Depth distribution of active bacteria and bacterial activity in lake sediment

Ann-Louise Haglund a,b,*; Peter Lantz b; Erik Törnblom b; Lars Tranvik b

a Department of Public Technology, Mälardalen University, Box 883, SE-721 23 Västerås, Sweden
b Department of Limnology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 20, SE-752 36 Uppsala, Sweden

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Abstract

The bacterial activity in sediments is often low considering the generally high bacterial abundance. Still, a large fraction of bacteria have been found active even in deep sediments. These findings suggest that sediment bacteria have comparatively low cell-specific production. We studied bacterial activity and the active fraction of bacteria in a lake sediment profile. Bacterial production and metabolism were measured by thymidine and leucine incorporation and by microcalorimetry. In addition to counts of total bacteria, we estimated the nucleoid-containing fraction of the bacteria by adding a destaining step to the DAPI staining method, and the live fraction using the Live/Dead BacLight bacterial viability kit. The bacterial activity and abundance decreased with sediment depth, while the proportion of active bacteria remained similar at all depths. Between 57 and 63% of the bacteria were scored viable, and 13–52% were scored as nucleoid-containing cells. Consequently, there was no accumulation of dead bacterial cells in deeper sediments. Cell-specific production of sediment bacteria may be severely underestimated if the active fraction of the sediment bacterial community is not considered during enumeration.

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1. Introduction

Sediment bacteria constitute an important component of the benthic food web as well as of nutrient cycling and decomposition of organic matter. Bacterial activity and biomass are generally highest near the sediment surface and decrease with depth (e.g. [1]). Considering the generally high abundance of bacteria in sediments, the bacterial activity therein is often low. This indicates that the cell-specific activity of sediment bacteria is low, or that only a small fraction of the bacterial community is metabolically active [2]. Several studies have reported relatively large proportions of active bacteria in sediments (e.g. [3]), which favors the hypothesis of low cell-specific productivity. Even in deep sediments, there are viable bacteria (e.g. [4,5]), however, the metabolic state of sediment bacteria in depth gradients is not well known.

* Corresponding author. Tel.: +46 (18) 471 27 18;
Fax: +46 (18) 53 11 34.
E-mail address: ann-louise.haglund@ebc.uu.se (A.-L. Haglund).

Fluorochromes such as acridine orange and 4’,6’-diamidino-2-phenylindole hydrochloride (DAPI, Sigma) may overestimate the number of bacteria due to staining of dead cells and non-bacterial particles [2]. In addition, all cells are not actively participating in production and growth, which may lead to underestimates of cell-specific growth rates using the traditional enumeration methods. There are several methods to distinguish live cells from dead cells but they have been used mostly on bacterioplankton.

In this study, we employed two different methods to determine the number of viable bacteria, neither of which has been used on freshwater sediment bacteria before. We used Zweifel and Hagström’s [6] method to detect cells containing nucleoids and the Live/Dead BacLight bacterial viability kit (Molecular Probes, Eugene, OR, USA) to separates live cells from dead cells. Zweifel and Hagström [6] added a destaining step to the traditional DAPI staining procedure to avoid unspecific staining of cells lacking nucleoids. The empty cells were referred to as ‘ghosts’ incapable of growth while the nucleoid-containing cells were considered viable and capable of metabolic activity.
The Live/Dead BacLight bacterial viability kit is a two-color fluorescence assay. Cells with intact membranes stain fluorescent green and are considered to be live while cells with damaged membranes stain fluorescent red.

Combining several methods for bacterial activity and abundance, we examined the changes in the distribution of viable bacteria as well as the total and cell-specific metabolic activity in depth profile of a sediment.

2. Materials and methods

2.1. Sampling

Seven sediment cores were taken in June 1999 at 12 m depth at an accumulation bottom in Lake Erken. Lake Erken is a relatively large mesotrophic lake situated 60 km north of Stockholm, Sweden (59°51′N, 18°35′E). The sediment cores were transported to the laboratory in darkness and stored at 4°C until sliced. The cores were sliced at 6-mm intervals. Eight layers were saved for further analysis, taken at depths below the sediment surface of 0–6, 12–18, 24–30, 48–54, 78–84, 102–108, 162–168, and 222–228 mm. The sampling site is in an area with a sediment accumulation rate of 6 mm year⁻¹ (G. Weyhenmeyer, personal communication). Consequently, a core represents sediment accumulated over approximately the last 60 years, taking the compaction of the sediment into account. The sediments were muddy and had an organic content of approximately 18% [7]. After slicing, the sediment was analyzed within 24 h. Samples for DAPI staining were taken immediately after slicing and fixed with 4% formaldehyde. The rest of the staining and analyses was performed on live sediment. Water content was determined and of carbon and nitrogen were measured using elemental microanalysis (LECO, CHNS 932) on all the layers in seven cores.

2.2. Abundance of live and dead bacteria

We counted live and dead bacteria in the sediment using the Live/Dead BacLight bacterial viability Kit, which contains a mixture of SYTO® 9 and propidium iodide. The SYTO® 9 stain is a nucleic acid stain with green fluorescence, which labels all cells with intact cell membranes. Propidium iodide penetrates those cells that have damaged cell membranes, where it causes a red fluorescence and a reduction in the SYTO® 9 fluorescence when both dyes are present. Thus, when excited with blue light, the live cells, with intact cell membranes, will appear green. Dead cells, with damaged cell membranes, will appear red.

Portions of 0.5 g of live sediment were transferred to glass vials and mixed with 4.5 ml of filter-sterilized (0.2 μm) water. The mixture was diluted 1000 times, and a 2.5-ml subsample was mixed with 2.5 ml of water and sonicated for 90 s at 30 W using an ultrasonic cell disrupter (Microson®). Filter-sterilized (0.2 μm) water was used in all dilutions. A 1:1 mixture of SYTO® 9 and propidium iodide was added (7.5 μl), and the sample was incubated for 15 min. Thereafter, it was filtered onto a 0.2-μm black polycarbonate filter (Micron Separations). The filter was dried and placed on a slide in mounting oil (Molecular Probes) together with the 0.2-μm cellulose acetate filter (Lida), used as a drain disc during filtration. Once mounted, filters were immediately counted using epifluorescence microscopy. A minimum of 400 cells, or at least 20 randomly chosen fields, was counted on each slide. Only those cells where the stain was clearly visible were counted. The fraction of live bacteria was calculated as the abundance of live cells divided by the total count obtained with the Live/Dead method.

2.3. Total and nucleoid-containing bacterial abundance and biomass

We used DAPI for total bacterial counts. A portion (0.1 g) of sediment was fixed with 9.9 ml of 4% filter-sterilized (0.2 μm) formaldehyde. This mixture was diluted 1000 times and a 5-ml subsample was taken and sonicated for 90 s as described above. DAPI stain was added to a final concentration of 10 mg l⁻¹ and incubated for 30 min. Filtration, mounting on slides and counting were performed as described above. The DAPI-stained cells were visualized in UV light using epifluorescence microscopy.

To estimate bacterial biomass, photos of slides with DAPI-stained bacteria were taken. Photos of samples from all eight layers from three cores were used. To be able to determine the size of bacteria, the photos and a micrometer scale were projected on the wall and 50 cells from each slide were measured. The bacterial biomass was then estimated using a washing step to remove unspecific DAPI staining [6] using warm (60–70°C, 1 ml) 2-propanol. Then the filter was dried for 5 min before mounting on a slide as described above. The fraction of nucleoid-containing bacteria was calculated as the abundance of nucleoid-containing cells divided by the total counts obtained with the DAPI method.

2.4. Bacterial activity

Bacterial production was estimated using [³H]thymidine and [¹⁴C]leucine incorporation, while heat production using direct calorimetry was used to determine total sediment metabolism.

For the [³H]thymidine incorporation, a mixture of labeled and unlabeled thymidine was used at a final concen-
tration of 1000 nM. Previous studies of Lake Erken sediment have shown thymidine saturation at 1000 nM [3]. By mixing labelled (81.0–83.0 Ci mmol⁻¹) [³H]thymidine (Amersham, TRK 686) and unlabelled thymidine (Sigma) the final specific activity obtained was 43.45–44.02 Ci mmol⁻¹.

Subsamples of sediment (0.2 g wet weight) were incubated in 10-ml centrifugation tubes. There were three replicates and one control for each sediment layer and three cores were analyzed. The controls were killed by adding 5 ml of 80% ethanol. Thereafter, [³H]thymidine was added to each tube, and the samples were incubated at in situ temperature (12°C) for 40 min. The incubations were terminated by adding 5 ml of 80% ethanol. Samples were kept cool (4°C) until further processing. The samples were processed according to Bell and Ahlgren [9] and the radioactivity was detected using a liquid scintillation counter (LKB Wallac 1219 Rackbeta). Bacterial production was estimated from thymidine incorporation rates using a conversion factor of 2.0×10¹⁸ cells mol⁻¹ thymidine [10].

Incorporation of [¹⁴C]leucine was measured following the procedure for sediments established by van Duyl and Kop [11] with a few adaptations. 0.2 g of sediment was incubated for 30 min with [¹⁴C]leucine (Amersham, CFB 67) (94 mCi mmol⁻¹) in 10-ml centrifuge tubes (final concentration 1000 nM). The incubation was performed in triplicate with one additional control killed with 5 ml of 80% ethanol. Incubation was terminated by adding 5 ml of 80% ethanol and the samples were stored at 4°C overnight. Samples were then centrifuged (7500 rpm, 8500×g) for 20 min, the supernatant was discarded and the pellet was washed with 5 ml of 80% ethanol. This procedure was repeated twice. The pellets were then washed with 5 ml of ice-cold trichloroacetic acid (5%), and after centrifugation (at 4°C) the supernatant was discarded. This procedure was repeated twice. 2 ml of 2 M NaOH was added to each pellet and the samples were placed in a boiling water bath for 2 h followed by centrifugation for 20 min (7500 rpm, 8500×g). 1 ml of the supernatant was then transferred to a scintillation vial and scintillation cocktail was added. The leucine concentration (1000 nM) was chosen based on a saturation experiment performed on sediment samples from the sampling site. Final concentrations in the saturation experiment ranged from 500 to 9000 nM.

We measured heat production of the total sediment metabolism using a heat conduction multichannel microcalorimeter (TAM 2277, Thermometric) according to Bostrom and Toornblom [12], with a modification in order to maintain anaerobic conditions. Instead of using a smaller amount of sediment and an overlying water phase, 3-ml ampoules were completely filled with sediment using a syringe. All measurements were performed at 12°C and heat production values were recorded after an equilibration time of 10 h.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to distinguish changes in the bacterial community with depth. Homogeneity of variances (Bartlett’s M²) and normal distribution of the dependent variable (Shapiro–Wilks test) were assured. When necessary, the data were log- or arc-sin-transformed to achieve normal distribution. Spearman rank correlation was used when relating bacterial activity and abundance to depth.

3. Results

The water content in the sediment surface layer (0–6 mm) was 95% and decreased to 86% in the deepest layer measured (222–228 mm) (Fig. 1A). The carbon content decreased from 10.3% at the surface to 7.7% of sediment dry weight in the deepest layer. The corresponding numbers for nitrogen content were 1.3% and 1.0%, respectively. The molar C:N ratio varied from 9.0 in the surface sediment to 8.7 at 48 mm depth and then it increased again to slightly over 9 in the deepest layer (Fig. 1B).

The total number of bacteria followed the same general trend with both staining methods (DAPI and Live/Dead). Bacterial abundance was highest at the sediment surface and decreased with depth (P<0.001; ρ=−0.84 and ρ=−0.77 for DAPI total counts and Live/Dead total counts, respectively). The total bacterial abundance of DAPI-stained bacteria was 11.0×10¹⁰ cells (g dry weight)⁻¹ at the sediment surface, and 2.0×10¹⁰ cells (g dry weight)⁻¹ in the deepest layer (Fig. 2A). When adding

![Fig. 1. (A) Water content (%) and (B) C:N ratio at different sediment depths (n=7 for each depth). Error bars indicate standard deviation.](https://academic.oup.com/femsec/article-abstract/46/1/31/470725)
the washing step with 2-propanol, the abundance decreased by more than 50%. The nucleoid-containing cell abundance at the surface was $4.3 \times 10^{10}$ cells (g dry weight)$^{-1}$ and in the deepest layer $0.5 \times 10^{10}$ cells (g dry weight)$^{-1}$ (Fig. 2A). The fraction of nucleoid-containing bacteria was not significantly different between depths ($P = 0.083$, ANOVA) and varied from 13 to 52% (Fig. 3) of the total counts obtained with DAPI staining.

Total counts of bacteria using the Live/Dead method were lower than DAPI counts. The total abundance decreased from $4.0 \times 10^{10}$ cells (g dry weight)$^{-1}$ in the most surficial sediment to $1.8 \times 10^{10}$ cells (g dry weight)$^{-1}$ in the deepest layer (Fig. 2B). The live cells were $2.5 \times 10^{10}$ cells (g dry weight)$^{-1}$ at the surface and decreased to $0.7 \times 10^{10}$ cells (g dry weight)$^{-1}$ in the deepest layer (Fig. 2B). The corresponding figures for the dead bacteria were $1.5 \times 10^{10}$ cells (g dry weight)$^{-1}$ and $0.5 \times 10^{10}$ cells (g dry weight)$^{-1}$, respectively (Fig. 2B). The fraction of live or dead bacteria was not significantly different between depths ($P = 0.31$, ANOVA) but was rather similar at all sediment depths. The live fraction ranged from 57 to 63% of the total cell abundance obtained with the Live/Dead method (Fig. 3).

DAPI staining resulted in considerably higher abundances compared to the Live/Dead method. This was visually apparent when the total DAPI counts were plotted against the Live/Dead total counts. At high abundances, the total DAPI counts were more than twice as large as the total Live/Dead counts (Fig. 4A). Interestingly, the nucleoid-containing cells were strongly related to the live bacterial cells ($r^2 = 0.86$) (Fig. 4B) although different total counts were used for the calculations. The slope of the regression was not significantly different from one ($P < 0.001$, t-test).

The bacterial activity generally decreased with depth ($P < 0.001$; $r = -0.80$ and $r = -0.68$ for thymidine and leucine incorporation, respectively, $P = 0.002$; $r = -0.53$ for heat production) (Fig. 5). The $[^3H]$thymidine incorporation had a steeper decrease compared to $[^14C]$leucine incorporation, as seen from the decrease in the ratio between leucine and thymidine incorporation with increasing depth (Table 1). The $[^3H]$thymidine incorporation was 50.1 pmol (g dry weight$^{-1}$ h$^{-1}$) at the surface and declined to 3.8 pmol (g dry weight$^{-1}$ h$^{-1}$) at 222 mm depth. The corresponding values for $[^14C]$leucine incorporation were 3.0 and 0.27 nmol (g dry weight$^{-1}$ h$^{-1}$), respectively. Heat production also decreased with depth but not in as pronounced a manner as the thymidine and leucine incorpo-
ration rates. At the surface heat production was 46.8 W (g dry weight)$^{-1}$ and at 222 mm depth it declined to 26.7 W (g dry weight)$^{-1}$. The ratio between leucine incorporation and heat production fluctuated somewhat but it did not decline with depth until below the 102-mm layer (Table 1).

The cell-specific bacterial activity, as calculated by dividing the carbon production (derived from leucine incorporation) by the total carbon biomass (DAPI counts and cell volume estimates), varied from 0.04 to 0.22 day$^{-1}$ and was not significantly correlated with depth ($P = 0.11$; $\rho = -0.29$) (Fig. 6A). When the biomass of nucleoid-containing cells (Fig. 6B) or live cells (Fig. 6C) was used instead of the total bacterial biomass, cell-specific activity was considerably higher. When the biomass of live cells was used the specific activity ranged between 0.12 and 0.83 day$^{-1}$, decreasing with depth ($P = 0.006$; $\rho = -0.47$) (Fig. 6C). Calculations with nucleoid-containing cells resulted in cell-specific activities from 0.31 to 0.74 day$^{-1}$ and there was no significant decrease with depth ($P = 0.75$; $\rho = 0.06$) (Fig. 6B).

### 4. Discussion

Bacterial abundance generally decreases with sediment depth [1,13,14]. Interestingly, we found no significant difference in the fraction of live or nucleoid-containing bacteria among depths (Fig. 3). Hence, there was no accumulation of dead bacterial cells with depth, a fact also reported by Miskin et al. [4]. Viable bacteria are present also in deep sediments of the oceans. Parkes et al. [5] found a constant percentage of bacterial cells in a dividing stage down to 518 m in the deep sediments of the Pacific Ocean. The fact that there was no difference in the fraction of live or nucleoid-containing bacteria with sediment depth indicates that grazing or viruses are not important regulators of bacterial mortality in these sediments. There are often high densities of protozoa in aquatic sediments (e.g. [15]). However, most grazing studies show a minor impact of flagellate grazing on the bacterial community.
(e.g. [16]). In addition, Hamels et al. [16] found that the mortality of flagellate grazing on bacterial mortality decreased with grain size of the sediment. Viruses have been found to be numerous in freshwater sediments but the virus-to-bacteria ratio has generally been close to one or lower, i.e. significantly lower than in pelagic environments [17,18]. Accordingly, the two mortality factors generally considered to be responsible for most of the mortality of bacteria in the water column may not be effective in the sediments. This may contribute to the persistence of intact, active cells throughout the studied sediment profile.

Apparently, the Live/Dead BacLight bacterial viability kit has not been used on sediment bacteria before. Luna et al. [19], who applied a similar method using SYBR Green I instead of SYTO 9, obtained live bacterial fractions between 26 and 30% in a coastal marine sediment profile. Metabolically active fractions of bacteria in surface sediments between 4 and 67% are reported using the tetrazolium salts 5-epyano-2,3-ditolyl tetrazolium chloride (CTC) [3,20,21] or 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride [22,23] and nalidixic acid [22]. In the study on the viability of prokaryote communities in a post-glacial profundal freshwater sediment profile, close to 100% of the bacteria in the sediment were active throughout the sediment core down to about 6 m, using the CTC method [4]. Since the CTC incubation was performed together with nutrient additions, this probably reflects the viable fraction of bacteria, rather than the fraction that is metabolically active in the sediment. The fraction of live bacteria obtained in this study (57–63%) was higher than most proportions of metabolically active bacteria reported in sediments, but since dormant bacteria with intact membranes should be included in the live fraction, this is not surprising.

Total DAPI counts were up to more than twice the total abundance with the Live/Dead staining (Fig. 4A). This is consistent with the reports of unspecific staining of bacteria lacking nucleoids and non-bacterial particles with DAPI [2,24], and DAPI staining of non-bacterial particles such as mucus, flagellate mucocysts and polysphosphate granules, some of which may be erroneously recorded as bacteria [25]. Destaining of the DAPI samples according to Zweifel and Hagström [6] resulted in large standard errors in our sediment samples (Fig. 3). The abundance of nucleoid-containing cells was often higher than the abundance of live cells, using the Live/Dead method, suggesting that some cells with broken cell membranes (scored dead with the Live/Dead staining) contained nucleoids. Similarly, Choi et al. [26] report a higher percentage of nucleoid-containing cells than live cells (Live/Dead method) in marine bacterioplankton. When adding peptone, the fraction of nucleoid-containing bacteria increased, although the total bacterial count was constant, which led to the conclusion that cells without visible nucleoids are not necessarily empty ghost cells [26]. Hence, nucleoid-containing bacteria, as detected after DAPI staining procedure, seem to include some cells that are not viable, while other cells that are viable are not detected by this procedure. We report fractions of nucleoid-containing cells between 13 and 52%. Luna et al. [19] found fractions between 1.5 and 6.2% in marine sediments. These considerably lower fractions in marine sediments may be attributed to lower nutrient concentrations in the marine environment compared to our freshwater sediment. The nucleoid-containing fraction increased upon nutrient enrichment [19] as also reported by Choi et al. [26].

Estimates of cell-specific growth rate and productivity are usually based on the total bacterial biomass and the total bacterial production. Since this and several other studies report that only a fraction of the total bacteria are viable or active and capable of growth, the cell-specific production obtained using the total biomass is most likely underestimated. We calculated cell-specific activity using three different biomasses, i.e. the total bacterial biomass (DAPI), the biomass of nucleoid-containing cells, and the biomass of live cells. The two latter calculations produce specific activity estimates up to eight times higher (Fig. 6). Hence, the cell-specific production of the viable fraction of the bacteria is considerably higher than implied from estimates based on total bacterial counts.

Like the bacterial abundance, total bacterial activity decreased with depth. Several studies from river sediments show a similar pattern [1,14,27]. Bacterial activity declined with depth independent of the method used, but the steepness of the decline differed. Thymidine incorporation declined most rapidly, which may reflect the shortcomings of this method for deeper anaerobic sediments. Many anaerobic bacteria lack the ability to incorporate externally added thymidine into their DNA [28,29], which leads to an underestimation of the bacterial activity in anaerobic environments. In contrast to thymidine, incorporation of leucine/thymidine ratio with depth may be due to an increasing relative contribution of anaerobic bacteria that are unable to incorporate thymidine. McDonough et al. [31] also report higher leucine/thymidine ratios in anoxic compared to oxic waters.

Direct calorimetry (heat production) provides non-specific estimates of the total metabolism of the sediment community. It measures the enthalpy changes in biological and chemical processes independently of their function and origin. Thus, the technique is especially useful with sediments that contain mixed communities of anaerobes, fermenters, and aerobes [32], but the method is seldom used due to the unspecificity in distinguishing between metabolically and chemically produced heat [33]. The leucine:heat ratio was fairly constant in the upper decimeter of the sediment, but considerably lower further down (Table 1). This implies that the deeper bacterial communities use more of their energy for respiration (and thus dissipate heat) than for protein production as compared to the bac-
teria in the overlying sediment. The C:N ratio was somewhat higher in deeper sediment, which indicates a more refractory carbon source [34]. As a consequence, the bacterial community at these depths should have lower cell-specific production. This is consistent with our estimates of productivity based on the total bacterial biomass and the live fraction of the bacteria, but not on the nucleoid-containing fraction (Fig. 6).

In conclusion, bacterial abundance and total activity decreased with increasing sediment depth, but the viable fraction of the total bacteria was similar throughout the sediment profile (Fig. 3). Thus, organic particles, recognizable as bacteria, seem to be lost during the diagenesis of organic carbon in the sediment rather than being accumulated. We report up to eight times higher cell-specific production for the viable bacterial community compared to traditional calculations using the total bacterial biomass.

Hence, estimates of productivity and specific growth rates of sediment bacteria may be highly biased if the non-viable fraction of the bacterial community is not considered.

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References


