and in the next future we will continue to acquire more information for a better characterisation of the various populations. Besides, we try to extend this approach to other groups of bacteria. For this purpose, we are open to suggestions from other OMEX partners, which physiological or taxonomic groups of bacteria might be most interesting for their specific interest.

Task III.3.5.
Objectives: to define role and importance of benthic fauna in recycling of carbon at different margins
Deliverables: month 36.
Results from the OMEX II phase in comparison with the OMEX I data published in the *Journal of Sea Research* 1999, 41: 73-86.

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