# Direct Counts of Bacteria in the Sediments of a North Carolina Salt Marsh

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Rublee, P. and B.E. Dornseif. 1978. Direct counts of bacteria in the sediments of a North Carolina salt marsh. *Estuaries* 1:188-191.



## Abstract:

The number of bacteria in sediments of a North Carolina salt marsh was determined by direct counts with epifluorescent illumination and acridine orange stain. Cell number decreased from 8.36-10.90 × $10^9$ cells/$cm^3$ of sediment at the surface to 2.19-2.58 × $10^9$ cells/$cm^3$ of sediment at a depth of 20 cm. No significant difference was found among four stations located on a transect which crossed the marsh and spanned subtidal to intertidal sediments.

## Article:

### *Introduction*

Although the ecology of estuarine bacteria has received increased attention in recent years, few studies have utilized direct techniques to assess bacterial populations. The majority of studies have utilized selective media to focus on specific physiological types of bacteria or to relate estimates of viable bacteria to physical and chemical conditions (e.g., Stevenson and Colwell 1973). Exceptions are the studies of the numbers of bacteria on the surface of pebbles by Batoosingh and Anthony (1971) and in intertidal sediments by Dale (1974), who utilized direct count techniques similar to those currently used to examine planktonic bacteria (Daley and Hobbie 1975; Hobbie and Rublee 1975; Ferguson and Rublee 1976; Hobbie et al. 1977).

Bacteria within salt marshes are assumed to play major roles, as for example in the breakdown and conversion of dead macrophytic tissues into utilizeable forms (i.e., bacterial biomass). Although most aboveground primary production of salt marsh macrophytes enters the estuarine detrital system (Teal 1962; Reimold et al. 1975), there is significant belowground production (Stroud 1976) which probably is decomposed within the marsh. Thus, saprophages may be a key component of the energy flow in marsh systems. Our study deals with the enumeration of bacteria in marsh sediments as a first step in quantitatively assessing the role of bacteria in the ecology of salt marshes.

This research was conducted at the National Marine Fisheries Service Beaufort Laboratory, Beaufort, N.C., through a cooperative arrangement between the Department of Zoology at North Carolina State University and the Beaufort Laboratory. Financial support was provided by the Office of Sea Grant, NOAA, U.S. Department of Commerce, under Grant Number 04-6-158-44054, and the State of North Carolina, Department of Administration. We also thank J. E. Hobbie, R. L. Ferguson and L. M. Cammen for their help and encouragement.

## Study Area and Methods

The Study area was a *Spartina alterniflora* marsh approximately 3 km from Beaufort Inlet, and within the Newport River Estuary of North Carolina. *Spartina* marshes in this area have an average above ground standing crop of 545 g dry wt/$m^2$ and an annual above-ground production of 650 g dry wt/$m^2$ (Williams and Murdoch 1969). Salinity of the overlying water ranges from about 22 to 36‰, and temperature of the surface sediments ranges from about 0°C to 35°C. Sediments below 1 cm are generally anaerobic and have more moderate

temperatures, varying from surface temperatures by as much as 11°C.

A transect with four stations was established across the marsh (Fig. 1). Station I was a subtidal station in the embayment bordering the marsh and had no macrophyte vegetation. Station 2, located approximately 12 m from the low tide line, was within the tallest *Spartina* in this marsh. Station 4, located near the high tide line, and Station 3, midway between Stations 2 and 4, were within areas of short *Spartina*.

Three cores were taken with a 4.45 cm internal diameter piston corer at each station on July 19, 1976. Each core was sectioned to sample 0-2, 4-6, 9-11, and 19-21 cm; the corresponding sections from the three cores were combined for sediment analyses. Particle size analysis was according to Morgans (1956), in which the sediment, oven-dried for 48 hr at 90°C, was passed through sieves according to the Wentworth Scale. The sediment grades were: granules (greater than 2.0000 mm), very coarse sand (1.0000-2.0000 mm), coarse sand (0.5000-1.0000 mm), medium sand (0.2500-0.5000 mm), fine sand (0.1250-0.2500 mm), very fine sand (0.0625-0.1250 mm), and silt and clay (less than 0.0625 mm). The weights of the fractions were plotted cumulatively according to Phi notation. Median grain size, MdØ, and Phi quartile deviation, QDØ, were calculated for each sediment sample.

Replicate portions of each sample also were analyzed for organic carbon by the Walkley-Black wet oxidation method (Morgans 1956) after sieving through a 1 mm mesh screen to remove most root material. None of the corrections suggested by Morgans were applied to the values obtained by this method. Additionally, an index of interstitial space and an estimate of surface area were calculated. The formula for interstitial space, spatial index = 1 × (MdØ) - 1 × [log (1% + % silt and clay)] - 1, is from Dornseif (1976). The estimate of surface area assumed a density of dry sediment material of 2.65 gm/cm$^3$ (Grim 1962; Buchanan and Kain 1972) and an average particle size for each of the seven sieve fractions (equal to a sphere with a diameter one-half the mesh opening).

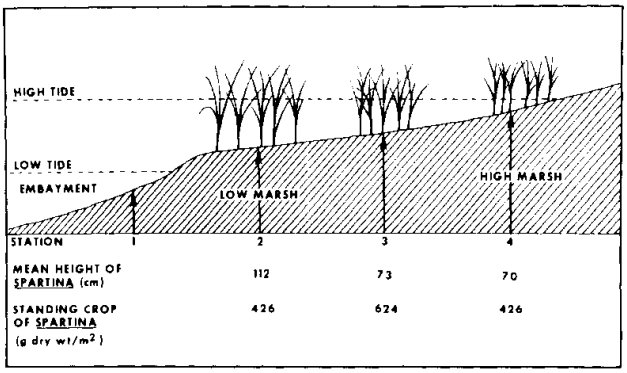


Fig. 1. Salt marsh transects showing relative location of stations and the standing crop and mean height of mature plants.

Replicate cores were taken for bacteria counts on 23 August 1976 for Stations 3 and 4, and two days later for Stations 1 and 2. Cores were placed on ice in the dark and taken to the laboratory where a 0.1 cm$^3$ sediment sample was taken by filling small aluminum planchets (Hewlett Packard #5080-5045) with sediment from each core at depths of 0, 5, 10, and 20 cm. Each sample was placed in a vial containing 20 ml of a filtered (0.2 μ Nuclepore filters) solution of Formalin in seawater (5% v/v) and all vials were stored in a refrigerator and counted within 48 hours. Before counting, each sample was further diluted with 100 ml filtered seawater and blended for 60 seconds at high speed in a Waring Blendor. A 0.5 ml subsample of the homogenate was removed and added to 1 ml of a prefiltered solution of acridine orange stain (0.01%). After a 60 second incubation the mixture was filtered onto a 0.2 μ Nuclepore filter which had been previously stained with Irgalan Black (Hobbie, et al. 1977). The filter then was examined under epifluorescent illumination at a magnification of 1250 ×. A minimum of ten fields or 200 cells were counted on each filter and the mean number of cells per field converted to number of cells/cm$^3$ of sediment. This method will miss cells on the underside of particles. A correction factor of 1.15 was used to account for this. The correction factor was determined on these samples by observing the area of the field covered by particles, the density of cells on the exposed surface of particles, and

by assuming that the distribution of cells was similar on the observed and the hidden surfaces of the particle. This procedure indicated that the counting method was 87% (S.E. 2%) efficient at counting bacterial cells in these sediments. Coefficient of variation of bacteria counts was 18.7%.

TABLE 1. Sediment properties and number of bacteria from the sediments of a North Carolina salt marsh.

| Station | Depth | MdØ | QdØ | % Silt and Clay | % Organic Carbon | Index of Space | Surface Area ($cm^2/cm^3$) | Sediment weight[a] | Bacteria ($10^9$ cells/$cm^3$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2.35 | 0.80 | 11.17 | 1.34 | 0.392 | 200 | 0.909 | 10.90 ± 1.20 |
| | 5 | 2.60 | 0.80 | 15.56 | 1.71 | 0.316 | 230 | 1.236 | 5.68 ± 0.84 |
| | 10 | 2.60 | 0.65 | 8.75 | 1.58 | 0.389 | 246 | 1.167 | 3.11 ± 0.66 |
| | 20 | 2.73 | 0.45 | 6.64 | 0.80 | 0.415 | 300 | 1.390 | 2.19 ± 0.43 |
| 2 | 0 | 2.55 | 0.90 | 17.00 | 4.10 | 0.312 | 112 | 0.621 | 9.91 ± 0.86 |
| | 5 | 2.60 | 0.60 | 13.77 | 3.37 | 0.329 | 163 | 0.948 | 5.06 ± 1.07 |
| | 10 | 2.67 | 0.58 | 12.33 | 3.21 | 0.334 | 188 | 1.056 | 2.45 ± 0.38 |
| | 20 | 2.76 | 0.28 | 8.16 | 1.27 | 0.377 | 234 | 1.042 | 2.28 ± 0.39 |
| 3 | 0 | 2.50 | 1.03 | 15.92 | 3.86 | 0.326 | 119 | 0.509 | 8.52 ± 0.00 |
| | 5 | 2.65 | 0.55 | 9.71 | 1.59 | 0.366 | 165 | 0.791 | 7.12 ± 0.89 |
| | 10 | 2.67 | 0.28 | 7.20 | 1.62 | 0.410 | 166 | 0.790 | 3.91 ± 0.91 |
| | 20 | 2.80 | 0.31 | 4.66 | 1.30 | 0.474 | 235 | 1.145 | 2.59 ± 0.83 |
| 4 | 0 | 0.06 | 1.33 | 5.53 | 10.62 | 20.450 | 26 | 0.245 | 8.36 ± 0.26 |
| | 5 | 1.50 | 1.53 | 3.37 | 0.57 | 1.040 | 81 | 0.662 | 6.50 ± 0.16 |
| | 10 | 2.60 | 0.78 | 5.82 | 0.19 | 0.461 | 281 | 1.493 | 4.40 ± 0.08 |
| | 20 | 2.75 | 0.95 | 18.67 | 1.37 | 0.281 | 262 | 0.969 | 2.20 ± 0.36 |

[a] g dry wt/$cm^3$ of wet sediment.

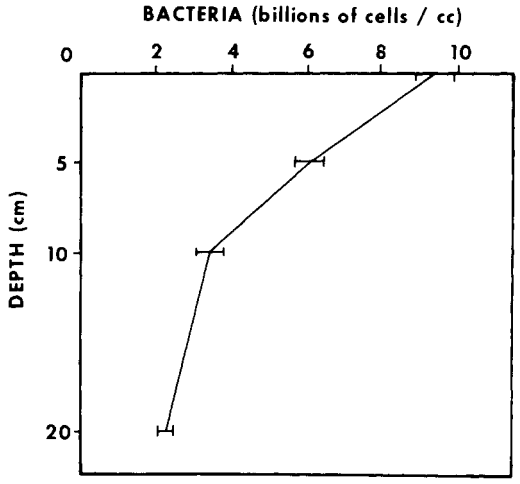


Fig. 2. Mean number of bacteria in sediments of four transect stations. Horizontal bars are ± one standard error.

## Results and Discussion

The highest and lowest number of bacteria were found at subtidal Station 1, 10.90 x $10^9$ cells/$cm^3$ at the sediment surface and 2.19 × $10^9$ cells/$cm^3$ at a depth of 20 cm. Although there appeared to be trends in the numbers along the transect (Table 1), an analysis of variance indicated that there was no significant difference between stations. Thus, the absence of macrophytes at the subtidal station did not appear to influence the distribution of bacteria that we found. The correlation of cell numbers with depth (Fig. 2) was highly significant ($r = -0.88$, $p < 0.0001$).

The use of the acridine orange direct count method with 0.2 μ Nuclepore filters reveals bacteria populations similar to those seen by scanning electron microscopy (Bowden 1977) and up to four orders of magnitude greater than viable count methods (Francisco, et al. 1973; Batoosingh and Anthony 1971). Comparison of our results with other studies will be restricted, therefore, to those which have used acridine orange stain and epifluorescent illumination to examine sediment bacteria. For example, Dale (1974) found the number of bacteria in intertidal sediments of Nova Scotia to range from 0.32-9.97 × $10^9$ cells/g dry wt of sediment at the sediment surface and 0.14-4.68 × $10^9$ cells/g dry wt at a 10 cm depth. A greater range in numbers, 0.10-55.0 × $10^9$ cells/g dry wt of sediment has been found in the highly organic and flocculent surface sediments of a freshwater tundra pond (Hobbie and Rublee 1975). Our numbers, when converted to a dry weight basis yield values ranging from 1.58 to 34.12 × $10^9$ cells, somewhat higher than those of Dale (1974) but within the range

reported by Hobbie and Rublee (1975). Any differences are probably the result of differences in location, salinity, and the fact that we used 0.2 μ pore size Nuclepore filters which retain more bacteria than the 0.45 μ pore size filters used in the other studies (Hobbie et al. 1977). Filially, bacterial counts in subtidal sediments of the Newport River estuary appear to have similar numbers to those we report (C. Shelton, North Carolina State University, pers. commun.).

Most stations had similar sediment properties (Table 1). With the exception of the surface and 5 cm depth of Station 4, all samples were characterized by well-sorted, medium to fine sand, with similar values for surface area and interstitial space. Organic carbon was low in the subtidal sediment (around 1%), and somewhat higher in Stations 2 and 3 (up to 4%). The surface and 5 cm depth of Station 4 had larger particles, less surface area, more interstitial space, and the highest value of organic carbon (over 10%). These differences, which were probably due to tidal action, did not appear to affect bacterial distribution.

Pearson correlation coefficients (Snedecor and Cochran 1971) were determined for bacteria ,numbers and sediment properties. The single strong correlation was a negative correlation of bacteria with depth. Other correlations were less important since first, they were not large, and second, they were strongly intercorrelated with depth. This is not surprising since the range of values of our sediment properties was narrow. Other studies which have demonstrated relationships of bacteria with sediment properties (e.g., ZoBell 1946; Dale 1974) have been based on broader ranges of data. Our values complement the positive relationship of bacteria to organic carbon reported by Dale (1974), but at similar values for mean grain size, our numbers of bacteria are one to two orders of magnitude higher.

The strong positive relationship of bacteria to surface area has been noted by many investigators (e.g., ZoBell 1946; Hargrave 1972). Reported density of bacteria ranges from one cell per 30 $\mu^2$ on the surface of decomposing turtle grass (Fenchel 1970) to one cell per 411 $\mu^2$ on the surface of marine sediment particles (Dale 1974). We found an average density of one cell per 5.31 $\mu^2$ (range of 0.31 to 13.73 $\mu^2$) on the surface of marsh sediment. This value is probably high since our methods yield a minimum estimate of surface area. Our values do, however, compare favorably with those of Zvyaginsev (1972), who found a density as high as one cell per 4 $\mu^2$ in terrestrial soils. This relationship requires further investigation, in particular with regard to the effect of bacterial density on decomposition in aquatic environments.

We have demonstrated that in this marsh the sediments maintain a standing crop of about $10^{10}$ bacteria/$cm^3$ in surface sediments and that, within similar sediments of this marsh in August, macrophyte vegetation did not appear to influence bacterial distribution. Whether the presence of macrophytes has an effect on distribution at other times or an effect on bacterial activity remains to be seen.

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