

Spatial variability in bacterial abundance and other microbial components in the NW Iberian margin during relaxation of a spring upwelling event

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ABSTRACT: The spatial distribution of heterotrophic bacterioplankton abundance (BA) and bacterial colony forming units (CFU) plus additional biological (abundance of autotrophic and heterotrophic pico- and nanoplankton, diatoms and chlorophyll *a* concentration), physical (temperature and salinity) and chemical (nutrient concentrations) variables were investigated along the Galician coast (NW Iberia) after the cessation of a strong spring upwelling event. BA and abundance of autotrophic and heterotrophic pico- and nanoplankton tended to increase with distance from the coast, while diatoms were more abundant near to the coast. Over 70% of the variance in BA could be explained by a regression equation with 3 variables, in which abundance of autotrophic nanoflagellates (61%) and abundance of heterotrophic nanoflagellates (7%) accounted for 68% of the total explained variability. The remaining 2% was related to chlorophyll *a* variations. Variability in CFU abundance (67%) was attributable to a negative relationship with salinity and to a lesser but significant degree by a positive relationship with diatom abundance. These data suggest that a number of mechanisms regulated bacterial abundance in the NW Iberian margin after spring upwelling: CFU was related to continental influence and diatoms, and BA was associated with the biomass of autotrophic nanoflagellates. The relationships between BA, autotrophic nanoflagellates (ANF) and chlorophyll *a* (Chl) in the oceanic samples suggest that a change from bottom-up to top-down control of BA would occur at concentrations higher than 2500 ANF ml⁻¹ and 1.72 mg Chl m⁻³.

KEY WORDS: Bacterial abundance · Phytoplankton · Upwelling relaxation · NW Iberia

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INTRODUCTION

Bacteria are important components of marine ecosystems, as they play a fundamental role in marine biogeochemical cycles by metabolising both autochthonous and allochthonous organic matter and converting particulate organic matter (POM) and dissolved organic matter (DOM) into bacterial biomass (Fuhrman 1992, Smith et al. 1992, del Giorgio & Cole 1998). Despite much recent progress in marine microbiology (Gasol & Duarte 2000, Li 2002, Li et al. 2004) and interest in the importance of microbial food web processes, the mechanisms that determine bacterial abundance in marine waters are still poorly under-

stood. Since coastal upwelling systems are among the areas with the highest POM and DOM production in the ocean (Wollast 1998, Álvarez-Salgado et al. 1999), an analysis of factors that influence bacterial abundance appears fundamental to improving our current understanding of these ecosystems.

Shelf waters of the Galician margin (NW Iberia) experience considerable hydrographic variability due to the influence of upwelling and the presence of 4 deep tectonic bays (Rías Baixas) (Fig. 1). These waters are thus ideal sites for studying factors that may affect bacterial abundance (e.g. Zdanowski & Figueiras 1997, 1999). Production in the NW Iberian margin is largely influenced by upwelling events that enhance primary

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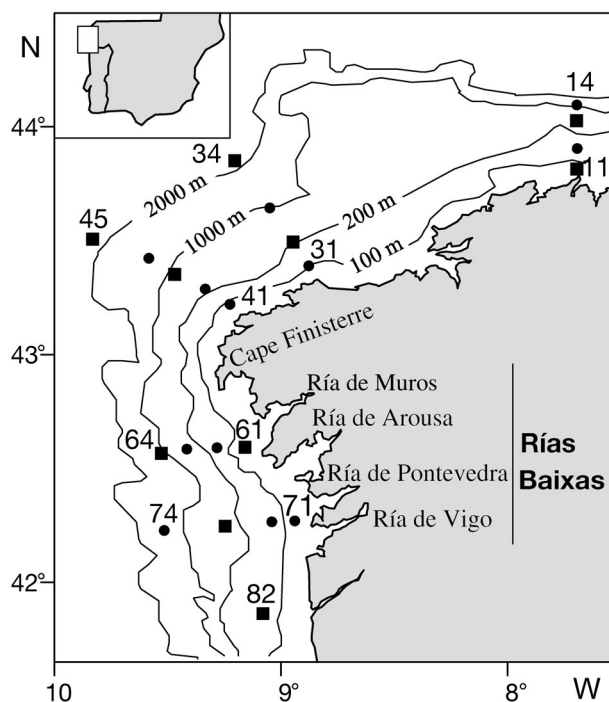


Fig. 1. Location of stations. Black squares represent stations at which colony forming unit (CFU) bacteria were grown. The shelf break coincides approximately with the 200 m isobath

production over the shelf (Tenore et al. 1995, Teira et al. 2001, Joint et al. 2002, Tilstone et al. 2003) and inside the Rías Baixas (Fraga 1976, Hanson et al. 1986, Tilstone et al. 1999, Moncoiffé et al. 2000), leading to the accumulation of DOM in surface waters (Alvarez-Salgado et al. 1999). As particulate and dissolved matter is exported to the shelf from the Rías Baixas (Prego et al. 1990, Alvarez-Salgado et al. 2000), bacterial abundance in the Galician shelf waters can be affected by the import of bacteria from the Rías or by *in situ* production. Bacterial secondary production is also affected by other biota and hydrographic variability in the region (Zdanowski & Figueiras 1997, 1999, Barbosa et al. 2001). Although some aspects of bacterial processes in Galician coastal waters, such as the bacteria response to upwelling pulses and their impact on DOC fluxes have been described (Hanson et al. 1986, Tenore et al. 1995, Barbosa et al. 2001, Morán et al. 2002, Valencia et al. 2003), there are few data relating bacterial abundance to other physical, chemical and biological variables. Bacterial abundance can be affected by the physical (e.g. temperature and salinity), chemical (e.g. nutrient concentrations) and biological (e.g. abundance of other microbial plankton components) environment. Therefore, studies relating these variables to bacterial abundance can provide information about the main factors controlling bacterial populations (Gasol & Duarte 2000, Li et al. 2004, Ning et al. 2005).

The study described here was carried out in Galician shelf and ocean waters in spring after a strong upwelling event had subsided, and coincided with the first phytoplankton blooms of the season. The main objective of this paper is to determine which factors account for distribution of bacterial abundance.

MATERIALS AND METHODS

Horizontal and vertical distributions of total bacterioplankton and the colony forming fraction were investigated off the Galician coast between 42 and 44°N, and from 7 to 10°W (Fig. 1), between 10 and 14 May 1991 during the GALICIA XI cruise aboard the RV 'Investigador-S'. Total bacterial abundance (BA) was determined at 21 stations on 5 cross-shore sections, while colony forming units (CFU) were enumerated at 9 of the 21 stations plus 1 other (Stn 82, Fig. 1). Samples for bacterial work were collected in an ethanol rinsed Van Dorn-type 5 l bottle at 0, 10, 20, 30, 50 and 80 m depth and, exceptionally, at 100 m where depth permitted. Thereafter, subsamples of ~100 ml were immediately transferred to sterile, opaque, screw-capped glass bottles and maintained at ~5°C until processed on board within 1 h.

Samples of 10 ml were fixed with buffered 0.2 µm filtered formaldehyde (2% final concentration) and stained with DAPI at 0.1 µg ml⁻¹ final concentration for 5 min (Porter & Feig 1980), and then filtered through 0.2 µm black Millipore-Isopore filters. The filters were frozen immediately and then analysed by epifluorescence microscopy within 2 mo of collection. BA and the abundance of *Synechococcus* type cyanobacteria (SYN), autotrophic and heterotrophic pico (<2 µm) and nanoflagellates (2 to 20 µm) were determined on the same sample. Autotrophic organisms were enumerated under blue light excitation where SYN were distinguished by their yellow colour and autotrophic pico- (APF) and nanoflagellates (ANF) by red-orange colour. Bacteria and heterotrophic pico- (HPF) and nanoflagellates (HNF) were enumerated under excitation with UV light. We are aware that mixotrophic flagellates could not be reliably identified with these count conditions. At least 300 bacteria and 100 cells of the other microbial components were counted. Diatoms (DIAT) were counted in 100 ml samples fixed with Lugol's iodine using an inverted microscope.

CFU were enumerated in 100 µl of seawater spread on Bacto Marine Agar 2216 (ZoBell 1941). Plates were incubated for up to 15 d at 24°C. From each sampling point, 3 replicates were performed, from which mean CFU abundance was calculated. The coefficient of variation was below 10%.

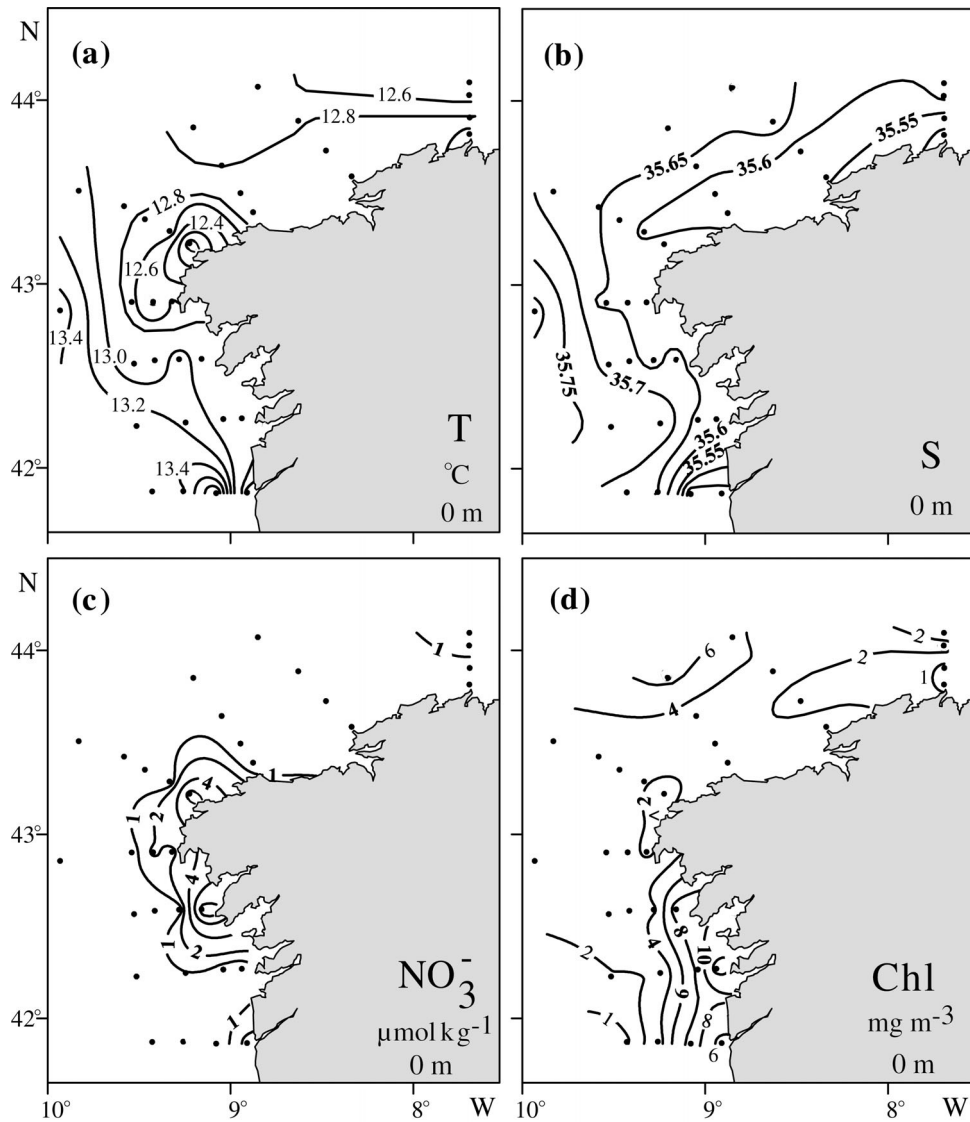


Fig. 2. Horizontal distributions at the sea surface of (a) temperature, (b) salinity, (c) nitrate concentration and (d) chlorophyll concentration

Seawater temperature (°C) and salinity (S) were determined by CTD. Chlorophyll *a* (Chl) concentration was determined by fluorometry (Yentsch & Menzel 1963) after low vacuum pressure filtration of 100 ml seawater samples through 25 mm Whatman GF/F filters and overnight pigment extraction in 90% acetone. Nutrients were measured in an auto-analyser; nitrate (NO₃) after reduction to nitrite (NO₂) through a Cu/Cd column (Hansen & Grasshoff 1983) with modifications after Mouriño & Fraga (1985), silica Si(OH)₄ and phosphates (PO₄) following Hansen & Grasshoff (1983) and ammonium (NH₄) according to Grasshoff & Johansen (1972). Additional CTD casts and Chl and nutrient determinations were conducted at other stations for mapping purposes (Figs. 2 & 3).

General trends in the spatial distribution of the variables along-shore (north to south transects), distance from land (position of the stations in transects) and depth of samples (surface to 100 m) were assessed with the aid of the Spearman rank order correlation analysis using discrete depth data. Product-moment correlation was employed to identify significant relationships among variables. The dependence of bacterial abundance on the other variables was assessed using regression analyses. Multiple regressions were used to identify which variables were most important in regulating BA and CFU variability. For regression analysis, the abundances and Chl values were log-transformed to correct for normal distributions. Multiple regressions

were run in a forward mode and tested at the 5% level of significance.

RESULTS

Hydrography and biology

Hydrographic conditions during the cruise were described by Castro et al. (1994). In summary, relaxation of an upwelling event caused the shoreward migration of surface water from oceanic stations, blocking the outflow of low salinity water (Fig. 2b) with high Chl values (Fig. 2d) from the Rías Baixas. Chl concentrations were also high ($>6 \text{ mg m}^{-3}$; Fig. 2d) at the surface of the oceanic stations in the northwest corner

of the sampling area where nutrients were depleted (Fig. 2c). Upwelling, however, was still detected in the vicinity of Cape Finisterre, where low temperatures, high nutrient and relatively low Chl concentrations were recorded in surface waters (Fig. 2a,c,d). Clear signals of intense upwelling remained at 50 m along a narrow band near the coast as indicated by temperature and nitrate concentration (Fig. 3a,b).

Biological, physical and chemical variables in the upper 100 m of the water column are presented in Table 1. Biological and chemical variability was high during the 4 d cruise. The greatest variability in the biological components occurred in diatom abundance (coefficient of variation, CV = 703%). Variability was also high (CV > 100%) for CFU abundance, other autotrophic components (SYN, APF, ANH) and Chl, while BA and HPF showed lower variation (CV < 42%). Variability of HNF (CV = 65%) was moderate. The range of variation of temperature was small ($<2^\circ\text{C}$) due to mixing forced by upwelling in a water column that was still weakly stratified. Salinity also showed little variability. Nutrient variability was influenced by the presence of upwelled waters at ~50 m with high nutrient levels (Fig. 3b) and their exhaustion in the surface (Fig. 2b).

Biological variability was not only due to variations with depth, since cross-shore and along-shore variations were considerable (Fig. 4). APF and ANF, which were positively correlated ($r = 0.86$, $p < 0.001$), were

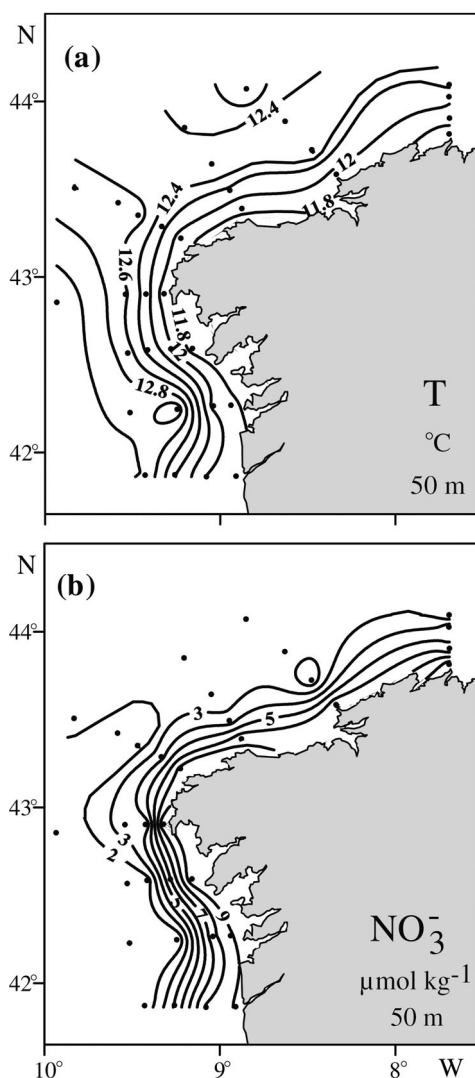


Fig. 3. Horizontal distributions at 50 m depth of (a) temperature and (b) nitrate concentration

Table 1. Mean, standard deviation (SD), and minima and maxima of bacterial abundance (BA), colony forming unit bacteria (CFU), diatoms (DIAT), *Synechococcus* 'type' cyanobacteria (SYN), autotrophic pico- and nanoflagellates (APF, ANF), heterotrophic pico- and nanoflagellates (HPF, HNF), chlorophyll *a* concentration (Chl), seawater temperature (T), salinity (S) and concentrations of ammonium (NH_4), nitrate (NO_3), phosphate (PO_4) and silica $\text{Si}(\text{OH})_4$ during the cruise GALICIA XI. CFU: $n = 35$, DIAT: $n = 147$, other variables: $n = 148$

Variable	Mean \pm SD	Minimum	Maximum
BA ($\times 10^6 \text{ ml}^{-1}$)	1.5 ± 0.6	0.5	2.8
CFU ($\times 10^2 \text{ ml}^{-1}$)	5.04 ± 8.21	0.62	43.47
DIAT ($\times 10^2 \text{ ml}^{-1}$)	12.22 ± 85.9	0	889.32
SYN ($\times 10^2 \text{ ml}^{-1}$)	16.43 ± 22.22	0.19	86.17
APF ($\times 10^2 \text{ ml}^{-1}$)	43.62 ± 44.04	0.93	259.33
ANF ($\times 10^2 \text{ ml}^{-1}$)	28.49 ± 28.91	0.18	147.38
HPF ($\times 10^2 \text{ ml}^{-1}$)	85.59 ± 35.65	14.29	188.13
HNF ($\times 10^2 \text{ ml}^{-1}$)	24.98 ± 16.23	2.6	79.62
Chl (mg m^{-3})	2.13 ± 2.18	0.07	12.71
T ($^\circ\text{C}$)	12.4 ± 0.4	11.5	13.3
S	35.653 ± 0.051	35.490	35.762
NH_4 ($\mu\text{mol kg}^{-1}$)	0.84 ± 0.37	0.26	2.05
NO_3 ($\mu\text{mol kg}^{-1}$)	3.13 ± 3.13	0	10.48
PO_4 ($\mu\text{mol kg}^{-1}$)	0.24 ± 0.19	0.036	0.67
$\text{Si}(\text{OH})_4$ ($\mu\text{mol kg}^{-1}$)	1.86 ± 1.10	0.34	5.15

more abundant in the open ocean than in shelf waters, and in the northern than in the western oceanic stations, where they significantly contributed to the high Chl values found there (see also Fig. 2d). SYN (data not shown), which were positively correlated with APF

and ANF ($r \geq 0.79$, $p < 0.001$) showed a similar distribution to that of APF + ANF. High Chl concentrations in the shelf waters in general (Fig. 4) and in front of the Rías Baixas in particular (Fig. 2) were due to diatoms (Fig. 4). Heterotrophs (HPF, HNF and bacteria) were

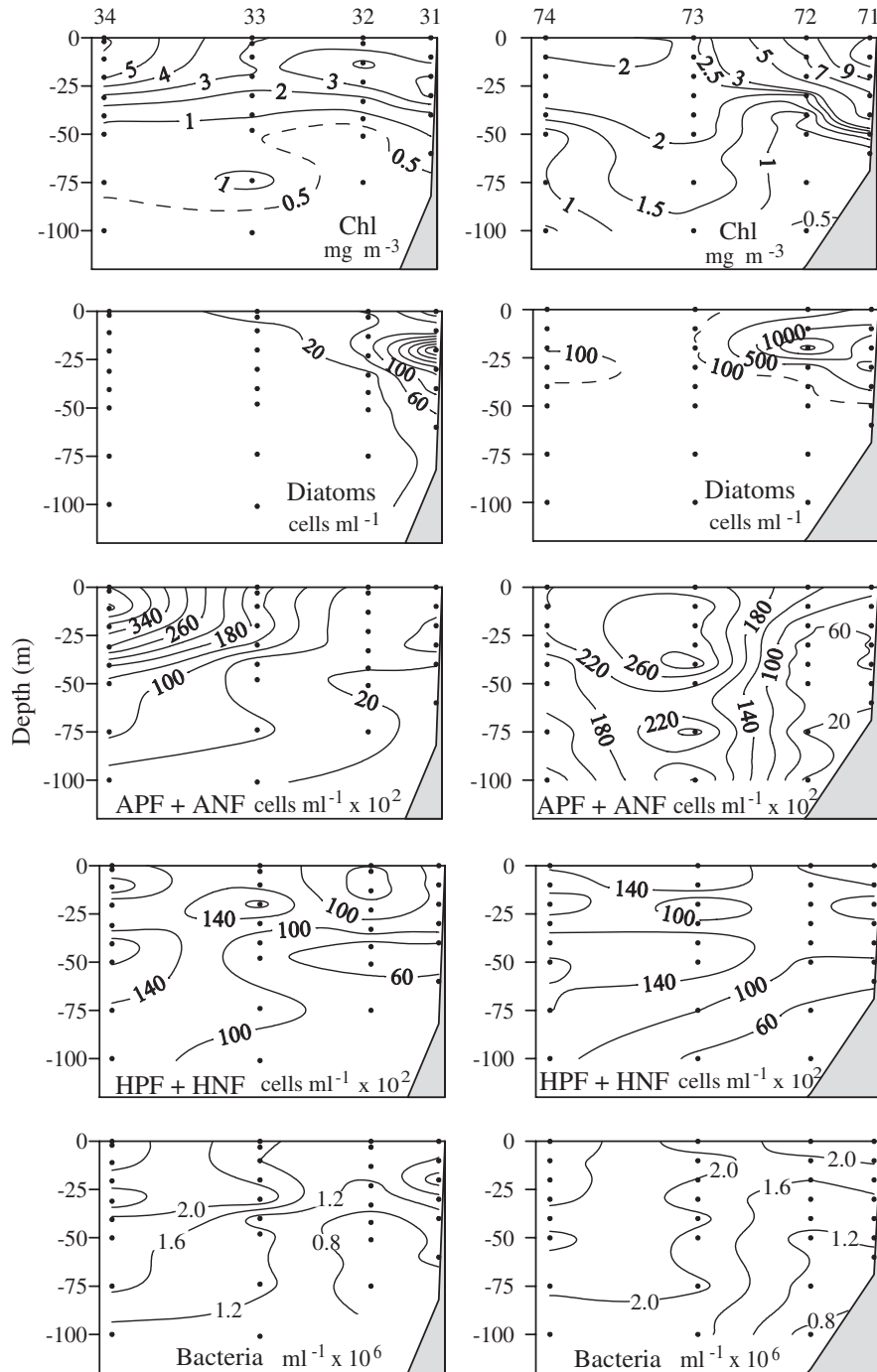


Fig. 4. Vertical distributions of chlorophyll a concentration (Chl) and abundances of diatoms, autotrophic pico- and nanoplankton (APF + ANF), heterotrophic pico- and nanoplankton (HPF + HNF) and bacteria, along 2 cross-shelf transects in the northern (Stns 31 to 34) and in the western (Stns 71 to 74) coast

more uniformly distributed than autotrophs (APF + ANF) (Fig. 4). HPF and HNF were positively correlated ($r = 0.52$, $p < 0.001$). In contrast, CFU (data not shown) showed higher abundance in coastal waters.

Table 2. Spearman rank order correlation matrix for the relationship between biological, physical and chemical variables with sampling points from north to south transects, distance from land and depth (surface to 100 m) during the cruise GALICIA XI. Variables: BA (bacterial abundance), CFU (colony forming bacteria), DIAT (diatoms), SYN (*Synechococcus* type cyanobacteria), APF (autotrophic picoflagellates), ANF (autotrophic nanoflagellates), HPF (heterotrophic picoflagellates), HNF (heterotrophic nanoflagellates), Chl (chlorophyll *a* concentration), T (seawater temperature), S (salinity), NH₄ (ammonium), NO₃ (nitrate), PO₄ (phosphate), Si(OH)₄ (silica). CFU: $n = 35$, DIAT: $n = 147$, other variables: $n = 148$. Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Variable	Transect (N-S)	Distance from land	Depth
BA	0.42***	0.42***	-0.42***
CFU	-0.38*	-0.78***	-0.12
DIAT	0.33***	-0.57***	-0.38***
SYN	0.22**	0.66***	-0.17**
APF	0.26**	0.57***	-0.34***
ANF	0.18*	0.49***	-0.47***
HPF	0.04	0.32***	-0.22**
HNF	0.25**	0.27**	-0.34***
Chl	0.26**	0.01	-0.71***
T	0.23**	0.44***	-0.58***
S	0.60***	0.55***	0.24**
NH ₄	0.06	0.25**	0.27***
NO ₃	0.02	-0.29***	0.66***
PO ₄	-0.03	-0.28***	0.67***
Si(OH) ₄	-0.21*	-0.24**	0.45***

The results of the Spearman rank order correlation analysis (Table 2) summarise these general trends in the distribution of the variables. BA, diatoms and salinity tended to increase from north to south. Six variables (SYN, APF, ANF, HNF, Chl and temperature) also shared a positive trend to increase from north to south, while CFU and Si(OH)₄ showed a negative trend (Table 2). Regardless of this along-shore gradient, coastal-ocean gradients were more obvious (Table 2). Whilst BA tended to increase with distance from the shore, CFU and diatoms decreased with distance from the coast. The rest of the biological variables, with the exception of Chl, also increased with distance from land. Temperature and salinity also increased with distance from the coast, while nutrients decreased; except for ammonium. BA and most of the biological variables decreased with depth. The uniform vertical distribution of CFU is remarkable. Salinity and nutrient concentrations increased and temperature decreased with depth.

Relationships between bacteria and other biological and environmental variables

Significant positive product-moment correlations (Table 3) were found between BA and pico-nano-plankton components plus Chl, temperature and salinity. Pico-nanoplankton and Chl were also positively correlated with temperature. Nutrients, ammonium excluded, showed negative correlations with BA, pico-nanoplankton and Chl. All pico-plankton components showed positive correlations among them. Diatoms, which were only weakly correlated with Chl and

Table 3. Pearson product-moment correlation matrix of biological, physical and chemical variables. BA (bacterial abundance), CFU (colony forming bacteria), DIAT (diatoms), SYN (*Synechococcus* type cyanobacteria), APF (autotrophic picoflagellates), ANF (autotrophic nanoflagellates), HPF (heterotrophic picoflagellates), HNF (heterotrophic nanoflagellates), Chl (chlorophyll *a* concentration), S (salinity), T (seawater temperature), NH₄ (ammonium), NO₃ (nitrate), PO₄ (phosphate), Si(OH)₄ (silica). CFU: $n = 35$, DIAT: $n = 147$, other variables: $n = 148$. Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	BA	CFU	DIAT	SYN	APF	ANF	HPF	HNF	Chl	S	T	NH ₄	NO ₃	PO ₄
CFU	-0.15													
DIAT	0.04	0.38*												
SYN	0.66***	-0.37*	-0.05											
APF	0.66***	-0.33	-0.01	0.85***										
ANF	0.71***	-0.25	0.04	0.79***	0.86***									
HPF	0.43***	0.05	0.04	0.16*	0.19*	0.27**								
HNF	0.50***	0.09	0.09	0.24**	0.25**	0.26**	0.52***							
Chl	0.47***	0.26	0.20*	0.10	0.35***	0.38***	0.19*	0.27**						
S	0.44***	-0.53**	-0.01	0.69***	0.54***	0.42***	0.06	0.12	0.00					
T	0.72***	-0.07	0.04	0.61***	0.57***	0.62***	0.35***	0.55***	0.33***	0.28***				
NH ₄	-0.05	-0.05	-0.17*	0.13*	0.06	-0.02	0.01	0.04	-0.16*	0.22**	0.01			
NO ₃	-0.60***	0.16	-0.08	-0.42***	-0.46***	-0.52***	-0.38***	-0.46***	-0.28***	-0.02	-0.86***	-0.02		
PO ₄	-0.62***	0.13	-0.10	-0.41***	-0.47***	-0.53***	-0.40***	-0.49***	-0.35***	-0.01	-0.86***	0.05	0.99***	
Si(OH) ₄	-0.54***	0.10	-0.13	-0.28***	-0.34***	-0.35***	-0.37***	-0.48***	-0.31***	-0.06	-0.78***	0.06	0.91***	0.92***

Table 4. Significant regressions ($p < 0.001$) relating bacterial abundance (BA, cells ml^{-1}) to other biological, physical and chemical variables. SYN (*Synechococcus* type cyanobacteria), APF (autotrophic picoflagellates), ANF (autotrophic nanoflagellates), HPF (heterotrophic picoflagellates), HNF (heterotrophic nanoflagellates), Chl (chlorophyll a concentration), S (salinity), T (seawater temperature), NO_3 (nitrate), PO_4 (phosphate), $\text{Si}(\text{OH})_4$ (silica). Cell abundance, cells ml^{-1} ; Chl, mg m^{-3} ; nutrients, $\mu\text{mol kg}^{-1}$. Cell abundances and Chl were log-transformed to compute regressions statistics. Number of samples = 148. The model II slope provides an estimate of the true slope when there is error in the independent variable

Independent variable	r^2	Slope \pm SE	Y-int. \pm SE	Model II slope
logSYN	0.45	0.19 ± 0.02	5.62 ± 0.05	0.28
logAPF	0.59	0.32 ± 0.02	5.04 ± 0.08	0.42
logANF	0.61	0.28 ± 0.02	5.25 ± 0.06	0.36
logHPF	0.22	0.44 ± 0.07	4.41 ± 0.27	0.94
logHNF	0.38	0.41 ± 0.04	4.78 ± 0.14	0.67
logChl	0.48	0.28 ± 0.02	6.10 ± 0.1	0.40
S	0.16	1.57 ± 0.3	-49.93 ± 10.64	3.93
T	0.51	0.33 ± 0.03	2.01 ± 0.34	0.46
NO_3	0.38	-0.04 ± 0.004	6.25 ± 0.02	-0.06
PO_4	0.40	-0.68 ± 0.07	6.30 ± 0.02	-1.09
$\text{Si}(\text{OH})_4$	0.31	-0.10 ± 0.01	6.32 ± 0.3	-0.18

ammonium, showed positive correlation with CFU. Salinity and SYN were negatively correlated with CFU.

Although regressions between BA and each one of the correlated variables were significant (Table 4), forward multiple stepwise regression identified only 3 significant biological variables (ANF, HNF, and Chl) that explained 70% of the BA variability (Table 5a). ANF alone accounted for 61% of the BA variability, while HNF and Chl explained 7 and 2%, respectively. Temperature, however, which alone accounted for 51% of the BA variability (Table 4), did not appear as a significant factor in the multiple regression. As BA could be regulated by the resources supplied by autotrophs and predation by HNF as suggested by the multiple regression, single pairs of the relationship between BA and the significant variables in the multiple regression were explored using the original non-transformed data. The relationships between BA and ANF and HNF were exponential, rising to a maximum value (Fig. 5a,b). BA and Chl showed 2 types of relationships (Fig. 5c); exponential for the ocean stations and linear for coastal waters. From the relationship between ANF and Chl (Fig. 5d) it can be deduced that the contribution of ANF to Chl was 8 times higher in the ocean with the absence of diatoms (2.14×10^9 ANF mg Chl^{-1}) than in the coastal waters with high diatom abundance (0.27×10^9 ANF mg Chl^{-1}).

The explained variability in CFU abundance (67%) could be described by a negative relationship with

Table 5. Significant multiple stepwise regressions of (a) logarithm of bacterial abundance (LogBA) and (b) logarithm of colony forming units (LogCFU). β : standardised coefficient of regression, p : level of significance of the slopes in the regression equation. LogANF (logarithm of ANF abundance, cells ml^{-1}), LogHNF (logarithm of HNF abundance, cells ml^{-1}), LogChl (logarithm of chlorophyll a concentration, mg m^{-3}), S (salinity), LogDiat (logarithm of diatom abundance, cells ml^{-1})

Variable	β	p	% explained variance
(a)			
LogBA (n = 148)			$r^2 = 0.70$, $p < 0.001$
LogANF	0.49 ± 0.07	< 0.001	61
LogHNF	0.27 ± 0.05	< 0.001	7
LogChl	0.21 ± 0.07	< 0.01	2
(b)			
LogCFU (n = 35)			$r^2 = 0.67$, $p < 0.001$
S	-0.53 ± 0.11	< 0.001	45
LogDiat	0.49 ± 0.11	< 0.001	22

salinity (45%), and to a lesser extent (22%) by a positive relationship with diatom abundance (Table 5b). Single relationships (Fig. 6a,b) accounted for similar percentages (40 to 45%) of CFU variability.

DISCUSSION

In general, estimates of BA averaging 10^6 ml^{-1} are similar to those found in shelf waters off the NW Iberian margin under upwelling conditions (Barbosa et al. 2001, Morán et al. 2002, Valencia et al. 2003) and in the Ría de Vigo (Zdanowski & Figueiras 1997, Morán et al. 2002). Conversely, estimates of CFU at each sampling point averaged 10^2 ml^{-1} , much lower (10 to 10^2 times) than in the Ría de Vigo in general (Zdanowski & Figueiras 1999). Total bacterial numbers (BA) varied less than CFU, as previously reported for the estuarine Ría de Vigo (Zdanowski & Figueiras 1997, 1999).

Control of heterotrophic bacterioplankton abundance

The cause of variations in bacterial biomass in the ocean is a controversial question, since both bottom-up and top-down control mechanisms might act simultaneously at different rates. Bacterial growth can be regulated by temperature and the availability of organic and inorganic nutrients (Cole et al. 1988, Currie 1990, White et al. 1991, Barbosa et al. 2001), whereas predation and viral lyses limit biomass (Sherr & Sherr 1987, Weimbauer & Peduzzi 1995, Proctor & Fuhrman 1999). Studies of factors regulating bacterial production in the Equatorial Pacific Ocean (Kirchman & Rich 1997) sug-

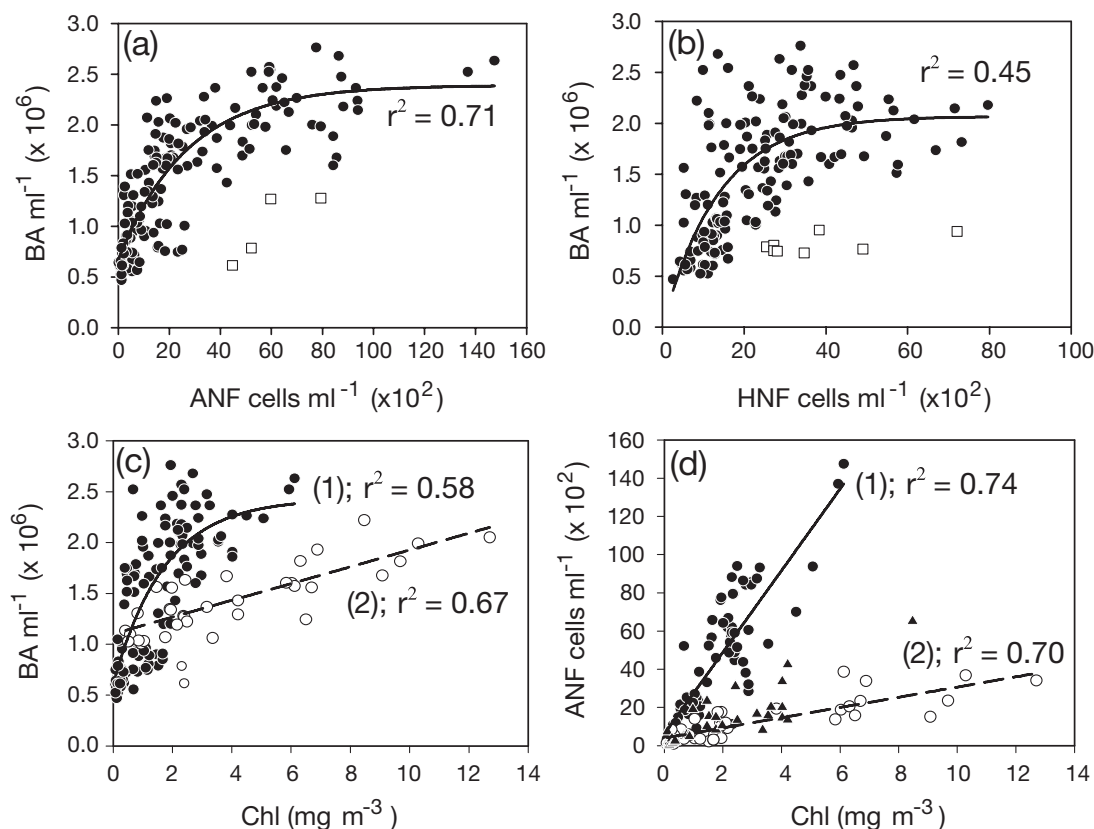


Fig. 5. Scatter plots of (a) ANF abundance vs. bacterial abundance, (b) HNF abundance vs. bacterial abundance, (c) chlorophyll *a* concentration vs. bacterial abundance and (d) chlorophyll *a* concentration vs. ANF abundance. Exponential relationships in (a) and (b) without the points marked with open squares are $Y = (0.65 \pm 0.07) + (1.64 \pm 0.09)[1 - \exp(-0.04 \pm 0.007)X]$ and $Y = (2.07 \pm 0.09)[1 - \exp(-0.07 \pm 0.009)X]$ respectively; the equations including these points, $Y = (0.65 \pm 0.08) + (1.52 \pm 0.1)[1 - \exp(-0.05 \pm 0.008)X]$, $r^2 = 0.62$ for (a) and $Y = (1.91 \pm 0.009)[1 - \exp(-0.08 \pm 0.01)X]$, $r^2 = 0.34$ for (b), are not substantially different. There were no differences between coastal and ocean samples in (a) and (b). In (c) and (d) (○) = coastal stations; (●) = ocean stations. Fitted lines in (c) are (1): $Y = (0.61 \pm 0.11) + (1.83 \pm 0.20)[1 - \exp(-0.58 \pm 0.17)X]$ and (2): $Y = (1.10 \pm 0.05) + (0.08 \pm 0.01)X$. Fitted lines in (d) are (1): $Y = (6.22 \pm 3.30) + (21.36 \pm 1.49)X$ and (2): $Y = (3.99 \pm 1.37) + (2.67 \pm 0.3)X$. Linear regressions in (d) correspond to ocean samples (●) with dominance of ANF and coastal samples (○) with dominance of diatoms; (▲) = samples that were not used for computations owing to no clear dominance of ANF or diatoms. Exponential relationships in (a), (b) and (c) $Y = Y_0 + a(1 - e^{-bX})$ allow estimation of the initial slopes for $X_{\rightarrow 0} = a \times b$, and the X value for saturated Y , $X_s = 1/b$

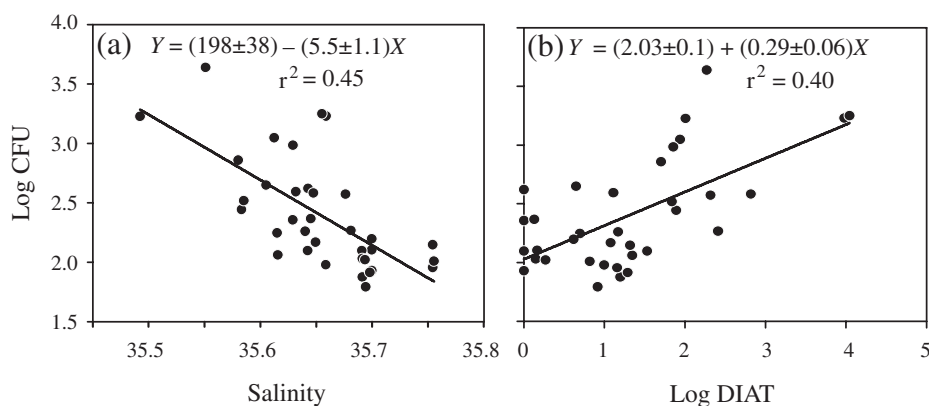


Fig. 6. (a) Salinity and (b) log of diatom abundance vs. log of abundance of colony forming units (CFU). Equations are linear regressions ($p < 0.001$)

gest that both temperature and DOM may control bacterial abundance; temperature affects the response time and affinity of bacteria for DOM. Donachie et al. (2001) showed that uptake rates of DOM are also affected by the type of available nitrogen. Ammonium significantly enhanced glucose uptake.

BA and abundance of most biological groups showed a tendency to increase southward and with distance from the shore (Table 2). Although greater accumulation of bacteria towards the south along the Galician coast may, in part, result from the offshore export of high primary production within the Rías Baixas, which is then advected south (Castro et al. 1994, 2000), the highest observed variability in bacterial abundance was related with coastal-ocean gradients. This might be related to the different aging of plankton communities along these coastal-ocean gradients, with fresh phytoplankton populations dominating in coastal waters where the influence of upwelling was stronger and the presence of a more evolved community offshore where trophic relationships would attain higher importance.

Although we found significant relationships between BA and several variables (Table 4), which could suggest that BA was regulated simultaneously by temperature and bottom-up (the relationships with phytoplankton components and Chl) and top-down processes (the relationships with HPF and HNF), the strong covariation among variables (Table 3) impedes such a direct explanation. The most obvious case was temperature, which accounted for 51% of the BA variability (Table 4) but also showed a positive covariation with almost all biological variables, and a negative covariation with nutrients (Table 3). Although temperature did not appear to be a significant factor in the explained variability in BA (Table 5), it may have indirectly affected variations in BA by promoting phytoplankton growth through the incipient stratification of surface waters, which then caused nutrient depletion.

The significant relationship between BA and phytoplankton (ANF, Chl) and HNF (61% of the BA variability explained by ANF; Table 5) suggests that bacterial abundance in the NW Iberian margin in spring during the cruise GALICIA XI was largely controlled by resource availability. Grazing control on bacterial abundance by HNF, as suggested by the multiple regression and by the relationship between HNF and BA (Fig. 5b), should be of lesser importance than the supply of resources, since only 7% of the BA variability could be explained by HNF in the multiple regression. Resources would be mainly supplied by piconanophytoplankton with lesser contribution by diatoms, as indicated by the slopes of the 2 equations describing the relationship between BA and Chl (Fig. 5c; 1.06×10^{12} bacteria mg Chl⁻¹ for ocean sam-

ples and 0.08×10^{12} bacteria mg Chl⁻¹ for coastal samples). These slopes suggest that ocean waters, where ANF dominated, were 10 times more efficient than coastal waters, with their diatom dominance, at supplying resources for bacterial growth. Although it could be argued that the lower slope might result from stronger grazing pressure on coastal bacteria (Barbosa et al. 2001), the relationship between HNF and BA when analysed for these samples separately ($\log BA = 4.76 \pm 0.19 + 0.41 \pm 0.06 \log HNF$) did not change significantly from that obtained from all samples (Table 4).

This may, then, suggest that there is a higher availability of resources for bacteria in the open ocean waters than in coastal waters. Resources would be supplied directly by phytoplankton through DOM exudation (Teira et al. 2001, Morán et al. 2002) and/or indirectly through DOM generated from trophic relationships (Joint et al. 2001). Higher ammonium and lower nitrate concentration at the oceanic stations (Table 2, Fig. 2c), where pico- and nanophytoplankton were more abundant, suggests that recycling of organic matter was in fact taking place. Recycling processes would partially occur by grazing, which in turn is considered an important source of DOM (Strom et al. 1997, Nagata 2000). In contrast, new nutrients injected by upwelling to coastal waters (Table 2, Fig. 2c, Fig. 3) would have favoured diatom growth over heterotrophy. Recently, Ning et al. (2005) also reported analogous results for the South China Sea, where they found a stronger coupling between bacterioplankton and SYN in offshore than in coastal waters.

Constant bacterial numbers around 2 to 2.5×10^6 bacteria ml⁻¹ at abundances higher than 2500 ANF ml⁻¹ (equation in Fig. 5a) and Chl concentrations higher than 1.72 mg m⁻³ in the ocean stations (equation 1 in Fig. 5c) correspond to those reported by Li et al. (2004) as the upper macroecological limit of bacterial abundance in the ocean, beyond which a transition from bottom-up to top-down control of BA would be expected. The exponential relationship between BA and HNF (Fig. 5b), with BA remaining virtually constant at $\sim 2 \times 10^6$ bacteria ml⁻¹ for the highest HNF abundances, suggests that at these HNF abundances all bacterial production would be removed by HNF. This would indicate a possible transition towards top-down control of bacteria at the highest BA. The same inference can be attained when the relationship between BA and HNF is analysed in the framework of the model proposed by Gasol (1994). According to this model, the importance of the bottom-up/top-down control of HNF/BA would be greater for a shorter distance between the actual position of the points and a line defining the maximum attainable HNF abundance (MAA) for a given BA. Fig. 7 shows this relationship, in

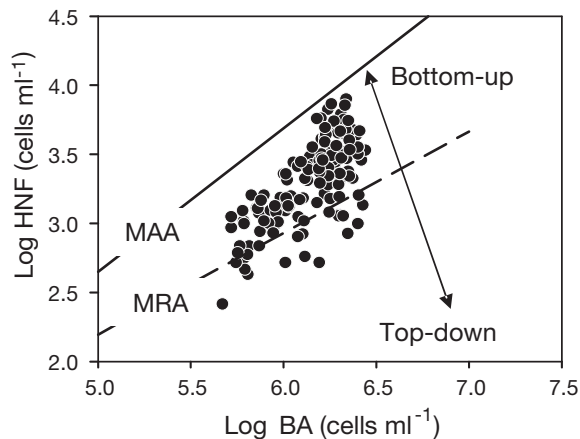


Fig. 7. Log of bacterial abundance (BA) vs. log of HNF abundance depicted in the framework proposed by Gasol (1994) for the assessment of top-down vs. bottom-up control of HNF abundance. MAA and MRA lines correspond to maximum attainable abundance and maximum realised abundance of HNF, respectively. Points close to the MAA line would indicate bottom-up control of HNF abundance and therefore top-down control of BA. Points on the MRA line and far below this line would point to a higher importance of top-down/bottom-up control of abundances of HNF/bacteria

which a shift in the relative position of the points is evident. Thus, while the points that defined low bacteria and HNF abundances situated well below the MAA line, several points that corresponded to high BA were placed close to it. This suggests a greater importance of top-down control of bacteria for the highest BA (Gasol 1994). Regardless of this, the use of resources other than bacteria by HNF (Gasol & Vaqué 1993, Tranvik et al. 1993) would also result in the points being closer to, or even above, the MAA line, implying that bacteria were controlled by factors other than HNF grazing. Although temperature can effectively limit bacterial growth through affecting the affinity of bacteria for DOM (Kirchman & Rich 1997), the action of grazers other than HNF and viral lyses should not be disregarded. Phagotrophy by ANF, which is usually more important at high bacterial densities (Sanders et al. 1990) and during low nutrient levels (Arenovski et al. 1995), would also contribute to this control of bacteria at these high abundances.

Control of CFU abundance

Comparing CFU with physical and biological variables revealed that low seawater salinity and high diatom abundance accounted for 67% of the variance in CFU. A decreasing shoreward trend in salinity might indicate the importance of terrestrial runoff in supplying the inshore with fresh water and fertilizers,

as well as with a significant fraction of halotolerant soil bacteria such as spore formers in the Ría de Vigo (Zdanowski & Figueiras 1997). However, CFU were more abundant in coastal waters where large diatoms dominated the phytoplankton in response to stronger upwelling. Previous studies in the Ría de Vigo (Zdanowski & Figueiras 1999) have also shown positive relationships between CFU and upwelling, when diatoms dominated the phytoplankton.

Although CFU is only a small fraction of total heterotrophic bacterioplankton (Table 1), Zdanowski & Donachie's (1993) experiments of seawater enrichment with particulate matter (detritus) showed a significant increase of the CFU:BA ratio. Thus a high abundance of CFU might indicate that bacteria are more able to exploit organic matter aggregates. The correlation of CFU with diatom abundance would therefore not be surprising, since large diatoms during spring or upwelling conditions may also form aggregates. We could conclude after Unanue et al. (1992) that CFU abundance follows patterns that differ from those of total bacterioplankton, which co-vary with DOM, while the number of CFU depends on the concentration of large particles of organic matter and less on changes in DOM.

CONCLUSIONS

Bacterial abundance in the NW Iberian margin in spring appeared to be largely controlled by the resources provided by phytoplankton, specifically from autotrophic nanoflagellates. Though diatoms had a lesser importance than autotrophic nanoflagellates in supplying resources for heterotrophic bacterioplankton, they showed a conspicuous relationship with colony forming units, which would suggest an association between diatoms and those bacteria that are more able to degrade large particulate matter. The relationship between the abundances of total heterotrophic bacterioplankton and autotrophic nanoflagellates in the oceanic waters suggested a shift from bottom-up to top-down control of bacterial abundance at autotrophic nanoflagellate abundances higher than 2500 cells ml⁻¹. This shift occurred at a Chl *a* concentration of approximately 2 mg m⁻³.

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