Original Article

Seasonal progression of microbial communities on the Faroe shelf

ANNI DJURHUUS1*, JÓHANNA JØRGENSEN1, HJÁLMAR HÁTÚN1, HØGNI HAMMERSHAIMB DEBES1 & DEBES HAMMERSHAIMB CHRISTIANSEN2

1Faroe Marine Research Institute, Tórshavn, Faroe Islands, and 2Food and Veterinary Agency, Tórshavn, Faroe Islands

Abstract
Microorganisms, such as phytoplankton and bacterioplankton, are affected by turnover rates of nutrients and show great fluctuations over seasons. In productive coastal areas, the biomass of bacterioplankton can be in the same range as that of phytoplankton. In these coastal areas the initiation and intensity of the spring bloom is highly variable between years. This variability is reflected in higher trophic levels and is therefore of major importance for ecosystems such as that of the Faroe Islands. However, one of the major unknown components is the bacterioplankton. We report a study on seasonal dynamics from March–September of nutrients, phytoplankton composition and their co-fluctuation with bacterial succession. For this purpose SAR11, Bacteroidetes, Roseobacter and cyanobacteria were relatively quantified using real-time PCR based on 16S DNA and total bacteria was assessed by epifluorescence microscopy. The phytoplankton species were identified using the inverted microscope technique. These data showed a pronounced diatom spring bloom and autumn bloom reflected by a corresponding decrease in nitrate and silicate ($R^2 = 0.72$ and 0.77, respectively). The cessation of the phytoplankton bloom did not, however, seem to be explained by nutrient limitation. Roseobacter bloomed during the phytoplankton spring bloom, while the other bacterial groups increased during low phytoplankton biomass. This suggests that algal substrate availability and environmental conditions provide the opportunity for bacterial communities to develop a post-spring bloom. This study reveals how planktonic bacteria adapt with their surroundings, enhancing the microbial loop post-spring bloom and providing a potentially important food resource for higher trophic levels.

Key words: Bacterioplankton, Faroe shelf, phytoplankton ecology, primary production, seasonal dynamics

Introduction
Although coastal areas only cover ~7% of the world’s oceans, they contribute to approximately 30% of global marine primary production (Gattuso et al. 1998). In these productive areas, the total biomass of bacterioplankton is in the same range as phytoplankton (Simon et al. 1992; Kirchman 2008), with bacterial abundance generally demonstrating a positive correlation with chlorophyll $a$, a proxy for primary produced biomass (Li et al. 2004). In environments where chlorophyll $a$ is low, or during low primary productivity intervals, prokaryotic biomass becomes an increasingly important component of the total planktonic biomass (Kallmeyer et al. 2012). In addition, microzooplankton make up an important component of the energy flow in the ecosystem. They graze on phytoplankton (Murrell et al. 2002) and cyanobacteria, and are an important food resource for higher trophic levels like mesozooplankton (Sipura et al. 2003).

In aquatic environments, studies have shown that microbial communities vary over different time scales (Fuhrman et al. 2006; Gilbert et al. 2012; Jones et al. 2012); however, seasonal fluctuations between phytoplankton and bacteria and the drivers behind these fluctuations are not well understood. Microbial community composition seems to reflect the environment and has shown community turnovers with changes in phosphate and silicate concentrations (Hatsoy et al. 2013). Phytoplankton communities also respond to changes in nutrient concentration at seasonal scales (Kent et al. 2007) and directly influence the microbial composition by excreting nutrients, such as dimethylsulphoniopropionate (DMSP)

*Correspondence: Anni Djurhuus, Faroe Marine Research Institute, Nóatún 1, FO-100 Tórshavn, Faroe Islands. E-mail: anni.djurhuus@gmail.com

Published in collaboration with the Institute of Marine Research, Norway

(Accepted 24 March 2015; first published online 10 July 2015)

© 2015 Taylor & Francis
and dissolved organic matter (DOM), supplying energy to bacteria (e.g. SAR11 and Roseobacter clades) (Azam & Malfatti 2007) and enforcing the microbial loop (Azam et al. 1983).

The heterotrophic bacterioplankton communities in near surface marine environments are most commonly dominated by α-Proteobacteria, γ-Proteobacteria and Bacteroidetes (Suzuki et al. 2001a; Morris et al. 2005; Fuhrman et al. 2006; Alonso-Sáez & Gasol 2007; West et al. 2008; Alonso Gutiérrez et al. 2009). From the α-Proteobacteria, the SAR11 and Roseobacter clades are usually very abundant. From the γ-Proteobacteria, the SAR86 and OM60/NOR5 clades are prominent components and from the phylum Bacteroidetes, the Cytophaga/Flavobacteria group is ubiquitous. Little is still known about the physiological properties of these groups, mainly because in most cases, no representative isolates are available and if they are, physiological and gene expression studies are scarce for various reasons, most often because organisms are difficult to grow (Giovannoni & Stingl 2005, 2007; Giebel et al. 2011). Amongst the photoautotrophic bacteria in marine pelagic environments, Prochlorococcus and Synechococcus (cyanobacteria) are most abundant.

Turbulence may increase the advective transport of nutrients to the cell surface of microorganisms and hence increase nutrient availability, especially to phytoplankton (Margalef 1978; Mann & Lazier 2006). This may be important for large (or chain-forming) diatoms, while small-celled species do not benefit significantly in terms of nutrient uptake from turbulence (Kiorboe 1993; Mann & Lazier 2006). For diatoms, turbulence or vertical mixing is a necessity in order to remain in the photic zone (Kiorboe 1993). The Faroe shelf is, because of its turbulent nature, an environment that favours diatoms. Diatoms usually dominate the phytoplankton species composition on the Faroe shelf, particularly during the spring bloom, but also later during the summer in years when nutrient concentrations remain high (Gaard et al. 1998). However, turbulence alone does not explain a dominance of diatoms in turbulent environments. Under high nutrient conditions, the diatoms must either have higher growth rates than the flagellates, lower mortality rates, or a combination of both (Gaard 1996).

Little is known about the microbial fraction from many marine systems and due to the relatively recent development of the field there are no historical data similar to that of phytoplankton from the Faroe Islands. The initiation of the seasonal spring bloom on the Faroe shelf is highly variable; it can differ inter-annually between late April and early June, while the intensity of the bloom biomass may vary by a factor of five between years (Steingrund & Gaard 2005). In these waters, a significant part of the annual primary production (60–70%) is produced during phytoplankton spring blooms. The variability in timing and intensity of the spring bloom is not well understood but is clearly reflected in higher trophic levels, e.g. weight and recruitment of commercially important fish species such as cod (Gadus morhua Linnaeus, 1758) and haddock (Melanogrammus aeglefinus Linnaeus, 1758), as well as of seabirds, and is therefore of major importance for the entire Faroese ecosystem (Steingrund & Gaard 2005).

When examining the dynamics of primary productivity of the Faroe shelf it is necessary to take the prokaryotic fraction of the ecosystem into account. It is likely that the prokaryotic microorganisms play a significant role in the unexplained variation of the seasonal production on the shelf area, and those are partial drivers of increases in primary and secondary consumers. Detailed knowledge of the drivers and components behind the observed variability in primary production for both eukaryotic and prokaryotic microorganisms is thus essential for understanding the ecosystem. The objective of this study was to give a description of phytoplankton and bacterial seasonality, progression and interactions with the environment. The seasonal change in microbial communities on the Faroe shelf is important due to the impact of fisheries in this region and the historical context, while giving a stronger interpretation of prokaryote dynamics relative to the major phytoplankton bloom species in the region.

In the present study, we examine the bacterial abundance and succession in relation to succession patterns in phytoplankton during a growing season from pre-spring bloom until post-autumn bloom. This is the first study to assess marine prokaryote with eukaryote ecology on the Faroe shelf, describing the seasonal dynamics of phyto- and bacterio-plankton throughout one growth season.

Materials and methods

Study area

The Faroe Islands are situated on the Greenland–Scotland Ridge. A tidal front, at 100–130 m bottom depth, provides a fair, although variable, degree of isolation between the small on-shelf (∼6700 km²) and off-shelf areas (Larsen et al. 2009). Very strong tidal currents prevail on the shelf, which mix the shelf water very efficiently. This results in a totally mixed water column from the surface to the bottom throughout the year without any summer stratification in the shallow areas (Gaard 1996; Gaard et al. 1998). The well mixed and relatively uniform neritic ecosystem is distinct from the oceanic environment outside the front (Gaard et al. 2002; Hansen et al.
both in phytoplankton and zooplankton production, abundance and species composition (Gaard 1996, 1999).

Hereafter, eukaryotic phytoplankton will be referred to as phytoplankton, while prokaryotic phytoplankton will be referred to as cyanobacteria.

Sample collection, nutrients and PAR

Samples were collected at 1 m depth with a polycarbonate water collector once a week for six months from April 2010 to September 2010 at the coastal station Gamlarøtt (G) at 61°57.655’N and 06°49.045’W. Station G is situated in the centre of the Faroe shelf and represents the relatively uniform Faroe shelf water mass (Gaard 1996; Debes et al. 2000). During the whole season Photosynthetically Available Radiation (PAR) was attained from the National Aeronautics and Space Administration’s (NASA) Moderate Resolution Imaging Spectroradiometer on the Aqua satellite (MODISA) project. The PAR data were averaged between the weeks of sampling on a 4 km resolution.

Nutrient samples were collected in acid-rinsed ammonia-free polycarbonate flasks. The samples were preserved in 12 drops of chloroform 100 ml⁻¹ and measured on an autoanalyser according to Grasshoff et al. (1999).

For chlorophyll a 2 l of seawater were filtered through 0.45 μm pore size GF/F filters (Whatman, Maidstone, UK). Pigments were extracted in acetone at 5°C for 24 hours and measured spectrophotometrically according to Parsons et al. (1984).

Phytoplankton

Water samples for phytoplankton species identification and enumeration were preserved with Lugol’s solution (1% final concentration) in dark glass bottles. The phytoplankton was identified with an inverted microscope after overnight settlement in 10 ml Utermöhl chambers (Utermöh 1958; Vaulot et al. 1989; Throndsen & Eikrem 2001; Vaulot et al. 2008). The phytoplankton was identified to generic and when possible to species level. The dimensions of each phytoplankton cell were measured and converted to carbon content based on simple geometrical shapes using a carbon conversion factor of 1.3 for armoured flagellates (Smetacek 1975) and 0.11 for all other phytoplankton (Strathmann 1967; Menden-Deuer & Lessard 2000).

Bacteria

Thirty millilitre water samples were prefiltered through 1.6 μm Millipore glass fibre filters (Whatman), and subsequently filtered through a 0.2 μm pore size membrane (Pall Gelman Inc.) in a Swinnex® filter holder (Millipore) using a 60 ml polypropylene syringe. By doing this, mostly free-living bacteria were included in the samples. The filters were placed in 1.5 ml microcentrifuge tubes, immersed in 200 μl of ATL lysis buffer (Tissue lysis buffer, Qiagen) and stored at 3°C for less than 1 week before DNA extraction. Sampling was carried out according to Suzuki et al. (2001b). One additional sample of 300 ml was filtered to generate a relatively high-DNA content sample, which enabled us to create a standard curve as reference for the relative abundance measures of the bacterial groups.

For further quantification, samples were collected every week for epifluorescence microscopy counts during the second half of the season. Aliquots of 5 ml were fixed to a final concentration of 1% glutaraldehyde at 3°C for 15 minutes and subsequently frozen to −80°C. Upon analysis the samples were prefiltered through a 1.6 μm pore size filter (Whatman, Maidstone, UK) and subsequently filtered on a 0.2 μm pore size filter (Whatman, Maidstone, UK), fixed in 2% formaldehyde and stained with 4’,6-diamidino-2-phenylindole (DAPI). When dry, the filter was placed on a microscope slide and 2 μl of SlowFade® Gold antifade reagent (Invitrogen™ Molecular Probes™) was positioned on a cover glass and at least 2000 cells were counted for each sample.

Genetic analysis

DNA extraction. DNA was extracted with the QIA-symphony DNA mini kit (Qiagen) as outlined by the producer. Briefly, 28 μl of Proteinase K (Qiagen) was added to each 1.5 ml microcentrifuge tube with a sample filter and 200 μl ATL buffer. The samples were incubated and vortexed in a heated block at 56°C and 900 rpm for 2–3 h. After incubation, the supernatant was transferred into 2 ml Sartsted tubes and DNA extracted/purified on the QIAsymphonySP robot (Qiagen) and diluted into 200 μl elution buffer (Qiagen).

Primer specificity of targeted prokaryotic groups. We used primer sets designed to target four phylogenetic groups of marine bacterioplankton: the SAR11 clade of the α-Proteobacteria, Roseobacter of the Roseobacter litoralis Shiba subgroup (RDP tln 2.28.1.8.1.1), the Cytophaga group (Bacteroidetes) (RDP tln 2.15.1.3) and Synechococcus of the cyanobacteria. Group specific primers are described in Suzuki et al. (2001b). Finally, we used the 16S universal primer set 341f-534r (Watanabe et al. 2001) in an attempt to amplify all bacteria (see Table I).
To test primer specificity and achieve optimum annealing temperatures for the polymerase chain reactions (PCR), four test samples were sequenced from various periods of the season. Conventional PCR was performed on these samples (GeneAmp® PCR system 2700, Applied Biosystems) using 2 μl 10xHotStar PCR buffer, 0.2 μl dNTP mix, 0.4 μl (10 μM) forward and reverse primers, 0.15 μl HotStarTaq polymerase (Qiagen: HotStarTaq®PCR (02/2008)) and RNase-free sterile water to a final volume of 20 μl. Cycling parameters were 94°C for 2 min, 25 cycles of 96°C for 10 s, 50°C for 5 s and 55–60°C for 4 min. Primer sets were tested with various annealing temperatures for the four bacterial groups (see Table I), and analysed for specificity by gel electrophoresis (Ultra Pure Gel Agarose, Invitrogen). Samples that yielded defined amplicons were purified with the Jetquick kit (Genomed) and sequenced using the BigDye® Terminator v.1.1 Cycle Sequencing kit (Applied Biosystems). The sequencing products were purified with ethanol precipitation diluted in formamide (Applied Biosystems) and electrophoresed on the 3100 Avant Genetic Analyser (Applied Biosystems). The chromatograms were analysed with the Sequencing Analysis 5.1 software (Applied Biosystems) and quality checked manually. Sequences from the four samples amplified at different annealing temperatures were compared using ClustalX and checked for group specificity using the software Basic Local Alignment Search Tools (BLAST). All sequences showed specificity to prokaryotic members within their own group, similarly to Suzuki et al. (2001b) and Watanabe et al. (2001).

Quantification of prokaryotes. PCR annealing temperatures yielding sequences with best quality (according to the chromatograms) and specificity for each bacterial group were used for quantification. All samples were quantified by real-time PCR on a 7500 Fast System (Applied Biosystems) with the SYBR-green kit (Qiagen: QuantiTect SYBR Green, real-time PCR Kit (08/2008)), with 10 μl reactions containing 5.0 μl 2 × QuantiTect SYBR Green RT-PCR Master mix, 0.5 μl (10 μM) forward and reverse primers and RNase-free sterile water to a final volume of 10 μl. Cycling parameters were 95°C for 15 min, 40 cycles of 94°C for 15 s, 55–60°C (annealing temperature, see Table I) for 30 s and 72°C for 30 s, followed by a dissociation step of 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. For the 16S universal primer set, an additional acquisition step was added (79°C) to avoid fluorescence from primer dimer. This step was not necessary for the other primer set, as was evident from the dissociation curves.

Due to a lack of resources, and thus not being able to clone or get counts of the specific bacterial groups, we produced 10-fold dilution series prepared on the high-DNA content reference sample (300 ml water filtered, 10 × amount of the other samples) with all primer sets. The slope of the dilutions was used for calculating relative abundance of the individual bacterial group. The PCR efficiency was calculated from the slope of the 10-fold dilution series with the formula: $E = 10^{-\frac{b}{m}}$, where $b$ is the slope (Pfaffl 2001). The bacterial group abundances from the samples were then calculated as DNA content relative to DNA content from the high-DNA reference dilution series. The relative DNA content in the samples was calculated from determining the threshold cycle ($C_t$) of the fluorescence from the real-time PCR run and calculating from the efficiency of the run. All calculations assume that the PCR efficiency of the amplicon of interest is constant over time. For each phylogenetic group of bacteria all the samples throughout the season were analysed in the same PCR plate, which minimized concentration variations due to differing PCR efficiency between runs. Real-time PCR efficiencies were higher than 100% (100% <= $E = 2$), and differed

| Primer target | Forward primer | Reverse primer | BP | AT (°C) |
|---------------|----------------|----------------|----|---------|
| Total bacteria | 341f | 534r | 193 | 55 |
| SAR11 | S11-433F | S11-588R | 155 | 60 |
| Bacteroidetes | CYT191F | CYT536R | 345 | 55 |
| Cyanobacteria | PHPICO191F | PHPICO420R | 229 | 60 |
| Roseobacter | ROS292F | ROS567R | 275 | 60 |

Table I. Primers used to target the four phylogenetic groups and total bacteria. BP is base pair length of the product and AT is annealing temperature.
between the phylogenetic groups, which prevented relative abundance comparison between groups due to detection variation for each PCR run. However, the overall trend and within-group abundance is comparable.

The reference dilution series was produced from an environmental sample rather than a known concentration, and because we do not know the abundance of bacteria in the reference sample, we do not have absolute abundances for the different bacterial groups during the season. Therefore, we used arbitrary units to express the amount of bacteria, which are a relative measure compared with the reference sample based on the $C_t$ level. The real-time PCR products from the reference dilution series were run on a DNA 1000 Assay Protocol (Agilent Technologies) to see if the real-time PCR fragments were specific and corresponded to the sequence base pair length according to the primer sets.

**Statistical analysis.** Phytoplankton community structure was assessed using a non-metric multidimensional scaling (NMDS) plot based on two dimensions using Bray–Curtis similarities, with 1000 random permutations to calculate the $P$ value. The data was grouped into spring bloom, no bloom (i.e. summer growth) and autumn bloom. The bloom was defined as a 5% increase from the yearly median of chlorophyll $a$ concentration (Henson & Thomas 2007). **Post hoc** analysis between the three groups, spring bloom, no bloom and autumn bloom, was done with an analysis of similarity (ANOSIM) test (Ramette 2007).

A canonical correlation analysis (CCA) was done to understand the physical drivers of the bacterial and phytoplankton community; a permutation test was performed to check significance of the CCA models. For all ordination analysis the R package ‘vegan’ was used (Oksanen et al. 2013) and the function ordielipse was used to fit a centroid to each group in the NMDS plot representing the 95% standard deviations of their averages. Data were analysed using R, version 3.0.1 (R Development Core Team 2008).

**Results**

**PAR, nutrients and chlorophyll $a$**

PAR increased from the start of the season to late July when it began decreasing again until the end of our survey. The most significant increase of PAR was in early April by more than 50%. In mid season from mid June to early August, when chlorophyll $a$ and phytoplankton abundance were at their lowest, PAR was at its highest at $\approx4000$ Einsteins m$^{-2}$ day$^{-1}$.

In the first sample, on 31 March 2010, the nutrients were at winter concentrations ($\approx12 \mu$M nitrate, see Gaard et al. 1998) and the chlorophyll $a$ concentration was low ($\approx0.4 \mu$g l$^{-1}$), indicating that the phytoplankton spring bloom had not yet initiated. The phytoplankton spring bloom started on 21 April when the chlorophyll $a$ concentration had increased to $>5\%$ of the yearly median (Henson & Thomas 2007), and reached a maximum of 4.43 $\mu$g l$^{-1}$ in late May. During this period, nitrate and silicate concentrations decreased more than 75% from 11.77 $\mu$M to 2.94 $\mu$M and 5.35 $\mu$M to 1.33 $\mu$M, respectively. In early June the chlorophyll $a$ concentration decreased and stayed at a low level during the summer, while the nutrient concentrations increased to 50% of the original concentration (Figure 1).

In late August a small autumn bloom was observed that persisted until mid-September. This bloom was, however, not as clearly reflected in the nutrient concentrations as the spring bloom. Otherwise, there was a strong negative correlation between chlorophyll $a$ and nitrate (Pearson’s $r = -0.72$, $P < 0.001$) and silicate (Pearson’s $r = -0.77$, $P < 0.001$) (see Figure 1). The phosphate concentrations ranged from 0.39 $\mu$M to 1.74 $\mu$M, fluctuations were large in the spring but stabilized over the summer with almost no fluctuations in the autumn (Figure 1). Maximum concentration of phosphate was in mid-May, simultaneous with a small peak in the silicate concentration and a small decrease in chlorophyll $a$. The phosphate did not show any

![Figure 1. Seasonal development of chlorophyll $a$ (green), silicate (blue), nitrate (red) and phosphate (orange) average concentrations collected weekly from early April–late September 2010. The figure shows an inverse trend between chlorophyll $a$ and nutrient concentrations.](image-url)
strong correlations to other nutrients, chlorophyll a, phytoplankton or bacteria.

**Phytoplankton**

Through the whole season 19 different groups of phytoplankton were observed (Supplementary Table SI). There were five different groups of diatoms that were clearly dominating the community at different times through the season (Figure 2). The rest of the phytoplankton groups, including only diatoms (Chaetoceros spp., Paralia sulcata (Ehrenberg) Cleve (Hasle & Syvertsen 1996), Nitzschia spp., Pseudonitzschia spp., Rhizosolenia spp. (Brightwell 1858), Striatella unstinctata (Lyngbye) C.Agardh, Tabellaria flocculosa (Roth) Küting, Thalassiosira spp.), were pooled and named ‘other diatoms’. Altogether only five dinoflagellates, at least four different species, were found and are not included in further analysis. *Coscinodiscus* spp. were the dominant phytoplankton during the initial phase of the spring bloom with cell counts exceeding $45 \times 10^3$ l$^{-1}$. The *Coscinodiscus* spp. cell sizes were on average large, > 3000 μm$^3$, but decreased to ~2300 μm$^3$ after the initial spring bloom in early May, but increased again to ~3300 μm$^3$ in mid-May. The variation in mean size is reflected in the carbon concentration of *Coscinodiscus* spp., where abundance decreased but cell size, and thus carbon content, increased. In mid-May, the carbon biomass of *Coscinodiscus* spp. peaked (Figure 2b), while the smaller *Leptocylindrus danicus* Cleve had increased in abundance to more than $130 \times 10^3$ l$^{-1}$ (Figure 2a), coinciding with the peak in chlorophyll a concentrations in early June. The *L. danicus* cells were small in size, ~200 μm$^3$ on average, and did not show much variation through the spring bloom, but they were more numerous than the *Coscinodiscus* spp. cells and thus accounted for a similar amount of carbon biomass (Table II and Supplementary Table SII). The total phytoplankton carbon biomass largely followed the same pattern as chlorophyll a throughout the whole season (Pearson’s $r = 0.93, P < 0.001$) (Figure 2b). Phytoplankton carbon content and chlorophyll a were highly correlated (Pearson’s $r = 0.93, P < 0.001$). Post spring bloom and during the autumn bloom, chlorophyll a concentrations were relatively high compared with carbon. Due to limitations in microscopy techniques when using 200× magnification, both cyanobacteria and small phytoplankton, such as flagellates, are not included in the carbon calculations. Therefore, carbon estimates are conservative and the chlorophyll to carbon ratio might be overestimated. Assuming that chlorophyll a measures fluorescence from all primary producing plankton, smaller organisms that are unidentifiable with a 200× magnification, such as cyanobacteria and micro-flagellates, are likely to constitute a relatively large fraction of the ‘absent’ carbon concentration after the spring bloom.

The ANOSIM and NMDS ordination plot substantiated the existence of three different phytoplankton phases with significant differences (ANOSIM: $R = 0.63, P < 0.001$) between the spring bloom, no bloom and autumn bloom (see Figure 3). The spring bloom was dominated by *Coscinodiscus* spp. and *L. danicus*, while the post-spring-bloom period was dominated by various diatomic species, all with low abundances. *Fragilaripopsis cylindrus* (Grunow) Krieger (Hällfors 2004) (average size ~100 μm$^3$) and *Eucampia zodiacus* Ehrenberg (average size ~330 μm$^3$) dominated the autumn bloom; however, there was also an increase in the otherwise scarce group ‘other diatoms’ during the *F. cylindrus* and *E. zodiacus* bloom. As mentioned above, ‘other diatoms’ was a mixture of several species and thus...
species with different niches, which had competitive advantages during the changing environment of the autumn bloom. Although the group ‘other diatoms’ as a whole was most abundant during the autumn bloom and before the spring bloom, there were two genera from this group, *Thalassiosira* spp. and *Chaetoceros* spp., both chain-forming, that bloomed during the spring bloom rather than the autumn bloom; however, neither of them were abundant (<1000 cells l\(^{-1}\)).

In Table II it is clear that the larger phytoplankton species, *Coscinodiscus* spp. and *E. zodiacus* had relatively higher biomass than they were abundant, especially compared with the relatively abundant *L. danicus* and *F. cylindrus*, which show relatively lower biomass, reflecting the importance of size when considering the contribution of biomass to the food web.

The CCA revealed that when PAR was high *L. danicus* was abundant. In addition, PAR also had some effect on *Coscinodiscus* spp. abundance, with both species falling within the dimensions of the spring-blooming phytoplankton (Figure 5). The environmental variables (constrained) explained 53% of the inertia of the phytoplankton and bacteria (unconstrained) with a statistically significant permutation test on the model (\(P = 0.005\)). PAR is negatively correlated to all nutrients, most likely due to the strong correlation to the abundant spring-blooming phytoplankton. The phytoplankton species abundant during the autumn bloom are mostly positively correlated to nitrate and silicate.

**Bacteria**

Correspondingly, studies in high latitude total bacterial abundance fluctuated greatly, and there was often more than two-fold variation between consecutive weeks (Figure 4a) (e.g. Iversen & Seuthe 2011). From May to mid-July, bacteria genetic

Table II. Cumulative percentage, from the whole sampling season of the six individual phytoplankton groups relative to total carbon biomass and total abundance.

|                         | *Coscinodiscus* | *Leptocylindrus danicus* | *Eucampia zodiacus* | *Fragilariopsis cylindrus* | Benthic algae | Other diatoms |
|-------------------------|-----------------|--------------------------|---------------------|---------------------------|---------------|--------------|
| % of total biomass (C)  | 34.00           | 32.64                    | 3.10                | 11.69                     | 4.15          | 14.42        |
| % of total abundance    | 14.69           | 36.00                    | 1.06                | 20.10                     | 10.92         | 17.24        |

Figure 3. NMDS ordination plot of the phytoplankton community composition grouped according to bloom period (Stress = 0.09). The shaded area represents the 95% confidence interval.
concentrations oscillated around a mean concentration (0.049). From highest to lowest abundance, the DAPI counts decreased ~80% from ~820 to ~160×10³ cells ml⁻¹ and based on the universal primer quantification there is a similar decrease from the highest concentration on 20 July to the lowest on 7 September (0.13 to 0.005, respectively). The same fluctuations are seen in the genetic data and DAPI counts (Pearson’s $r = 0.90; P < 0.001$) (Figure 4a), indicating that the data based on real-time PCR is representative for the total bacteria community. After the lowest concentration in September, the bacterial abundance increased again to just above mean concentration.

The cyanobacteria and SAR11 blooms peaked on 20 July, coinciding with the total bacteria peak and the chlorophyll $a$ minimum (Figure 4b) (Pearson’s $r = 0.79, P < 0.001$ and Pearson’s $r = 0.86, P < 0.001$, respectively). The cyanobacteria concentration in the peak is an order of magnitude higher (1.18E-5) than its average concentration (1.0E-06) during the season. The SAR11 concentrations were low in the start of the season until the end of June and then increased considerably for four consecutive weeks and peaked with a concentration of 0.015 (mean concentration = 0.004) (Figure 4). The rapid increase in SAR11 followed the cessation of the phytoplankton spring bloom. SAR11 concentrations dropped from the highest peak to below mean in one week and varied around mean concentration for the rest of the season.

Four distinct peaks, all of which took a few weeks to build up and only one week to collapse, characterized the fluctuations in Bacteroidetes concentration, with a significant correlation to total bacterial concentration (Pearson’s $r = 0.48, P < 0.05$). The third peak coincides with the total bacteria, cyanobacteria and SAR11 peaks and chlorophyll $a$ minimum (Figure 4). Mean concentration was 0.0026 and the highest concentration on 25 May was 0.0051, an increase of ~50%. On 7 September, after the autumn peak, the lowest Bacteroidetes concentration occurred, coinciding with the decreased concentrations of all the bacterial groups.

Unlike the other bacterial groups, Roseobacter appeared to bloom from mid-May until late July, with the highest peak on 8 June at 1.2E-4. The
fluctuation in the *Roseobacter* group is different from the total bacteria, not showing any significant relationships between them (Pearson’s $r = 0.28$, $P = 0.22$) (Figure 4). The initiation of the *Roseobacter* bloom coincided with decreasing nitrate and silicate concentrations and the peak coincides with the lowest nitrate concentration. For the rest of the season, *Roseobacter* levels remained low.

On the CCA we can see that all the bacterial groups fall in the centre of the plot; however, *Roseobacter* and *Bacteroidetes* are a little closer to PAR, which is negatively correlated to silicate and nitrate (Figure 5), and *Roseobacter* can be observed closer to phosphate than any of the other bacterial groups. All groups appear to be virtually equally affected or not affected by the environmental parameters measured in this study. As mentioned above, the permutation tests on the CCA (Figure 5) displayed a significant model ($P = 0.005$). *Roseobacter* fell within the same dimensions as *Leptocylindrus danicus* and the corresponding spring-bloom samples and was mostly affected by PAR. Most bacterial species showed some correlation with silicate, most likely due to the increase of silicate after the spring bloom following the succession of bacteria after cessation of the phytoplankton bloom.

**Discussion**

**Phytoplankton**

Our findings support what has been reported in previous literature about phytoplankton succession and seasonal fluctuations (Gaard et al. 1998). In addition, this study provides a foundation with new relevant information about the, so far, unknown prokaryote fraction of the marine ecosystem around the Faroe Islands. During the initial phase of the spring-bloom, the group of ‘other diatoms’, i.e. a combination of several species, constituted a large fraction (~50%) of the phytoplankton population, demonstrating that several species are able to benefit from advantageous conditions present during the spring season. As spring blooms formed, the nitrate and silicate concentrations co-fluctuated inversely with the phytoplankton, as was expected when the bloom was completely dominated by diatoms (Townsend et al. 1994). As a group, diatoms have a Redfield ratio of $C:Si:N:P = 106:15:16:1$ (Brzeziński 1985), and nitrate concentrations never reached a critical level and consequently did not limit growth. Silicate concentration was low during most of the bloom period, and when it fell below 2 $\mu$M, which is considered limiting for diatoms as a group (Egge & Aksnes 1992; Jacobsen et al. 1995), the peak chlorophyll $a$ concentration decreased and the phytoplankton species composition changed from dominance by the large *Coscinodiscus* to the smaller *Leptocylindrus danicus*. Towards the shift from *Coscinodiscus* to *L. danicus* the former cells became smaller, representing the plasticity within the *Coscinodiscus* genus as the genus adapts to the scarcity of nutrients by increasing its relative cell surface to cell volume ratio. The *Coscinodiscus* group was not identified to species level due to limitations in techniques available. A scanning electron microscope could have revealed further oscillations between species within the *Coscinodiscus* genus; however, we believe that the overall impact of the genus is still acceptable for interpretation of the ecological data.

The sequential bloom of different phytoplankton species occurs because the different optimal nutrient concentrations for uptake or other environmental influences cause growth peaks at differing times. The observed shifts from large to small phytoplankton cells most likely occurred due to a competitive advantage of the smaller cells, such as those of *L. danicus*, when nutrients became limiting (Kierboe 1993). As nitrate never fell below growth limiting concentrations but silicate did, the latter is considered a more likely limiting nutrient than the former. Both silicate and nitrate increased immediately following the spring bloom, disrupting nutrient availability causing growth limitation of diatoms during the summer. Availability of nutrients infers active supply, likely via inflow from tidal movements and physical mixing with the oceanic environment outside the front, implying that the Faroe shelf is not completely isolated from the open ocean surroundings despite the persistent tidal front. Thus, the cessation of the phytoplankton bloom suggests a bottom-up physical control through water mass dynamics (i.e. horizontal loss mechanisms) (Elíassen et al. 2005; Hansen et al. 2005; Debes et al. 2008b) on the phytoplankton abundance and progression or through biological effects from cell lysis by bacterial and virus infections (Sutcliffe 2005).

The CCA (Figure 5) clearly demonstrates how *Coscinodiscus* spp. and *L. danicus* are associated with the spring bloom and that they heavily rely on nitrate, silicate and PAR. *Coscinodiscus* spp. were more negatively correlated with silicate and *L. danicus* with nitrate, suggesting differences in substrate adaptation between the two genera, with the initial *Coscinodiscus* peak being completely limited by silicate. Diatoms usually dominate the phytoplankton community on the Faroe shelf, but when silicate is limited, the balance may shift towards dominance of small flagellates or mixture with dinoflagellates (Debes et al. 2008b). However, in years with high nutrient concentrations during the summer, diatoms
may continue to dominate in terms of biomass (Gaard et al. 1998), which is the pattern seen in 2010, where the nutrient concentrations after the spring bloom increased by over 50% of winter concentrations. During 2010 the dinoflagellates were always found in low abundance and comprise <0.1% of the total phytoplankton abundance and biomass. Dinoflagellates usually do not dominate on the shelf, most likely because of the strong tidal currents and vertical mixing.

It is crucial to keep in mind that our measurements of phytoplankton chlorophyll a only give an estimate of the standing stock and not the production. Although chlorophyll a and carbon concentrations decreased as the summer approached, it is not evident that the phytoplankton production ceased. Nonetheless, the increase in nutrients (or rather the lack of a decrease in nutrients) indicates that the new primary production declined and never reached the same magnitude as was previously achieved during the rest of our study period. This was especially important after the first few weeks of the phytoplankton spring bloom, when the zooplankton abundance most likely had increased, and therefore the potential grazing impact on the phytoplankton also increased (Gaard et al. 1998). Detailed measurements of ingestion rates at stage level of different copepod species on the Faroe shelf revealed a maximum daily grazing impact of only 3.4% of the phytoplankton standing stock during a spring bloom (Debes et al. 2008a). However, in the same study the authors showed a substantially higher grazing impact by the zooplankton community later during the season when the abundance of bacteria in this study was the greatest. The initiation of the autumn bloom might be facilitated by a decrease in grazers, while the cessation of the autumn bloom is most likely due to light limitation. The autumn bloom is composed of different species than the spring bloom; the autumn bloom phytoplankton community is more mixed, but dominated by *Fragilariopsis cylindrus* and *Eucampia zodiacus*, which are much smaller than species of the genus *Coscinodiscus*. The very large-sized *Coscinodiscus* and *Striatella unipunctata* were present during the autumn bloom at very low abundances, but it seems that the conditions in the autumn are not favourable for these species, while *F. cylindrus* and *E. zodiacus* apparently have some traits, e.g. endurance of low light intensities, which give them a competitive advantage. Another possible reason for the observed succession is that large species are still being more heavily grazed upon and that the dominant species are more resistant to grazing.

**Bacteria**

Bacteria are consumed by ciliates and other heterotrophs; these heterotrophs are consumed by smaller zooplankton, which incorporate bacterially derived nutrients into the planktonic food web, known as the microbial loop (Azam et al. 1983). Teeling et al. (2012) showed that distinct populations of bacteria specialize in successive decomposition of algal-derived organic matter. Algal substrate availability further provided a series of ecological niches in which specialized populations could bloom. In all the samples from 27 April–21 September, all the phylogenetic groups of bacteria were found. During summer on 20 July, in between the phytoplankton spring and autumn bloom, there is a clear proliferation of the total bacteria, SAR11, *Bacteroidetes* and cyanobacteria. Bacterial DAPI counts correspond well to total bacterial abundance based on the 16S rRNA gene variations. This indicates that primer set 341f/534r represents the total bacterial population, which is within the estimated bacterial abundance in seawater (Kirchman 2008). We were unable to obtain relative or absolute quantities of any bacterial groups other than total bacteria from the DAPI counts. Ramakers et al. (2003) proposed an alternative software for quantification and PCR efficiency of the samples from real-time PCR, which is based on linear regressions of the amplification curve. For more accurate calculations of the abundances of the phylogenetic groups, the software proposed in Ramakers et al. (2003) would be a good alternative; however, we would argue that because of the close correlation between DAPI counts and the total bacteria from the universal primers, our data are a reflection of the real trend.

During our sampling season, the cyanobacterial bloom, after the phytoplankton spring bloom had ceased, probably constitutes a relatively large fraction of primary production and carbon fixation during low phytoplankton abundance. In temperate eutrophic environments, low concentrations (<10% of bulk chlorophyll) of cyanobacteria in relation to phytoplankton blooms are repeatedly observed (Neufeld et al. 2008; Schattenhofer 2009), yet they may play a key role during low phytoplankton stock or heavy grazing. During the cyanobacteria bloom, small-scale variations in phytoplankton carbon content and variations in cyanobacteria abundance show a negative relationship. This indicates a niche overlap and hence competitive exclusion of cyanobacteria when diatoms are abundant.

Throughout the sampling season, it is apparent that when total bacteria abundance increases, both SAR11 and *Bacteroidetes* are generally abundant as well. *Bacteroidetes* have been reported several times in relation to phytoplankton blooms (Suzuki
et al. 2001b; Fandino et al. 2005; Neufeld et al. 2008). In this study, the Bacteroidetes group had two distinct peaks during the phytoplankton spring bloom, which can be assigned to the phytoplankton Coscinodiscus spp. and Leptocylindrus danicus, respectively (Figures 2, 4). In the sample on 1 June, the abundance of both Coscinodiscus spp. and Bacteroidetes had decreased drastically. In the subsequent week the L. danicus peak decayed and Bacteroidetes peaked again and successively decreased rapidly, indicating that the Bacteroidetes community responds to different phytoplankton species. Members of the Bacteroidetes are thought to be the main consumers of high-molecular-weight dissolved organic matter released during the waning of phytoplankton blooms (Kirchman 2008). Similar to Bacteroidetes, the significant increase in SAR11 could be due to higher concentrations of a previously limiting nutrient released by decaying phytoplankton. It has been established that SAR11 depends on exogenous sulphur (Tripp et al. 2008), but because of its long generation time, the SAR11 peak might be a consequence of a slow but steady increase in SAR11 abundance after the phytoplankton spring bloom.

Marañón et al. (2007) showed that small phytoplankton or bacteria are unlikely to dominate phytoplankton blooms, simply because they have lower intrinsic growth rates than larger species. This characteristic, together with niche overlap, would explain one of the reasons most bacterial abundances were highest in our samples, while the phytoplankton chlorophyll a and carbon were at their lowest.

The only bacterial group that showed a bloom with the phytoplankton was Roseobacter. The Roseobacter bloom started a couple of weeks later than the start of the phytoplankton spring bloom. This outcome could be due to an increase in DOC content because of phytoplankton decay. This is supported by Giebel et al. (2011), who found a positive correlation between phaeopigments, a proxy for decaying phytoplankton, and RCA bacteria, an abundant clade of the Roseobacter group. Alderkamp et al. (2006) found similar outcomes during a phytoplankton bloom in the North Sea. Furthermore, the negative correlation between nitrate and Roseobacter from this study could indicate that Roseobacter has a competitive advantage in low inorganic nutrient conditions. Attempts in Roseobacter cultivation show that unknown organic compounds (from marine broth) are vital for successful growth of RCA (Giebel et al. 2011). Therefore, it is more likely that the correlation between Roseobacter and nitrate arises because both are affected by phytoplankton growth/decay. The specificity of the Roseobacter primer set from Suzuki et al. (2001a) used in this study has been reanalysed by Giebel et al. (2011), who found that it does not target all Roseobacter genera and even a few non-Roseobacter phyotypes. Furthermore, Fandino et al. (2005) used a modified version of primer CYT191F and CYT536R from Suzuki et al. (2001a). Therefore, it is likely that some Roseobacter strains have not been detected in this study, but the data still give us an approximation of the abundance of the Roseobacter clade.

Biotic and abiotic influence on microbial progression

Variations in early development of the spring bloom may be explained by the variable exchange between shelf and off-shelf waters (Eliasen et al. 2005; Hansen et al. 2005). In years with a strong exchange, nutrients are regularly imported onto the shelf, which should stimulate the primary production, causing an increase in nutrients after a spring bloom as seen in 2010 with the influx of nutrients after the phytoplankton bloom. Strong exchange rates can enhance the physical transport of phytoplankton out of the shelf area, decreasing the chlorophyll a concentrations. Therefore, a strong exchange should result in a lower chlorophyll concentration (Rasmussen et al. 2014).

The North Atlantic subpolar gyre has declined since the early 1990s (Häkkinen & Rhines 2004), leading to a lower contribution of nutrient-rich subarctic waters from the west and an increased contribution of nutrient-poor subtropical waters from the Bay of Biscay region to the mixing region west of the British Isles (Hätün et al. 2005). This has gradually reduced silicate concentrations in the Atlantic waters along the slope from the Faroe–Shetland region to the Barents Sea (Rey 2012). In the southern Nordic Seas, the pre-bloom (winter) concentrations of silicate have declined from around 6 µM to about 4.5 µM in 2010 (Rey 2012). Realizing that silicate becomes limiting for diatom growth at about 2 µM (Egge & Aksnes, 1992), this might imply a ~38% reduction in the potential diatom production. The pre-bloom silicate concentrations on the Faroe shelf have also declined slightly (Sólva Jacobsen, January 2015, personal communication) and increasingly stronger interruptions and fluxes in the spring bloom (as inferred from the on-shelf chlorophyll a concentrations) have been observed during the last decade (Faroe Marine Research Institute 2015). We suggest that these interruptions are associated with community shifts from dominantly large and fast-growing diatoms to either smaller diatoms or non-diatom primary producing communities, due to silicate limitation. This could
specifically be in situations where the first diatom species to dominate the community relies heavily on the silicate, causing its concentration to become limiting for further growth of that same species.

Steingrund & Gaard (2005) used an index for estimation of the new primary production based on nitrate reduction from late winter until late June, plus the influx of nutrients from the surrounding oceanic environment. Even though the chlorophyll a concentrations in 2010 were not high compared with other years (Hansen et al. 2005; Debes et al. 2008b), the new primary production index was ~10.8 in 2010, which is above the average of 9.1 (~160gCm$^{-2}$y$^{-1}$) for the period 1997–2010. This result implies that the chlorophyll a and phytoplankton biomass measurements were affected by loss, such as zooplankton grazing. Some regenerated production probably also occurred from recycled nutrients such as organic materials (e.g. urea) and from bacterial transformation (Galloway et al. 2004). All phylogenetic groups of bacteria showed some response to the phytoplankton spring bloom, either after decay or during the absence of competition from phytoplankton. The phytoplankton predominantly facilitated heterotrophic bacterial growth apart from the small peak in cyanobacteria. The coupling between prokaryotic and eukaryotic microorganisms and the dynamics of secondary production is essential for consideration in the microbial loop, which, through a series of trophic relationships between diverse organisms, makes up the marine food web (Azam et al. 1983). In ecosystems, or during periods dominated by picoplankton (bacteria), the trophic pathway to mesozooplankton production is relatively inefficient and the mesozooplankton become food-limited during low phytoplankton productivity. On the Faroe shelf, bacteria show an immense variation and abundance, which may have a great effect on the ecosystem. Bacteria and phytoplankton communities are the basis of the ecosystems on which all higher trophic levels rely. The sheer numbers of bacteria indicate that they have an effect on food supply and nutrient remineralization. Fouilland et al. (2014) showed that under high nutrient conditions 50–66% of the carbon released by phytoplankton was reused in bacterial production, implying a strong dependency on freshly produced phytoplankton exudates during the initial phase leading to phytoplankton blooms. Thus, the bacterial biomass is directly dependent on the phytoplankton bloom, which then recycles nutrient by degrading the phytoplankton cells. *Roseobacter* and *Bacteroidetes* were the two groups most abundant during this phase and most likely rely heavily on the fresh phytoplankton growth (Figure 5). However, grazing and viral lysis (20–40% of the total stock) within the microbial food web play a larger role in sustaining bacterial production than carbon from phytoplankton exudates (Suttle 2005; Fouilland et al. 2014). Also, low nutrient conditions, such as during summer, prove that bacterial production relies more on organic matter released from strong grazing pressure on microorganisms (phytoplankton and prokaryotes), indicating the important role of the microbial carbon recycling in such conditions. This creates a seasonal flux leading from phytoplankton to heterotrophic bacteria, enabling the flux of nutrients to complete the microbial loop.

Our study appears to be in agreement with previous field studies suggesting that heterotrophic bacteria are closely associated with phytoplankton blooming species in coastal marine environments (Rooney-Varga et al. 2005). For further studies, flow cytometry and next-generation sequencing would be an improved way to analyse the microorganisms, thus allowing for a higher taxonomic resolution of the overall ecology of the marine bacteria in relation to phytoplankton. In addition, it would be ideal to relate these data to the production of both phytoplankton and bacteria to quantify the variability within each group.

This study contributes new and fundamentally important information about the processes within and interactions between the bacterial community and primary producers on the Faroe shelf. It provides information about the key parameters that contribute to the ongoing endeavour to understand the functioning of this rather complex ecosystem.

**Acknowledgements**

The authors would like to acknowledge the Food and Veterinary Agency, especially Ann Siri Borg Hentze, Kristín Baldvinsdóttir and Marita Næs, for their helpful cooperation and assistance with all the genetic research. From the Faroe Marine Research Institute we would like to thank Sólvi Jacobsen, Dánjál Petur Høigaard and Petur Steingrund for encouragement and Eilíf Gaard for help with phytoplankton identification and intellectual input. Fróðskaparsetur Føroya is acknowledged for academic encouragement and Eilíf Gaard for help with phytoplankton exudates. Mikalsen for taking an interest in our project.

**Supplementary material** (Table SI, SII)

The supplementary material for this article is available via the Supplemental tab of the article’s online page at http://dx.doi.org/10.1080/17451000.2015.1041532

**References**

Agardh CA. 1832. *Conspectus criticus diatomacearum. Lundae [Lund]: Literis Berlingianus Part 4:49–66.**
Alder kamp AC, Sintes E, Herndl GJ. 2006. Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer. Aquatic Microbial Ecology 45:237–46.

Alonso Gutiérrez J, Lekunberri I, Teira E, Gasol JM, Figueras A, Novoa B, et al. 2009. Bacterioplankton composition in the upwelling system of ‘Ría de Vigo,’ NW Spain. FEMS Microbiology Ecology 70:493–505.

Alonso–Siez L, Gasol JM. 2007. Seasonal variation in the contribution of different bacterial groups to the uptake of LMW–DOM compounds in NW Mediterranean coastal waters. Applied Environmental Microbiology 73:3528–35.

Azam F, Malfatti F. 2007. Microbial structuring of marine ecosystems. Nature Reviews Microbiology 7:582–91.

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10:257–63.

Brightwell T. 1858. Remarks on the genus Rhyzosolenia of Ehrenberg. Quarterly Journal of Microscopical Science 6:93–95.

Breznizki MA. 1985. The Si:C:N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables. Journal of Phyiology 21:347–57.

Clevé PT. 1889. Peliagisk Diatomeer från Kattegat. In: Petersen CGJ, editor. Det Videnskabelige Udbytte af Kanonbaaden – 1883–86. Copenhagen: A.F. Høst & Søn, p 53.

Debes H, Eliasen K, Gaard E. 2008a. Seasonal variability in copepod ingestion and egg production on the Faroe shelf. Hydrobiologia 600:247–65.

Debes H, Gaard E, Hansen B. 2008b. Primary production on the Faroe Shelf: Temporal variability and environmental influences. Journal of Marine Systems 74:686–97.

Egge JK, Asnes DL. 1992. Silicate as regulating nutrient in phytoplankton competition. Marine Ecology Progress Series 83:281–89.

Eliasen SK, Gaard E, Hansen B, Larsen KMH. 2005. A horizontal Sverdrup mechanism may control the spring bloom around small oceanic islands and over banks. Journal of Marine Systems 56:352–62.

Fandino LB, Riemann L, Steward GF, Azam F. 2005. Population dynamics of Cytophaga–Flavobacteria during marine phytoplankton blooms analyzed by real-time quantitative PCR. Aquatic Microbial Ecology 40:251–57.

Faroe Marine Research Institute. 2015. http://www.hav.fo/index.php?option=com_content&view=article&id=15&Itemid=120 (accessed 28 January 2015). (in Faroese and English)

Fouilland E, Tolosa I, Bonnet D, Bouvier C, Bouvy M, Got P, Hallegraeff GM. 2008. The ISME Journal 5:8–19.

Gilbert JA, Steele JA, Caporaso JG, Steinbrück L, Reeder J, Temperton B, et al. 2012. Defining seasonal marine microbial community dynamics. The ISME Journal 6:298–308.

Giovannoni SJ, Sturl U. 2005. Molecular diversity and ecology of microbial plankton. Nature 437:343–48.

Giovannoni S, Stirling U. 2007. The importance of culturing bacterioplankton in the ‘omics’ age. Nature Reviews Microbiology 5:820–26.

Grasshoff K, Kremling K, Ehrhardt M, editors. 1999. Methods of Seawater Analysis. 3rd edition. Weinheim, Germany: Wiley-VCH. 632 pages.

Häkkinen S, Rhines PB. 2004. Decline of subpolar North Atlantic circulation during the 1990s. Science 304:555–59.

Hålffors G. 2004. Changes of Baltic Sea phytoplankton species (including some heterotrophic protestan groups). Baltic Sea Environment Proceedings 95:1–210.

Hansen B, Eliasen SK, Gaard E, Larsen K. 2005. Climatic effects on plankton and productivity on the Faroe Shelf. ICES Journal of Marine Science 62:1224–32.

Hasle GR, Syvertsen EE. 1996. Marine diatoms. In: Tomás CR, editor. Identifying Marine Phytoplankton. San Diego: Academic Press, p 5–38.

Hatoxy SM, Martiny J BH, Sachdeva R, Steele J, Fuhrman JA, Martiny AC. 2013. Beta diversity of marine bacteria depends on temporal scale. Ecology 94:1898–904.

Hätun H, Sandø AB, Drange H, Hansen B, Valdimarsson H. 2005. Influence of the Atlantic subpolar gyre on the thermohaline circulation. Science 309:1841–44.

Henson SA, Thomas AC. 2007. Interannual variability in timing of bloom initiation in the California Current System. Journal of Geophysical Research 112:C08007. 12 pages.

Iversen KR, Seutele L. 2011. Seasonal microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): I. Heterotrophic bacteria, picoplankton and nanoflagellates. Polar Biology 34:731–49.

Jacobsen A, Egge JK, Heimdal BR. 1995. Effects of increased concentration of nitrate and phosphate during a spring bloom experiment in mesocosm. Journal of Experimental Marine Biology and Ecology 187:239–51.

Jones SE, Cadkin TA, Newton RJ, McMahan KD. 2012. Spatial and temporal scales of aquatic bacterial beta diversity. Frontiers in Microbiology 3:x198. 10 pages.

Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D' Hondt S. 2012. Global distribution of microbial abundance and biomass in subsurface sediment. Proceedings of the National Academy of Sciences 109:16213–16.

Kent AD, Yannarell AC, Rusak JA, Triplett BW, McMahan KD. 2007. Synchrony in aquatic microbial community dynamics. The ISME Journal 1:38–47.

Kierboe T. 1993. Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Advances in Marine Biology 29:1–72.

Kirchman DL, editor. 2008. Microbial Ecology of the Oceans. Chichester, UK: Wiley. 620 pages.

Kützing FT. 1844. Die Kieselalgen des Kaspischen Meeres, der Schwarzen See, der Adriatik, der Ägäis und der Tarent mediterraneorum. Copenhagen: Haubits & Søn, 354 pages.

Lefèvre P, Trouet B, Beal D, Leppar G, Stal L-J. 2014. Bacterial carbon dependence on freshly produced LMW-DOM compounds in NW Mediterranean coastal marine sediments. Annual Review of Ecology and Systematics 45:1–31.

Sherman K, Skjoldal H-R, editors. Large Marine Ecosystems of the North Atlantic. Amsterdam: Elsevier, 345 pages.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, et al. 2004. Nitrogen cycles: Past, present, and future. Biogeochemistry 70:153–226.

Gatto JP, Frankignoulle M, Wollast R. 1998. Carbon and carbonate metabolism in coastal aquatic ecosystems. Annual Review of Ecology and Systematics 29:405–34.

Giebel H-A, Kalhoefner D, Lemen A, Thole S, Gahl-Janssen R, Simon M, Brinkhoff T. 2011. Distribution of Roseobacter RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. The ISME Journal 5:1–19.

Häkkinen S, Rhines PB. 2004. Decline of subpolar North Atlantic circulation during the 1990s. Science 304:555–59.
Tenacibaculum amylolyticum gen. nov. with comb. nov., and description of Tenacibaculum sp. nov. and comb. nov. and sp. nov.

Schattenhofer M. 2009. Distribution of Major Bacterioplankton Groups in the Atlantic Ocean. Doctoral Thesis. University of Bremen, Germany. 178 pages.

Simon M, Cho BC, Azam F. 1992. Significance of bacterial biomass in lakes and the ocean: Comparison to phytoplankton biomass and biogeochemical implications. Marine Ecology Progress Series 86:103–10.

Sipura J, Lores E, Snyder RA. 2003. Effect of copepods on estuarine microbial plankton in short-term microcosms. Aquatic Microbial Ecology 33:181–90.

Smetacek V. 1975. Die Sukzession des Phytoplankton der westlichen Kieler Bucht. Doctoral Thesis. University of Kiel, Germany. 131 pages.

Steingrund P, Giard E. 2005. Relationship between phytoplankton production and cod production on the Faroe Shelf. ICES Journal of Marine Science 62:163–76.

Strathmann RR. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnology and Oceanography 12:411–18.

Suttle CA. 2005. Viruses in the sea. Nature 437:356–61.

Suzuki M, Nakagawa Y, Harayama S, Yamamoto S. 2001a. Phylogenetic analysis and taxonomic study of marine Cytophaga-like bacteria: Proposal for Tenacibaculum gen. nov. with Tenacibaculum maritimum comb. nov. and Tenacibaculum ovalvum comb. nov., and description of Tenacibaculum mesophilum sp. nov. and Tenacibaculum amolyticum sp. nov. International Journal of Systematic and Evolutionary Microbiology 51:1639–52.

Suzuki MT, Preston CM, Chavez FP, Delong EF. 2001b. Quantitative mapping of bacterioplankton populations in seawater: Field tests across an upwelling plume in Monterey Bay. Aquatic Microbial Ecology 24:117–27.

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Benneke CM, et al. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science 336:608–11.

Throndsen J, Eikrem W. 2001. Marine mikroalger i farger. Oslo: Almater Forlag AS. 188 pages. (in Norwegian)

Townsend DW, Cammen LM, Holligan PM, Campbell DE, Pettigrew NR. 1994. Causes and consequences of variability in the timing of spring phytoplankton blooms. Deep Sea Research I 41:747–65.

Tripp HJ, Kitzer JB, Schwabach MS, Dacey JWH, Wilhelm LJ, Giovannoni SJ. 2008. SAR11 marine bacteria require exogenous reduced sulphur for growth. Nature 452:741–44.

Utermöhl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitteilung der Internationale Vereinigung für Theoretische und Angewandte Limnologie 9:1–38.

Vaulot D, Courtes C, Partensky F. 1989. A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry 10:629–35.

Vaulot D, Eikrem W, Viprey M, Moreau H. 2008. The diversity of small eukaryotic phytoplankton (≤ 3μm) in marine ecosystems. FEMS Microbiology Reviews 32:795–820.

Watanabe K, Kodama Y, Harayama S. 2001. Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. Journal of Microbiological Methods 44:253–62.

West NJ, Obernosterer I, Zemb O, Lebaron P. 2008. Major differences of bacterial diversity and activity inside and outside of a natural iron-fertilized phytoplankton bloom in the Southern Ocean. Environmental Microbiology 10:738–56.

Editorial responsibility: Hongyue Dang