

Relationships between the abundance of bacteria and other biota and the hydrographic variability in the Ría de Vigo, Spain

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ABSTRACT: Hydrographic variability driven by changes in wind speed and direction and by meteorological parameters, such as incoming solar radiation, fluvial discharges, and temperature, affects biological standing stocks (e.g. of bacteria) in the marine environment. We studied the effects of such parameters in the Ría de Vigo (Galicia, Spain), between October 1990 and August 1991, where downwelling in winter results from predominantly poleward winds, whilst in summer upwelling is driven by winds travelling towards the equator. Total bacterial abundance was strongly affected by physical factors in the Ría de Vigo. Almost 60% of the variance in bacterial abundance in the surface layer could be explained by an equation relating logTC [log(total count of heterotrophic bacterioplankton)] to incoming solar radiation, water temperature, run-off and coastal upwelling, where solar radiation, water temperature and moderate upwelling are all positive terms, while run-off and strong upwelling act negatively. Consideration of biological variables such as chlorophyll, ciliates and heterotrophic flagellates did not further explain the variance. Physical factors, however, only accounted for 40% of the total variance in bacterial abundance at the bottom of the photic layer. The inclusion of biological variables in the regression equations increased the explained variance to 55%; chlorophyll was the most important of these variables and was positively related to logTC. This indicates that bacterial populations in the surface layer are mainly controlled by physical factors with a weak phytoplankton influence; however, a stronger coupling between primary production and bacterial growth must exist at the bottom of the photic layer. Physical variables also explained 60 to 65% of the variance in cultivable spore-forming saprophytic bacteria, but, in contrast to effects on total bacterial numbers, run-off had a positive effect while moderate upwelling had a negative influence on spore-forming numbers. This suggests that terrestrial run-off has some ecological importance through the enrichment of nearshore bacterial populations with cells of terrestrial origin.

KEY WORDS: Upwelling · Run-off · Bacteria · Phytoplankton · Protozooplankton · Hydrographic variables · Estuarine ecosystem · Ría de Vigo

INTRODUCTION

Ría de Vigo (Spain) is a typical large maritime Galician bay covering 176 km² and containing 3317 × 10⁶ m³ of water (Fraga & Margalef 1979). It attains a depth of 40 m in the central part of its U-shaped cross section (Fig. 1), the site chosen for regular bacteriological sampling (Stn 3b). As with the other 3 rías in this area

(Muros, Arousa and Pontevedra), the bay penetrates the Galician coast in a northeasterly direction, in this case to a distance of 33 km. The bay opens widely to the west, and its hydrology is influenced by both Atlantic Ocean upwelling (mainly in summer) and by freshwater run-off as a function of the rainfall in the drainage basin (mainly in winter). The mouth of the ría is partially closed by the Cíes Islands, and therefore opens to the ocean through 2 'channels', the southern one of which is 5 km wide and 67 m deep and the northern some 2.5 km wide and 23 m deep. In contrast

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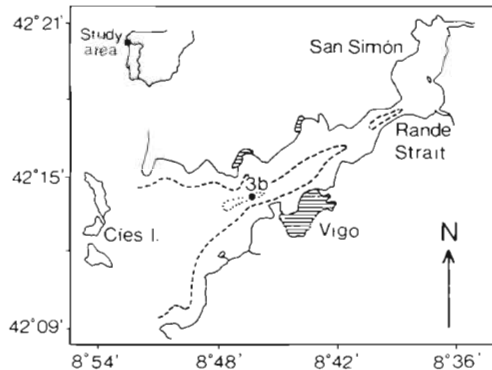


Fig. 1 Ría de Vigo (northwest corner of Iberian peninsula) showing the sampling station (3b). Dashed and dotted lines indicate the location of the 20 and 40 m isobaths, respectively

to the main body of the Ría de Vigo, the bay's inner reaches (San Simón Inlet) are partially separated from the main body and protected from direct oceanic influence by a narrow channel (Rande Strait) of 700 m width.

The hydrological characteristics of the Ría de Vigo are well documented (Fraga & Margalef 1979, Prego et al. 1990, Prego & Fraga 1992, Rios et al. 1992, Alvarez-Salgado et al. 1993, Figueiras et al. 1994), as are its physico-chemical characteristics (Prego 1990). In general there are 2 layers of water in the Ría de Vigo: the lower and more saline layer enters as subsurface oceanic water that compensates for the less saline water flowing out at the surface. In summer, prevailing coastal northerly winds promote coastal upwelling which introduces biogenic salts into the Ría and forces an outflow of surface waters. These upwelled waters migrate from the ocean across the bottom, ascending and infiltrating the very stable upper layer, and in so doing provide it with a steady supply of nutrients. During spring, when vertical stratification is not as strong as in summer, upwelled water penetrates the upper layer, mixing the water of the 2 layers. Downwelling also produces an homogenous water column, but in this case by reversing the circulation. Surface coastal water is advected into the Ría and pushed down in its interior. In winter, a classical positive estuarine circulation develops, driven by the less saline outgoing water derived from terrestrial run-off (Fraga & Margalef 1979, Prego et al. 1990). Consequently, it is expected that bacteria from this system will be under strong hydrological, meteorological and biological pressures.

Due to the unique ability of heterotrophic bacteria to utilise soluble compounds in very low concentrations (Morita 1984), they are a basic component of the marine food web. Transferring dissolved organic matter (DOM) to their own cells, they initiate energy flow through bacterivores (Fuhrman & McManus 1984, Ras-

soulzadegan & Sheldon 1986, Garrison & Gowing 1993) to higher trophic levels (Williams 1981, Azam et al. 1983, Ducklow 1983). Moreover, by converting particulate organic matter (POM) to both soluble and detrital forms (Karl & Knauer 1984, Zdanowski 1988), heterotrophic bacteria play an important role in the regeneration of many types of organic matter in the pelagic zone of oceanic ecosystems.

Galician coastal waters have very high primary and secondary production, and the microbial loop is important in the food web in these waters (Hanson et al. 1986, Blanton et al. 1987, Tenore et al. 1995). Few data exist on bacteria in the Galician rías' ecosystems (Fierro et al. 1976a, b, Anonymous 1987) and these are mainly concerned with bacteriological investigations related to pollution, epidemiology, and public hygiene. So far there are no data on the status of bacteria in the trophic structure or on the dependence of bacteria upon seasonal cycles in the Galician marine ecosystem. As an initial step, we have commenced bacteriological studies in the Ría de Vigo with the aim of describing aspects of ecological interactions between marine heterotrophic bacteria and other auto- and heterotrophic microorganisms, abiotic components, and hydrological parameters. As it is generally accepted that the primary habitat of most aerobic spore-forming bacteria is the soil (Alexander 1971) and as terrestrial run-off is important in the Ría de Vigo, this fraction of bacteria was also considered.

MATERIAL AND METHODS

Investigations were conducted between 31 October 1990 and 19 August 1991 at Stn 3b (Fig. 1) in the central part of the Ría de Vigo; this station has been used for regular oceanographic sampling since 1987. Sampling was conducted between 09:00 and 10:00 h without consideration of tidal state, viz. effects of tide on circulation and mixing; these are negligible when compared with those imposed by upwelling-downwelling cycles and run-off in Galician rías (Figueiras et al. 1994, Rosón et al. 1995). Water samples for bacterial work were collected with an ethanol-rinsed Van Dorn type 5 l plastic bottle from the surface (0 m), from the middle layer (bottom of the photic layer, characterised by 1% of total light reaching the surface), and from the bottom layer (about 5 m above the bottom). The first part of the water in the sampler was discarded by flushing through the tap. Thereafter, sub-samples (ca 100 ml) were immediately transferred to sterile, opaque, screw-capped glass bottles and maintained at ca 5°C until processed in the laboratory, within 2 to 3 h.

The total count (TC) of heterotrophic bacterioplankton was determined (bi-weekly for the surface and

middle layers, and once a week for the bottom layer) in water samples fixed with formalin 4% final concentration by epifluorescence microscopy on 0.2 μm black Millipore polycarbonate filters, using DAPI (4'-6-diamidino-2-phenylindole) (Porter & Feig 1980). Both total (Cyan) and dividing (CyanD) cyanobacteria were counted in parallel to heterotrophic bacterioplankton, after Davis & Sieburth (1982). Heterotrophic picoflagellates (HPF) ($\leq 2 \mu\text{m}$) and nanoflagellates (HNF) (between 2 and 20 μm) were also counted in these same samples (i.e. the DAPI sample). Ciliates (Cil) were counted in samples fixed with Lugol's iodine using an inverted microscope. Cyan, CyanD, HPF, HNF and Cil were determined in the surface and middle layers only.

Heat-resistant spore-forming aerobic bacteria were described in terms of Colony Forming Units (CFUs): 10 ml of water was incubated at 80°C for 10 min, then mixed with 10 ml of double-strength Bacto Marine Agar 2216 (ZoBell 1941) and poured into petri dishes (Fenchel & Hemmingsen 1974). Media were prepared using aged seawater. Water samples were homogenised aseptically immediately before inoculation (Ultra Turrax for 30 s) whilst maintained in an ice bath. Plates were incubated in darkness for up to 15 d at 24°C.

Temperature (Temp), salinity (Sal), and density (Dens) were determined by CTD casts. The upwelling index for the previous 3 d plus the sampling day (Upw4) was calculated using geostrophic winds off Cape Finisterre derived from surface pressure (3 times d^{-1}) according Bakun (1973). Run-off (Qr4) was calculated following the equation proposed by Ríos et al. (1992), taking into account rainfall over the preceding 3 d and using a retention coefficient of 0.75. Mean incoming solar radiation of the preceding 3 d plus sampling day (Qs4) was estimated by Mosby's formula (Dietrich et al. 1980). Chlorophyll *a* (chl *a*) was determined fluorimetrically (Yentsch & Menzel 1963). Nutrients were determined in an auto-analyser. Nitrate (NO_3), after reduction to nitrite through a Cu/Cd column, was determined according to Hansen & Grasshoff (1983) modified by Mouriño & Fraga (1985). Silicate (SiOH_4) was analysed following Hansen & Grasshoff (1983). Ammonium (NH_4) was determined according to Grasshoff & Johannsen (1972).

RESULTS

Bacterioplankton (DAPI-TC)

On the basis of incoming solar radiation (Qs4) the study period was divided into winter (lowest Qs4: November to February), and summer (highest Qs4: March to August) (Fig. 2). Total bacterial counts ranged from 4.9×10^5 to $6.2 \times 10^6 \text{ ml}^{-1}$ for the whole

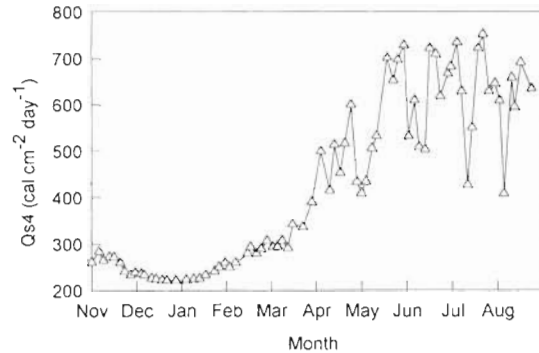


Fig. 2. Seasonal variations in incoming solar radiation (Qs4) in Ría de Vigo. Means from previous 3 d plus sampling day

period (November to August), with minimal values in the winter and maximal values in the summer. Distinct seasonal trends in terms of TC increases were noted at all depths (Fig. 3d), with the greatest increase at the surface (Fig. 3a, d), where the mean value in summer exceeded that in winter by a factor of 2; TC in the middle and bottom layers during summer exceeded winter values by only 1.3 and 1.5 times, respectively (Fig. 3b–d). Bacterial numbers decreased with depth. In terms of mean values in the middle and bottom layers, these were 1.6 and 1.8 times lower, respectively, than at the surface.

Considerable fluctuations in TC occurred in each month and at each depth (Fig. 3). Fluctuations were higher in summer than in winter, and were greatest during summer at the surface where the maximum: minimum ratio was 8.7. The lowest ratio (1.5) was observed during winter in bottom samples.

Hydrological properties of the water column [primarily characterised by 4 parameters: density, temperature, run-off in the drainage basin, and the upwelling index] also fluctuated considerably (Fig. 4). The most dramatic change in density (Fig. 4a) occurred during winter (January) with a steep drop at the surface, from 27.2 (20 December) to 9.4 (11 January), while relatively high values were recorded in the middle layer (26.8 to 23.7). The lowest density values (26) in the bottom layer for the whole period of observations were also recorded during this period. The abrupt change in density was not reflected in temperature (Fig. 4b), indicating that run-off (Fig. 4c) was the factor responsible. These observations are also consistent with the correlation between run-off (i.e. high) in the drainage basin (up to $100 \text{ m}^3 \text{ s}^{-1}$) and the very strong downwelling (to less than $-2000 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ of coast) over this period of about 3 wk (Fig. 4c, d). During the development of this first strong downwelling (throughout December and January), TC in each layer decreased (Fig. 3a–c), reaching minimum values in the 3 layers. By the end of

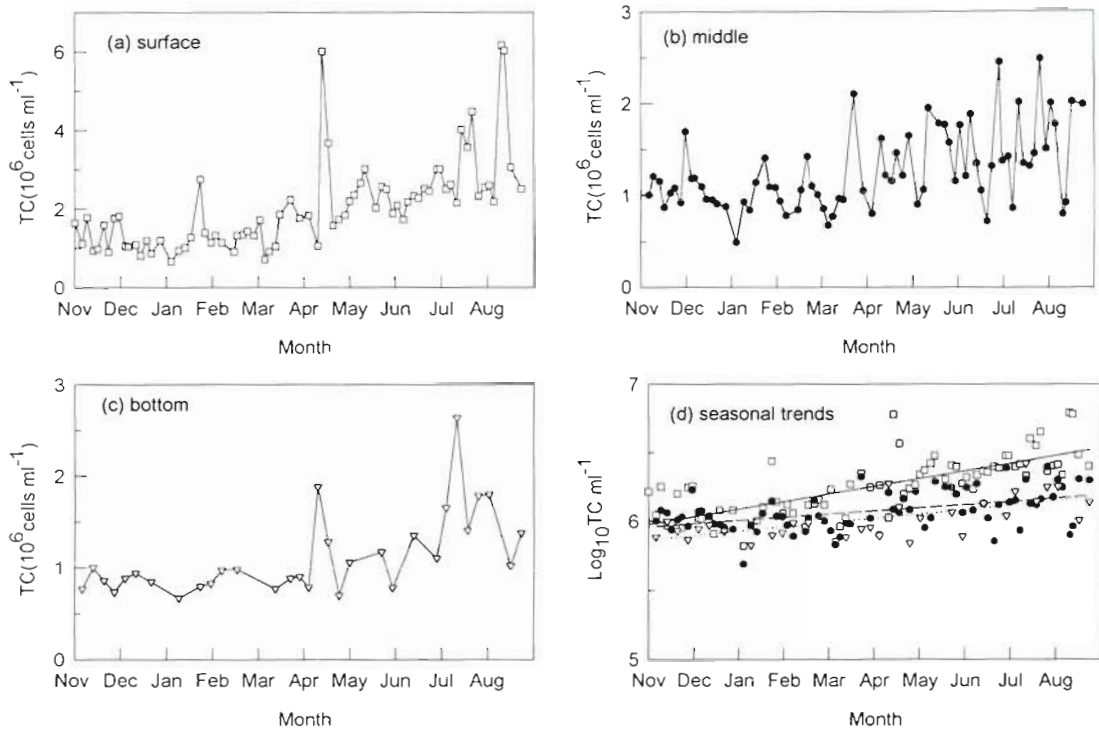


Fig. 3. Total bacterioplankton numbers (DAPI-TC) in the (a) surface, (b) middle (bottom of the photic layer) and (c) bottom layers of Ria de Vigo (Stn 3b) and (d) their seasonal trends, as revealed by the following regression equations: surface (\square , —), $\log_{10}TC = 5.979 + 0.0019^{***} \text{ Julian Day}$, $r^2 = 0.54^{***}$, $n = 75$; middle (\bullet , - - -), $\log_{10}TC = 5.959 + 7.9 \times 10^{-4}^{***} \text{ Julian Day}$, $r^2 = 0.23^{***}$, $n = 74$; bottom (∇ , ·····), $\log_{10}TC = 5.865 + 0.0011^{***} \text{ Julian Day}$, $r^2 = 0.42^{***}$, $n = 31$. $^{***}p < 0.001$

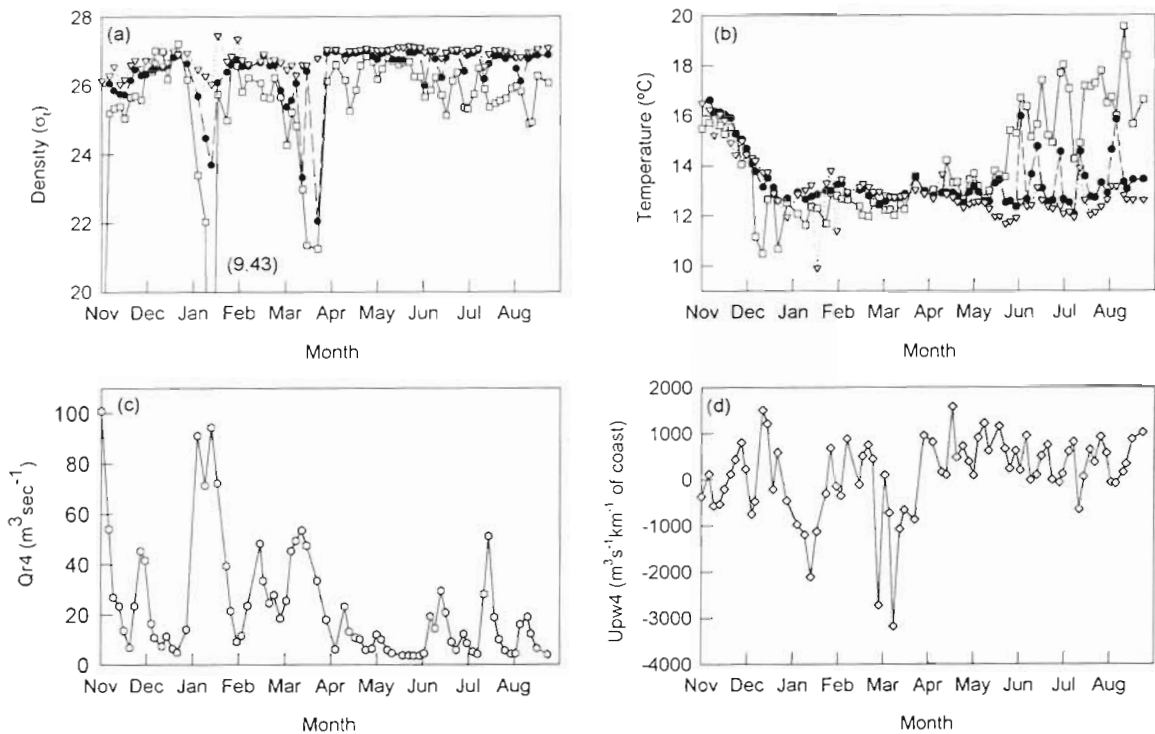


Fig. 4. Seasonal variations in (a) density and (b) temperature in the 3 layers at Stn 3b (\square : surface, \bullet : middle, ∇ : bottom) and in (c) runoff (Qr4) and (d) upwelling index (Upw4). Qr4 and Upw4 are averaged values from the 3 previous days plus sampling day

January, coinciding with the decay of these hydrological phenomena and with a downwelling to upwelling transition (Fig. 4d), TC increased at the 3 depths (Fig. 3a–c), reaching high levels considering the winter conditions. In spite of this event there were no significant correlations between TC and density or between TC and upwelling at any depth during winter (Table 1). A similar situation, although of a lesser order, occurred during the March upwelling-downwelling-upwelling transition, when density dropped to 21.2 at the surface (Fig. 4a). From late March, the upwelling index fluctuated mainly within positive values (up to $1586 \text{ m}^3 \text{ s}^{-1} \text{ km}^{-1}$ of coast in April), with the only exception in July (Fig. 4d), when relatively high run-off in the drainage basin (Fig. 4c) occurred. During summer, as in winter, no significant positive correlation was observed between TC and density at the 3 depths, but a significant positive correlation with upwelling was found at the surface, at the same time that Qr4 negatively correlated with the total bacterial counts at the surface and in the middle layer (Table 1). The high

Table 1. Correlation coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between total bacterial counts (TC) and other hydrographical and biological variables [incoming solar radiation (Qs4), water temperature (Temp), upwelling index (Upw4), run-off (Qr4), water density (Dens), chl a (Chl), cyanobacteria numbers (Cyan), numbers of dividing cyanobacteria (CyanD), heterotrophic picoflagellates (HPF), heterotrophic nanoflagellates (HNF), and ciliates (Cil)] during winter (W) and summer (S) in 3 layers of Ria de Vigo (Stn 3b)

Variables	Season	Surface	Middle (1% of light)	Bottom
TC-Qs4	W	0.22	0.19	0.42
	S	0.54***	0.26	0.12
TC-Temp	W	0.20	0.19	0.04
	S	0.68***	0.48***	0.44
TC-Upw4	W	0.06	0.17	0.38
	S	0.40**	0.14	-0.05
TC-Qr4	W	0.02	-0.33	-0.65*
	S	-0.40**	-0.35*	0.05
TC-Dens	W	0.02	0.24	0.11
	S	-0.07	-0.04	-0.38
TC-Chl	W	0.20	0.48**	0.06
	S	0.18	0.51***	0.61**
TC-Cyan	W	0.24	0.44*	
	S	0.43**	0.15	
TC-CyanD	W	0.37*	0.46*	
	S	0.47**	0.04	
TC-HPF	W	-0.07	0.01	
	S	-0.07	-0.11	
TC-HNF	W	0.01	0.02	
	S	0.10	0.17	
TC-Cil	W	0.01	0.04	
	S	0.51***	0.55***	
No. of samples	W	n = 30	n = 29	n = 12
	S	n = 45	n = 44	n = 19

(surface, middle), and moderate (bottom) positive correlations between TC and water column temperature in summer (Table 1) should also be noted. On the other hand, as would be expected, Qs4 and surface temperature were strongly positively related ($r = 0.69$, $p < 0.001$, $n = 45$).

Relationships between bacterial concentrations and other biotic components were also observed. There was a significant positive correlation between TC and chl a (Table 1) in the middle layer during winter and summer and in the bottom layer only in summer. The high correlation during summer occurred in spite of strong fluctuations in chl a concentrations (maximum: minimum ratio was 44.2 in summer, compared to 19.2 in winter; Fig. 5). Total cyanobacteria and dividing cyanobacteria correlated with TC at the surface and in

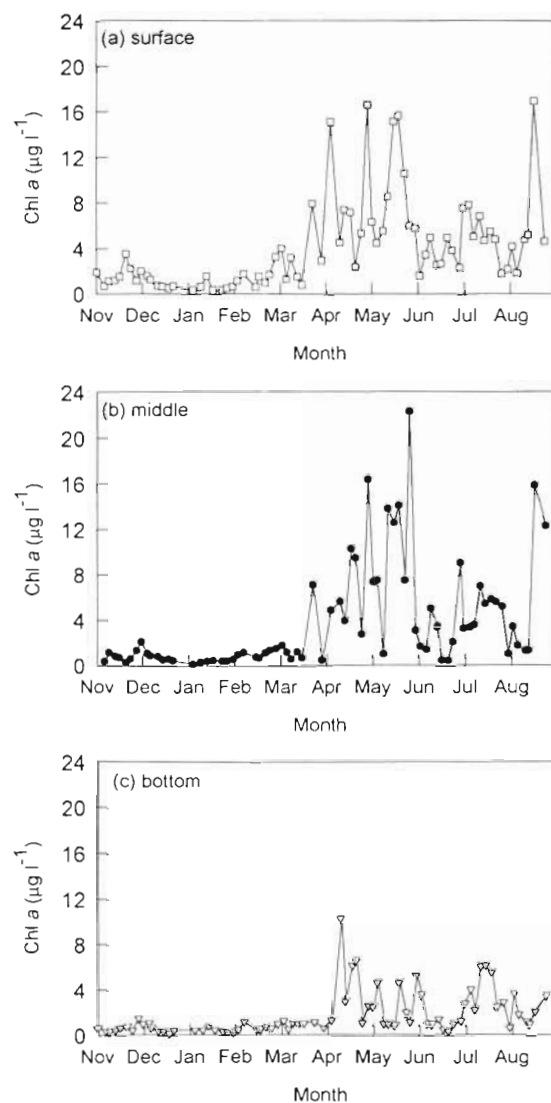


Fig. 5. Seasonal variations in chl a concentration in the (a) surface, (b) middle and (c) bottom layers at Stn 3b

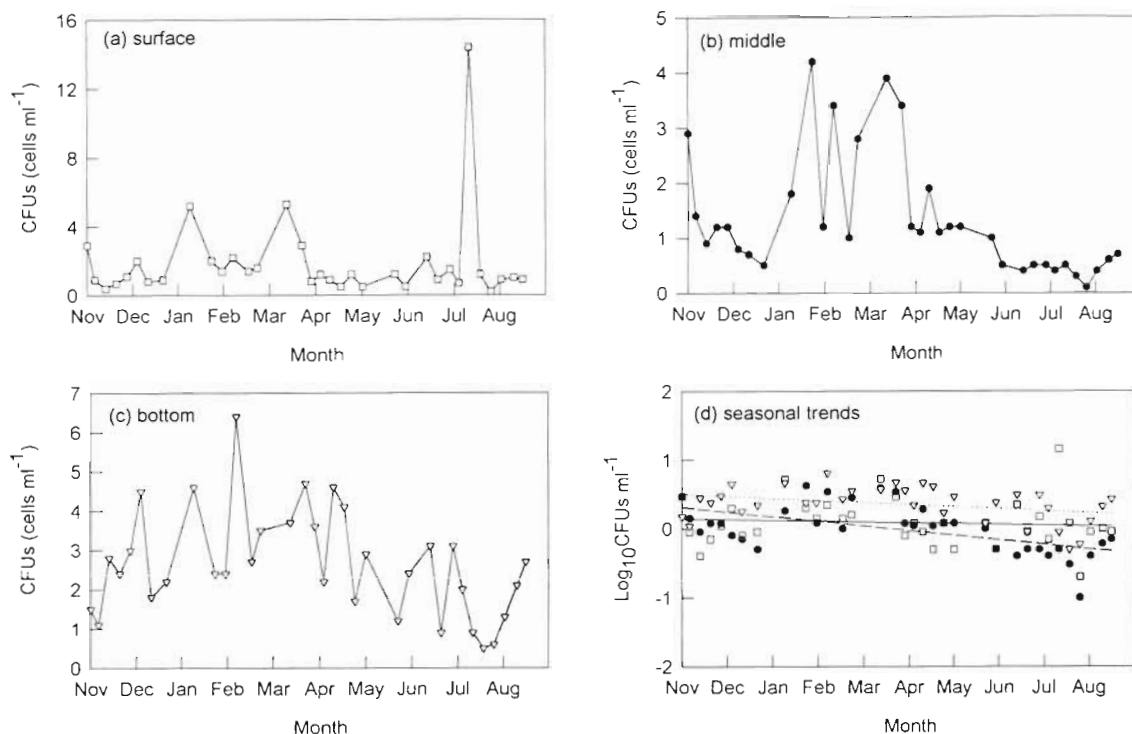


Fig. 6. Number of aerobic spore-forming bacteria (CFUs) in the (a) surface (b) middle and (c) bottom layers of Ria de Vigo (Stn 3b) and (d) their seasonal trends revealed by the following regression equations: surface (\square , —), $\log_{10}\text{CFU} = 0.147 - 3.94 \times 10^{-4}$ Julian Day, $r^2 = 0.01$, $n = 34$; middle (\bullet , - - -), $\log_{10}\text{CFU} = 0.290 - 2.13 \times 10^{-3}$ Julian Day, $r^2 = 0.28^{**}$, $n = 34$; bottom (∇ , ····), $\log_{10}\text{CFU} = 0.5 - 0.001 \cdot$ Julian Day, $r^2 = 0.12^{**}$, $n = 34$. * $p < 0.05$, ** $p < 0.01$

the middle layer. Strong TC versus ciliate correlation was also observed in summer in both layers (Table 1). Conversely, no statistically significant correlations with heterotrophic pico- and nanoflagellates were found (Table 1).

Spore-forming bacteria (CFUs)

Heat-resistant spore-forming bacteria represented only a very small fraction (0.0001%) of the total bacterioplankton (DAPI-TC). They also fluctuated considerably with greatest variations in number at the surface (maximum:minimum ratio = 72) and lowest variations at the bottom (maximum:minimum = 13) (Fig. 6).

Only surface CFU numbers increased slightly over the winter to summer transition (avg 1.68 CFU ml⁻¹ in winter, and 1.94 CFU ml⁻¹ in summer). However, this increase was strongly influenced by the extremely high summer value of 14.4 CFU ml⁻¹ on 8 July (Fig. 6a), when there was relatively high run-off (Fig. 4c). Other summer surface values were actually rather low (avg 1.29 CFU ml⁻¹) and tended to decrease from winter to summer (Fig. 6a). In the middle and bottom layers, a tendency for CFU numbers to decrease from winter to summer was also recorded (Fig. 6b-d).

Table 2. Correlation coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between spore-forming bacterial counts (CFUs) and other hydrological and biological variables in the surface layer of Ria de Vigo (Stn 3b). See Table 1 for abbreviations

Variables	Season	Surface
CFUs-Qs4	W	-0.21
	S	-0.43
CFUs-Upw4	W	-0.40
	S	-0.74***
CFUs-Qr4	W	0.58**
	S	0.70***
CFUs-Sal	W	-0.55*
	S	-0.45*
CFUs-Dens	W	-0.50
	S	-0.40
CFUs-NO ₃	W	0.84***
	S	0.42
CFUs-NH ₄	W	0.27
	S	0.20
CFUs-Si(OH ₄)	W	0.59*
	S	0.43
CFUs-Cyan	W	-0.84***
	S	0.09
CFUs-TC	W	0.17
	S	-0.22
No of samples	W	n = 14
	S	n = 20

A significant positive correlation between CFUs and Qr4 was found in the surface layer during both the winter and summer periods (Table 2). A positive correlation was also observed between CFUs and nitrate and silicate but only during winter. Conversely, there was negative correlation with salinity in winter and summer, with upwelling in summer, and cyanobacteria in winter (Table 2).

DISCUSSION

Physical and biological control of bacterioplankton population

Variability in bacterial number is a typical feature for most marine coastal habitats. In the Ría de Vigo which opens wide to the Atlantic Ocean, the coefficient of variation ranged from 34% in the middle layer to 56.3% at the surface. This variability was similar to that found in other very different ecosystems, for example the brackish waters of the Kiel Bight in the western Baltic Sea (Zimmermann 1977), where water exchange is severely hampered by narrow and shallow straits, or in the deep but strongly mixed cold waters of Admiralty Bay in West Antarctica (Donachie 1995).

When calculated on the basis of mean winter and mean summer values, the summer bacterioplankton (TC) population at the surface was on average numerically larger than the winter population. Nevertheless, when TC fluctuations at the surface are considered over a short period of time (3 to 4 d, on the basis of bi-weekly measurements) counts changed much more dramatically, e.g. by a factor of ca 6 in April, or 3 in August (Fig. 3a). This means that the general trend in seasonal terms (winter-summer transition) is for TC to increase, although there are short-term fluctuations during this time. Similar situations, although with smaller ranges, were noted in the middle and bottom layers.

The water column's physical structure was shown to have considerable influence on bacterioplankton populations in the photic layer. Thus, during winter and in spite of the high run-off associated with strong downwelling in January (Fig. 4a, c, d), the correlation between TC at the surface and TC in the middle layer was positive and significant ($r = 0.62$, $p < 0.001$, $n = 30$), even though mean TC values were 1.2 times higher at the surface (Fig. 3a, b). On the contrary, during the summer period there was no significant correlation between TC at the surface and in middle layer. These facts suggest that winter bacterioplankton communities from the surface and middle layers were not totally separated by a discontinuity (thermoclines or halo-

clines) that could prevent mixing. During summer, however, both layers were apparently separated by such a discontinuity based on thermal stratification (Fig. 4a, b). On the other hand, significant correlations between TC and other biological variables, such as chl *a*, cyanobacteria, and ciliates (Table 1), indicate that there exists some control by, or coupling of total bacterial numbers with, other biological components in the system.

On the basis of the multiple stepwise regression between logTC and solar radiation (Qs4), temperature (Temp), upwelling (Upw4), run-off (Qr4), and density (Dens), we can presume mechanistic connections among the physical factors and standing stock of bacteria (Tables 3A & 4A). In the light of these data, throughout this study, which included a strong winter to summer transition, the increasing Qs4 was the principal physical factor controlling bacterial standing

Table 3. Multiple stepwise regressions between logTC and (A) physical and (B) physical and biological variables in the surface layer in Ría de Vigo (Stn 3b). Results are presented for data collected during the full period of this study ($n = 75$), and separately for winter ($n = 30$) and summer ($n = 45$). β : standardized coefficient of regression; p : level of significance of the slopes in the regression equations. See Table 1 for abbreviations

Variables	Winter and summer		Winter		Summer	
	β	p	β	p	β	p
(A)						
Qs4	0.53	0.000	0.216	0.251		
Temp	0.26	0.013			0.52	0.000
Upw4	0.09	0.273			0.28	0.089
Dens					-0.26	0.108
Qr4					-0.23	0.23
Regression equations:						
W&S $\logTC = 5.58 + 6.95 \times 10^{-4} Qs4 + 2.63 \times 10^{-2} Temp$, $r^2 = 0.58$						
S $\logTC = 5.55 + 0.053 Temp + 7.2 \times 10^{-5} Upw4$, $r^2 = 0.47$						
(B)						
Qs4	0.74	0.000				
Temp					0.63	0.000
Upw4					0.35	0.023
Dens			-0.50	0.05	-0.27	0.060
Chl					0.20	0.14
logCyanD	0.21	0.01	0.74	0.007		
logHNF					0.18	0.15
Regression equations:						
W&S $\logTC = 5.88 + 9.17 \times 10^{-4} Qs4 + 0.079 \logCyanD$, $r^2 = 0.61$						
W $\logTC = 6.54 + 0.18 \logCyanD - 0.017 Dens$, $r^2 = 0.26$						
S $\logTC = 5.55 + 0.053 Temp + 7.2 \times 10^{-5} Upw4$, $r^2 = 0.47$						

Table 4. Multiple stepwise regressions between logTC and (A) physical and (B) physical and biological variables in the middle layer (bottom of the photic layer) in Ria de Vigo (Stn 3b). Results are presented for data collected during the full period of this study (n = 75), and separately for winter (n = 30), and summer (n = 45); β : standardized coefficient of regression; p: level of significance of the slopes in the regression equations. See Table 1 for abbreviations

Variables	Winter and summer		Winter		Summer	
	β	p	β	p	β	p
(A)						
Qs4	0.46	0.000			0.23	0.177
Temp	0.27	0.009			0.47	0.000
Qr4	-0.32	0.014	-0.33	0.083	-0.39	0.025
Dens	-0.24	0.05			-0.30	0.063
Regression equations:						
W&S $\logTC = 6.57 + 3.6 \times 10^{-4} Qs4 + 0.036 Temp - 0.002 Qr4 - 0.04 Dens, r^2 = 0.37$						
S	$\logTC = 5.03 + 0.087 Temp - 0.0039 Qr4, r^2 = 0.37$					
(B)						
Chl	0.55	0.000	0.40	0.036	0.45	0.001
logCyan	0.29	0.019	0.44	0.025		
Temp	0.22	0.028			0.39	0.003
logCil			-0.27	0.15	0.29	0.03
Qs4	0.38	0.001				
Regression equations:						
W&S $\logTC = 5.48 + 0.017 Chl + 2.98 \times 10^{-4} Qs4 + 0.064 \logCyan + 0.029 Temp, r^2 = 0.54$						
W	$\logTC = 5.88 + 0.11 \logCyan + 0.084 Chl - 0.068 \logCil, r^2 = 0.40$					
S	$\logTC = 5.10 + 0.013 Chl + 0.069 Temp + 0.068 \logCil, r^2 = 0.56$					

stock, not only at the surface (Table 3A), but also in the middle layer (Table 4A), with some negative influence from run-off and high density water on total bacteria counts in the middle layer (Table 4A). After the transition to summer, the period of regular high solar radiation, the correlation between Qs4 and TC disappeared (surface layer) or lost its high significance (middle layer), and temperature became the main factor controlling bacterial population. It should also be noted that logTC was slightly but still positively related to upwelling during summer in the surface layer, and negatively related to run-off and density in the middle layer.

When all biological variables were added to the multiple regression analysis, the general pattern of relations between logTC and physical factors in the surface layer did not differ appreciably from that described above. The weak relationships between logTC and chl a (Chl), dividing cyanobacteria (CyanD) or heterotrophic nanoflagellates (HNF) which did appear were well below the level of significance in summer,

only being relatively important for CyanD in winter (Table 3B). In the middle layer, however, biological factors (Chl and Cyan) appeared to be statistically significant throughout the study at the same time that Qs4 and temperature remained important (Table 4B). During summer, cyanobacteria and Qs4 were replaced by ciliates. Moreover, in this middle layer, determination coefficients (r^2) increased considerably when biological variables were added and analysed in tandem with physical variables, whereas in the surface layer (with the exception of winter period, with very low r^2), they were similar in both cases, i.e. with or without biological variables. This fact seems to derive from the varying reactions of heterotrophic bacteria in habitats under different levels of stress from physical factors such as solar radiation, temperature, run-off, upwelling, etc.

It can be concluded that the surface layer was more controlled by physical factors, whereas in the middle layer (bottom of the photic zone) biological factors played a role at least similar to that of physical factors. Solar radiation and water temperature promote bacterial growth, while run-off and strong coastal upwelling (as reflected through density influence) dilute the bacterial population in the ria. A slight positive influence of coastal upwelling on the surface bacterial population can be derived from regression equations (Table 3), probably through both promoting phytoplankton growth and bringing nutrients to the surface layer during summer.

The nature of the strong positive correlation between heterotrophic bacteria and Qs4 is undoubtedly related to increases in phytoplanktonic primary production (cf. highly significant correlation between Chl a and Qs4; $r = 0.56$, $p < 0.001$, $n = 74$), of which ca 30 to 50% is utilised by heterotrophic bacteria in the marine environment (Williams 1981, Azam et al. 1983, Ducklow 1983, Hagstrom 1984). Another factor that may be acting in the ecosystem and which can be invoked to partially explain the correlation between logTC and Qs4 is ultraviolet (UV) radiation. Photolysis by UV-B in sunlight of fluvially derived humic substances (which are rather resistant to microbial breakdown) produces substances such as pyruvate, acetaldehyde, formaldehyde and glyoxylate, which are easily broken down or assimilated by bacteria (Mopper & Zhou 1990). The rate of formation of low molecular weight breakdown products is directly proportional to light intensity at 300 nm and to the concentration of humus; in lakes, this ranges from 7 to 22 mg C m⁻² d⁻¹ (de Haan 1993). Moreover, there are indications that in seas and oceans, photolysis of carbon fixed by phytoplankton is greater (5 to 40%) than the photolysis of humus (Laane et al. 1985).

The availability of large quantities of bacterial biomass as a source of food in pelagic habitats is restricted, above all, to other ecologically important and diverse microbial groups, such as protozooplankton (Wright & Coffin 1984). Important questions remain, however, insofar as how observed relationships between bacteria and protozoan standing stocks can testify to predator-prey dependence in terms of trophic relations. Which is more important in the control of bacterial abundance: bacterivory or losses connected more with hydrology? Gasol & Vaqué (1993) demonstrated a weak relationship between bacterial and nanoflagellate abundance across a wide diversity of aquatic systems. This fits our findings of a lack of significant correlation between DAPI-TC and HNF (Table 1). On the other hand, the significant correlation between DAPI-TC and Cil (Table 1) seems to support their suggestion that organisms other than HNF are important predators of bacteria. A considerable body of experimental evidence indicates that ciliates, and particularly microciliates smaller than 30 μm (Rivier et al. 1985, Rassoulzadegan & Sheldon 1986), constitute a link between dissolved organic carbon, bacteria, and higher trophic levels in aquatic food chains.

Run-off, upwelling and spore-forming bacteria

Aerobic spore-forming bacteria (CFUs) can be passively transported through streams or groundwater to areas in which they can grow. During studies in Antarctica, Zdanowski (1995) found that ca 12.8% of saprophytic bacteria in a glacial stream infiltrating soil and running into Admiralty Bay were halotolerant. Such translocation from soil to the Ría de Vigo can occur during run-off into the drainage basin. Despite their low numbers, the presence of viable spores, especially during periods of high run-off, support the alien status in seawater of this (CFUs) bacterial fraction.

The patterns of correlation between CFUs and physical variables in the surface layer revealed by both simple (Table 2) and multiple stepwise (Table 5) regression differ distinctly to those for TC (Tables 1, 3 & 4). Amongst others, the most prominent contrasts, i.e. positive correlation of CFUs with Qr4 but negative with upwelling and temperature versus negative correlation of TC with Qr4 but positive with upwelling and temperature, emphasises the existence of quite distinct mechanisms driving the development of both bacterial populations.

The highest CFU numbers, recorded in July in the surface layer, should be regarded with special interest as they occurred when all primary and secondary production is usually driven by upwelling. At this time

Table 5. Multiple stepwise regressions between logCFU (spore-forming aerobic bacteria) and Qs4, Temp, Upw, Qr4, and Dens in the surface layer in Ría de Vigo (Stn 3b). Results are presented for data collected during the full period of this study ($n = 34$), and separately for winter ($n = 14$) and summer ($n = 20$); β : standardized coefficient of regression; p : level of significance of the slopes in the regression equations. See

Table 1 for abbreviations

Variables	Winter and summer		Winter		Summer	
	β	p	β	p	β	p
Upw4	-0.55	0.001	-0.35	0.129	-0.61	0.018
Temp	-0.47	0.012	-0.59	0.016		
Qr4	0.39	0.024	0.62	0.014	0.51	0.052
Qs4	0.37	0.076				
Dens					0.38	0.104
Regression equations:						
W&S	logCFU = $0.82 - 2.8 \times 10^{-4}$ Upw4 - 0.077 Temp + 6.3×10^{-3} Qr4 + 0.7×10^{-3} Qs4, $r^2 = 0.56$					
W	logCFU = $1.12 + 6.4 \times 10^{-3}$ Qr4 - 0.089 Temp - 1.4×10^{-4} Upw4, $r^2 = 0.66$					
S	logCFU = $-3.03 - 3.7 \times 10^{-4}$ Upw4 + 0.016 Qr4 + 0.12 Dens, $r^2 = 0.65$					

there is also the greatest bacterial production in the soil, so terrestrial run-off into the drainage basin can inoculate surface waters of the Ría de Vigo with larger numbers of spore-forming bacteria than during the winter.

These findings throw light on the considerable impact of terrestrial run-off in supplying and coastal upwelling in diluting the Ría de Vigo with such a fraction of bacteria as important as the spore formers. We base our hypothesis regarding the potential importance of this fraction on the study of physiological and biochemical properties of isolates, which show extremely high ecophysiological potential (Zdanowski & Figueiras unpubl.). It must be pointed out that in this study only the aerobic, heat-resistant spore-forming bacteria were counted. This study may indicate, however, the possibility of introduction of other bacteria, for example low-thermo-resistant or strictly anaerobic spore-formers, the latter being especially important for sanitary reasons.

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