UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE AND MATHEMATICS

SCHOOL OF OCEAN AND EARTH SCIENCES

BACTERIOPLANKTON DYNAMICS IN SURFACE WATERS OF THE NORTH-EASTERN (SUB-)TROPICAL ATLANTIC OCEAN AFFECTED BY AEOLIAN DUST

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"Anybody? No?! Dust."

Marjorie Dawes

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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By Polly Hill

The microbial community dominates biogeochemical cycling of the ocean, affecting global climate. The impact of physical disturbance of near surface microbial populations was studied in the north-eastern tropical and subtropical Atlantic Ocean. This region lies beneath easterly trade winds, resulting in strong perturbations in terms of wind driven mixing and Aeolian dust deposition.

Firstly, the region's surface water bacterioplankton community was compared with adjacent regions in terms of metabolic activity, by measuring the uptake rates of radioactively labelled amino acids (^{3}H-leucine and ^{35}S-methionine) as a proxy for bacterial production. Remarkably, there was little variation in uptake rates between the two Atlantic (sub-)tropical gyres. Rates reflected regional photosynthetic biomass, except in the study region. The bacterioplankton community of this region was less metabolically active than that of the oligotrophic north Atlantic gyre, despite ocean colour data identifying the region as productive. The region's uniqueness is probably related to the episodic Saharan dust inputs experienced.

To test whether dust deposition controls microbial community structure, surface communities were compared, using flow cytometry and fluorescence *in situ* hybridisation, between two winter periods when either wind-driven mixing or dust deposition occurred. Wind-driven mixing was associated with domination by the ubiquitous SAR11 clade of *Alphaproteobacteria*, whereas key primary producers, *Prochlorococcus* cyanobacteria, numerically dominated during calmer conditions. Phytoplankton-associated *Bacteroidetes* and *Synechococcus* cyanobacteria were most abundant during turbulent conditions. *Gammaproteobacteria*, encompassing opportunistic species, were the only group to benefit from dust inputs; thus dust deposition seems to have a minor influence on the region's bacterioplankton community compared to wind mixing, suggesting community change following dust storm events may be linked to nutrients delivered by wind mixing, as much as from dust.

To test further whether changes in the SAR11 and *Prochlorococcus* populations varied between years due to wind- or dust-related perturbation, a method based on ^{35}S-methionine uptake was developed for measuring the metabolic response of these groups to Aeolian dust, whilst excluding wind impacts. Subsurface seawater samples were treated with freshly collected dust, added directly or indirectly as a "leachate" after its rapid dissolution in deionised water. *Prochlorococcus* and SAR11 cells were sorted by flow cytometry to determine their group-specific responses. Both *Prochlorococcus* and SAR11 were metabolically impaired by the addition of dust, which may explain the low metabolic activity observed in the region (mentioned above). Although SAR11 showed minor positive responses to dust leachate additions, leachate proved detrimental to *Prochlorococcus*. Thus dust dissolution *in situ* appears to be more deleterious to *Prochlorococcus* than SAR11 and hence could initiate a compositional shift in the indigenous bacterioplankton, suggesting the observed switch from SAR11- to *Prochlorococcus*-domination following dust deposition (mentioned above) was indeed a result of an alternative stimulus, most likely wind stress.

In conclusion, whereas dust deposition may prove beneficial to bacterioplankton species with high nutrient demands, such as some *Gammaproteobacteria*, it does not appear to affect the ambient dominant bacterioplankton groups of the northeast (sub-)tropical Atlantic to the same degree as wind-driven perturbations. Furthermore, large dust deposition events may prove detrimental to ambient populations, resulting in low community metabolic activity.

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DECLARATION OF AUTHORSHIP

I, Polly Hill, declare that the thesis entitled 'Bacterioplankton dynamics in surface waters of the north-eastern (sub-)tropical Atlantic Ocean affected by Aeolian dust' and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published as:

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Chapter One

Introduction to the study region and project

1.1 Nutrient limitation in the North Atlantic subtropical gyre

The main factors influencing marine microbial communities are supply of labile dissolved nutrients, protozoan predation and lysis by viruses (Thingstad & Lignell, 1997). In the oligotrophic North Atlantic subtropical gyre, nutrient bioavailability is generally considered to be the most important limiting factor of microbial abundance and production, thus it is the influence of nutrients with which this thesis is concerned.

Carbon provides the backbone for most classes of macromolecules, thus on a dry weight basis a typical bacterial cell is ~50% carbon (Madigan & Martinko, 2006). Nitrogen is required for nucleic acids, proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy. Phosphorus is primarily used for the production of nucleic acids, phospholipids, and high energy compounds (e.g. adenosine triphosphate, ATP).

The growth of autotrophic organisms is fundamentally constrained by inorganic nutrients and surface irradiance. In the oligotrophic subtropical gyre, irradiance tends not to be limiting above the pycnocline, but inorganic macronutrients – nitrate and phosphate – are perpetually at 'limiting' concentrations. The term 'limiting' refers to the fact that nutrients are present at concentrations insufficient to allow phytoplankton blooms to occur, such as those that form following stratification of temperate waters in spring. Phytoplankton in the north Atlantic gyre have been shown experimentally to be primarily limited by nitrate, followed by phosphate (Graziano *et al.*, 1996, Moore *et al.*, 2008), with a westwards trend for phosphate limitation (Sohm & Capone, 2010), possibly due to nitrogen fixation (Wu *et al.*, 2000).

Heterotrophic bacterioplankton preferentially assimilate organic nutrients. In the oligotrophic gyre, where low inorganic nutrient concentrations lead to low production of dissolved organic nutrients by phytoplankton, heterotrophic bacteria can also become

nutrient limited. Thus, there is evidence that bacterial production in the gyre can also be limited by phosphate (Cotner *et al.*, 1997, Rivkin & Anderson, 1997) or co-limited by inorganic nitrogen (ammonia + nitrate) and phosphate (Mills *et al.*, 2008).

Due to its perpetually oligotrophic status, the North Atlantic subtropical gyre is considered to be a stable environment. This is not true. Although aspects of the gyre's core vary minimally seasonally and inter-annually, in terms of bacterial and photosynthetic biomass for example (Heywood *et al.*, 2006, Tarran *et al.*, 2006), the extent of the gyre, and thus the extent of these characteristics, shows substantial seasonality: in boreal summer months the oligotrophic gyre spans almost the entire ocean between Florida and northwest Africa, yet in winter it retreats to about a quarter of the size (Hardman-Mountford *et al.*, 2008, Oliver & Irwin, 2008).

1.2 Microbial life in the oligotrophic gyre

Despite the so-called nutrient-limitation of the oligotrophic gyre, each millilitre of seawater contains one million prokaryotes. Thus there are populations of microorganisms for whom this region provides the ideal environment, where they can grow and divide at a leisurely pace without being overcrowded by larger, faster growing, acquisitive cells. The blue sunlit waters of the oligotrophic gyre are characterised by a microbial community that is highly specialised to the stable environment of perpetual nutrient depletion.

1.2.1 The heterotrophic community

A great diversity of heterotrophic bacterioplankton has been observed (Giovannoni & Stingl, 2005); however, the majority of those inhabiting surface waters of the open ocean can be affiliated to the *Alphaproteobacteria*, with smaller contributions from the *Gammaproteobacteria* and *Bacteroidetes* groups (Alonso-Sáez *et al.*, 2007, Alonso-Sáez & Gasol, 2007, Schattenhofer, 2009). *Alphaproteobacteria* are a major component of all marine environments (Fuchs *et al.*, 2005, Mary *et al.*, 2006a, Mary *et al.*, 2006b, Topping *et al.*, 2006). A major contributor to *Alphaproteobacteria* is the ubiquitous SAR11 clade (Giovannoni *et al.*, 1990), which is estimated to contribute 24-55% of oceanic prokaryotic cells and ~12% of total marine prokaryotic biomass (Morris *et al.*, 2002). SAR11 is unique in its conservative

nature of contributing about one-third to surface water bacterioplankton communities in the open ocean (Morris *et al.*, 2002, Malmstrom *et al.*, 2004, Alonso-Sáez *et al.*, 2007, Schattenhofer, 2009), which it is able to do by existing as temporally and spatially partitioned, phylogenetically distinct subgroups (Field *et al.*, 1997, Morris *et al.*, 2005, Carlson *et al.*, 2009).

1.2.2 The phototrophic community

With the advent of flow cytometry, cyanobacteria and picoeukaryote groups can be studied with relative ease and thus a wealth of information now exists at relatively high spatial and temporal resolution. The picoeukaryotes encompass a broad group of picophytoplankton and as the dominant group of eukaryote algae in the open ocean they are responsible for up to 44% of primary production (Jardillier *et al.*, 2010). The diversity of this group is not so well understood as that of the cyanobacteria. Cyanobacteria belonging to the genera *Prochlorococcus* (Chisholm *et al.*, 1988) and *Synechococcus* (Waterbury *et al.*, 1979) are the numerically dominant oxygenic phototrophs in subtropical oceans (Tarran *et al.*, 2001, Scanlan & West, 2002, Scanlan, 2003, Heywood *et al.*, 2006) where they are estimated to contribute up to 60% of primary production (Chisholm *et al.*, 1988, Li, 1994, Vaulot *et al.*, 1995).

Prochlorococcus is associated with, although not restricted to, regions of inorganic nutrient depletion where it is typically at concentrations of 10^4 – 10^5 mL^{-1} (Zubkov *et al.*, 1998, Zubkov *et al.*, 2000a, Heywood *et al.*, 2006). *Synechococcus* is also present in oligotrophic regions, typically at concentrations an order of magnitude lower than *Prochlorococcus*, but is more numerically abundant towards mesotrophic regions where it reaches concentrations of over 10^4 mL^{-1} (Zubkov *et al.*, 2000b, Heywood *et al.*, 2006). *Prochlorococcus* inhabit, and often dominate, oceanic waters within the 40°S to 45°N latitude belt, from ultraoligotrophic surface waters down to the pycnocline, by existing as phylogenetically distinct ecotypes or clades, which are adapted to distinct ranges of temperature and nutrient bioavailability (Moore *et al.*, 1998, West & Scanlan, 1999, West *et al.*, 2001, Scanlan & West, 2002, Johnson *et al.*, 2006). *Synechococcus* also has distinct clades that are partitioned temporally and spatially (Ahlgren & Rocap, 2006).

1.2.3 Adaptation to life in the oligotrophic gyre

Despite limiting concentrations of nutrients in surface waters of oligotrophic gyres, large populations of *Prochlorococcus* (DuRand *et al.*, 2001, Heywood *et al.*, 2006) and SAR11 bacterioplankton (Giovannoni *et al.*, 1990, Schattenhofer *et al.*, 2009) persist. Adaptive strategies for survival in the oligotrophic gyre have included a minimisation of cellular nutrient requirement, maximisation of efficiency in nutrient uptake, use of alternative nutrient sources, and utilisation of solar energy. Both SAR11 and the cyanobacteria have evolved mechanisms by which to utilise energy from the perpetual subtropical sun. Genes for proteorhodopsin, a light-driven photon pump (Béjà *et al.*, 2000), are expressed by the SAR11 strain *Pelagibacter ubique* (Giovannoni *et al.*, 2005a). The potential for SAR11 to use proteorhodopsin for harvesting light could reduce their cellular energy consumption. Cyanobacteria use sunlight for the photosynthetic fixation of inorganic carbon, thereby avoiding limitation by organic carbon.

In the north Atlantic subtropical gyre, *Prochlorococcus* and SAR11 are the major competitors for bioavailable phosphate, followed by *Synechococcus* (Zubkov *et al.*, 2007), suggesting they are particularly well adapted to taking up the nutrient at low concentration. Indeed, *Prochlorococcus*, *Synechococcus* and SAR11 have a high proportion of proteins involved in the transport of nutrients across the cell membrane, particularly for phosphorus acquisition (Sowell *et al.*, 2009). Furthermore, unlike heterotrophic bacterioplankton, *Prochlorococcus* and *Synechococcus* (Van Mooy *et al.*, 2006, Sowell *et al.*, 2009, Van Mooy *et al.*, 2009), can use sulpholipids, rather than phospholipids, in the cell membrane under phosphorus stress.

SAR11 isolates and strains of *Prochlorococcus* and *Synechococcus* are able to use organic phosphorus forms (Rippka *et al.*, 2000, Moore *et al.*, 2005, Sowell *et al.*, 2009), thus avoiding phosphate limitation. *Synechococcus* and *Prochlorococcus* will also grow on organic nitrogen sources and their degradation products (Rippka *et al.*, 2000, Moore *et al.*, 2002) in addition to inorganic nitrate (Moore *et al.*, 2002, Casey *et al.*, 2007, Martiny *et al.*, 2009). The use of organic nutrients (Zubkov *et al.*, 2003, Malmstrom *et al.*, 2005, Zubkov & Tarran, 2005), gives cyanobacteria the opportunity to compete with heterotrophic bacteria for nutrients that are energetically more attractive (e.g. Zubkov *et al.*, 2003). SAR11 can assimilate both organic and inorganic nitrogen and phosphorus (Sowell *et al.*, 2009), making them equally

competitive in this respect. Amino acids are an important source of nutrition for *Prochlorococcus* (Zubkov *et al*., 2003, Zubkov *et al*., 2004, Mary *et al*., 2008a) and SAR11 (Malmstrom *et al*., 2004, Mary *et al*., 2006b, Mary *et al*., 2008b), but less so for *Synechococcus* (Zubkov *et al*., 2003). As well as putting these groups at an advantage in nitrate depleted waters, the assimilation of amino acids removes the energy requirement for the reduction of inorganic nitrogen, and for building proteins since many amino acids will be used directly.

It has been suggested that the reduced genome of *Prochlorococcus* (Kettler *et al*., 2007), *Synechococcus* (Dufresne *et al*., 2008) and SAR11 (Giovannoni *et al*., 2005b) allows a smaller cell size and thus reduces nutrient requirement for genome replication and cell division. *Prochlorococcus* is the smallest known oxygenic phototroph (0.5 – 0.7 µm diameter) with an estimated carbon content of 29-53 fg carbon per cell (Campbell & Nolla, 1994, Zubkov *et al*., 1998, Zubkov *et al*., 2000b) compared to 100 to 400 fg carbon per *Synechococcus* cell (Burkill *et al*., 1993, Campbell & Nolla, 1994, Zubkov *et al*., 1998, Zubkov *et al*., 2000b). SAR11 is among the smallest free-living bacteria (Rappé *et al*., 2002) containing an estimated 5.8 fg carbon per cell (Tripp *et al*., 2008). As well as minimising cellular nutrient requirements, a small cell size results in a high surface area-to volume ratio, which may aid nutrient acquisition at nanomolar concentration. Being small may also reduce self-shading, and predation pressure since small cells tend to be grazed less than large bacteria (Jürgens & Matz, 2004).

1.3 Life at the edge

Although phylogenetically closely related (Urbach *et al*., 1992), *Prochlorococcus* and *Synechococcus* vary in size, nutrient physiology and, thus, distribution. The highly specialised nature of *Prochlorococcus* to life in the oligotrophic gyres has been at the expense of adaptability and competitiveness in changing environments (Dufresne *et al*., 2005), thus it becomes outcompeted in regions of elevated nutrient concentration. *Synechococcus* is present in oligotrophic regions, but more numerically abundant towards mesotrophic regions (Olson *et al*., 1988) where it reaches concentrations of over 10^4 mL^{-1} (Zubkov *et al*., 2000b, Heywood *et al*., 2006) and, as such, is an appropriate biomarker for the boundary of oligotrophic gyres (Zubkov *et al*., 2000a). The inverse relationship between *Prochlorococcus* and *Synechococcus* related to nutrient bioavailability has been

illustrated through time-series sampling in a seasonal region of the Sargasso Sea (Figure 1.1, from DuRand *et al.*, 2001). Picoeukaryotes also become more numerically abundant in regions of nutrient inputs (Tarran *et al.*, 2006).

The edges of the subtropical gyres are characterised by elevated nutrient bioavailability with consequential increases in *Synechococcus* and picoeukaryote abundance (Olson *et al.*, 1990, Heywood *et al.*, 2006, Zwirglmaier *et al.*, 2007). This increase in phytoplankton biomass is reflected in chlorophyll *a* concentration, and thus gyre boundaries are visible from space using ocean colour satellites (Hardman-Mountford *et al.*, 2008, Hirata *et al.*, 2008).

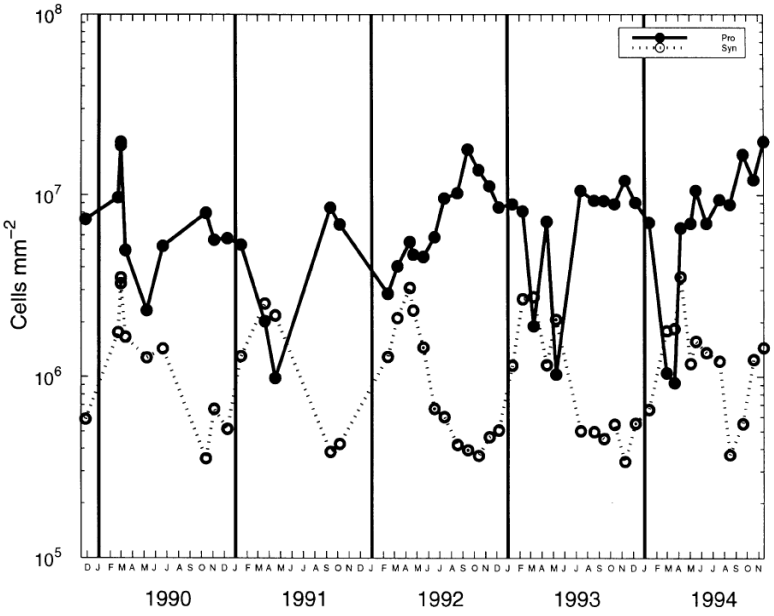


Figure 1.1: Taken from DuRand *et al.* (2001). Time series of integrated cell concentrations (cells mm^{-2}) for *Prochlorococcus* and *Synechococcus*. Data are integrated over the upper 200 m. Solid symbols and solid line represent *Prochlorococcus*, open symbols and dotted line represent *Synechococcus*.

1.4 Atmospheric perturbation of the gyre

Atmospheric forcings affect the surface water microbial community in three ways (Gargett & Marra, 2002):

1 Wind driven mixing results in the transfer of nutrients across the pycnocline to the surface layers. Wind also transports mineral dust and anthropogenic particles, which become deposited on the ocean surface.

2 Stratification of the water column resulting from solar radiation affects nutrient availability and light quality in the surface waters.
3 Atmospheric transfer of heat leading to latitudinal variation in surface water temperature affects growth rates.

This thesis is primarily concerned with the first point, regarding wind driven perturbation of surface seawater microbial communities. In the subtropical gyre, where there is little seasonal variation in solar radiation, wind may produce the greatest physical force of perturbation.

Perturbation of the oligotrophic gyre typically results in the introduction of nutrients. Topography also generates turbulence (Gregg & Sanford, 1980), which results in a biologically heterogeneous region around the Cape Verde islands on the south-eastern fringe of the North Atlantic subtropical gyre.

1.4.1 Wind-driven mixing & upwelling

'Forced convection' is the mixing that results from wind stress across the sea surface (Yamazaki & Osborn, 1988). There is a direct relationship between wind speed and the intensity of turbulence generated in the upper ocean (Oakey & Elliott, 1982) with a striking contrast in turbulence generated by low and high wind speeds (Dillon & Caldwell, 1980). Wind shear, in conjunction with the Coriolis force, can introduce significant quantities of nutrients to the surface layer from beneath the pycnocline and has potential to fuel highly productive blooms. In mesotrophic regions there is a shift from *Prochlorococcus* domination toward *Synechococcus* dominance. Where productivity is very high, even *Synechococcus* become displaced by large eukaryotic phytoplankton with comparatively high nutrient requirements, such as diatoms. These hotspots of productivity are clearly visible in ocean colour data.

Bacterial production is often coupled to primary production, since organic exudates of phytoplankton fuel bacterial growth. The *Gammaproteobacteria* and *Bacteroidetes* are associated with regions of nutrient pulses associated with upwelling and photosynthetic biomass (Suzuki *et al.*, 2001, Alonso-Sáez *et al.*, 2007, Teira *et al.*, 2008, Schattenhofer, 2009, Gómez-Pereira *et al.*, 2010). As with the picocyanobacteria, tiny SAR11 cells can become outcompeted in regions of high productivity by these larger, fast-growing cells (Fuchs *et al.*, 2005, Mary *et al.*, 2006b).

1.4.2 Atmospheric dust deposition

In providing a significant source of new nutrients to surface waters of the open ocean (Graham & Duce, 1982, Patterson & Settle, 1987, Duce *et al.*, 1991), atmospheric dust plays a key role in regulating oceanic primary production in some regions, affecting ocean biogeochemistry with resultant climatic and dust production feedback effects. Global dust production is estimated at 1700 Tg per year, with two-thirds from North Africa and 26% reaching the oceans (Figure 1.2; Jickells *et al.*, 2005). With the potential for increased desertification, leading to enhanced dust fluxes (Rosenfeld *et al.*, 2001), understanding dust impacts on oceanic biogeochemistry becomes increasingly important.

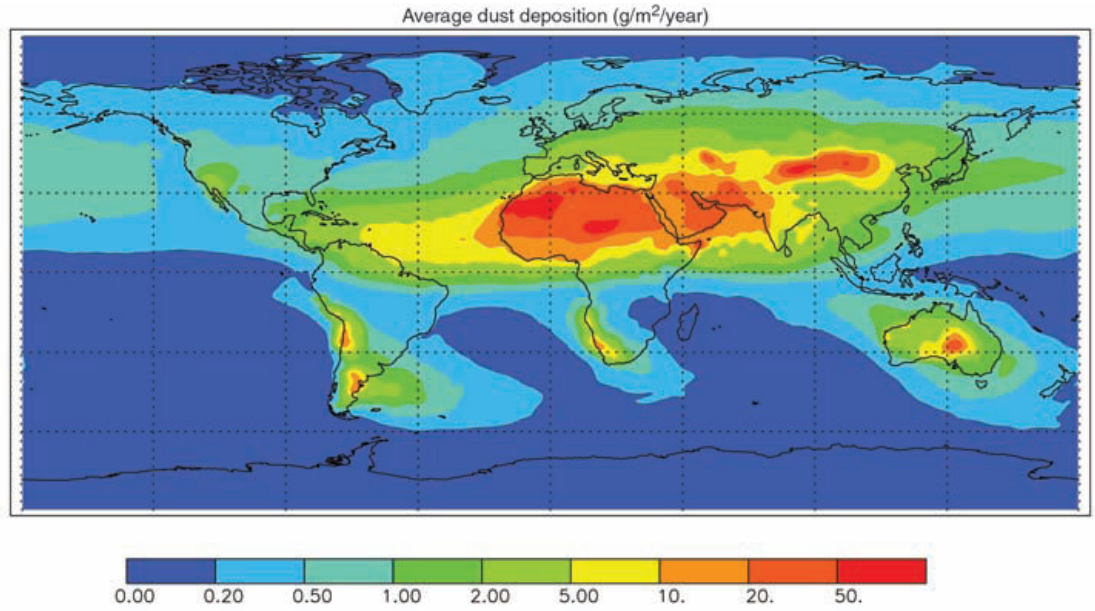


Figure 1.2: Taken from (Jickells, et al., 2005). Dust fluxes to the world oceans.

Iron has received much attention as a micro nutrient responsible for limiting primary production in vast oceanic regions (Boyd *et al.*, 2007). Dissolved iron concentrations in the open ocean are generally below 0.5 nM, and typically much lower (Johnson *et al.*, 1997, Bowie *et al.*, 2003, Rijkenberg *et al.*, 2008). Each year an estimated 16 Tg of iron is deposited into the oceans through atmospheric deposition (Jickells *et al.*, 2005), compared to 625-962 Tg of fluvial particulate iron and 1.5 Tg fluvial dissolved iron (Poulton & Raiswell, 2002). Due to low dissolution of iron in seawater, it is estimated that only 1-4% of deposited iron

will dissolve (Jickells & Spokes, 2001, Baker *et al.*, 2003, Bonnet & Guieu, 2004, Baker *et al.*, 2006a) and this solubility varies with aerosol source (Spokes *et al.*, 1994, Baker *et al.*, 2006b, Koven & Fung, 2006), particle size (Hand *et al.*, 2004, Baker *et al.*, 2006b) and means (wet or dry) of deposition (Ridame & Guieu, 2002). One study suggests that large dust deposition events may in fact act as a sink for surface ocean dissolved iron (Wagener *et al.*, 2010).

As well as being the main source of iron to oceanic surface water (Moore *et al.*, 1984, Duce, 1986, Duce & Tindale, 1991), desert dust has also been shown to supply phosphorus (Ridame & Guieu, 2002) and, when atmospherically processed, nitrogen (Baker *et al.*, 2006a). However, there remains little consensus on the absolute contribution of these nutrients as a result of the sporadic and variable nature of dust deposition events, the diverse range of dust sources, particle size and variable dissolution of dust-derived nutrients (Graham & Duce, 1982, Savoie *et al.*, 1989, Jickells & Spokes, 2001, Ridame & Guieu, 2002, Baker *et al.*, 2003, Jickells *et al.*, 2005, Baker *et al.*, 2006b).

Current understanding of Saharan dust impacts on microbial communities

Research into the impacts of Saharan dust events has hitherto focused on the Mediterranean region, with few studies conducted in the North Atlantic (Blain *et al.*, 2004, Mills *et al.*, 2004, Duarte *et al.*, 2006, Moore *et al.*, 2006). The majority of studies have used dust addition incubations; although some interesting observations were made of dust deposition leading to increased bacterioplankton abundance and activity in the Mediterranean Sea (Herut *et al.*, 2005, Bonilla-Findji *et al.*, 2010) and an increase in bacterial abundance in a Mediterranean lake (Pulido-Villena *et al.*, 2008a).

Incubation experiments have generally confirmed observations that heterotrophic bacteria respond to dust addition with increased production (Herut *et al.*, 2005, Lekunberri *et al.*, 2010) and abundance (Pulido-Villena *et al.*, 2008b, Lekunberri *et al.*, 2010). Saharan dust has also been shown to stimulate phytoplankton biomass and primary production (Blain *et al.*, 2004, Mills *et al.*, 2004, Bonnet *et al.*, 2005, Herut *et al.*, 2005, Duarte *et al.*, 2006, Moore *et al.*, 2006, Lekunberri *et al.*, 2010), but decrease phytoplankton community diversity (Pulido-Villena *et al.*, 2008a), and cause a shift from *Prochlorococcus* to *Synechococcus* domination (Herut *et al.*, 2005), or from a community rich in cyanobacteria to a community of diatoms and nanoflagellates (Duarte *et al.*, 2006, Lekunberri *et al.*, 2010).

Saharan dust also appears to stimulate nitrogen fixation by supplying diazotrophs with iron and phosphate (Lenes *et al*., 2001, Mills *et al*., 2004).

Contradictions to these generalities occur, however. Paytan *et al*. (2009) showed stimulation of *Prochlorococcus* abundance by dust addition to seawater samples from the Red Sea, and Duarte *et al*. (2006) did not observe dust stimulation of bacterial production in the northeast Atlantic. In summary, dust deposition appears to benefit eukaryotic phytoplankton and nitrogen fixing bacteria by supplying otherwise limiting nutrients. However, benefits to members of the non-nitrogen-fixing bacterioplankton community are not definitive. The impacts on this group may depend on the study region and season, and the dust's origin.

Hitherto, dust addition incubations have lasted between two and five days, thus a bacterial response to dust addition cannot be distinguished from a response to phytoplankton growth or the breakdown of other microbial cells. For example, Lekunberri *et al*. (2010) measured an increase in heterotrophic bacterioplankton abundance after a large dust addition (500 mg L^{-1}), but this was concurrent with the demise of the *Synechococcus* community, which may have released more desirable nutrients than the dust. Accounts of dust deposition *in situ* may provide a more realistic view of dust impacts on the microbial community; however, as discussed, the winds on which dust is carried have their own environmental impacts, which may mistakenly be attributed to dust-derived nutrients.

The ever growing understanding of the diversity of Bacteria, and the discoveries of increasingly specialised life strategies, suggests the classical measurements of bacterial abundance and production, and treating Bacteria as a black box, is largely redundant. Yet, to date, few studies have measured bacterioplankton community change in response to dust (Reche *et al*., 2009, Lekunberri *et al*., 2010) and none in a quantitative manner. Furthermore, whereas there have been many measurements of bacterial community production in response to dust addition, no measurements have been made of the metabolic response of specific bacterioplankton groups. Thus I aimed to measure microbial community structure change in response to dust deposition using quantitative methods (Chapter Three), and the specific metabolic response of dominant bacterioplankton groups to dust addition (Chapters Four and Five).

1.5 Methods in microbial ecology

A diverse range of techniques are employed in marine microbial ecology. The response of microbial communities to environmental perturbation is typically studied as a change in cell abundance, community structure and/or production.

1.5.1 Cell abundance: Flow cytometry

Flow cytometry generates cell abundance data with greatly increased throughput compared to microscopy, and minimises errors by counting large numbers of cells (thousands per second) with high precision (Olson *et al*., 1993). Cell concentration is calculated by an internal standard of fluorescent beads of known concentration (e.g. Olson *et al*., 1988) or by injecting samples at a known and constant speed (e.g. Zubkov *et al*., 2000b). One of the most powerful advantages of flow cytometry is the option to sort groups of cells based on their optical characteristics, to obtain pure samples (Olson *et al*., 1993, Zubkov *et al*., 2003).

The cyanobacteria and a broad group of picoeukaryotes can be identified and enumerated from unstained samples, based on their characteristic autofluorescent and side scatter signatures (Olson *et al*., 1993). The majority of *Synechococcus* strains have a unique signature due to their double fluorescence: orange for phycoerythrin and red for chlorophyll. *Prochlorococcus* cells contain divinyl chlorophylls *a* and *b*, maximising absorption of the blue light of open ocean environments (Chisholm *et al*., 1988). *Prochlorococcus* cells collected from depth are visible but cells from the surface often have too little pigment for detection in unstained samples (Chisholm *et al*., 1988), which can lead to underestimation. However, they form a distinct group if a nucleic acid stain is applied (Zubkov *et al*., 2000b), which can give a more representative value in surface samples, particularly in oligotrophic waters where the *Prochlorococcus* population dominates.

1.5.2 Community structure: Fluorescence *in situ* hybridisation

The majority of marine bacteria cannot be identified by their morphology, thus microbial populations are generally identified using genomic markers, e.g. ribosomal RNA (rRNA) (Pace *et al*., 1985, Amann *et al*., 1990). rRNA is routinely used because it is highly

conserved within species of Bacteria (Woese, 1987). Fluorescence *in situ* hybridisation (FISH) with rRNA-targeted probes allows phylogenetic identification and quantification of cells within mixed samples. The method works by hybridising natural samples with probes of oligonucleotide sequences specific to a target group. These probes contain a fluorescent molecule, or the means by which to attach a fluorescent molecule, such that each hybridised cell can be seen by epifluorescence microscopy. By counter-staining with a DNA stain, e.g. 4',6-diamidino-2-phenylindole (DAPI), it is possible to count the proportion of all cells that belong to the target group.

The main advantage of FISH is that it is not dependent on polymerase chain reaction (PCR)-amplified gene fragments, as many molecular methods are (e.g. gene cloning and fingerprinting). PCR amplifies genes, which some prokaryotes often contain in multiple copies for maximised reproduction rates. In streamlined communities this approach can be successful, but if fast growing species, such as opportunistic *Gammaproteobacteria*, are present their abundance can be overestimated. Thus, unlike PCR-dependent methods, FISH is a more accurate method for determining the exact abundance of cells in natural samples. FISH has its own limitations, however. Unequal permeabilisation of cells and variation in ribosome abundance and accessibility of the probe binding site all affect the labelling of cells, which can leave some cells unidentified despite hybridisation with the appropriate probes. There is also an element of human error involved as cells are generally counted manually by microscopy, and it is up to the counter to decide what counts as a hybridised cell. Despite these limitations, FISH does provide a quantitative means by which to identify and compare community diversity between samples within a reasonable timeframe and budget.

1.5.3 Bacterial activity: Uptake rates of radioactively labelled amino acids

Bacterial production is the rate of synthesis of biomass by heterotrophic bacterioplankton. It is estimated by the rate of cell division, DNA production - estimated by ^{3}H-Thymidine incorporation (Fuhrman & Azam, 1980, Fuhrman & Azam, 1982) - or protein synthesis.

Protein synthesis is generally estimated from the uptake rate of radioactively labelled amino acids, typically leucine. The majority of studies have used the method of Kirchman *et al.* (1985), or variations thereof. Briefly, 20 nM ^{14}C- or ^{3}H-labelled L-Leucine is added to seawater samples and incubated for 30-240 minutes. The radioactivity retained in

trichloroacetic acid (TCA)-insoluble protein is taken as leucine incorporation. In the oligotrophic open ocean, where nutrient concentration and uptake are low, saturating the community with a nutrient has the potential to affect the bacterioplankton community; for example, eliciting the switching on of additional transporters, which would not necessarily reflect the status quo. However, by adding isotopically labelled molecules at and below the ambient concentration, this affect should be avoided and we should measure the initial linear portion of the uptake curve, thereby giving an estimate of ambient uptake rates.

The dilution bioassay approach employed in this study was published by Wright & Hobbie (1966) to estimate ambient uptake of glucose and acetate by freshwater bacteria. It was later adapted to measure microbial assimilation of amino acids (Ferguson & Sunda, 1984, Zubkov *et al*., 2003). The technique has been criticised because of the multiphasic kinetics of nutrient uptake at concentrations above ambient (Azam & Hodson, 1981), but is still considered relevant if low concentration additions are made (Williams, 1973, Ferguson & Sunda, 1984, Zubkov & Tarran, 2005). This technique also estimates concentration more reliably than chemical methods, which can easily become contaminated (Ferguson & Sunda, 1984). By estimating concentration and uptake rate simultaneously, turnover time can be deduced, thus providing additional ecologically relevant information.

1.6 Aims and objectives

Despite increasing data on atmospheric dust fluxes and their impacts on ocean biogeochemistry, there remains little quantitative appreciation of how dust deposition affects ambient near surface bacterioplankton communities directly. Thus the aim of this thesis is to comprehensively evaluate the effect of dust deposition on ambient near-surface oceanic bacterioplankton communities of the heavily dust impacted northeast subtropical and tropical Atlantic Ocean. Data for the thesis were collected by myself during five research cruises, with contributions from Mikhail Zubkov, Isabelle Mary, Ross Holland and Duncan Purdie (Table 1.1).

Table 1.1: Summary of data reported in this thesis. Data were collected by Polly Hill, Mikhail Zubkov, Isabelle Mary, Ross Holland and Duncan Purdie. Data presented are amino acid concentration, uptake rate and turnover time (AA bioassay), chlorophyll-a concentration (Chl-a), microbial community composition from flow cytometry (FCM) and community structure from Catalysed Reported Deposition Fluorescence in situ Hybridisation (CARD-FISH).

Cruise	Date	Data	Chapter	Produced by
AMT-13	Sep-Oct 2003	AA bioassay	2	M Zubkov
AMT-17	Oct-Nov 2005	AA bioassay	2	M Zubkov
PO332	Jan-Feb 2006	Chl-*a*, FCM, CARD-FISH	3 & 4	P Hill
BATS225	July 2007	AA bioassay	2 & 4	P Hill, I Mary
TRYNITROP	Nov-Dec 2007	AA bioassay	2 & 4	P Hill
D326	Jan-Feb 2008	AA bioassay, CARD-FISH	2-5	P Hill
		FCM	3	R Holland
		Chl-*a*	3	D Purdie
D338	April-May 2009	AA bioassay	2 & 4	P Hill

The near surface bacterioplankton community of the study region is characterised in terms of metabolic activity, and compared with adjacent oceanic provinces, in Chapter Two.

In Chapter Three the impact of dust deposition *in situ* is described in terms of bacterioplankton community composition, and caution given for the misinterpretation of observations assigned to dust deposition events concurrent with high wind speeds.

Chapter Four describes problems associated with dust addition incubations for the study of bacterioplankton community responses to dust deposition, and the steps taken to develop a method that eliminates some of these problems.

In Chapter Five, bacterioplankton metabolic activities are used to assess the immediate impacts of dust addition to ambient communities of the region. The group-specific metabolic responses of SAR11 and *Prochlorococcus* are studied individually to assess whether the integral measurement of bacterial production may give misleading results.

Finally, Chapter Six draws together and highlights the conclusions reached as a result of this study.

1.7 References

Ahlgren NA & Rocap G (2006) Culture isolation and culture-independent clone libraries reveal new marine *Synechococcus* ecotypes with distinctive light and N physiologies. *Applied and Environmental Microbiology* **72**: 7193-7204.

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González JM, Vaqué D, Agustí S & Gasol JM (2007) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquatic Microbial Ecology* **46**: 43-53.

Alonso-Sáez L & Gasol JM (2007) Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. *Applied and Environmental Microbiology* **73**: 3528-3535.

Amann RI, Krumholz L & Stahl DA (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *Journal of Bacteriology* **172**: 762-770.

Azam F & Hodson RE (1981) Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. *Marine Ecology Progress Series* **6**: 213-222.

Baker AR, Kelly SD, Biswas KF, Witt M & Jickells TD (2003) Atmospheric deposition of nutrients to the Atlantic Ocean. *Geophysical Research Letters* **30**: 2296, doi:10.1029/2003GL018518.

Baker AR, Jickells TD, Biswas KF, Weston K & French M (2006a) Nutrients in atmospheric aerosol particles along the Atlantic Meridional Transect. *Deep Sea Research II* **53**: 1706-1719.

Baker AR, Jickells TD, Witt M & Linge KL (2006b) Trends in the solubility of iron, aluminium, manganese and phosphorus in aerosol collected over the Atlantic Ocean. *Marine Chemistry* **98**: 43-58.

Béjà O, Aravind L, Koonin EV, Suzuki MT, Hadd A, Nguyen LP, Jovanovich SB, Gates CM, Feldman RA, Spudich EN & DeLong EF (2000) Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* **289**: 1902-1906.

Blain S, Guieu C, Claustre H, Leblanc K, Moutin T, Queguiner B, Ras J & Sarthou G (2004) Availability of iron and major nutrients for phytoplankton in the northeast Atlantic Ocean. *Limnology and Oceanography* **49**: 2095-2104.

Bonilla-Findji O, Gattuso J-P, Pizay M-D & Weinbauer MG (2010) Autotrophic and heterotrophic metabolism of microbial planktonic communities in an oligotrophic

coastal marine ecosystem: seasonal dynamics and episodic events. *Biogeosciences Discussions* **7**: 2033-2064.

Bonnet S & Guieu C (2004) Dissolution of atmospheric iron in seawater. *Geophysical Research Letters* **31**: L03303, doi:10.1029/2003GL018423.

Bonnet S, Guieu C, Chiaverinin J, Ras J & Stock A (2005) Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system. *Limnology and Oceanography* **50**: 1810-1819.

Bowie AR, Achterberg EP, Blain S, Boye M, Croot PL, de Baar HJW, Laan P, Sarthou G & Worsfold PJ (2003) Shipboard analytical intercomparison of dissolved iron in surface waters along a north-south transect of the Atlantic Ocean. *Marine Chemistry* **84**: 19-34.

Boyd PW, Jickells T, Law CS, Blain S, Boyle EA, Buesseler KO, Coale KH, Cullen JJ, de Baar HJW, Follows M, Harvey M, Lancelot C, Levasseur M, Owens NPJ, Pollard R, Rivkin RB, Sarmiento J, Schoemann V, Smetacek V, Takeda S, Tsuda A, Turner S & Watson AJ (2007) Mesoscale iron enrichment experiments 1993-2005: synthesis and future directions. *Science* **315**: 612-617.

Burkill PH, Leakey RJG, Owens NJP & Mantoura RFC (1993) *Synechococcus* and its importance to the microbial foodweb of the northwestern Indian Ocean. *Deep Sea Research II* **40**: 773-782.

Campbell L & Nolla HA (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnology and Oceanography* **39**: 954-961.

Carlson CA, Morris R, Parsons R, Treusch AH, Giovannoni SJ & Vergin K (2009) Seasonal dynamics of SAR11 populations in the euphotic and mesopelagic zones of the northwestern Sargasso Sea. *ISME* **3**: 283-295.

Casey JR, Lomas MW, Mandecki J & Walker DE (2007) *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. *Geophysical Research Letters* **34**: L10604, doi:10.1029/2006GL028725.

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB & Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**: 340-343.

Cotner JB, Ammerman JW, Peele ER & Bentzen E (1997) Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquatic Microbial Ecology* **13**: 141-149.

Dillon TM & Caldwell DR (1980) The Batchelor spectrum and dissipation in the upper ocean. *Journal of Geophysical Research* **85**: 1910-1916.

Duarte CM, Dachs J, Llabrés M, Alonso-Laita P, Gasol JM, Tovar-Sánchez A, Sañudo-Wilhelmy S & Agustí S (2006) Aerosol inputs enhance new production in the

subtropical northeast Atlantic. *Journal of Geophysical Research* **111**: G4, doi:10.1029/2005JG000140.

Duce RA (1986) The impact of atmospheric nitrogen, iron, phosphorus and iron species on marine biological productivity. *The role of air-sea exchange in geochemical cycling* (Buat-Menard P, eds.), pp. 497-529. Reidel, Dordrecht.

Duce RA, Liss PS, Merrill JT, Atlas EL, Buat-Menard P, Hicks BB, Miller JM, Prospero JM, Arimoto R, Church TM, Ellis W, Galloway JN, Hansen L, Jickells TD, Knap AH, Reinhardt KH, Schneider B, Soudine A, Tokos JJ, Tsunogai S, Wollast R & Zhou M (1991) The atmospheric input of trace species to the world ocean. *Global Biogeochemical Cycles* **5**: 193-259.

Duce RA & Tindale NW (1991) Atmospheric transport of iron and its deposition in the ocean. *Limnology and Oceanography* **36**: 1715-1726.

Dufresne A, Garczarek L & Partensky F (2005) Accelerated evolution associated with genome reduction in a free-living prokaryote. *Genome Biology* **6**: R14.

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, Paulsen IT, Tandeau de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post AF, Hess WR & Partensky F (2008) Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. *Genome Biology* **9**: R90.

DuRand MD, Olson RJ & Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep Sea Research II* **48**: 1983-2003.

Ferguson RL & Sunda WG (1984) Utilization of amino acids by planktonic marine bacteria: importance of clean technique and low substrate additions. *Limnology and Oceanography* **29**: 258-274.

Field KG, Gordon D, Wright T, Rappé M, Urbach E, Vergin K & Giovannoni SJ (1997) Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. *Applied and Environmental Microbiology* **63**: 63-70.

Fuchs BM, Woebken D, Zubkov MV, Burkill P & Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. *Aquatic Microbial Ecology* **39**: 145-457.

Fuhrman JA & Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia. *Applied and Environmental Microbiology* **39**: 1085-1095.

Fuhrman JA & Azam F (1982) Thymidine incorporation as a measure of heterotrophic production in marine surface waters: evaluation and field results. *Marine Biology* **73**: 79-89.

Gargett A & Marra J (2002) Effects of upper ocean physical processes (turbulence, advection, and air-sea interaction) on oceanic primary production. *Biological-physical interactions in the sea* (Robinson AR, McCarthy JJ & Rothschild BJ, eds.), pp. 19-49. John Wiley & Sons, Inc., New York.

Giovannoni SJ, Britschgi TB, Moyer CL & Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **345**: 60-63.

Giovannoni SJ, Bibbs L, Cho J-C, Stapels MD, Desiderio R, Vergin KL, Rappé MS, Laney S, Wilhelm LJ, Tripp HJ, Mathur EJ & Barofsky DF (2005a) Proteorhodopsin in the ubiquitous marine bacterium SAR11. *Nature* **438**: 82-85.

Giovannoni SJ & Stingl U (2005) Molecular diversity and ecology of microbial plankton. *Nature* **437**: 343-348.

Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M, Rappé MS, Short JM, Carrington JC & Mathur EJ (2005b) Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309**: 1242-1245.

Gómez-Pereira PR, Fuchs BM, Alonso C, Oliver MJ, van Beusekom JEE & Amann R (2010) Distinct flavobacterial communities in contrasting water masses of the North Atlantic Ocean. *The ISME Journal*.

Graham WF & Duce RA (1982) The atmospheric transport of phosphorus to the western North Atlantic. *Atmospheric Environment* **16**: 1089-1097.

Graziano LM, Geider RJ, Li WKW & Olaizola M (1996) Nitrogen limitation of North Atlantic phytoplankton: analysis of physiological condition in nutrient enrichment experiments. *Aquatic Microbial Ecology* **11**: 53-64.

Gregg MC & Sanford TB (1980) Signatures of mixing from the Bermuda slope, the Sargasso sea, and the Gulf stream. *Journal of Physical Oceanography* **10**: 105-127.

Hand JL, Mahowald NM, Luo C, Chen Y, Siefert RL, Subramaniam A & Fung I (2004) Estimates of atmospheric-processed soluble iron from observations and a global mineral aerosol model: Biogeochemical implications. *Journal of Geophysical Research* **109**: D17205, doi:10.1029/2004JD004574.

Hardman-Mountford N, Hirata T, Richardson KA & Aiken J (2008) An objective methodology for the classification of ecological pattern into biomes and provinces for the pelagic ocean. *Remote Sensing of Environment* **112**: 3341-3352.

Herut B, Zohary T, Krom MD, Mantoura RFC, Pitta P, Psarra S, Rassoulzadegan F, Tanaka T & Thingstad TF (2005) Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Sea Research II* **52**: 3024-3040.

Heywood JL, Zubkov MV, Tarran GA, Fuchs BM & Holligan PM (2006) Prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean: Evaluation of inter-annual variability. *Deep Sea Research II* **53**: 1530-1547.

Hirata T, Aiken J, Hardman-Mountford N, Smyth TJ & Barlow RG (2008) An absorption model to determine phytoplankton size classes from satellite ocean ocolour. *Remote Sensing of Environment* **112**: 3153-3159.

Jardillier L, Zubkov MV, Pearman J & Scanlan DJ (2010) Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. *The ISME Journal* **4:** 1180-1192.

Jickells TD & Spokes LJ (2001) Atmospheric iron inputs into the oceans. *The biogeochemistry of iron in seawater* (Turner DR & Hunter KA, eds.), pp. 85-121. John Wiley & Sons, Chichester.

Jickells TD, An ZS, Andersen KK, Baker AR, Bergametti G, Brooks N, Cao JJ, Boyd PW, Duce RA, Hunter KA, Kawahata H, Kubilay N, laRoche J, Liss PS, Mahowald N, Prospero JM, Ridgwell AJ, Tegen I & Torres R (2005) Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* **308**: 67-71.

Johnson KS, Gordon RM & Coale KH (1997) What controls dissolved iron concentrations in the world ocean? *Marine Chemistry* **57**: 137-161.

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS & Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* **311**: 1737-1740.

Jürgens K & Matz C (2004) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie van Leeuwenhoek* **81**: 413-434.

Kettler GC, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferriera S, Johnson J, Steglich C, Church GM, Richardson P & Chisholm SW (2007) Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. *PLoS Genetics* **3**: e231.

Kirchman D, K'nees E & Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology* **49**: 599-607.

Koven CD & Fung I (2006) Inferring dust composition from wavelength-dependent absorption in Aerosol Robotic Network (AERONET) data. *Journal of Geophysical Research D: Atmospheres* **111**: D14205, doi:10.1029/2005JD006678.

Lekunberri I, Lefort T, Romero E, Vázquez-Domínguez E, Romera-Castillo C, Marrasé C, Peters F, Weinbauer M & Gasol JM (2010) Effects of a dust deposition event on coastal marine microbial abundance and activity, bacterial community structure and ecosystem function. *Journal of Plankton Research* **32**: 381-396.

Lenes JM, Darrow BP, Cattrall C, Heil CA, Callahan M, Vargo GA, Byrne RH, Prospero JM, Bates DE, Fanning KA & Walsh JJ (2001) Iron fertilization and *Trichodesmium* response on the West Florida shelf. *Limnology and Oceanography* **46**: 1251-1277.

Li WKW (1994) Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting. *Limnology and Oceanography* **39**: 169-175.

Madigan MT & Martinko JM (2006) *Brock Biology of Microorganisms*. Prentice-Hall, Upper Saddle River, NJ.

Malmstrom RR, Kiene RP, Cottrell MT & Kirchman DL (2004) Contribution of SAR11 bacteria to dissolved dimethylsulfoniopropionate and amino acid uptake in the North Atlantic Ocean. *Applied and Environmental Microbiology* **70**: 4129-4135.

Malmstrom RR, Kiene RP, Vila M & Kirchman DL (2005) Dimethylsulfoniopropionate (DMSP) assimilation by *Synechococcus* in the Gulf of Mexico and northwest Atlantic Ocean. *Limnology and Oceanography* **50**: 1924-1931.

Martiny AC, Kathuria S & Berube PM (2009) Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. *Proceedings of the National Academy of Science* **106**: 10787-10792.

Mary I, Cummings DG, Biegala IC, Burkill PH, Archer SD & Zubkov MV (2006a) Seasonal dynamics of bacterioplankton community structure at a coastal station in the western English Channel. *Aquatic Microbial Ecology* **42**: 119-126.

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH & Zubkov MV (2006b) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* **45**: 107-113.

Mary I, Garczarek L, Tarran GA, Kolowrat C, Terry MJ, Scanlan DJ, Burkill PH & Zubkov MV (2008a) Diel rhythmicity in amino acid uptake by *Prochlorococcus*. *Environmental Microbiology* **10**: 2124-2131.

Mary I, Tarran GA, Warwick PE, Terry MJ, Scanlan DJ, Burkill PH & Zubkov MV (2008b) Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiology Ecology* **63**: 36-45.

Mills MM, Ridame C, Davey M, La Roche J & Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292-294.

Mills MM, Moore CM, Langlois R, Milne A, Achterberg EP, Nachtigall K, Lochte K, Geider RJ & La Roche J (2008) Nitrogen and phosphorus co-limitation of bacterial productivity and growth in the oligotrophic subtropical North Atlantic. *Limnology and Oceanography* **53**: 824-834.

Moore CM, Mills MM, Milne A, Langlois R, Achterberg EP, Lochte K, Geider RJ & La Roche J (2006) Iron limits primary productivity during spring bloom development in the central North Atlantic. *Global Change Biology* **12**: 626-634.

Moore CM, Mills MM, Langlois R, Milne A, Achterberg EP, La Roche J & Geider RJ (2008) Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean. *Limnology and Oceanography* **53**: 291-305.

Moore LR, Rocap G & Chrisholm SW (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* **393**: 464-467.

Moore LR, Post AF, Rocap G & Chisholm SW (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnology and Oceanography* **47**: 989-996.

Moore LR, Ostrowski M, Scanlan DJ, Feren K & Sweetsir T (2005) Ecotypic variation in phosphorus-acquisition mechanisms within marine picocyanobacteria. *Aquatic Microbial Ecology* **39**: 257-269.

Moore RM, Milley JE & Chatt A (1984) The potential for biological mobilisation of trace elements from Aeolian dust and its importance in the case of iron. *Oceanologica acta* **7**: 221-228.

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA & Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806-810.

Morris RM, Vergin KL, Cho JC, Rappé MS, Carlson CA & Giovannoni SJ (2005) Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series Study site. *Limnology and Oceanography* **50**: 1687-1696.

Oakey NS & Elliott JA (1982) Dissipation within the surface mixed layer. *Journal of Physical Oceanography* **12**: 171-185.

Oliver MJ & Irwin AJ (2008) Objective global ocean biogeographic provinces. *Geophysical Research Letters* **35**: L15601, doi:10.1029/2008GL034238.

Olson RJ, Chisholm SW, Zettler ER & Armbrust EV (1988) Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. *Deep Sea Research I* **35**: 425-440.

Olson RJ, Chisholm SW, Zettler ER & Armbrust EV (1990) Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnology and Oceanography* **35**: 45-58.

Olson RJ, Zettler ER & Durand MD (1993) Phytoplankton analysis using flow cytometry. *Handbook of methods in aquatic microbial ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds.), pp. 175-186. Lewis Publishers, Boca Raton, Florida.

Pace NR, Stahl DA, Lane DJ & Olsen GJ (1985) The analysis of natural microbial populations by ribosomal RNA sequences. *Advances in Microbial Ecology* **9**: 1-55.

Patterson CC & Settle DM (1987) Review of data on eolian fluxes of industrial and natural lead to the land and seas in remote regions on a global scale. *Marine Chemistry* **22**: 137-162.

Paytan A, Mackey KRM, Chen Y, Lima ID, Doney SC, Mahowald N, Labiosa R & Post AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences* **106**: 4601-4605.

Poulton SW & Raiswell R (2002) The low-temperature geochemical cycle of iron: from continental fluxes to marine sediment deposition. *American Journal of Science* **302**: 774-805.

Pulido-Villena E, Reche I & Morales-Baquero R (2008a) Evidence of an atmospheric forcing on bacterioplankton and phytoplankton dynamics in a high mountain lake. *Aquatic Sciences* **70**: 1-9.

Pulido-Villena E, Wagener T & Guieu C (2008b) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* **22**: GB1020, doi:10.1029/2007GB003091.

Rappé MS, Connon SA, Vergin KL & Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418**: 630-633.

Reche I, Ortega-Retuerta E, Romera O, Pulido-Villena E, Morales-Baquero R & Casamayor EO (2009) Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs. *Limnology and Oceanography* **54**: 869-879.

Ridame C & Guieu C (2002) Saharan input of phosphate to the oligotrophic water of the open western Mediterranean Sea. *Limnology and Oceanography* **47**: 856-869.

Rijkenberg MJA, Powell CF, Dall'Osto M, Nielsdottir MC, Patey MD, Hill PG, Baker AR, Jickells TD, Harrison RM & Achterberg EP (2008) Changes in iron speciation following a Saharan dust event in the tropical North Atlantic Ocean. *Marine Chemistry* **110**: 56-67.

Rippka R, Coursin T, Hess W, Lichtle C, Scanlan DJ, Palinska KA, Iteman I, Partensky F, Houmard J & Herdman M (2000) *Prochlorococcus marinus* Chisholm *et al*., 1992 subsp. *pastoris* subsp. nov. strain PCC 9511, the first axenic chlorophyll a_2/b_2-containing cyanobacterium (*Oxyphotobacteria*). *International Journal of Systematic and Evolutionary Microbiology* **50**: 1833-1847.

Rivkin RB & Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. *Limnology and Oceanography* **42**: 730-740.

Rosenfeld D, Rudich Y & Lahav R (2001) Desert dust suppressing precipitation: A possible desertification feedback loop. *Proceedings of the National Academy of Sciences* **98**: 5975-5980.

Savoie DL, Prospero JM & Saltzman ES (1989) Non-sea-salt sulfate and nitrate in Trade Wind aerosols at Barbados: Evidence for long-range transport. *Journal of Geophysical Research* **94**: 5069-5080.

Scanlan DJ & West NJ (2002) Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiology Ecology* **40**: 1-12.

Scanlan DJ (2003) Physiological diversity and niche adaptation in marine *Synechococcus*. *Advances in Microbial Physiology* **47**: 1-64.

Schattenhofer M (2009) Distribution of major bacterioplankton groups in the Atlantic Ocean. PhD Thesis, University of Bremen, Bremen.

Schattenhofer M, Fuchs BM, Amann R, Zubkov MV, Tarran GA & Pernthaler J (2009) Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. *Environmental Microbiology* **11**: 2078-2093.

Sohm JA & Capone DG (2010) Zonal differences in phosphorus pools, turnover and deficiency across the tropical North Atlantic Ocean. *Global Biogeochemical Cycles* **24**: GB2008, doi:10.1029/2008GB003414.

Sowell SM, Wilhelm LJ, Norbeck AD, Lipton MS, Nicora CD, Barofsky DF, Carlson C, Smith RD & Giovannoni SJ (2009) Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. *The ISME Journal* **3**: 93-105.

Spokes LJ, Jickells TD & Lim B (1994) Solubilisation of aerosol trace metals by cloud processing: a laboratory study. *Geochimica et Cosmochimica Acta* **58**: 3281-3287.

Suzuki MT, Preston CM, Chavez FP & DeLong EF (2001) Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay. *Aquatic Microbial Ecology* **24**: 117-127.

Tarran GA, Zubkov MV, Sleigh MA, Burkill PH & Yallop M (2001) Microbial community structure and standing stocks in the NE Atlantic in June and July of 1996. *Deep Sea Research II* **48**: 963-985.

Tarran GA, Heywood JL & Zubkov MV (2006) Latitudinal changes in the standing stocks of nano- and picoeukaryotic phytoplankton in the Atlantic Ocean. *Deep Sea Research II* **53**: 1516-1529.

Teira E, Gasol JM, Aranguren-Gassis M, Fernández A, González J, Lekunberri I & Álvarez-Salgado XA (2008) Linkages between bacterioplankton community composition, heterotrophic carbon cycling and environmental conditions in a highly dynamic coastal ecosystem. *Environmental Microbiology* **10**: 906-917.

Thingstad TF & Lignell R (1997) Theoretical models for control of bacterial growth rate, abundance, diversity and carbon demand. *Microbial Ecology* **13**: 19-27.

Topping JN, Heywood JL, Ward P & Zubkov MV (2006) Bacterioplankton composition in the Scotia Sea, Antarctica, during the austral summer of 2003. *Aquatic Microbial Ecology* **45**: 229-235.

Tripp HJ, Kitner JB, Schwalbach MS, Dacey JWH, Wilhelm LJ & Giovannoni SJ (2008) SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* **452**: 741-744.

Urbach E, Roberson DLR & Chisholm SW (1992) Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. *Nature* **355**: 267-270.

Van Mooy BAS, Rocap G, Fredricks HF, Evans CT & Devol AH (2006) Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *Proceedings of the National Academy of Sciences* **103**: 8607-8612.

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblizek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS & Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* **458**: 69-72.

Vaulot D, Marie D, Olson RJ & Chisholm SW (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. *Science* **268**: 1480-1482.

Wagener T, Guieu C & Leblond N (2010) Effects of dust deposition on iron cycle in the surface Mediterranean Sea: results from a mesocosm seeding experiment. *Biogeosciences Discussions* **7**: 2799-2830.

Waterbury JB, Watson SW, Guillard RRL & Brand LE (1979) Widespread occurrence of a unicellular, marine planktonic cyanobacterium. *Nature* **277**: 293-294.

West NJ & Scanlan DJ (1999) Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. *Applied and Environmental Microbiology* **65**: 2585-2591.

West NJ, Schönhuber WA, Fuller NJ, Amann RI, Rippka R, Post AF & Scanlan DJ (2001) Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by *in situ* hybridization using 16S rRNA-targeted oligonucleotides. *Microbiology* **147**: 1731-1744.

Williams PJlB (1973) The validity of the application of simple kinetic analysis to heterogeneous microbial populations. *Limnology and Oceanography* **18**: 159-165.

Woese CR (1987) Bacterial evolution. *Microbiology Reviews* **51**: 221-271.

Wright RT & Hobbie JE (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* **47**: 447-464.

Wu J, Sunda W, Boyle EA & Karl DM (2000) Phosphate depletion in the western north Atlantic Ocean. *Science* **289**: 759-762.

Yamazaki H & Osborn TR (1988) Review of oceanic turbulence: implications for biodynamics. *Toward a theory on biological-physical interactions in the world ocean* (Rothschild BJ, eds.), pp. 215-234. Kluwer Academic Publishers, Dordrecht.

Zubkov MV, Sleigh MA, Tarran GA, Burkill PH & Leakey RJG (1998) Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S. *Deep Sea Research I* **45**: 1339-1355.

Zubkov MV, Sleigh MA, Burkill PH & Leakey RJG (2000a) Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. *Journal of Plankton Research* **22**: 685-711.

Zubkov MV, Sleigh MA, Burkill PH & Leakey RJG (2000b) Picoplankton community structure on the Atlantic Meridional transect: a comparison between seasons. *Progress in Oceanography* **45**: 369-386.

Zubkov MV, Burkill PH, Tarran GA, Fuchs BM & Amann R (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Applied and Environmental Microbiology* **69**: 1299-1304.

Zubkov MV, Tarran GA & Fuchs BM (2004) Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *FEMS Microbiology Ecology* **50**: 153-161.

Zubkov MV & Tarran GA (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. *Aquatic Microbial Ecology* **40**: 241-249.

Zubkov MV, Mary I, Woodward EMS, Warwick PE, Fuchs BM, Scanlan DJ & Burkill PH (2007) Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environmental Microbiology* **9**: 2079-2089.

Zwirglmaier K, Heywood JL, Chamberlain K, Woodward EMS, Zubkov MV & Scanlan DJ (2007) Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. *Environmental Microbiology* **9**: 1278-1290.

Chapter Two

Similarity in microbial amino acid uptake in surface waters of the North and South Atlantic (sub-)tropical gyres

2.1 Introduction

The oligotrophic gyres occupy 40% of the Earth's surface area, and are considered to be expanding due to global warming leading to increased vertical stratification in the open ocean (McClain *et al*., 2004, Polovina *et al*., 2008). The more we understand about the biogeochemistry of these vast regions, the better equipped we will be to predict the consequences of this expansion. Compared to temperate and coastal zones, the oligotrophic gyres of the Atlantic Ocean are homogeneous in pigment concentration and microbial assemblage (Heywood *et al*., 2006, Hirata *et al*., 2008, Aiken *et al*., 2009). This conservative nature of the oligotrophic gyres makes them relatively simple to study at the provincial scale, and to characterise for the purpose of mathematical models. The application of average conditions of variables indicative of production has been suggested useful for the characterisation of distinct biogeographic provinces (Longhurst, 1998, Zubkov *et al*., 2000b). By applying mean production values to the oceanic gyres, only the areal extent of the gyres would be required to estimate overall provincial production. The extent of oceanic gyres can now be achieved at high spatial resolution from satellite observations (Hardman-Mountford *et al*., 2008, Hirata *et al*., 2008, Oliver & Irwin, 2008).

Considerable datasets have already been gathered on the standing stocks of various components of the bacterioplankton and phytoplankton community throughout the Atlantic Ocean (Zubkov *et al*., 2000a, Heywood *et al*., 2006, Tarran *et al*., 2006), but there are few cross-province studies of microbial activity (Zubkov *et al*., 2000b, Hoppe *et al*., 2002, Hoppe *et al*., 2006, Gasol *et al*., 2009). Hoppe *et al.* (2002) found that the ratio of bacterial carbon demand to primary production was positively correlated with seawater temperature along a north-south transect through the Atlantic Ocean, as was the ratio of bacterioplankton production to primary production. Furthermore, the warmer latitudes have been demonstrated to be net heterotrophic (Hoppe *et al*., 2002, Gasol *et al*., 2009), with bacterial production dependent on the supply of nutrients across the thermocline

(Gasol *et al*., 2009). However, despite crossing through a range of provinces, including the open ocean and African upwelling regions, with variable leucine uptake rates (Hoppe *et al*., 2002, Hoppe *et al*., 2006), these studies did not attempt to compare bacterial production between regions of distinct trophic status. Gasol *et al.* (2009) compared bacterial production measurements between regions and sampling periods, however, the regions were static and were not determined according to distinct water masses or any biological feature, which may explain some of the inter-province and inter-cruise variability observed.

Bacterial production is generally determined from the uptake rates of saturating concentrations of amino acids which, as mentioned in Chapter One, may give misleading results. Alternatively, microbial activity can be estimated at ambient amino acid concentration by adding radioactively-labelled amino acids in concentrations at and below ambient. The dilution bioassay approach designed by Wright & Hobbie (1966) and later used for the assessment of amino acid uptake (Fuhrman & Ferguson, 1986, Zubkov & Tarran, 2005), has been employed in this study, rather than a bulk bacterial production measurement (Kirchman *et al*., 1985). This technique also estimates ambient amino acid concentration, and is believed to be more accurate than estimates by chemical methods (Berman & Bronk, 2003), and minimises contamination. Thus it provides information on concentration and demand simultaneously. Although amino acid uptake rates could be converted to bacterial production measurements, this is not necessary to compare seasonal and provincial variance in microbial activity.

Uptake rates of amino acids are a reliable indicator of metabolic activity of the whole bacterioplankton community because amino acids are assimilated by cyanobacteria (Zubkov *et al*., 2003) as well as heterotrophic bacterioplankton. Dissolved free amino acids are a good model for studying biologically available dissolved organic matter (DOM) in seawater. Measuring the uptake rate of different amino acids simultaneously gives an indication of the relative importance of each amino acid within each biogeographic province, which can provide further information on the microbial community physiology.

The aim of this compilation was to study variability in bacterioplankton activity in the Atlantic (sub-)tropical gyres. By defining distinct biogeographic provinces, variation within the gyres was compared with more mesotrophic and productive regions of the Atlantic Ocean, such as the Equatorial and Mauritanian upwelling regions. The

applicability of a mean value was assessed based on the variability in uptake rates observed within each province.

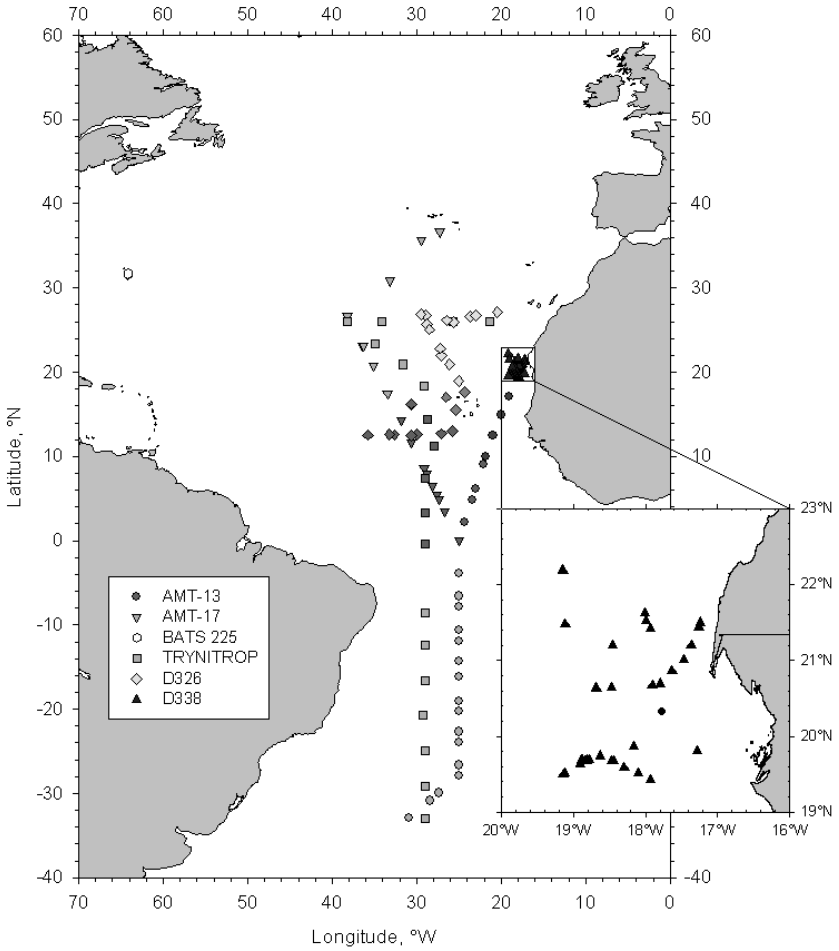


Figure 2.1: Sampling locations, divided between cruises: AMT-13 (September-October 2003, southbound), AMT-17 (October-November 2005), BATS (July 2007), TRYNITROP (November-December 2007, southbound), D326 (January-February, 2008), D338 (April-May 2009). The degree of shading of the symbols indicates biogeographic provinces from most oligotrophic (white) to most productive (black).

2.2 Method

General sampling methods

Seawater was collected during six research cruises to the Atlantic Ocean from 2003 to 2009 (Table 2.1 and Figure 2.1). Surface seawater samples were generally collected from 3-7 m depth in 20 L Niskin bottles mounted on a CTD profiler with a stainless steel or titanium

frame. Seawater was decanted into 1 L acid-cleaned glass thermos flasks or polycarbonate bottles using acid-soaked silicone tubing. To increase sampling frequency during the BATS cruise, samples were mostly collected using an acid-cleaned polypropylene bucket, which was lowered manually from the side of the ship. Radioactively labelled amino acid dilution bioassays were initiated within 1 h of sampling.

The northern gyre was sampled predominantly in the centre and in the east, with only the Bermuda Atlantic Time-series Study (BATS) station in the Sargasso Sea sampled in the west (Figure 2.1). The proximity of BATS to the Gulf Stream results in a region characterised by high eddy energetics (Steinberg *et al.*, 2001). Furthermore, it lies in a transition zone between the oligotrophic Sargasso Sea and relatively eutrophic waters from the north (Steinberg *et al.*, 2001). The latitudinal movement of these water bodies results in seasonality at the BATS site more typical of temperate regions that those of the North Atlantic oligotrophic gyre.

Table 2.1: Summary of research cruises. Provinces sampled are abbreviated as follows: North Atlantic Gyre (NG), South Atlantic Gyre (SG), Bermuda Atlantic Time-series Study (BATS), North-East Subtropical Atlantic (NESA), Cape Verde (CV), Equatorial region (EQ), and Mauritanian Upwelling (MU). Data from AMT-13 and AMT-17 were provided by Mikhail Zubkov; some data from BATS225 were provided by Isabelle Mary.

Cruise	Research vessel	Sampling period	Provinces	Sampled by
JR91, AMT-13	RRS *James Clark Ross*	Sep-Oct 2003	MU, SG	M Zubkov
D299, AMT-17	RRS *Discovery*	Oct-Nov 2005	NG, EQ	M Zubkov
BATS225	RV *Atlantic Explorer*	July 2007	BATS	P Hill, I Mary
TRYNITROP	RV *Hespérides*	Nov-Dec 2007	NG, EQ, SG	P Hill
D326	RRS *Discovery*	Jan-Feb 2008	NESA, CV, EQ	P Hill
D338, ICON	RRS *Discovery*	April-May 2009	MU	P Hill

Radioactively labelled amino acid dilution bioassays

Ambient concentrations and turnover rates of amino acids were estimated using a bioassay technique of radiotracer dilution (Wright & Hobbie, 1966) with untreated live samples. Leucine and methionine uptake rates were measured during all studies, and tyrosine uptake was measured during AMT-13 and D338. The method published for the

AMT cruises (Zubkov & Tarran, 2005, Mary *et al.*, 2008b, Zubkov *et al.*, 2008) was also used for subsequent cruises.

Briefly, L-[4,5-^{3}H]leucine (specific activity 4-6 TBq mmol^{-1}) was added in a series of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 nM concentration in oceanic regions, or 0.2 - 2.0 nM in the more productive Mauritanian upwelling region (D338). The L-[3,5-^{3}H]tyrosine (specific activity 2 TBq mmol^{-1}) was generally added in the range 0.1 - 2.0 nM. The L-[^{35}S]methionine (specific activity >37 TBq mmol^{-1}) was added at a standard concentration of 0.1 nM during the AMT cruises, or 0.05 nM on all other cruises, and diluted with unlabelled methionine in the range 0.1 - 2.0 nM. Radioactive chemicals were purchased from Amersham Biosciences (Little Chalfont, UK) or its successor GE Healthcare (Little Chalfont, UK), or Hartmann Analytic (Brunswick, Germany).

Triplicate samples (1.6 mL) for each amino acid and at each concentration were incubated in 2 mL polypropylene screw cap vials at *in situ* temperature. One sample from each concentration was fixed at 10, 20 and 30 min by the addition of 20% paraformalydehyde (1% v/w final concentration). Due to the short incubation times, it was not possible to work in the dark; however, incubations were kept in dim indirect light. Fixed cells were filtered onto 0.2 µm polycarbonate membrane filters soaked in the corresponding non-labelled amino acid solution to reduce adsorption of tracer. Filtered samples were washed twice with 4 mL deionised water. Radioactivity of samples was measured as disintegrations per minute (DPM) by liquid scintillation counting (Tri-Carb 3100TR, Perkin Elmer, Beaconsfield, UK, or Wallac WinSpectral 1414; Tri-Carb 2900TR, Perkin Elmer).

Amino acid uptake rates were calculated at each addition concentration as the gradient of the linear regression of community assimilated radioactivity (DPM) against incubation time (Figure 2.2a). The time it would have taken the community to assimilate all the added amino acid was then plotted against added concentration (Figure 2.2b); ambient uptake rate was determined from the slope of its linear regression and presented with the standard error of the regression. Ambient concentration is estimated as the intercept on the x-axis (at which turnover time is equal to zero). The ambient turnover time is thus derived as the uptake rate divided by ambient concentration; that is, the turnover time when addition concentration is equal to zero.

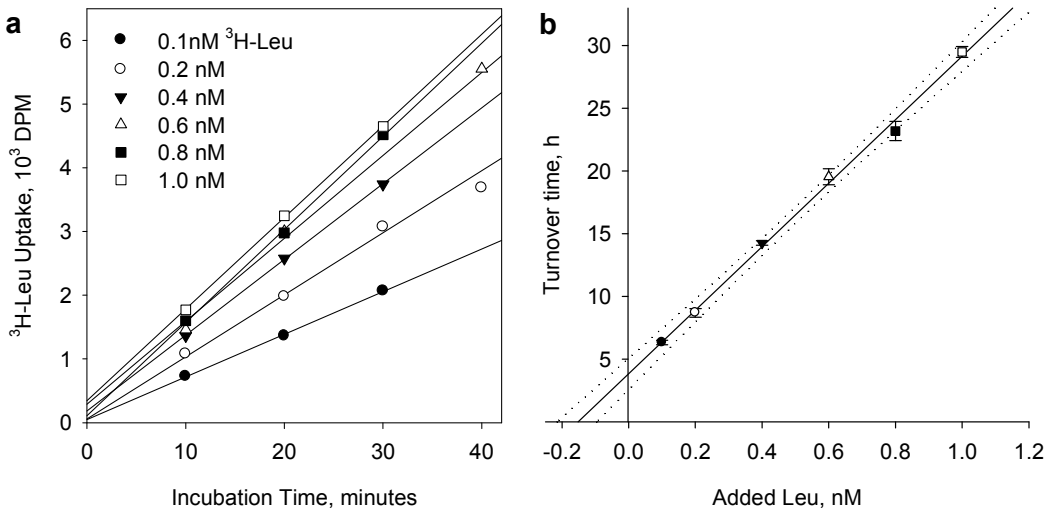


Figure 2.2: Example of the results from a ^{3}H-leucine (^{3}H-Leu) dilution bioassay estimation of concentration and turnover rates of ambient leucine (Leu) carried out during cruise D326 in January 2007. Figure (a) shows the time series of ^{3}H-Leu at each added concentration; regression lines give a measurement of uptake rate at each concentration. Figure (b) shows the relationship between added ^{3}H-Leu and turnover time (derived from Figure a, ± standard error of the regression) with the 95% confidence intervals. The y-axis intercept of the regression in Figure b gives an estimate of turnover time at ambient Leu concentration. Ambient Leu concentration is estimated as the x-axis intercept (Figure b).

Determination of biogeographic provinces

The extent of the oceanic gyres was resolved for each sampling period to allow for boundary migration. Boundaries were determined from MODIS ocean colour data as the point at which chlorophyll *a* (Chl-*a*) concentration exceeded 0.1 mg m^{-3}, thus the gyres were characterised by oligotrophic, blue water. Intermediate regions, such as the equatorial region, were distinguished as containing 0.1-0.25 mg Chl-*a* m^{-3}. Mesotrophic, generally coastal, regions were identified as those areas where Chl-*a* concentration exceeded 0.25 mg m^{-3}, since this coincides with a decline in the picoplankton community (Hirata *et al.*, 2008, Aiken *et al.*, 2009). The MODIS composite images produced for each sampling period and used for the provincial allocation of data are given in Figure2.3.

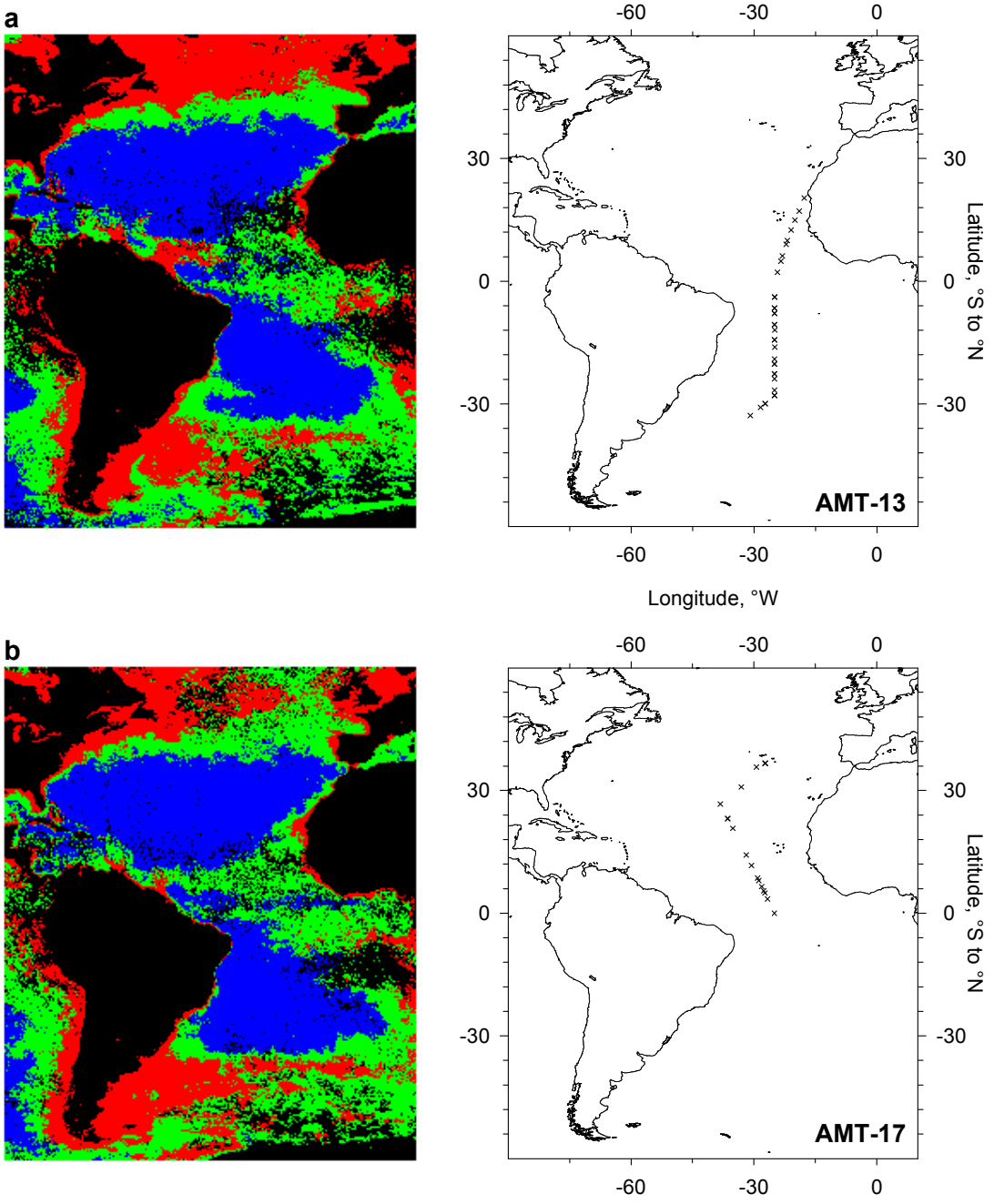


Figure 2.3: Composite ocean colour images taken from MODIS (Moderate Resolution Imaging Spectroradiometer) and sampling stations for cruises (a) AMT-13 (September and October 2003) and (b) AMT-17 (October and November 2005). Chlorophyll *a* values indicated from the MODIS images: Blue, Chl-*a* <0.1 mg m^{-3}; Green, Chl-*a* 0.1-0.25 mg m^{-3}; Red, Chl-*a* >0.25 mg m^{-3}. MODIS composite images were supplied by the NERC Earth Observation Data Acquisition and Analysis Service (NEODAAS).

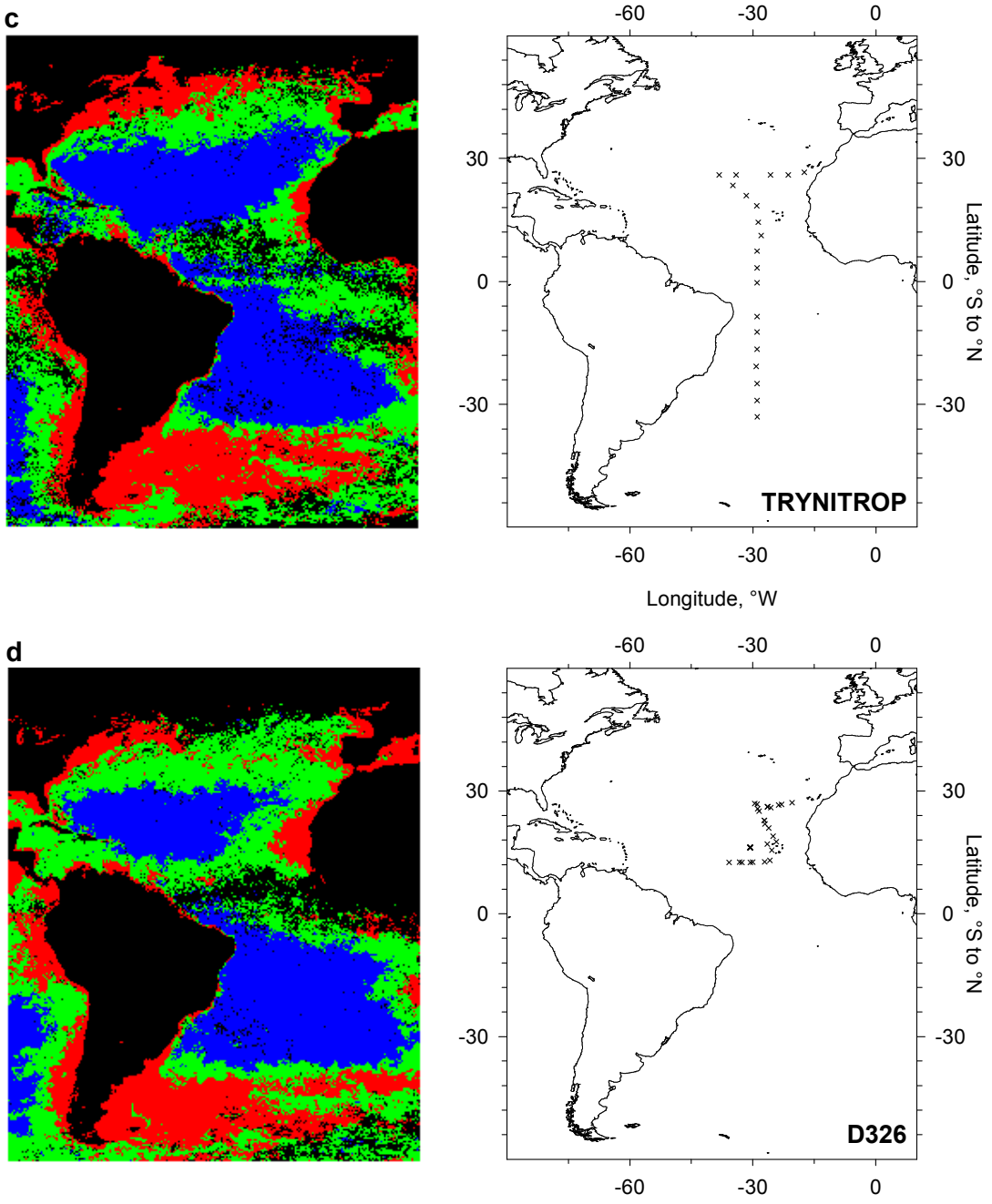


Figure 2.3 continued: MODIS Ocean colour images and sampling stations for cruises (c) TRYNITROP (November and December 2007) and (d) D326 (January and February 2008). Chlorophyll *a* values indicated from the MODIS images: Blue, Chl-*a* <0.1 mg m^{-3}; Green, Chl-*a* 0.1-0.25 mg m^{-3}; Red, Chl-*a* >0.25 mg m^{-3}. MODIS composite images were supplied by the NERC Earth Observation Data Acquisition and Analysis Service (NEODAAS).

Few satellite-derived Chl-*a* concentrations were available from the exact sampling locations and times for amino acid bioassays due to perpetual cloud cover, but we believe these boundaries are relevant due to the homogenous distribution of pigments in the gyres (Aiken *et al.*, 2009). Boundaries were verified by a step-wise increase or decrease in chlorophyll fluorescence measured at the point of sampling, and by signatures of distinct water masses from temperature-salinity diagrams. No attempts have been made to compare chlorophyll fluorescence data between cruises due to a lack of cross ship calibration.

Data analysis

Unprocessed amino acid uptake data are presented as the regression slope of the measurement ± standard error (SE). Provincially averaged data are presented as mean ± standard deviation (SD) since variation is central to the study. Coefficient of variance was calculated as (SD/mean) x 100, and is thus presented as a percentage. Variance is compared between provinces using the F-test. Following the F-test for equality of variance, means were compared using the student's *t*-test for matched or unmatched pairs depending on datasets compared. A one-way ANOVA followed by a Tukey-Kramer means comparison test ($\alpha = 0.05$) was used to compare three or more sampling periods or regions. Where data within a province did not differ between sampling periods data were combined. Where there were only one or two provincial data points from a particular sampling period, data were included if they lay within three standard deviations of the provincial mean.

2.3 Results

2.3.1 Temporal within-province variation

The Oligotrophic (Sub-)tropical Gyres

The regions identified in this study as North and South Atlantic Gyres are essentially the biogeographic provinces identified by Longhurst (1998) as the North and South Atlantic Tropical Gyral Provinces. The North Atlantic Gyre (NG) was sampled during October-November 2005, and November 2007 (Table 2.1), during which periods the mixed layer depths (54 ± 7 and 65 ± 8 m, respectively) were comparable. Variation in methionine uptake rates and mean values measured in the NG were similar between sampling periods (Table 2.2). Variation in leucine uptake rate differed slightly between sampling

periods, due to a particularly high uptake rate measured at the edge of the gyre (25.24 nmol L^{-1} h^{-1}), which is clearly an outlying point on Figure 2.4a. Despite this high uptake rate at the gyre boundary, most likely a response to increased production where the two water bodies met, mean uptake rates did not differ significantly (Table 2.2 and Figure 2.4a). However, this does highlight the importance of defining a reliable method by which to determine gyre boundaries before mean values can be applied throughout. Although the value lies within three standard deviations of the mean for the period during which it was sampled (25.27 nmol L^{-1} h^{-1}), it lies outside three sigmas of the mean value for the NG, and thus the value is excluded from further analyses to avoid overestimation of the mean uptake value for the oligotrophic gyre.

Table 2.2: Mean leucine (Leu) and methionine (Met) uptake rates (pmol L^{-1} h^{-1}) measured during different sampling periods in the North and South Atlantic Gyres. Mean uptake rates and variation are compared between sampling periods. Results of the F-test (F) for equality of variance and the student's *t*-test (*t*) for comparing the means of independent samples are presented; the number of observations (n) is given in parenthesis. *p<0.05.

	North Gyre				South Gyre			
	Oct 2005	F	*t*	Nov 2007	Oct 2003	F	*t*	Dec 2007
Leu	13.0 ± 6.3 (9)	>*	=	12.2 ± 2.7 (7)	13.4 ± 2.5 (11)	=	=	12.9 ± 3.3 (7)
Met	8.2 ± 4.1 (8)	=	=	10.0 ± 1.9 (7)	11.0 ± 3.3 (18)	=	=	10.3 ± 2.9 (7)

Table 2.3: Mean (±SD) leucine (Leu) and methionine (Met) uptake rates (pmol L^{-1} h^{-1}) measured in the North and South Atlantic Gyres. Results of the F-test (F) for equality of variance and the student's *t*-test (*t*) for comparing mean uptake rates between the two gyres are presented. The number of observations, n, is given in parenthesis. All variation was found to be insignificant (p>0.1).

	South Gyre	F	*t*	North Gyre
Leu	13.2 ± 2.7 (18)	=	=	11.8 ± 3.7 (15)
Met	10.8 ± 3.2 (25)	=	=	8.7 ± 3.2 (15)

The mixed layer depth in the South Atlantic Gyre (SG) was comparable between the two sampling periods in the region, September-October 2003 (115 ± 22 m) and December 2007 (131 ± 50 m). Mean and variance of leucine and methionine uptake rates were also similar between the two periods (Table 2.2 and Figure 2.4a). Variation in uptake rates was similar

between the North and South gyres for both amino acids, as were mean values (Table 2.3). Therefore, data from the two gyres were combined to give overall mean uptake rates of 12.6 ± 3.2 pmol Leu L^{-1} h^{-1} and 10.0 ± 3.3 pmol Met L^{-1} h^{-1} for the Atlantic Ocean oligotrophic gyres.

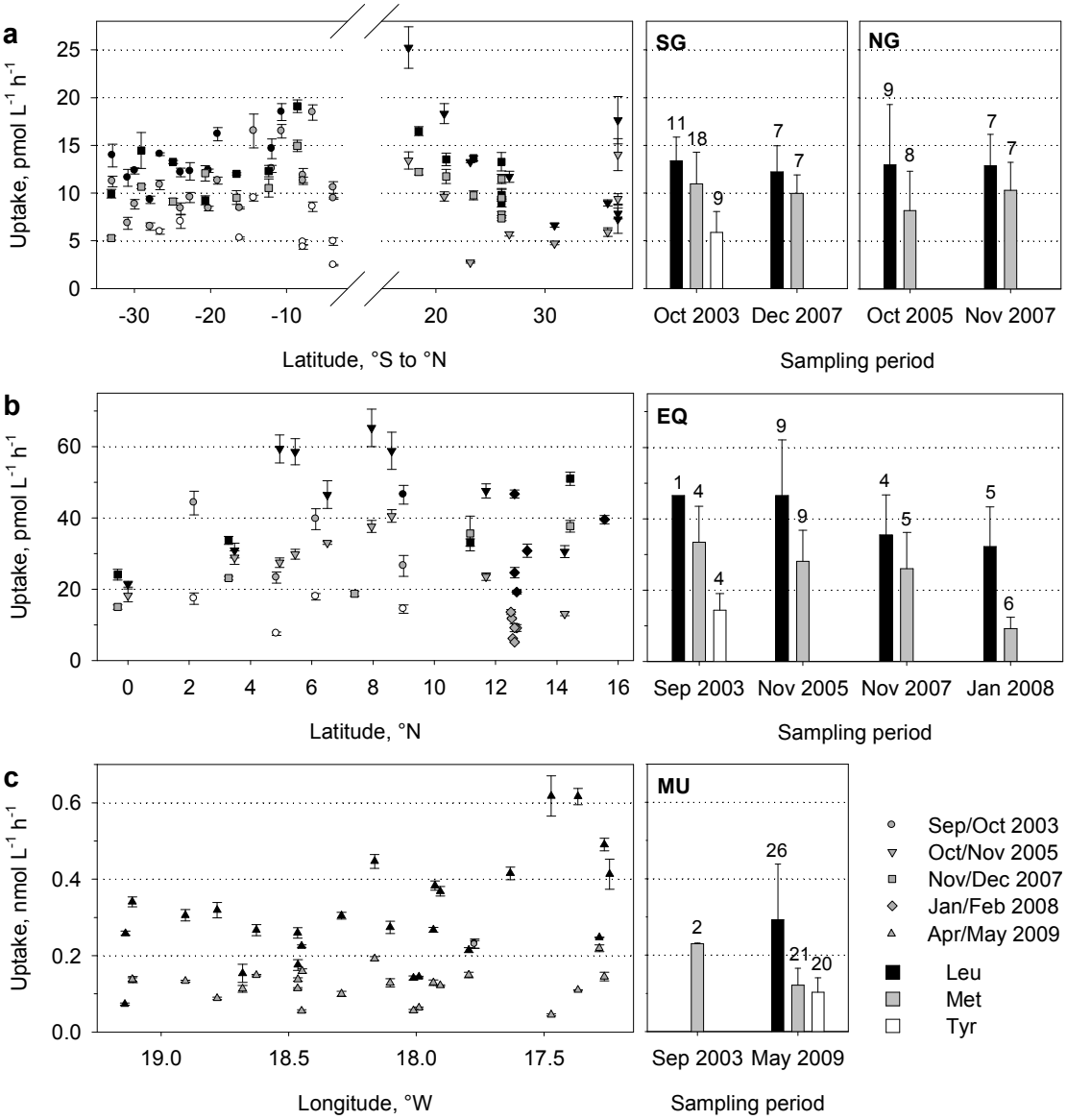


Figure 2.4: Leucine (black), Methionine (grey) and Tyrosine (white) uptake rates measured in (a) the North (NG) and South (SG) Atlantic Gyres, (b) the Equatorial region (EQ), and (c) the Mauritanian Upwelling (MU). Original data are presented ± standard error of measurements in the left hand scatter plots; uptake is plotted against degrees of latitude (a and b) or longitude (c). Mean values for each cruise are presented ± standard deviation for the stated number of observations, or ± standard difference where n = 2 (right hand bar charts). Note that uptake is reported as pmol L^{-1} h^{-1} for NG, SG and EQ, and nmol L^{-1} h^{-1} for the MU region.

The North East (Sub-)tropical Atlantic

During January and February 2008 (cruise D326) a heterogeneous region was sampled in the northeast tropical and (sub-)tropical Atlantic. Although the sampling region covered what is generally considered to be the north Atlantic (sub-)tropical gyre, ocean colour data showed Chl-*a* concentration exceeded 0.1 mg m^{-3} throughout (Figure 2.3). This was confirmed by ship-board Chl-*a* analysis of filtered seawater samples analysed by fluorescence (DA Purdie, unpublished data). Therefore, the region could not be divided by ocean colour data alone. Temperature-salinity diagrams for all CTD stations sampled indicated three distinct regions, which have been termed the North-East Subtropical Atlantic (NESA), and the Cape Verde (CV) and Equatorial (EQ) regions (Appendix I). The NESA region had mixed layer depths of between 100 and 150 m, with a modest temperature gradient between the upper and lower layers. Waters influenced by the equatorial region had warm, shallow mixed layers of 35 to 70 m, overlying much cooler waters of the north equatorial counter-current. In between these distinct provinces was an intermediate region, which was influenced by the topography of the Cape Verde Islands. There was significant variation in the uptake rates of leucine ($F = 27$, $df = 23$, $p<0.0001$) and methionine ($F = 26$, $df = 27$, $p<0.0001$) between the three water masses sampled.

Table 2.4: Mean (±SD) leucine (Leu) and methionine (Met) uptake rates (pmol L^{-1} h^{-1}) measured in the North-East Subtropical Atlantic (NESA, Jan-Feb 2008) and at the Bermuda Atlantic Time-series Study (BATS) station in the Sargasso Sea (July 2007). Mean uptake rates and variation measured in the NESA and BATS provinces are compared with those from the North Gyre (NG, final column). The results of the F-test (F) for equality of variance and the student's *t*-test (*t*) are presented. The number of observations, n, is given in parenthesis. Where mean or variance is significantly different from the NG, *p<0.05, **p<0.01, ***p<0.0001.

Province		Uptake rate	F	*t*	Uptake rate, NG
NESA	Leu	8.9 ± 2.7 (12)	=	<*	11.8 ± 3.7 (15)
	Met	5.3 ± 1.9 (13)	<*	<***	8.7 ± 3.2 (15)
BATS	Leu	3.1 ± 1.9 (16)	<*	<**	11.8 ± 3.7 (15)
	Met	4.0 ± 2.3 (15)	=	<***	8.7 ± 3.2 (15)

In the CV region, uptake rates (29.0 ± 9.2 pmol Leu L^{-1} h^{-1} and 13.2 ± 5.3 pmol Met L^{-1} h^{-1}) were between those of the NG and the EQ region. Interestingly, despite apparently higher

photosynthetic biomass in the region as shown in ocean colour images (Figure 2.3d), both leucine and methionine uptake rates were significantly lower in the NESA region than in the NG (Table 2.4). This is an important observation because the region here identified as the North-East Subtropical Atlantic is a region that is often considered to be part of the North Atlantic Gyre. During January and February 2008 the region clearly was not within the oligotrophic gyre and, had these observations been included with those from the gyre, they would have decreased the mean uptake rates.

Sargasso Sea

On the western side of the North Atlantic Ocean is the Bermuda Atlantic Time-series Study (BATS) station, located within the Sargasso Sea. The BATS station was sampled in July 2007; the water column was thermally stratified with a comparatively fresh surface layer as is typical for the time of year (Steinberg *et al*., 2001). The mixed layer depth was shallower than 20 m. Of the 16 samples collected at this station, two were collected in Niskin bottles from 7 m and the others from the surface (<1 m) using a bucket. The data obtained from Niskin-collected samples (4.9 and 2.1 pmol Leu L^{-1} h^{-1}; 3.7 and 6.1 pmol Met L^{-1} h^{-1}) lay within one standard deviation of the mean of those from bucket samples (3.1 ± 1.9 and 3.8 ± 2.4 pmol L^{-1} h^{-1} for leucine and methionine uptake, respectively), thus data are considered comparable.

Leucine and methionine uptake rates measured at this site were significantly lower than those measured in the NG (Table 2.4). In this instance the BATS station is considered unrepresentative of the NG and the data are discussed independently. The considerably lower uptake rates measured at BATS highlight the seasonality of the region as a result of its proximity to the transition zone between the gyre and eutrophic waters from the north. Interestingly, methionine uptake rates measured in the Sargasso Sea were not significantly different to those measured in the NESA region (Table 2.4; t = 1.5, df = 25, p = 0.1), suggesting that regions on the fringes of the gyre may also be comparable.

Equatorial region

The region identified in this study as the Equatorial (EQ) region is the biogeographic province identified by Longhurst (1998) as the Western Tropical Atlantic Province. The region was sampled during four periods – September 2003, October 2005, November 2007 and January 2008 – and was characterised by a shallow mixed layer depth of typically 30-70 m. Leucine uptake rates in the EQ region did not vary significantly between cruises

(Figure 2.4b; ANOVA, F = 2.1, df = 17, p = 0.16); whereas methionine uptake varied due to low uptake rates measured in January 2008 (Figure 2.4b; ANOVA: F = 8.8, df = 23, p = 0.001).

Mauritanian Upwelling

The main sampling period in the Mauritanian Upwelling (MU) region was April-May 2009, although two samples in the region were analysed for methionine uptake in September 2003 (Figure 2.4c). Surface seawater samples were collected from areas with a range of depths, from the source of the upwelling (65 m) to the deep sea (3000 m). Temperature in the region was positively correlated with longitude west ($r = 0.92$, $p<0.0001$, $N = 32$), confirming upwelling at the coast and offshore drift. Unlike methionine and tyrosine, leucine uptake was negatively correlated with temperature ($r = -0.65$, $p<0.0002$, $N = 27$), indicating highest microbial activity at the source of upwelling, and a decline with distance from the source.

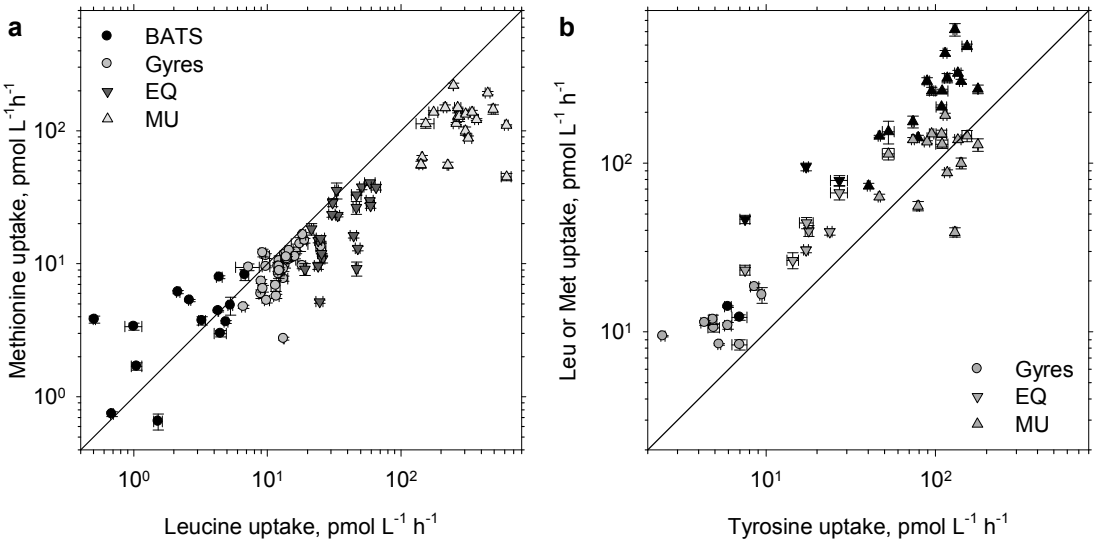


Figure 2.5: Leucine and methonine uptake rates are presented for those samples where both amino acids were measured simultaneously (a). Data have been divided between provinces: Bermuda Atlantic Time-series Study in the Sargasso Sea (BATS) in the Sargasso Sea, the North and South (sub-)tropical gyres (Gyres), the Equatorial region (EQ) and the Mauritanian Upwelling (MU). Tyrosine uptake against leucine (black) or methionine (grey) uptake for each province sampled for tyrosine uptake (b). The error bars indicate standard errors of the measurements. Note the log scale on both axes. An equality line, where uptake rates are equal, is given as a reference.

2.3.2 Variation between amino acids and biogeographic provinces

Figure 2.5a includes all data from samples that were analysed for leucine and methionine uptake simultaneously, providing further visual evidence that data from within the same province cluster together. Uptake data from the BATS station diverge from those from the oligotrophic gyres, with some very low uptake rates. EQ data are mostly distinct from the gyre data, and data from the MU region are completely distinct from all other provinces due to very high uptake rates of an order of magnitude higher than measured elsewhere.

There was also strong provincial variation in amino acid concentration and turnover times, for which the BATS and MU regions were the most distinctive (Figure 2.6). Leucine concentration was most variable, and generally higher, at BATS and in the MU region. Methionine concentration was generally highest at BATS, and similar between the Gyres, and EQ and MU regions. Turnover time of these two amino acids decreased in the order BATS>Gyres>EQ>MU, although the data from the Gyres and EQ region are very similar, particularly for methionine. Turnover time at BATS was unusually high due to high concentrations and very low uptake rates. Tyrosine showed less provincial variation in turnover times, but was found in higher concentrations in the MU region.

Table 2.5: Mean (± SD) leucine (Leu) and methionine (Met) uptake rates (pmol L^{-1} h^{-1}) for each biogeographic province identified: The Bermuda Atlantic Time-series Study site in the Sargasso Sea (BATS), the oligotrophic Atlantic gyres (Gyres), the Equatorial region (EQ) and the Mauritanian Upwelling (MU). Results of the student's t-test for matched samples are presented for the comparison of regional Leu and Met uptake rates. The number of observations, n, is given in parenthesis. Correlations between Leu and Met uptake rates in each sampling region are also presented, and provincially averaged Met:Leu uptake ratios. *p<0.05, **p<0.01, ***p<0.0001.

Region	Leu	*t*	r	Met	Met:Leu uptake
BATS	3.1 ± 1.9 (16)	=	0.64**	4.0 ± 2.3 (15)	1.45
Gyre	12.6 ± 3.2 (34)	>***	0.64***	10.0 ± 3.3 (40)	0.76
EQ	40.4 ± 14.2 (19)	>***	0.68**	23.4 ± 11.8 (24)	0.60
MU	293 ± 145 (26)	>***	0.02	131 ± 52 (23)	0.44

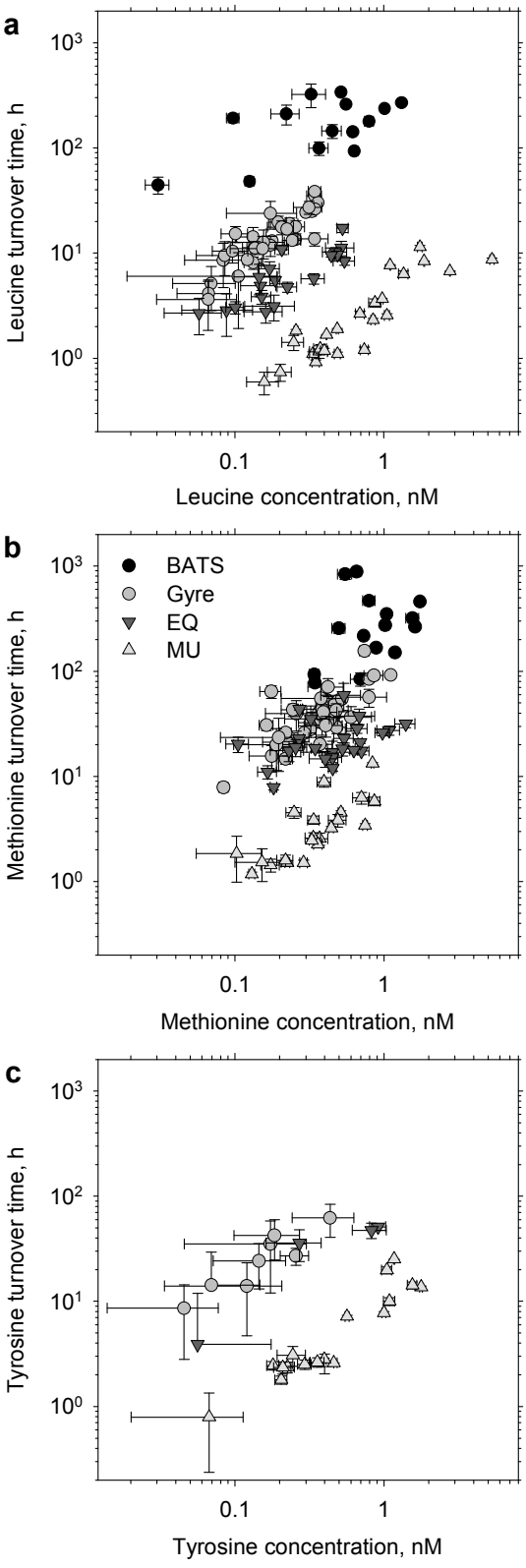


Figure 2.6: Scatter plot comparison of (a) leucine, (b) methionine and (c) tyrosine concentrations versus their corresponding turnover times. The error bars indicate standard errors of the measurements. The figure legend applies to all plots. Note the log scale on both axes.

Leucine and methionine uptake rates were compared for each sample that was analysed for both amino acids, using the *t*-test for matched pairs. Leucine was taken up significantly more quickly than methionine in the oligotrophic gyres, and the EQ and MU regions (Table 2.5). Conversely, the rate of methionine uptake at BATS was higher than leucine uptake, but not significantly so (t = 2.1, df = 13, p = 0.055; Table 2.5). Tyrosine was generally the slowest of the measured amino acids to be taken up, except in the productive MU region where it was often taken up more quickly than methionine (Figure 2.5b).

Perhaps the most interesting result from this study is that there is a clear deviation of Met:Leu uptake data from the 1:1 line towards the extreme ends of production, i.e. in the BATS and MU regions (Figure 2.5a). In fact, on a linear scale, the slope of the regression for all data points is 0.3. The average Met:Leu uptake values for each study region are given in Table 2.5 and imply a relative importance of methionine in oligotrophic regions compared to productive regions.

Despite differences between the leucine and methionine uptake rates, they were always positively correlated (64-68%), except in the MU region (Table 2.5). Tyrosine was more strongly influenced by the other two amino acids; uptake was correlated with leucine (r = 0.8, n = 21, $p<0.0001$) and methionine (r = 0.8, n = 31, $p<0.0001$) uptake, for which the slopes of the linear regressions were 2.5 and 0.8, respectively. This result provides further evidence of the importance of methionine in low nutrient environments and leucine in productive and/or upwelling regions, and suggests that either tyrosine is particularly important in the MU region, or methionine is not.

2.3.3 Variance within biogeographical provinces

Variance was normalised between regions by dividing by the mean to give the coefficient of variance (Figure 2.7). Regional variance in leucine uptake rates increased in the order Gyres<EQ<MU<BATS. The order was different for variation in methionine uptake due to the low values observed in the EQ region during January and February 2008; however, when data from this period were excluded variance increased in the same order. Interestingly, this is the opposite order to that for sampling area: BATS<MU<EQ<Gyres.

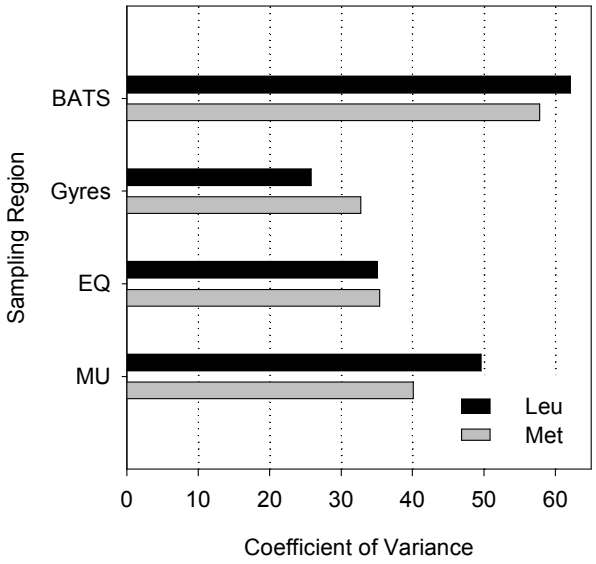


Figure 2.7: Comparison of the coefficient of variance for leucine and methionine uptake rates in each biogeographic province identified: The Bermuda Atlantic Time-series Study site in the Sargasso Sea (BATS), the oligotrophic Atlantic gyres (Gyre), the Equatorial region (EQ) and the Mauritanian Upwelling (MU). Unusually low methionine uptake rates measured during January & February 2008 were excluded from the calculation.

2.4 Discussion

The movement of gyre boundaries between sampling periods, as was visible in ocean colour images (Figure 2.3), was reflected in significant changes in amino acid assimilation rates, for example, between the North Atlantic Gyre and the North-East Subtropical Atlantic region. This suggests that, in addition to characteristic pigments (Hirata *et al.*, 2008, Aiken *et al.*, 2009), bacterial activity is a sensitive marker of gyre boundaries and distinct water masses. A correlation between leucine and methionine uptake in the South Atlantic Gyre has been reported previously (Zubkov *et al.*, 2004). The additional data in this study confirm that this correlation is also true for the Northern Gyre, Equatorial region and the BATS station, thus confirming the validity of using ^{35}S-methionine uptake rates to assess bacterioplankton activity in open ocean regions. The lack of a relationship between the two amino acids in the Mauritanian Upwelling region, however, suggests it is necessary to apply caution whilst interpreting methionine uptake data in highly productive regions.

The addition of radioactively labelled amino acid tracers allows estimation of ambient amino acid uptake rates at ambient concentration, in contrast to maximum uptake rates that are determined by saturation techniques (e.g. Alonso-Sáez *et al.*, 2007b). Due to the sparse dataset generated for ambient uptake in the study area, most of which lies herein, data are generally discussed in the context of potential uptake rates and bacterial

production estimated by saturation with upwards of 20 nM leucine. Leucine uptake rates measured throughout the open Atlantic Ocean in winter (Hoppe *et al.*, 2002, Hoppe *et al.*, 2006) were roughly twice the ambient rates measured in this study. This two-fold difference may be due to methodological differences, rather than a halving in bacterial activity, since there was no significant change in activity between the studies described herein. Thus, the discussion will focus on patterns of uptake and variance, as opposed to absolute values of uptake.

Amino acid uptake data for each biogeographical province sampled was comparable between sampling periods regardless of season, with the one exception of the Equatorial region, where low methionine uptake rates were measured during January 2008. Uptake rates were representative of the regions in which they were collected, in that amino acids were taken up slowly in the oligotrophic gyres, twice as quickly in the mesotrophic equatorial region and 20-fold faster in the productive upwelling region, thus reflecting the general pattern of variation observed in primary production (Poulton *et al.*, 2006), with which bacterial production is generally considered linked. The only exception to this was the low uptake rates observed in the NESA region despite Chl-*a* concentration in the 0.1-0.25 mg m^{-3} range, implying a dynamic environment in which the bacterioplankton community is not correlated with the primary producers, as has been reported previously (Gasol *et al.*, 2009). Despite incompatibility of methods between studies, spatial variability in amino acid uptake rates throughout the open Atlantic Ocean was generally similar to previous reports of leucine uptake or bacterial production, and showed the same low variance within the gyres (Zubkov *et al.*, 2000b, Hoppe *et al.*, 2002, Hoppe *et al.*, 2006), with more seasonal variation in the Equatorial region (Zubkov *et al.*, 2000b). The data presented herein were mostly collected in early to late (Boreal) autumn. The Hoppe *et al.* (2002, 2006) studies were conducted in winter, and Zubkov *et al.* (2000b) report from the spring and early autumn, which suggests that the spatial variability in microbial activity is seasonally consistent.

The Mauritanian upwelling region showed high variation in amino acid uptake rates despite sampling a relatively small area (~6 $x10^4$ km^2) compared to the area sampled in the two gyres (~4 $x10^6$ km^2). The heterogeneity of upwelling regions is visible in satellite infrared images, where cool upwelled water is seen at the coast, warmer waters offshore, and an intermediate region of eddies and plumes where the two bodies mix (Mann & Lazier, 2006). A gradient from high leucine uptake at the source of upwelling, decreasing

as water moved westwards offshore and nutrients become depleted, was indicated by the inverse correlation between leucine uptake and seawater temperature. Such a gradient has also been reported for an upwelling source further up the coast at 27.69°N, between northwest Africa and the Canary coastal transition zone, with a 20-fold decrease in leucine uptake between stations at the source of upwelling and stations off the coast of Gran Canaria (Baltar *et al.*, 2007). With such a gradient, it is perhaps unsurprising that, in agreement with previous studies (Cuevas *et al.*, 2004, Alonso-Sáez *et al.*, 2007a, Alonso-Sáez *et al.*, 2007b), the data herein suggest substantial variation in bacterial metabolic activity associated with coastal upwelling. Similar spatial heterogeneity in bacterioplankton abundance has been observed over distances of only 12 km in the Celtic Sea (Martin *et al.*, 2005). Heterogeneity is evidently typical of dynamic regions, thus bacterioplankton production cannot sensibly be described for such biogeographic provinces by one mean value.

The Atlantic oligotrophic gyres show low biological heterogeneity in terms of chlorophyll *a* concentrations (Aiken *et al.*, 2009); however, photosynthetic biomass has been shown to vary between some passages along similar Atlantic Meridional Transects (Poulton *et al.*, 2006). Primary production rates are not so variable between sampling periods in the Atlantic (Poulton *et al.*, 2006), although high variation has been observed in the southern gyre (Marañón *et al.*, 2003). In agreement with previous studies (Zubkov *et al.*, 2000b, Hoppe *et al.*, 2002, Hoppe *et al.*, 2006) leucine uptake was similar between the two gyres. Leucine and methionine uptake rates reflected that of *Prochlorococcus* abundance along meridional transects through the Atlantic (Zubkov *et al.*, 1998, Heywood *et al.*, 2006), perhaps because a considerable proportion of the uptake of these amino acids in oceanic regions within the 40° latitude belt can be attributed to *Prochlorococcus* (Zubkov *et al.*, 2004, Zubkov & Tarran, 2005, Michelou *et al.*, 2007, Mary *et al.*, 2008a). Of the biogeographic provinces sampled within this study, the variability of amino acid uptake rates was lowest in the oligotrophic gyres.

The highest variability in uptake rates was observed at the BATS station in July 2007. Since this was one station sampled repeatedly, the high coefficient of variance must have been a result of the 2-5-hourly sampling regime. However, a diel cycle, such as that exhibited by *Prochlorococcus* cells (Vaulot *et al.*, 1995, Mary *et al.*, 2008a), was not observed, perhaps due to minimal surface water *Prochlorococcus* concentrations during summer (Olson *et al.*, 1990, DuRand *et al.*, 2001, Michelou *et al.*, 2007). This was also the location of

the lowest uptake rates of the study, suggesting limitation of the bacterioplankton community. Although bacterial production at BATS is typically at its highest in July, this is due to a subsurface peak, rather than high bacterial production at the surface (Steinberg *et al*., 2001). Suttle *et al.* (1991) measured over four times higher leucine uptake rates in surface waters in the region in November compared to July. Leucine and methionine concentrations in surface water were some of the highest measured (0.51 ± 0.35 nM and 0.73 ± 0.27) suggesting immeasurable inorganic, rather than organic, nutrients were limiting production, which is typical during summer stratification (Lipschultz, 2001), when surface phosphate concentration is generally <5 nM (Wu *et al*., 2000).

The clear Met:Leu uptake gradient from ultra-oligotrophic to productive regions (Table 2.5) suggests a specific requirement for sulphur-containing methionine in low nutrient environments, particularly at the BATS site where methionine was often taken up more quickly than leucine. Phytoplankton in the Sargasso Sea have adapted to phosphorus stress (Lomas *et al*., 2004) by using non-phosphorus lipids (Van Mooy *et al*., 2006, Van Mooy *et al*., 2009). Strains of *Prochlorococcus* grown in culture under phosphate depletion were shown to substitute a high proportion of phospholipids with sulpholipids, reducing their cellular P demand by up to 43% (Van Mooy *et al*., 2009), and increasing their sulphur requirement, suggesting a specific sulphur requirement in this region. The genome of *Prochlorococcus marinus* SS120 encodes for sulphate assimilation and reduction (Dufresne, *et al*., 2003); however, Mary *et al.* (2008b) showed that flow cytometrically sorted *Prochlorococcus* took up more methionine than leucine at some locations in the Atlantic Ocean. Furthermore, the SAR11 clade of *Alphaproteobacteria* requires exogenous reduced sulphur for growth (Tripp *et al*., 2008). Considering the dominance of SAR11 and *Prochlorococcus* bacteria in oligotrophic waters (Chisholm *et al*., 1988, Morris *et al*., 2002) an increased methionine demand in such regions might be expected.

In contrast, methionine uptake was only about one-third the rate of leucine uptake off the coast of Mauritania, perhaps since SAR11 and *Prochlorococcus* are not favoured by upwelling conditions (Fuchs *et al*., 2005, Alonso-Sáez *et al*., 2007a). Mary *et al.* (2006) suggested SAR11 are less productive in the Mauritanian Upwelling region, compared to the open Atlantic Ocean, due to competition with faster growing bacteria for organic nutrients. Indeed, cell-specific production was highest in surface waters close to the source of upwelling and decreased with distance off the northwest African coast (Baltar *et al*., 2007) and *Gammaproteobacteria*, usually fast growing cells, were favoured by upwelling

conditions off the coast of Mauritania (Alonso-Sáez *et al.*, 2007a). Thus it seems that fast growing bacteria inhabiting productive upwelling regions do not have the methionine requirement of *Prochlorococcus* and SAR11, but receive sufficient sulphur from alternative sources. Conversely, tyrosine was taken up more quickly than methionine at some stations in the Mauritanian Upwelling region, unlike other regions studied, suggesting that the upwelling community has adapted mechanisms by which to utilise components of the CDOM, since it tends to be abundant in such regions and thus could provide a substantial nutrient supply.

Leucine and methionine uptake rates in the north and south Atlantic gyres did not vary significantly between sampling periods. Likewise, somewhat remarkably, uptake rates were comparable between the two gyres, suggesting that one mean value of uptake rate for leucine and methionine (12.6 ± 3.2 and 10.0 ± 3.3 pmol L^{-1} h^{-1}, respectively) could be used to characterise the gyres, without a need for routine measurements. Since sampling was done during boreal autumn and winter only, further sampling in these regions during spring and summer months will be necessary to test for significant seasonality. However, relatively stable bacterioplankton biomass and production data in the two gyres between spring and autumn sampling periods (Zubkov *et al.*, 2000b, Heywood *et al.*, 2006), in addition to comparative uptake rates between gyres despite experiencing different seasons, suggest that the effect of seasons may be minimal.

2.5 References

Aiken J, Pradhan Y, Barlow R, Lavender S, Poulton A, Holligan P & Hardman-Mountford N (2009) Phytoplankton pigments and function in the Atlantic Ocean: a decadal assessment, 1995-2005. *Deep Sea Research II* **56**: 899-917.

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González JM, Vaqué D, Agustí S & Gasol JM (2007a) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic Ocean. *Aquatic Microbial Ecology* **46**: 43-53.

Alonso-Sáez L, Gasol JM, Arístegui J, Vilas JC, Vaqué D, Duarte CM & Agustí S (2007b) Large-scale variability in surface bacterial carbon demand and growth efficiency in the subtropical northeast Atlantic Ocean. *Limnology and Oceanography* **52**: 533-546.

Baltar F, Arístegui J, Gasol JM, Hernández-León S & Herndl GJ (2007) Strong coast-ocean and surface-depth gradients in prokaryotic assemblage structure and activity in a coastal transition zone region. *Aquatic Microbial Ecology* **50**: 63-74.

Berman T & Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquatic Microbial Ecology* **31**: 279-305.

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB & Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**: 340-343.

Cuevas LA, Daneri G, Jacob B & Montero P (2004) Microbial abundance and activity in the seasonal upwelling area off Concepción (~36°S), central Chile: a comparison of upwelling and non-upwelling conditions. *Deep Sea Research II* **51**: 2427-2440.

Dufresne A, Salanoubat M, Partensky F, Artiguenave F, Axmann IM, Barbe V, Duprat S, Galperin MY, Koonin EV, Le Gall F, Makarova KS, Ostrowski M, Oztas S, Robert C, Rogozin IB, Scanlan DJ, de Marsac NT, Weissenbach J, Wincker P, Wolf YI & Hess WR (2003) Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. *Proceedings of the National Academy of Sciences* **100:** 10020-10025.

DuRand MD, Olson RJ & Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep Sea Research II* **48**: 1983-2003.

Fuchs BM, Woebken D, Zubkov MV, Burkill P & Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. *Microbial Ecology* **39**: 145-157.

Fuhrman JA & Ferguson RL (1986) Nanomolar concentrations and rapud turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. *Marine Ecology Progress Series* **33**: 237-242.

Gasol JM, Vázquez-Domínguez E, Vaqué D, Agustí S & Duarte CM (2009) Bacterial activity and diffusive nutrient supply in the oligotrophic Central Atlantic Ocean. *Aquatic Microbial Ecology* **56**: 1-12.

Hardman-Mountford N, Hirata T, Richardson KA & Aiken J (2008) An objective methodology for the classification of ecological pattern into biomes and provinces for the pelagic ocean. *Remote Sensing of Environment* **112**: 3341-3352.

Heywood JL, Zubkov MV, Tarran GA, Fuchs BM & Holligan PM (2006) Prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean: Evaluation of inter-annual variability. *Deep Sea Research II* **53**: 1530-1547.

Hirata T, Aiken J, Hardman-Mountford N, Smyth TJ & Barlow RG (2008) An absorption model to determine phytoplankton size classes from satellite ocean ocolour. *Remote Sensing of Environment* **112**: 3153-3159.

Hoppe H-G, Gocke K, Koppe R & Begler C (2002) Bacterial growth and primary production along a north-south transect of the Atlantic Ocean. *Nature* **416**: 168-171.

Hoppe H-G, Gocke K, Koppe R & Kraus G (2006) Changing bacterioplankton growth characteristics on a large spatial scale: oligotrophic versus mesotrophic ocean. *Marine Ecology Progress Series* **323**: 21-33.

Kirchman D, K'nees E & Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology* **49**: 599-607.

Lipschultz (2001) A time-series assessment of the nitrogen cycle at BATS. *Deep Sea Research II* **48**: 1897-1924.

Lomas MW, Swain A, Shelton R & Ammerman JW (2004) Taxonomic variability of phosphorus stress in Sargasso Sea phytoplankton. *Limnology and Oceanography* **49**: 2302-2310.

Longhurst A (1998) *Ecological geography of the sea*. Academic Press, London.

Mann KH & Lazier JRN (2006) *Dynamics of marine ecosystems: biological-physical interactions in the oceans*. Blackwell, Oxford.

Marañón E, Mouriño B, Behrenfeld MJ, González N & Zubkov MV (2003) High variability of primary production in oligotrophic waters of the Atlantic Ocean: Uncoupling from phytoplankton biomass and size structure. *Marine Ecology Progress Series* **257**: 1-11.

Martin AP, Zubkov MV, Burkill PH & Holland RJ (2005) Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian time-series. *Biology Letters* **1**: 366-369.

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH & Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* **45**: 107-113.

Mary I, Garczarek L, Tarran GA, Kolowrat C, Terry MJ, Scanlan DJ, Burkill PH & Zubkov MV (2008a) Diel rhythmicity in amino acid uptake by *Prochlorococcus*. *Environmental Microbiology* **10**: 2124-2131.

Mary I, Tarran GA, Warwick PE, Terry MJ, Scanlan DJ, Burkill PH & Zubkov MV (2008b) Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiology Ecology* **63**: 36-45.

McClain CR, Signorini SR & Christian JR (2004) Subtropical gyre variability observed by ocean-color satellites. *Deep Sea Research II* **51**: 281-301.

Michelou VK, Cottrell MT & Kirchman DL (2007) Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in the north Atlantic Ocean. *Applied and Environmental Microbiology* **73**: 5539-5546.

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA & Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806-810.

Oliver MJ & Irwin AJ (2008) Objective global ocean biogeographic provinces. *Geophysical Research Letters* **35**: L15601, doi:10.1029/2008GL034238.

Olson RJ, Chisholm SW, Zettler ER, Altabet MA & Dusenberry JA (1990) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. *Deep Sea Research I* **37**: 1033-1051.

Polovina JJ, Howell EA & Abecassis M (2008) Ocean's least productive waters are expanding. *Geophysical Research Letters* **35**: L03618, doi:10.1029/2007GL031745.

Poulton AJ, Holligan PM, Hickman A, Kim Y-N, Adey TR, Stinchcombe MC, Holeton C, Root S & Woodward EMS (2006) Phytoplankton carbon fixation, chlorophyll-biomass and diagnostic pigments in the Atlantic Ocean. *Deep Sea Research II* **53**: 1593-1610.

Steinberg DK, Carlson CA, Bates NR, Johnson RJ, Michaels AF & Knap AH (2001) Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. *Deep Sea Research II* **48**: 1405-1447.

Suttle CA, Chan AM & Fuhrman JA (1991) Dissolved free amino acids in the Sargasso Sea: Uptake and respiration rates, turnover times, and concentrations. *Marine Ecology Progress Series* **70**: 189-199.

Tarran GA, Heywood JL & Zubkov MV (2006) Latitudinal changes in the standing stocks of nano- and picoeukaryotic phytoplankton in the Atlantic Ocean. *Deep Sea Research II* **53**: 1516-1529.

Tripp HJ, Kitner JB, Schwalbach MS, Dacey JWH, Wilhelm LJ & Giovannoni SJ (2008) SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* **452**: 741-744.

Van Mooy BAS, Rocap G, Fredricks HF, Evans CT & Devol AH (2006) Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *Proceedings of the National Academy of Sciences* **103**: 8607-8612.

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblizek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS & Webb EA (2009) Phytoplankton in

the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* **458**: 69-72.

Vaulot D, Marie D, Olson RJ & Chisholm SW (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. *Science* **268**: 1480-1482.

Wright RT & Hobbie JE (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology* **47**: 447-464.

Wu J, Sunda W, Boyle EA & Karl DM (2000) Phosphate depletion in the western north Atlantic Ocean. *Science* **289**: 759-762.

Zubkov MV, Sleigh MA, Tarran GA, Burkill PH & Leakey RJG (1998) Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S. *Deep Sea Research I* **45**: 1339-1355.

Zubkov MV, Sleigh MA, Burkill PH & Leakey RJG (2000a) Picoplankton community structure on the Atlantic Meridional transect: a comparison between seasons. *Progress in Oceanography* **45**: 369-386.

Zubkov MV, Sleigh MA, Burkill PH & Leakey RJG (2000b) Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. *Journal of Plankton Research* **22**: 685-711.

Zubkov MV, Burkill PH, Tarran GA, Fuchs BM & Amann R (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Applied and Environmental Microbiology* **69**: 1299-1304.

Zubkov MV, Tarran GA & Fuchs BM (2004) Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *FEMS Microbiology Ecology* **50**: 153-161.

Zubkov MV & Tarran GA (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. *Aquatic Microbial Ecology* **40**: 241-249.

Zubkov MV, Tarran GA, Mary I & Fuchs BM (2008) Differential microbial uptake of dissolved amino acids and amino sugars in surface waters of the Atlantic Ocean. *Journal of Plankton Research* **30**: 211-220.

Chapter Three

Microbial Community Structure in the Vicinity of the Cape Verde Islands: Distinguishing Effects of Wind-Mixing and Dust Deposition

3.1 Introduction

A significant proportion of primary production in the tropical ocean is produced by the cyanobacterial portion of the bacterioplankton community (Goericke & Welschmeyer, 1993, Li, 1994, Partensky *et al.*, 1999). Various physical processes have been shown to impact on near surface bacterioplankton communities, such as upwelling and wind-driven turbulence (Troussellier *et al.*, 1993, Kerkhof *et al.*, 1999, Herut *et al.*, 2005, Alonso-Sáez *et al.*, 2007). Aeolian dust deposition also has the potential to affect microbial communities (Herut *et al.*, 2005, Reche *et al.*, 2009); with increased desertification potentially leading to a rise in the amount of dust deposited into the oceans (Rosenfeld *et al.*, 2001), the impacts of dust deposition on bacterioplankton communities becomes increasingly important when considering future climate change. All these processes are often concomitant and result in enrichment of nutrients in the surface photic layer, making it difficult to establish the primary factor affecting bacterioplankton communities. However, the episodic nature of dust storms offers an opportunity to disentangle the effects of these processes if an appropriate oceanic region and season are chosen.

The north-eastern (sub-)tropical Atlantic Ocean is an ideal place for a comparative study. It is an oligotrophic to mesotrophic region bordered to the west by the oligotrophic gyre, to the south by the equatorial convergence, and to the east by the north-western African coast containing two archipelagos, the Canary and Cape Verde Islands. To the north of Cape Verde is a region remote from coastal upwelling and this leads to low nutrient, stratified conditions. In contrast, water around the Cape Verde Islands is influenced by the Canary Current which flows south-westwards from the Canary Island Archipelago, entraining with it water upwelled along the African coast (Pelegrí *et al.*, 2006). The equatorial region to the south of Cape Verde is influenced by both the south-westwards flow of the Canary Current through the Cape Verde islands, and the westwards flow of the North Equatorial Current (Pelegrí *et al.*, 2006), which also entrains waters from the

African coastal zone. A significant contribution of new nutrients to surface water in this region comes from Aeolian dust that is transported from arid regions of Africa, mainly the Sahara desert, on trade winds (Jickells, 1995, Baker *et al.*, 2007). Annual deposits of 5-20 g dust per square metre are typical for the region (Jickells *et al.*, 2005).

Using group-specific oligonucleotide probes to identify major bacterial groups by Catalysed Reported Deposition Fluorescence *in situ* Hybridisation (CARD-FISH), Schattenhofer *et al*. (2009) showed that regional variation of bacterioplankton community structure could be detectable at the high taxonomical level. Previous studies have suggested that Bacteria belonging to the class *Bacteroidetes* may be associated with productive regions (Suzuki *et al.*, 2001, Alonso-Sáez *et al.*, 2007, Gómez-Pereira *et al.*, 2010) due to an association with phytoplankton (Gómez-Pereira *et al.*, 2010). The *Gammaproteobacteria* have been shown to respond to nutrient pulses associated with upwelling systems (Alonso-Sáez *et al.*, 2007, Teira *et al.*, 2008) and dust deposition to freshwater (Reche *et al.*, 2009). Winter mixing favours eukaryotic algae and *Synechococcus*, and leads to a decline in *Prochlorococcus* abundance in the Red Sea (Lindell & Post, 1995), but the impact of wind-driven mixing on the heterotrophic bacterioplankton community structure has not been investigated in detail. Furthermore, the impact dust deposition has on the composition of oceanic bacterioplankton communities has not been studied quantitatively.

Therefore, the aim of this study was to examine the bacterioplankton community response to dust deposition events in a heavily dust-impacted oceanic region, by drawing comparisons between periods of no dust deposition, with periods of significant dust deposition, and to distinguish impacts of dust deposition from other processes, such as wind-driven mixing and island-induced upwelling.

3.2 Method

3.2.1 Study area and sampling

The study was conducted in the eastern (sub-)tropical North Atlantic Ocean (Figure 3.1) during January and February 2006 onboard Research Vessel *Poseidon* (PO332) and during January and February 2008 onboard Royal Research Ship *Discovery* (D326). As mentioned

in Chapter Two, the sampling region was not within the oligotrophic gyre, but was a heterogeneous region on its eastern edge, influenced by different water masses which were visible from temperature-salinity plots; the same was true of the area during the 2006 sampling period (Appendices III and IV). The region covered three distinct sub-regions identified in Chapter Two: the North-East Subtropical Atlantic (NESA, north of ~20°N), and the Cape Verde (CV, ~14-20°N) and Equatorial (EQ, below ~14°N) regions.

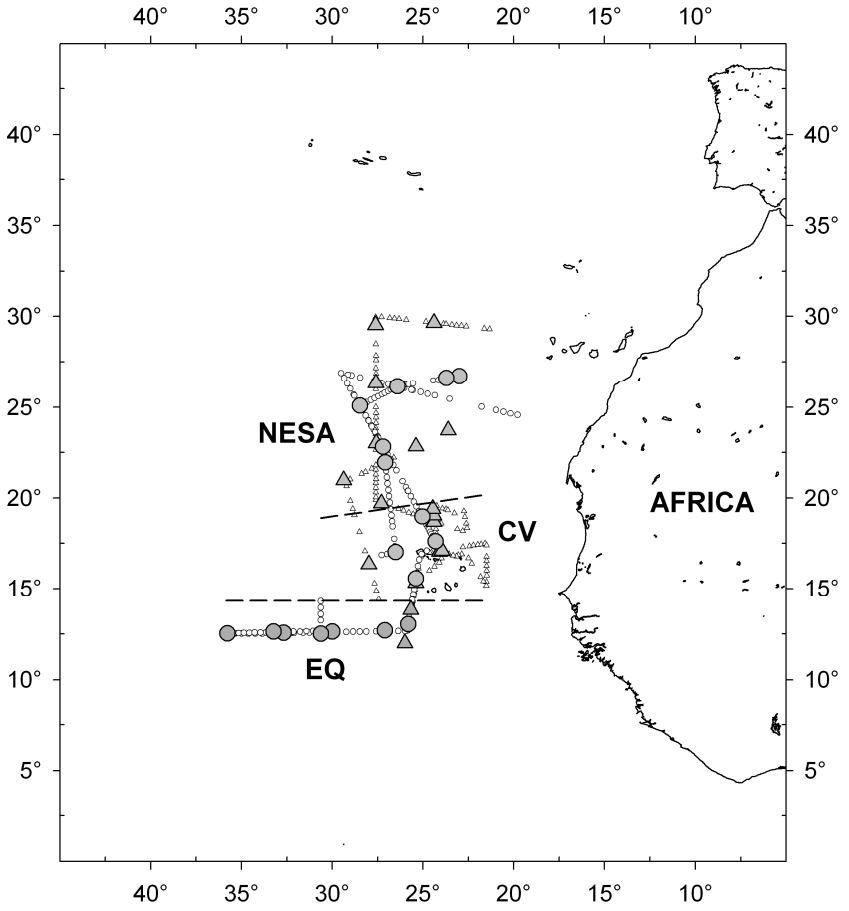


Figure 3.1: Origins of samples analysed for (white symbols) bacterioplankton abundance and chlorophyll *a* concentration and (grey symbols) community structure, from (triangles) 2006 and (circles) 2008. Data have been divided between the three sub-regions identified: the North-East Subtropical Atlantic (NESA), and the Cape Verde (CV) and Equatorial (EQ) regions.

Surface seawater samples were collected for CARD-FISH analysis from a depth of 5-20 m in Niskin bottles mounted on a stainless steel (2006) or titanium (2008) frame with a conductivity, temperature, depth (CTD) profiler. Seawater was collected from the non-toxic underway supply, at approximately 5 m depth, every two hours for analysis of bacterioplankton abundance by flow cytometry and chlorophyll *a* (Chl-*a*) concentration by fluorometry. In both years over 150 samples were analysed by flow cytometry and fluorometry and 17 samples by CARD-FISH (Figure 3.1).

No major dust storms were encountered during the research cruise in 2006. In 2008 two dust storms were encountered during the study (January 17-19 and 25-28), which deposited dust over the study area and up to the British Isles. The dust storms were visible in satellite images (Figure 3.2) and reached atmospheric dust concentrations of up to 0.43 mg m^{-3} (Claire Powell, personal communication). During the dust storms there was a brown haze around the ship that reduced visibility, and the ship became covered in a layer of red-brown dust; back trajectories showed the dust was of Saharan origin (Appendix III). In 2008 the CV region was sampled before the dust deposition events (January 13-16), and briefly following dust deposition (January 28-29). The EQ region was sampled during and after the first dust event (January 16-24). The NESA region was sampled before (January 7-13) and after (January 29-February 2) dust events, allowing for a comparison of the bacterioplankton community pre- and post-dust; however, all NESA samples for CARD-FISH analysis were collected following dust deposition. Within the NESA region samples were collected along a transect between 24.9°N; 28.4°W and 22.7°N; 27.2°W, which was sampled once at the beginning of the study (January 11-12 2008), and again 18 days later.

3.2.2 Chlorophyll *a*

Surface seawater samples of 500 mL and duplicate samples of 250 mL were filtered through MF300 glass fibre filters in 2006 and 2008, respectively, for later analysis by fluorometry using the method of Welschmeyer *et al.* (1994). Extracts were analysed in a TD-700 Turner Designs fluorometer, calibrated with fresh chlorophyll *a* standard from *Anacystis nidulans* (Sigma, UK).

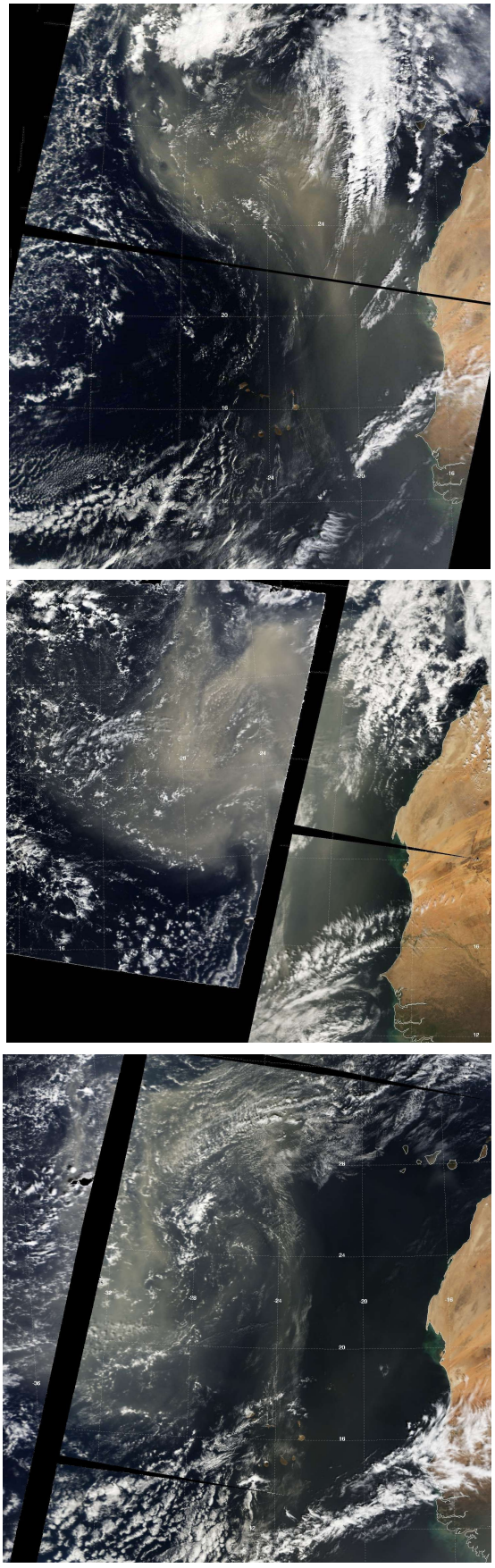


Figure 3.2: Satellite images of the dust storm encountered between January 25 and 28 2008. Images courtesy of Tom Church, University of Delaware, USA.

3.2.3 Flow cytometry

Cyanobacteria groups, *Prochlorococcus* and *Synechococcus*, and picoeukaryotes were enumerated by flow cytometry, allowing high spatio-temporal coverage of concentrations of these groups in surface waters. In both years 1.6mL seawater samples were fixed with paraformaldehyde (PFA, 1% final concentration) within 30 minutes of collection. In 2006, samples were stored at -80°C until analysis ashore because it was logistically impossible to analyse samples onboard. In 2008, samples were analysed onboard by Ross Holland. All samples were processed by flow cytometry (FACSCalibur, Becton Dickenson, BD Biosciences, Oxford, UK). Cell concentrations were calculated from sample flow rate, which was determined by addition of a known concentration of fluorescent latex beads as an internal standard (Zubkov & Burkill, 2006).

The total bacterioplankton population was counted after staining with the nucleic acid stain SYBR® Green I (Marie *et al.*, 1997). The cyanobacterial group *Synechococcus*, and a broad group of picoeukaryotes were identified by their characteristic autofluorescence (Olson *et al.*, 1993). In 2006 *Prochlorococcus* abundance was also estimated from unstained samples, because cells had sufficient autofluorescence for detection by flow cytometry. However, in 2008 *Prochlorococcus* autofluorescence was insufficient in a significant proportion of samples, therefore their abundance was estimated from SYBR® Green I stained samples (Zubkov *et al.*, 2000, Heywood *et al.*, 2006). The autofluorescent cells were subtracted from total cell abundance to give the remaining 'heterotrophic bacterioplankton' that represent all prokaryotes that lack autofluorescent pigment, both Bacteria and Archaea.

3.2.4 CARD-FISH

In 2006 samples were fixed with PFA (1% final concentration) overnight at 4°C, filtered onto polycarbonate membrane filters (0.2 µm pore size, 47 mm diameter) and stored in Petri dishes at -80°C until processing. In 2008 PFA-fixed samples were stained with SYBR® Green I and sorted into high and low nucleic acid (HNA and LNA, respectively) containing cells by flow cytometry (Mary *et al.*, 2006) so that the LNA community could be identified for a parallel study (Chapter Five). The sorted cells were filtered directly onto polycarbonate membrane filters (0.2 µm, 13 mm) and stored at -20°C until

processing. The SYBR® Green I stain was washed off the cells during the sorting process (Mary *et al.*, 2006, Zubkov *et al.*, 2007).

Table 3.1: Oligonucleotide probes used in study; target phylogenetic group, sequence, formamide concentration (FA) used for hybridisation buffer (at 46°C), and data source.

Probe	Target organisms	Sequence (5′- 3′) of probe	FA[a]	Source
EUB338/I	Eubacteria	GCTGCCTCCCGTAGGAGT	35	(Amann *et al.*, 1990)
EUBII	Eubacteria	GCAGCCACCCGTAGGTGT	35	(Daims *et al.*, 1999)
EUBIII	Eubacteria	GCTGCCACCCGTAGGTGT	35	(Daims *et al.*, 1999)
NON338	Control to EUB	ACTCCTACGGGAGGCAGC	35	(Wallner *et al.*, 1993)
GAM42a[b]	*Gammaproteobacteria*	GCCTTCCCACATCGTTT	35	(Manz *et al.*, 1992)
ALF968	*Alphaproteobacteria*	GGTAAGGTTCTGCGCGTT	35	(Glöckner *et al.*, 1999)
SAR11 486	SAR11 clade	GGACCTTCTTATTCGGGT	25	(Fuchs *et al.*, 2005)
CF319a	*Bacteroidetes*	TGGTCCGTGTCTCAGTAC	35	(Manz *et al.*, 1996)
405Syn	*Synechococcus*	AGAGGCCTTCATCCCTCA	40	(West *et al.*, 2001)
405Pro	*Prochlorococcus*	AGAGGCCTTCGTCCCTCA	40	(West *et al.*, 2001)
645HLI	High-light *Pro* 1	ACCATACTCAAGCCGATC	35	(West *et al.*, 2001)
645HLII	High-light *Pro* 2	ACCATACTCAAGCCTTTC	30	(West *et al.*, 2001)

[a] formamide (FA) concentration (wt/vol) in CARD-FISH hybridisation buffer

[b] including an unlabelled competitor probe Bet42a (5′-GCCTTCCCACTTCGTT-3′); see Manz et al. (1992) for details

Samples from each cruise were processed using a sensitive CARD-FISH protocol (Pernthaler *et al.*, 2002), with modifications according to Mary *et al.* (2006). Additionally, in this study filter sections were submerged in buffer-probe mix on microscope slides and incubations were done at 46°C. Group-specific oligonucleotide probes were employed to identify major bacterial phyla (*Bacteroidetes*) and classes (*Alphaproteobacteria* and *Gammaproteobacteria*), as well as more specific clades (SAR11) and ecotypes (High light adapted *Prochlorococcus*) to assess community change in more detail. The probes that were applied to samples are detailed in Table 3.1. CARD-FISH preparations were counter-stained with DAPI (1µg mL^{-1}). Filter sections were inspected and cells counted under a Zeiss Axioplan II motorised epifluorescence microscope (Carl Zeiss, Germany), equipped with a 100x UV plan Apochromat objective and excitation/emission filters 360/420 for DAPI and 490/515 for fluorescein isothiocyanate (FITC). For each sample and probe,

about 300 cells were counted per sample from at least six fields of view, and the mean value used for data analysis. All probe-specific counts were recorded as the percentage of cells stained by DAPI and referred to as 'community structure'.

CARD-FISH data from 2008, which were divided into HNA and LNA groups, were transformed into percentage of total community structure by correcting for the contribution of HNA and LNA groups to total bacterioplankton community, thus enabling a direct comparison of data between years. The HNA and LNA percentages were determined from flow cytometry data for those samples.

3.2.5 Data analysis

CellQuest software (Becton Dickinson Biosciences) was used for operating the flow cytometer and analysis of flow cytometric data. Outliers were removed from the flow cytometry database if they lay outside three standard deviations of the mean. The base of the mixed layer was determined as the depth at which the potential density, sigma-t, in the upper layer changed by 0.01 kg m^{-3}. All data are presented as mean ± standard error. Mean values were compared using the student's *t*-test for unmatched samples, following the F-test for equality of variance.

3.3 Results

3.3.1 Study region division, hydrography and meteorology

In order to highlight microbial community variation attributable to either physical mixing processes or Aeolian inputs, the dataset was divided between the three sub-regions identified. Between CTD stations, an order of magnitude change in *Synechococcus* abundance was used to support regional break down since this is a sensitive gauge of nutrient bioavailability (Partensky *et al.*, 1999, Zubkov *et al.*, 2000), which is another indicator of distinct water masses. Cruise data were compared between regions, and regional data compared between sampling periods.

In both years the CV and EQ regions were characterised by a shallow mixed layer depth, in contrast to a deep mixed layer in the NESA region (Table 3.2). The EQ region showed

the most pronounced pycnocline, followed by CV and NESA regions. In both years, there was regional variation in physical parameters. The EQ region was the warmest region, and NESA the coolest. Mean temperature varied significantly between all regions and sampling periods, and may explain some of the biological variation.

Table 3.2: Environmental variation between regions and sampling periods; data are presented as mean ± SE, or ± standard difference for mixed layer depth (MLD) in the EQ region of 2006. The *t*-test compares cruise data between adjacent regions (*t*-test columns), and regional data between sampling periods (*t*-test rows). The end column is a comparison between the EQ and NESA regions. The numbers of observations (n) of MLD is stated in parenthesis. For all other variables n>64 (NESA), n>107 (CV) and n>15 (EQ) in 2006, and n=86 (NESA), n=32 (CV) and n=55 (EQ) in 2008. *p<0.05, **p<0.01, ***p<0.0001.

Variable	Year	NESA	*t*-test	CV	*t*-test	EQ	*t*-test
MLD, m	2006	106 ± 10 (11)	>***	32 ± 5 (9)	=	15 ± 1 (2)	<**
	t-test	=		=		<**	
	2008	127 ± 4 (14)	>***	36 ± 5 (10)	=	50 ± 4 (8)	<***
Temperature, °C	2006	20.91 ± 0.18	<***	22.65 ± 0.06	<***	24.00 ± 0.12	>***
	t-test	<***		>**		<***	
	2008	21.82 ± 0.07	<***	22.29 ± 0.07	<***	24.59 ± 0.05	>***
Salinity	2006	36.94 ± 0.02	>***	36.07 ± 0.05	>***	35.99 ± 0.04	<***
	t-test	<***		<***		=	
	2008	37.29 ± 0.02	>***	36.63 ± 0.05	>***	35.97 ± 0.03	<***
Wind speed, ms^{-1}	2006	16.02 ± 0.6	=	16.12 ± 0.6	=	15.38 ± 0.5	=
	t-test	>***		>***		>***	
	2008	9.31 ± 0.35	=	10.29 ± 0.44	>***	8.36 ± 0.31	<*
Wind direction, °	2006	87 ± 2	>***	44 ± 5	=	41 ± 3	<***
	t-test	>***		=		=	
	2008	74 ± 2	>***	54 ± 2	>*	46 ± 2	<***

Absolute wind speed was variable between regions only during 2008, and highly variable between sampling periods. Wind speed was almost double in 2006 compared to the 2008 campaign in all three regions (Table 3.2), with much of the study conducted during force

6-9 winds, the equivalent of strong breeze to strong gale. Wind direction did not vary between sampling periods in the CV and EQ regions (Table 3.2), blowing north-easterlies in both regions in both years. In the NESA, mean wind direction was more easterly than in the other regions, particularly in 2008.

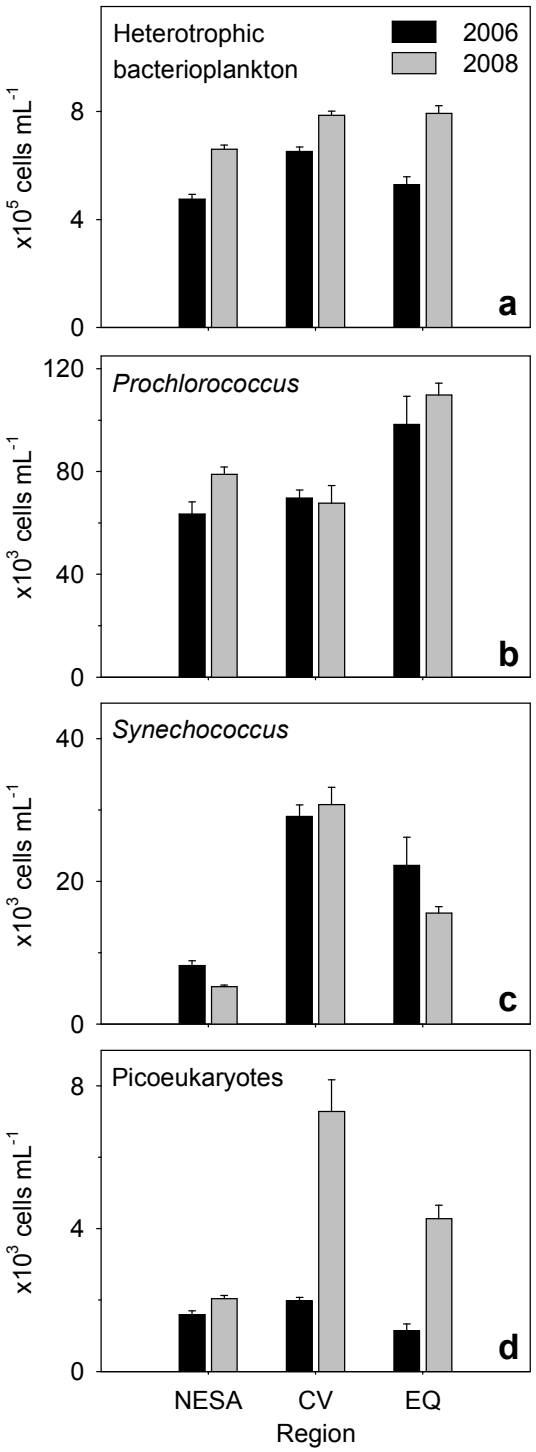


Figure 3.3: Regional variation in abundance of (a) heterotrophic bacterioplankton, (b) *Prochlorococcus*, (c) *Synechococcus*, and (d) picoeukaryotes, in (black) 2006 and (grey) 2008. Data are expressed as mean ± SE.

3.3.2 The heterotrophic community

Overall, there were 19% fewer heterotrophic bacterioplankton in 2006 than in 2008 (5.8 ± 0.1 and 7.2 ± 0.1 x10^5 cells mL^{-1}, respectively; t = 7.2, df = 319, p<0.0001); however, this difference may be the result of freezing samples in 2006. Heterotrophic bacterioplankton were most abundant in the CV region during 2006, and in the CV and EQ regions in 2008 (Table 3.3 and Figure 3.3a). Data from the NESA transect sampled twice during January 2008 show a 27% increase in heterotrophic bacterioplankton abundance towards the end of the study (Table 3.4). When all data from the region are compared between pre- and post-dust deposition a more substantial 39% increase was observed.

Table 3.3: Regional heterotrophic bacterioplankton (10^5 cells mL^{-1}), and phytoplankton (10^3 cells mL^{-1}) abundance, and chlorophyll *a* concentration (Chl-*a*, mg m^{-3}) during each sampling period; data are presented as mean ± SE. The *t*-test compares cruise data between regions (*t*-test columns), and regional data between sampling periods (*t*-test rows). The end column is a comparison between the EQ and NESA regions. In 2006, n>48 (NESA), n>87 (CV) and n>18 (EQ). In 2008, n>70 (NESA), n>17 (CV) and n> 45 (EQ). *p<0.05, **p<0.01, ***p<0.0001.

Variable	Year	NESA	*t*-test	CV	*t*-test	EQ	*t*-test
Het. Bpl.	2006	4.75 ± 0.19	<***	6.52 ± 0.16	>**	5.30 ± 0.29	=
	t-test	<***		<***		<***	
	2008	6.55 ± 0.20	<***	7.86 ± 0.15	=	7.93 ± 0.29	>***
Prochlorococcus	2006	63.5 ± 4.7	=	69.8 ± 3.1	<**	98.4 ± 11.0	>*
	t-test	<*		=		=	
	2008	79.6 ± 3.5	=	67.8 ± 6.8	<**	110 ± 4.7	>***
Synechococcus	2006	8.2 ± 0.7	<***	29.1 ± 1.6	=	22.2 ± 3.9	>*
	t-test	>***		=		=	
	2008	4.60 ± 0.3	<***	30.8 ± 2.4	>***	15.5 ± 0.9	>***
Picoeukaryotes	2006	1.59 ± 0.11	<**	1.98 ± 0.09	>**	1.15 ± 0.18	<**
	t-test	<**		<***		<***	
	2008	2.01 ± 0.11	<***	7.28 ± 0.89	>**	4.28 ± 0.38	>***
Chl-*a*	2006	0.26 ± 0.01	=	0.23 ± 0.01	<*	0.27 ± 0.01	=
	t-test	>***		<**		>**	
	2008	0.18 ± 0.01	<***	0.31 ± 0.02	>***	0.22 ± 0.01	>***

Bacteria probes (EUBI-III) identified 75-92% and 84-94% of DAPI-stained cells during 2006 and 2008, respectively. In both years the *Alphaproteobacteria* class of the *Proteobacteria* were the dominant heterotrophic group, comprising half of the DAPI-stained cells in 2006, and about one-third in 2008 (Table 3.5 and Figure 3.4a). In both years the class was dominated by the SAR11 clade, 86 ± 2% and 79± 2% of *Alphaproteobacteria* in 2006 and 2008, respectively (Figure 3.4b). In fact, in 2006 SAR11 was the dominant of all bacterioplankton groups, comprising 2.4-fold more prokaryotes than the second most dominant group, *Prochlorococcus* cyanobacteria. *Bacteroidetes* was the second most dominant heterotrophic group throughout the region in 2006 and around CV in 2008 (Table 3.5 and Figure 3.4c). In the NESA and EQ regions of 2008 (i.e. those impacted by dust deposition) *Gammaproteobacteria* dominated (5.2- and 1.9-fold, respectively) over *Bacteroidetes* (Table 3.5 and Figure 3.4d).

Table 3.4: Abundance of cyanobacteria, picoeukaryotes and heterotrophic bacterioplankton (x10^3 cells mL^{-1}), and chlorophyll *a* (Chl-*a*) concentration, measured along a transect in the NESA region pre- (n = 9) and post- (n = 8) dust deposition in 2008. Mean values are presented ± SE and compared between sampling periods with the *t*-test. *p<0.01, **p<0.001.

Group	Pre-dust	*t*-test	Post-dust
Prochlorococcus	117 ± 10	>**	63 ± 8
Synechococcus	3.6 ± 0.4	<*	5.0 ± 0.3
Picoeukaryotes	1.6 ± 0.07	<**	2.8 ± 0.19
Het. Bpl.	640 ± 24	<**	814 ± 31
Chl-*a* µg L^{-1}	0.18 ± 0.01	=	0.17 ± 0.02

The heterotrophic community structure of 2006 did not differ regionally, except for a larger *Gammaproteobacteria* community in the EQ region compared to the NESA region (Table 3.5). Conversely, the community of 2008 showed significant variation in *Gammaproteobacteria* and *Bacteroidetes* groups between the CV and NESA regions (Table 3.5, Figure 3.4c and d); *Bacteroidetes* were more prominent in the CV region, whereas *Gammaproteobacteria* constituted a higher proportion of the NESA community. In both sampling years, the proportion of cells affiliated to *Alphaproteobacteria* and SAR11 was stable between regions (Table 3.5, Figures 3.4a and b).

Table 3.5: Regional CARD-FISH data are presented as mean percentage of DAPI-stained cells hybridised in each region ± SE, or standard difference (EQ, 2006). The *t*-test compares cruise data between adjacent regions (*t*-test columns), and regional data between sampling periods (*t*-test rows). The end column is a comparison between the EQ and NESA regions. In 2006 n = 8 (NESA), 7 (CV) and 2 (EQ); in 2008 n = 8 (NESA), 4 (CV) and 7 (EQ). *p<0.05, **p<0.01, ***p<0.0001.

Variable	Year	NESA	*t*-test	CV	*t*-test	EQ	*t*-test
Alphaproteobacteria	2006	48.72 ± 2.03	=	48.39 ± 2.41	=	52.04 ± 1.24	=
	t-test	>**		>*		>*	
	2008	35.30 ± 2.28	=	32.17 ± 8.10	=	36.33 ± 3.38	=
SAR11	2006	41.75 ± 1.63	=	40.49 ± 1.92	=	45.32 ± 0.83	=
	t-test	>**		=		>**	
	2008	30.90 ± 2.20	=	23.46 ± 8.25	=	28.53 ± 2.31	=
Bacteroidetes	2006	9.48 ± 1.33	=	8.76 ± 0.82	=	11.67 ± 3.11	=
	t-test	>**		=		>*	
	2008	4.72 ± 0.33	<*	8.59 ± 1.22	=	6.25 ± 0.81	=
Gammaproteobacteria	2006	4.64 ± 0.57	=	5.47 ± 0.82	=	8.36 ± 1.21	>*
	t-test	<**		=		=	
	2008	22.45 ±3.96	>**	4.53 ± 1.19	=	12.87 ± 3.04	=
Prochlorococcus	2006	17.40 ± 2.31	=	20.19 ± 2.71	=	11.41 ± 2.39	=
	t-test	<***		=		<**	
	2008	39.98 ± 1.15	>*	20.60 ± 3.76	<***	42.86 ± 2.35	=
Prochlorococcus HLI	2006	10.86 ± 2.53	>*	2.80 ± 0.51	=	2.95 ± 2.31	=
	t-test	>**		=		=	
	2008	0.66 ± 0.50	=	2.08 ± 0.50	>**	0.42 ± 0.25	=
Prochlorococcus HLII	2006	13.14 ± 1.70	=	10.46 ± 1.37	=	7.46 ± 3.68	=
	t-test	<**		=		<*	
	2008	23.72 ± 2.53	=	16.65 ± 3.79	=	26.50 ± 3.79	=

Community structure around CV did not differ significantly between sampling periods, except that a smaller proportion of the community were affiliated to the *Alphaproteobacteria* in 2008 (Table 3.5). *Alphaproteobacteria* and SAR11 groups were more dominant in 2006 than 2008. In the EQ region, *Gammaproteobacteria* were the only group not to differ between the two years. All groups varied significantly between studies in the

NESA region; one of the more striking changes was a 4.8-fold increase in *Gammaproteobacteria* from 2006 to 2008 (Figure 3.4d).

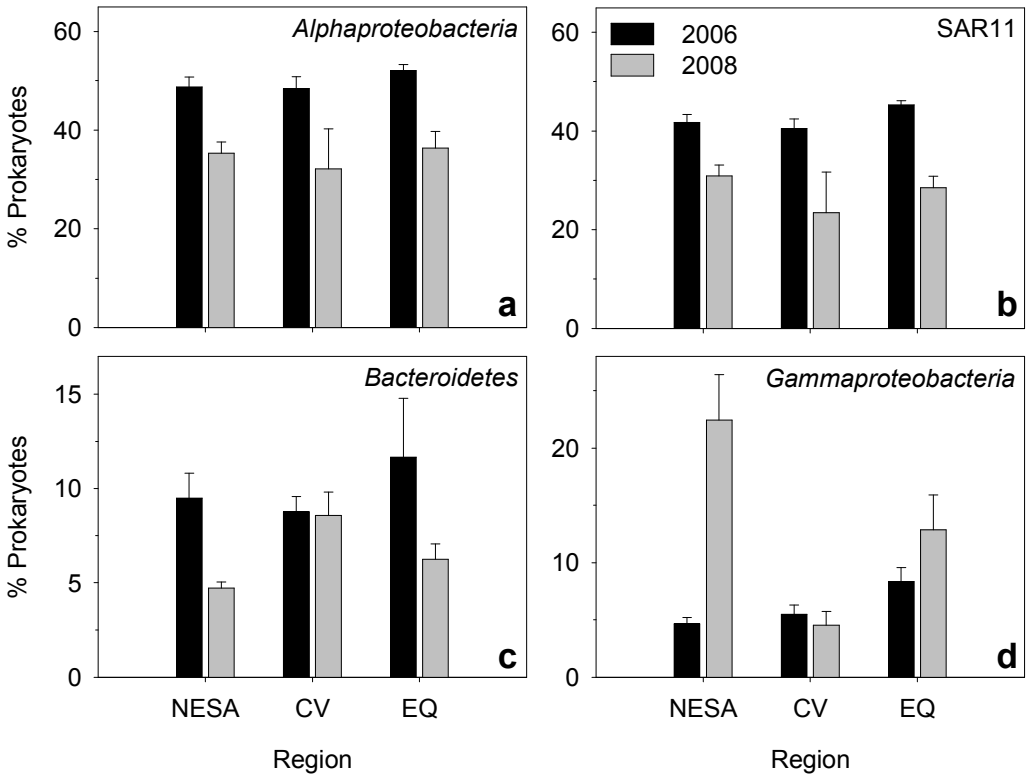


Figure 3.4: CARD-FISH analysis of community structure. The percentage of prokaryote cells assigned to (a) *Alphaproteobacteria*, (b) SAR11, (c) *Bacteroidetes* and (d) *Gammaproteobacteria* are presented for (black) 2006 and (grey) 2008. Data are presented as mean ± standard error or (EQ, 2006) standard difference. In 2006 n = 8 (NESA), 7 (CV) and 2 (EQ); in 2008 n = 8 (NESA), 4 (CV) and 7 (EQ).

3.3.3 The photoautotrophic community

Prochlorococcus abundance was measured by flow cytometry, and the proportion of cells belonging to the *Prochlorococcus* group, and its high light adapted ecotypes, was also measured by CARD-FISH. *Prochlorococcus* numerically dominated the autotrophic community throughout the region during both sampling periods. *Prochlorococcus* abundance varied regionally in both years, except between the NESA and CV regions (Table 3.3 and 3.3b). Abundance did not vary significantly between years in the CV and EQ regions, but there were 24% more in the NESA region in 2008 compared to 2006 (Table 3.3 and Figure 3.3b).

In 2006 *Prochlorococcus* was generally the second most dominant bacterioplankton group after SAR11; in 2008 *Prochlorococcus* dominated the bacterioplankton community in the NESA and EQ regions, constituting 40 ± 1% and 43 ± 2% of prokaryotes, respectively, which were significantly higher proportions than those measured in the same regions in 2006 (Table 3.5 and Figure 3.5a). In the CV region *Prochlorococcus* constituted 20% of cells in both years.

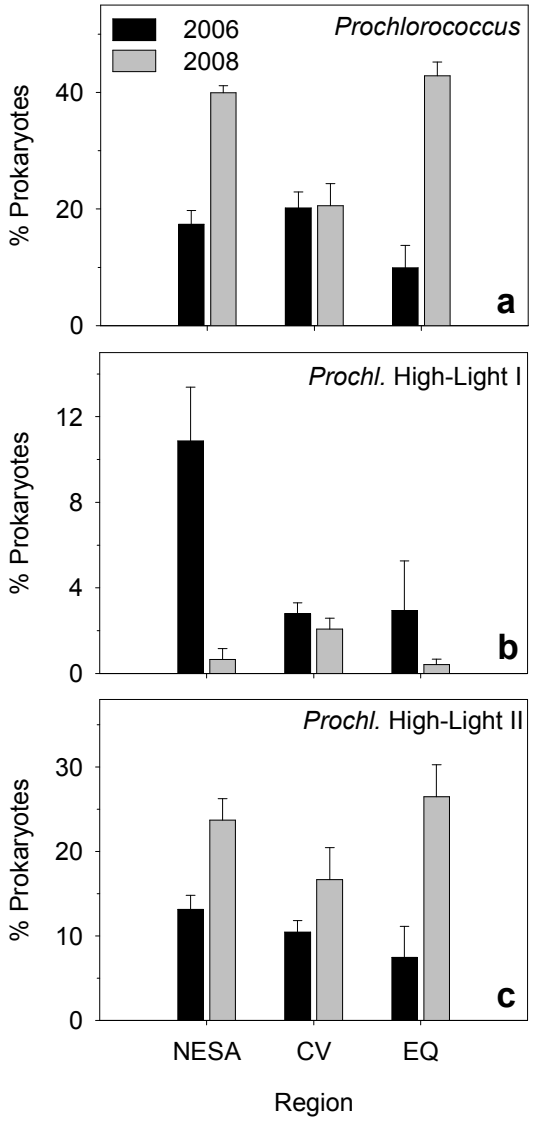


Figure 3.5: Proportion of prokaryotes identified as (a) *Prochlorococcus*, (b) *Prochlorococcus* High-Light I and (c) *Prochlorococcus* High-Light II. Samples were analysed by CARD-FISH using probes 645Pro, 405HLI and 405HLII, respectively.

The *Prochlorococcus* high light adapted group II ecotype (HLII) dominated the *Prochlorococcus* community throughout the study region in both years (Table 3.5 and Figure 3.5c). Despite apparent differences, the proportion of HLI and HLII around CV did

not vary significantly between years. However, in 2008 in the NESA and EQ regions – those impacted by dust deposition - there was a smaller proportion of HLI and greater proportion of HLII, as compared to 2006 (Figure 3.5 b and c). *Prochlorococcus* HLI were a more prominent group in 2006 than in 2008, particularly in the NESA region (Table 3.5 and Figure 3.5b). Most *Prochlorococcus* cells were assigned to HLII in 2008 (Figure 3.5c) and less than 2% of *Prochlorococcus* cells were attributed to HLI.

Synechococcus abundance varied regionally in both years, with substantial peaks in the CV region and troughs in the NESA region (Table 3.3 and Figure 3.3c). *Synechococcus* abundance did not differ between years except in the NESA region, where they were more numerous in windy 2006 compared to dusty 2008. Picoeukaryote abundance also differed between regions in both years and peaked in the CV region (Table 3.3 and Figure 3.3d). Abundance was significantly higher in 2008, predominantly in the CV and EQ regions. Despite the variation in phytoplankton abundance between regions, Chl-*a* concentration only showed substantial regional variation in 2008, when regional concentration reflected variation in *Synechococcus* and Picoeukaryote abundance (Table 3.3).

Data from the transect in the NESA region sampled pre- and post-dust deposition in January 2008 show that *Synechococcus* and picoeukaryote abundances had increased significantly towards the end of the study, whereas *Prochlorococcus* had decreased (Table 3.4). Furthermore, when all samples from the northern region were divided into pre- and post-dust deposition, the same trend was observed. Despite this, Chl-*a* concentration did not vary significantly between sampling periods.

3.4 Discussion

The SAR11 clade of *Alphaproteobacteria* dominated the microbial community during the 2006 survey when gale conditions were experienced. Conversely, *Prochlorococcus* dominated throughout much of the region in 2008 when conditions were calmer, blowing moderate to strong breeze. The lack of dust deposition experienced in 2006 resulted in 'clean' conditions whereby underlying influences on microbial community structure and composition could be determined, without the impact of dust-derived nutrient additions. However, the strong winds of 2006 meant that these were not true control conditions to

the dust deposition experienced in 2008. Since dust storms are brought about by wind events, the impacts of dust deposition and wind-driven perturbation are compared.

Heterotrophic bacterioplankton community structure in the CV region generally did not vary significantly between sampling years. Conversely, all heterotrophic groups varied between sampling periods in the NESA region, which was unaffected by the islands but experienced a contrast in wind speed and Aeolian dust deposition between sampling periods. Considering there were two years between sampling periods, the similar community structure of the heterotrophic bacterioplankton in the CV region suggests that the influence of the Cape Verde island topography is more significant than any other factor in the region.

Synechococcus abundance peaked in the CV region in both years, as did picoeukaryote abundance and Chl-*a* concentration in 2008, implying the region was relatively mesotrophic compared to the other regions (Partensky *et al.*, 1999, Zubkov *et al.*, 2000). In 2008 *Bacteroidetes* were identified mostly in the CV region followed by the EQ region, reflecting the regional distribution of Chl-*a*, picoeukaryotes and *Synechococcus*. Islands have been shown to increase production in large surrounding areas (Simpson, *et al.*, 1982). Thus increased abundance of these groups around the Cape Verde Islands could be a response to increased concentrations of dissolved organic material resulting from island-induced eddies or the relatively high phytoplankton abundance, as has previously been found in other regions (Eilers *et al.*, 2001, Alonso-Sáez *et al.*, 2007, Teira *et al.*, 2008, Gómez-Pereira *et al.*, 2010).

The NESA transect sampled before and after dust deposition showed an increase in *Synechococcus* and picoeukaryote abundance following dust deposition, which is a similar response to that observed of Mediterranean microbial communities following Saharan dust addition (Herut *et al.*, 2005). Despite the increase in *Synechococcus* and picoeukaryote abundance, Chl-*a* concentration did not indicate an increase in phytoplankton biomass following dust deposition in 2008, suggesting that the increase in those phytoplankton groups was counteracted by the decrease in *Prochlorococcus* abundance. However, given that around a quarter of *Prochlorococcus* cells pass through the glass fibre filters used for sampling Chl-*a* (see Chapter Four, Figure 4.2), the conserved Chl-*a* concentration between sampling periods perhaps reflects a lack of response from larger autotrophic cells. Dust

addition in the Mediterranean Sea also showed no effect on Chl-*a* concentration despite stimulation of the photosynthetic community (Bonnet *et al.*, 2005). Furthermore, a recent study showed Chl-*a* concentration decreased in response to dust addition in the northern Red Sea, along with *Synechococcus* and picoeukaryote abundance (Paytan *et al.*, 2009). Considering *Synechococcus* were more abundant in windy 2006, and both *Synechococcus* and picoeukaryotes were most abundant in the CV region, it appears that these groups benefit more from wind- and island-induced mixing than from dust-derived nutrient inputs.

In contrast to other regions, *Prochlorococcus* did not vary between years in the CV region in terms of abundance, and the proportion of the bacterioplankton community affiliated to *Prochlorococcus* and the two high-light adapted ecotypes. This again suggests that the Cape Verde islands have a greater effect on microbial communities than wind-driven perturbation or dust deposition. Previous studies have shown an increase (Paytan *et al.*, 2009) and a decrease (Herut *et al.*, 2005) in *Prochlorococcus* abundance in response to dust inputs. In this study *Prochlorococcus* abundance decreased following dust deposition in 2008, and dust addition also led to a decrease in *Prochlorococcus* metabolic activity (Chapter Five). Thus it is unlikely that the elevated *Prochlorococcus* abundance in the NESA region of 2008, compared to 2006, was due to dust deposition. Further to this, the *Prochlorococcus* HLII ecotype, identified in oligotrophic environments (West *et al.*, 2001, Schattenhofer *et al.*, 2009), almost entirely dominated the *Prochlorococcus* community in 2008; whereas HLI, with relatively high nutrient requirements (Johnson *et al.*, 2006), was most abundant during windy 2006. Thus the enhanced wind-driven mixing and associated nutrients that perhaps supported higher *Prochlorococcus* ecotype HLI, SAR11 and *Synechococcus* abundance in 2006 was likely the cause of the overall decline in *Prochlorococcus* abundance the same year. Conversely, the relatively calm conditions of 2008 gave *Prochlorococcus* an opportunity to increase its abundance beyond the previously dominant SAR11 clade.

The increase in heterotrophic bacteria abundance following dust deposition concurs with previous findings in a high mountain lake (Pulido-Villena *et al.*, 2008a) and the western Mediterranean Sea (Pulido-Villena *et al.*, 2008b, Bonilla-Findji *et al.*, 2010). Considering *Gammaproteobacteria* were associated with the dust impacted NESA and EQ regions of 2008, these additional heterotrophic bacteria may have been opportunistic

Gammaproteobacteria, that are known to respond to sudden nutrient pulses (Eilers *et al.*, 2000, Fuchs *et al.*, 2000, Pinhassi & Berman, 2003, Allers *et al.*, 2007, Teira *et al.*, 2008). Indeed, a recent study of Mediterranean lakes and reservoirs suggests an association of *Gammaproteobacteria* with Saharan dust deposition (Reche *et al.*, 2009). The NESA region, which was sampled after both dust events, allowing up to thirteen days for the microbial community to respond to the first storm and five days to the second, had the largest proportion of *Gammaproteobacteria* in any population sampled. Thus the data suggest that the *Gammaproteobacteria* were the only group to benefit from the dust storms. Considering dust deposition resulted in a loss of *Prochlorococcus* cells, it seems likely that the *Gammaproteobacteria* population growth was in response to organic exudates from these decaying cells.

In summary, the surface bacterioplankton community of the north-eastern tropical and subtropical Atlantic was dominated by SAR11 in the windy winter months of 2006, whereas during the calmer conditions of early 2008 *Prochlorococcus* dominated. Phytoplankton-associated *Bacteroidetes* were associated with productive regions, whereas opportunistic *Gammaproteobacteria* seemed to multiply in response to dust deposition in 2008. The results from this study imply dust deposition has a lesser influence on bacterioplankton community structure than wind-driven mixing and upwelling due to island topography. Observations of dust deposition impacts on microbial communities *in situ* should, therefore, be interpreted with caution.

3.5 References

Allers E, Gómez-Consarnau L, Pinhassi J, Gasol JM, Simek K & Pernthaler J (2007) Response of *Alteromonadaceae* and *Rhodobacteriaceae* to glucose and phosphorus manipulation in marine mesocosms. *Environmental Microbiology* **9**: 2417-2429.

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González JA, Vaqué D, Agustí S & Gasol JM (2007) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic. *Aquatic Microbial Ecology* **46**: 43-53.

Amann R, Binder BJ, Olson RJ, Chisholm SW, Devereux R & Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology* **56**: 1919-1925.

Baker AR, Weston K, Kelly SD, Voss M, Streu P & Cape JN (2007) Dry and wet deposition of nutrients from the tropical Atlantic atmosphere: Links to primary productivity and nitrogen fixation. *Deep Sea Research I* **54**: 1704-1720.

Bonilla-Findji O, Gattuso J-P, Pizay M-D & Weinbauer MG (2010) Autotrophic and heterotrophic metabolism of microbial planktonic communities in an oligotrophic coastal marine ecosystem: seasonal dynamics and episodic events. *Biogeosciences Discussions* **7**: 2033-2064.

Bonnet S, Guieu C, Chiaverinin J, Ras J & Stock A (2005) Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system. *Limnology and Oceanography* **50**: 1810-1819.

Daims H, Bruhl A, Amann R, Schleifer KH & Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology* **22**: 434-444.

Eilers H, Pernthaler J & Amann R (2000) Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. *Applied and Environmental Microbiology* **66**: 4634-4640.

Eilers H, Pernthaler J, Peplies J, Glöckner FO, Gerdts G & Amann R (2001) Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. *Applied and Environmental Microbiology* **67**: 5134-5142.

Fuchs BM, Zubkov MV, Sahm K, Burkill PH & Amann R (2000) Changes in community composition during dilution cultures of marine bacterioplankton as assessed by flow cytometry and molecular biology techniques. *Environmental Microbiology* **2**: 191-201.

Fuchs BM, Woebken D, Zubkov MV, Burkill P & Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. *Aquatic Microbial Ecology* **39**: 145-457.

Glöckner FO, Fuchs BM & Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridisation. *Applied and Environmental Microbiology* **65**: 3721-3726.

Goericke R & Welschmeyer NA (1993) The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass and primary production in the Sargasso Sea. *Deep Sea Research I* **40**: 2283-2294.

Gómez-Pereira PR, Fuchs BM, Alonso C, Oliver MJ, van Beusekom JEE & Amann R (2010) Distinct flavobacterial communities in contrasting water masses of the North Atlantic Ocean. *The ISME Journal*.

Herut B, Zohary T, Krom MD, Mantoura RFC, Pitta P, Psarra S, Rassoulzadegan F, Tanaka T & Thingstad TF (2005) Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Sea Research II* **52**: 3024-3040.

Heywood JL, Zubkov MV, Tarran GA, Fuchs BM & Holligan PM (2006) Prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean: Evaluation of inter-annual variability. *Deep Sea Research II* **53**: 1530-1547.

Jickells T (1995) Atmospheric inputs of metals and nutrients to the oceans: their magnitude and effects. *Marine Chemistry* **48**: 199-214.

Jickells TD, An ZS, Andersen KK, Baker AR, Bergametti G, Brooks N, Cao JJ, Boyd PW, Duce RA, Hunter KA, Kawahata H, Kubilay N, laRoche J, Liss PS, Mahowald N, Prospero JM, Ridgwell AJ, Tegen I & Torres R (2005) Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* **308**: 67-71.

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS & Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* **311**: 1737-1740.

Kerkhof LJ, Voytek MA, Sherrell RM, Millie D & Schofield O (1999) Variability in bacterial community structure during upwelling in the coastal ocean. *Hydrobiologia* **401**: 139-148.

Li WKW (1994) Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: Measurements from flow cytometric sorting. *Limnology and Oceanography* **39**: 169-175.

Lindell D & Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. *Limnology and Oceanography* **40**: 1130-1141.

Manz W, Amann R, Ludwig W, Wagner M & Schleifer KH (1992) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria - Problems and solutions. *Systematic and Applied Microbiology* **15**: 593-600.

Manz W, Amann R, Ludwig W, Vancanneyt M & Schleifer KH (1996) Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria - Problems and solutions. *Systematic and Applied Microbiology* **15**: 593-600.

Marie D, Partensky F, Jacquet S & Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology* **63**: 186-193.

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH & Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* **45**: 107-113.

Olson RJ, Zettler ER & Durand MD (1993) Phytoplankton analysis using flow cytometry. *Handbook of methods in aquatic microbial ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds.), pp. 175-186. Lewis Publishers, Boca Raton, Florida.

Partensky F, Hess WR & Vaulot D (1999) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews* **63**: 106-127.

Paytan A, Mackey KRM, Chen Y, Lima ID, Doney SC, Mahowald N, Labiosa R & Post AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences* **106**: 4601-4605.

Pelegrí JL, Marrero-Díaz A & Ratsimandresy AW (2006) Nutrient irrigation of the North Atlantic. *Progress in Oceanography* **70**: 366-406.

Pernthaler A, Pernthaler J & Amann R (2002) Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology* **68**: 3094-3101.

Pinhassi J & Berman T (2003) Differential growth response of colony-forming alpha- and gamma-Proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Applied and Environmental Microbiology* **69**: 199-211.

Pulido-Villena E, Reche I & Morales-Baquero R (2008a) Evidence of an atmospheric forcing on bacterioplankton and phytoplankton dynamics in a high mountain lake. *Aquatic Sciences* **70**: 1-9.

Pulido-Villena E, Wagener T & Guieu C (2008b) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* **22**: GB1020, doi:10.1029/2007GB003091.

Reche I, Ortega-Retuerta E, Romera O, Pulido-Villena E, Morales-Baquero R & Casamayor EO (2009) Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs. *Limnology and Oceanography* **54**: 869-879.

Rosenfeld D, Rudich Y & Lahav R (2001) Desert dust suppressing precipitation: A possible desertification feedback loop. *Proceedings of the National Academy of Sciences* **98**: 5975-5980.

Schattenhofer M, Fuchs BM, Amann R, Zubkov MV, Tarran GA & Pernthaler J (2009) Latitudinal distribution of prokaryoplankton populations in the Atlantic Ocean. *Environmental Microbiology* **11**: 2078-2093.

Simpson JH, Tett PB, Argote-Espinoza ML, Edwards A, Jones KJ & Savidge G (1982) Mixing and phytoplankton growth around an island in a stratified sea. *Continental Shelf Research* **1:** 15-31.

Suzuki MT, Preston CM, Chavez FP & DeLong EF (2001) Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay. *Aquatic Microbial Ecology* **24**: 117-127.

Teira E, Gasol JM, Aranguren-Gassis M, Fernández A, González J, Lekunberri I & Álvarez-Salgado A (2008) Linkages between bacterioplankton community composition, heterotrophic carbon cycling and environmental conditions in a highly dynamic coastal ecosystem. *Environmental Microbiology* **10**: 906-917.

Troussellier M, Cahet G, Lebaron P & Baleux B (1993) Distribution and dynamics of bacterial production in relation to wind perturbations in a Mediterranean lagoon. *Limnology and Oceanography* **38**: 193-201.

Wallner G, Amann R & Beisker W (1993) Optimizing fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* **14**: 136-143.

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll-a in the presence of chlorophyll-b and pheopigments. *Limnology and Oceanography* **39**: 1985-1992.

West NJ, Schönhuber WA, Fuller NJ, Amann RI, Rippka R, Post AF & Scanlan DJ (2001) Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by *in situ* hybridization using 16S rRNA-targeted oligonucleotides. *Microbiology* **147**: 1731-1744.

Zubkov MV, Sleigh MA, Burkill PH & Leakey RJG (2000) Picoplankton community structure on the Atlantic Meridional transect: a comparison between seasons. *Progress in Oceanography* **45**: 369-386.

Zubkov MV & Burkill PH (2006) Syringe pumped high speed flow cytometry of oceanic phytoplankton. *Cytometry Part A* **69A**: 1010-1019.

Zubkov MV, Mary I, Woodward EMS, Warwick PE, Fuchs BM, Scanlan DJ & Burkill PH (2007) Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environmental Microbiology* **9**: 2079-2089.

Chapter Four
Development of a method for the experimental study of ambient bacterioplankton community response to dust additions

4.1 Introduction

Whilst studying natural ecosystems it is not always possible to distinguish direct dust impacts from indirect, secondary responses. Although observations are important in the study of dust impacts (Herut *et al.*, 2005, Pulido-Villena *et al.*, 2008), high wind speeds commonly associated with dust deposition may influence the bacterioplankton community to the same or greater extent than the deposited dust itself (see Chapter Three). This, and the sporadic nature of dust events, has meant that the study of dust impacts on the marine environment has typically involved dust addition experiments.

Dust addition incubations are often done in parallel with nutrient bioassays to decipher which nutrients from within the dust may be of benefit to microorganisms (Mills *et al.*, 2004, Moore *et al.*, 2006). Dust addition studies have used sifted Saharan soils (Mills *et al.*, 2004, Bonnet *et al.*, 2005, Moore *et al.*, 2006, Davey *et al.*, 2008, Pulido-Villena *et al.*, 2008) or 'atmospherically processed' aerosol collected as wet (Pulido-Villena *et al.*, 2008, Lekunberri *et al.*, 2010) or dry (Herut *et al.*, 2005, Duarte *et al.*, 2006, Moore *et al.*, 2006, Paytan *et al.*, 2009, Reche *et al.*, 2009) deposition.

The benefit of using sifted soils is the abundance of material available for chemical analyses and bioassay studies. However, during atmospheric transport, dust particles are exposed to cycles of ranging pH, which alters the solubility of metals within the dust (Spokes *et al.*, 1994). For example, dust dissolution experiments have shown that iron may be more soluble from atmospherically processed dust than sifted soils (Moore *et al.*, 2006). Furthermore, atmospheric transport gives dust the opportunity to mix with pollution, leading to higher nitrogen (Baker *et al.*, 2006) and lead (Patterson & Settle, 1987, Guieu *et al.*, 2002) content. Thus the impact of sifted soils may be far removed from that of atmospherically processed dust.

A comparison of sifted Saharan soil with the particulate fraction of Saharan rain suggested that the atmospherically processed dust had a more stimulatory effect on bacterial abundance than the same concentration of soil (Pulido-Villena *et al.*, 2008). However, it is not possible to compare atmospheric dust with its end-member, in terms of their impacts on bacterioplankton, because the dust that is deposited in the oceans is a mixture of soils from spatially diverse sources, which vary between dust deposition events.

Table 4.1: Summary of research cruises involved in method development.

Cruise	Dates	Region	Experimental work done
RV *Poseidon* 'PO332'	Jan-Feb 2006	NE (sub-) tropical Atlantic (Figure 4.1)	Addition of atmospherically processed 'Barbados dust'. Microbial response measured as change in cell abundance and Chlorophyll *a* concentration.
RV *Atlantic Explorer* 'BATS 225'	July 2007	Bermuda Atlantic Time-series Study site (BATS)	Tested suitability of Swinnex units as incubation vessels. Addition of dust collected from Cape Verde atmospheric observatory. Addition of leachate produced from 'M4' dust.
RV *Hespérides* 'TRYNITROP'	Nov-Dec 2007	Tenerife to Buenos Aires (Figure 2.1)	Tested suitability of filter funnels as incubation vessels. Addition of dust collected at sea. Addition of leachate produced from dust collected at sea.
RRS *Discovery* 'D326'	Jan-Feb 2008	NE (sub-) tropical Atlantic (Figure 2.1)	Addition of dust collected at sea. Addition of leachate from dust collected at sea. Tested toxicity of polypropylene filters on flow cytometrically sorted bacterioplankton groups.
RRS *Discovery* 'D338'	Apr-May 2009	Mauritanian upwelling (Figure 2.1)	Tested for toxicity of polypropylene filters in 1.6 mL seawater samples.

This study aimed to characterise the impact of dust deposition on surface seawater microbial communities, mimicking natural conditions as closely as possible, by using atmospherically processed dust. The collection of deposited dust is problematic: dust collected from rainwater will have lost its readily soluble fraction, as will dust that is washed from collection nets using ultrapure water. Thus the ultimate aim was to collect dust for additions, without the need for dissolution prior to incubations.

To replicate natural conditions as closely as possible, atmospherically processed dust was added to natural seawater samples. The study was done during five research cruises to the (sub-) tropical northeast Atlantic Ocean (Table 4.1). Despite receiving an estimated 5-20 g dust m^{-2} per year (Jickells *et al.*, 2005), few studies have explored the impacts of dust deposition in this region.

There has been one study between 21 and 26°N, off the coast of north-western Africa (Duarte *et al.*, 2006), and one between 3 and 11°N, in equatorially affected waters (Mills *et al.*, 2004, Davey *et al.*, 2008), but none, to my knowledge, in the intermediate region. Although both studies measured the change in abundance of the main phytoplankton groups, only Duarte *et al.* (2006) measured bacterial abundance and the metabolic response of the bacterioplankton community.

The ultimate aim of this study was to characterise the instantaneous metabolic response of dominant bacterioplankton groups to the addition of freshly collected atmospherically processed dust, in a region heavily impacted by Saharan dust storms.

4.2 48 h Dust Addition Incubation Experiments

The initial attempt to study how dust deposition affects microbial communities used dust of Saharan origin that had been collected in nets in Barbados and rinsed off using deionised water. This 'Barbados dust' was used because it was representative of dust that is deposited into the North Atlantic Ocean. Furthermore, it was available in large quantities enabling many variables to be measured from dust addition incubations.

Seven 48 h dust addition incubation experiments were conducted during a four week cruise onboard RV *Poseidon*, from January 26th to February 23rd 2006 (Figure 4.1). The aim was to measure the impact of dust addition on the microbial community in terms of the

change in bacterial production, chlorophyll *a* concentration and cell abundance of major microbial groups. Although the more sensitive radioactively-labelled amino acid tracers would have been the preferred method, bacterial production was estimated by ^{15}N-leucine uptake because the RV *Poseidon* is not licensed to carry radioactive chemicals.

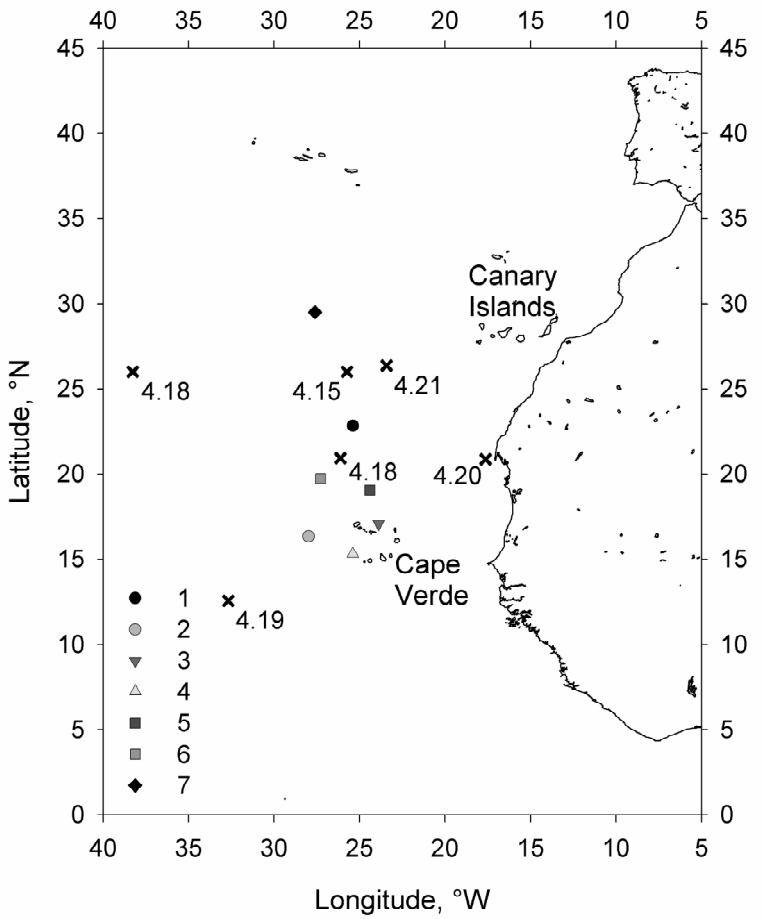


Figure 4.1: Sampling locations of seawater used in each of the seven incubation experiments. Crosses indicate origins of seawater samples used for subsequent method development. Each position is labelled with its corresponding figure number.

4.2.1 Method

Experimental Design

The first three experiments were initiated between 23:00 and 01:00 h, and the remaining four between 05:00 and 07:00 h. Twenty-four acid-washed 4.4 L polycarbonate bottles were rinsed three times and filled with surface seawater collected using a trace metal clean pumping system and towed torpedo fish. Triplicate bottles were filled for T = 0 measurements of ambient conditions, initial 24 h community production, dust addition treatments and controls (Table 4.2). The initial 24 h community production measurements were used because a long incubation period is necessary to allow the bacterial community to assimilate sufficient ^{15}N-leucine to be detected by mass spectrometry, which is insensitive compared to liquid scintillation counting of radioactive isotopes.

Table 4.2: Summary of how each incubation bottle was employed.

Bottle	T = 0	T = 24h	T = 48h
T = 0, 1	Filtered	-	-
T = 0, 2	Filtered	-	-
T = 0, 3	Filtered	-	-
Initial 1	^{15}N-leucine added	Filtered	-
Initial 2	^{15}N-leucine added	Filtered	-
Initial 3	^{15}N-leucine added	Filtered	-
Control 1	-	^{15}N-leucine added	Filtered
Control 2	-	^{15}N-leucine added	Filtered
Control 3	-	^{15}N-leucine added	Filtered
+Dust 1	Dust added	^{15}N-leucine added	Filtered
+Dust 2	Dust added	^{15}N-leucine added	Filtered
+Dust 3	Dust added	^{15}N-leucine added	Filtered

The ship was moving during the incubation bottle filling period so the water composition may have varied between bottles. Therefore T = 0 bottles were filled at the beginning, middle, and end of the filling period to measure variation over the water collection region. These bottles were sampled immediately for chlorophyll *a* concentration and cell abundance.

Incubation bottles were placed in on-deck incubators maintained at ambient temperature by means of a continuous supply of surface seawater. Light was attenuated to 20% surface irradiance using blue filters; during hours of darkness the incubators were covered with thick black plastic to exclude light from the ship.

Additional bottles were incubated in parallel for the measurement of ^{13}C-bicarbonate and $^{15}N_2$ gas uptake, and nitrate, phosphate, aluminium and iron concentrations in all treatments and controls. These data are not reported here, however, chlorophyll *a* concentration and cell abundance was measured in these additional bottles when enough volume remained from other analyses, the results of which are combined with data from the bottles described.

^{15}N-leucine uptake

The three Initial, Control and +Dust bottles were labelled with 100µl of 1 mM ^{15}N-leucine solution (final concentration 22.73 nM). The Initial bottles were labelled at T = 0 and terminated by filtration after 24 h. At the start of the incubation, 8 mg (final concentration

~2 mg L^{-1}) of Barbados dust was added to the +Dust bottles. The Control and +Dust bottles were placed in the incubators at T = 0 without tracer. At T = 24 h all +Dust and Control bottles were removed from the incubators and 22.73 nM ^{15}N-leucine was added. The bottles were returned to the incubator and removed for sampling 24 h later (T = 48 h).

Following 24 h incubation in the presence of ^{15}N-leucine, 3.5 L subsamples were filtered through pre-combusted (450^{o}C, 4h) Whatman GF/F filters to measure tracer uptake. Filters were rinsed with filtered seawater, then placed in Petri slides and dried at 60^{o}C for 24 h.

Samples would have been analysed for ^{15}N content by mass spectrometry, however, cell abundances in the seawater that passed through the GF/F filters were high (Figure 4.2), with up to 63, 5 and 12 % of *Prochlorococcus*, *Synechococcus* and picoeukaryote cells, respectively, passing through. The cells retained on the GF/F filters were not considered to be representative of the ambient community so mass spectrometric analysis was not conducted.

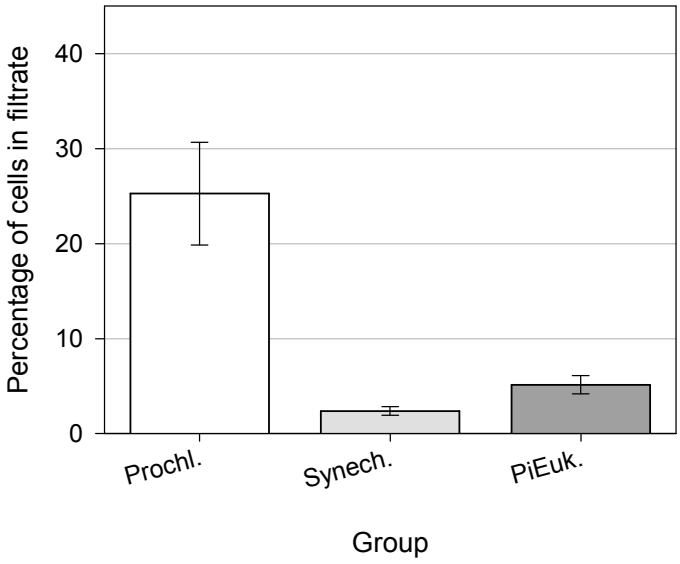


Figure 4.2: The percentage of *Prochlorococcus* (Prochl.), *Synechococcus* (Synech.) and picoeukaryote (PiEuk.) cells that passed through a GF/F filter used for measuring ^{15}N-leucine uptake. Data are from incubation three and are shown as mean ± standard error (n = 9).

Flow cytometry

Triplicate 1.6 mL seawater samples were fixed with paraformaldehyde (PFA), 1% w/v final concentration, in sterile 2 mL polypropylene screw cap vials. Samples were fixed in the dark at 4°C for 24 h then stored at -80^{o}C. Samples from incubations three, four and five were analysed for cell abundance by flow cytometry (FACSCalibur, Becton Dickinson, Oxford, UK).

Cells were enumerated using multi-fluorescent latex beads (6.4 x 10^5 mL^{-1}) as an internal standard (Zubkov & Burkill, 2006). The cyanobacteria groups *Prochlorococcus* spp. and *Synechococcus* spp. were identified in unstained samples by their characteristic autofluorescence (Olson *et al.*, 1993). Picoeukaryotes were identified as the larger pigment-containing cells.

The nucleic acid stain SYBR Green I (Marie *et al.*, 1997) was applied to subsamples to count all bacterioplankton cells on a plot of side scatter against green fluorescence. The term 'heterotrophic bacterioplankton' here refers to all cells that showed no autofluorescence, and thus includes Archaea. Flow cytometry data was processed using CellQuest software (Becton Dickinson, Oxford, UK).

Chlorophyll a

Subsamples of 500 mL were filtered through Fisherbrand MF 300 glass fibre filters and stored at -80°C until analysed in batches on board using the method of Welschmeyer *et al.* (1994). Samples were extracted in 7 mL of 90% acetone at 4°C for 24 h. Extracts were analysed in a TD-700 Turner Designs fluorometer, calibrated with fresh chlorophyll *a* standard from *Anacystis nidulans* (Sigma, UK). Samples from all incubations were analysed for chlorophyll *a* concentration. The use of 0.7 µm retention glass fibre filters underestimates chlorophyll *a* concentration because many picophytoplankton cells pass though (Figure 4.2).

Statistical analysis

Cell abundance and chlorophyll *a* concentration were compared between Control and +Dust treatments at T = 48 h, using the Student's *t*-test for independent samples, following an F-test for the equality of variance. Control and treatment were considered to be significantly different when $p<0.05$.

4.2.2 Results & Discussion

In each incubation *Prochlorococcus* abundance increased between T = 0 and 24 h followed by a decrease between T = 24 and 48 h, with no significant effect of added dust as compared with the control (Figure 4.4 & Table 4.3). There was considerable variation between the incubations; in incubation number three *Prochlorococcus* concentration returned to the time zero concentration after 48 h, whereas they increased in incubation

number four (~100%) and decreased in incubation number five (~30%). *Synechococcus* numbers also varied between incubations, although there was a general decrease in *Synechococcus* concentration throughout the incubations, with no significant effect of dust in any incubation (Figure 4.4 & Table 4.2).

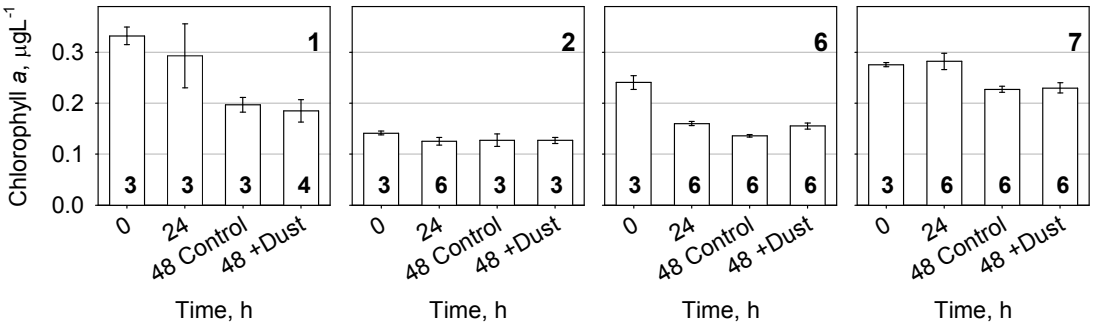


Figure 4.3: Chlorophyll *a* data from the four dust addition incubations without corresponding cell abundance data; mean ± standard error for the number of observations indicated on each bar.

Picoeukaryotes did not show a consistent pattern between incubations, except for showing no significant response to the dust in any incubation (Figure 4.4 & Table 4.3). Chlorophyll *a* concentration generally remained constant throughout the incubation period or decreased (Figures 4.3 & 4.4). A slight increase in concentration was only shown in the +Dust treatment of incubation six (Table 4.3).

Heterotrophic bacteria concentration remained fairly stable throughout each of the incubations and, again, showed no response to dust (Figure 4.4 & Table 4.3).

During the 48 h incubations, the bacterioplankton community responded to enclosure, showing more dramatic changes in abundances than would be expected *in situ*. Previous incubation experiments have seen a crash in *Prochlorococcus* (Herut et al., 2005) and *Synechococcus* populations (Lekunberri et al., 2010; Paytan et al., 2009) within two to three days, suggesting these cyanobacteria do not respond well to periods of enclosure. Had there been a response to the dust addition, it seems unlikely it would have been a response from the original community. Thus, a 48 h incubation period is considered too long to study the response of the ambient microbial community to dust addition.

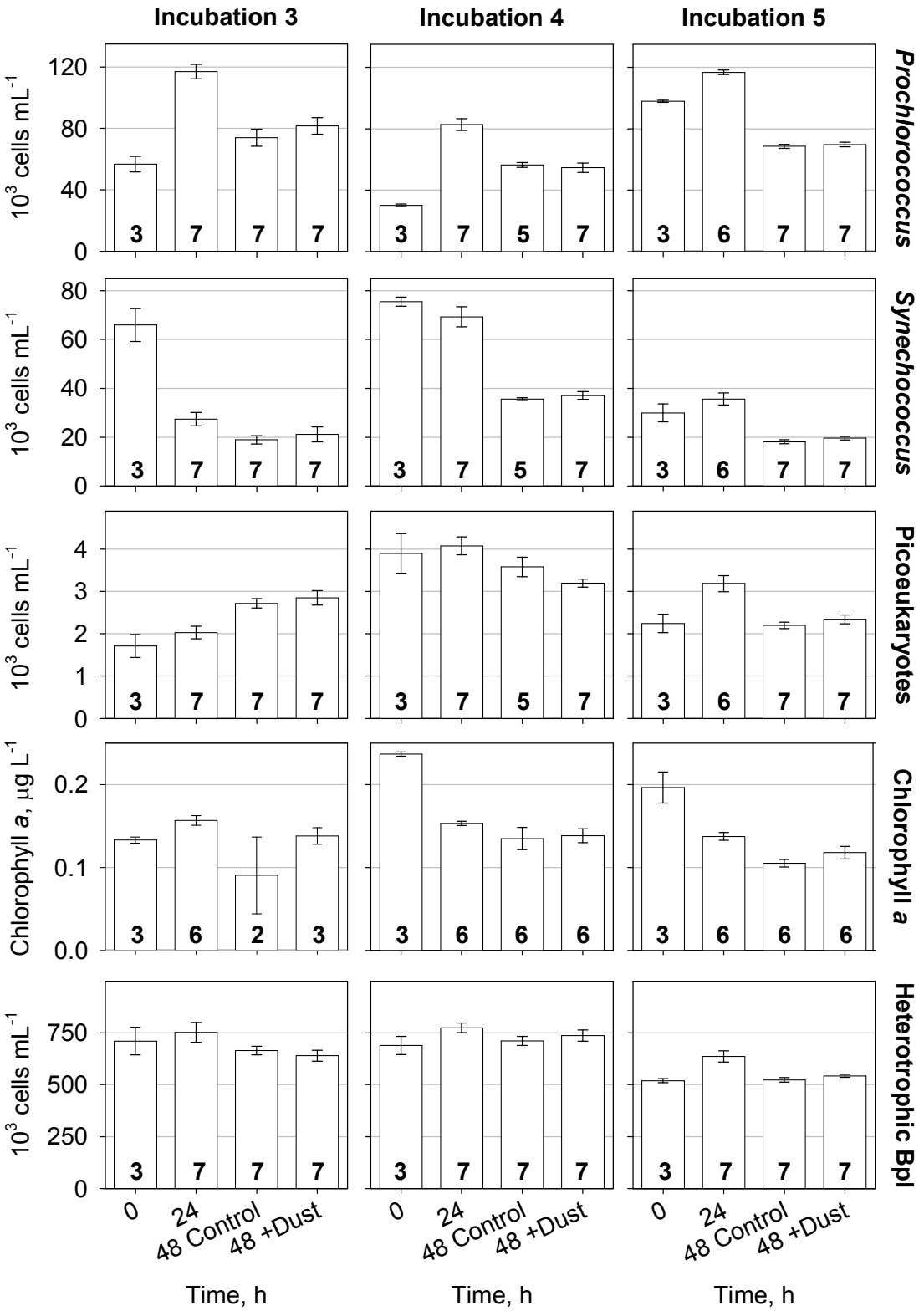


Figure 4.4: Cell abundance and chlorophyll *a* concentration data from three dust addition incubations. Data are presented as mean ± standard error for the number of observations indicated on each bar, or ± standard difference where n = 2.

Table 4.3: Results of the F-test for equality of variance and Student's *t*-test for independent samples comparing cell abundance and chlorophyll *a* concentration data between +Dust treatments and controls. * Denotes a significant impact of dust addition at the 5% level.

Inc	F	p	*t*	df	p
Prochlorococcus					
3	0.003	0.95	1.0	12	0.34
4	4.8	0.05	0.5	10	0.66
5	0.003	0.96	0.6	12	0.57
Synechococcus					
3	1.1	0.32	0.6	12	0.53
4	11.7	0.01	0.8	7.5	0.43
5	0.9	0.76	1.3	12	0.22
Picoeukaryotes					
3	0.2	0.69	0.6	12	0.54
4	2.5	0.14	1.7	10	0.12
5	1.0	0.34	1.1	12	0.27
Heterotrophic bacterioplankton					
3	0.3	0.61	0.7	12	0.48
4	0.8	0.38	0.7	10	0.50
5	2.2	0.16	1.5	12	0.17
Chlorophyll *a*					
1	1.7	0.25	0.4	5	0.69
2	1.6	0.28	0.03	4	0.98
3	6.9	0.06	1.0	4	0.37
4	0.5	0.48	0.2	10	0.84
5	1.0	0.35	1.5	10	0.18
6	10.1	0.01	2.9	6.5	0.03*
7	0.9	0.37	0.2	10	0.83

The lack of response to dust addition may be due to slow dissolution of nutrients. The incubations were too short to measure a release of nutrients from the dust. Therefore, it seems unfeasible to combine microbial and chemical analyses in the same study in this way. Some previous studies have shown changes in bacterioplankton and picophytoplankton groups in response to dust additions within 48 h (Lekunberri et al., 2010; Paytan et al., 2009; Pulido-Villena et al., 2008), but not all (Bonnet et al., 2005). Increasing the incubation period further will result in the development of a community further removed from ambient. Instead, shorter incubations with readily available dust-derived nutrients were considered necessary to study the response of ambient bacterioplankton to dust addition, which requires a more sensitive method for measuring bacterioplankton response than changes in cell abundance.

4.3 Dust Leachate Addition Experiments

It was proposed that 'dust leachate' could be added to seawater samples to avoid long incubation periods required for the dissolution of dust-derived nutrients. Leaching nutrients from dust, then adding aliquots of this leachate to seawater, means that dust-derived nutrients are immediately available to the microbial community.

The first dust leachate addition incubations were done onboard RV *Atlantic Explorer* at the Bermuda Atlantic Timer Series (BATS) station (31°40' N, 64°10' W) during July 2007 (Cruise no. BATS 225). Leachate was produced from 'M4' dust, which is atmospherically processed dust collected on board RRS *Discovery* during a Saharan dust storm in the Gibraltar strait region (36°50'N; 09°05'W) on 9-10 April 1982 (Murphy, 1985). Dust leachate was produced by suspending dust in sterile filtered Milli-Q water for two weeks. An additional dust leachate was produced using dust from the same sample that had been combusted to remove organic material to test whether bacterioplankton respond to dust-derived organic (and inorganic) or inorganic nutrients.

During the BATS cruise the microbial response to leachate additions was assessed as the change in bacterial production, which increases before cell abundance increases. The rate of ^{3}H-leucine uptake was used as a proxy of production in these experiments. Radioactive isotopes are more sensitive tracers than stable isotopes so shorter incubation periods and smaller seawater volumes can be used. Furthermore, polycarbonate filters, which efficiently retain bacterioplankton cells, can be used for liquid scintillation counting.

4.3.1 Method

M4 dust was combusted (450°C, 4h) to remove organic residues. Prior to the cruise weighed aliquots of ~20 mg combusted (inorganic) or uncombusted (organic) dust were suspended in sterile-filtered Milli-Q water in HCl cleaned 35 mL PTFE bottles (400 mg L^{-1} final concentration). The dust bottles and a control bottle of sterile filtered Milli-Q water were placed on a stirring plate for two weeks. Bottles were taken to Bermuda and kept at 4°C for 48 h while the dust settled. The dust-free supernatant is termed 'leachate'. Surface seawater was collected using an acid cleaned polypropylene bucket. Four 1.5 mL seawater samples were used for each leachate addition and control incubation. 10 µL of leachate or Milli-Q control were added to each sample, which equated to a 2.65 mg L^{-1} final dust concentration. ^{3}H-leucine was added at 0.4 nM final concentration and

incubations were terminated at T = 5, 10, 20 and 30 minutes with 1% PFA. Fixed samples were filtered onto 0.2 µm pore size polycarbonate filters and rinsed three times with 4 mL Milli-Q water. Samples were placed in 10 mL scintillation vials with 4 mL scintillation cocktail (Goldstar, Meridian, UK). Activity was measured by liquid scintillation counting at the Bermuda Institute of Ocean Sciences (BIOS; Perkin Elmer, Tri Carb 2900TR) and is presented as disintegrations per minute (DPM).

Statistical analysis

The amount of radioactivity taken up by the community at each time point was converted to DPM mL^{-1} min^{-1} by subtracting the y-intercept, then dividing by the incubation period (5-30 min) and volume (1.5 mL). Thus four uptake rates were achieved for each treatment and control in all incubations. Uptake rates measured in dust addition incubations were compared to those from controls using the student's *t*-test for independent samples, following the F-test for equality of variance.

4.3.2 Results & Discussion

Some examples of results from the leachate addition incubations are presented in Figure 4.5. There was no clear stimulation of bacterioplankton metabolism following addition of either leachate (Table 4.4), nor was there a consistent difference between the two dust leachates used.

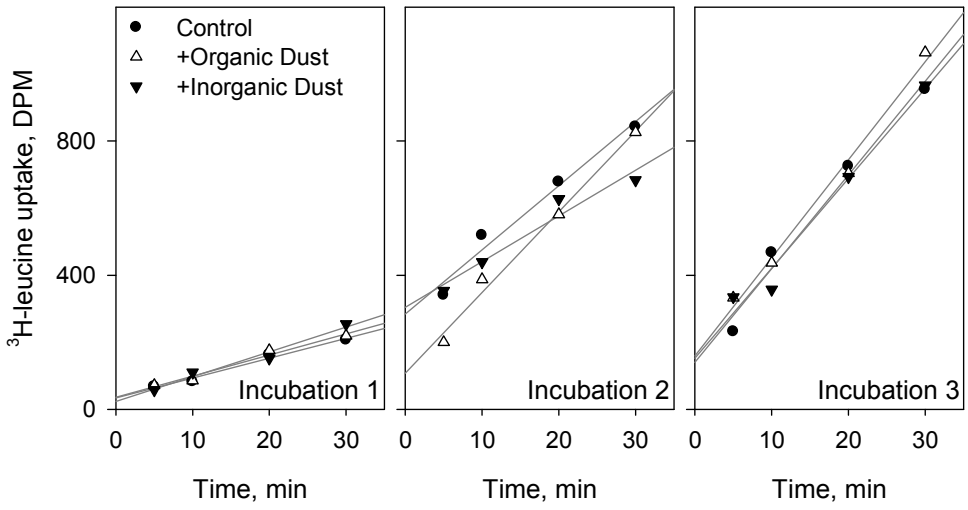


Figure 4.5: Examples of results from three dust leachate addition incubations conducted at the BATS station in July 2007. Leachate was produced from untreated (Organic) or combusted (Inorganic) dust. An addition of Milli-Q water was used as a control for dilution (Control).

Table 4.4: Uptake rates (DPM mL^{-1} min^{-1}) measured in the three dust leachate addition incubations shown in Figure 4.5 (mean ± standard error). Results of the student's *t*-test comparing uptake rates measured in +Dust treatments with controls. The F-test showed variance to be equal between all treatments and controls. df = 6 for all comparisons.

	Control	Organic Dust			Inorganic Dust		
Inc	Mean	Mean	*t*	p	Mean	*t*	p
1	3.9 ± 0.3	4.2 ± 0.3	0.6	0.6	4.9 ± 0.3	2.2	0.1
2	12.2 ± 1.7	15.7 ± 1.3	1.6	0.2	8.7 ± 0.9	1.8	0.1
3	17.9 ± 2.1	20.0 ± 1.1	0.9	0.4	18.5 ± 2.2	0.2	0.8

Considering immeasurable concentrations of nutrients at the surface are typical for the BATS region in summer (Lipschultz, 2001, Steinberg *et al*., 2001), and ambient leucine and methionine uptake rates were low for the northern Atlantic gyre (3.1 ± 1.9 and 4.0 ± 2.3 pmol L^{-1} h^{-1}, respectively; Chapter Two), dust-derived nutrients would be expected to stimulate bacterioplankton community production and, hence, leucine uptake. It is, therefore, considered unlikely that significant concentrations of nutrients were leached from the M4 dust.

4.4 Collection of fresh dust

Results from the above experiments, together with personal communication with Alex Baker (University of East Anglia) and Bill Landing (Florida State University) suggested that the Barbados and M4 dust may have become inert over years of storage, and therefore was not releasing the concentrations of nutrients they originally would. Furthermore, the 48 h incubations were influencing the microbial community, which will affect how the community responds to additions.

A method was proposed to collect dust from the atmosphere which could then be added directly to natural seawater samples. Ideally, dust would be collected during a dust deposition event and added to seawater samples collected from outside of the dust deposition region that has not already been in contact with dust and therefore most likely to show a response. This proved to be impractical, however. Instead, freshly collected dust was added to seawater from the same region, but collected from 20 m since water from this depth should have a similar microbial community composition to surface

seawater (Heywood *et al*., 2006) but would not be immediately affected by dust deposition *in situ*.

In Chapter Two the uptake rate of radioactively labelled amino acids was shown to be a sensitive method for detecting changes in nutrient availability, so this method was chosen for the detection of bacterioplankton response to dust addition. Due to the sensitivity of the measurement, short incubations and small seawater sample volumes could be used, which gave confidence that ample dust could be collected to simulate natural dust deposition events.

To minimise both loss of collected material and contamination, it was proposed to collect the dust and run seawater incubations within the same filter unit. Polypropylene Millipore Swinnex® filter holders (Figure 4.6) were chosen because the 25 mm units have an air space of ~1.5 mL in which seawater can be incubated. However, this approach involves incubating seawater samples on top of the filter onto which dust is collected, which proved problematic.

Figure 4.6: Swinnex® filter holder.

Polycarbonate filters were considered the most appropriate membrane due to their smooth surface, regular pore size, low extractabilities and low absorbance of radioactive tracer. Furthermore, polycarbonate filters have previously been used for the addition of atmospheric dust to seawater incubations (Paytan *et al*., 2009). However, the use of Cu^{2+} ions in pore production can make the filters toxic to some bacteria, so protocols for the acid cleaning of polycarbonate filters were tested.

Initially, cultured bacteria were used to test the experimental design then, whilst at sea, the opportunity was used to test the method further using natural surface seawater communities. While bacterial cultures are an important tool in preliminary experimental research, the ultimate aim was the study of natural communities.

4.4.1 Method

Bacterioplankton cultures

Methods were trialled in the laboratory using cultured *Vibrio* or *Prochlorococcus*. *Vibrio* cells were grown on Difco™ marine agar plates for up to seven days, until large colonies were visible. Cells were scraped off then suspended in 0.2 µm filtered seawater, collected previously from the Atlantic Ocean, and filtered (0.8 µm) to remove cell aggregates. *Vibrio* cells were enumerated by flow cytometry and diluted to a concentration of about 10^6 cells mL^{-1} using filtered seawater.

Prochlorococcus cultures were also used because they are one of the most dominant bacterioplankton groups in the study region and they can be sensitive to metals (e.g. copper, Mann *et al.*, 2002). *Prochlorococcus* were grown in PCR-S11 medium (Rippka *et al.*, 2000) by Isabelle Mary (currently at Blaise Pascal University, France). The *Prochlorococcus* cultures were not axenic.

General incubation procedure

Control incubations were done in sterile 2 mL screw cap polypropylene vials in parallel with test treatments, which were either done in 2 mL polypropylene vials, with the addition of a filter, or in Swinnex® filter holders with a filter.

Swinnex® filter holders were acid cleaned and autoclaved prior to use. Hydrochloric acid (10% HCl) and Trichloroacetic acid (5% TCA) were compared for acid cleaning and showed no difference in terms of *Vibrio* activity (paired sample *t*-test: t = 1.0, df = 3, p = 0.4; t = 1.2, df = 3, p = 0.2, for HCl and TCA, respectively). TCA was chosen because it can be fumed easily from the units whereas HCl, if not rinsed sufficiently, can prove detrimental to bacterioplankton cells.

Swinnex® filter holders were supported using luer lock stop cocks placed into hypodermic needles in silicone bungs. Stop cocks create a cushion of air to prevent seawater draining through the filter during incubation. Samples to be incubated in Swinnex® filter holders were prepared in 2 mL vials before their immediate transfer into the units. This ensured the tracer was well mixed with the seawater sample.

The radioactive amino acid tracer ^{3}H-leucine was added to 1-1.6 mL seawater samples at a standard concentrations in the range of 0.4 – 1.0 nM. Typically, three to six samples were

incubated for each control and treatment, and one or two samples were terminated by cell fixation every five, ten or fifteen minutes up to between 20 and 60 minutes.

Cell fixation prevents further tracer uptake and avoids cell breakage during filtration. Samples incubated within vials were typically fixed with 1% PFA but this could not be used within the reusable Swinnex® filter holders because PFA does not easily wash off. TCA was proposed as an alternative because it is commonly used when measuring ^{3}H-leucine uptake (e.g Kirchman *et al*., 1985) and it is easily evaporated, allowing complete removal. TCA at 1% final concentration was sufficient to fix cells.

Fixed cells from vial incubations were filtered through 0.2 µm polycarbonate filters, supported by 0.2 µm nitrocellulose filters. Samples incubated in Swinnex® filter holders were filtered through the 0.2 µm polycarbonate filters within the units. Filters were washed three times with Milli-Q water. Filtered TCA-fixed samples were kept at 60°C over night to fume off any traces of TCA, which might otherwise cause quenching.

Filtered samples were placed in scintillation vials with 3 or 4 mL scintillation cocktail. Radioactivity of samples was measured by liquid scintillation counting (NOCS: Wallac Quantalus 1220; BIOS: Perkin Elmer Tri-Carb 2900TR; RV *Hespérides*: Wallac WinSpectral 1414; RRS *Discovery*: Perkin Elmer Tri-Carb 3100) and reported as counts per minute (CPM) or DPM.

Cleaning polycarbonate filters

Polycarbonate filters were suspended in either 10% HCl or 5% TCA on a stirring plate for 24 h. Filters were rinsed four times, autoclaved, and suspended for 24 h in Milli-Q water. Filters were autoclaved again then dried at 60°C over night. The impact of different filter cleaning methods on bacterial metabolism was tested by incubating *Vibrio* or *Prochlorococcus* cells in the absence (control) or presence of untreated filters or filters cleaned with 10% HCl or 5% TCA.

Acid cleaned filters did not affect the activity of cultured *Vibrio* cells (Figure 4.7 & Table 4.5), but *Prochlorococcus* cells appeared to be inhibited, particularly by HCl-cleaned filters (Figure 4.8 & Table 4.5). However, the presence of an unclean filter did not affect *Prochlorococcus* activity, suggesting that the filters were not sufficiently rinsed of the acids, particularly HCl which would not have evaporated like TCA. Acid cleaning

polycarbonate filters was not beneficial, thus it was decided to accept the level of purity as they were supplied.

Table 4.5: Comparison of ^{3}H-leucine uptake between control incubations and incubations in the presence of untreated or acid cleaned filters. Results from the Student's *t*-test for paired (*Vibrio*) or independent (*Prochlorococcus*) samples.

Filter	*Vibrio*			*Prochlorococcus*		
treatment	*t*	df	p	*t*	df	p
Untreated	0.8	4	0.5	1.4	8	0.2
10% HCl	0.1	4	0.9	27	7	<0.0001*
5% TCA	0.9	4	0.4	5.5	8	0.001*

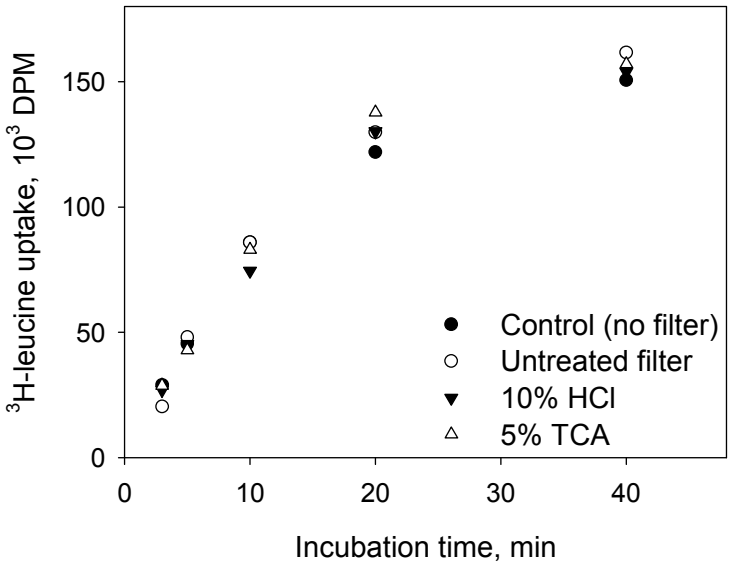


Figure 4.7: ^{3}H-leucine uptake by cultured *Vibrio* cells incubated in 2 mL polypropylene screw cap vials in the absence (control) or presence of an untreated or acid cleaned (10% HCl or 5% TCA) filter.

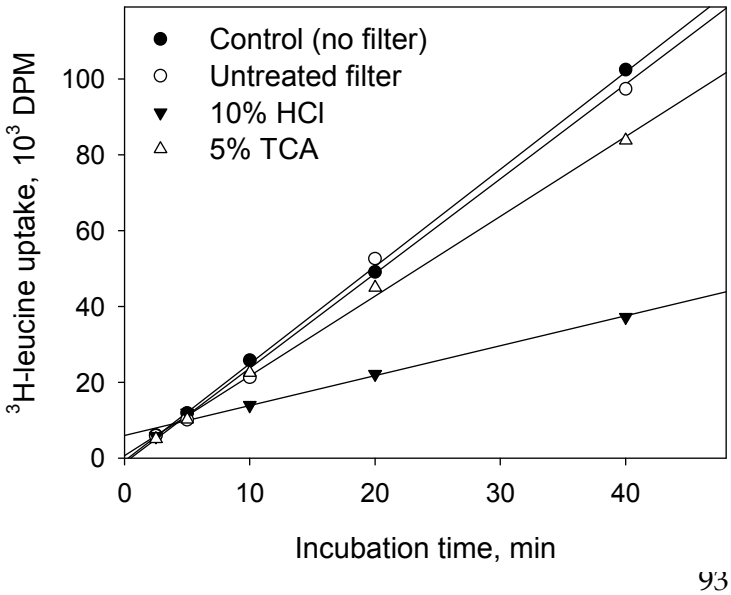


Figure 4.8: ^{3}H-leucine uptake by cultured *Prochlorococcus* spp. cells incubated in 2 mL polypropylene screw cap vials in the absence (control) or presence of an untreated or acid cleaned (10% HCl or 5% TCA) filter.

The suitability of Swinnex® filter holders as an incubation chamber was tested using cultured *Vibrio* cells. The uptake rate of ^{3}H-leucine by *Vibrio* cells incubated on polycarbonate filters in Swinnex® filter holders was similar to that measured in 2 mL polypropylene vials (Paired sample *t*-test: t = 0.5, df = 4, p = 0.7; Figure 4.9).

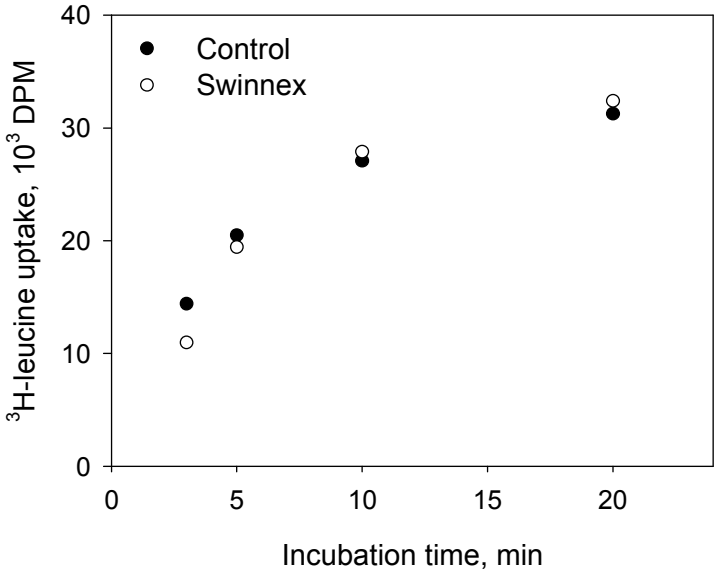


Figure 4.9: ^{3}H-leucine uptake by *Vibrio* cells incubated on polycarbonate filters in Swinnex® filter holders (Swinnex) or within polypropylene vials (Contol).

Dust collection apparatus

Replicate dust samples are needed for this time series incubation method, so a 24-port filtration unit (Figure 4.10) was employed to collect aerosol onto a number of filters simultaneously. Air was drawn through the filter units by means of a rotary vein vacuum pump. Due to the size of the Swinnex® filter holders, a maximum of twelve could fit on the filtration unit at once; the remaining ports were plugged.

Figure 4.10: The filtration unit used to collect replicate aerosol samples. Twelve Swinnex® filter holders are in place.

Urban aerosol collection

Urban aerosol was used to test the method prior to oceanic field studies. In October 2006 the filtration unit was trialled in Regent's Park, London, during an atmospheric chemistry study with Manuel Dall'Osto of the University of Birmingham (currently at IDEA-CSIS, Barcelona). Vacuum pressure across all ports was uniform (Manuel Dall'Osto, personal communication), confirming that the filtration setup will collect replicate aerosol samples.

Two sets of twelve aerosol samples were collected in Regent's Park. One set sampled for 3 h and the other 18 h. During the 3 h sampling period the rate of air flow remained at 3.6 L min^{-1}, filtering ~250 L of air. During the 18 h sampling period air flow decreased from 3.6 to 3.2 L min^{-1}, filtering an estimated 3670 L of air through each filter.

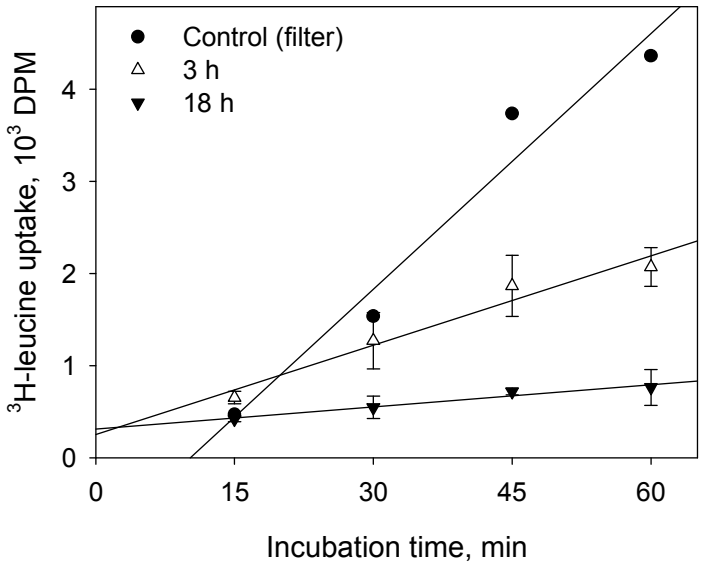


Figure 4.11: Impact of London aerosol on the uptake of ^{3}H-leucine by bacterioplankton community in aged seawater, collected from the north Atlantic the previous year. The control contained a polycarbonate filter without aerosol sample. Aerosol samples were collected for 3 h or 18 h. Uptake in the presence of aerosol is presented as mean ± standard error (n = 3).

Aerosol samples were removed from the Swinnex® filter holders and placed in sterile 2 mL screw cap polypropylene vials so that the holders could be re-used. The aerosol addition incubations were done in these vials, in the laboratory, using 'aged seawater' that had been collected previously from the Atlantic Ocean. Control incubations were in the presence of unused (without aerosol) filters, also in polypropylene vials. Incubations were run in triplicate, so three samples were fixed at each time point of 15, 30, 45 and 60 min. Incubations were terminated by fixation with 1% PFA.

The collected aerosol samples had a measureable impact on the seawater bacterioplankton community activity (Figure 4.11). Bacterioplankton exposed to London aerosol took up ^{3}H-leucine more slowly than control samples (t = 5, df = 3, p = 0.016, for both aerosol samples). Given the heavy metal component of urban aerosols, this negative impact on the bacterioplankton community metabolism is not surprising. This was nevertheless a useful exercise in terms of testing the aerosol sampling equipment and the use of radioactively labelled amino acids as a means of measuring the immediate metabolic response of marine bacterioplankton to aerosol additions.

Atmospheric dust collection on Cape Verde

An identical aerosol sampling system was deployed at the Cape Verde Atmospheric Observatory (16°41'46 N, 24°52'02 W; Figure 4.12) to collect dust characteristic of that being carried westwards from Africa on trade winds and deposited into the northeast Atlantic Ocean. Four sets of twelve replicate dust samples were collected in June 2007. Varied sampling periods resulted in 170 (sample one), 660 (sample two), 1320 (sample three) or 2650 (sample four) L of air being sampled.

The atmospheric dust loading at the time of each sampling period is not known; however, assuming it remained fairly uniform throughout the two day sampling period, dust loading on the filters would have increased with sampling duration. Swinnex® filter holders containing dust samples were sealed using Luer-Lok® components until used the following month.

Figure 4.12: The Cape Verde Atmospheric Observatory. Dust samples were collected from the top of the 30 m tower. Photograph provided by the University of York.

The impact of 'Cape Verde dust' on natural bacterioplankton of the Sargasso Sea was tested during a study at the BATS station in July 2007. Surface seawater samples were collected in an acid cleaned bucket and incubated with dust samples within the Swinnex® filter holders. Incubations were run in triplicate so three samples were terminated at each of four time points (5, 10, 20, 30 min). Controls were done in Swinnex® filter holders containing polycarbonate filters that had not been sampling dust; these were not replicated. Dust samples one & four and two & three were incubated with the same seawater sample and so shared controls.

The addition of Cape Verde dust generally reduced the uptake of ^{3}H-leucine by bacterioplankton of the Sargasso Sea (Figure 4.13). There was little effect with the addition of dust sample number one, which was presumably the smallest of the dust additions, but dust samples collected over longer sampling periods had a negative impact on bacterioplankton metabolism. The 40% reduction in ^{3}H-leucine uptake with the addition of dust sample number four was statistically significant (t = 3, df = 14, $p<0.01$). These results suggest it is necessary to sample about 2000-3000 L of air, outside of major dust storms, to collect sufficient dust to elicit a response in bacterioplankton metabolism.

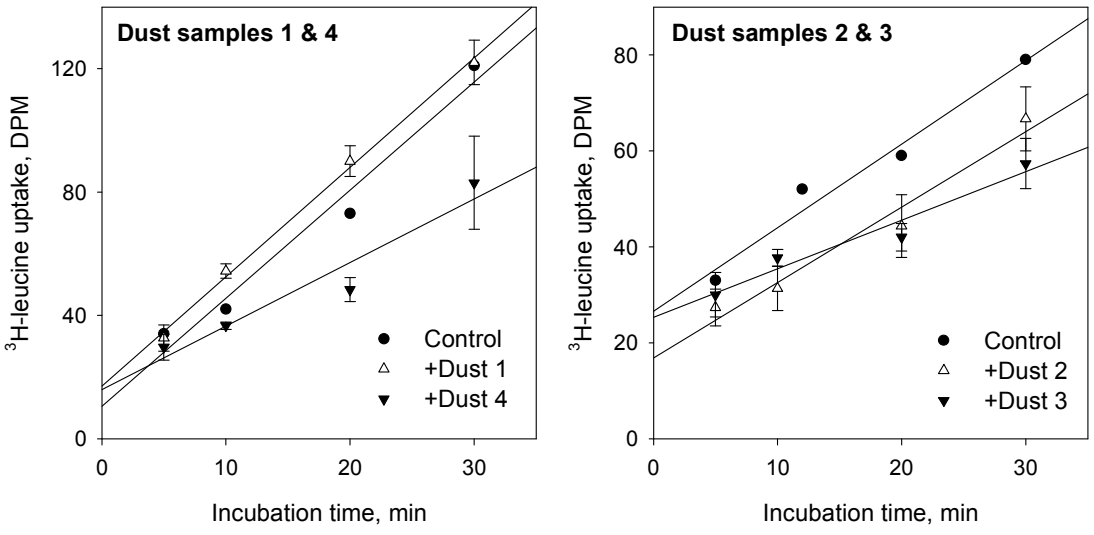
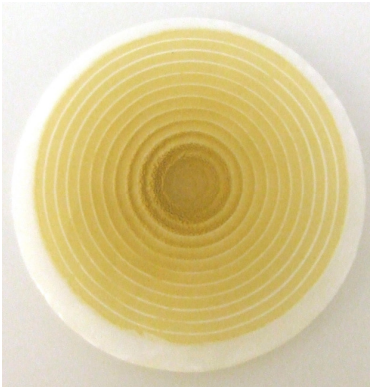


Figure 4.13: Impact of Cape Verde dust on bacterioplankton ^{3}H-leucine uptake at the Bermuda Atlantic Time-series station. 170, 660, 1320 and 2650 L of air was sampled for dust samples one, two, three and four, respectively. For the +Dust treatment, uptake is given as mean ± standard error (n = 3).

Atmospheric dust collection at sea

The aerosol sampling system was deployed on the monkey island of RV *Hespérides* during November and December 2007 and RRS *Discovery* during January and February 2008 (Figure 4.14). Atmospheric dust was collected throughout each cruise; the pump was switched off whilst the ships were stationary or travelling with the wind, to avoid contamination from the ships' stacks.

Figure 4.14: Example of a Saharan dust sample collected during D326.



Seawater was collected in 20 L Niskin bottles mounted on a stainless steel (RV *Hespérides*, 5 m) or titanium (RRS *Discovery*, 20 m) frame with a CTD profiler. Seawater samples were incubated in Swinnex® filter holders with freshly collected dust. Parallel incubations in Swinnex® filter holders without dust samples acted as controls. Incubations within 2 mL polypropylene vials were used as controls for the effect of the Swinnex® filter holders.

Dust addition had a negative impact on bacterioplankton ^{3}H-leucine uptake in incubations conducted on RV *Hespérides* ($t = 12$, $df = 14$, $p<0.0001$; Figure 4.15) and RRS *Discovery* ($t = 4$, $df = 13$, $p = 0.002$; Figure 4.16) compared to controls. However, the Swinnex® filter holders also impaired metabolism in these incubations ($t = 9$, $df = 6$, $p = 0.0001$, and $t = 36$, $df = 10$, $p<0.0001$, respectively).

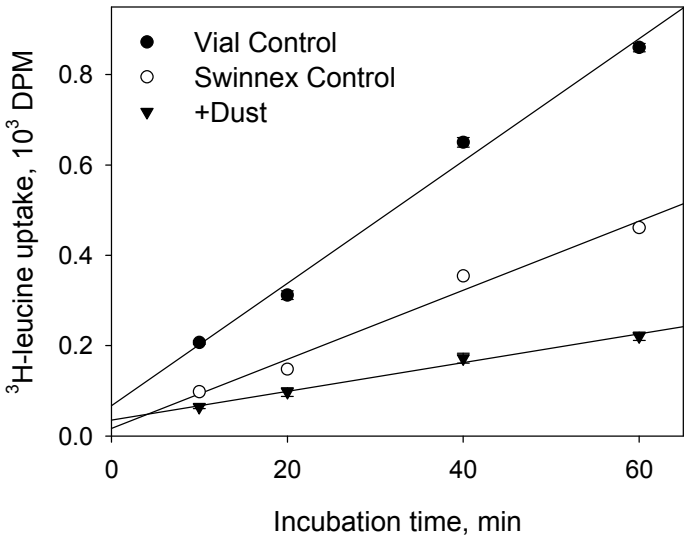


Figure 4.15: Impact of dust on the rate of ^{3}H-leucine uptake by the bacterioplankton community. Dust and seawater collected onboard RV *Hespérides*. Filters collected dust from 8000 L of air. Surface (5 m) seawater was collected from 25°59′51 N; 25°44′04 W (Figure 4.1). The rate of tracer uptake was compared between seawater samples in 2 mL polypropylene vials (Vial Control), in Swinnex® filter holders containing unused polycarbonate filters (Swinnex Control) and in Swinnex® filter holders used to sample dust onto polycarbonate filters (+Dust). For the +Dust treatment, uptake is given as mean ± standard error (n = 3).

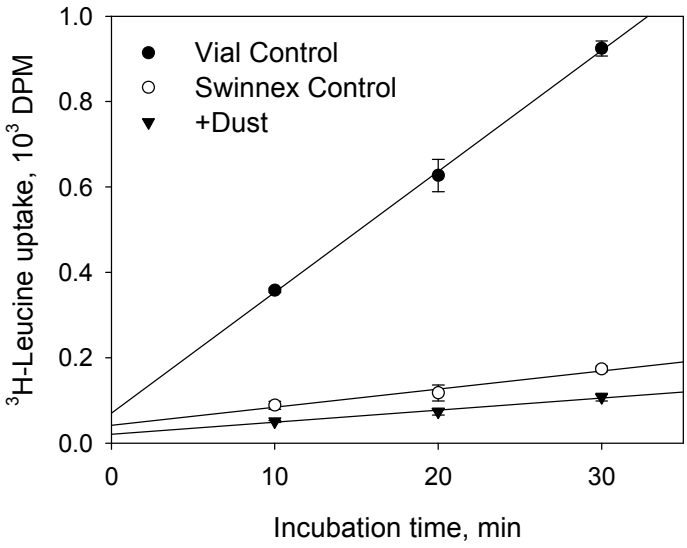


Figure 4.16: Impact of dust on the rate of ^{3}H-leucine uptake by the natural seawater bacterioplankton community. Dust and seawater collected onboard RRS *Discovery*. Filters sampled 16000 L air collecting an estimated 2.7 µg dust (incubation concentration 2.7 mg L^{-1}). Seawater was collected from 20 m depth at 20°55′49 N; 26°07′13 W (Figure 4.1). The rate of tracer uptake was compared between samples in 2 mL polypropylene vials (Vial Control), Swinnex® filter holders containing clean polycarbonate filters (Swinnex Control) and Swinnex® filter holders used to collect dust onto polycarbonate filters (+Dust). Uptake is given as mean ± standard error (n = 3).

An alternative approach was tested onboard RV *Hespérides* to avoid the use of Swinnex® filter holders. The method was the same but used Whatman 20 mL polypropylene filter funnels (Figure 4.17) to collect and incubate dust samples. The same polycarbonate filters were used in these filter funnels, but they were supported by 0.45 µm nitrocellulose filters. Incubations done within these units were terminated by fixation with 1% TCA so that they could be re-used.

Figure 4.17: Whatman 20 mL polypropylene filter funnels were tested for their suitability as dust collection and incubation vessels. Image supplied with permission of Whatman International – part of GE Healthcare.

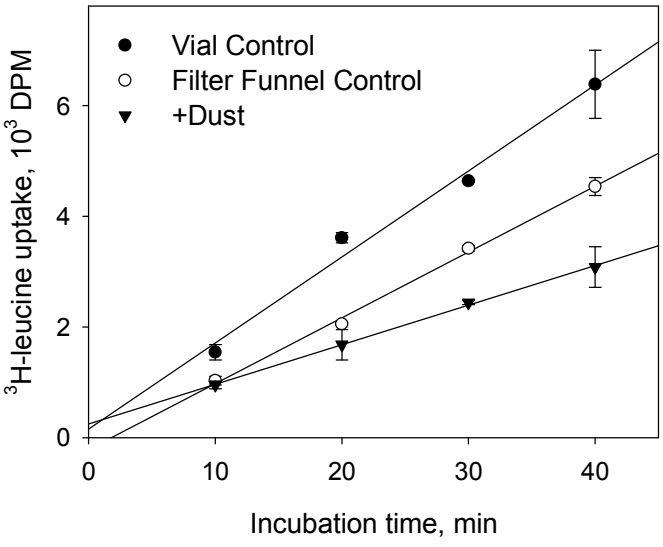


Figure 4.18: Testing the feasibility of filter units for dust collection and incubations onboard RV *Hespérides*. The rate of ^{3}H-leucine uptake was compared between seawater samples incubated in 2 mL polypropylene vials (Vial Control), in filter funnels containing clean polycarbonate filters (Filter Funnel Control) and in filter funnels used to sample dust onto polycarbonate filters (+Dust). Surface (5 m) seawater was collected from 26°00′01 N; 38°15′54 W (Figure 4.1). Filters collected dust from 5700 L air. Uptake is presented as mean ± standard difference (n = 2).

Dust addition impaired the metabolism of bacterioplankton incubated in filter funnels compared to controls ($t = 8$, $df = 15$, $p<0.0001$; Figure 4.18); however, the filter funnels also reduced ^{3}H-leucine uptake ($t = 5$, $df = 7$, $p = 0.002$).

Although lab trials had shown that polycarbonate filters did not negatively affect cultured *Vibrio* or *Prochlorococcus*, natural samples were proving more sensitive. To determine whether it was the polycarbonate filter or the Swinnex® filter holder that was impairing bacterioplankton metabolism, a further trial of polycarbonate filters was done in 2 mL polypropylene vials using natural seawater samples collected onboard RRS *Discovery*. These results suggest that, although atmospheric dust addition is detrimental to bacterioplankton metabolism ($t = 7.6$, $df = 13$, $p <0.0001$), some of this is due to inhibition by the polycarbonate filter ($t = 4.8$, $df = 8$, $p = 0.001$; Figure 4.19). At this stage it became apparent that the wetting agent, polyvinylpyrrolidone (PVP), which makes naturally hydrophobic polycarbonate hydrophilic, may prove problematic when working with live oceanic cells. As a polymer PVP is biologically inert, however, monomers cleaved from the compound could prove toxic. Aluminium oxide and PTFE filters were tested as alternatives but these also affected bacterioplankton metabolism. However, polypropylene filters showed promise ($t = 0.2$, $df = 4$, $p = 0.9$; Figure 4.20).

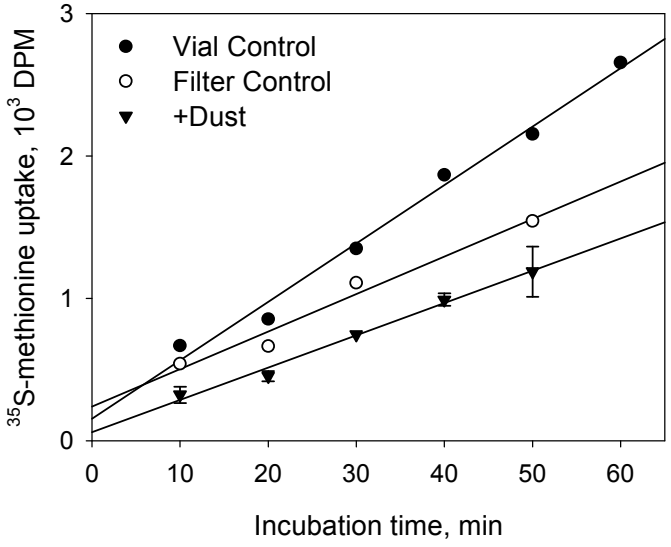


Figure 4.19: Testing the suitability of polycarbonate filters for the collection and incubation of dust samples onboard RRS *Discovery*. 5100 L of air was sampled collecting an estimated 10 µg dust, resulting in a final concentration of 6.2 mg L^{-1} in 1.6 mL incubations. Seawater was collected from a depth of 20 m at 12°33'57 N; 32°40'35 W (Figure 4.1). All incubations were done in 2 mL polypropylene vials. The rate of ^{3}H-leucine uptake was compared between seawater samples with no filter (Vial Control), with an unused polycarbonate filter (Filter Control) or with a dust sampled polycarbonate filter (+Dust). Samples were filtered through different filters to those present during incubation. For the +Dust treatment, mean ± standard difference is presented (n = 2).

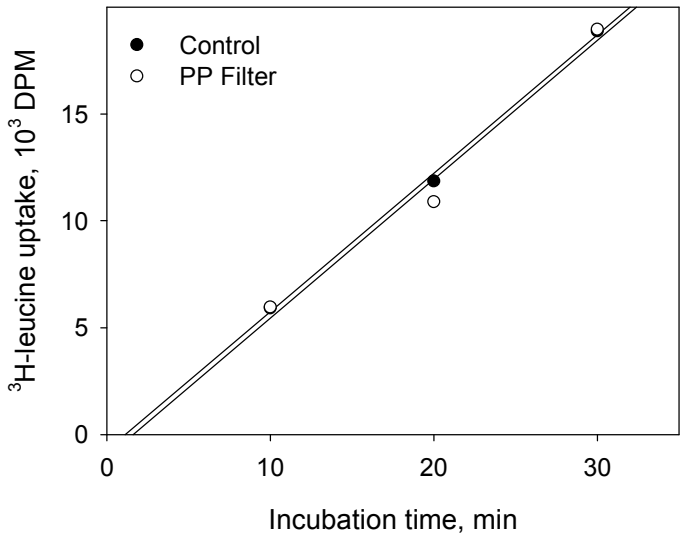


Figure 4.20: Testing the impact of polypropylene (PP) filters on ^{3}H-leucine uptake of bacterioplankton incubated in 2 mL PP vials. Surface (5 m) seawater was collected from 20°51′53 N; 17°37′56 W (Figure 4.1) onboard RRS *Discovery*.

The effect of polypropylene filters on bacterioplankton activity was tested further onboard *RV* Discovery cruise D326 (Table 4.1). At the time, only 47 mm polypropylene filters were available so incubations were done in 35 mL PTFE bottles with 34 mL seawater samples. A control bottle incubated in parallel had no filter but otherwise was treated in the same way. Bottles were placed in on-deck incubators screened to permit 20% surface irradiance and cooled to *in situ* temperature. An 18 h incubation period allowed the bacterioplankton community time to respond to the filter.

Incubating larger volumes of seawater meant additional analyses were possible. To ascertain which, if any, bacterioplankton group was affected by the presence of the polypropylene filter, individual groups were sorted by flow cytometry. Subsamples were removed from the incubation bottles following 6 h of incubation and incubated for 2 h with 0.2 nM ^{35}S-methionine before fixation by 1% PFA. ^{35}S-methionine was used rather than ^{3}H-leucine because a tracer with high specific activity is necessary to detect cellular uptake.

Aliquots of 500 µL were removed throughout the incubation period (t = 2, 4, 6, 8, 18 h) and the rate of 50 pM ^{35}S-methionine uptake compared between treatment (+PP filter) and control (e.g. Figure 4.21). The polypropylene filter appeared to have no impact on bacterioplankton community metabolism after 2 h ($t = 0.5$, $df = 4$, $p = 0.6$) or 18 h ($t = 2.6$, $df = 4$, $p = 0.06$).

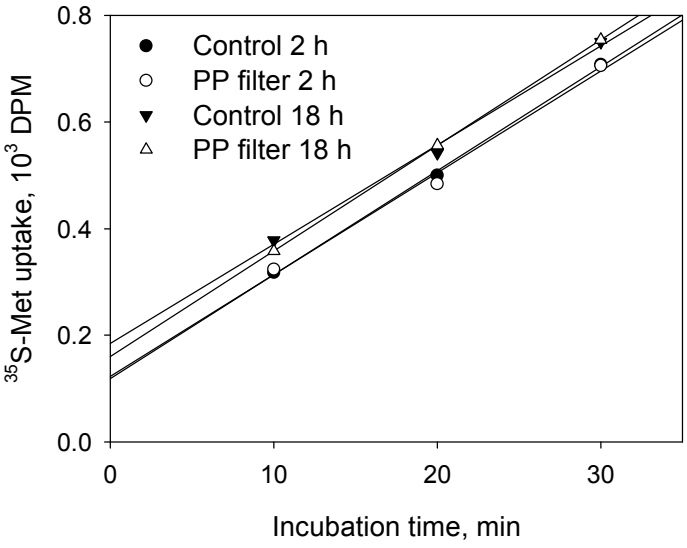


Figure 4.21: The uptake of ^{35}S-methionine uptake by bacterioplankton community after 2 h and 18 h of incubation in the absence (Control) and presence of a polypropylene filter (PP filter). The seawater sample was taken from 26°21′05 N; 23°25′29 W (Figure 4.1).

To test the suitability of polypropylene filters further, the response of specific bacterioplankton groups was examined. *Synechococcus* and *Prochlorococcus* cells were sorted from unstained samples based on their characteristic autofluorescence (Olson *et al.*, 1993). Low nucleic acid (LNA) containing bacterioplankton were sorted from samples stained with the nucleic acid dye SYBR Green I (Marie *et al.*, 1997) using a plot of side scatter against green fluorescence. Uptake by average bacterioplankton cells was determined by sorting random cells from the whole group of stained bacterioplankton cells.

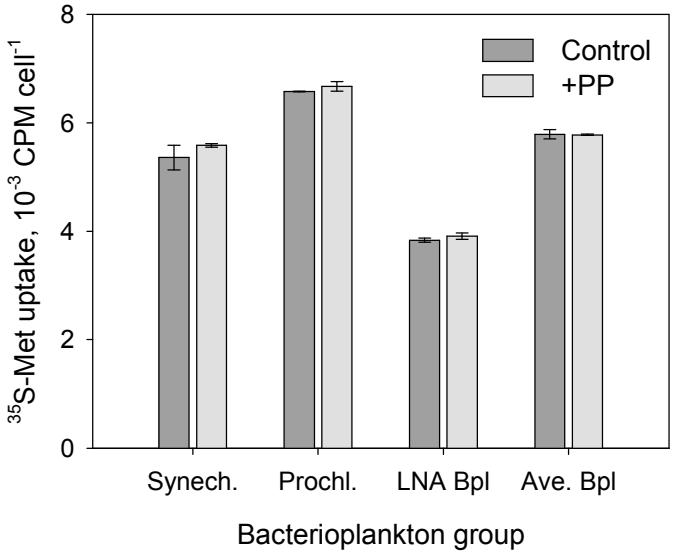


Figure 4.22: Cellular uptake of ^{35}S-methionine by major bacterioplankton groups that were sorted by flow cytometry: *Synechococcus* (Synech.), *Prochlorococcus* (Prochl.), average bacterioplankton (Ave. Bpl, whole bacterioplankton group sorted non-specifically) and low nucleic acid containing bacterioplankton (LNA Bpl). Seawater was collected from 26°21′05 N; 23°25′29 W (Figure 4.1). Cellular uptake is reported as mean ± standard error for the activity retained by each cell (n = 3).

Of the bacterioplankton groups studied, none showed evidence of a response to the filter (Figure 4.22 & Table 4.6). Polypropylene is hydrophobic so bacterioplankton do not come into direct contact with the filter, but the dust becomes dislodged and suspended in the seawater. Thus polypropylene filters could be recommended for dust collection and addition to bacterioplankton samples.

Table 4.6: Results of the Student's *t*-test for independent samples comparing cellular ^{35}S-methionine uptake, in presence and absence of a PP filter, by individual bacterioplankton groups. Df = 4 for all comparisons.

Bacterioplankton group	*t*	p
Synechococcus	1.0	0.4
Prochlorococcus	1.1	0.3
LNA containing	1.0	0.4
Average bacterioplankton	0.1	0.9

Dust leachate addition incubations

Dust addition proved detrimental to bacterioplankton metabolism. Further experiments were done using dust leachate produced from freshly collected dust. The aim was to make additions of dust-derived nutrients at known concentrations. To ensure nutrients were leached from dust samples, a method described by Buck *et al*. (2006) was followed, which has been shown to dissolve 6 ± 5% and 9 ± 8% of aerosol iron in seawater and ultrapure water leaches, respectively.

Matt Patey (NOCS) collected dust samples onto 0.4 µm pore size, 47 mm polypropylene filters whilst at sea then filtered 100 mL of either 0.2 µm filtered seawater or Milli-Q water (during the TRYNITROP and D326 cruises, respectively) through the filter, under vacuum, in approximately 10 seconds. By measuring the inorganic nutrient concentration in the leachate it was possible to control the concentration of those nutrients added; however, this was only possible during D326. Filtered seawater was used initially to avoid osmotic shock of cells by fresh water additions. However, it was later considered that by using seawater the addition of nutrients may not be measureable over the ambient nutrient concentrations.

Leachate additions of 10-32 µL were made to 1.6 mL seawater samples collected from 5 m (TRYNITROP) or 20 m (D326). During D326 the additions equated to about a ten-fold increase in ambient N and P concentrations, however, it was not possible to measure

nutrient concentration in the leachates produced during TRYNITROP. The bacterioplankton metabolic response to leachate addition was measured as a change in the rate of radioactively labelled amino acid (^{3}H-leucine or ^{35}S-methionine) uptake compared to controls, during 30 minute incubations.

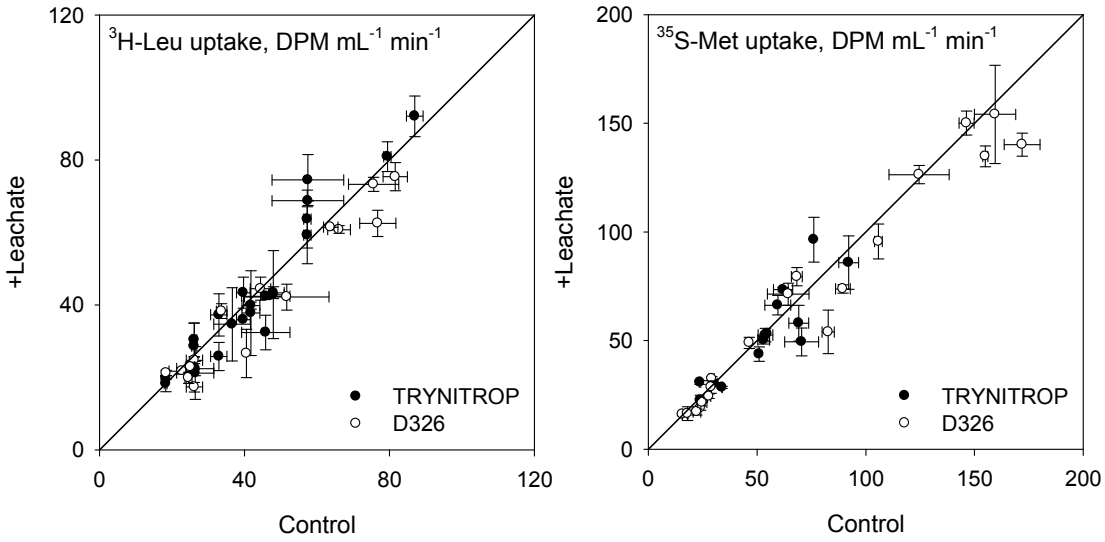


Figure 4.23: Results of leachate addition incubations during two cruises. The rate of ^{3}H-leucine or ^{35}S-methionine uptake in controls and in the presence of leachate are plotted with the standard error of the measurement (n = 3). During the TRYNITROP cruise dust leachate was produced using 0.2 µm filtered seawater, whereas during D326 Milli-Q water was used. Leachate was produced from freshly collected dust. A unity line, where the rate of tracer uptake in controls equals that in the treatment, is shown.

There was no obvious impact of leachate addition on the uptake rate of ^{3}H-leucine (paired *t*-test: $t = 1.6$, df = 37, p = 0.13) or ^{35}S-methionine (paired *t*-test: $t = 1.8$, df = 32, p = 0.08) within the 30 minute incubations (Figure 4.23). This gave rise to two theories: 30 minutes may not be long enough for bacterioplankton to respond to the dust-derived nutrients; there may be some groups that are benefiting but their response is masked when studying the community as a whole.

4.5 Conclusions

Studying the impacts of atmospheric dust deposition on oceanic microbial communities was not as straightforward as initially anticipated. Sifted Saharan soils are not entirely

representative of dust that is deposited onto the ocean, but it is difficult to collect substantial quantities of dust from the atmosphere. The atmospherically processed dust that was available in sufficient quantities did not affect the microbial community, in part because rinsing dust off collection nets removed a proportion of soluble nutrients, but perhaps mostly due to its years of storage.

Recently collected dust is necessary to elicit a response from the bacterioplankton community, but it is difficult to quantitatively add dust that is collected at sea. An aerosol sampling system was developed that was able to collect up to twelve replicate dust samples simultaneously, enabling time series dust addition incubations, but dust needed to be added with the filter onto which it was collected. Swinnex® filter holders were shown to be unsuitable as incubation chambers, but were nevertheless used for collecting dust.

Polycarbonate filters could not be used for the delivery of dust to incubation vessels. Polycarbonate filters contain copper and also a wetting agent, polyvinylpyrrolidone (PVP), which may harm some bacterioplankton. Although acid cleaning is an option, this is difficult to standardise, and remaining acid residues may compromise bacterioplankton cell integrity.

Polypropylene filters did not affect the metabolism of natural open ocean bacterioplankton communities, including *Prochlorococcus* cyanobacteria and LNA containing bacterioplankton, the majority of which belong to the *Alphaproteobacteria* clade SAR11 (Chapter Five), which are the dominant groups in the study region. Polypropylene vials are routinely used so the material is known to be suitable for working with marine bacterioplankton. Thus polypropylene filters were ultimately chosen for dust collection and addition experiments (Chapter Five).

Forty-eight hours was too long an incubation period for studying the ambient community response, however, 30 minutes may have been too short to measure a response to leachate addition. It was therefore subsequently decided to follow the metabolic response of the bacterioplankton community at 2-hourly intervals during 24 h incubations. The use of 34 mL seawater samples, from which subsamples can be removed, also allows the study of individual bacterioplankton group responses to dust and leachate additions by flow cytometric sorting.

4.6 References

Baker AR, Jickells TD, Biswas KF, Weston K & French M (2006) Nutrients in atmospheric aerosol particles along the Atlantic Meridional Transect. *Deep Sea Research II* **53**: 1706-1719.

Bonnet S, Guieu C, Chiaverinin J, Ras J & Stock A (2005) Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system. *Limnology and Oceanography* **50**: 1810-1819.

Buck CS, Landing WM, Resing JA & Lebon GT (2006) Aerosol iron and aluminium solubility in the northwest Pacific Ocean: Results from the 2002 IOC cruise. *Geochemistry, Geophysics, Geosystems* **7**: Q04M07, doi:10.1029/2005GC000977.

Davey M, Tarran GA, Mills MM, Ridame C, Geider RJ & LaRoche J (2008) Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. *Limnology and Oceanography* **53**: 1722-1733.

Duarte CM, Dachs J, Llabrés M, Alonso-Laita P, Gasol JM, Tovar-Sánchez A, Sañudo-Wilhelmy S & Agustí S (2006) Aerosol inputs enhance new production in the subtropical northeast Atlantic. *Journal of Geophysical Research* **111**: G4, doi:10.1029/2005JG000140.

Guieu C, Loe-Pilot M-D, Ridame C & Thomas C (2002) Chemical characterisation of the Saharan dust end-member: Some biogeochemical implications for the western Mediterranean Sea. *Journal of Geophysical Research* **107**: D15, doi:10.1029/2001JD000582.

Herut B, Zohary T, Krom MD, Mantoura RFC, Pitta P, Psarra S, Rassoulzadegan F, Tanaka T & Thingstad TF (2005) Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Sea Research II* **52**: 3024-3040.

Heywood JL, Zubkov MV, Tarran GA, Fuchs BM & Holligan PM (2006) Prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean: Evaluation of inter-annual variability. *Deep Sea Research II* **53**: 1530-1547.

Jickells TD, An ZS, Andersen KK, Baker AR, Bergametti G, Brooks N, Cao JJ, Boyd PW, Duce RA, Hunter KA, Kawahata H, Kubilay N, laRoche J, Liss PS, Mahowald N, Prospero JM, Ridgwell AJ, Tegen I & Torres R (2005) Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* **308**: 67-71.

Kirchman D, K'nees E & Hodson R (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Applied and Environmental Microbiology* **49**: 599-607.

Lekunberri I, Lefort T, Romero E, Vázquez-Domínguez E, Romera-Castillo C, Marrasé C, Peters F, Weinbauer M & Gasol JM (2010) Effects of a dust deposition event on coastal marine microbial abundance and activity, bacterial community structure and ecosystem function. *Journal of Plankton Research* **32**: 381-396.

Lipschultz (2001) A time-series assessment of the nitrogen cycle at BATS. *Deep Sea Research II* **48**: 1897-1924.

Mann EL, Ahlgren N, Moffett JW & Chrisholm SW (2002) Copper toxicity and cyanobacteria ecology in the Sargasso Sea. *Limnology and Oceanography* **47**: 976-988.

Marie D, Partensky F, Jacquet S & Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology* **63**: 186-193.

Mills MM, Ridame C, Davey M, La Roche J & Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292-294.

Moore CM, Mills MM, Milne A, Langlois R, Achterberg EP, Lochte K, Geider RJ & La Roche J (2006) Iron limits primary productivity during spring bloom development in the central North Atlantic. *Global Change Biology* **12**: 626-634.

Murphy KJT (1985) The trace metal chemistry of the Atlantic aerosol. Thesis, Liverpool, UK, University of Liverpool.

Olson RJ, Zettler ER & Durand MD (1993) Phytoplankton analysis using flow cytometry. *Handbook of methods in aquatic microbial ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds.), pp. 175-186. Lewis Publishers, Boca Raton, Florida.

Patterson CC & Settle DM (1987) Review of data on eolian fluxes of industrial and natural lead to the land and seas in remote regions on a global scale. *Marine Chemistry* **22**: 137-162.

Paytan A, Mackey KRM, Chen Y, Lima ID, Doney SC, Mahowald N, Labiosa R & Post AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences* **106**: 4601-4605.

Pulido-Villena E, Wagener T & Guieu C (2008) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* **22**: GB1020, doi:10.1029/2007GB003091.

Reche I, Ortega-Retuerta E, Romera O, Pulido-Villena E, Morales-Baquero R & Casamayor EO (2009) Effect of Saharan dust inputs on bacterial activity and community

composition in Mediterranean lakes and reservoirs. *Limnology and Oceanography* **54**: 869-879.

Rippka R, Coursin T, Hess W, Lichtle C, Scanlan DJ, Palinska KA, Iteman I, Partensky F, Houmard J & Herdman M (2000) *Prochlorococcus marinus* Chisholm *et al*., 1992 subsp. *pastoris* subsp. nov. strain PCC 9511, the first axenic chlorophyll a_2/b_2-containing cyanobacterium (*Oxyphotobacteria*). *International Journal of Systematic and Evolutionary Microbiology* **50**: 1833-1847.

Spokes LJ, Jickells TD & Lim B (1994) Solubilisation of aerosol trace metals by cloud processing: a laboratory study. *Geochimica et Cosmochimica Acta* **58**: 3281-3287.

Steinberg DK, Carlson CA, Bates NR, Johnson RJ, Michaels AF & Knap AH (2001) Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. *Deep Sea Research II* **48**: 1405-1447.

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll-a in the presence of chlorophyll-b and pheopigments. *Limnology and Oceanography* **39**: 1985-1992.

Zubkov MV & Burkill PH (2006) Syringe pumped high speed flow cytometry of oceanic phytoplankton. *Cytometry Part A* **69A**: 1010-1019.

Chapter Five

Differential responses of *Prochlorococcus* and SAR11-dominated bacterioplankton groups to atmospheric dust inputs in the tropical Northeast Atlantic Ocean

Hill, PG, Zubkov, MV & Purdie, DA (2010) *FEMS Microbiology Letters* **306:** 82-89 (Appendix IV)

5.1 Introduction

Desert dust consists of soil particles that are lifted into the atmosphere when high winds occur over dry and sparsely vegetated land (Mahowald *et al.*, 2005). With dust production estimated at about 1700 Tg year^{-1} (Jickells *et al.*, 2005) and potentially increasing desertification (Rosenfeld *et al.*, 2001), the effect of dust deposition on indigenous microbial communities of the surface ocean can be significant. Desert dust, and its associated nutrients, can play a key role in regulating primary production (Guieu *et al.*, 2002, Bonnet *et al.*, 2005, Herut *et al.*, 2005, Moore *et al.*, 2006) and bacterial production (Herut *et al.*, 2005, Pulido-Villena *et al.*, 2008b) in the open ocean, as well as bacterioplankton and phytoplankton dynamics in lakes and reservoirs (Pulido-Villena *et al.*, 2008a, Reche *et al.*, 2009).

Generally, studies have shown atmospheric dust deposition to be beneficial to bacterioplankton communities. Saharan dust addition incubations have indicated stimulation of bacterial production in a Spanish reservoir (Reche *et al.*, 2009) and the Mediterranean basin (Herut *et al.*, 2005, Lekunberri *et al.*, 2010), nitrogen fixation in the tropical north Atlantic (Mills *et al.*, 2004) and bacterial abundance in a high mountain lake (Pulido-Villena *et al.*, 2008a) and the western Mediterranean Sea (Pulido-Villena *et al.*, 2008b, Lekunberri *et al.*, 2010). However, the bacterial communities of the north-western Mediterranean Sea (Bonnet *et al.*, 2005) and subtropical northeast Atlantic (Duarte *et al.*, 2006) showed little or no response to dust addition. Observations of dust deposition *in situ* have also indicated a positive response of bacterial abundance in a Mediterranean lake (Pulido-Villena *et al.*, 2008a) and in the western Mediterranean Sea (Pulido-Villena *et al.*, 2008b, Bonilla-Findji *et al.*, 2010), and bacterial activity in the Mediterranean Sea (Herut *et al.*, 2005, Bonilla-Findji *et al.*, 2010).

More specifically, *Synechococcus* abundance increased and *Prochlorococcus* abundance decreased in response to dust addition in the eastern Mediterranean basin (Herut *et al.*, 2005), whereas the opposite was observed in the Gulf of Aqaba in the northern Red Sea (Paytan *et al.*, 2009). The *Synechococcus* population also soon disappeared following dust addition in the north-western Mediterranean (Lekunberri *et al.*, 2010).

There is a need to assess the response of individual populations of the bacterioplankton community to dust deposition. The aim of this study, therefore, was to assess metabolic responses of key groups of oceanic bacterioplankton to dust deposition. The study focussed on two bacterioplankton groups: the *Prochlorococcus* cyanobacteria and SAR11 clade of *Alphaproteobacteria*, because in the (sub-)tropical open ocean, the bacterioplankton community is often dominated by *Prochlorococcus* (Chisholm *et al.*, 1988), and the globally ubiquitous and abundant SAR11 (Morris *et al.*, 2002).

The metabolic response of these bacteria was studied because microbial metabolism, or production, is more sensitive to environmental change than abundance (Gasol & Duarte, 2000). The (sub-)tropical north-eastern Atlantic region was chosen because this region is regularly exposed to high Saharan dust inputs, 5-20 g m^{-2} of dust per year (Jickells *et al.*, 2005), yet few studies on the subject have been conducted there (Mills *et al.*, 2004, Duarte *et al.*, 2006). Dust addition incubations were used to exclude factors associated with dust events, such as high wind speeds and surface cooling, which may lead to favourable conditions for cell growth (McGillicuddy & Robinson, 1997, Singh *et al.*, 2008, Chapter Three). Additions of freshly collected dust or dust 'leachate' (Buck *et al.*, 2006) were made in parallel to natural seawater samples.

5.2 Materials and methods

Sampling region and sample collection

The experimental work was done during the RRS *Discovery* cruise D326 in the eastern (sub-)tropical North Atlantic Ocean (Figure 5.1) during January and February 2008. During 25-28 January a major dust deposition event occurred, whilst the ship was in the southwest of the study region, making the sky brown and covering the ship in a layer of red-brown dust. The dust cloud was clearly visible in satellite images (Chapter Three, Figure 3.2) and back trajectories for these dates show that the air mass came from the Sahara region (Appendix III).

Seawater samples were collected using a trace metal clean technique from 20 m depth, to minimise iron contamination from the ship's hull, using a rosette of 20 L Niskin bottles mounted on a titanium frame with a CTD profiler (Sea-Bird Electronics) in polyoxymethylene plastic and titanium casing. Samples were decanted into 1 L HCl-cleaned polycarbonate bottles. The experiments commenced within an hour of sampling.

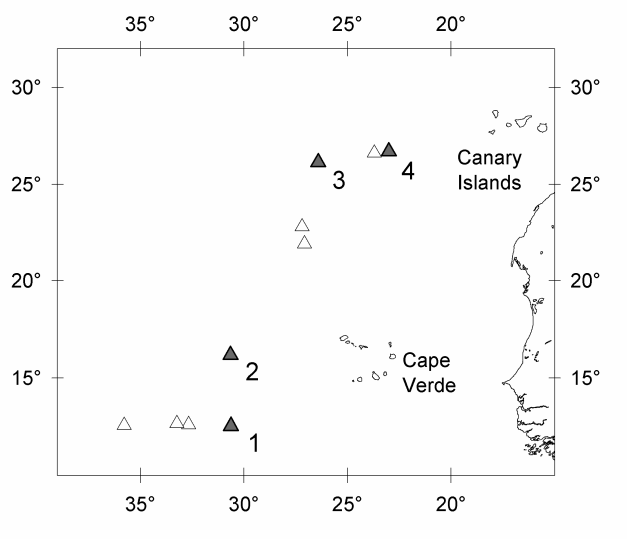


Figure 5.1: Sample collection locations (all triangles) for cellular ^{35}S-Met uptake by *Prochlorococcus* and LNA bacterioplankton cells, and FISH analysis of bacterioplankton community structure. Black triangles also indicate where seawater for dust and leachate addition incubations was collected, with indication of incubation number.

Dust collection and leachate production

Dust samples were collected daily, at sea, onto polypropylene filters (47 mm, 0.45 µm Sterlitech, USA). Rotary vein vacuum pumps filtered aerosol at 25-30 L min^{-1} for periods of typically 24 h although this was reduced to 4-6 h during the major dust event on 25-28 January. The instantaneous dissolution of metals and nutrients was simulated by quickly passing 100 mL of deionised water (milli-Q) through the dust-loaded filter (Buck *et al.*, 2006) and the leachate subsampled into sterile 2 mL polypropylene vials.

Experimental procedure

Bacterioplankton response to dust and leachate additions was determined by time-course sampling during incubations lasting 24 h. Four incubations were done, two in the southwest of the region and two in the north (Figure 5.1). 34 mL seawater samples were incubated in HCl-cleaned 35 mL PTFE bottles with dust, leachate, or no (control) additions. Dust was added with the polypropylene filter onto which it was collected; additions were calculated post-cruise to be 0.3 mg L^{-1} (incubation 1), 1.5 mg L^{-1} (incubation 2) or 4.7 mg L^{-1} (incubations 3 & 4). A further control of a blank polypropylene filter was used to ensure bacterioplankton response was due to the dust and not the filter. Leachate additions of 700 µL supplied 100 nM inorganic N and 10 nM P to all incubations.

Bottles were placed in on-deck incubators screened to permit 20% surface irradiance and cooled to *in situ* temperature.

The uptake rate of ^{35}S-methionine (^{35}S-Met) was measured at t = 0, 2, 4, 6, 24 h to determine the bacterioplankton community metabolic response to treatments (+Leachate or +Dust) as compared to controls. Two incubations were also sampled at t = 8 h. At t = 0 and 6 h, samples were taken to measure cellular uptake by sorted bacterioplankton groups. A further eight t = 0 h samples were collected throughout the cruise to measure cellular uptake in response to natural dust deposition in the ocean. Parallel to that, untreated samples were fixed with paraformaldehyde (PFA, 1 % final concentration) and stored at -80°C prior to analysis of bacterioplankton community structure.

^{35}S-Met uptake rates

Throughout the 24 h dust and leachate addition incubations, uptake rates of 50 pM ^{35}S-Met (1175 Ci mmol^{-1}, Perkin Elmer, UK) by total bacterioplankton was measured using time series (10, 20 and 30 min) incubations with 500 µL subsamples. Subsamples were fixed with 1% PFA and filtered onto 0.2 µm polycarbonate membrane filters. The radioactivity retained on filters was measured using a liquid scintillation counter (Tri-Carb 3100, Perkin Elmer, UK) on board the ship and is presented in becquerels (Bq).

At t = 0 and 6 h, three 1.6 mL replicate seawater samples were incubated with 0.2 nM ^{35}S-Met for 2 h to compare bacterioplankton metabolic response to ambient dust deposition (t = 0 h), and dust and leachate addition, as compared to controls, in incubation bottles (t = 6 h). Samples were fixed with 1% PFA and stored at -80°C until sorted by flow cytometry to determine group-specific ^{35}S-Met cellular uptake. ^{35}S-Met dilution bioassays (Zubkov *et al.*, 2003) were done in parallel to all experiments to estimate ambient methionine concentration, uptake rates and turnover times. These data are discussed in Chapter Two.

Analysis of bacterioplankton groups

Bacterioplankton samples were analysed by flow cytometry (FACSCalibur, BD Biosciences, Oxford, UK). *Prochlorococcus* cyanobacteria were identified and flow sorted from unstained samples using their characteristic red autofluorescence (Olson *et al.*, 1993). Bacterioplankton cells were stained with the nucleic acid stain SYBR Green I (Marie *et al.*, 1997) and the cells with low nucleic acid (LNA) and high nucleic acid (HNA) content (Li *et al.*, 1995, Gasol *et al.*, 1999) were separated using a plot of side scatter (90° right angle

light scatter) against green (FL1) fluorescence. Although the SAR11 clade of *Alphaproteobacteria* cannot be discriminated specifically by flow cytometry, they dominate the LNA bacterioplankton group (Mary *et al.*, 2006, Schattenhofer, 2009), which can be sorted.

The isotopically labelled LNA bacterioplankton and *Prochlorococcus* cells were flow sorted as previously described (Zubkov *et al.*, 2004, Mary *et al.*, 2006). Radioactivity retained by known numbers of sorted cells from the two groups examined was measured by an ultra-low level liquid scintillation counter (1220 Quantulus, Wallac, Finland) ashore and is presented as mBq per cell. In order to assess ^{35}S-Met adsorption to dust, 5000 dust particles were sorted in parallel to microbial cells. The radioactivity of the dust particles was indistinguishable from background measurements, indicating insignificant adsorption of ^{35}S-Met to dust.

Bacterioplankton cells in samples collected for community structure analysis were sorted into the HNA and LNA groups. Cells were collected directly onto 0.2 µm pore size polycarbonate membrane filters (Millipore, Isopore™) and analysed by FISH using the method described in Chapter Three, and the probes detailed in Table 5.1.

Table 5.1: Results of FISH analysis of LNA and HNA containing bacterioplankton groups, expressed as percentages of prokaryotes (mean ± SD of at least six fields of view). Probes applied were EUB338I-III (Amann *et al.*, 1990, Daims *et al.*, 1999), SAR11-441 (Morris *et al.*, 2002), 405Pro (West *et al.*, 2001) and 645HLII (West *et al.*, 2001).

	Sample Origin		LNA		HNA			
Date	Lat, °N	Long, °W	EUB	SAR11	EUB	Pro	HLII	SAR11
20/01	12°33′56	32°40′34	94 ± 4	86 ± 4	90 ± 3	75 ± 8	36 ± 6	0 ± 0
21/01	12°31′50	35°46′51	94 ± 4	76 ± 6	88 ± 4	59 ± 9	42 ± 6	0 ± 0
22/01	12°37′13	33°14′06	89 ± 4	60 ± 5	94 ± 6	69 ± 9	68 ± 5	0.3 ± 0.6
23/01	12°30′19	30°37′31	94 ± 2	83 ± 9	87 ± 5	62 ± 7	48 ± 3	0 ± 0
25/01	16°11′13	30°38′37	89 ± 5	39 ± 7	96 ± 2	74 ± 3	49 ± 9	0 ± 0
30/01	21°56′50	27°04′52	No data	64 ± 5	93 ± 5	70 ± 4	44 ± 5	0 ± 0
30/01	22°49′02	27°11′50	87 ± 4	80 ± 7	95 ± 3	72 ± 4	31 ± 3	0.3 ± 0.8
01/02	26°08′52	26°24′48	No data	79 ± 8	92 ± 3	66 ± 4	54 ± 7	0.3 ± 0.7
02/02	26°36′26	23°42′29	92 ± 6	79 ± 4	94 ± 3	64 ± 4	32 ± 3	0.3 ± 0.8
02/02	26°42′32	23°00′46	No data	83 ± 3	93 ± 4	75 ± 7	54 ± 5	0 ± 0
Mean			**91 ± 3**	**73 ± 14**	**92 ± 3**	**68 ± 6**	**46 ± 12**	**0.1 ± 0.2**

Statistical analysis

All data are presented as means ± standard deviation for the stated number of independent observations. Statistical significance at $p<0.05$ was determined using the Student's *t*-test for paired or unpaired samples depending on compared datasets.

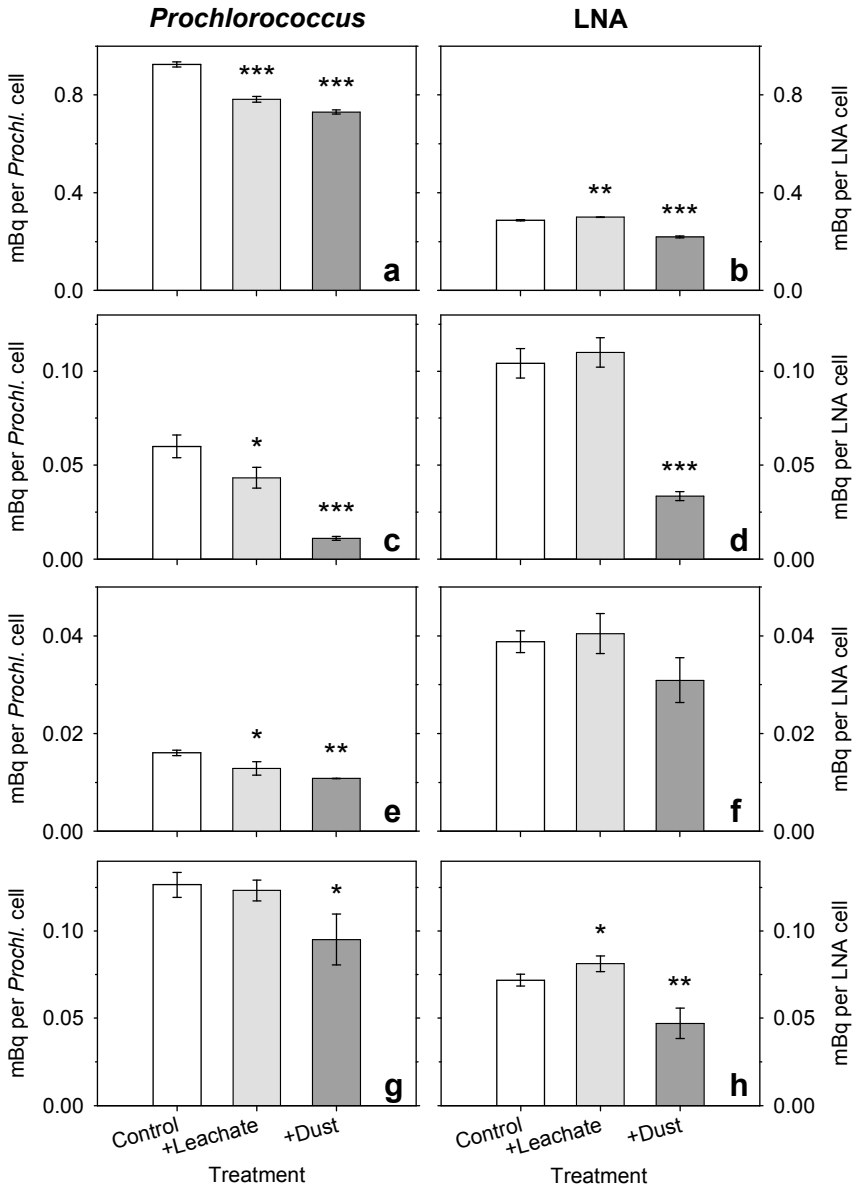


Figure 5.2: Uptake of ^{35}S-Met (mBq) per *Prochlorococcus* (left) and Low Nucleic Acid (LNA, right) bacterioplankton cell after 6 h incubations with no addition (control), or in the presence of dust leachate or dust (mean ± SD; N = 3). Dust additions were (a & b) 0.3 mg L^{-1} in incubation 1, (c & d) 1.5 mg L^{-1} in incubation 2, (e & f) 4.7 mg L^{-1} in incubation 3 and (g & h) 4.7 mg L^{-1} in incubation 4. Treatments were compared to controls using Student's *t*-test; statistical significance is indicated as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.0001$. df = 4 for all comparisons.

5.3 Results

The SAR11 clade of *Alphaproteobacteria* dominated the LNA group at 73 ± 14% of prokaryotes (Table 5.1). The unidentified fraction of the LNA group could not be phylogenetically affiliated using other probes including Gam42a (identifying *Gammaproteobacteria*), 405Pro (*Prochlorococcus*) or 645LL (low light adapted *Prochlorococcus*).

Prochlorococcus dominated the HNA bacterioplankton at 68 ± 6% of prokaryotes (Table 5.1). The majority of *Prochlorococcus* cells belonged to the High-Light adapted ecotype II (Table 5.1). A maximum of 2% of prokaryotes were identified by 645HLI as the High-Light adapted ecotype I, with the majority of samples containing none. No more than one or two HNA cells were identified as SAR11 in each sample, with the majority containing none (Table 5.1).

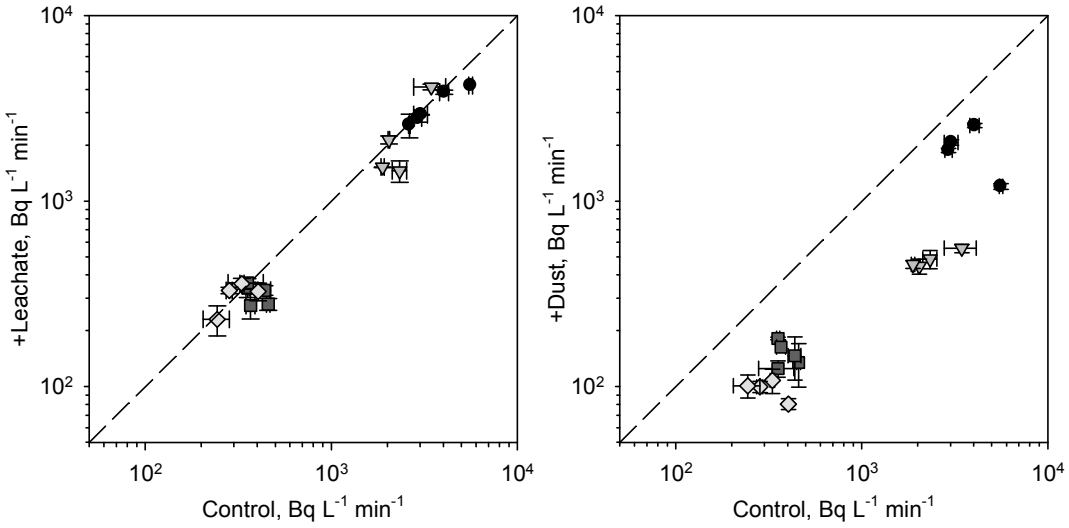


Figure 5.3: Impact of dust leachate (a) and dust (b) addition on rate of bacterioplankton community ^{33}S-Met uptake (Bq L^{-1} min^{-1}) at 2 hourly intervals throughout initial 6-8 h, and after 24 h of incubation experiments. Symbols indicate data from individual incubations. All data are presented as mean ± SE.

In experimental incubations, ^{35}S-Met uptake by LNA bacterioplankton cells increased by 4-13% in the presence of leachate (as compared to controls) in each of the four incubations,

and the increase was statistically significant in two (Figure 5.2). Conversely, *Prochlorococcus* cells took up significantly less ^{35}S-Met in the presence of dust leachate (3-28% less than in controls) in each of the experiments (Figure 5.2). Yet in unsorted samples the bacterioplankton community was mostly unaffected by the addition of dust leachate at each time point (4 or 5 per incubation) sampled throughout the four incubations (paired *t*-test, $p > 0.1$, $n = 18$; Figure 5.3a).

The effect of direct dust addition (not as a leachate) was more dramatic; ^{35}S-Met uptake by both *Prochlorococcus* and LNA bacterioplankton decreased during all incubations by 21-82% and 20-68% of the control, respectively (Figure 5.2). Dust addition also negatively impacted the bacterioplankton community as a whole (Figure 5.3b).

During the dust deposition event, LNA bacterioplankton took up significantly more ^{35}S-Met per cell than *Prochlorococcus*, paired *t*-test, $p<0.005$, $n = 3$, suggesting reduced metabolic activity of *Prochlorococcus* and/or enhanced metabolic activity of the LNA bacterioplankton (Figure 5.4). Outside of the dust event, *Prochlorococcus* cells took up more ^{35}S-Met than the LNA cells.

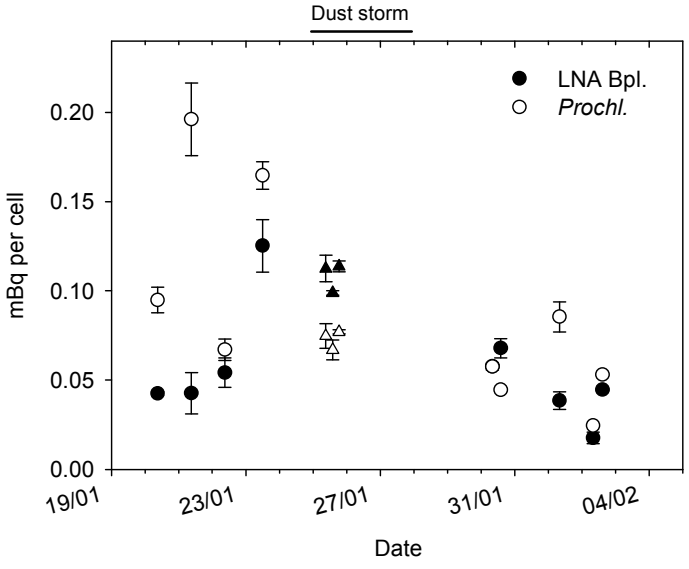


Figure 5.4: Cellular uptake of ^{35}S-Met by untreated *Prochlorococcus* (*Prochl*.) and LNA bacterioplankton (LNA Bpl.) cells throughout the cruise period. Uptake was measured as mBq per cell and displayed as mean ± SD (N = 3 for each sample). Samples were incubated with 0.2 nM ^{35}S-Met for 2h. The period of dust deposition is indicated by a black line and triangular symbols.

5.4 Discussion

The bacterioplankton metabolic response to dust additions was measured by comparing cellular uptake rates of radiolabelled methionine, as a proxy for bacterioplankton

production. Methionine was used because it is available with a ^{35}S label, which gives it a higher specific activity than the more traditional ^{14}C and tritium labelled leucine tracers used previously (e.g. Herut *et al*., 2005), which increases the sensitivity of the flow sorting technique. *Prochlorococcus* and SAR11 have been shown to actively take up ^{35}S-Met (Zubkov *et al*., 2003, Mary *et al*., 2006); indeed, *Prochlorococcus* has exhibited preference for methionine over leucine (Mary *et al*., 2008) despite leucine requirement for all proteins.

The data presented suggest that the LNA bacterioplankton, but not *Prochlorococcus*, benefited metabolically from dust leachate additions. This differential result was hidden when observing the community response as a whole, which suggested no stimulation or suppression of bacterial metabolism. The varying degree of stimulation of LNA bacterioplankton by leachate within the four incubations was presumably due to variation in ambient methionine uptake rates, as indicated by ^{35}S-Met bioassays that were conducted in parallel (4.2-17.7 pmol L^{-1} h^{-1}, Chapter Two).

In agreement with previous observations, the SAR11 clade of *Alphaproteobacteria* dominated the LNA bacterioplankton, yet was not identified within the HNA bacterioplankton. This coverage of 72 ± 15% LNA prokaryotes is similar to that achieved in one previous study (Schattenhofer, 2009), but higher than others (Mary *et al*., 2006, Zubkov *et al*., 2007), probably because the cells were more metabolically active, allowing more hybridisations to occur. The remaining fraction of LNA bacterioplankton cells could be identified as Bacteria while they could not be affiliated to other groups, including *Gammaproteobacteria* and *Prochlorococcus*. The difficulty in identifying the LNA group in open ocean samples (Mary *et al*., 2006, Schattenhofer, 2009) suggests that they could belong to the SAR11 clade but differ in their cellular ribosomal content.

Dust may introduce organic carbon (Duarte *et al*., 2006, Pulido-Villena *et al*., 2008b) which could benefit heterotrophic SAR11 cells more than phototrophic *Prochlorococcus* cells. It may also alleviate limitation of microbial growth by inorganic N or P (Rivkin & Anderson, 1997, Caron *et al*., 2000); some *Prochlorococcus* strains can assimilate these inorganic nutrients (Casey *et al*., 2007). Indeed, a strain of *Prochlorococcus* found in the Red Sea, which is relatively insensitive to metal toxicity compared to strains from the Atlantic, has been shown to increase in abundance following inorganic nutrient and Saharan dust additions (Paytan *et al*., 2009). However, the majority of *Prochlorococcus* cells in samples from the present study belonged to the High-Light adapted ecotype II (Table 5.1), which

are well adapted to oligotrophic environments (West *et al.*, 2001, Johnson *et al.*, 2006, Zubkov *et al.*, 2007, Zwirglmaier *et al.*, 2007). No more than 2% of HNA prokaryotes were identified as High-Light adapted ecotype I (HLI), which has a relatively high nutrient requirement compared to HLII (Johnson *et al.*, 2006). Given that the study region was dominated by HLII, it seems unlikely that the *Prochlorococcus* population would have benefited from dust-derived nutrients.

Ecotypes of both *Prochlorococcus* and SAR11 have maximised their ability to take up nutrients efficiently at very low nutrient concentrations. Their resultant streamlined genomes lack many of the regulatory proteins found in most marine bacteria for regulating uptake of N, P and Fe (Rocap *et al.*, 2003, García-Fernández *et al.*, 2004, Giovannoni *et al.*, 2005, Martiny *et al.*, 2006, Martiny *et al.*, 2009), which could make it difficult for these groups to regulate nutrient uptake at substantially elevated concentrations. Thus, deposition of high quantities of nutrients and metals in dust may be toxic to these groups. *Prochlorococcus*, for example, have been shown to be particularly sensitive to copper (Mann *et al.*, 2002). Herut *et al.* (2005) also report a decline in the *Prochlorococcus* community in response to Saharan dust in Mediterranean waters. Furthermore, studies have shown that SAR11 is not abundant in mesotrophic regions compared to low nutrient environments (Fuchs *et al.*, 2005, Alonso-Sáez *et al.*, 2007) which implies a disadvantage of this clade in regions of high nutrient availability.

Direct dust addition to seawater suppressed metabolism of both *Prochlorococcus* and LNA cells, and this negative impact was clear also at the bacterioplankton community level. Conversely, dust additions to reservoir water showed an increase in bacterial production after 48 h incubation, although there was evidence that this was due to the introduction of air-borne *Gammaproteobacteria* associated with the dust particles (Reche *et al.*, 2009). A comparison of cellular methionine uptake by the two flow sorted bacterioplankton groups in control samples suggests that LNA bacterioplankton benefited and/or *Prochlorococcus* were inhibited by dust deposition in the field (Figure 5.4). These observations support our experimental findings that small increases in dust-derived nutrients have a detrimental impact on *Prochlorococcus* in the region. It seems plausible, therefore, that ambient bacterioplankton communities suffer from large dust events, whereas opportunistic bacteria multiply rapidly, leading to increased bacterial production.

In summary, this study suggests differential responses of major bacterioplankton groups to dust-derived nutrients, which are hidden when studying the bacterioplankton community as one entity. However, the cause of these differential responses of the *Prochlorococcus* and LNA bacterioplankton groups requires further investigation.

5.5 References

Alonso-Sáez L, Arístegui J, Pinhassi J, Gómez-Consarnau L, González JA, Vaqué D, Agustí S & Gasol JM (2007) Bacterial assemblage structure and carbon metabolism along a productivity gradient in the NE Atlantic. *Aquatic Microbial Ecology* **46**: 43-53.

Amann R, Binder BJ, Olson RJ, Chisholm SW, Devereux R & Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology* **56**: 1919-1925.

Bonilla-Findji O, Gattuso J-P, Pizay M-D & Weinbauer MG (2010) Autotrophic and heterotrophic metabolism of microbial planktonic communities in an oligotrophic coastal marine ecosystem: seasonal dynamics and episodic events. *Biogeosciences Discussions* **7**: 2033-2064.

Bonnet S, Guieu C, Chiaverinin J, Ras J & Stock A (2005) Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system. *Limnology and Oceanography* **50**: 1810-1819.

Buck CS, Landing WM, Resing JA & Lebon GT (2006) Aerosol iron and aluminium solubility in the northwest Pacific Ocean: Results from the 2002 IOC cruise. *Geochemistry, Geophysics, Geosystems* **7**: Q04M07, doi:10.1029/2005GC000977.

Caron DA, Lin Lim E, Sanders RW, Dennett MR & Berninger UG (2000) Responses of bacterioplankton and phytoplankton to organic carbon and inorganic nutrient additions in contrasting oceanic ecosystems. *Aquatic Microbial Ecology* **22**: 175-184.

Casey JR, Lomas MW, Mandecki J & Walker DE (2007) *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. *Geophysical Research Letters* **34**: dpo: 10.1029/2006GL028725.

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB & Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. *Nature* **334**: 340-343.

Daims H, Bruhl A, Amann R, Schleifer KH & Wagner M (1999) The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology* **22**: 434-444.

Duarte CM, Dachs J, Llabrés M, Alonso-Laita P, Gasol JM, Tovar-Sánchez A, Sañudo-Wilhelmy S & Agustí S (2006) Aerosol inputs enhance new production in the subtropical northeast Atlantic. *Journal of Geophysical Research* **111**: G4, doi:10.1029/2005JG000140.

Fuchs BM, Woebken D, Zubkov MV, Burkill P & Amann R (2005) Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. *Aquatic Microbial Ecology* **39**: 145-457.

García-Fernández JM, Tandeau de Marsac N & Diez J (2004) Streamlined regulation and gene loss as adaptive mechanisms in *Prochlorococcus* for optimized nitrogen utilization in oligotrophic environments. *Microbiology and Molecular Biology Reviews* **68**: 630-638.

Gasol JM, Zweifel UL, Peters R, Fuhrman JA & Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Applied and Environmental Microbiology* **65**: 4475-4483.

Gasol JM & Duarte CM (2000) Comparative analysis in aquatic microbial ecology: how far do they go? *FEMS Microbiology Ecology* **31**: 99-106.

Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M, Rappé MS, Short JM, Carrington JC & Mathur EJ (2005) Genome streamlining in a cosmopolitan oceanic bacterium. *Science* **309**: 1242-1245.

Guieu C, Loe-Pilot M-D, Ridame C & Thomas C (2002) Chemical characterisation of the Saharan dust end-member: Some biogeochemical implications for the western Mediterranean Sea. *Journal of Geophysical Research* **107**: D15, doi:10.1029/2001JD000582.

Herut B, Zohary T, Krom MD, Mantoura RFC, Pitta P, Psarra S, Rassoulzadegan F, Tanaka T & Thingstad TF (2005) Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Sea Research II* **52**: 3024-3040.

Jickells TD, An ZS, Andersen KK, Baker AR, Bergametti G, Brooks N, Cao JJ, Boyd PW, Duce RA, Hunter KA, Kawahata H, Kubilay N, laRoche J, Liss PS, Mahowald N, Prospero JM, Ridgwell AJ, Tegen I & Torres R (2005) Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* **308**: 67-71.

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EMS & Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* **311**: 1737-1740.

Lekunberri I, Lefort T, Romero E, Vázquez-Domínguez E, Romera-Castillo C, Marrasé C, Peters F, Weinbauer M & Gasol JM (2010) Effects of a dust deposition event on coastal

marine microbial abundance and activity, bacterial community structure and ecosystem function. *Journal of Plankton Research* **32**: 381-396.

Li WKW, Jellett JF & Dickie PM (1995) DNA distributions in planktonic Bacteria stained with TOTO or TO-PRO. *Limnology and Oceanography* **40**: 1485-1495.

Mahowald NM, Baker AR, Bergametti G, Brooks N, Duce RA, Jickells TD, Kubilay N, Prospero JM & Tegen I (2005) Atmospheric global dust cycle and iron inputs to the ocean. *Global Biogeochemical Cycles* **19**: GB4025, doi:10.1029/2004GB002402.

Mann EL, Ahlgren N, Moffett JW & Chrisholm SW (2002) Copper toxicity and cyanobacteria ecology in the Sargasso Sea. *Limnology and Oceanography* **47**: 976-988.

Marie D, Partensky F, Jacquet S & Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Applied and Environmental Microbiology* **63**: 186-193.

Martiny AC, Coleman ML & Chisholm SW (2006) Phosphate acquisition genes in *Prochlorococcus* ecotypes: evidence for genome-wide adaptation. *Proceedings of the National Academy of Sciences* **103**: 12552-12557.

Martiny AC, Huang Y & Weizhong L (2009) Occurrence of phosphate acquisition genes in *Prochlorococcus* cells from different ocean regions. *Environmental Microbiology* **11**: 1340-1347.

Mary I, Heywood JL, Fuchs BM, Amann R, Tarran GA, Burkill PH & Zubkov MV (2006) SAR11 dominance among metabolically active low nucleic acid bacterioplankton in surface waters along an Atlantic meridional transect. *Aquatic Microbial Ecology* **45**: 107-113.

Mary I, Tarran GA, Warwick PE, Terry MJ, Scanlan DJ, Burkill PH & Zubkov MV (2008) Light enhanced amino acid uptake by dominant bacterioplankton groups in surface waters of the Atlantic Ocean. *FEMS Microbiology Ecology* **63**: 36-54.

McGillicuddy DJ & Robinson AR (1997) Eddy-induced nutrient supply and new production in the Sargasso Sea. *Deep Sea Research I* **44**: 1427-1450.

Mills MM, Ridame C, Davey M, La Roche J & Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292-294.

Moore CM, Mills MM, Milne A, Langlois R, Achterberg EP, Lochte K, Geider RJ & La Roche J (2006) Iron limits primary productivity during spring bloom development in the central North Atlantic. *Global Change Biology* **12**: 626-634.

Morris RM, Rappé MS, Connon SA, Vergin KL, Siebold WA, Carlson CA & Giovannoni SJ (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420**: 806-810.

Olson RJ, Zettler ER & Durand MD (1993) Phytoplankton analysis using flow cytometry. *Handbook of methods in aquatic microbial ecology* (Kemp PF, Sherr BF, Sherr EB & Cole JJ, eds.), pp. 175-186. Lewis Publishers, Boca Raton, Florida.

Paytan A, Mackey KRM, Chen Y, Lima ID, Doney SC, Mahowald N, Labiosa R & Post AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences* **106**: 4601-4605.

Pulido-Villena E, Reche I & Morales-Baquero R (2008a) Evidence of an atmospheric forcing on bacterioplankton and phytoplankton dynamics in a high mountain lake. *Aquatic Sciences* **70**: 1-9.

Pulido-Villena E, Wagener T & Guieu C (2008b) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* **22**: GB1020, doi:10.1029/2007GB003091.

Reche I, Ortega-Retuerta E, Romera O, Pulido-Villena E, Morales-Baquero R & Casamayor EO (2009) Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs. *Limnology and Oceanography* **54**: 869-879.

Rivkin RB & Anderson MR (1997) Inorganic nutrient limitation of oceanic bacterioplankton. *Limnology and Oceanography* **42**: 730-740.

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER & Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature* **424**: 1042-1047.

Rosenfeld D, Rudich Y & Lahav R (2001) Desert dust suppressing precipitation: A possible desertification feedback loop. *Proceedings of the National Academy of Sciences* **98**: 5975-5980.

Schattenhofer M (2009) Distribution of major bacterioplankton groups in the Atlantic Ocean. PhD Thesis, University of Bremen, Bremen.

Singh RP, Prasad AK, Kayetha VK & Kafatos M (2008) Enhancement of oceanic parameters associated with dust storms using satellite data. *Journal of Geophysical Research* **113**: doi:10.1029/2008/JC004815.

West NJ, Schönhuber WA, Fuller NJ, Amann RI, Rippka R, Post AF & Scanlan DJ (2001) Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by *in situ* hybridization using 16S rRNA-targeted oligonucleotides. *Microbiology* **147**: 1731-1744.

Zubkov MV, Burkill PH, Tarran GA, Fuchs BM & Amann R (2003) High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Applied and Environmental Microbiology* **69**: 1299-1304.

Zubkov MV, Tarran GA & Fuchs BM (2004) Depth related amino acid uptake by *Prochlorococcus* cyanobacteria in the Southern Atlantic tropical gyre. *FEMS Microbiology Ecology* **50**: 153-161.

Zubkov MV, Mary I, Woodward EMS, Warwick PE, Fuchs BM, Scanlan DJ & Burkill PH (2007) Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environmental Microbiology* **9**: 2079-2089.

Zwirglmaier K, Heywood JL, Chamberlain K, Woodward EMS, Zubkov MV & Scanlan DJ (2007) Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. *Environmental Microbiology* **9**: 1278-1290.

Chapter Six
Conclusions of the project

At the beginning of this project in 2005, a few studies had attempted to assess Saharan dust impacts on microbial communities in the North Atlantic (Blain *et al*., 2004, Mills *et al*., 2004) and the Mediterranean Sea (Bonnet *et al*., 2005, Herut *et al*., 2005), and these focussed on phytoplankton biomass with some mention of bacteria abundance (Bonnet *et al*., 2005, Herut *et al*., 2005) and production (Herut *et al*., 2005). Since then, additional studies have added to our knowledge of dust impacts on microorganisms. Dust addition has generally led to increased rates of primary production and phytoplankton biomass (e.g. Herut, *et al*., 2005; Duarte, *et al*., 2006). The bacterial community response has been less consistent; however, this could in part be due to fewer studies of bacterial dynamics. Bacterial production has either increased (Herut *et al*., 2005, Lekunberri *et al*., 2010) or remained unaffected (Duarte *et al*., 2006). Furthermore, dust addition has led to both an increase and decrease in the abundance of dominant cyanobacteria groups *Prochlorococcus* and *Synechococcus* (Herut, *et al*., 2005; Duart,e *et al*., 2006; Paytan, *et al*., 2009).

The problem in comparing individual studies, as with any field of research, is the lack of methodological consistency. Some variation will be due to spatial distribution of studies, since different regions typically have different nutrient regimes and ambient microbial communities. For example, in the Mediterranean Sea dust addition stimulated growth of picoeukaryotes and *Synechococcus* abundance whereas *Prochlorococcus* populations diminished (Herut *et al*., 2005), yet the exact opposite was observed in the Red Sea (Paytan *et al*., 2009). However, given that Aeolian dust is blown over a great area of the world's oceans, it is certainly worth studying impacts in various affected regions.

Further variation comes from the addition of different dust types. Experiments have used dust from the source, i.e. sifted Saharan sand termed 'Saharan end-member', or wet or dry deposited atmospheric dust. Dust becomes chemically altered during transportation, thus atmospheric dust is more representative of that received by the oceans. For example, Moore *et al*. (2006) compared the dissolution of iron from the same quantity of sifted soil and aerosol dust and found that significantly more iron was leached from the latter.

Unfortunately, however, collection of this material is not easy, particularly in the quantities required for thorough analysis. Wet deposited dust has been added as the particulate fraction of Saharan rain (Pulido-Villena *et al.*, 2008, Lekunberri *et al.*, 2010), but a high proportion of the water-soluble fraction will have already been lost in the rain water. Dry deposited dust was originally washed from plastic sheets onto which it settled, which poses similar problems with nutrient leaching. More recently, dry atmospheric dust has been collected onto filters (Paytan, *et a*l, 2009; Hill, *et al*., 2010), which is a cleaner method and retains all nutrients and metals, and is thus considered the most appropriate method. The extent of the microbial response also depends upon the quantity of dust added (Herut *et al.*, 2005). Again, due to high variability in natural dust deposition events, it is worthwhile experimenting with ranging concentrations, so long as they are within a naturally occurring range. Lekunberri *et al.* (2010), however, used concentrations of 50 and 500 mg L^{-1}, which are considerably higher than the ranges previously used, 0.2-2 mg L^{-1}, which are believed to be more representative of natural dust storm events.

The main problem with dust addition experiments has been the dilemma of incubation duration. The desire to measure a multitude of parameters simultaneously, some of which take days to exhibit measureable change, has lead to incubations lasting two to five days, although this also allows time for nutrients to leach from the dust particles. Long incubations are often designed to measure the eukaryotic phytoplankton response to dust-derived nutrients (e.g. Duarte, *et al.*, 2006). Given the considerably shorter turnover of bacterial communities, and their role as recyclers of organic matter, such long incubations are inappropriate for studying the bacterioplankton community response. When studying bacterial dynamics within bottles over periods of days, it is not possible to distinguish direct effects of dust from secondary effects, such as a response to dust-induced increases in phytoplankton biomass. Bacteria themselves will rapidly respond to a change in nutrient availability, thus the current study aimed to conduct dust addition incubations of up to 30 minutes. In case the added dust did not leach nutrients immediately, dust leachate, produced from atmospheric dust collected on filters, was also added to parallel incubations so that dust-derived nutrients were readily available to the microbial community. Thus, these experiments were not inhibited by the time taken for nutrients to dissolve from dust particles.

The study region for the research presented in this thesis, the northeast (sub-)tropical Atlantic Ocean, was chosen because it is a region regularly impacted by dust deposition,

at an estimated rate of 5-20 g m^{-2} y^{-1} (Jickells *et al.*, 2005). During the study the region's microbial community was distinct from that of the oligotrophic subtropical gyre in terms of metabolic activity. Satellite ocean colour data confirm it is a dynamic region that moves into the gyre in summer and out in the winter (Oliver & Irwin, 2008), when this study was done. As with all (sub-)tropical oceanic environments, the ambient autotrophic bacterioplankton community of the region was dominated by *Prochlorococcus*, the most numerically abundant oceanic primary producer, followed by *Synechococcus*, and the microheterotrophs were largely of the ubiquitous SAR11 clade of *Alphaproteobacteria*. Ideally the study would have included more measurements from the oligotrophic open ocean, where microbial communities are stable in terms of abundance and metabolism, and thus may have shown a clearer response. Living on the dynamic edge of the gyre, the community would already have been in a state of change. This would have made some cells vulnerable to further perturbation, in particular those with a compromised stress response as a result of genome streamlining, such as *Prochlorococcus*.

From physical, chemical and biological parameters measured during this study the area could be divided into three sub-regions of distinct water masses influenced by wind stress and dust deposition, the Cape Verde topography, and the Canary and equatorial currents. Spatial and temporal abundance data suggested distribution of the main microbial groups was dependent upon wind-induced perturbations. SAR11 dominated during turbulent conditions whereas *Prochlorococcus* dominated during calmer conditions. *Synechococcus* were also more numerous during more turbulent conditions. Data indicated that wind has an important role in shaping microbial community structure. Considering climate change threatens to bring enhanced storm activity, this may be an important avenue of further research.

Previous dust addition studies have not determined group-specific metabolic responses of bacterioplankton communities. Bacterial production has been estimated from the uptake rate of the amino acid leucine. Leucine is incorporated by the dominant autotrophic bacterioplankton groups – *Prochlorococcus* and *Synechococcus* – as well as heterotrophic bacterioplankton. Furthermore, the heterotrophic bacterioplankton community includes a great diversity of organisms with distinct physiologies and optimum growth conditions, which we cannot assume will respond in the same manor when faced with dust. Although far from complete, our present knowledge of the diversity of marine microorganisms is enough to be certain that the treatment of the bacterioplankton

community as one entity is simply insufficient. Thus, to show that dust addition influences bacterial production, either positively or negatively, provides little useful information. This fact was proven in the current work by employing flow cytometric sorting to assess the metabolic response of individual bacterioplankton groups. The groups studied in detail were *Prochlorococcus* and SAR11. These were chosen because they are the most abundant organisms in the region and thus, presumably, the most significantly impacted by dust addition, but also because they represent both the autotrophic and heterotrophic community. As a whole, the bacterioplankton community seemed relatively unaffected by dust leachate addition, yet SAR11 sometimes benefitted, and *Prochlorococcus* always suffered, metabolically. Thus, studying the specific metabolic activities of SAR11 and *Prochlorococcus* cells in response to dust addition showed subtleties in individual group response that are hidden when studying the community as one entity.

Direct dust addition had an immediate and devastating impact on the metabolism of the ambient microbial community, including both *Prochlorococcus* and SAR11, most likely the result of a higher concentration of toxic compounds in the dust additions than was present in the leachate. Flow cytometry data showed a significant halving in *Prochlorococcus* abundance following dust deposition *in situ*. Herut *et al.* (2005) agree that *Prochlorococcus* communities suffer significantly from dust addition. Conversely, Paytan *et al.* (2010) report an increase in *Prochlorococcus* abundance following dust addition to seawater from the Red Sea; the point they brush over, however, is that this follows a loss of over 90% of the population in the first two days. Thus the newly blooming *Prochlorococcus* cells could be a different strain from the original, most likely fuelled by organic exudates from the picoeukaryote, *Synechococcus* and *Prochlorococcus* cells that died in the same bottle. Either way, it is optimistic to attribute this thriving community to dust alone. Studies that present data from numerous dust addition experiments often find few consistencies in biological responses. It is worth bearing this in mind when evaluating the results from single experiments such as this. Of the heterotrophic bacterioplankton groups quantified, *Gammaproteobacteria* was the only one to show a potential increase in abundance in response to dust deposition. If the dominant bacterial groups were harmed as suggested by dust addition incubations, the organic exudates released by these decaying cells could have fuelled the substantial *Gammaproteobacteria* growth observed following dust deposition. In summary, therefore, the results from these experiments and observations have shown both a direct negative, and an indirect positive, impact of dust

deposition on near surface bacterioplankton communities, and these impacts are group specific.

6.1 References

Blain S., Guieu C., Claustre H., Leblanc K., Moutin T., Queguiner B., Ras J. & Sarthou G. (2004) Availability of iron and major nutrients for phytoplankton in the northeast Atlantic Ocean. *Limnology and Oceanography* **49**: 2095-2104.

Bonnet S., Guieu C., Chiaverinin J., Ras J. & Stock A. (2005) Effect of atmospheric nutrients on the autotrophic communities in a low nutrient, low chlorophyll system. *Limnology and Oceanography* **50**: 1810-1819.

Duarte C. M., Dachs J., Llabrés M., Alonso-Laita P., Gasol J. M., Tovar-Sánchez A., Sañudo-Wilhelmy S. & Agustí S. (2006) Aerosol inputs enhance new production in the subtropical northeast Atlantic. *Journal of Geophysical Research* **111**: G4, doi:10.1029/2005JG000140.

Herut B., Zohary T., Krom M. D., Mantoura R. F. C., Pitta P., Psarra S., Rassoulzadegan F., Tanaka T. & Thingstad T. F. (2005) Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep Sea Research II* **52**: 3024-3040.

Hill, PG, Zubkov, MV, & Purdie, DA (2010) Differential responses of *Prochlorococcus* and SAR11-dominated bacterioplankton groups to atmospheric dust inputs in the tropical Northeast Atlantic Ocean. *FEMS Microbiology Letters*, **306:** 82-89.

Jickells T. D., An Z. S., Andersen K. K., Baker A. R., Bergametti G., Brooks N., Cao J. J., Boyd P. W., Duce R. A., Hunter K. A., Kawahata H., Kubilay N., laRoche J., Liss P. S., Mahowald N., Prospero J. M., Ridgwell A. J., Tegen I. & Torres R. (2005) Global iron connections between desert dust, ocean biogeochemistry, and climate. *Science* **308**: 67-71.

Lekunberri I., Lefort T., Romero E., Vázquez-Domínguez E., Romera-Castillo C., Marrasé C., Peters F., Weinbauer M. & Gasol J. M. (2010) Effects of a dust deposition event on coastal marine microbial abundance and activity, bacterial community structure and ecosystem function. *Journal of Plankton Research* **32**: 381-396.

Mills M. M., Ridame C., Davey M., La Roche J. & Geider R. J. (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292-294.

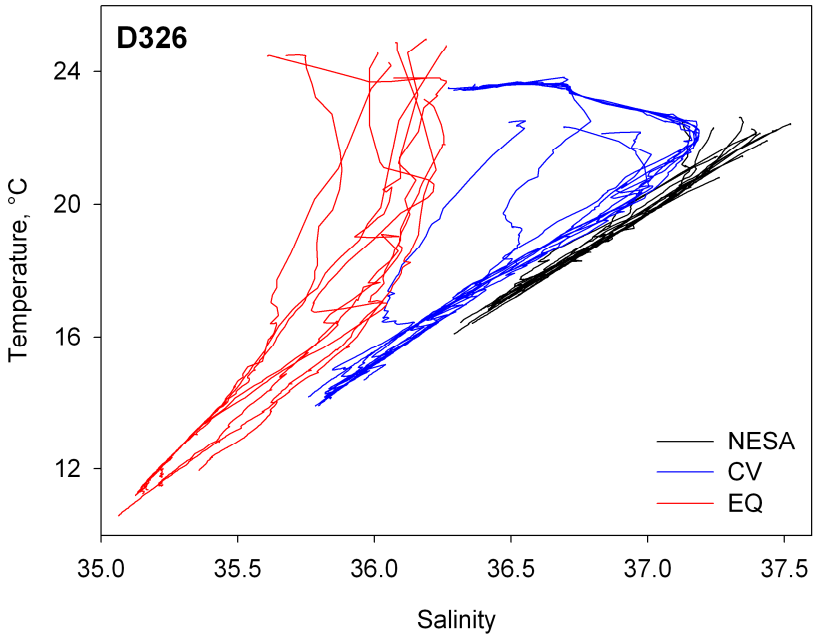
Oliver M. J. & Irwin A. J. (2008) Objective global ocean biogeographic provinces. *Geophysical Research Letters* **35**: L15601, doi:10.1029/2008GL034238.

Paytan A., Mackey K. R. M., Chen Y., Lima I. D., Doney S. C., Mahowald N., Labiosa R. & Post A. F. (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *Proceedings of the National Academy of Sciences* **106**: 4601-4605.

Pulido-Villena E., Wagener T. & Guieu C. (2008) Bacterial response to dust pulses in the western Mediterranean: Implications for carbon cycling in the oligotrophic ocean. *Global Biogeochemical Cycles* **22**: GB1020, doi:10.1029/2007GB003091.

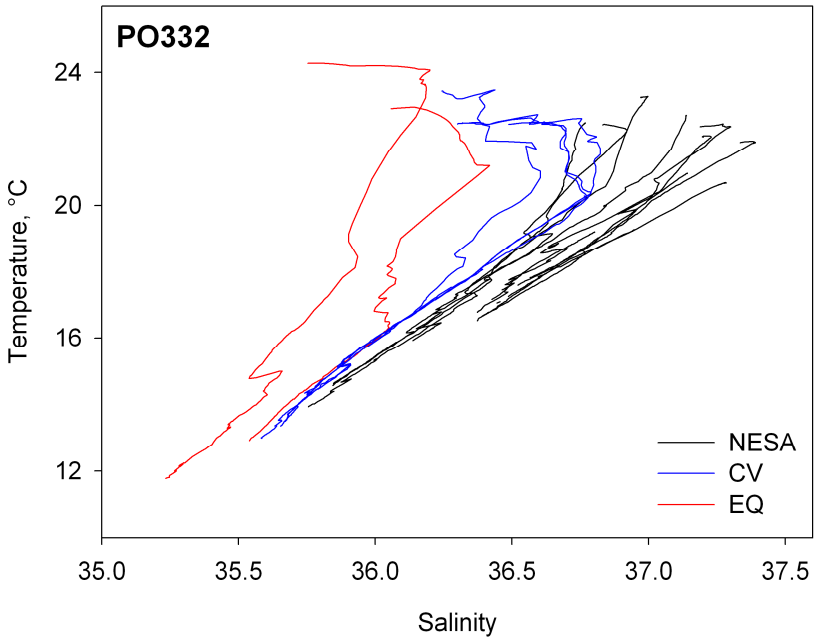
Appendix I

Temperature-salinity plots from CTD profiles collected during UK SOLAS cruise D326 onboard RRS *Discovery*, January-February 2008. T-S plots have been divided according to signature, such that the study region was divided between the North-East Subtropical Atlantic (NESA) and the Cape Verde (CV) and Equatorial (EQ) regions.



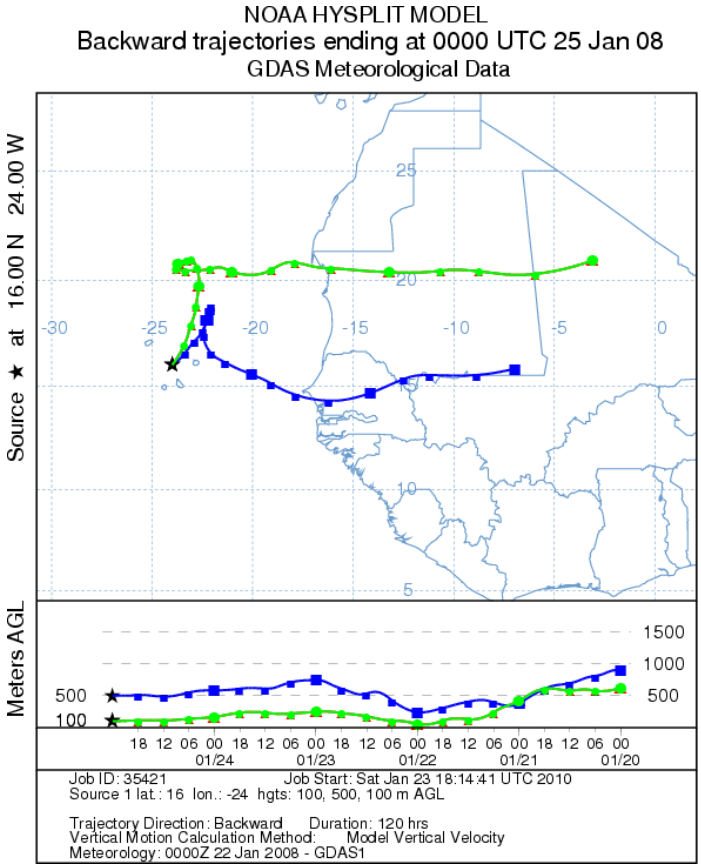
Appendix II

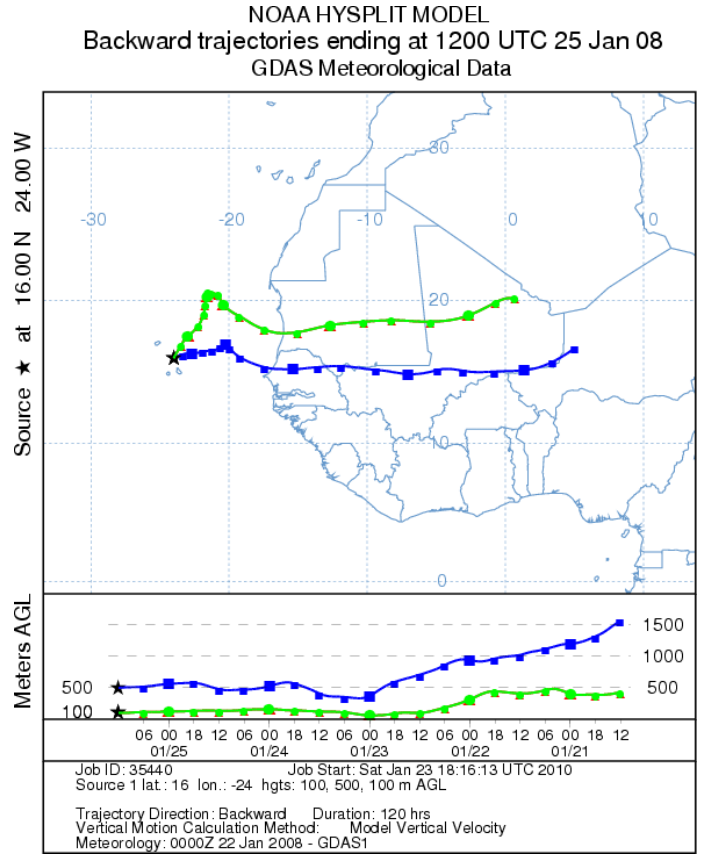
Temperature-salinity plots from CTD profiles collected during UK SOLAS cruise PO332 onboard RV *Poseidon*, January-February 2006. T-S plots have been divided according to signature, such that the study region was divided between the North-East Subtropical Atlantic (NESA) and the Cape Verde (CV) and Equatorial (EQ) regions.



Appendix III

Examples of back trajectories for the second dust storm during January 2008 show its Saharan origin. Courtesy of Manuel Dall'Osto, currently at IDEA-CSIS, Barcelona.





Appendix IV

Hill, PG, Zubkov, MV & Purdie, DA (2010) Differential responses of *Prochlorococcus* and SAR11-dominated bacterioplankton groups to atmospheric dust inputs in the tropical Northeast Atlantic Ocean. *FEMS Microbiology Ecology Letters* **306:** 82-89.
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