

# Bacterial colonization of seston particles in brackish waters (Ria de Aveiro, Portugal)

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**ABSTRACT:** The frequency of attached bacteria, as percentage of total bacteria, in estuarine water of the Ria de Aveiro was determined over a seasonal range of temperature, seston, BOD, salinity and water depth. The frequency was inversely related to temperature only in the marine zone of the lagoon. No other associations could be established with environmental factors. The broad spring-summer peak of total bacteria was not apparent in attached bacteria which showed an erratic temporal profile with an average frequency of 9 % (range 1 to 49 %) of total planktonic bacteria. The bacterial coverage per unit area of particle surface was densest in small particles. The density of coverage decreased sharply to values corresponding to 22, 5 and 2 % in particles >3 to 10, >10 to 40 and >40 to 140  $\mu\text{m}$  in diameter respectively compared to the density of coverage of the >1 to 3  $\mu\text{m}$  size-class. Colonized seston and sediment particles exhibited similar bacterial numbers per particle of each size-class and did not show tidal, spatial or seasonal patterns of variation. It is suggested that bacteria only seldom attach to particles in the water column of this lagoon and that resuspension of bottom sediments is the main factor governing the frequency of colonized particles in surface water

## INTRODUCTION

Planktonic bacteria can be either free-living or attached to suspended particles. The frequency of these 2 fractions may vary dramatically from less than 1 % to about 98 % of total bacteria (reviewed by Iriberry et al. 1987). No obvious reason explains this variation although different factors have been associated with high frequencies of the attached state, e.g. water turbidity (Goulder 1977), salinity (Palumbo et al. 1984), temperature, light, turbulence, nutrients (reviewed by Lopez 1980) and the abundance of organically rich seston (Kondratieff & Simmons 1985). This state can be advantageous in natural environments (Pedrós-Alio & Brock 1983) and protective against stress (Harvey et al. 1982). Bacterial attachment to particles has been linked with increased capacity for heterotrophic activity (Hanson & Wiebe 1977) leading to high rates of mineralization (Goulder 1977) and biomass production (Fergusson & Rublee 1976) and/or to the accumulation of secreted materials (Paerl 1975). Loosdrecht et al. (1990) conclude, however, that the diverse metabolic changes do not always follow a general increase in activity after bacterial

adhesion. The importance of the attached state is associated, on the other hand, with the flow of bacterial biomass in the food web as the free-living and the attached bacteria may be predated selectively by different species (Albright et al. 1987, Sibbald & Albright 1988).

We studied bacterial colonization of particles in the Ria de Aveiro with 2 main objectives: (1) to relate the observed frequency of attached bacteria with a set of environmental variables often mentioned as important in other aquatic ecosystems; (2) to assess the distribution of the bacteria on 4 size-classes of particles in the range >1 to 140  $\mu\text{m}$  in diameter, this being relevant to their potential contribution to the diet of different predators.

## MATERIALS AND METHODS

**Study sites.** The Ria de Aveiro, on the western coast of Portugal (8° 44' W, 40° 39' N), is a tidal lagoon with an area of 47 km<sup>2</sup> connected to the Atlantic by a narrow opening. It exchanges with the sea a volume of water of 25 to 90  $\times 10^6$  m<sup>3</sup> between tides of 1 to 3 m

amplitude (Hall 1982). Several rivers and streams carry fresh water into the lagoon, the River Vouga being the main input. Fig. 1 shows the location of 16 sampling stations, one (P11) in this river. Within the Ria proper we distinguish the Canal de Mira (Stns M1, M2 & M3), which has almost no industrial activity, from all the other channels which show different degrees of industrial and domestic pollution (P2 to P20). Table 1 summarizes the hydrographical variables and values of labile organic matter (BOD) and seston.

**Sampling method.** Near-surface water samples used for bacterial counts were collected at about 0.30 m in sterile glass containers and were immediately fixed in 1.2 % formaldehyde. Sediments were sampled with a Van Veen type dredge. A sediment subsample of about

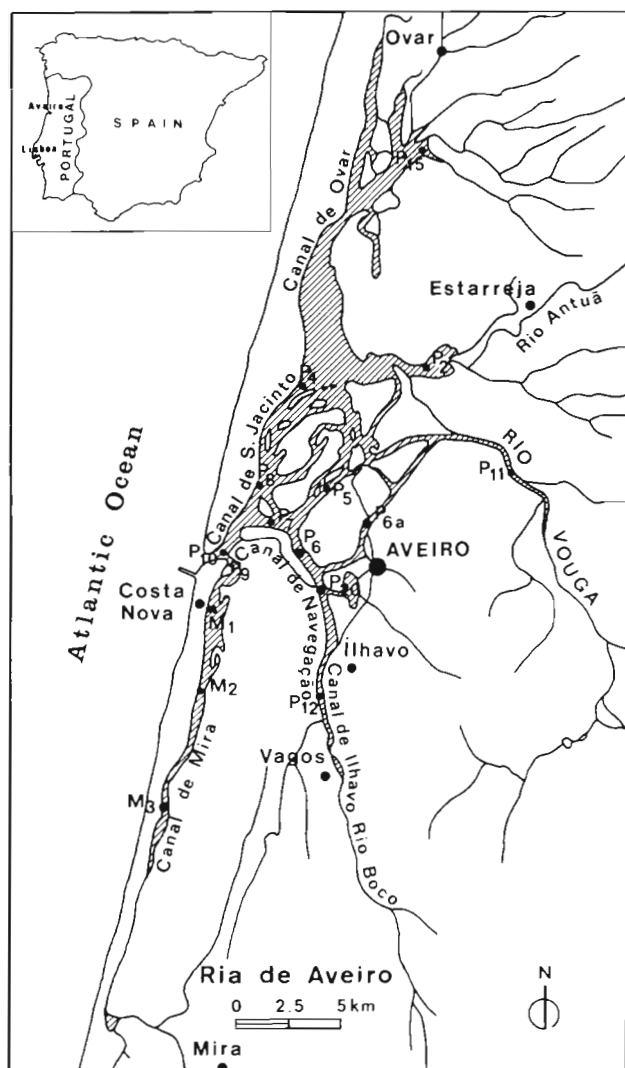


Fig. 1 Location of 16 sampling stations in the Ria de Aveiro, Portugal: in the Canal de Mira (Stns M1, M2, M3); in the mouth of the Ria (Stn P10), in other channels (Stns P2, P4, P5, P6, P7, P8, P9, P12, P15, P20) and in the River Vouga (Stn P11)

200 g wet weight, aseptically removed from the undisturbed top layer (about 0.5 cm thick), was collected into a sterile bottle. For seston dry weight and organic matter determinations, a 5 l sample of surface water was collected in plastic jars. Sampling was usually at high tide during the morning and followed the tidal flux to the different stations. Sampling was every 2 mo at Stns P2 to P20 and monthly at Stns M1 to M3.

**Direct bacterial counts.** Acridine orange direct counts of bacteria were performed in water samples and dilutions of sediment suspensions according to Hobbie et al. (1977). Three replicates per sample were observed and a minimum of 20 optical fields (or 350 total cells) were counted for each. The number of observed bacteria attached to seston and sediment particles was doubled to account for hidden cells underneath the particles. Epifluorescence observation was performed using a Leitz Laborlux microscope equipped with a 3-Ploempak system and FITC 12 filters.

**Particle frequency.** Surface water volumes of 500 ml were taken from the 5 l samples and immediately frozen at  $-20^{\circ}\text{C}$  in polyethylene bottles. Within 1 mo the frozen subsamples were gently melted during 10 h at room temperature and 100 ml aliquots were freeze-dried (Dura Dry, FTS Systems Inc.) to obtain a final 5-fold particle concentration. The concentrated suspensions were analysed for particle size (up to  $140\ \mu\text{m}$  diameter) and frequency (percentage) of the different particle sizes in a laser light diffraction granulometer (Malvern, 2600 C) with a 100 mm focal length cell as described by Brown & Felton (1985) and Rebola et al. (1988). Distilled water filtered through  $0.2\ \mu\text{m}$  pore polycarbonate filters was used to calibrate the granulometer and for particle-suspension dilutions. Sample dilution, if necessary, was performed under gentle magnetic stirring for 5 min.

**Seston dry weight and organic matter content.** Subsamples of 500 ml of water were filtered, in triplicate, through glass fibre filters (Schleicher & Schuell, no. 6). The filters were dried at  $60^{\circ}\text{C}$  to constant weight. The same filters were used for organic matter determinations by combustion at  $550^{\circ}\text{C}$  for 4 h (Rodier 1971).

**Hydrographical characteristics.** Salinity and temperature were determined with a WTW LF196 conductivity meter and dissolved oxygen with a WTW OXI 96 oxygen meter. Current Meter (Braystoke) determinations of maximum water velocity were done by Silva (1989).

**Biological oxygen demand (BOD).** Oxygen was titrated by the modified Winkler method (Strickland & Parsons 1972) shortly after sampling and after 5 d incubation in the dark at  $20^{\circ}\text{C}$ . Titrations on three 50 ml water volumes taken from the same Winkler bottle were averaged for the determination of oxygen consumption.

Table 1 Water characteristics at the sampled stations (1989 to 1990). Mean, standard deviation and range values (in parentheses) at high tide, and maximum water velocity

Station	Salinity (g l <sup>-1</sup> )	Dissolved O <sub>2</sub> (mg l <sup>-1</sup> )	Temperature (°C)	BOD (mg l <sup>-1</sup> )	Seston (mg l <sup>-1</sup> )	Depth (m)	Water vel. (cm s <sup>-1</sup> )
P2	25.8 ± 9.6 (8.8 – 36.2)	6.8 ± 1.7 (4.1 – 9.7)	17.5 ± 5.0 (18.4 – 24.6)	1.6 ± 0.8 (0.4 – 3.1)	25.2 ± 47.8 (5 – 176)	0.5 – 2.0	72.7
P4	32.2 ± 4.0 (26.7 – 35.8)	8.5 ± 1.5 (7.7 – 10.6)	18.4 ± 3.3 (12.6 – 21.8)	0.8 ± 0.4 (0.3 – 1.3)	6.2 ± 2.3 (3 – 10)	1.0 – 3.0	–
P5	31.2 ± 9.6 (7.3 – 35.8)	7.9 ± 1.2 (4.8 – 9.6)	16.4 ± 1.6 (13.4 – 18.4)	1.3 ± 1.5 (0.3 – 4.7)	7.2 ± 4.5 (2 – 16)	2.0 – 4.0	–
P6	33.4 ± 3.2 (26.0 – 35.8)	7.7 ± 1.0 (6.5 – 9.5)	16.4 ± 1.7 (13.7 – 18.5)	1.4 ± 1.1 (0.0 – 3.2)	5.0 ± 2.9 (2 – 10)	4.0 – 6.0	–
P6a	32.1 ± 3.3 (27.0 – 35.7)	7.1 ± 0.8 (5.7 – 8.6)	17.0 ± 2.9 (12.1 – 21.2)	1.3 ± 0.7 (0.0 – 2.2)	6.6 ± 3.5 (3 – 16)	3.0 – 5.0	74.3
P7	35.0 ± 1.5 (30.8 – 35.8)	8.2 ± 0.6 (6.9 – 9.0)	16.4 ± 1.3 (13.6 – 18.1)	1.1 ± 0.8 (0.0 – 2.7)	8.4 ± 5.8 (2 – 17)	9.0 – 11.0	–
P8	34.8 ± 1.1 (32.5 – 35.8)	8.5 ± 0.7 (6.8 – 9.2)	16.2 ± 1.5 (13.6 – 18.1)	1.0 ± 0.6 (0.1 – 1.9)	11.0 ± 10.7 (2 – 31)	8.0 – 10.0	–
P9	35.0 ± 1.2 (31.4 – 35.8)	8.4 ± 0.9 (7.0 – 10.1)	16.2 ± 1.5 (13.6 – 18.0)	1.4 ± 1.5 (0.0 – 5.2)	11.0 ± 11.6 (1 – 41)	5.0 – 7.0	–
P10	35.4 ± 0.4 (34.4 – 35.9)	8.8 ± 0.9 (7.7 – 10.3)	16.1 ± 1.5 (13.6 – 18.0)	1.0 ± 0.7 (0.1 – 1.9)	14.0 ± 16.2 (2 – 62)	11.0 – 13.0	187.1
P11	<2.0 ± 0.0 (<2.0 – 0.0)	4.9 ± 2.8 (1.0 – 8.5)	17.6 ± 6.2 (8.0 – 27.6)	3.9 ± 3.6 (0.3 – 10.1)	10.4 ± 4.8 (4 – 22)	0.5 – 2.5	96.2
P12	28.4 ± 6.8 (17.0 – 36.0)	6.5 ± 1.6 (4.1 – 8.7)	17.2 ± 4.5 (9.1 – 23.8)	1.3 ± 0.9 (0.0 – 2.5)	17.5 ± 13.5 (4 – 52)	0.8 – 2.0	118.3
P15	23.3 ± 10.9 (6.9 – 36.5)	7.8 ± 4.2 (0.2 – 14.5)	19.0 ± 5.4 (9.2 – 26.3)	4.3 ± 1.5 (2.3 – 6.6)	25.3 ± 63.2 (3 – 226)	0.5 – 2.0	–
P20	33.3 ± 2.5 (30.2 – 35.8)	7.4 ± 0.7 (6.8 – 8.7)	16.9 ± 2.0 (13.1 – 18.5)	0.8 ± 0.7 (0.0 – 2.8)	5.8 ± 3.1 (2 – 10)	4.0 – 6.0	–
M1	27.4 ± 5.6 (14.5 – 34.5)	7.7 ± 1.0 (6.0 – 10.5)	16.1 ± 4.1 (10.0 – 24.0)	1.6 ± 0.5 (0.3 – 2.8)	40.5 ± 13.4 (7 – 59)	1.5 – 3.0	–
M2	17.0 ± 9.9 (1.0 – 32.0)	8.1 ± 1.5 (4.8 – 10.2)	16.8 ± 5.8 (9.0 – 30.0)	2.2 ± 1.1 (0.7 – 4.5)	26.6 ± 12.8 (3 – 39)	1.5 – 2.5	–
M3	8.3 ± 7.7 (0.0 – 24.5)	9.1 ± 2.4 (5.2 – 13.0)	17.6 ± 6.0 (10.0 – 30.0)	4.7 ± 3.2 (0.9 – 13.4)	22.2 ± 16.9 (3 – 76)	0.3 – 1.5	–

**Statistical analysis.** Correlation coefficients, Student's exact test and Fisher's approached test (Fisz 1963), were applied to evaluate the correlations between the frequency of attached bacteria and different properties of the estuarine water.

## RESULTS

### Abundance and frequency of adhered bacteria

At 16 stations over 2 yr (1989 and 1990), the abundance of adhered bacteria (NB<sub>ad</sub>) varied within the limits 1 to 20 × 10<sup>5</sup> cells ml<sup>-1</sup>, representing 1 to 49 % of the total bacteria number (Table 2). The overall mean of the frequency of adhered bacteria as a percentage of total bacteria (FB<sub>ad</sub>) was 9 %, the annual mean at individual stations varying between 4 and 20 %. There

was no marked temporal pattern in the fluctuation of NB<sub>ad</sub> although there was a tendency towards decreased values in summer and autumn. The 2 yr average values of FB<sub>ad</sub> at the contrasting Stns P10 (marine) and P11 (freshwater) were, respectively, 14 and 7 %, values that did not reflect the large difference in salinity. At the other stations, with mean salinities ranging from 8.3 to 35.0 ‰ (Table 1), no relationship could be detected between salinity and NB<sub>ad</sub> or FB<sub>ad</sub>. Mean annual water temperatures varied from 16 to 19 °C at different stations (Table 1). The frequency of attached bacteria varied inversely (5 % significance level) with temperature at the stations with salinities above 31 ‰. Water depth at the 16 stations (Table 1) varied from <1 to 13 m (Capitania do Porto de Aveiro pers. comm.). There were no correlations between depth or maximum water velocity (Silva 1989) and NB<sub>ad</sub> or FB<sub>ad</sub>.

Table 2. Mean annual values, with range and coefficient of variation, of the abundance and frequency of adhered bacteria in the surface water of the Ria de Aveiro and of the River Vouga at high tide

Station	Year	Number			Frequency		
		Mean	Range	CV	Mean	Range	CV
P2	1989	8	1–19	103	10	2–20	82
	1990	3	2–5	41	4	1–11	86
P4	1989	–	–	–	–	–	–
	1990	2	1–3	54	5	1–14	112
P5	1989	3	1–8	74	10	5–23	66
	1990	1	1–2	39	4	1–8	67
P6	1989	4	1–7	59	11	6–18	49
	1990	2	1–3	55	5	1–9	65
P6a	1989	6	1–16	99	12	3–25	71
	1990	2	1–4	68	5	2–9	49
P7	1989	4	1–8	90	12	5–22	67
	1990	2	1–5	87	5	1–7	55
P8	1989	7	2–20	104	16	8–34	59
	1990	–	–	–	–	–	–
P9	1989	5	1–9	57	19	1–37	63
	1990	2	1–2	37	6	1–14	73
P10	1989	5	1–12	89	20	6–38	56
	1990	2	1–3	62	8	1–17	87
P11	1989	3	1–5	61	8	2–20	93
	1990	2	1–7	84	6	1–13	54
P12	1989	6	2–11	69	10	3–15	51
	1990	7	2–16	84	12	2–39	115
P15	1989	2	1–5	74	5	1–15	112
	1990	4	1–9	61	5	3–9	51
P20	1989	–	–	–	–	–	–
	1990	2	1–5	70	9	1–27	122
M1	1989	4	1–7	47	12	3–35	64
	1990	3	1–11	106	7	1–22	91
M2	1989	7	1–17	77	12	3–32	64
	1990	6	2–16	60	12	2–49	109
M3	1989	8	1–21	64	11	1–24	67
	1990	6	2–14	79	10	2–31	108

We tried to relate the frequency of attached bacteria at Stns M1 to M3 with the abundance of seston (dry weight) and with its organic content. Fig. 2 shows these 3 variables from October 1989 to December 1990. Generally, the percentage of organic matter in the particles increased with seston dry weight, indicating the organic nature of seston peaks. In May, however, the organic matter component of the particles increased sharply in spite of the scarcity of seston. The oscillations in  $FB_{ad}$  followed neither the fluctuations in the seston dry weight nor those in its organic content.

Along the Canal de Mira the sediments are of medium-grain sands with less than 0.4 % organic matter (Stns M1 & M2) and silt-clay deposits with 1.8 % organic matter (Stn M3). The total (free and attached) bacteria number (TBN) and  $FB_{ad}$  values in surface water and in the top 0.5 cm layer of the sediments are shown in Table 3.  $FB_{ad}$  in the sediments ranged from 41 to 96 % but showed no clear variation with the type of deposit.

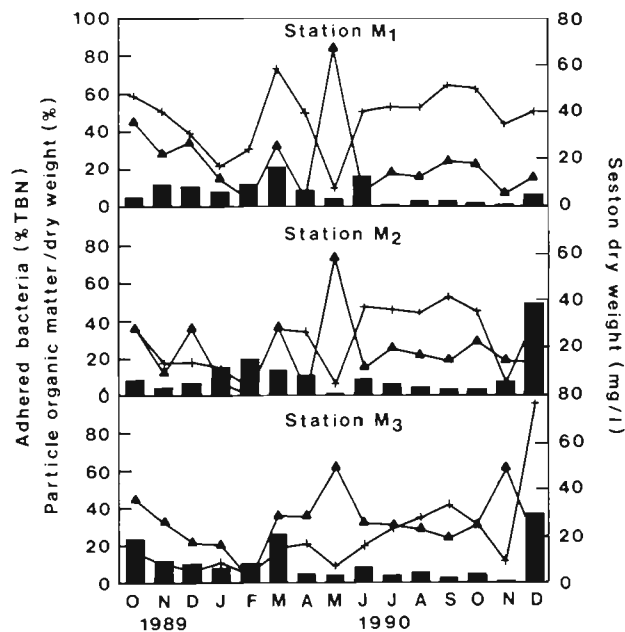


Fig. 2. Comparison of the frequency of adhered bacteria with the abundance of seston and its relative content in organic matter TBN: total number of bacteria in surface water. Bars:  $FB_{ad}$  (frequency of adhered bacteria); +: seston dry weight;  $\blacktriangle$ : percentage of organic matter in seston

The influence of tide on  $NB_{ad}$  of the surface water was assessed during 6 tidal cycles, 3 at Stn M1 and 3 at Stn M3. The data in Fig. 3 show that only in June did low tide seem to produce, at these 2 stations, a large increase (2- to 3-fold) in  $NB_{ad}$ .

#### Bacterial colonization of seston and sediment particles

Fig. 4 shows the variation in the mean number of bacteria on sediment and suspended particles from the 3 stations in the Canal de Mira. Particles were arranged in 4 size-classes, > 1 to 3, > 3 to 10, > 10 to 40 and > 40 to 140  $\mu\text{m}$ . The mean number of bacteria per particle of the 2 first classes was fairly constant both spatially and temporally and was not affected by tide. In surface waters, large particles only rarely supported bacterial cells. The data show that colonized particles  $\leq 3 \mu\text{m}$  supported mean numbers of up to 6 bacterial cells, most frequently 2. Irrespectively of tide, the mean number of bacteria per colonized particle in surface water (634 particles) and sediments (2862 particles) was very similar, averaging 2 to 3 cells (particles > 1 to 3  $\mu\text{m}$ ), 5 to 6 cells (particles > 3 to 10  $\mu\text{m}$ ), 13 to 21 cells (particles > 10 to 40  $\mu\text{m}$ ) and 40 to 59 cells (particles > 40 to 140  $\mu\text{m}$ ), as summarized in Table 4. The largest variation in mean bacterial counts per colonized particle in each size-class corresponded to a factor of 3 in the smaller particles and

Table 3. Total bacterial number (TBN) and frequency of bacteria adhered to particles (FB<sub>ad</sub>, as percentage of TBN) in the top layer of sediments and in the surface water (Canal de Mira, 1988)

Date (1988)	Surface water				Sediment			
	High tide		Low tide		High tide		Low tide	
	TNB ( $\times 10^6 \text{ ml}^{-1}$ )	FB <sub>ad</sub> (%)	TNB ( $\times 10^6 \text{ ml}^{-1}$ )	FB <sub>ad</sub> (%)	TNB ( $\times 10^8 \text{ gdw}^{-1}$ )	FB <sub>ad</sub> (%)	TNB ( $\times 10^8 \text{ gdw}^{-1}$ )	FB <sub>ad</sub> (%)
<b>Station M1</b>								
Jun	6.2	12	8.4	4	1.1	59	0.8	60
Jul	6.6	0	8.6	13	0.7	82	0.3	41
Aug	5.4	10	10.6	21	0.4	76	0.9	69
Sep	6.3	3	6.6	11	1.5	88	0.8	77
Oct	8.3	28	5.4	3	0.3	65	0.3	70
Nov	2.5	1	4.0	17	0.8	93	1.3	94
Average	5.9	9	7.3	12	0.8	77	0.7	69
<b>Station M2</b>								
Jun	6.7	4	12.0	10	1.9	74	7.5	96
Jul	8.3	14	8.7	22	6.3	95	0.7	64
Aug	8.5	12	11.0	15	0.2	37	11.2	88
Sep	11.0	38	15.5	13	1.0	77	0.3	57
Oct	9.3	11	9.5	8	0.6	64	0.4	42
Nov	5.1	8	4.9	13	0.9	69	0.6	79
Average	8.2	15	10.3	14	1.8	69	3.5	71
<b>Station M3</b>								
Jun	7.4	1	1.1	4	230.8	96	135.5	96
Jul	6.1	8	7.5	3	115.0	63	158.4	66
Aug	7.0	6	7.4	8	75.2	72	116.7	70
Sep	9.2	12	19.2	68	79.5	75	69.3	54
Oct	5.9	20	6.9	2	155.9	80	91.9	80
Nov	5.6	35	5.2	15	98.1	83	426.0	95
Average	6.9	14	7.9	17	125.8	78	166.3	77

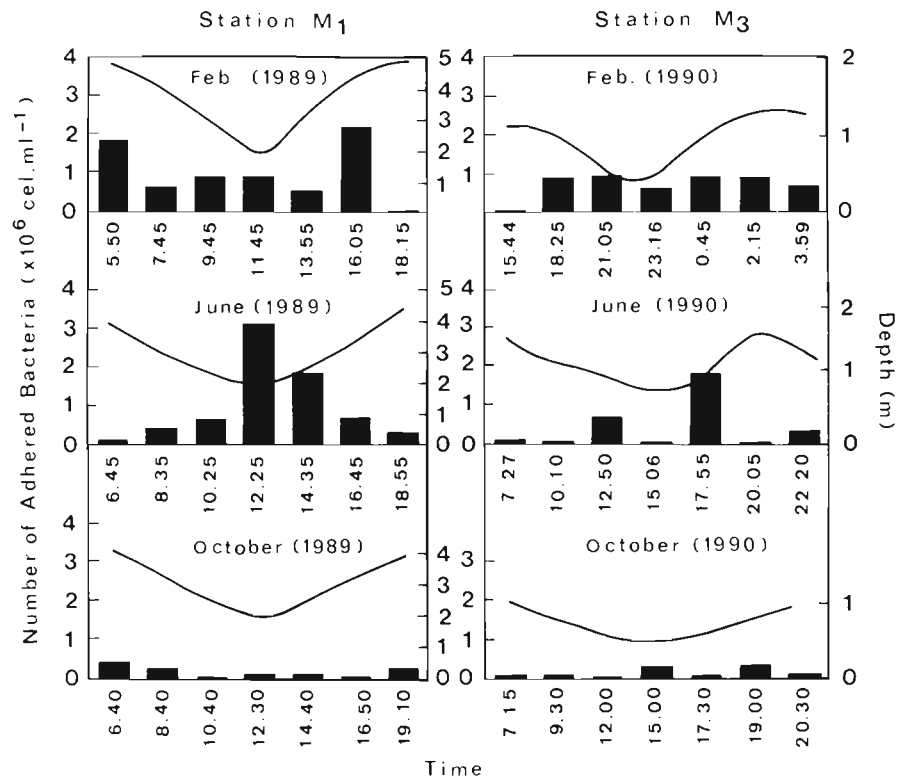


Fig. 3. Tidal fluctuations in number of adhered bacteria in the surface water of the Canal de Mira. Symbols: Bars: NB<sub>ad</sub> (number of adhered bacteria); —: water depth

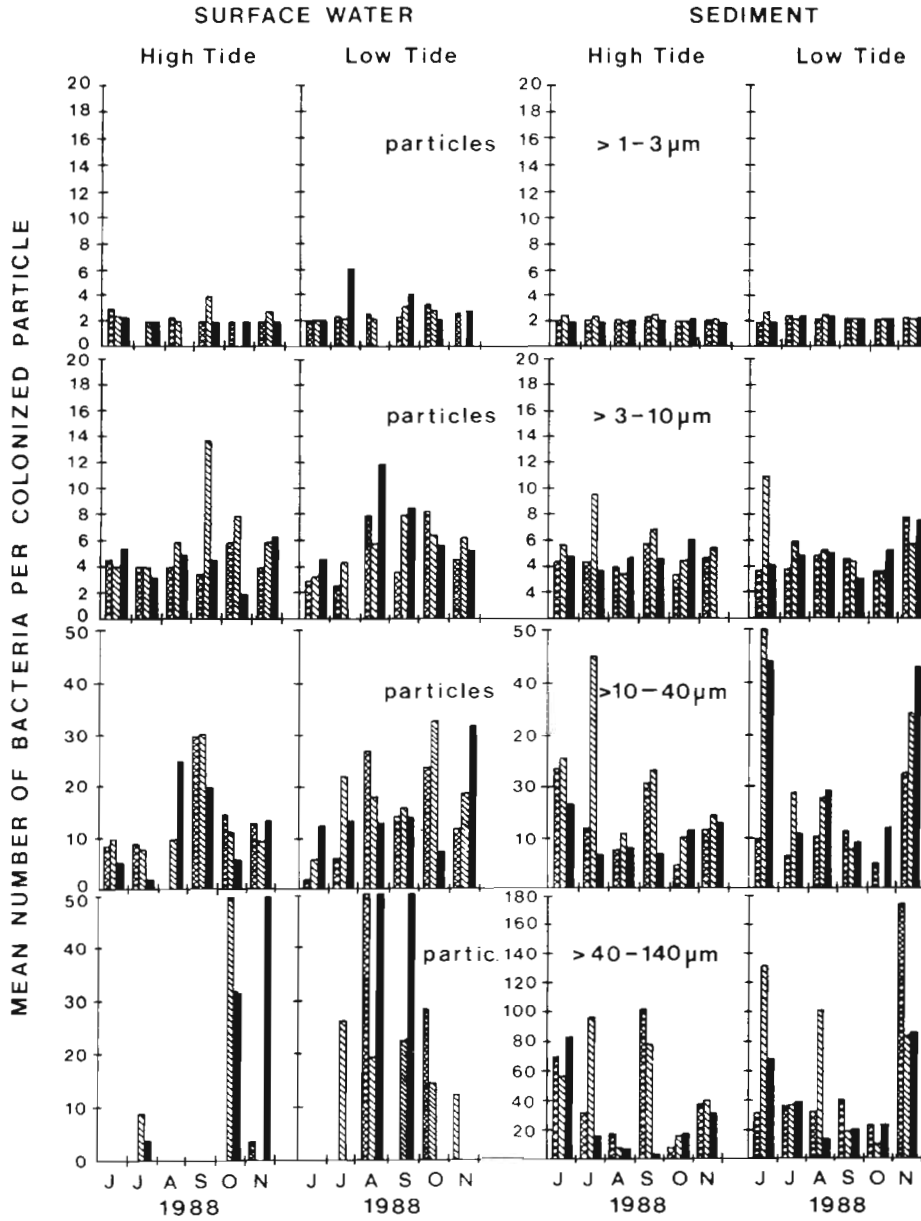


Fig. 4. Spatial and temporal variation of mean number of bacteria on colonized seston and sediment particles in the Canal de Mira. Stn M1; Stn M2; Stn M3

increased successively to factors of about 4, 14 and 24 in the following size-classes (Table 4). The increase in the variation of mean bacterial coverage did not reflect the variation in surface area within each particle size-class

which corresponds to a factor of 9 in the first 2 classes and to factors of 16 and 12 in the third and fourth classes. This suggests that bacterial colonization of large particles is more erratic than that of small ones.

Table 4. Bacterial coverage of colonized particles in the Canal de Mira (June to November 1988)

Particle size-class (μm)	Average number of bacteria per colonized particle (range)			
	Near-surface water		Sediment	
	High tide	Low tide	High tide	Low tide
>1-3	2 (2.0-4.0)	3 (2.0-6.0)	2 (2.0-2.5)	2 (2.0-2.7)
>3-10	5 (2.0-13.7)	6 (2.7-12.0)	5 (3.4-9.6)	5 (3.0-11.0)
>10-40	13 (2.0-30.5)	16 (2.0-33.0)	15 (5.0-45.1)	21 (5.2-79.9)
>40-140	45 (4.0-150.0)	59 (12.0-192.0)	40 (4.0-102.0)	54 (10.0-174.8)



Table 5. Contribution of bacteria on particles of different sizes to the total number of attached bacteria in surface water of the Canal de Mira (January 1989 to December 1990)

Particle size-class ( $\mu\text{m}$ )	Fraction of all colonized particles (mean % $\pm$ SD)	Fraction of total adherent bacteria (mean % $\pm$ SD)
>1-3	18.9 $\pm$ 9.4	7.2 $\pm$ 6.8
>3-10	55.7 $\pm$ 8.5	41.3 $\pm$ 18.1
>10-40	24.4 $\pm$ 10.7	44.8 $\pm$ 21.4
>40-140	1.0 $\pm$ 1.2	6.7 $\pm$ 16.3

The importance of particle-size in the extent of bacterial colonization was assessed in 2 ways: through the relative frequency of each class of colonized particles (as percentage of total number of colonized particles) and through the index of relative particle coverage ( $I$ ) which is a measure of the density of coverage. The index is the ratio (%) of the observed and expected mean bacterial coverage, the expected mean being derived from mean number of bacteria on the smaller particles and surface-area ratios. In a total of 5532 colonized particles observed from January 1989 to December 1990, the most frequent were in the size-class >3 to 10  $\mu\text{m}$  in diameter, accounting for 55.7 % of the total number of particles (Table 5). The less frequent (1.0 %) was the >40 to 140  $\mu\text{m}$  class. In spite

Table 6. Relative bacterial coverage of colonized particles in the surface water of the Canal de Mira (January 1989 to December 1990). SR: surface-area ratio; n: average number of bacteria observed per particle and standard deviation; N: theoretical number of bacteria per particle derived from the observed mean colonization of the smaller particles and SR;  $I$ : index of relative coverage ( $I = 100 \times n/N$ )

Particle size-class ( $\mu\text{m}$ )	Surface-area ratio (SR)	Observed colonization (n)	Derived colonization (N)	Index of relative coverage (%) ( $I$ )
>1-3	1	2.4 $\pm$ 0.6	2.4	100
>3-10	11	5.9 $\pm$ 3.3	26.4	22
>10-40	156	17.5 $\pm$ 11.5	374.4	5
>40-140	2020	77.5 $\pm$ 104.0	4848.0	2

of the large variation (20 to 90 %) in the relative frequency of the total (colonized and non-colonized) particles equal to or smaller than 40  $\mu\text{m}$ , their contribution to  $NB_{ad}$  was always above 70 % and their contribution to the number of colonized particles close to 100 % (Fig. 5). The  $I$  value decreased about 5 and 20 times within this size range (Table 6). These numbers, taken together, indicate that the efficiency of the bacterial process of colonization is inversely related to particle size.

## DISCUSSION

In fresh and ocean waters  $FB_{ad}$  is usually below 50 % and frequently below 20 % of total bacterial counts (Iriberni et al. 1987). In estuaries and salt marshes the attached state can be either rare (Zimmermann 1977), predominant (Goulder 1977) or of varying predominance depending on season (Wilson & Stevenson 1980) or on salinity (Bell & Albright 1981). In the Ria de Aveiro and the incoming water of the River Vouga, we found that, in spite of the contrasting hydrographical features of the 16 stations, only seldom was  $FB_{ad}$  above 20 %, with the 2 yr averages ranging from 5 to 16 %. The different environmental factors measured, some of which were previously associated with increased bacterial attachment in other water bodies, could not be linked to pronounced variation in the extent of colonization of seston particles in the Ria de Aveiro (Tables 1 & 2). These results agree with those of other authors (Zimmermann 1977, Yoon & Rosson 1990). The average frequency of small particles (>1 to 40  $\mu\text{m}$ ) in the water of Stns M1 to M3 was 43 % of total particles, implying that, here, suspended particles are generally larger than in the Humber Estuary (NE England) where that fraction was greater than 87 % and where attached bacteria predominated (Goulder 1977). This

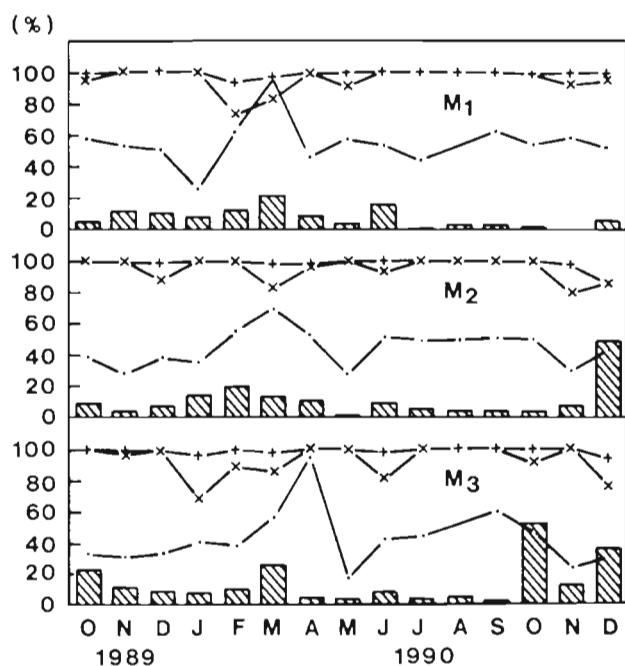


Fig. 5. Small particle frequency and colonization in surface water (Canal de Mira). —•—: frequency of >1 to 40  $\mu\text{m}$  particles; +: contribution of particles >1 to 40  $\mu\text{m}$  to the total number of colonized particles; x: contribution of >1 to 40  $\mu\text{m}$  particles to  $NB_{ad}$ ; ▨:  $NB_{ad}$

may, however, not be relevant as our results do not show a direct relationship between  $FB_{ad}$  values and frequency of >1 to 40  $\mu\text{m}$  particles (colonized plus non-colonized) as percentage of the total number of particles (Figs. 2 & 5).

Wilson & Stevenson (1980) and Yoon & Rosson (1990) observed increased abundance of sestonic bacteria during ebbing and low tides. This increase was also observed in the Ria de Aveiro but was not a constant feature throughout the year (Fig. 3).

In the Ria de Aveiro, TBN commonly increases by a factor of 2 during spring and summer, the factor being larger (up to 5) in brackish inner waters than in stations near the mouth (Alcântara et al. 1991).  $FB_{ad}$  tended, however, to decrease during the warmer months, although the temporal fluctuations did not show a regular pattern (Table 2), as was also noticed by Zimmermann (1977) and Iriberry et al. (1987) but contrasts with observations by Wilson & Stevenson (1980) and Pedrós-Alio & Brock (1983).

Attached bacteria were predominant in surface sediments which agrees with previous results reviewed by Rheinheimer (1981). In spite of a sharp increase of bacterial density in fine sediments, as observed by ZoBell (1938), the average value (73 %) of  $FB_{ad}$  was roughly similar in sandy and silty deposits (Table 3).

The contribution of bacterial carbon and nitrogen to the diet of particle feeders has been a matter of controversy. Different authors emphasize selection by predators feeding on bacteria attached to detritus (Hanson & Wiebe 1977), or the importance of bacteria-produced extracellular mucopolysaccharides (Hobbie & Lee 1980), while others dispute a significant nutritious value of bacteria on particles (Allison & Sutherland 1987). Nevertheless feeding on attached bacteria has been well established for some species of the nano- and microzooplankton (Sibbald & Albright 1988). The relative extent of bacterial coverage of different particles has, then, direct implications on the flux of carbon and nitrogen to higher levels of the food web through detritivorous chains.

The efficiency (frequency and extent) of particle colonization can be envisaged as depending on 2 complex factors. First, bacterial adherence, a factor which may be related to particle abundance (Goulder 1977, Yoon & Rosson 1990), particle quality or shape (DeFlaun & Mayer 1983), water velocity and nature of the bacterial envelope. The efficiency of adherence would be expected to be reflected in the frequency of colonized particles. This is  $\leq 50\%$  in surface waters (Hanson & Wiebe 1977, Iriberry et al. 1987). The fact that, irrespective of the abundance of seston and of its organic content at the 16 stations the  $FB_{ad}$  value was constantly low (Tables 1 & 2) indicates that the bacterial capacity for adherence in the water of Ria de

Aveiro and River Vouga is generally low. From the lack of evidence for the involvement of relevant environmental factors on  $FB_{ad}$  we conclude that this low capacity is probably an inherent property of planktonic bacteria, and that sestonic bacteria are mainly of sedimentary origin. This assumption is supported by the similarity of bacterial coverage in sediment and suspended particles on a per-particle-basis (Fig. 4, Table 4). It is also supported by the bias in bacterial colonization of small particles (>1 to 40  $\mu\text{m}$ ) irrespective of the relative frequency of particles in the water (Fig. 5).

The efficiency of the colonization process would also depend on the bacterial capacity for growth, that is, the rate of bacterial multiplication and spreading on the particle surface. Analysis of the extent of bacterial colonization of particles of different sizes revealed that the average number of bacteria per particle, in the size-range of >1 to 140  $\mu\text{m}$ , varied from 2 to 192. Colonized particles  $\leq 40\ \mu\text{m}$  showed 2 to 80 bacterial cells, agreeing with results by Fergusson & Rublee (1976) and Marsh & Odum (1979), but contrasting with the values of 2.9 to 6.4 cells per particle obtained by Iriberry et al. (1987) in coastal waters. Considering the particles of the 4 size-classes that may be of interest to detritivorous species, ranging from ciliates (Albright et al. 1987) and heterotrophic flagellates (Sibbald & Albright 1988) to bivalves (Edwards 1987), the index of relative particle coverage drops sharply to 2 % in particles >40 to 140  $\mu\text{m}$ , as shown in Table 6. This decrease agrees with Kondratieff & Simons (1985) and Edwards (1987) but is contrary to the results of Marsh & Odum (1979) who found increased bacterial coverages in larger particles. If the index reflects bacterial growth capacity – the second complex factor proposed as governing the efficiency of colonization – it may be higher in small particles for several reasons: (1) smaller particles are more intensively grazed and nutrients liberated during grazing encourage bacterial growth (Morrison & White 1980); (2) particle surfaces are conditioned by adherent bacteria through the production of mucopolysaccharides (Hobbie & Lee 1980) which, tending to promote bacterial growth and to encompass a larger fraction of the surface area in the case of small particles, increases bacterial coverage; (3) particles tend to fracture along lines or areas of bacterial growth and enzymatic attack, originating small portions of denser colonization as well as sparsely colonized particle fractions of larger dimensions.

## CONCLUSION

The similarity of bacterial coverage of seston and sediment particles of the same size-class, as well as the lack of association of the number of adhered bacteria



(NB<sub>ad</sub>) and particle frequency in surface water, suggest that most of the suspended adhered bacteria have their origin in resuspension of the top layer of the sediments. Considering the higher density of bacterial coverage of small particles shown by the index of relative particle coverage, we suggest that particle size may play an indirect role on bacterial growth on particles.

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