Vol. 370: 87–95, 2008
doi: 10.3354/meps07582

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 28

Prokaryotic community dynamics in the sedimentary microenvironment of the demosponge *Tentorium semisuberites* from deep Arctic waters

Nadia V. Quéric[1,3,*], Jesus M. Arrieta[2], Thomas Soltwedel[1], Wolf E. Arntz[1]

[1]Alfred Wegener Institute for Polar and Marine Research, New Technologies/Underwater Vehicles and Deep-sea Technology, Am Handelshafen 12, 27515 Bremerhaven, Germany

[2]Mediterranean Institute for Advanced Studies (IMEDEA), Natural Resources Department, Miquel Marquès 21, 07190 Esporles, Mallorca, Illes Balears, Spain

[3]*Present address:* Department of Geosciences, Geobiology, University of Göttingen, Goldschmidtstrasse 3, 37077 Göttingen, Germany

ABSTRACT: The sedimentary microenvironment of a sessile epibenthic deep-sea species, the small demosponge *Tentorium semisuberites,* has been investigated to determine its effect on the distribution, physiology and community structure of benthic bacteria and archaea. The upper sediment layers (0 to 2 cm) in the immediate sponge vicinity were characterized by an increased bacterial colonisation with cell abundances on average 3 times higher than those in reference sediments. Similar results were obtained for bacterial secondary production, measured by simultaneous incorporation of the radioactive-labeled substrates ^{3}H-thymidine and ^{14}C-leucine. Our data show a high heterogeneity of deep-sea sediments with a pronounced patchy distribution of particulate organic carbon (POC), and a significant enrichment of POC in the sediments next to *T. semisuberites.* Cell-specific ^{3}H-thymidine and ^{14}C-leucine incorporation rates indicate that the quality rather than the quantity of POC around sponges may lead to the observed increase in cell abundances and protein synthesis. Terminal restriction fragment length polymorphism (T-RFLP) analysis revealed that the sponges support a specific benthic bacterial and archaeal community with some unique OTUs (Operational Taxonomic Units), while other OTUs were entirely missing from its surrounding microenvironment. Our data indicate that the small demosponge *T. semisuberites* causes highly productive patches as hot spots of biochemical cycling, potentially increasing habitat heterogeneity in deep-sea sediments.

KEY WORDS: Biogenic structures · Benthic prokaryotic community · Small-scale heterogeneity · Deep-sea sediments · Demospongiae

INTRODUCTION

Biogenic structures, which are common and conspicuous features of deep-sea sediments, form microhabitats that may contribute to high species diversity in the deep sea (Jumars & Eckman 1983, Thistle 1983). By resuspension or translocation of sediment, macrobenthic organisms can affect early diagenesis and the evolution of the sedimentary record in surface sediments, with lasting effects on benthic community dynamics (Aller 1982, Findlay et al. 1990, Huettel & Gust 1992). The hydrodynamic energy at abyssal water depths is generally too low to erode the seabed or the infauna, thus allowing a long persistence of features originating from macrofaunal restructuring of the upper sediment layers. Interacting with near-bottom currents, these biogenic sediment structures continue to alter the sedimentary record and constitute an evident source of deep-sea habitat heterogeneity for smaller biota (Gage 1996 and references therein), including microbial com-

*Email: nqueric@gwdg.de

munities. Protruding polychaete tubes (Carey 1983), robust and thus persisting mud concretions such as dwellings of cirratulid polychaetes (Thistle & Eckman 1990), pits (Yager et al. 1993) and leftovers of large food falls (Smith et al. 1998) can affect local particle deposition by creating characteristic vortex patterns and turbulences. Acting as a trap for vertically sinking and horizontally transported particles, any type of small-scale, topographic alteration may lead to an accumulation of nutrients, with the potential to locally stimulate the productivity of benthic microorganisms. Soltwedel & Vopel (2001) reported on an accumulation of bacteria and meiofauna in the immediate vicinity of the deep-sea demosponge *Thenea abyssorum*. This sponge even supported a nematode community significantly different from that in reference sediments. The genera *Areolaimus* and *Southerniella* contributed most to the total average dissimilarity between all pairs of inter-group samples (E. Grünberger pers. comm.). By contrast, the sedimentary microenvironment of the deep-sea anthozoan *Bathyphellia margaritacea* did not differ significantly from nearby reference sediments, neither in meiofaunal community composition (H.–H. Koopmann pers. comm.), nor in bacterial abundance (Soltwedel & Vopel 2001). These observations indicate that changes in activity and/or community composition of small benthic biota may be related to the shape of obstacles at the sediment–water interface alone. In addition to such passive mechanisms of particle deposition, sponges actively create a characteristic small-scale flow regime around them that enhances particle capture (Witte et al. 1997). They modify chemical gradients within the sediment by generating characteristic depositional patterns and thereby create a specific microclimate.

Until now, studies have overlooked the potential of small, epibenthic sponges to support a specific prokaryotic community which is significantly different from that of undisturbed nearby sediments. Addressing the questions on physiological and phylogenetic microbial responses, we investigated the sedimentary microenvironment of the demosponge *Tentorium semisuberites* as a common representative of the sessile epifauna in deep Arctic waters (Barthel & Tendal 1993). The aim was to test the following hypotheses: compared to reference sediments (lacking protruding biogenic structures), the adjacent, sedimentary microenvironment of *T. semisuberites* is characterized by (1) increased bacterial densities, (2) enhanced bacterial activity exhibiting different synthesis patterns and (3) a specific prokaryotic (bacterial and archaeal) community structure.

MATERIALS AND METHODS

Sampling. During an expedition with the RV 'L'Atalante' in summer 2001, sediment samples were taken from the deep-sea long-term observatory HAUSGARTEN in the eastern Fram Strait, Arctic Ocean (Soltwedel et al. 2005) at ca. 2500 m bottom depth. Targeted sampling was performed using pushcores (60 mm in diameter, 40 cm in length), manipulated by the ROV 'Victor 6000'. The sedimentary microenvironment of 4 sponges was compared to nearby (0.5 to 1 m distance) reference sediments lacking apparent biogenic structures (Fig. 1). Sampling locations of the different sample pairs (sponge, reference) were up to 12 m away from each other. Each sponge was carefully removed from the sediment surface and immediately preserved in 4% formaldehyde for identification and fluorescent *in situ* hybridization (FISH) analysis (Pape et al. 2006). The upper 2 cm of sediment of each pushcore were subsequently sliced, leaving out a 0.5 cm border to avoid any smear-effects caused by pushcor-

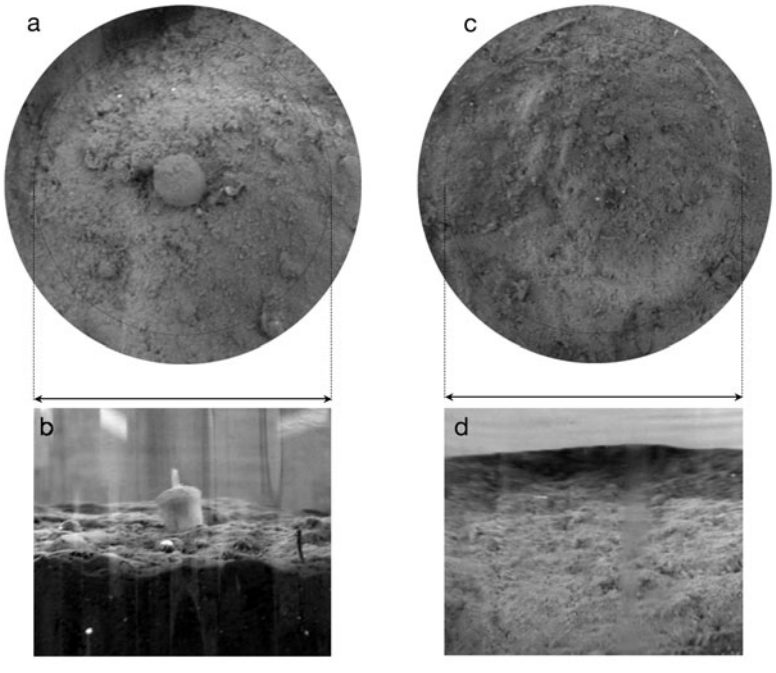

Fig. 1. (a,b) Pushcore profiles of the sedimentary microenvironment of the demosponge *Tentorium semisuberites* compared to (c,d) macroscopically plain reference sediments as (a,c) supervision and (b,d) side-view. Dashed lines indicate the subsampling areas. Pictures were taken under atmospheric pressure conditions

ing. After homogenization, the surface sediment of each core was split for the assessment of bacterial abundance, prokaryotic production measurements and fingerprinting analyses; leftovers were frozen for dry and ash-free dry weight measurements. To focus on parametric differences between sponge and reference sediments, samples were treated as pairs (sample pairs 1 to 4).

Test organism. Our test organism, *Tentorium semisuberites* (Schmidt, 1870), belongs to the Demospongiae (Order Hadromerida, Family Polymastiidae and Genus *Tentorium*; Vosmaer, 1885). These typically mushroom-shaped sponges can be easily identified by a smooth surface and usually 1 to 6 short, tubular papillae on top. They occur at depths from 26 to 2800 m in the Arctic Ocean, the White Sea and the northern Atlantic Ocean (Hansson 1999). Although the specimens used in the present study were generally 1 to 2 cm in diameter (under atmospheric pressure conditions), they can grow to up to 3.55 cm in height and 3 cm in width.

Sediment characteristics. Sediment water content was determined by weight loss of wet sediment samples (48 h at 80°C). Estimates of particulate organic carbon (POC) concentration were based on sediment dry weight after combustion (4 h at 580°C).

Prokaryotic abundance. Sediment subsamples (2 g sediment wet weight) were preserved with 4% formaldehyde and stored at 4°C until further processing. After sonication (100 W, 30 s burst), sample dilutions (10^4 final dilution) were stained with 4′6-diamidino-2-phenylindole (DAPI) at a final concentration of 1 µg ml^{-1}. Bacteria and archaea were counted under UV excitation (Olympus-WU filter set; BP 360-730/DM 400/BA 420). The number of microscopic fields was adjusted to maintain an enumeration standard error of <5%.

Prokaryotic production measurements. A dual-labelling protocol for the incorporation of Methyl-[^{3}H] thymidine (65 Ci $mmol^{-1}$) and L-[^{14}C] leucine (262 mCi $mmol^{-1}$) (Amersham International) was applied as described previously (Quéric & Soltwedel 2007). To evaluate cell-specific activities (mol $cell^{-1}$ h^{-1}), the incorporation rates of ^{3}H-thymidine (DNA synthesis, TdR) and ^{14}C-leucine (protein synthesis, Leu) were divided by respective cell abundances. Only data pairs of each subsample were included in calculations.

Terminal restriction fragment length polymorphism (T-RFLP) analysis. Immediately after retrieval, undiluted subsamples (5 g sediment wet weight) were frozen at −80°C. Total prokaryotic community DNA was extracted from sediments according to Zhou et al. (1996) and subsequently purified by using the WIZARD DNA Clean Up System (Promega). We used the eubacteria-specific 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′), obtaining a 1.503 bp product of the bacterial 16S rDNA (Lane 1991). For the amplification of archaeal 16S rDNA, a separate PCR reaction was performed with the specific primers 21F (5′-TTC CGG TTG ATC CYG CCG GA-3′) and 958R (5′-YCC GGC GTT GAM TCC AAT T-3′), giving a 915 bp product of the archaeal 16S rDNA gene (DeLong 1992). Synthesized by Interactiva, forward primers were 5′-labeled with the phosphoramidite fluorochrome 5-carboxy-fluorescein (5-FAM) and reverse primers with 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxyfluorescein (6-JOE). PCR reactions and cycling conditions, as well as restriction digests (HhaI and MspI), purification and separation of restriction fragments were performed following Moeseneder et al. (2001). A dual channel setup was used for the simultaneous detection of FAM- and JOE-labeled fragments (GeneScan 3.1, PE Applied Biosystems). Peak size determination of fragments, representative for different operational taxonomic units (OTUs), was done by comparison with the internal GeneScan-2500 (Tamra) size standard (PE Applied Biosystems) through the use of the local southern size-calling method. Peaks with areas <1% of the total peak area per electropherogram were excluded from statistical analysis. T-RFLP fingerprints generated with each of the 2 enzymes HhaI and Msp I were pooled.

The Dice similarity index was applied to presence (1) and absence (0) data of aligned bacterial and archaeal OTUs (±1 bp), respectively, which puts more weight on joint occurrences than on mismatches. The unweighted pair group method (UPGMA; Liu et al. 1997) was applied to determine similarities between T-RFLP fingerprints of all samples, using the software package PAlaeontological STatistics (PAST) version 1.65 (palaeo-electronica.org/2001_1/past/issue1_01.htm). Remaining data of sponge and reference samples were tested for significant differences by applying 2-way analyses of variance (ANOVA) and Bonferroni post-hoc tests using GraphPad Prism 4 (GraphPad Software).

RESULTS

Sediment characteristics and bacterial distribution

Data on POC concentration and sediment porosity are given in Table 1. A 2-way ANOVA showed that 93.4% of the variance in POC content was due to the different sampling locations, whereas a small (5.4%) but similarly significant ($p < 0.0001$) fraction depended on the presence of sponges. In every sample pair, the microenvironment of *Tentorium semisuberites* featured a significant enrichment (Bonferroni: $p < 0.05$ to 0.001) of POC (mean = 461.52 ± 72.6 µg g^{-1}) compared to reference sediments

Table 1. Concentration of particulate organic carbon (POC) and sediment water content (%H_2O), as well as mean cell-specific uptake rates of H^3-thymidine (TdR) and C^{14}-leucine (Leu) in sediments around the sponge *Tentorium semisuberites* (S) and in reference sediments (C). Numbers 1 to 4 identify sample pairs (e.g. S1 and C1)

Sample pair	POC (µg g^{-1} dry weight)	%H_2O	H^3-TdR (µmol $cell^{-1}$ h^{-1})	C^{14}-Leu (µmol $cell^{-1}$ h^{-1})
S 1	191.4 ±11.1	64 ± 1.2	0.04 ± 0.00	4.8 ± 0.4
S 2	600.8 ± 23.2	62.7 ± 0.9	0.11 ± 0.01	7.3 ± 1.2
S 3	485.9 ± 15.4	63.3 ± 1.1	0.11 ± 0.00	8.3 ± 0.4
S 4	568 ± 12.6	63.6 ± 1.2	0.10 ± 0.01	7.8 ± 0.8
C 1	93.4 ± 6.3	63 ± 0.9	0.07 ± 0.02	3.3 ± 0.9
C 2	533.2 ± 17.2	62.3 ± 0.6	0.12 ± 0.01	7.3 ± 0.5
C 3	445.3 ± 11.3	62.3 ± 0.6	0.14 ± 0.01	7.4 ± 0.7
C 4	455.3 ± 9.5	62.3 ± 1.1	0.11 ± 0.00	6.0 ± 0.5

(381.81 ± 81.7 µg g^{-1}). The sediment water content of all samples did not vary significantly (63.37 ± 0.5% H_2O).

Fig. 2 shows the mean bacterial abundances in the microenvironment around each sponge in relation to the corresponding reference sample. A 2-way ANOVA showed that 78.7% of the variance in cell abundances was significantly linked to the presence of sponges ($p \leq 0.0001$), whereas a small (9.5%) but also significant ($p < 0.002$) fraction of variance was caused by the location of the sample pairs. A Bonferroni post-hoc test showed that sediments in the immediate vicinity of all 4 sponges supported significantly ($p \leq 0.001$) higher bacterial cell abundances (5.33 ± 0.59 × 10^8 cells g^{-1}) compared to reference sediments (2.12 ± 0.79 × 10^8 cells g^{-1}).

Thymidine and leucine incorporation

Thymidine (TdR) incorporation rates in the sponge surroundings (4.81 ± 1.7 fmol g^{-1} h^{-1}) were significantly higher (Bonferroni: $p \leq 0.001$) than in reference sediments (2.54 ± 1.2 fmol g^{-1} h^{-1}) (Fig. 3a). Results showed that 56.10% of the variance in TdR uptake was due to the different sampling locations, while 40.44% was explained by the presence of sponges. As a measure for protein synthesis, leucine (Leu) incorporation rates followed a similar trend with higher values in the sponge vicinity (0.38 ± 0.11 pmol g^{-1} h^{-1}) compared to references (0.14 ± 0.07 pmol g^{-1} h^{-1}) (Fig. 3b). Bonferroni comparisons showed that Leu uptake rates were significantly higher near sponges ($p < 0.0001$), explaining 67.4% of the variance, while only 26.66% was due to the different sampling locations. Molar ratios between Leu and TdR uptake rates were significantly higher ($p < 0.05$ to 0.001) in the sponge vicinity (82.65 ± 18.9) than in reference sediments (52.31 ± 6.9) (Fig. 3c). While the sponges accounted for 52.13% of the variation, the sampling location had no significant effect on molar Leu:TdR ratios.

The cell-specific TdR incorporation rates in sponge sediments ranged from 0.04 to 0.11 µmol $cell^{-1}$ h^{-1} and were lower than in reference samples (Table 1). A 2-way ANOVA showed that 76.36% of the variance was significantly linked to local sediment patches ($p \leq 0.0001$), while a smaller (15.35%) but nevertheless

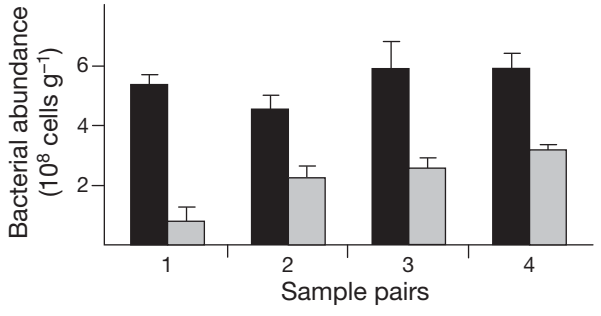

Fig. 2. Bacterial abundance (mean values, n = 3, 0 to 2 cm upper sediment layer) in the close sedimentary vicinity of 4 sponges (black bars) in relation to the nearby reference sediment (grey bars) for Sample pairs 1 to 4

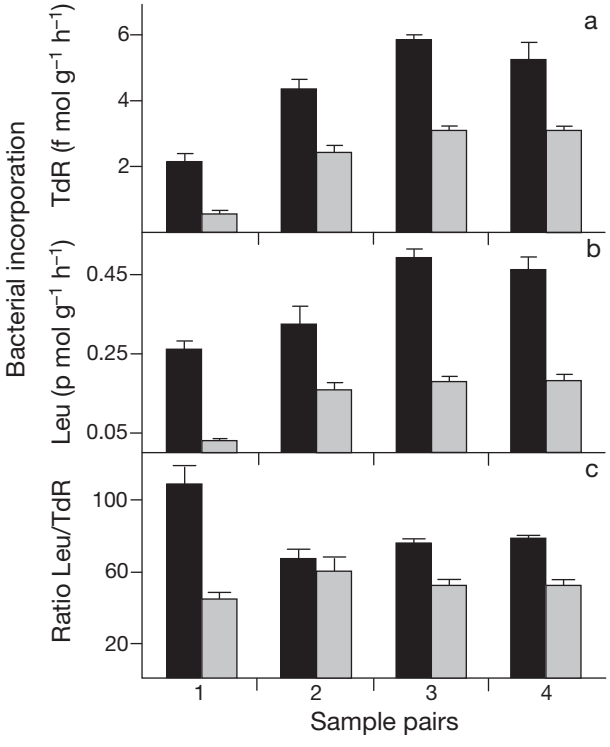

Fig. 3. (a) Bulk ^{3}H-thymidine (TdR) and (b) ^{14}C- leucine (Leu) incorporation rates (mean values, n = 3, 0 to 2 cm upper sediment layer) and (c) respective leucine/thymidine ratio in the sedimentary microenvironment of 4 sponges (black bars) in relation to corresponding reference sediments (grey bars) for Sample pairs 1 to 4

significant fraction of variance was due to the presence of *Tentorium semisuberites*. A Bonferroni test on the sample pairs showed that this decrease was significant only for sample pairs 1 and 3 ($p \leq 0.01$). Cell-specific Leu incorporation rates were higher in sponge sediments (4.78 to 8.34 µmol cell^{-1} h^{-1}) than in reference sediments (3.35 to 7.37 µmol cell^{-1} h^{-1}) (Table 1). This explains only 9.46% of the significant ($p = 0.0027$) variation in cellular Leu uptake, whereas 74.56% of the variation was linked to local sediment patchiness ($p < 0.0001$). Cell-specific Leu incorporation rates were exponentially correlated with POC concentrations (Pearson: $p \leq 0.005$).

Prokaryotic community patterns

The 2 restriction enzymes used to determine the OTU numbers in both sediment types (sponge environment and reference) gave a different yield for bacterial and archaeal fragments. Pooling the data from forward and reverse primers, 76 bacterial OTUs were gained by restriction with MspI, compared to 70 fragments by HhaI. Exactly 108 bacterial OTUs were included in statistical analyses. Of 176 MspI- and 100 HhaI-restricted archaeal fragments, only a total of 39 OTUs displayed acceptable peak areas. Both the bacterial and archaeal communities contained ca. 50% of fragments which were consistently present in all samples. The sampling location accounted for 66.16% (bacteria) and 70.04% (archaea) of the total variation.

UPGMA analysis of bacterial communities (Dice similarity index, cophenetic correlation coefficient = 0.767) indicated that assemblages from sediments around sponges clustered together to some extent (Fig. 4a). Sample pair 2 showed a close relation between bacteria occurring in the sediment next to the sponge and in the respective reference. For archaeal communities, the cophenetic correlation coefficient was 0.855 (Fig. 4b), indicating that the UPGMA dendrogram does not distort the original structure of the input data. The clustering pattern points to a certain difference between archaeal assemblages in sediments influenced by *Tentorium semisuberites* compared to reference sediments.

Sediments close to *Tentorium semisuberites* exclusively contained at least 6 bacterial (Fig. 5a) and 4 archaeal (Fig. 5b) OTUs. Interestingly, reference sediments contained 9 unique bacterial OTUs, which were not detected at all near the sponges (Fig. 5c). By contrast, all of the archaeal OTUs from reference sediments were also detected near the sponges.

Based on combined data from HhaI and MspI restriction digests, only the ratio of bacterial OTUs in sediments near sponges versus references was significantly correlated with respective POC concentrations (Pearson: $p < 0.005$), whereas bacterial and archaeal OTUs based on MspI restriction gave a highly significant correlation with POC concentrations (both $p < 0.0001$).

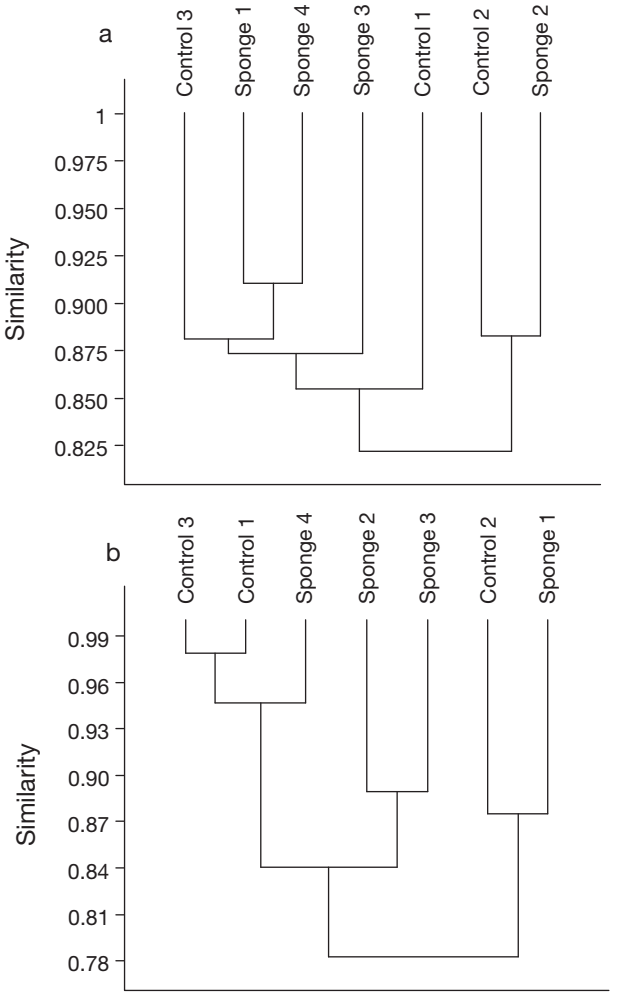

Fig. 4. Unweighted pair group (UPGMA) dendrogram of (a) bacterial and (b) archaeal operational taxonomic units (OTUs) in the microenvironment around *Tentorium semisuberites* (Sponge) compared to nearby reference sediments (Control). Numbers 1 to 4 indicate pairs of sponge- and nearby reference sediments

DISCUSSION

Patchiness of POC in deep-sea sediments

Our data indicate a high heterogeneity of the sediments analysed, with a pronounced patchiness of POC concentrations, since 93.4% of the variance was

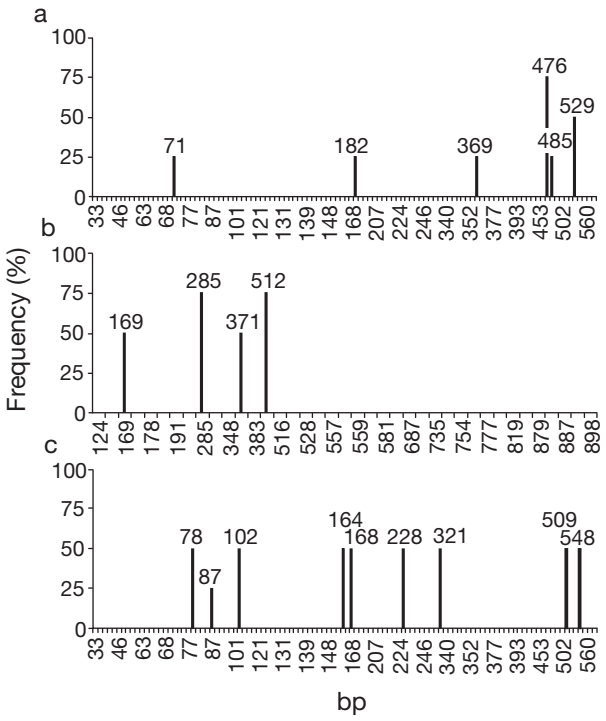

Fig. 5. Frequency (%) of (a) bacterial and (b) archaeal operational taxonomic units (OTUs) exclusively occurring in the sedimentary microenvironment of *Tentorium semisuberites* compared to plain reference sediments, containing unique bacterial OTUs not occurring near the (c) sponges. Values indicate the respective fragment length size (bp)

due to sample locality. It is needless to mention that we cannot generalize the results from a relatively small sample set in explaining the heterogeneity of the deep-sea benthic ecosystem. The trophic state of the deep sea depends on the input of organic material from the euphotic zone. Continuously degraded during sinking (Cho & Azam 1988), the quantity and quality of organic carbon reaching the deep seafloor is determined by residence time in the water column and water depth (Tholosan et al. 1999, Turley & Stutt 2000, Davey et al. 2001). Exposed to lateral transport mechanisms, the distribution of organic particles is prone to high regional differences (Boetius & Damm 1998, Soltwedel et al. 2000). In deep Arctic waters, the patchiness of sediment-bound organic particles is reinforced by melting processes of sea ice during summer (Schewe & Soltwedel 2003). Together with a release of stored terrigenous material, ice-edge-induced particle fluxes lead to a pulsed input of rapidly sinking phytodetrital aggregates. Both processes combined bring about a pronounced POC patchiness at the deep seafloor, which is, as proven by our data from HAUSGARTEN, independent of water depth.

Impact of *Tentorium semisuberites* on benthic POC content and prokaryotic activity

Despite the local sediment patchiness, we found a significant enrichment of POC in the sediments surrounding *Tentorium semisuberites*. Sponges may locally contribute to the total sediment POC content through biodeposition. In the deep Greenland and Norwegian Seas, for example, biodeposition rates — adding up to 10% of the vertical particle flux — were found for a poriferan community (Witte et al. 1997). Viewing sponges as obstacles, a passive interaction with near-bottom currents, which alters the particle deposition and erosion processes at small scales, becomes evident. Differential settling of particles is a function of particle size and of microscale flow patterns around epibenthic structures (Carey 1983, Eckman 1985, Friedrichs et al. 2000). Additionally, their feeding current may affect microscale flow patterns around sponges, whereas active particle sorting is a function of pore size (Witte et al. 1997, Kowalke 1999). Actively or passively enhanced settlement of particles may therefore result in an accumulation of POC in the vicinity of sponges. Similarly, transient bacterial cells may be trapped within the vascular system, attached to the sponge surface or deposited through downward vortexing at the lee-side of the sponge (Witte et al. 1997). Compared to other species within the Demospongiae, an obvious reduction of choanocytes in number and size has been observed for *T. semisuberites* (Witte 1995). This feature indicates the importance of 'microbial farming' (Pape et al. 2006 and references therein) and thus a mainly passive (bottom-current dependent) deposition of particles in the sponge surroundings. However, the sponge shown in Fig. 1 clearly deviates from *in situ* dimensions. As the specimen shrunk to an undefined degree during retrieval, the sponge's impact on the surrounding sediment surface might be spatially overestimated.

With cell numbers around *Tentorium semisuberites* up to 3 times higher than in reference sediments, our data fit well with previous findings on bacterial accumulation around the similarly small-growing, closely related demosponge *Thenea abyssorum* (Soltwedel & Vopel 2001). The authors found a significantly higher total bacterial biomass in the surrounding sediments compared to reference sediments. Their observation resulted from either a higher total cell abundance ($80.4 \pm 17.6 \times 10^8$ cells 5 cm^{-3}) compared to references ($53.2 \pm 6.2 \times 10^8$ cells 5 cm^{-3}), a higher mean biomass per cell (28.9 fg C $cell^{-1}$) around *T. abyssorum*, or a combination of both.

However, the POC enrichment in the sediments surrounding *Tentorium semisuberites* is not sufficient to justify the 3-fold increase in cell abundance and a

4-fold increase in Leu uptake. Focussing on the molar Leu:TdR ratios, the sampling location seemed to have no effect on the physiology of sediment-inhabiting prokaryotes. But in sediments next to the sponge, bacteria invested a significantly higher proportion of cellular resources in biomass synthesis (4 times higher than in reference sediments) than in cell division rates (2 times higher than in reference sediments). The observed shift between protein and DNA synthesis indicates an 'unbalanced' community growth, which may be related to low ambient temperatures (Tibbles 1996) and/or different substrate supply (Chin-Leo & Kirchman 1990), leading back to the passive and active mechanisms for an enhanced settlement of particles around sponges. Not only the quantity but, probably more so, the quality of the POC around *T. semisuberites* may lead to such increased cell abundances and protein synthesis. The cellular TdR and Leu uptake rates indicate that the increased secondary production was chiefly a function of higher bacterial biomass. Still, cellular Leu incorporation remained higher in sediments close to the sponges. The exponential correlation with the POC concentrations found supports the idea of a different carbon quality in these sediments. Biodeposition processes around sponges may result in a higher bioavailability of POC (Witte 1995). Similar mechanisms could also explain the discrepancies between bacterial cell abundance and cellular biomass found around *Thenea abyssorum* (Soltwedel & Vopel 2001).

The presence of slowly dividing, but actively metabolising cells might also lead to the assumption that bacterivory was low. At least grazers such as nematodes, which occur in great numbers in sediments next to *Tentorium semisuberites,* did not seem to select larger cells to ingest (Hasemann 2006). Interestingly, an increase of sponge-induced bulk TdR rates was solely based on a higher bacterial abundance, since cell-specific incorporation was decreasing in the microenvironment of sponges compared to reference sediments. Similar to an increase of Leu uptake, a reduction of cell division rates might be linked to a different prokaryotic community structure consisting of variable physiological types of prokaryotes in sediments near *T. semisuberites.*

Impact of *Tentorium semisuberites* on the benthic prokaryotic community structure

Nutritional differences may give rise to a different prokaryotic community composition (Cottrell & Kirchman 2000). It can be assumed that the organic material deriving from surface waters differs significantly in composition from the metabolic products released by sponges, i.e. fecal pellets (Findlay et al. 1990). Parallel to POC enrichment near the sponges, UPGMA analysis indicated a certain clustering of both the bacterial and archaeal communities in sediments affected by *Tentorium semisuberites*. A clear determination, however, could not be made, most probably due to the heterogeneity of sampling locations, which also became manifest in regionally high POC content variations. A connection of *T. semisuberites* with cell abundances, of course, has to be treated with caution. As DAPI stains both bacteria and archaea, we cannot infer specific information on the archaeal cell distribution. A comparison of POC concentrations and Leu and TdR incorporation data supports the idea of a different carbon quality. This also appears to have an effect on the prokaryotic community structure, as bacterial OTUs mainly correlated with the POC concentrations. The effect of *T. semisuberites* on prokaryotic carbon metabolism, however, basically seemed to be related to its specific bacterial community structure, as demonstrated by the similar incorporation rates in Sample pair 2. This suggests that archaea play a minor role in taking up amino acids such as Leu (Ouverney & Fuhrman 2000), but highlights the urgent need for further research on the prokaryotic physiological potential. A combination of microautoradiography with FISH (Lee et al. 1999) would allow a detailed identification of bacteria and archaea with respect to individual cell activity. The detection of signals in deep-sea sediments, however, might be hampered through masking by silt and clay (Schallenberg et al. 1989).

Both types of sediment (near sponges and references) did not differ significantly in their ribotype richness (number of OTUs), all samples combined accounting for a total of 108 bacterial OTUs and 39 archaeal OTUs. Nevertheless, T-RFLP patterns show that *Tentorium semisuberites* supports a specific microenvironment with some unique bacterial and archaeal OTUs which did not appear in reference sediments. In addition to a possible physiological coherence as discussed above, one might speculate on an influence of symbiotic prokaryote–sponge associations. Most reports of such associations refer to bacteria that are, in some cases, specific to their host (Preston et al. 1996, Friedrich et al. 1999). Symbiotic bacteria can account for up to 40% of the sponge biomass (Vacelet & Donadev 1977), exceeding that of seawater by 2 or 3 orders of magnitude (Friedrich et al. 2001). FISH analysis combined with electron microscopy indicated that all 4 demosponges whose sedimentary microenvironment was investigated in the present study harboured an exclusively methanotrophic archaeal consortium (Pape et al. 2006). Transient bacteria were solely settled on the external side of the pinacoderm. We do not yet know whether associated bacterial or archaeal cells are actively released, which would imply mixing with

the benthic prokaryotic community in the sponge's surroundings. On the other hand, we have to be aware of decompression effects during retrieval, since there was a clear deformation of the sponge shapes compared to *in situ* observations prior to sampling. Therefore, we cannot exclude the possibility of an artificial release of prokaryotes from the sponge interior and a resulting contamination of the surrounding sediments.

However, a total of 9 bacterial OTUs that were detected in reference sediments were completely absent around the sponges. Hence, *Tentorium semisuberites* seems to have an inhibitory effect on certain bacteria usually occurring in these deep-sea sediments. On the one hand, this species is supposed to filter sediment pore water due to its partially buried lifestyle (Hoffmann et al. 2007), which raises the question of whether the bacteria which were absent from sediments next to the sponge might be part of the sediment pore water community. On the other hand, demosponges are known for their high potential of antimicrobial activity (Thompson et al. 1985). Hence, it is very likely that *T. semisuberites* exudes antimicrobial compounds into the surrounding sediments, which implies an active selection for and against certain microbes from the surrounding environment. This possible inhibitory effect of *T. semisuberites*, however, demands further investigation.

To put more emphasis on the passive physical effects of sponges on small-scale particle deposition, a long-term mimic experiment (sponge-shaped, plastic simulations) was deployed during summer 2002 in close vicinity to our sampling area. Similar mimic experiments have already been successfully conducted in deep-sea environments to reveal the abiotic effect of biogenic structures on copepod communities (Thistle & Eckmann 1990). These experiments are expected to corroborate the results on prokaryotic community dynamics presented here and enable us to differentiate between physically-mediated (deposition and erosion) and biologically-mediated effects (feeding current and release of excretion products) on benthic community response.

Acknowledgements. We thank the operational team of the ROV 'Victor 6000' (IFREMER/GENAVIR) and the officers and crew of RV 'L'Atalante' for their support during the expedition in summer 2001. K. v. Juterzenka and F. Hoffmann helped to identify the sponges. The comments of M. Bergmann and suggestions of 4 anonymous reviewers helped to improve this paper. This is publication 'awi-n17165' of the Alfred Wegener Institute for Polar and Marine Research.

LITERATURE CITED

Aller RC (1982) The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: McCall PL, Tevesz MSS (eds) Animal–sediment relations. Plenum Publishing, New York, p 53–102

Barthel D, Tendal OS (1993) The sponge association of the abyssal Norwegian–Greenland Sea: species composition, substrate relationships and distribution. Sarsia 78:83–96

Boetius A, Damm E (1998) Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. Deep-Sea Res I 45:239–275

Carey DA (1983) Particle resuspension in the benthic boundary layer induced by flow around polychaete tubes. Can J Fish Aquat Sci 40:301–308

Chin-Leo G, Kirchman DL (1990) Unbalanced growth in natural assemblages of marine bacterioplankton. Mar Ecol Prog Ser 63:1–8

Cho BC, Azam F (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. Nature 332:441–443

Cottrell MT, Kirchman DL (2000) Natural assemblages of marine *Proteobacteria* and members of the *Cytophaga–Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692–1697

Davey KE, Kirby RR, Turley CM, Weightman AJ, Fry JC (2001) Depth variation of bacterial extracellular enzyme activity and population diversity in the northeastern North Atlantic Ocean. Deep-Sea Res II 48:1003–1017

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci 89:5685–5689

Eckman JE (1985) Flow disruption by an animal-tube mimic affects sediment bacterial colonization. J Mar Res 43: 419–435

Findlay RH, Trexler MB, White DC (1990) Response of a benthic microbial community to biotic disturbance. Mar Ecol Prog Ser 62:135–148

Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence *in situ* hybridization (FISH). Mar Biol 134:461–470

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the mediterranean sponge *Aplysina aerophoba.* FEMS Microbiol Ecol 38:105–113

Friedrichs M, Graf G, Springer B (2000) Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar Ecol Prog Ser 192:219–228

Gage JD (1996) Why are there so many species in deep-sea sediments? J Exp Mar Biol Ecol 200:257–286

Hansson HG (1999). NEAT (North East Atlantic Taxa): Scandinavian marine Porifera (Spongiaria) check-list. Available at: www.tmbl.gu.se/libdb/taxon/neat_pdf/NEAT*Porifera.pdf

Hasemann C (2006) Small-scale heterogeneity in the Arctic deep sea: impact of small coldwater-sponges on the diversity of benthic nematode communities. Rep Polar Mar Res 527:1–294

Hoffmann F, Sauter E, Sachs O, Røy H, Klages M (2007) Oxygen distribution in *Tentorium semisuberites* and in its habitat in the Arctic deep sea. In: Custódio M, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation, sustainability, Série Livros. Museu Nacional, Rio de Janeiro, p 379–382

Huettel M, Gust G (1992) Impact of bioroughness on interfacial solute exchange in permeable sediments. Mar Ecol Prog Ser 89:253–267

Jumars PA, Eckman JE (1983) Spatial structure within deep-sea benthic communities. In: Rowe G (ed) The sea. John Wiley, New York, p 399–451

Kowalke J (1999) Filtration in Antarctic ascidians — striking a balance. J Exp Mar Biol Ecol 242:233–244

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. John Wiley, New York, p 115–176

Lee N, Nielsen PH, Andreasen KH, Juretschko S, Nielsen JL, Schleifer KH, Wagner M (1999) Combination of fluorescent *in situ* hybridization and microautoradiography — a new tool for structure–function analyses in microbial ecology. Appl Environ Microbiol 65:1289–1297

Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Appl Environ Microbiol 63:4516–4522

Moeseneder MM, Winter C, Arrieta JM, Herndl GJ (2001) Terminal-restriction fragment length polymorphism T-RFLP screening of a marine archaeal clone library to determine the different phylotypes. J Microbiol Methods 44:159–172

Ouverney CC, Fuhrman JA (2000) Marine planktonic Archaea take up amino acids. Appl Environ Microbiol 66: 4829–4833

Pape T, Hoffmann F, Quéric NV, von Juterzenka K, Reitner J, Michaelis W (2006) Dense populations of Archaea associated with the demosponge *Tentorium semisuberites* Schmidt, 1870 from Arctic deep-waters. Polar Biol 29: 662–667

Preston CM, Wu KY, Molinski TF, DeLong EF (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. Proc Natl Acad Sci 93: 6241–6246

Quéric NV, Soltwedel T (2007) Impact of small-scale biogenic sediment structures on bacterial distribution and activity in Arctic deep-sea sediments. PSZNI Mar Ecol 28:66–74

Schallenberg M, Kal J, Rasmussen JB (1989) Solution to problems in enumerating sediment bacteria by direct counts. Appl Environ Microbiol 55:1214–1219

Schewe I, Soltwedel T (2003) Benthic response to ice-edge-induced particle flux in the Arctic Ocean. Polar Biol 26: 610–620

Smith CR, Maybaum HL, Baco AR, Pope RH and others (1998) Sediment community structure around a whale skeleton in the deep Northeast Pacific: macrofaunal, microbial and bioturbation effects. Deep-Sea Res II 45:335–364

Soltwedel T, Vopel K (2001) Bacterial abundance and biomass in response to organism-generated habitat heterogeneity in deep-sea sediments. Mar Ecol Prog Ser 219:291–298

Soltwedel T, Mokievsky V, Schewe I (2000) Benthic activity and biomass on the Yermak Plateau and in adjacent deep-sea regions northwest of Svalbard. Deep-Sea Res I 47: 1716–1785

Soltwedel T, Bauerfeind E, Bergmann M, Budaeva N, and others (2005) HAUSGARTEN: multidisciplinary investigations at a deep-sea, long-term observatory in the Arctic Ocean. Oceanography 18:46–61

Thistle D (1983) The role of biologically produced habitat heterogeneity in deep-sea diversity maintenance. Deep-Sea Res 30:1235–1245

Thistle D, Eckman JE (1990) The effect of biologically produced structure on the benthic copepods of a deep-sea site. Deep-Sea Res 37:541–554

Tholosan O, Garcin J, Bianchi A (1999) Effects of hydrostatic pressure on microbial activity through a 2000 m deep water column in the NW Mediterranean Sea. Mar Ecol Prog Ser 183:49–57

Thompson JE, Walker RP, Faulkner DJ (1985) Screening and bioassays for biologically-active substances from forty marine sponge species from San Diego, California, USA. Mar Biol 88:11–21

Tibbles BJ (1996) Effects of temperature on the incorporation of leucine and thymidine by bacterioplankton and bacterial isolates. Aquat Microb Ecol 11:239–250

Turley CM, Stutt ED (2000) Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean. Limnol Oceanogr 45:419–425

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Ecol 30:301–314

Witte U (1995) Reproduktion, Energiestoffwechsel und Biodepositionsleistung dominanter Porifera aus der Tiefsee des Europäischen Nordmeeres. Bericht Sonderforschungsbereich 313, Vol 58, University of Kiel, p 1–98

Witte U, Brattegard T, Graf G, Springer B (1997) Particle capture and deposition by deep-sea sponges from the Norwegian-Greenland Sea. Mar Ecol Prog Ser 154:241–252

Yager PL, Nowell ARM, Jumars PA (1993) Enhanced deposition to pits: a local food source for benthos. J Mar Res 51:209–236

Zhou J, Brunns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62: 316–322

Editorial responsibility: Otto Kinne, Oldendorf/Luhe, Germany

Submitted: July 30, 2007; Accepted: May 23, 2008
Proofs received from author(s): October 10, 2008