Seasonal Distribution of Bacteria in Salt Marsh Sediments in North Carolina

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Abstract:
The number and size of bacteria at four depths (0-1, 5-6, 10-11, and 20-21 cm) in a North Carolina salt marsh were minotored by direct counts for 13 months. The number of bacteria reached a maximum of about $1{\cdot}4 \times 10^{10}$ cells cm^{-3} at the sediment surface in October, corresponding to the period of *Spartina alterniflora* die-back. Cell numbers were lowest and most consistent throughout the year at the 20 cm depth of sediment. Cell volumes averaged $0{\cdot}2\ \mu m^3$ at the marsh surface and decreased with depth. Mean standing crop of bacteria to a depth of 20 cm of sediment was about 14 g bacterial carbon m^{-2}. In surface sediments bacteria contribute up to 15% and algae up to 10% of total living microbial biomass as estimated by adenosine triphosphate (ATP). Bacteria were the major biomass component at sediment depths of 5, 10 and 20 cm. At all depths the microbial community contributes < 4% total organic carbon and < 8 % of total nitrogen.
Keywords: bacteria; salt marshes; microorganisms; intertidal environment

Article:
Introduction
The role of microorganisms in natural systems includes decomposition, nutrient regeneration and cycling, and production of particulate matter. Such roles are of particular importance in highly productive systems where direct grazing of primary production by herbivores is not important, such as estuarine salt marshes. In these systems detrital food webs are well developed and have been the focus of many studies (e.g. Fenchel & Jørgensen, 1977).

Bacteria are a key component of the detrital microbial community because of their rapid colonization and high metabolic activity in association with detrital material (Fenchel & Jørgensen, 1977; Morrison et al., 1977; Rublee, 1978). Few studies have looked at the bacteria directly, however, and most of our information on standing crop is from indirect estimates of the total microbial biomass from measurement of ATP (e.g. Christian et al., 1975) or constituents of procaryotic cell walls such as muramic acid (e.g. Morrison et al., 1977). This paper reports the results of a 13-month study of the standing crop of bacteria in a salt marsh and comparisons with both the microalgal and total microbial biomass.

Methods
The study site was a 100-m^2 plot in a *Spartina alterniflora* marsh located approximately 3 km from Beaufort Inlet and within the Newport River estuary, North Carolina. Characteristics of this marsh included a salinity range of the water over the marsh from about 22 to 30‰, dependent upon rainfall, river flow and stage of the tide (amplitude approximately 1 m). Temperature of the surface sediments was similar to air temperature throughout the year and ranged from 0 to 35 °C. Subsurface sediments had more moderate temperature ranges and they were generally anaerobic within several millimeters of the surface. Sediments were composed of fine sand with 5-17% silt and clay, and organic carbon content decreased from 4% in surface sediments to about 1% at 20 cm (Rublee & Dornseif, 1978). Analysis of surface sediments with a CHN analyser yielded total nitrogen values of about 0•33% of dry weight.

Salt marsh sediments were sampled monthly at 0-1, 5-6, 10-11 and 20-21 cm depths from May 1975 until June 1976. Samples were analysed for bacteria by direct counts, for total microbial biomass by ATP in all months except May 1975, and for algal biomass by chlorophyll *a* determination in all samples after July 1975. Generally on each sampling date four sediment cores were taken to a depth of at least 25 cm by a hand-held piston corer with removable sleeve (internal diameter of 4•45 cm). Cores were taken at or near low tide. Two cores were used for ATP and chlorophyll *a* analysis and the remaining two for bacterial enumeration, Cores for ATP and chlorophyll a were subsampled (0•2 cm^3) in the field immediately after collection. At four depths (0-1, 5-6, 10-11 and 20-21 cm) the core was sectioned and subsamples were taken by filling two aluminum planchets (each 0•1 cm^3) with sediment. Replicates were taken at each depth from each core for both ATP and chlorophyll *a* determinations. Subsamples for ATP were placed in small plastic vials with several pieces of dry ice for rapid freezing. Vials were then stored on dry ice until extracted (within 24 h). The subsamples for chlorophyll *a* were placed in plastic vials with several drops of a $MgCO_3$ slurry and approximately 5 ml of acetone and shaken vigorously. Vials were then stored on dry ice, moved to the laboratory and placed in a freezer (-20 °C) until analysed.

TABLE 1. Efficiency of recovery of added bacterial ATP from sediment samples

Depth (cm)	Lyophilyzed bacterial cells		Wet bacterial cells	
	% recovered	S.D.	% recovered	S.D.
0–1	16·1	2·3	47·5	33·6
5–6	31·9	16·0	34·5	9·8
10–11	39·2	6·6	32·7	10·3
20–21	39·5	5·3	45·0	12·5

Total microbial biomass (defined here to include all microbiota and most meiofauna) was determined by ATP analysis following the Tris extraction method of Ferguson & Murdoch (1975). This extraction method was tested for a recovery efficiency on one occasion by adding known amounts of ATP (in the form of lyophilized bacterial cells and in a replicate set of samples in the form of freshly harvested wet bacterial cells) to the samples after they were returned to the laboratory, but prior to extraction with Tris buffer. The recovery of added ATP determined by this method varied with the standard used (Table 1), but are in the same range as those reported by Ferguson & Murdoch (1975). Low recovery in shallow sediments was likely due to high organic carbon, silt and clay content of these sediments which would reduce recoveries of ATP (cf. Ferguson & Murdoch, 1975). As a result, values at each depth reported here are corrected for the lower efficiency determined by the two standards at that depth. Note, however, that there is no absolute test of efficiency for ATP extraction from natural samples.

Recently, Karl (1980) discussed extraction methods for ATP in sediments and recommends use of cold acids or organic solvents rather than boiling buffers for sediments. Subsequently compared recovery of added reagent ATP in samples from this site by Tris extraction and by cold phosphoric acid extraction (Karl & Craven, 1980). Reagent ATP was added to triplicate subsamples either before or after the extraction. In both cases, after subtraction of ambient ATP levels, there was no difference in recovery if reagent ATP was added before or after extraction ($P < 0•05$, t-test). The acid extraction yielded a higher recovery of reagent ATP (87%) than the Tris (61%). When the concentration of ATP in the sediments was calculated, based on these recovery efficiencies, the acid extraction method yielded twice the concentration of ATP. If however, the efficiency determined earlier for the lyophilized bacterial cells is used, then both methods indicate similar concentrations of ATP in these sediment samples. Thus, Tris extraction did appear an appropriate method for these samples. Note, however, that there is no absolute test for *extraction* efficiency (as opposed to *recovery* efficiency) and the caution raised by Karl (1980) should be emphasized: each extraction method varies with the characteristics of the sediment and recovery should be carefully tested at each study site. In general, errors on the order of 5-25% are not unexpected. Since error in the interpretation of results—at least in the use of ATP as a biomass indicator—may encompass a greater range (up to orders of magnitude—cf. Karl, 1980), such errors may be acceptable if the resultant data are used with caution.

Benthic algal biomass was determined from fluorometric assay of chlorophyll a in sediment samples. The

procedure followed that described by Ferguson & Murdoch (1975), except that the samples were extracted for 1-3 weeks at -20 °C in the acetone mixture rather than by mechanical means. These methods give essentially equivalent results (R. Ferguson, personal communication), although probably neither method yields a complete extraction of chlorophyll. Algal biomass estimations were made by multiplying chlorophyll values by 3o (Ferguson & Murdoch, 1975).

The cores for bacterial enumeration by epifluorescent microscopy were returned to the laboratory on ice, within the removable sleeve of the coring device. In the laboratory the cores were removed from the sleeves and subsamples were taken from the same depths as for ATP and chlorophyll samples. Generally one core was sampled in duplicate and a single sample was taken from the other. Occasionally more replicates were taken from the same core, or more cores were sampled to assure that variation within and between cores was adequately estimated. Each subsample consisted of 0•1 cm^3 of wet sediment which was placed in a plastic vial containing a filtered solution of 19 ml seawater and 1 ml formalin. Vials were then capped, shaken to disperse the sample and stored in a refrigerator at about 4 °C until they were counted, usually within 3 days of collection. All preserving solution was filtered on the day of addition to samples through a 0•2-µm pore size Nuclepore filter. A 5% formalin solution (v/v) preserved bacteria in the sample for at least 7 days in preliminary tests.

The procedure for counting the bacteria is a modification of the acridine orange direct count (AODC) used for planktonic samples (Hobble et al., 1977) and described in Rublee & Dornseif (1978). In addition to counting the number of cells in a sample, the average cell size for most samples was determined. A minimum of 100 cells from random fields in each sample were observed and placed within one of five categories:

(i) cocci < 0•5 µm in diameter;

(ii) cocci 0•5-1•0 µm in diameter;

(iii) cocci > 1•0 µm in diameter;

(iv) rods approximately 0•5 × 1•0 µm;

(v) cells which did not fit in any of the first four categories and whose dimensions were noted individually.

Most cells fit in categories (i), (ii) or (iv). The average cell size was then determined by assuming that the mean volume for each of the first four categories was 0•033, 0•221, 0•524 and 0•246 $µm^3$, respectively, and by calculating the volumes for individuals in category (v). Bacterial biomass estimations were made using conversion factors of 0•099 g C cm^{-3} bacterial biovolume (Ferguson & Rublee, 1976) and 0•033 g N cm^3 bacterial biovolume (Doetsch & Cook, 1973).

Results and discussion

The number of bacteria was highest in the fall at the surface of the salt marsh sediments, ranging from 1•4 × 10^{10} cells cm^{-3} of sediment at 0-1 cm during October to 0•1 × 10^{10} cells cm^{-3} of sediment at a depth of 20-21 cm in August [Figure 1(a)]. In all months the number of bacteria at 5-6 cm was about 60% of the surface value and exhibited a similar seasonal variation. At sediment depths of 10-11 and 20-21 cm the numbers of bacteria averaged 35 and 22% of the surface value respectively (Table 2), but displayed relatively little seasonal variation. The coefficient of variation for all counts was 29%. The numbers of bacteria are similar to those found in other sediment studies which have used the AODC method. For example, studies in the Newport River estuary have demonstrated numbers of bacteria in other locations within this marsh and in subtidal sediments that are similar to those reported here (Rublee & Dornseif, 1978; Shelton, 1979). Similar, but higher bacterial numbers have also been found in a Massachusetts *Spartina* marsh with a higher (30%) organic carbon content of sediments (J. E. Hobbie, J. Helfrich, Marine Biological Laboratory, Woods Hole, Massachusetts, personal communication). Dale (1974) reported numbers of bacteria in intertidal sediments of Nova Scotia to range from 0•03 to 1•00 × 10^{10} cells g^{-1} dry weight of sediment at the sediment surface. On a dry weight basis the numbers

found in this study range from 0•8 to 2•2 × 10^{10} cells g^{-1} dry weight.

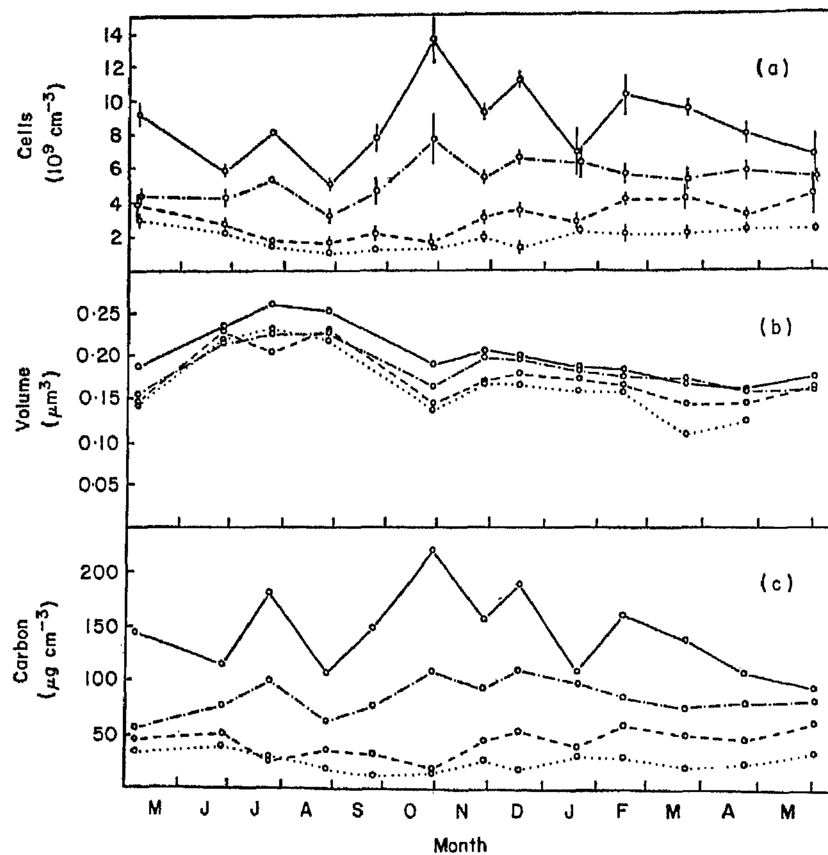

Figure 1. Bacterial characteristics of a North Carolina salt marsh. (a) Number of bacteria, (b) average bacterial cell size and (c) bacterial standing crop. ——, surface sediment samples (0–1 cm); — · —, 5–6 cm depth; - - -, 10–11 cm depth; . . . ., 20–21 cm depth.

TABLE 2. Mean yearly values (±1 S.D.) for some bacterial characteristics of a North Carolina salt marsh

Depth (cm)	Number (10^9 cells cm^{-3})	Volume (μm^3)
0–1	8·5±2·1	0·20
5–6	5·4±1·0	0·19
10–11	3·1±1·0	0·18
20–21	1·9±0·7	0·18

Analysis of variance of bacterial numbers indicated strong depth and seasonal components as well as a significant interaction term. The decrease in the number of bacteria with depth has been noted by many investigators and need not be discussed further except to note that the seasonal trends at the surface and 5-cm depths of sediment are similar and much more variable than the trends at 10- and 20-cm depths (Figure 1). The variance in the upper sediments suggests a seasonal pattern related to nutrient input, while the relatively stable number of bacteria at depth suggests less strong seasonal influences. The input of particulate and dissolved carbon from macrophytes has been well documented (e.g. Reimold et al., 1975) and its utilization the subject of many studies. The high numbers of bacteria found in marsh sediments in the fall are likely a reflection of nutrient input (both dissolved and particulate) following the die-back of *Spartina alternillora* in the fall.

Bacterial volume also varied significantly with depth and season [Figure 1(b)]. The maximum average cell volume was observed in the summer, o•26 μm^3, at the marsh surface, and the minimum volume, 0•09 μm^3, was found at the 20 cm depth of sediment in spring. There was a significant correlation of cell volume with temperature (r = 0•62, $P < 0.05$). The average cell volume in the surface sediments was 0•20 μm^3 (Table 2), which is 2-4 times larger than the average planktonic cell volume (Ferguson & Rublee, 1976; Bowden, 1977).

The estimates of bacterial biomass [Figure 1(c)] reflect the strong influence of cell numbers and size estimates

in their calculation. The seasonal pattern is less pronounced but indicates high standing crop in both summer and fall. The highest values for bacterial standing crop were found in late summer and fall and reached a maximum of 221 µg bacterial C cm^{-3} of sediment at 0-1 cm depth in October. Minimum values occurred in early summer (93 µg bacterial C cm^{-3} of sediment). Standing crop decreased with depth; the average value at 20-21 cm was only 17% of the surface value. An integrated value of the average standing crop of bacteria over the top 20 cm of sediment is approximately 14 g C m^{-2} or about 40 g dry weight m^{-2}. Dale (1974) found 5•5-21•5 g dry weight m^{-2} in subtidal sediments of Nova Scotia.

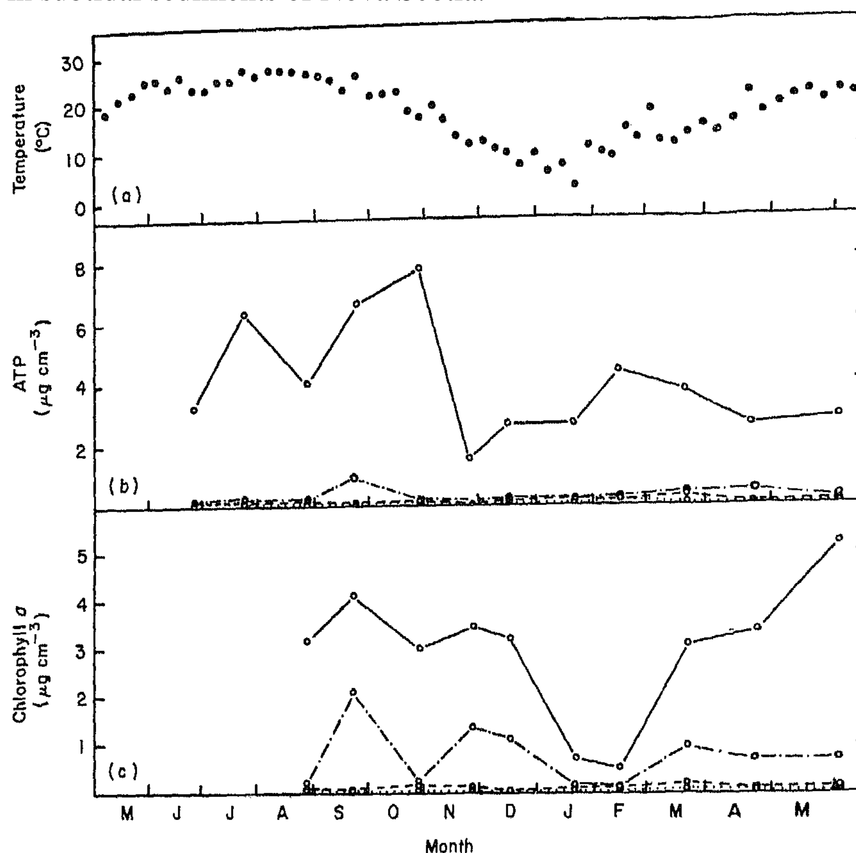

Figure 2. (a) Temperature, (b) recoverable ATP and (c) chlorophyll *a* content of North Carolina salt marsh sediments. Representations of samples from different depths as in Figure 1.

ATP and chlorophyll *a* decreased with depth of sediment and exhibited high values during the fall months (Figure 2). ATP attained a maximum value of 7•77 µg ATP cm^{-3} of sediment at the surface of the marsh in October. In all months the ATP content of the sediments decreased with depth. The peak chlorophyll *a* value was found on the marsh surface in June (5•2 µg chl. *a* cm^{-3} of sediment) and chlorophyll decreased rapidly with depth. Similar amounts of ATP have been reported in subtidal estuarine sediments in North Carolina and in salt marsh sediments of South Carolina and Georgia (see Karl, 198o). Chlorophyll *a* values are in the range reported in the literature for estuarine sediments (Ferguson & Murdoch, 1975), but are somewhat lower than those reported for some Massachusetts marsh sediments (Estrada at al., 1974).

Mean yearly estimates for biomass characteristics suggest that bacteria contribute significantly to total microbial biomass which includes bacteria, algae, protozoans and meiofauna (Table 3). At the sediment surface (0-1 cm) bacterial standing crop accounts for approximately 15% of the total microbial standing crop as determined by the ATP method if a conversion factor of 250 is used (see Karl, 198o). Percentage contribution of bacterial carbon at depths below the surface is somewhat greater than 100%, and suggest that the direct count method overestimated bacterial biomass, ATP was underestimated, or the conversion factor of 250 for ATP to carbon is inappropriate. It is likely that cell sizes were overestimated, and dead bacterial cells are included in this counting method although the proportion is probably small due to rapid decomposition of microbial cells. Errors in the ATP determination were discussed previously. It is also likely that in a natural system where growth of microbes may not be optimum and in fact large portions of the populations may be inactive or non-

viable (Postgate, 1973), the conversion factor is inaccurate. Karl (198o) has discussed the range of conversion factors for ATP to biomass and if a larger value of 500, as suggested by Ausmus (1973) for soil microorganisms, is used, then values of total microbial biomass are larger than those estimated for bacterial biomass. Use of this factor also reduces the estimate of bacterial contribution to around 8% of the total microbial community in surface sediments.

TABLE 3. Estimated contribution of microbial and bacterial standing crop to total sediment organic carbon and nitrogen in a North Carolina salt marsh

Depth (cm)	Microbial C[a] (mg C cm^{-3})	Bacterial C[b] (mg C cm^{-3})	Sediment C[c] (mg C cm^{-3})	Microbial N[d] (mg N cm^{-3})	Bacterial N[b] (mg N cm^{-3})	Sediment N[e] (mg N cm^{-3})
0–1	1·013	0·147	25·40	0·170	0·044	2·19
5–6	0·080	0·088	31·90			
10–11	0·023	0·047	33·80			
20–21	0·010	0·014	13·20			

[a] ATP × 250 (Karl, 1980).
[b] Estimated from direct counts—see text.
[c] Values from Rublee & Dornseif (1978) determined by the Walkley–Black wet oxidation method.
[d] ATP × 24 (Christian *et al.*, 1975).
[e] Determined by CHN analyser.

A conversion of the mean chlorophyll *a* value, 3•0 μg cm^{-3}, to algal biomass by a conversion factor of 3o (Ferguson & Murdoch, 1975), indicates that the benthic algae contribute about 10% of the total standing crop at the surface. This is probably an underestimate because of the method used to determine chlorophyll *a* and because the missing chlorophyll data is from summer months which would probably raise the mean value. In any case, the standing crop of algae below the surface appears to be small and does not seem to be an important contributor to microbial standing crop at depth. The remaining microbial carbon at the surface is presumably composed of an array of organisms including fungi, protozoans and small metazoans.

The contribution of total microbes and bacteria to sediment carbon and nitrogen pools is not large (Table 3). In this study the contribution of the total microbiota (determined by ATP) was about 4% of the sediment organic carbon value and less than 8% of the sediment nitrogen. The contribution of bacteria as estimated in this study is even less important, about 0•6% of sediment organic carbon and less than2z% of the nitrogen at the sediment surface. These values also compare well with those reported for a Georgia salt marsh (Christian et al., 1975) and for shallow subtidal sediments (Ernst, 1970; Ferguson & Murdoch, 1975) where the contribution of microbes to sediment organic carbon and sediment nitrogen was in the range of 0•05--2•98%.

The apparently small contribution of the bacteria and total microbial community to organic carbon and nitrogen content of sediments may not give a true picture of their importance, however, since standing crop measures are often poor indicators of metabolic activity. This is particularly important with respect to microorganisms due to their potentially high metabolic rates and to their potential as a selected food source for deposit feeders. Thus their true contributions to the community in terms of production and nutrient cycling can only be assessed accurately with measures of activity in addition to standing crop values.

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