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Bacterial survival governed by organic carbon release from senescent oceanic phytoplankton

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Abstract

Bacteria recycle vast amounts of organic carbon, playing key biogeochemical and ecological roles in the ocean. Bacterioplankton dynamics are expected to be dependent on phytoplankton primary production, but there is a high diversity of processes (e.g. sloppy feeding, cell exudation, viral lysis) involved in the transference of primary production to dissolved organic carbon available to bacteria. Here we show cell survival of heterotrophic bacterioplankton in the subtropical Atlantic Ocean to be determined by phytoplankton extracellular carbon release (PER). PER represents the fraction of primary production released as dissolved organic carbon, and changes in the PER variability was explained by phytoplankton cell death, with the communities experiencing the highest phytoplankton cell mortality showing a larger proportion of extracellular carbon release. Both PER and the percent of dead phytoplankton cells increased from eutrophic to oligotrophic waters, while heterotrophic bacteria communities, including 60 to 95 % of living cells (%LC), increased from the productive to the most oligotrophic waters. The percentage of living heterotrophic bacterial cells increased with increasing phytoplankton extracellular carbon release, across oligotrophic to productive waters in the NE Atlantic, where lower PER have resulted in a decrease in the flux of phytoplankton DOC per bacterial cell. The results highlight phytoplankton cell death as a process influencing the flow of dissolved photosynthetic carbon in the NE Atlantic Ocean, and demonstrated a close coupling between the fraction of primary production released and heterotrophic bacteria survival.

1 Introduction

Heterotrophic bacteria (HB) play a key ecological role in the cycling of carbon and nutrients in aquatic systems (Cole et al., 1988; Fuhrman, 1992; Ducklow, 2000) been the major consumers of dissolved organic matter (DOM) in the ocean (Sherr and Sherr, 1994; Azam, 1998). HB recycle organic carbon through respiratory processes and

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channel significant amounts of dissolved organic carbon (DOC) to higher levels of the pelagic food webs via the microbial loop (Williams and Le, 1981; Azam et al., 1983; Sherr and Sherr, 1988). The availability of DOC is a major constraint for heterotrophic bacterial dynamics, influencing a range of processes including HB growth efficiency, respiration or cell activity (Kirchman et al., 1991; Carlson and Ducklow, 1996; Herndl et al., 1997; Kirchman, 1997; Kirchman et al., 2004). Indeed, a high percentage of bacterial cells are either metabolically inactive or dead in natural marine plankton communities (Choi et al., 1996; Smith and del Giorgio, 2003).

Phytoplankton, in turn, is the main source of DOC to support bacterial dynamics, linking phytoplankton and bacterial dynamics in the ocean. Phytoplankton release DOC as a result of cell lysis or direct exudation (Nagata, 2000), and about 50 % of daily primary production can be released by phytoplankton as DOC (Karl et al., 1998), potentially providing a source of carbon to HB. Extracellular release or production of dissolved organic carbon (P_{DOC}) by phytoplankton is a process mostly dependent on phytoplankton health (Fogg, 1977; Sharp, 1977), which is mediated by nutrient availability (Myklestad, 1977; Obernosterer and Herndl, 1995), incident UV and PAR radiation (Berges and Falkowski, 1998; Llabrés and Agustí, 2006), and viral infection (Mühling et al., 2005). Phytoplankton cell death results in cell lysis (Brussaard et al., 1995; Agustí et al., 1998; Agustí and Duarte, 2000) and would lead to the release of recently labelled carbon compounds. Veldhuis et al. (2001) reported that senescent or dying cells, meaning with a reduced viability (increased membrane permeability) still photosynthesized although photosynthetic activity dropped by as much as 60 % relative to that of the viable cells. Recent studies provided evidences that increasing phytoplankton mortality in oligotrophic waters leaded to increasing P_{DOC} among senescent or dying natural populations (Agustí and Duarte, 2013; Lasternas et al., 2013), accounting thus for the large fraction of the photosynthetic carbon channelled through bacteria characteristic of oligotrophic marine communities. This subsequent release of the cellular contents could play, therefore, an important role in driving P_{DOC} and the associated DOC supply to bacteria. Yet, the possible relationship between phytoplankton cell status and P_{DOC},

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on the one hand, and the status of heterotrophic bacterial cells, on the other, has not yet been tested.

The status bacterial cells depends on a large number of processes but is ultimately dependent on the functioning of membrane proton pumps and the integrity of the cell membrane that indeed defines the viability of bacteria (Shapiro, 2008). The analysis of bacterial cell-membrane integrity allows the discrimination between living and dying cells and has been introduced in recent studies assessing the environmental factors driving bacterial survival (Alonso-Sáez et al., 2007; Gasol et al., 2009; Morán and Calvo-Diaz, 2009; Lasternas et al., 2010). These new approaches allow the characterisation of bacterial status at the individual-cell level and offer, as identified by Gasol et al. (2008), great potential to further our understanding on the variability of bacterial activity in aquatic systems, beyond the insights derived from previous approaches based on the examination of bulk assemblage properties.

Here we examine the status and survival of heterotrophic bacteria in the subtropical Atlantic and test their hypothesised relationships with the status of photosynthetic plankton cells and the release of dissolved organic carbon. Phytoplankton and bacteria cell health status were investigated by quantifying the percentage of living and dying cells in communities across a range of oceanographic conditions from highly oligotrophic to productive waters in the NE subtropical Atlantic.

2 Materials and methods

2.1 Study site and sampling

This study was conducted in the subtropical NE Atlantic Ocean section during the RODA 2 cruise on board R/V *Hespérides*, from 2 February to 27 February of 2007. A total of 24 stations were sampled, nine stations in the north Atlantic gyre area (Zone 1), eight placed in the vicinity of the Canary Current region (Zone 2) and eight stations in the area influenced by the Mauritania's upwelling (Zone 3; Fig. 1). At each station,

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vertical profiles of temperature, salinity, and fluorescence down to 200 m depth were performed using a Seabird 911 Plus conductivity-temperature-depth (CTD) system. Seawater samples were collected in 12 L Niskin bottles mounted on a General Oceanics rosette sampler from 7 depths from the surface to 200 m. Samples for nutrient analysis (phosphate, nitrate + nitrite, ammonium and silicate) were collected down to 200 m. Samples for the determination of the dissolved inorganic phosphate concentrations and the nitrate + nitrite concentrations were kept frozen until analyzed in a Bran + Luebbe AA3 autoanalyzer following standard spectrophotometric methods (Hansen and Koroleff, 1999) and ammonium concentrations were measured spectrofluorometrically within 1 h of collection following Keìrouel and Aminot (1997).

2.2 Primary production and percentage of extracellular release (PER)

In situ total and particulate primary production (TPP and PPP) was measured using 14C additions (Steemann-Nielsen, 1952). Seawater sampled at 5 depths including the surface (5 m), two intermediate depths, the DCM and an ultimate depth below the DCM, was delivered into transparent (light) and obscure (black masking tape-covered) polycarbonate bottles (150 mL), and inoculated with 80 µCi activity of a $NaH^{14}CO_3$ working solution. Inoculated bottles were set up at respective depths along a mooring buoy and incubated in-situ for 4 h. For each sample, two aliquots of 5 mL (replicates) were introduced in scintillation vials (20 mL) for the determination of total labelled organic carbon production (TPP); the sum of ^{14}C incorporated into POC (particulate organic carbon) and released as DOC (dissolved organic carbon). The remaining volume was filtered through 0.22 µm mesh membrane filters (cellulose membrane filters) of 25 mm to determine particulate primary production (PPP > 0.22 µm). To remove inorganic 14C, the liquid samples were acidified with 100 µL of 10 % HCl and shaken for 12 h, while the filters were fumed with concentrated HCl (37 %) for 12 h. Then, 10 mL and 5 mL of scintillation cocktail (Packard Ultima Gold XR) were respectively added to the TPP and PPP vials, and the disintegrations per minute were counted after 24 h with a scintillation counter (EG&G/Wallac). The dissolved organic carbon production by phytoplankton

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(P_{DOC}) was calculated as the difference between total and particulate primary production (Morán et al., 2001) and the percentage of the production released extracellular by phytoplankton (PER = 100 P_{DOC}/TPP) was calculated.

2.3 Bacterioplankton abundance and viability

At each station, the proportion of living heterotrophic bacteria was quantified from seawater sampled at up to 7 depths. To do so, we used the Nucleic Acid Double Staining (NADS) (Grégori et al., 2001) flow cytometric protocol. This technique consists on the use of two nucleic acid fluorescent dyes, SYBR Green I (SG1; Molecular Probes) and Propidium Iodide (PI; Sigma Chemical Co.). Bacterial membranes are permeable to SG1, independently of the cell viability, resulting in green fluorescence when stained. However, living or viable cells with intact plasmic membranes are impermeable to PI. Thus only compromised or damaged cells are stained with PI (Barbesti et al., 2000), showing red fluorescence as described in Falcioni et al. (2008). Subsamples were analyzed immediately after collection. Samples (1 mL) were stained with 10 µL of Propidium iodide (PI, 1 mg mL^{-1} stock solution), reaching a final concentration of 10 µg mL^{-1} and incubated for 30 min in a dark room. Then, 10 µL of SYBR Green I (10-fold dilution of 10 000 × commercial solution in dimethyl sulfoxide) was added to subsamples and incubated for 10 more minutes. SG1 and PI fluorescence were detected using a FACSCalibur Flow Cytometer (Beckton Dickinson©) in the green (FL1) and the red (FL3) cytometric channels, respectively. Bivariate plots of green vs. red fluorescence (FL1 vs. FL3) allowed for discrimination of live (green fluorescent, impermeable to PI) from dead cells (red fluorescent membrane-compromised cells, stained by PI and SG1). Bacterial concentration was calculated using a 1 µm diameter fluorescent bead solution (Polysciences Inc.) as an internal standard. Total heterotrophic bacterial abundance was calculated as the sum of red and green fluorescent cell abundance, while living bacterial cell abundance was determined from the green fluorescent cell counts.

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2.4 Phytoplankton communities and viability of populations

Water samples of 200 mL were filtered through Whatmann GF/F filters to estimate total chlorophyll *a* concentration (Chl *a*), and extracted for 24 h in 90 % acetone before fluorometric determination (Turner Designs fluorometer) following Parsons et al. (1984). Samples for the quantification of nano- and microphytoplankton abundance was sampled at the surface (5 m) and the deep chlorophyll maximum (DCM) at each station. Samples of 2–3 L were concentrated into 50–70 mL samples using a Millipore cell concentration chamber. This concentration system has been used in previous studies (Alonso-Laíta and Agustí, 2006; Lasternas et al., 2010; Lasternas and Agustí, 2010) with accurate results for microphytoplankton, and no effect on the viability or other cell properties (i.e. movement for flagellated cells, integrity of frustules, etc.). 10 mL aliquots (duplicates) of the concentrated sample were filtered onto 2 µm pore-size black polycarbonate filters, fixed with gluteraldehyde (1 % final concentration) and stored frozen at −80 °C until counting. Phytoplankton cells were counted using an epifluorescence microscope (Zeiss© Axioplan Imaging), and were classified into 3 major groups: flagellates, dinoflagellates and diatoms, which were then separated into pennate and centric. Autotrophic picoplankton abundance was assessed using Flow Cytometry. At each station, duplicate 2 mL fresh samples from 7 depths were counted on board (duplicated counts) using a FACSCalibur Flow Cytometer (Beckton Dickinsoné). An aliquot of a calibrated solution of 1 µm diameter fluorescent beads (Polysciences Inc.) was added to the samples as an internal standard for the quantification of cell concentration. The red (FL3, bandpass filter > 670 nm), green (FL1 bandpass filter 530 nm) and orange (FL2, bandpass filter 585 nm) fluorescence, and forward and side scattering signals of the cells and beads were used to detect picoplankton populations of *Synechococcus*, *Prochlorococcus* and autotrophic picoeukaryotes (Marie et al., 2005). The proportion of dead cells in the autotrophic communities examined was quantified by applying the cell digestion assay (CDA), a cell membrane permeability test known as consisting on the exposure of the phytoplankton communities to an enzymatic cocktail (DNAse

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and Trypsin, 30). Both enzymes are able to enter the cytoplasm and digests cells with compromised membranes (dead or dying cells), which are removed from the sample. The cells remaining in the sample after the CDA are living cells, those with intact membranes (Agustí and Sanchez, 2002), which are then counted by flow cytometry or epifluorescence microscope, as described above. The percentage of dead cells was calculated from the ratio between the concentration of dead cells (total concentration minus the concentrations of living cells) and total population abundance, which includes both living and death cells (Agustí and Sanchez, 2002).

2.5 Statistics

Spearman's rank coefficients were used to determine correlation coefficients between variables that departed from normality (Siegel and Castellan, 1988). The statistical significance of the differences between average values was tested using Student's *t* test, with a critical *p* value of 0.05. Heterotrophic bacteria survival, as the percent of living heterotrophic bacterial cells, were grouped by 20 % PER bins to examine a relationships between %LHB and PER. Linear regression analyses were applied to raw and binned PER data.

3 Results

The waters studied included three distinct oceanographic zones (Fig. 1) including the oligotrophic subtropical Atlantic Ocean, which presented significantly warmer and saltier waters and low nutrient concentration (Table 1); waters influenced by the NW African upwelling system, characterized by cooler and fresher waters and higher dissolved nutrient concentration (Table 1); and the transitional system around the Canary Islands, influenced by the Canary current, exhibiting intermediate temperature, salinity and nutrient concentration (Table 1).

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Total primary production (TPP) declined from the waters influenced by the upwelling, which exhibited the highest values to the most oligotrophic zone, which presented the lowest rates (Table 1). Dissolved primary production (P_{DOC}) was positively related to total primary production (TPP; $\log P_{DOC} = -0.501 + 0.92$ (± 0.09) $\log$ TPP, $R^2 = 0.68$, $P < 0.001$, $N = 45$), and tended to increase as total primary production increased, but with a slope gently lower than 1, indicating that P_{DOC} tended to be proportionally lower in productive waters (Table 1). Thus, the percentage of extracellular release (PER), which varied greatly across the study (Table 1), was greatest in the most oligotrophic waters sampled and declined towards more productive waters (Table 1).

Nano-microphytoplankton communities were present along the study site, and showed higher abundance at the DCM than at the surface waters, with slightly higher abundance within Zone 3, area influenced by the upwelling system (Table 2). Autotrophic flagellates dominated the microphytoplanktonic community throughout the study (Table 2) and presented relatively uniform abundance within the studied zones. Diatoms were poorly abundant within Zone 1 (Table 2) represented almost solely by the pennate genera *Nitzschia* spp., but showed a consistent increase in abundance at the waters influenced by the upwelling (Zone 3, Table 2), with the centric genera *Thalassiosira* sp. and *Chaetoceros* sp. being the most abundant. Dinoflagellates, primarily represented by the naked form *Gymnodinium* spp., displayed low abundance across the cruise (Table 2) and were principally located in surface waters.

Prochlorococcus spp. was the most abundant, during the cruise (Fig. 2), presented significant higher values than both populations of *Synechococcus* spp. and picoeukaryotes at Zones 1 and 2, and decreased at waters associated to the upwelling system (Zone 3). Within this zone, *Synechococcus* spp. abundance surpassed that of *Prochlorococcus* spp. (Fig. 2). Picoeukaryotes's abundance was relatively uniform (about 10^3 cells mL^{-1}) between the 3 zones of study, with maximum values observed at the intermediate zone of the Canary current (Zone 2, Fig. 2). Heterotrophic bacteria presented significantly higher abundance at the oligotrophic zone (Zone 1) and lower ones at zone 2 (Fig. 2).

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The proportion of dead phytoplankton cells (%DC) varied greatly across communities (Fig. 3, Table 1). Diatoms dominated the communities in the upwelling area (Table 2), where they showed a low proportion of dead cells, with the highest percentage of dead diatom cells observed in oligotrophic waters (Fig. 3). *Prochlorococcus* spp., the dominant picophytoplanktonic taxa (Table 2), was less abundant and presented a higher proportion of dead cells in the upwelling zone (Figs. 2 and 3). The oligotrophic zone presented highest phytoplankton mortality (Table 1) associated to highest PER rates. The variability in the percent extracellular carbon release in each station was closely dependent on the status of the photosynthetic community, as reflected in a linear increase in the percent extracellular carbon release with an increase in the percent of dead cells in the photosynthetic community (Fig. 4).

Heterotrophic bacteria communities included 60 to 95 % of living cells across communities, with the average % of living heterotrophic bacteria cell being higher than that of autotrophic picoplankton (Student's *t* test, $P < 0.0001$). While bacterioplankton presented highest abundance in the upwelled waters (Fig. 5, Table 2), the percentage of heterotrophic living bacteria was lowest in the most productive waters and increased towards more oligotrophic waters (Fig. 5, Table 1). By dividing the production of dissolved organic carbon by phytoplankton and the bacterial abundance, we obtained the flux of P_{DOC} per bacterial cell (pg C. bacterial cell^{-1}) and could appreciate that availability in DOC for heterotrophic bacteria were higher in the oligotrophic waters (Fig. 5) than at the other zone of the study. The percentage of living heterotrophic bacterioplankton cells increased with increasing proportion of extracellular dissolved organic carbon released ($R^2 = 0.83$, $P < 0.005$, Fig. 6).

4 Discussion

The results presented here provide evidence of a close coupling between heterotrophic bacterial survival and the release of recently photosynthesized carbon by phytoplankton in the NE subtropical Atlantic. The results presented also suggest a mechanistic

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pathway linking phytoplankton cell death with high extracellular carbon release and a subsequent increase in the percentage of living heterotrophic bacteria cells. These results confirm the power of approaches based on assessments at the single-cell level (Agustí and Sanchez, 2002; Bidle and Falkowki, 2004; Gasol et al., 2008; Lasternas et al., 2010) to resolve the relationships between the status of phytoplankton cells and that of heterotrophic bacteria, mediated by the extracellular release of organic carbon.

Previous attempts at testing the relationship between P_{DOC} release and bacterial production remained elusive and variable among systems. In open ocean sites, bacterial production and dissolved primary production (DPP) are often tightly linked (Morán et al., 2001; Antarctic off shore waters), while in coastal (Morán et al., 2002a, NE Atlantic coastal system and Morán et al., 2002b, NW Mediterranean) or eutrophic sites (Morán et al., 2002b, Antarctic coastal) persists a lack of linkage. Our study provides, to the best of our knowledge, the first demonstration of a direct relationship between recently released labile photosynthate, the preferred carbon source for HB (Norrman et al., 1995), and the survival of heterotrophic bacteria.

A gradient in phytoplankton productivity and community structure from the African upwelling region to the oligotrophic region offshore has been previously reported for the subtropical NE Atlantic (Teira et al., 2003; Pelegrí et al., 2005; Alonso-Laíta and Agustí, 2006), including an increase in phytoplankton mortality rates and the proportion of dead phytoplankton cells along this gradient (Agustí et al., 2001; Alonso-Laíta and Agustí, 2006). The results presented here confirm these findings, with phytoplankton cell viability decreasing from upwelling-influenced waters to oligotrophic waters, particularly for diatoms, which showed a two-fold reduction in the percent of living cells from the upwelling to the oligotrophic waters. However, the patterns displayed by the different populations conforming the phytoplankton community were complex, as phytoplankton show intricate and differentiated niches of cell viability depending on cell size, irradiance, nutrient concentration and temperature (Berges and Flakowski, 1998; Agawin et al., 2000; Agustí 2004; Alonso-Laíta and Agustí, 2006; Agustí and Llabrés, 2007; Lasternas et al., 2010). The percentage of dead cells tended to increase with decreas-

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ing cell size, with more than 40 % dead cells generally found in the picophytoplankton community, consistent with the reported increase in mortality rates with decreasing cell size (Marbá et al., 2007). Although picophytoplankton communities are typically dominant in oligotrophic waters (Agawin et al., 2000), they showed high variability in cell viability in the most oligotrophic waters sampled here. Surface populations are exposed to high PAR and UV radiation, resulting in high %DC of *Prochlorococcus* spp., which is strongly sensitive to high solar radiation (Llabrés and Agustí, 2006; Agustí and Llabrés, 2007; Llabrés et al., 2010), whereas *Synechococcus* is typically stressed by low light at deep layers but shows higher cell survival in surface waters (Agustí 2004; Llabrés and Agustí, 2006). In addition, the high cell mortality of *Prochlorococcus* sp. in the upwelling waters is consistent with the incapacity of *Prochlorococcus* sp. to use nitrate (Moore et al., 2002) and with the decline in cell viability in waters below 21 °C (Alonso-Laíta and Agustí, 2006).

The patterns of cell survival of the natural phytoplankton populations described here provided compelling evidence that the variation in the proportion of dissolved organic carbon release is driven by phytoplankton cell mortality in the subtropical NE Atlantic Ocean. In agreement with previous studies, communities in unproductive oligotrophic waters tended to release as DOC a higher fraction of their total primary production compared to more productive, nutrient-rich upwelling waters (Teira et al., 2001; Morán et al., 2002a). However, despite the lower PER in productive waters, the magnitude of total organic carbon released by the community was higher, because total primary production was much higher in the upwelling zone. Similarly, whereas the proportion of phytoplankton cells dying in eutrophic waters was low, the total dead biomass in the upwelling region was much larger than that in oligotrophic waters, independently the phytoplankton size fraction. The larger phytoplankton mortality lead to a higher release of primary production as dissolved organic carbon, which, in turn, can support a larger biomass and carbon flux through bacteria in the upwelling zone compared to the oligotrophic waters.

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Within the upwelling-influenced area of the NE Atlantic Ocean, bacterial communities have been identified to be carbon-limited (Alonso-Sáez et al., 2007). In our study, we found a significantly higher bacterial abundance in upwelling-influenced waters, consistent with the higher release of DOC from phytoplankton, declining towards the oligotrophic waters offshore. The PER was, however, lowest at the upwelling-influenced area and, accordingly, the supply of dissolved organic carbon per bacterial cell was lowest at the upwelling-influenced area. Indeed, the patterns in the survival of bacteria cells was consistent with the supply of dissolved organic carbon per cell, both being highest in the oligotrophic, compared to the more productive, waters. This is in agreement with the carbon limitation of the bacterial community in the upwelling-influenced waters reported by Alonso-Saez et al. (2007). High bacterial viability was observed in the oligotrophic waters, where phytoplankton released a much higher proportion of their production as DOC, resulting in a higher flux of P_{DOC} per bacterial cell. This finding is also in agreement with reports of a strong dependence of bacteria on algal extracellular production in open-ocean environments, while bacterial carbon demand was not related to algal P_{DOC} in coastal and productive systems (Morán et al., 2002a, b). In oligotrophic areas, allochthonous organic matter from lateral transfer or atmospheric inputs can be an alternative source of carbon to autochthonous production (del Giorgio et al., 1997; Arístegui et al., 2003; Herndl et al., 2008). The lability of atmospheric sources of organic carbon has not yet been established (Dachs et al., 2005), but organic inputs from riverine sources and the majority of the DOC pool in oceanic systems are mostly refractory (Raymond and Bauer, 2001). Accordingly, DOC freshly released by phytoplankton is the source of carbon supporting the most efficient assimilation by bacteria in the oligotrophic ocean (Coveney and Wetzel, 1989; Norrman et al., 1995).

Our results support high phytoplankton cell death in the oligotrophic ocean, consistent with previous findings (Agustí 2004; Alonso-Laíta and Agustí, 2006; Lasternas et al., 2010, 2013), and demonstrates that high phytoplankton cell death in the open oligotrophic areas of the NE Atlantic results in a large release of DOC relative to primary production, providing a significant flux of labile carbon, that results in high heterotrophic

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bacteria survival, as demonstrated by the relationship between HB viability and PER presented here.

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Table 1. Average ± SE hydrological properties, nutrients and chlorophyll *a* concentration, primary production rates, percentage of extracellular release (PER), health status of phytoplankton and HB and DOC flux per bacteria cell quantified at the three zones. The average values for the zones connected by same letter are not significantly different ($p < 0.05$), and those for the zones connected by different letter are significantly different ($p < 0.05$).

Mean ± SE	Oligotrophic	Intermediate	Upwelling
Temperature (°C)	21.46 ± 0.16^{A}	19.15 ± 0.16^{C}	19.94 ± 0.22^{B}
Salinity (PSU)	37.21 ± 0.04^{A}	36.87 ± 0.03^{B}	36.74 ± 0.04^{C}
Dissolved inorganic Nitrogen ($\mu mol\,N\,L^{-1}$)	0.31 ± 0.06^{B}	0.75 ± 0.15^{B}	2.28 ± 0.41^{A}
Ammonium ($\mu mol\,N\,L^{-1}$)	0.10 ± 0.01^{B}	0.11 ± 0.01^{AB}	0.13 ± 0.01^{A}
Phosphate ($\mu mol\,P\,L^{-1}$)	0.21 ± 0.03^{AB}	0.09 ± 0.02^{B}	0.33 ± 0.03^{A}
Chlorophyll ($mg\,Chl\,a\,m^{-3}$)	0.28 ± 0.02^{B}	0.37 ± 0.04^{AB}	0.48 ± 0.05^{A}
Total primary production ($mg\,C\,m^{-3}\,h^{-1}$)	0.70 ± 0.10^{A}	0.96 ± 0.13^{A}	1.14 ± 0.20^{A}
Dissolved organic carbon production by Phytoplankton ($mg\,C\,m^{-3}\,h^{-1}$)	0.58 ± 0.09^{A}	0.64 ± 0.10^{A}	0.41 ± 0.09^{A}
PER	81.9 ± 1.9^{A}	64.4 ± 4.7^{B}	41.3 ± 7.9^{C}
Phytoplankton dead cells (%DC)	51.9 ± 4.2^{A}	39.1 ± 2.7^{B}	44.1 ± 4.4^{B}
Heterotrophic living bacteria (%HLB)	85.7 ± 1.1^{A}	79.9 ± 0.9^{B}	74.8 ± 1.0^{C}
Flux of DOC per bacteria cell ($pg\,C\,cell^{-1}\,h^{-1}$)	1.82 ± 0.42^{A}	1.59 ± 0.24^{AB}	0.81 ± 0.21^{B}

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Table 2. Average ± SE of the nano-microphytoplankton abundances in the three zones.

Mean (cells L^{-1}) ± SE	Oligotrophic	Intermediate	Upwelling
Nano-microphytoplankton	$2.22 \pm 0.31 \times 10^3$	$2.83 \pm 0.38 \times 10^3$	$5.89 \pm 1.54 \times 10^3$
Flagellates	$1.09 \pm 0.22 \times 10^3$	$1.53 \pm 0.11 \times 10^3$	$2.78 \pm 0.74 \times 10^3$
Diatoms	$5.18 \pm 0.76 \times 10^2$	$8.41 \pm 1.26 \times 10^2$	$2.23 \pm 0.72 \times 10^3$
Dinoflagellates	$6.09 \pm 0.15 \times 10^2$	$4.63 \pm 1.68 \times 10^2$	$8.77 \pm 3.02 \times 10^2$

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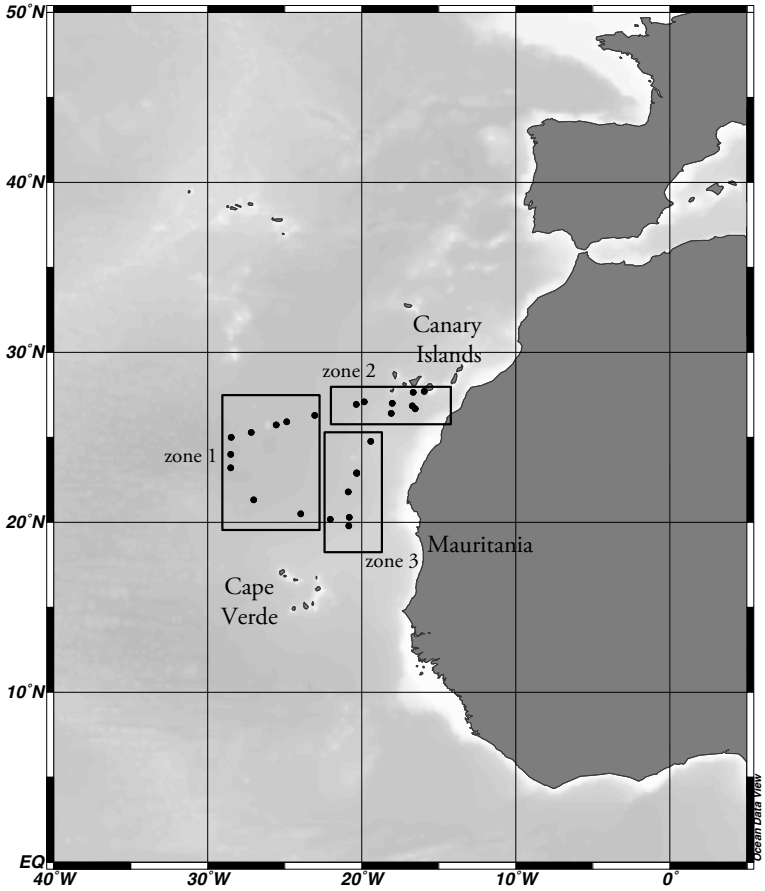

Fig. 1. Areas of sampling and stations occupied during the RODA cruise. Zones 1, 2 and 3 correspond to three singular hydrographical and biological conditions: Oligotrophic tropical Atlantic zone (Zone 1); Intermediate Gran Canaria Current area (Zone 2), Upwelling associated waters (Zone 3).

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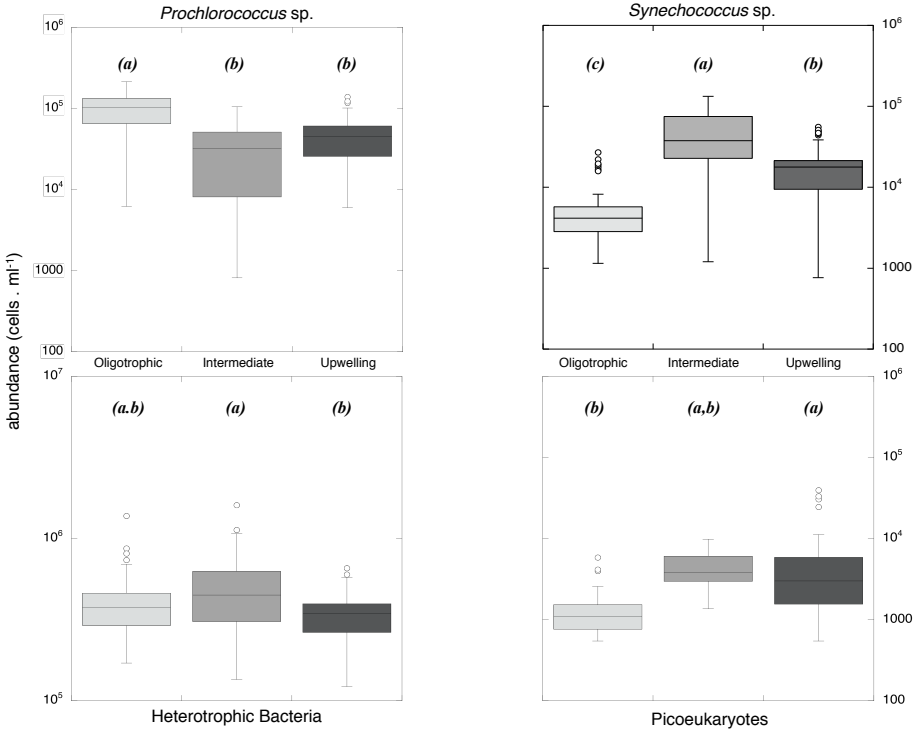

Fig. 2. Box plots showing the abundance distribution of the picoplankton populations within systems. The boxes show the lower and upper quartiles, median, minimum and maximum values, and outliers. Numbers correspond to averaged (± SE) abundances within systems. The boxes connected by same letter are not significantly different ($p < 0.05$).

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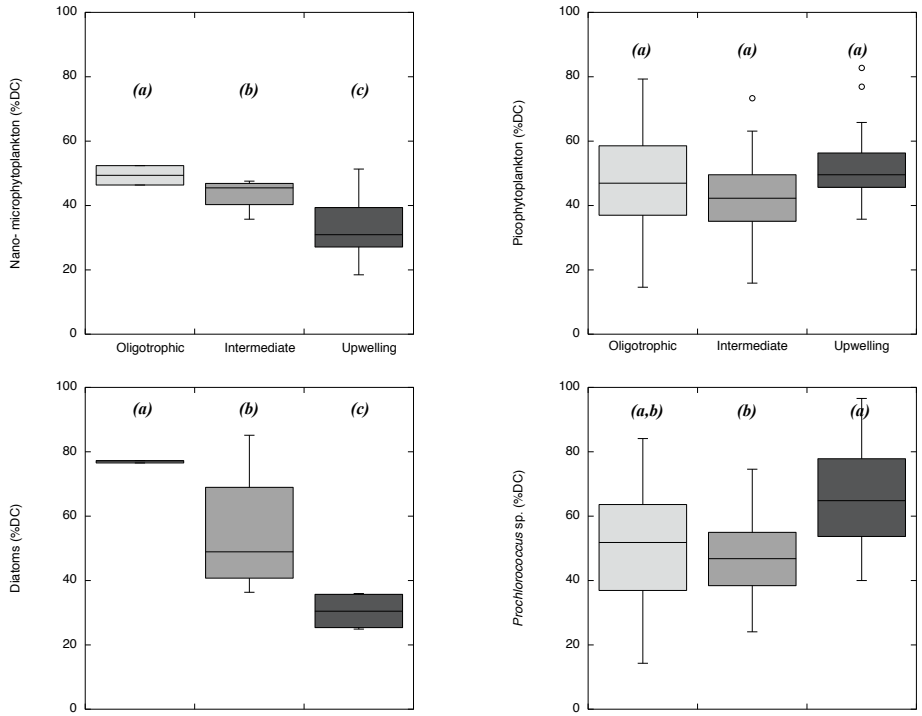

Fig. 3. Box plots showing the distribution percentage of dead cells (%DC) of the different phytoplankton groups in the sampled zones. The boxes present the lower and upper quartiles, median, minimum and maximum values, and outliers. The boxes showing the same letter do not have significantly different mean values (*t* test, $p > 0.05$).

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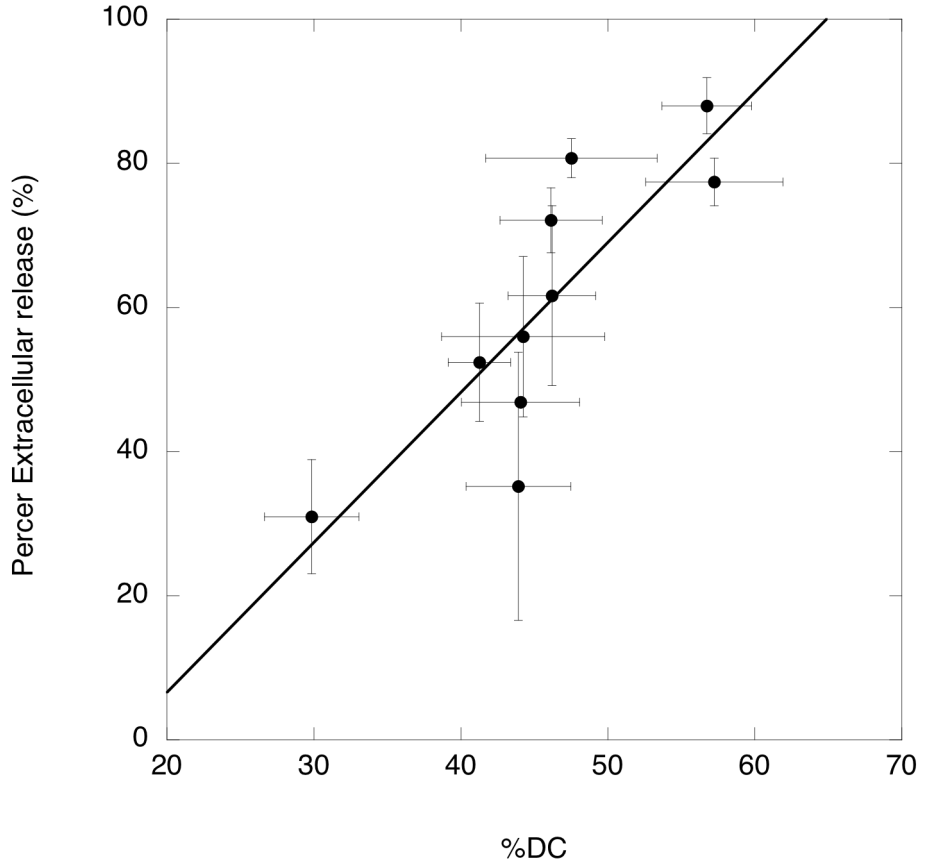

Fig. 4. The relationship between the percentage of dead phytoplankton cells (%DC) and the proportion of extracellular carbon release (PER), averaged by stations across the study. The full line represents the fitted regression equation: PER = −35.03 + 2.08 (±0.49) phytoplankton %DC ($R^2 = 0.69$, $P = 0.0029$, $N = 10$).

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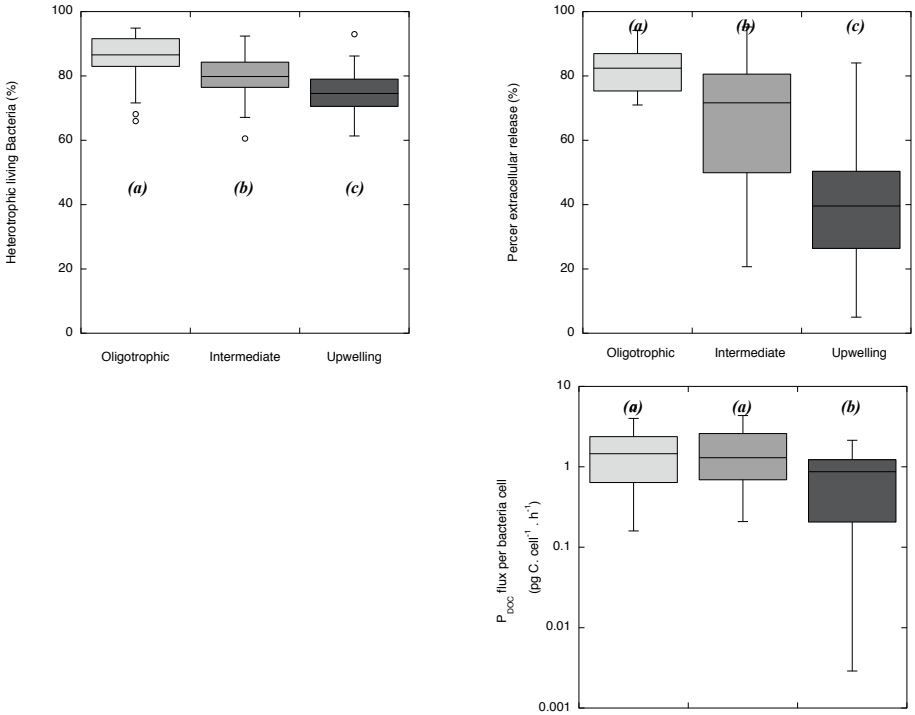

Fig. 5. Box plots showing the distribution of the percentage of living bacteria cells (%HLB), the distribution of PER and the variation of fluxes of PDOC per bacteria at the three sampled zones. The boxes present the lower and upper quartiles, median, minimum and maximum values, and outliers. The boxes showing the same letter do not have significantly different mean values (t test, $p > 0.05$).

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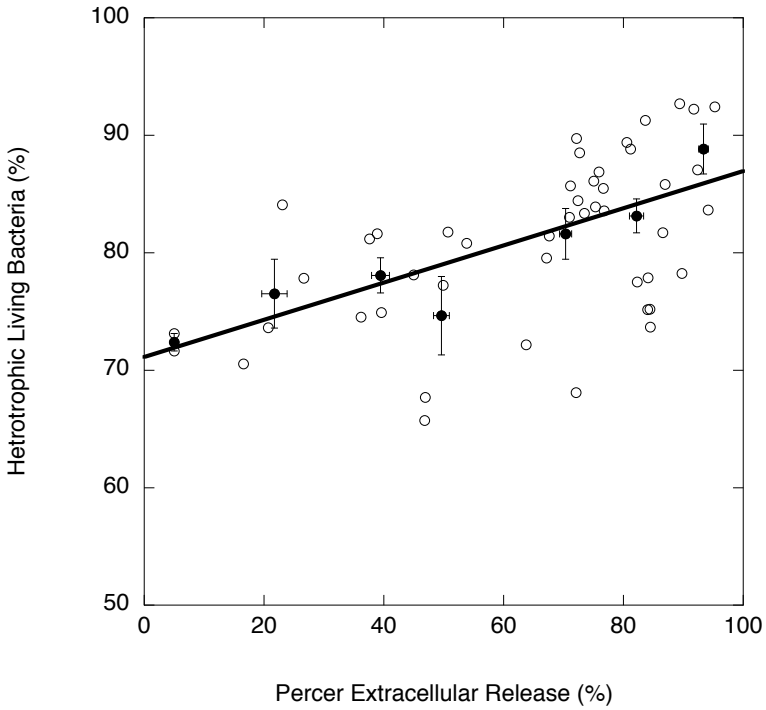

Fig. 6. The relationship between the percentage of living heterotrophic bacteria and the percent extracellular dissolved organic carbon release. The open and full symbols represent the individual and average (± SE) percentage of living bacteria binned by 20 % PER intervals. The doted line represents the fitted regression equation between the percent living heterotrophic bacteria average across 20 % PER bins and PER: (%LC) = 71.6 + 0.16 PER (R^2 = 0.83, $P < 0.005$, $N = 7$).

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