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Planktonic Bacteria in the Humber Estuary; Seasonal Variation in Population Density and Heterotrophic Activity

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Abstract

Seasonal variation in population density and heterotrophic activity (as maximum potential rate, V_{max}, for glucose mineralization) of bacteria attached to suspended solids, of free bacteria and of total bacteria was examined in the water of the Humber Estuary, north-east England. At the principal site, which was studied over 2 yr, values of density and activity of attached and total bacteria showed marked seasonal periodicity, being low in summer and high from autumn to spring. This was a consequence of density of attached bacteria being dependent on the concentration of suspended solids, which was high in winter and low during summer. Values of density and activity of free bacteria, which were less than those of attached bacteria, showed no seasonal pattern but fluctuated irregularly and were independent of concentration of suspended solids. At two further sites, which were studied over 1 yr only, the density of bacteria varied seasonally in a similar manner, but the activity of attached and total bacteria showed no distinct seasonal pattern - this may, however, be due to limitations of the data rather than to fundamental differences between the sites studied.

Introduction

Planktonic bacteria in estuaries are responsible for the heterotrophic breakdown of organic matter; this originates from diverse sources. Autochthonous organic material is made available by phytoplankton, macroalgae and estuarial fauna. Allochthonous material is brought in from the sea by tides, originates in rivers and their catchments, and may be discharged as organic pollutants. These sources are all liable to seasonal variation, as also are the physical and chemical factors which might influence bacteria (e.g. concentration of suspended solids, temperature, salinity). It follows that there is likely to be seasonal variation in the population density and heterotrophic activity of planktonic bacteria in estuaries. Such variation is described in the literature, but no common pattern has emerged.

Seasonal variation in population density has, for example, been recorded in the Inner Kiel Fjord (FRG) where, in 1973, the density of viable bacteria (i.e., those which can be grown on an agar medium), at 2 m depth, reached a summer peak in August-September (Gocke, 1978). In 1974, however, separate peaks occurred in May and October with low levels in winter and mid-summer (Rheinheimer, 1977), but the density of total bacteria (obtained by direct counting using epifluorescence microscopy) reached high levels in April-June but not in the autumn (Zimmermann, 1977). In the Saelenvann Estuary in Norway, Indrebø *et al.* (1979) found pronounced summer maxima in density of viable bacteria in surface water over 3 successive years. In contrast, Moshiri *et al.* (1979) reported that bacterial density in Bayou Texar Estuary, Florida (USA) (obtained by direct counting with an inverted microscope) fluctuated irregularly over a 10-month period, with maxima in July and January-February.

Similarly, different patterns of seasonal variation in heterotrophic activity have been reported. In the Inner Kiel Fjord in 1973, at 2 m depth, Gocke (1978) found that V_{max}, for assimilation of glucose, aspartate and acetate into bacterial biomass, attained a single late-summer peak in August-September. In 1974, however, high values were recorded in April and August-September, with low levels in winter and mid-summer (Gocke, 1977). Crawford *et al.* (1974) measured V_{max} for uptake (assimilation + mineralization) of glucose and a range of amino-acids in the Pamlico River Estuary, North Carolina (USA), and found highest values in summer. Likewise, Zubkoff and Warinner (1977) noted that V_{max} for glucose assimilation in the York River Estuary, Virginia (USA) was highest in summer. In contrast, when Albright (1977) measured V_{max} for glucose uptake in the Fraser River Estuary in British Columbia (Canada),

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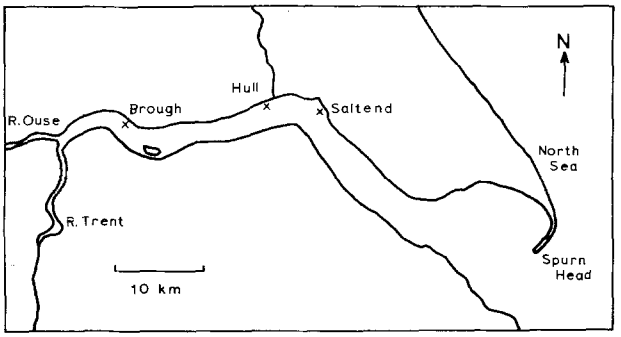

Fig 1. The Humber Estuary, north-east England; Crosses indicate approximate positions of sampling sites

Table 1. Density of attached and free bacteria (10^6 ml^{-1}) obtained from direct counts on non-centrifuged and centrifuged samples from the Humber, at Hull, February 1979 to January 1980

Date	Non-centrifuged sample		Centrifuged sample	
	Attached	Free	Attached	Free
26 February	12.3	0.70	0.25	0.77
26 March	13.6	0.68	0.16	1.19
20 April	7.08	0.84	0.16	0.96
15 May	6.61	1.16	0.34	1.48
11 June	4.31	0.99	0.17	1.72
10 July	4.14	1.36	0.11	1.68
1 August	2.89	1.40	0.04	1.78
30 August	3.61	1.67	0.05	1.53
25 September	12.1	1.50	0.09	2.81
19 October	8.28	1.33	0.09	1.30
27 November	26.4	1.78	0.08	2.17
17 December	21.0	2.13	0.16	1.70
22 January	15.2	1.08	0.09	1.08

he found low values in summer and high values in winter and early spring. Also, Moshiri *et al.* (1979) recorded highest levels of glucose assimilation rate in November and March in Bayou Texar Estuary, Florida (USA).

More information about seasonal variation of bacteria in estuaries is needed before any general understanding can be reached. The purpose of the present paper is to record the results of an investigation into seasonal variation in population density and heterotrophic activity of planktonic bacteria in the Humber Estuary.

This estuary, in north-east England, is a major British estuary which drains a catchment of 26,000 km^2, which represents ca. 20% of England. It extends 64 km eastwards from the confluence of the Rivers Ouse and Trent to the North Sea at Spurn Head (Fig. 1) and is ca. 300 km^2 in area. About 1.7×10^9 m^3 of sea water enters the estuary during a spring tide and ca. 1.1×10^9 m^3 during a neap tide. Average fresh-water flow is ca. 2.1×10^7 m^3 d^{-1}, with spate values of $> 13.0 \times 10^7$ m^3 d^{-1} (Denman, 1979). Concentrations of suspended solids are extremely high. Mean concentrations for the whole estuary range from 330 mg l^{-1} at summer neap-tides to 1900 mg l^{-1} at winter spring-tides (Anonymous, 1970). Goulder (1976, 1977) found that most ($> 75\%$) of the suspended-solids particles at Hull are aggregates, and that the most frequent size category (usually $> 60\%$) is 5 to 14 μm, while $< 20\%$ of the particles exceed 20 μm.

Planktonic bacteria in the Humber are of special interest because most are attached to suspended-solids particles; usually only a small minority is free-living in the water (Goulder, 1976, 1977; Goulder *et al.*, 1979a, 1980). This is in marked contrast to the Inner Kiel Fjord (FRG) where the concentration of suspended solids at 2 m depth (annual mean of only 4.1 mg l^{-1}: Lenz, 1977), is very much less than in the Humber and where attached bacteria make up, on average, only 2.9% of the total bacteria (Zimmermann, 1977). The planktonic bacteria in the Humber are divided into 2 groups (i.e., attached or free), on the basis of their occupation of two distinct micro-habitats; therefore, we have measured separately the population density and heterotrophic activity of these 2 groups. Seasonal variations of some physico-chemical factors which might influence density and heterotrophic activity of bacteria, and concentration of phytoplankton chlorophyll *a* and phaeophytin, were also examined.

Materials and Methods

Sampling and Sample Treatment

Sampling was at Hull at a site off Albert Dock (National Grid Reference TA 093 277), ca. 37 km upstream of Spurn Head, at about monthly intervals from January 1978 to January 1980. To allow comparison with other sites, samples were also taken at Saltend (TA 157 271), ca. 7 km downstream of Hull, and at Brough (SE 938 260), ca. 16 km upstream of Hull, but only over 1 yr (January 1978 to January 1979). Sites are shown on Fig. 1. Surface water was collected from jetties at slack high-water in a clean polythene bucket and transferred to a sterile 10 litre glass container.

To obtain a volume of sample substantially free of attached bacteria, 1 litre was centrifuged (15 min at 400 relative centrifugal force), and the liquid fraction was then decanted. The effectiveness of removal of attached bacteria is demonstrated in Table 1, which shows the density of attached and free bacteria for non-centrifuged and centrifuged samples taken at Hull between February 1979 and January 1980. These results were obtained by direct counting, using epifluorescence microscopy (see "Counting of Bacteria") and indicate that, on average, centrifugation removed 98.2% of the attached bacteria. The attached bacteria which did remain were attached to a few residual, very small (< 5 μm) particles. This separation technique is less effective than screening through 3 μm membrane filters (Goulder, 1977), but is much quicker. Table 1 also shows apparent frequent increases in density of free bacteria in the centrifuged sample. This could partly be caused by some attached bacteria becoming dislodged during centri-

fugation. However, it is more probable that some free bacteria were hidden behind particles of suspended solids during counting of the non-centrifuged samples; this conclusion is reinforced by the fact that a similar increase was noted when particles were removed by screening rather than by centrifugation (Goulder, 1977).

Counting of Bacteria

Attached bacteria were routinely counted in the non-centrifuged sample and free bacteria in the centrifuged sample. Bacteria were counted using epifluorescence microscopy after staining with acridine orange (100 mg l^{-1} for 10 min) and concentrating by means of vacuum filtration on the surface of black, 0.22 μm pore-size, Millipore membrane filters (Jones and Simon, 1975). The procedure and microscope used were as described by Goulder (1976). The bacteria appear bright green; hence, free bacteria contrast well with the black membrane, and attached bacteria with the apparently red surfaces of the particles. During counting, the particles of suspended solids were flattened against the membrane as a result of vacuum filtration; hence, only half the surface area of the particles was visible under incident-light illumination, the lower surfaces being hidden. It follows that only those bacteria on the upper surfaces were counted; hence, we doubled counts of attached bacteria before calculation of density, to allow for bacteria on the lower, hidden, surfaces of particles. Twenty or 30 microscope fields were counted and the density (number per unit volume) of bacteria was calculated from the arithmetic mean. To obtain 95% confidence intervals, log transformation of counts was necessary (Elliott, 1971: pp 91-92). The confidence intervals are therefore strictly around the geometric means, hence their apparently marked asymmetry.

Determination of Heterotrophic Activity

V_{max} for glucose mineralization (i.e., the potential mineralization rate per unit volume) and turnover time for total bacteria were determined using the non-centrifuged sample. V_{max} of free bacteria was measured using the centrifuged sample. An estimate of V_{max} for attached bacteria alone was obtained by subtracting V_{max} of free bacteria from V_{max} of total bacteria.

The technique used was the modification by Harrison *et al.* (1971) of the method of Wright and Hobbie (1966). With this modification, ^{14}C glucose mineralization to $^{14}CO_2$ is measured, but not ^{14}C assimilation into bacterial biomass; the procedure was as described by Goulder (1976, 1980). Briefly, three replicate 25 ml sub-samples were incubated at each of 5 added-glucose concentrations (A) in the range 3 to 16 $\mu g\ l^{-1}$. All incubations were at 10 °C and duration (t) was 1 or 2 h. The incubations were stopped by addition of 2 ml of 2.5M H_2SO_4, and the $^{14}CO_2$ evolved was collected as described by Harrison *et al.* (1971). A blank incubation, to which acid was added at zero time, was also run at each substrate concentration to allow for any non-biological $^{14}CO_2$ evolution. V_{max} and turnover-time values, with 95% confidence intervals, were obtained from linear regression of t/f against A (f being the fraction of ^{14}C glucose which is mineralized). The F-test was used to test the significance of linear regression (Sokal and Rohlf, 1969: pp 430-436). In 94 out of 110 determinations, there was significant regression at $P < 0.05$; in 5 further cases $P > 0.05$ but < 0.1. In 11 cases, however (mostly samples from Brough), $P > 0.1$, so these latter data were rejected.

The average rate at which individual bacteria are capable of mineralizing glucose (V_{max} per bacterium) was calculated for Hull samples. V_{max} per bacterium, as an average for all bacteria present, was obtained by dividing V_{max} for total bacteria by the density of total bacteria (i.e., attached bacteria in non-centrifuged sample + free bacteria in centrifuged sample). V_{max} per bacterium for attached bacteria alone equalled V_{max} of attached bacteria divided by the density of attached bacteria; V_{max} per bacterium of free bacteria equalled V_{max} of free bacteria divided by the density of free bacteria.

Counting of bacteria and determination of heterotrophic activities were usually completed within 4 to 8 h of sampling.

Measurement of Environmental Variables

To determine concentration of suspended solids, three replicate 0.5 or 1.0 litre sub-samples were filtered through pre-weighed Whatman GF/C glass-fibre filters which were dried overnight at 100 °C and then reweighed. Particulate organic matter was obtained from weight loss on heating the above filters at 400 °C for 0.5 h. This temperature was used since Jaffé and Walters (1977) found that weight loss from Humber sediments at 400 °C is chiefly a result of oxidation of organic matter, whereas above 400 °C weight loss because of dehydroxylation of clay minerals is important. Surface-water temperature was measured using a mercury-in-glass thermometer, and salinity was measured with a salinity meter.

Phytoplankton chlorophyll *a* and phaeophytin were determined spectrophotometrically. Three replicate 1.0 litre sub-samples were filtered (Whatman GF/C) and pigments were extracted overnight, at 1 ° to 3 °C, into 90% acetone. Optical density at 665 and 750 nm was measured before and after acidification with dilute HCl, and concentrations of chlorophyll *a* and phaeophytin were calculated from the equations of Lorenzen (1967).

Correlation coefficients (r) were calculated after transformation of all raw values (x), using the log x transformation or log $(x + 1)$ when zero values occurred. A two-tailed significance test was always used.

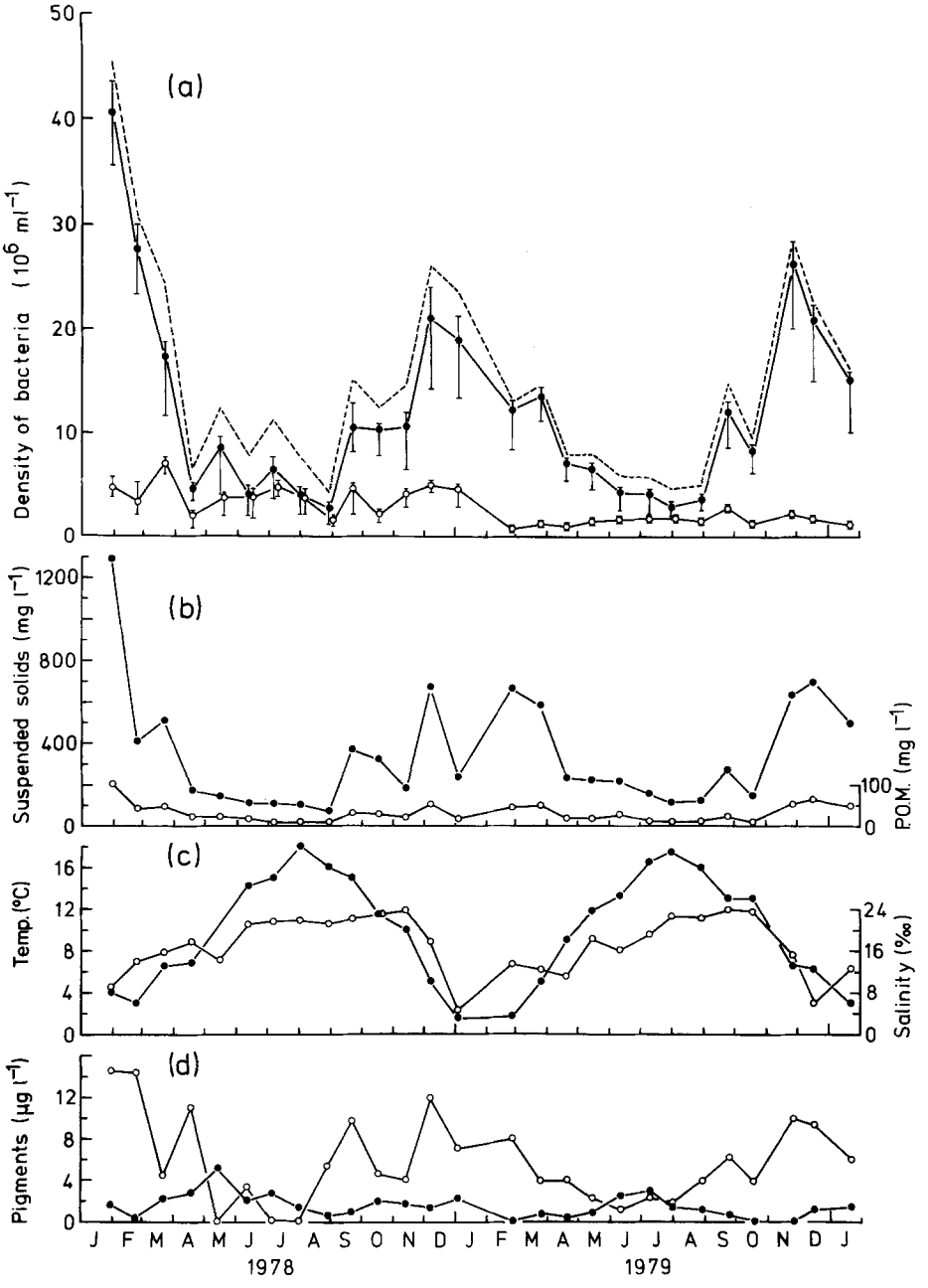

Fig. 2. Samples from Hull, January 1978 to January 1980. (a) Density of attached bacteria (filled circles) free bacteria (open circles) and total bacteria (dashed line); vertical lines indicate 95% confidence intervals. (b) Concentration of suspended solids (filled circles) and particulate organic matter (open circles). (c) Water temperature (filled circles) and salinity (open circles). (d) Concentration of phytoplankton chlorophyll *a* (filled circles) and phaeophytin (open circles)

Results

Data from Hull (January 1978 to January 1980)

The density of attached bacteria at Hull showed marked seasonal periodicity, being low in summer and higher in the autumn to spring period (Fig. 2a). The minimum density was 2.8×10^6 ml^{-1} in August 1978; the maximum was 40.7×10^6 ml^{-1} in January 1978.

Free bacteria, in contrast, showed no seasonal pattern, but fluctuated irregularly in the range 0.8 to 7.0×10^6 ml^{-1} (Fig. 2a). The density of free bacteria was always less than that of attached bacteria, the difference being greatest in the winter months. There was no significant correlation between density of free bacteria and that of attached bacteria ($r = 0.29$, $n = 27$, $P > 0.05$).

The density of total bacteria at Hull is also included in Fig. 2a. The range was from 4.3×10^6 ml^{-1} in August 1978 to 45.4×10^6 ml^{-1} in January 1978. Seasonal fluctuation of total bacteria closely followed that of attached bacteria since most bacteria were attached.

Values of V_{max} for total bacteria (i.e., from non-centrifuged samples) at Hull also showed obvious seasonal fluctuation, with low values in summer and generally higher ones over the autumn to spring period (Fig. 3a). The range was from 0.09 µg l^{-1} h^{-1} in June 1978 to 6.1 µg l^{-1} h^{-1} in November 1979. Turnover times which were due to total bacteria (Fig. 3b) tended,

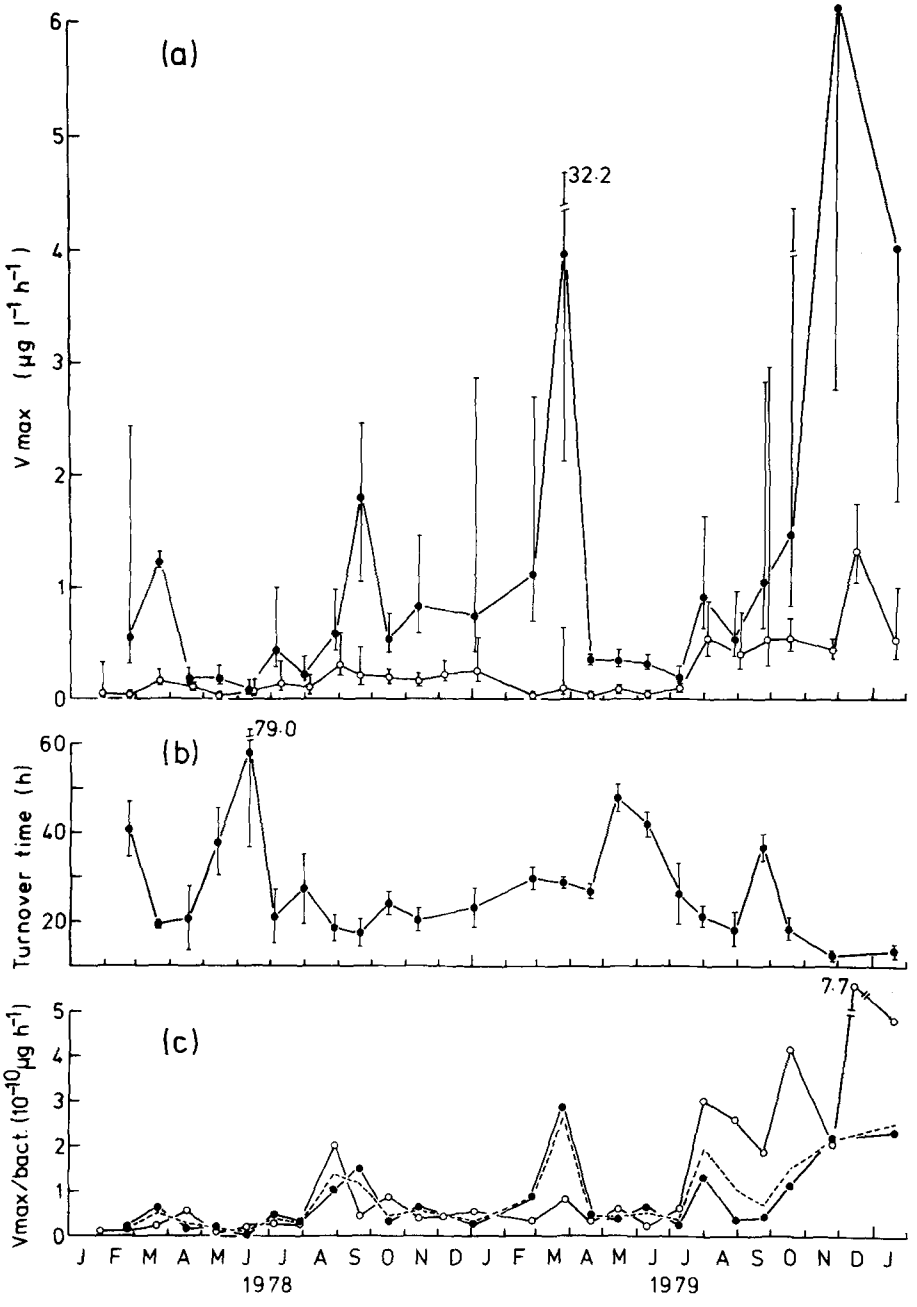

Fig. 3. Samples from Hull, January 1978 to January 1980. (a) V_{max} of total bacteria (filled circles) and free bacteria (open circles); the excess of V_{max} of total bacteria over that of free bacteria provides an estimate of V_{max} of attached bacteria. (b) Turnover time relating to total bacteria. (c) V_{max} per bacterium for attached bacteria (filled circles), free bacteria (open circles) and total bacteria (dashed line). Vertical lines indicate 95% confidence intervals (on two occasions, the upper confidence interval to V_{max} of total bacteria could not be calculated for technical reasons and hence is omitted)

with some exceptions, to be long in early summer (indicating low heterotrophic activity) and shortest in winter. The range was from 12.5 h in November 1979 to 58.0 h in June 1978. Overall, there was a significant negative correlation between V_{max} and turnover time ($r = -0.63, n = 24, P < 0.001$).

Values of V_{max} for free bacteria (i.e., from centrifuged samples) were usually much lower than those of total bacteria, the range being 0.03 to 1.3 $\mu g\, l^{-1}\, h^{-1}$, and they fluctuated somewhat irregularly (Fig. 3a). Relatively high values sometimes coincided with high values of V_{max} for total bacteria (e.g. August 1979 to January 1980), but sometimes low values were recorded when V_{max} of total bacteria was high (e.g. February to April 1979). Overall, however, there was a significant correlation between V_{max} of free bacteria and that of total bacteria ($r = 0.50, n = 24, P < 0.05$).

Values of V_{max} for attached bacteria at Hull are represented in Fig. 3a by the difference between V_{max} of total bacteria and V_{max} of free bacteria. These values are approximate because they are derived from the other two V_{max} values, both of which are subject to error (note 95% confidence intervals in Fig. 3a). The V_{max} of attached bacteria, however, usually exceeded that of free bacteria, and showed marked seasonal variation, tending to be low in summer and higher over the autumn to

spring period. The minimum value obtained was 0.01 $\mu g\ l^{-1}\ h^{-1}$ in June 1978 and the maximum was 5.7 $\mu g\ l^{-1}\ h^{-1}$ in November 1979.

Values of V_{max} per bacterium for attached, free, and for total bacteria at Hull fluctuated irregularly (Fig. 3c). The ranges obtained were wide: 0.02 to 2.9×10^{-10} $\mu g\ h^{-1}$ for attached bacteria; 0.08 to 7.7×10^{-10} $\mu g\ h^{-1}$ for free bacteria; 0.12 to 2.7×10^{-10} $\mu g\ h^{-1}$ for total bacteria. V_{max} per bacterium of attached bacteria was not consistently more or consistently less than that of free bacteria; each was greater on half of the 24 occasions when values for both were available.

The concentration of suspended solids showed pronounced seasonal variation (Fig. 2b). Concentrations were low in summer (minimum 74 mg l^{-1} in August 1978) and high in the autumn to spring period (up to 1290 mg l^{-1} in January, 1978). The concentration of particulate organic matter, which ranged from 9 to 102 mg l^{-1} (Fig. 2b), was also greatest in winter and was closely dependent on concentration of suspended solids ($r = 0.96$, $n = 27$, $P < 0.001$). Seasonal variation of temperature and salinity is shown in Fig. 2c. Temperature ranged from 1.5 °C to 18 °C, and salinity (at high tide) from 4.6 to 23.6 ‰ (lowest salinities are in winter when fresh-water flow is greater).

Chlorophyll *a* concentration, which was usually less than that of phaeophytin (Fig. 2d), was relatively high in summer when conditions presumably most favoured phytoplankton photosynthesis (maximum 5.1 $\mu g\ l^{-1}$ in May 1978) and low in winter. Phaeophytin concentration in contrast, was low in summer and higher in winter (maximum 14.5 $\mu g\ l^{-1}$ in January 1978). Phaeophytin concentration was closely related to concentration of suspended solids ($r = 0.65$, $n = 27$, $P < 0.001$); hence, high winter levels could be a result of winter resuspension of sunken inactive phytoplankton and possibly macrophyte debris and zooplankton faecal pellets, along with the bulk of the suspended-solids material.

Data from Saltend and Brough (January 1978 to January 1979)

The density of attached bacteria at Saltend (Fig. 4a) and Brough (Fig. 5a) during 1978 was, as at Hull, low in summer and high early and late in the year. The densities recorded tended to be lower than at Hull, the mean (and range) being 5.9 (0.4 to 16.6) $\times 10^6$ ml^{-1} at Saltend and 9.4 (3.9 to 20.1) $\times 10^6$ ml^{-1} at Brough, compared to 13.5 (2.8 to 40.7) $\times 10^6$ ml^{-1} over the same period (January 1978 to January 1979) at Hull.

The density of free bacteria, as at Hull, fluctuated irregularly (Figs. 4a and 5a) but, unlike at Hull, it was just significantly correlated with density of attached bacteria (at Saltend $r = 0.55$, $n = 14$, $P < 0.05$; at Brough $r = 0.58$, $n = 13$, $P < 0.05$). The mean (and range) of densities were similar to those at Hull: 3.1 (1.4 to 5.2) $\times 10^6$ ml^{-1} at Saltend and 3.9 (1.2 to 6.4) $\times 10^6$ ml^{-1} at Brough, compared to 3.9 (1.5 to 7.0) $\times 10^6$ ml^{-1} for the same period at Hull.

The density of attached bacteria at these sites usually exceeded that of free bacteria. This was most obvious early and late in the year. There were, however, exceptions in the summer (i.e., at Saltend in May, July and twice in August; at Brough in May).

The density of total bacteria at Saltend (Fig. 4a) and Brough (Fig. 5a) tended to be lower than at Hull. This was a result of lower numbers of attached bacteria. The mean (and range) for total bacteria was 9.0 (1.8 to 20.5) $\times 10^6$ ml^{-1} at Saltend and 13.3 (6.0 to 26.5) $\times 10^6$ ml^{-1} at Brough, compared to 17.4 (4.3 to 45.4) $\times 10^6$ ml^{-1} for the same period at Hull. The seasonal pattern for total bacteria at Saltend and Brough, as at Hull, closely followed that of attached bacteria.

The range of V_{max} values for total bacteria at Saltend (Fig. 4e) and Brough (Fig. 5e) during 1978 was not notably different from at Hull over the same period (0.07 to 1.0 $\mu g\ l^{-1}\ h^{-1}$ at Saltend and 0.2 to 1.6 $\mu g\ l^{-1}\ h^{-1}$ at Brough, compared to 0.09 to 1.8 $\mu g\ l^{-1}\ h^{-1}$ at Hull), although the higher values were much less than those found at Hull in the following year (Fig. 3a). At Saltend and Brough, however, unlike at Hull, there was no distinct seasonal pattern. For example, high values at Saltend were found in August and December, and low ones from January to June, while at Brough high levels occurred in March and July, with low levels from April to June. The range of turnover times, due to total bacteria at Saltend (Fig. 4f) and Brough (Fig. 5f) was rather wider than over the same period at Hull (11.8 to 127.5 h at Saltend and 17.6 to 78.3 h at Brough, compared with 17.4 to 58.0 h at Hull). Unlike at Hull, there was no tendency for long turnover-times to be a summer feature. Turnover time at Saltend, as at Hull, was negatively correlated with V_{max} ($r = -0.84$, $n = 14$, $P < 0.001$). This was not the case at Brough ($r = -0.64$, $n = 9$, $P > 0.05$); here, however, many values were missing.

V_{max} values for free bacteria at Saltend (Fig. 4e) and Brough (Fig. 5e) were at times higher than over the same period at Hull. The ranges were 0.03 to 1.1 $\mu g\ l^{-1}\ h^{-1}$ at Saltend and 0.09 to 0.9 $\mu g\ l^{-1}\ h^{-1}$ at Brough, compared to 0.03 to 0.3 $\mu g\ l^{-1}\ h^{-1}$ at Hull. A peculiarity of the Saltend data is that, on 5 occasions, V_{max} of free bacteria apparently equalled or exceeded that of total bacteria. This is not logically possible. The explanation is that each V_{max} value determined is a statistic which is an estimate of the true V_{max}. The width of the 95% confidence interval provides an indication of the likely accuracy of each estimate. When the estimate of V_{max} of free bacteria exceeded that of total bacteria, the confidence intervals were wide and showed considerable overlap (Fig. 4e). At neither site did the V_{max} of free bacteria show any distinct seasonal pattern. As at Hull, both sites displayed a significant correlation between V_{max} of free bacteria and that of total bacteria (at Saltend $r = 0.92$, $n = 14$, $P < 0.001$; at Brough $r = 0.82$, $n = 9$, $P < 0.01$).

Values of V_{max} for attached bacteria can be estimated from Figs. 4e and 5e. At Saltend, the values ranged from apparently zero to 0.4 $\mu g\ l^{-1}\ h^{-1}$ and were

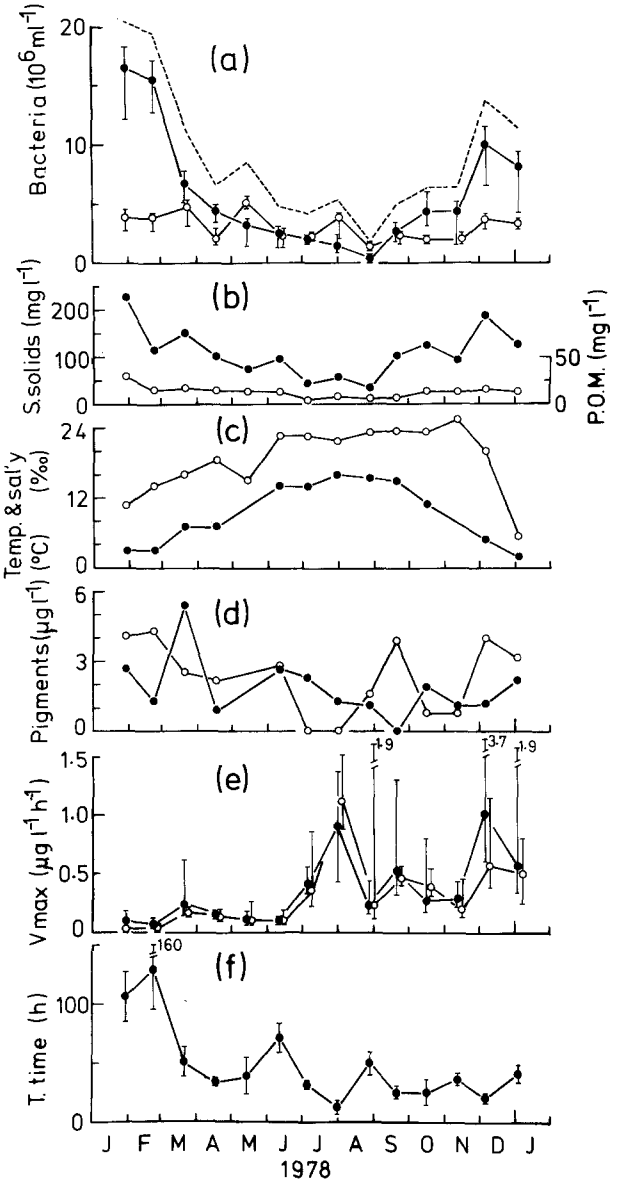

Fig. 4. Samples from Saltend, January 1978 to January 1979. (a) Density of attached bacteria (filled circles), free bacteria (open circles) and total bacteria (dashed line). (b) Concentration of suspended solids (filled circles) and particulate organic matter (open circles). (c) Water temperature (filled circles) and salinity (open circles). (d) Concentration of phytoplankton chlorophyll *a* (filled circles) and phaeophytin (open circles). (e) V_{max} of total bacteria (filled circles) and free bacteria (open circles). (f) Turnover time relating to total bacteria. Vertical lines indicate 95% confidence intervals

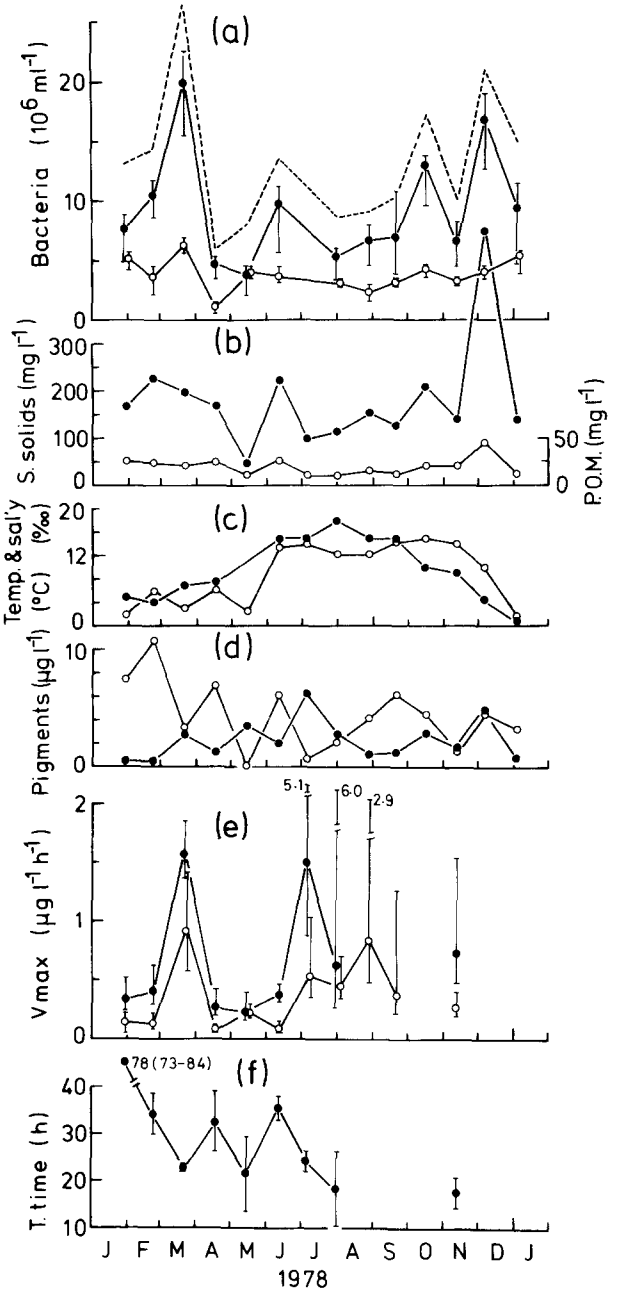

Fig. 5. Samples from Brough, January 1978 to January 1979. Symbols as in Fig. 4

low in comparison with Hull over the same period (i.e., 0.01 to 1.6 μg l^{-1} h^{-1}). In contrast to Hull samples, there was no seasonal pattern, and V_{max} of attached bacteria apparently exceeded that of free bacteria on only 1 out of 14 occasions. At Brough, V_{max} for attached bacteria also fluctuated irregularly - but there several values were missing (Fig. 5e). The range was apparently zero to 1.0 μg l^{-1} h^{-1}, and values more closely resembled those at Hull. Also, V_{max} for attached bacteria exceeded that of free bacteria on 6 out of the 9 occasions when both values were available.

Seasonal variation in the concentration of suspended solids at Saltend (Fig. 4b) and Brough (Fig. 5b) was similar to that at Hull, with high values early and late in the year and low ones in summer, except for an anomalously high result at Brough in June. Highest

values, particularly at Saltend, were less than at Hull. The mean (and range) was 111 (35 to 228) mg l^{-1} at Saltend and 183 (47 to 537) mg l^{-1} at Brough, compared to 337 (74 to 1290) mg l^{-1} over the same period at Hull. The concentration of particulate organic matter ranged from 5 to 30 mg l^{-1} at Saltend and 10 to 45 mg l^{-1} at Brough and, as at Hull, closely followed that of suspended solids (at Saltend $r = 0.89$, $n = 14$, $P < 0.001$; at Brough $r = 0.85$, $n = 14$, $P < 0.001$). The temperature range (Figs. 4c and 5c) at Saltend (2° to 16° C) and Brough (1° to 18°C) was similar to that at Hull. Salinity (Figs. 4c and 5c), as at Hull, was high in summer. Salinities at Saltend (5.4 to 25.4 ‰) were slightly greater than at Hull over the same period (i.e., 4.6 to 23.5 ‰). However, those at Brough (1.5 to 14.9 ‰), were markedly lower.

Chlorophyll *a* and phaeophytin fluctuated irregularly at both sites (Figs. 4d and 5d). Phaeophytin concentration, as at Hull, was usually greater than that of chlorophyll *a*, although this was less pronounced at Saltend. The mean (and range) of chlorophyll *a* concentration at Saltend was 1.9 (0 to 5.4) $\mu g\, l^{-1}$, and at Brough 2.3 (0.5 to 6.3) $\mu g\, l^{-1}$; that of phaeophytin was 2.3 (0 to 4.3) $\mu g\, l^{-1}$ and 4.4 (0 to 10.7) $\mu g\, l^{-1}$. These values may be compared with 1.9 (0.3 to 5.1) $\mu g\, l^{-1}$ for chlorophyll *a* and 6.4 (0 to 14.5) $\mu g\, l^{-1}$ for phaeophytin at Hull over the same period.

Discussion

The density of attached bacteria at Hull was closely dependent upon the concentration of the suspended solids to which they were attached (Fig. 2, Table 2). The coefficient of determination (r^2) equalled 0.76 - hence up to 76% of the variation in density might have been caused by variation in the concentration of suspended solids. A similar relationship was also found at Saltend and Brough (Figs. 4 and 5, Table 2). It follows that the number of attached bacteria per unit weight of solids was fairly constant at each site and that the marked seasonal variation in density was a result of seasonal variation in the concentration of suspended solids. This concentration, at any particular time, is a function of the interaction of several physical variables (e.g. temperature, freshwater flow, tidal range, wind conditions). Physical conditions in the Humber are such that high concentrations of suspended solids occur in winter (Anonymous, 1970). It follows that seasonal variation in density of attached bacteria (and total bacteria, since most bacteria are attached) is principally an indirect result of variation in those physical factors which control suspended solids, rather than a direct result of biological processes. Presumably, in summer, when density of attached bacteria is low, a large proportion of the solid particles with bacteria attached is to be found in the superficial benthic deposits.

A similar dependence of density of attached bacteria on concentration of suspended solids was also found over a single neap-spring-neap tidal cycle at Corporation Pier, Hull (ca. 0.75 km downstream of the Albert Dock site). Here, high values of attached bacteria and suspended solids coincided at spring tides (Goulder, 1976).

Significant correlations were sometimes found between density of attached bacteria and some environmental variables other than suspended solids (i.e.,

Table 2. Relationships between density and V_{max} of bacteria and environmental variables; samples from the Humber at Hull (January 1978 - January 1980) and Saltend and Brough (January 1978 - January 1979). Values are correlation coefficients; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; NS = $P > 0.05$; a minus sign indicates a negative correlation; n = number of pairs of data available

	Suspended-solids concentration	Particulate organic matter	Temperature	Salinity	Chlorophyll *a* concentration	Phaeophytin concentration
Hull						
Density of attached bacteria (n = 26 or 27)	0.87***	0.84***	− 0.71**	− 0.57***	NS	0.61***
Density of free bacteria (n = 26 or 27)	NS	NS	NS	NS	0.48*	NS
V_{max} of total bacteria (n = 23 or 24)	0.67***	0.55**	NS	NS	− 0.54**	0.45*
V_{max} of attached bacteria (n = 23 or 24)	0.73***	0.61**	− 0.44*	NS	− 0.49*	NS
V_{max} of free bacteria (n = 26 or 27)	NS	NS	NS	NS	NS	NS
Saltend						
Density of attached bacteria (n = 12 to 14)	0.88***	0.85***	− 0.88***	− 0.58*	NS	0.57*
Density of free bacteria (n = 12 to 14)	NS	0.54*	− 0.58*	NS	NS	NS
V_{max} of total bacteria (n = 12 to 14)	NS	NS	NS	NS	NS	NS
V_{max} of attached bacteria (n = 8 or 9)	NS	NS	NS	NS	NS	NS
V_{max} of free bacteria (n = 12 to 14)	NS	− 0.58*	NS	NS	NS	NS
Brough						
Density of attached bacteria (n = 12 or 13)	0.77**	0.57*	NS	NS	NS	NS
Density of free bacteria (n = 12 or 13)	NS	NS	NS	NS	NS	NS
V_{max} of total bacteria (n = 8 or 9)	NS	NS	NS	NS	NS	NS
V_{max} of attached bacteria (n = 8 or 9)	NS	NS	NS	NS	NS	NS
V_{max} of free bacteria (n = 10 or 11)	NS	NS	NS	NS	NS	NS

particulate organic matter, temperature, salinity and phaeophytin concentration: Table 2). It is likely, however, that these were not causal relationships since: (1) the negative correlations with temperature and salinity are explained by temperature and fresh-water flow (which controls salinity) being among the physical factors which determine concentration of suspended solids; (2) the correlations with particulate organic matter and phytoplankton phaeophytin are probably a result of these two variables being fairly constant components of suspended solids; (3) there are no significant correlations (apart from two exceptions at Saltend) between density of free bacteria and these environmental variables (Table 2) - such correlations would probably be found if these variables did markedly influence bacterial numbers in the estuary.

It is probable that the density of attached bacteria (and hence also total bacteria) tended to be lower at Saltend and Brough, than at Hull, because the concentrations of suspended solids were lower in these areas (cf. Figs. 4, 5 with Fig. 2). There was, however, some suggestion that the extent of colonization of suspended solids was greater at Saltend than at Brough or Hull. Linear regression analysis of density of attached bacteria against concentration of suspended solids gave a regression coefficient of 7.6×10^7 bacteria per milligram of solids at Saltend, compared to 2.9×10^7 bacteria mg^{-1} at Hull and 2.8×10^7 bacteria mg^{-1} at Brough.

The fact that the excess of attached over free bacteria was greatest during winter (Figs. 2a, 4a, 5a) was presumably a result of this being the time of highest concentration of suspended solids. Conversely, the occasions at Saltend and Brough when density of free bacteria exceeded that of attached bacteria (Figs. 4a, 5a) were a result of exceptionally low concentrations of suspended solids.

The density of free bacteria, in contrast to that of attached bacteria, was independent of suspended solids (Fig. 2a, 4a, 5a; Table 2), and consequently showed no obvious seasonal periodicity. A significant correlation was obtained between density of free bacteria and chlorophyll *a* concentration at Hull (Table 2). Relationships between numbers of planktonic bacteria and phytoplankton biomass have been found in fresh-waters (e.g. Coveney *et al.*, 1977; Jones, 1977), but there the chlorophyll levels were much higher than in the Humber. It is not certain that the comparatively low Humber phytoplankton crops are capable of releasing sufficient soluble organic compounds to stimulate an increase in bacterial density, nor is it known whether such compounds make up a significant proportion of total dissolved organic matter in the Humber. At Saltend, the density of free bacteria was significantly correlated with particulate organic matter and (negatively) with temperature (Table 2): ecological interpretation of these correlations is, however, difficult.

The use of V_{max} for glucose mineralization as an indicator of heterotrophic activity has several potential disadvantages: (1) V_{max} represents potential rate, at a non-limiting substrate concentration; the actual flux, however, is a variable proportion of V_{max}, e.g. 6 to 69% in Lake Ontario, Canada (Wood and Chua, 1973). (2) Glucose is only one of many organic substrates which are utilized by bacteria (it is assumed that glucose metabolism reflects total metabolism). (3) Only mineralization is measured, and this is not a constant proportion of total glucose uptake, e.g. 8 to 22% of mineralization plus assimilation in the Humber at East Clough, ca. 4 km downstream of the Brough site (Goulder *et al.*, 1979b); also, Hoppe (1978) cites a range of mean values, taken from several authors and diverse aquatic sites, of 22 to 49%. (4) All V_{max} determinations in this study were made at 10 °C, to allow comparison. V_{max} in the field will, however, vary with water temperature [a Q_{10} of 2.2 was obtained by Wright and Hobbie (1966) using a bacterial culture isolated from Lake Erken, Sweden].

In spite of these disadvantages, the difference between the high winter and low summer V_{max} values for attached, and total, bacteria at Hull (Fig. 3a) were so great (the maximum value for total bacteria was 68 times the minimum) that it may be concluded that there probably is genuine seasonal variation in heterotrophic activity.

The high V_{max} values for attached and total bacteria found in winter at Hull are probably a result of high density of attached (and therefore total) bacteria, and hence are an indirect result of high winter concentrations of suspended solids. There were significant corelations between V_{max} and density of attached bacteria ($r = 0.69$, $n = 24$, $P < 0.001$) and between V_{max} and density of total bacteria ($r = 0.54$, $n = 24$, $P < 0.01$).

Significant correlations were also found at Hull between V_{max} of total and attached bacteria and some environmental variables (Table 2), but these are probably not causal relationships. The correlations with concentration of suspended solids, particulate organic matter, and phaeophytin may be a result of these variables being independently correlated with density of attached bacteria. The negative correlation between V_{max} of attached bacteria and temperature is explained by temperature being negatively correlated with suspended solids and hence with density of attached bacteria. The negative correlation of V_{max} for attached and total bacteria with chlorophyll *a* concentration is presumably a result of low summer density of attached bacteria coinciding by chance with the high summer concentration of chlorophyll *a*.

The values of V_{max} for total and attached bacteria at Saltend and Brough did not reflect the seasonal pattern found at Hull (Figs. 4e and 5e). The only significant correlation of V_{max} with bacterial density was at Brough, between V_{max} and density of total bacteria ($r = 0.72$, $n = 8$, $P < 0.05$). Also, unlike at Hull, no correlations were found between V_{max} of total or attached bacteria and those environmental variables which did show seasonal periodicity (Table 2).

At first sight, therefore, seasonal variation of heterotrophic activity at Saltend and Brough appears to be rather different from that at Hull. The information available, however, does not allow a firm conclusion

that the situation is fundamentally different from that at Hull because (1) there are several potential disadvantages (listed above) in the use of V_{max} as an indicator of heterotrophic activity; (2) much less information is available than for Hull - sampling was over only 1 yr, also several V_{max} determinations did not produce usable results; (3) there were some obvious anomalies, when a low V_{max} value for total bacteria coincided with a high bacterial density, e.g. at Saltend in January-February 1978 (Fig. 4).

Values of V_{max} of free bacteria fluctuated irregularly at all 3 sites. Non-overlap of 95% confidence intervals (Figs. 3a, 4e, 5e) indicated significant differences between the values measured. There was, therefore, genuine temporal variation in V_{max} and hence, possibly, in heterotrophic activity of free bacteria. No significant correlations were found, however, between V_{max} and density of free bacteria (at Hull $r = -0.03$, $n = 27$, $P > 0.05$; at Saltend $r = -0.21$, $n = 14$, $P > 0.05$; at Brough $r = 0.28$, $n = 10$, $P > 0.05$). Nor, generally, were correlations found between V_{max} of free bacteria and environmental variables (Table 2). The only exception was a significant negative correlation with concentration of particulate organic matter at Saltend; it is difficult, however, to explain this in biological terms. Given the data available, we cannot suggest any reasons for the variation in V_{max} of free bacteria. It is mentioned above, however, that significant correlations were present, at all 3 sites, between V_{max} of free bacteria and V_{max} of total bacteria. It is possible, therefore, that the component of variability in V_{max} of total bacteria, which was related to factors other than density of attached bacteria, was caused by the same unidentified factors which brought about variation in V_{max} of free bacteria.

The extreme variation in V_{max} per bacterium found at Hull (Fig. 3c) is not unusual in aquatic habitats (Goulder, 1979). The values quoted are approximate because they are ratios calculated from two values, both subject to error. Nevertheless, although there was no seasonal pattern, the extreme variation suggests that there was real temporal change in the V_{max} per bacterium values of attached, free, and total bacteria. This was presumably a result of temporal variation in factors which we have not investigated, e.g. physiological state of cells, proportion of cells capable of utilizing glucose, mean cell-size, taxonomic composition of the bacterial flora. The observation that V_{max} per bacterium of attached bacteria was not necessarily greater than that of free bacteria agrees with results obtained from fewer samples at Corporation Pier, Hull (Goulder, 1977), and suggests that the heterotrophic potential of individual attached bacteria is not generally greater than that of free bacteria. It follows that, for individual bacteria, the attached habit may not be significantly more favourable than the free-living habit.

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Literature Cited

Albright, L. J.: Heterotrophic bacterial dynamics in the lower Fraser River, its estuary and Georgia Strait, British Columbia, Canada. Mar. Biol. *39*, 203-211 (1977)

Anonymous: Silt movement in the Humber Estuary, 8 pp. Southall, Middx.: British Transport Docks Board Research Station 1970. (Res. Stn. Rep. No. R. 221)

Coveney, M. F., G. Cronberg, M. Enell, K. Larsson and L. Olofsson: Phytoplankton, zooplankton and bacteria-standing crop and production relationships in a eutrophic lake. Oikos *29*, 5-21 (1977)

Crawford, C. C., J. E. Hobbie and K. L. Webb: The utilization of dissolved free amino acids by estuarine microorganisms. Ecology *55*, 551-563 (1974)

Denman, N. E.: Physical characters of the Humber. Publs nat. Envir. Res. Coun., Swindon (Ser. C) *20*, 5-8 (1979)

Elliott, J. M.: Some methods for the statistical analysis of samples of benthic invertebrates. Scient. Publs Freshwat. biol. Ass. *25*, 1-144 (1971)

Gocke, K.: Heterotrophic activity. *In*: Microbial ecology of a brackish-water environment, pp 198-222. Ed. by G. Rheinheimer. Berlin: Springer-Verlag 1977

Gocke, K.: Untersuchungen über den Jahreszyklus der heterotrophen Aktivität in der Kieler Förde. Arch. Hydrobiol. *82*, 123-141 (1978)

Goulder, R.: Relationships between suspended solids and standing crops and activities of bacteria in an estuary during a neap-spring-neap tidal cycle. Oecologia *24*, 83-90 (1976)

Goulder, R.: Attached and free bacteria in an estuary with abundant suspended solids. J. appl. Bact. *43*, 399-405 (1977)

Goulder, R.: V_{max} per bacterium and turnover rate per bacterium for glucose mineralization in natural waters. Curr. Microbiol. *2*, 365-368 (1979)

Goulder, R.: Seasonal variation in heterotrophic activity and population density of planktonic bacteria in a clean river. J. Ecol. *68*, 349-363 (1980)

Goulder, R., A. S. Blanchard, P. J. Metcalf and B. Wright: Inhibition of estuarine bacteria by metal refinery effluent. Mar. Pollut. Bull. *10*, 170-173 (1979a)

Goulder, R., A. S. Blanchard, P. L. Sanderson and B. Wright: A note on the recognition of pollution stress in populations of estuarine bacteria. J. appl. Bact. *46*, 285-289 (1979b)

Goulder, R., A. S. Blanchard, P. L. Sanderson and B. Wright: Relationships between heterotrophic bacteria and pollution in an industrialized estuary. Wat. Res. *14*, 591-601 (1980)

Harrison, M. J., R. T. Wright and R. Y. Morita: Method for measuring mineralization in lake sediments. Appl. Microbiol. *21*, 698-702 (1971)

Hoppe, H.-G.: Relations between active bacteria and heterotrophic potential in the sea. Neth. J. Sea Res. *12*, 78-98 (1978)

Indrebø, G., B. Pengerud and I. Dundas: Microbial activities in a permanently stratified estuary. I. Primary production and sulfate reduction. Mar. Biol. *51*, 295-304 (1979)

Jaffé, D. and J. K. Walters: Intertidal trace metal concentrations in some sediments from the Humber Estuary. Sci. total Envir. *7*, 1-15 (1977)

Jones, J. G.: The effect of environmental factors on estimated viable and total populations of planktonic bacteria in lakes and experimental enclosures. Freshwat. Biol. *7*, 67-91 (1977)

Jones, J. G. and B. M. Simon: An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. J. appl. Bact. *39*, 317-329 (1975)

Lenz, J.: Seston and its main components. *In*: Microbial ecology of a brackish-water environment, pp 37–60. Ed. by G. Rheinheimer. Berlin: Springer-Verlag 1977

Lorenzen, C. J.: Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. *12*, 343–346 (1967)

Moshiri, G. A., W. G. Crumpton and N. G. Aumen: Dissolved glucose in a bayou estuary, possible sources and utilization by bacteria. Hydrobiologia *62*, 71–74 (1979)

Rheinheimer, G.: Regional and seasonal distribution of saprophytic and coliform bacteria. *In*: Microbial ecology of a brackish-water environment, pp 121–137. Ed. by G. Rheinheimer. Berlin: Springer-Verlag 1977

Sokal, R. R. and F. J. Rohlf: Biometry. The principles and practice of statistics in biological research, 776 pp. San Francisco: W. H. Freeman & Co. 1969

Wood, L. W. and K. E. Chua: Glucose flux at the sediment-water interface of Toronto Harbour, Lake Ontario, with reference to pollution stress. Can. J. Microbiol. *19*, 413–420 (1973)

Wright, R. T. and J. E. Hobbie: Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology *47*, 447–464 (1966)

Zimmermann, R.: Estimation of bacterial number and biomass by epifluorescence microscopy and scanning electron microscopy. *In*: Microbial ecology of a brackish-water environment, pp 103–120. Ed. by G. Rheinheimer. Berlin: Springer-Verlag 1977

Zubkoff, P. L. and J. E. Warinner: The heterotrophic potential assay as an indicator of environmental quality. *In*: Physiological responses of marine biota to pollutants, pp 425–437. Ed. by J. Vernberg, A. Calabrese, F. Thurberg and W. Vernberg. New York: Academic Press 1977

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