Active bacterioplankton community response to dissolved 'free' deoxyribonucleic acid (dDNA) in surface coastal marine waters.

Joe D Taylor[1,4], Kimberley E Bird[1,2,] Claire E Widdicome[3] & Michael Cunliffe[1,2]

[1]Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth.

[2]Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, Plymouth University, Drake Circus, Plymouth.

[3]Plymouth Marine Laboratory, Prospect Place, Plymouth.

[4]School of Environment and Life Sciences, University of Salford, Salford.

Corresponding author: Michael Cunliffe
Marine Biological Association of the United Kingdom,
The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK.
E: micnli@mba.ac.uk

Running title: Marine bacterioplankton assimilation of free DNA

Keywords: DNA, bacterioplankton, stable-isotope probing, DON, DOC, DOP

Abstract

Seawater contains dissolved 'free' DNA (dDNA) that is part of a larger <0.2 μm pool of DNA (D-DNA) including viruses and uncharacterised bound DNA. Previous studies have shown that bacterioplankton readily degrade dDNA and culture-based approaches have identified several potential dDNA-utilising taxa. This study characterised the seasonal variation in D-DNA concentrations at Station L4, a coastal marine observatory in the Western English Channel, and linked changes in concentration to cognate physicochemical and biological factors. The impact of dDNA addition on active bacterioplankton communities at Station L4 was then determined using 16S rRNA high-throughput sequencing and RNA Stable Isotope Probing (RNA SIP) with ^{13}C-labelled diatom-derived dDNA. Compared to other major bacterioplankton orders, the Rhodobacterales actively responded to dDNA additions in amended microcosms and RNA SIP identified two Rhodobacterales populations most closely associated with the genera *Halocynthiibacter* and *Sulfitobacter* that assimilated the ^{13}C-labelled dDNA. Here we demonstrate that dDNA is a source of dissolved organic carbon for some members of the major bacterioplankton group the Marine Roseobacter Clade. This study enhances our understanding of roles of specific bacterioplankton taxa in dissolved organic matter cycling in coastal waters with potential implications for nitrogen and phosphorus regeneration processes.

Introduction

Phytoplankton growth, death and lysis releases large amounts of dissolved organic matter (DOM) (Agustí & Duarte, 2013), which is a vital resource for heterotrophic bacterioplankton through the microbial loop (Azam *et al.*, 1983). In addition to carbon for bacterioplankton growth, DOM also provides accessible nitrogen and phosphorus, particularly after phytoplankton blooms when inorganic nutrients are at lower concentrations (Smyth *et al.*, 2010). The dynamics of the production and subsequent bacterioplankton cycling of DOM remain poorly understood, in part, because many of the complex specific DOM compounds are currently poorly characterised (McCarthy *et al.*, 1993, McCarthy *et al.*, 1997, Benner, 2002).

A distinct component of the seawater DOM pool are nucleic acids such as deoxyribonucleic acid (DNA). Of the total seawater DNA pool that is operationally defined as <0.2 μm (D-DNA), much is dissolved 'free' DNA (dDNA) as well as DNA viruses and uncharacterised bound DNA (Brum, 2005). DNA is ubiquitous throughout the marine environment with variable concentrations (Table 1) (DeFlaun *et al.*, 1987, Karl & Bailiff, 1989). Studies in the Aegean Sea have shown that seawater DNA concentrations are higher during summer months (June to October) than winter months (November to May), coinciding with periods of increased primary productivity (Weinbauer *et al.*, 1993, Weinbauer *et al.*, 1995). Seawater DNA concentrations are also higher in surface coastal waters than in open ocean and deeper waters (DeFlaun *et al.*, 1987, Boehme *et al.*, 1993, Weinbauer *et al.*, 1995, Brum, 2005), suggesting that DNA has a strong temporal variation related to plankton biomass and activity.

Previous studies have shown that dDNA is readily degraded and assimilated by marine bacterioplankton (Maeda & Taga, 1974, Paul *et al.*, 1987, Paul *et al.*, 1988), however few studies have identified specific bacterioplankton taxa actively involved in the cycling of dDNA. Lennon (2007) isolated dDNA-utilising bacteria from seawater collected from Eel Pond (Woods Hole, MA) using dDNA-containing growth media and identified several genera, including *Vibrio*, *Alteromonas*, *Pseudoaltermonas* and *Roseobacter*. Comparion between the

isolates indicated that different taxa are adapted to better utilise either low-molecular-weight or high-molecular weight dDNA pools depending on the original isolation conditions.

From a biogeochemical perspective, nucleic acids and associated degradation products (e.g. purines and pyrimidines) make important contributions to seawater carbon, nitrogen and phosphorus DOM pools, with C:N:P ratios that exceed that of typical “Redfield” plankton (Berman & Bronk, 2003, Karl & Björkman, 2015). Bacterioplankton cycling of these compounds can therefore result in nitrogen and phosphorus regeneration processes taking place that produce substrates that are more widley accessible to the plankton communities, such as urea (Berg & Jorgensen, 2006). For example, studies at Station ALOHA in the North Pacific Subtropical Gyre have suggested that dDNA can provide a major component of the total biologically avaiable phosphorus demand throughout the water column (Brum, 2005).

The aims of this study were to quantify seawater D-DNA concentrations in surface coastal waters at Station L4 sampling site in the Western English Channel over a spring-summer transition and to assess the relationships between changes in concentration with physiochemical and biological factors. This study also focused on dDNA component of the D-DNA pool by identifying specific bacterioplankton taxa able to utilise phytoplankton-derived dDNA using dDNA-amended seawater microcosms and RNA Stable Isotope Probing (RNA SIP) with ^{13}C-labelled diatom dDNA.

Materials and Methods

Seawater D-DNA quantification

Seawater samples were collected weekly between 04/03/2014 and 21/06/2014 from Station L4 (Lat 50.15 Lon -4.13) in the Western English Channel (Supplementary Figure 1) from 5 m depth. 50 mL samples (n 3) were gently filtered through a 0.2 µm Minisart® cellulose acetate membrane filter (Sartorius, UK) into sterile aged (> 5 years old) plastic bottles containing 50 µL 1 M tetrasodium ethylenediaminetetraacetic acid (EDTA) (Brum *et al.*, 2004), stored in the dark at 4 °C and returned to the laboratory for analysis within 2 hours.

D-DNA (combined <0.2 µm; dissolved 'free' DNA, DNA viruses and uncharacterised bound DNA) was quantified fluorometrically using the method described by Brum *et al.* (2004). 15 mL samples were concentrated using Amicon Ultra Centrifugal Filter units (ultra-15 MWCO 10k Da, Sigma Aldrich, UK) centrifuged for between 10-20 min at 4000 *g* and 25 °C, reducing the volume to 1 mL. 10 mL Tris-EDTA buffer (TE buffer: 10 mM Tris-HCl, 1 mM disodium EDTA, pH 7.5, autoclaved) was added to the concentrate before further centrifugation to a final volume of 250 µL. The concentrated D-DNA was quantified using the QuantiFluor dsDNA System (Promega, UK) using a slight modification of the manufacturer's instructions, with 5 µL concentrated DNA samples added to 45 µL of DNA-binding dye and quantified using a calibrated QuantiFluor® (Promega, UK).

Phytoplankton and bacterioplankton abundance

Seawater samples were also collected from Station L4 from a depth of 10 m using a 10 L Niskin bottle, with 200 mL removed and immediately preserved with 2 % (final concentration) acid-Lugol's iodine solution (Throndsen, 1978) and 200 mL preserved with neutral formaldehyde (4 % final concentration) (Widdicombe et al., 2010). Samples were analysed using the Utermöhl technique (Utermöhl, 1931) according to guidance procedures within "Water quality –Guidance standard for routine microscopic surveys of phytoplankton using inverted microscopy (Utermöhl technique)" (BS EN 15204:2006). Samples were acclimatised to room temperature to ensure a random distribution of cells in the settlement chambers.

Following cell re-suspension and separation through gentle rotation of samples bottles in a figure-of-eight movement, a subsample volume of either 50 or 100 mL (depending on cell density) was transferred to a plankton settling chamber. Cells were identified, where possible, to species level according to published literature. Abundance of high and low nucleic acid bacteria were assessed using flow cytometry from the un-preserved seawater samples using protocols outlined in Tarran & Bruun (2015).

^{13}C-labelled dDNA production

Chaetoceros contortus PLY550 was selected as a candidate for dDNA production as the genus *Chaetoceros* is bloom forming at Station L4 (Widdicombe *et al.*, 2010). Axenic cultures (1 L) of *C. contortus* were grown in artificial seawater media (Berges *et al.*, 2001) with f/2 nutrients (Guillard & Ryther, 1962). To produce ^{13}C-labelled dDNA, the media was modified by adding $NaH^{13}CO_3$ (5 mM). The cultures were maintained sealed and incubated at 15 °C for 14 days under a 16:8 hours light dark cycle regime at an intensity of 85.3 µmol photons s^{-1} m^{-2}, with daily mixing by inversion.

Cultures were harvested by centrifugation at 4000 *g* for 20 min and the cell pellets stored at -80 °C. dDNA was extracted from the cell pellets using the DNeasy Blood & Tissue Kit (Qiagen, UK). Resulting dDNA extracts were RNase treated to remove potential co-extracted RNA (RNase 1, Promega, UK) as per the manufacturer's instructions. The dDNA solution was made up to 1 mL using Nuclease free water and concentrated to 250 µL in Amicon Ultra Centrifugal Filter units (ultra-15 MWCO 10k Da) by centrifuging at 4000 *g* for 5-10 min. The resulting 10k Da fraction (consistent with the high molecular weight fraction quantified from environmental samples) was recovered and stored at -80 °C.

Experimental setup

Surface seawater (5 m depth, 60 L) was collected on 28/04/2014 from Station L4, pre-filtered through 100 µm mesh to remove large grazing zooplankton and aliquoted (2 L) into acid-washed sterilised glass 5 L conical flasks. To assess enrichments of specific taxa due to the

addition of dDNA the treatments were a no addition control and an addition of dDNA (dDNA amended treatment), a further treatment of ^{13}C-labelled DNA was used to conduct RNA SIP experiments. The unlabelled dDNA amended treatments were also used as a ^{12}C control. All treatments were conducted in triplicate (n 3). Ambient DNA concentrations were quantified and diatom DNA added to a final concentration of 1.29 µg L^{-1} (3 x the ambient concentration at the time of sampling) in both the ^{12}C and ^{13}C dDNA amended treatments. The microcosms were incubated in the dark at 13 °C (seawater temperature at Station L4 at the time of sampling) and aerated continually. At time point T0 before the addition of dDNA (0 hrs) and after 24 hrs, 660 mL seawater from each microcosm was filtered through a 0.2 µm cellulose nitrate membrane filter (Whatman, UK) and the filters stored at -80 °C.

RNA was extracted from the filters in 1 mL Tri-reagent (Sigma Aldrich, UK). The extracted aqueous phase was added to 500 µL chloroform isoamyl alcohol (24:1) before vortexing and centrifuging at 10,000 *g* for 1 min. The resulting upper layer was recovered and the RNA precipitated with an equal volume of isopropanol and ammonium acetate (1:10) with 1 µL of 20 µg $µL^{-1}$ molecular grade glycogen. The extracted RNA was cleaned using the RNeasy MinElute Cleanup Kit (Qiagen, UK) and treated twice with RQ1 RNase-Free DNase (Promega, UK) using the manufacturers' protocol. Absence of DNA was confirmed by a negative PCR result using general bacterial primers as outlined below and the RNA stored at -80 °C.

RNA Stable Isotope Probing (RNA SIP)

RNA SIP was performed using cesium tri-fluroacetate (CeTFA) gradients following established protocols (Whiteley *et al.*, 2007, Taylor *et al.*, 2013, Taylor & Cunliffe, 2017). Gradients (5.1mL) were prepared with 500 ng RNA, 1.75 mL formamide, 4.78 mL of a 2 g mL^{-1} CeTFA solution and molecular grade water to 5.1 mL. This produced a starting density of 1.8 g mL^{-1}. The tubes were centrifuged at 165,196 *g* (41,000 rpm) for 50 hrs in an ultracentrifuge (Beckman Coulter Optima L-100 XP ultracentrifuge rotor VTi 65.2) and gradients were fractionated into 420 µL fractions using displacement with sterile molecular

178 grade water (Whiteley *et al.*, 2007). Fraction density was determined from Refractive Index
179 measured using digital refractometer (Bellingham Stanley). To the remaining gradient
180 solution an equal volume of isopropanol and 1 µL of 20 µg μL^{-1} glycogen was added and
181 precipitated for 1 hr at -20°C. The tubes were centrifuged for 30 min at 12000 *g* and then
182 washed with 500 µL 75 % ethanol. The RNA pellets were air dried in a laminar flow hood, re-
183 suspended in RNase free water (20 µL) and the RNA quantified using the QuantiFluor® RNA
184 system (Promega, UK). Characterisation of all 'light' to 'heavy' SIP fractions was performed
185 by quantification of buoyant densities and DGGE analysis of RT-PCR amplified 16S rRNA
186 transcripts (see below). By comparing buoyant densities and DGGE fingerprints from
187 unlabelled control incubations with labelled incubations, incorporation of ^{13}C-labelled dDNA
188 into the bacterioplankton communities was determined (Whiteley *et al.*, 2007, Taylor *et al.*,
189 2013, Taylor & Cunliffe, 2017). Specific 'heavy' gradient fractions from both the ^{12}C control
190 and ^{13}C-labelled incubations were selected for further analysis using high-throughput
191 sequencing as described below.
192
193 *Controlling for amplification of bacterial DNA from dDNA additions*
194 Using DNA as an added substrate means there is a potential that, with improper care, we
195 could amplify organisms from our added material in downstream processes. The main step
196 we took was to use RNA instead of DNA for diversity analysis. We took a series of other
197 steps to ensure that our dDNA additions were not amplified in downstream processes. The
198 cultures of diatoms used were axenic (treated with antibiotics and filtered and washed
199 through 3 µM filter) to minimise bacterial contamination of the dDNA additions. DNA
200 extraction from diatoms was twice RNase treated and the lack of RNA confirmed using the
201 QuantiFluor® RNA System (Promega, UK). From the experiment, RNA was extracted using
202 tri-reagent, which minimises DNA contamination (Pinto *et al.*, 2009). Extracted RNA was
203 twice treated with DNase (30 min incubation) and the negative results confirmed by PCR of
204 a bacteria 16S rRNA gene, as well as performing quantification of both DNA and RNA using
205 a the respective QuantiFluor® high sensitivity kits. Furthermore, had any DNA been carried

over into the ultra-centrifugation, RNA and DNA have different buoyant densities and would therefore have been separated out. In sequencing of the no addition controls and T0 natural community we were able to determine that operational taxonomic unites (OTUs) relating to the experiments were present in the original seawater sample and also in the control experiment that had no additions.

16S rRNA high-throughput sequencing and bioinformatics

RNA was reverse transcribed using the Omniscript Reverse Transcription kit according to the manufactures' instructions (Qiagen, UK) with the reverse primer PROK1492R (GGW TAC CTT GTT ACG ACT T) (Suzuki *et al.*, 2000). The V4 region of the bacterial 16S rRNA gene was amplified using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso *et al.*, 2011) in reactions using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) and ~10 ng cDNA. PCR conditions were as follows: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. Sequencing was performed on an Ion Torrent PGM (Life technologies, USA). Libraries were prepared using the Ion Xpress fragment library kit (Life technologies, USA) with template preparation using the Ion OneTouch2 400 bp v2 Template kit (Life technologies, USA). Sequencing was carried out using an Ion 400 bp sequencing kit on a 318v2 chip all accordance with the manufacturer's instructions. Signal processing and base calling was carried out using the TorrentSuite v4.4 software.

Sequences were analysed using a combination of USEARCH v7.0.1090 (32 Bit) (Edgar, 2010) and QIIME v1.8.0 (Caporaso *et al.*, 2010) as described previously (Taylor & Cunliffe, 2017). Multiplexed files had barcodes removed and were quality filtered (low quality = expected error >0.5 and short sequences <200bp), length truncated (200bp) and converted to FASTA files. The FASTA files were de-replicated, abundance sorted and singleton sequences removed. OTUs were clustered using the UPARSE clustering algorithm (Edgar, 2013). Chimeras were then filtered using UCHIME (Edgar *et al.*, 2011) and the Gold

database (Edgar, 2010) as a reference. OTUs were mapped back to the original reads and an OTU table produced. Taxonomy was assigned to OTUs using the uclust method in QIIME v1.8.0 against a curated Greengenes reference database (release 13_8) (DeSantis *et al.*, 2006). Before further analysis, singletons were removed as well as chloroplast plastid and archaeal sequences. OTU tables were then rarefied to 25,000 sequences per library. Weighted and un-weighted UniFrac distance matrices (Lozupone *et al.*, 2011) were used to generate 2D principal component analysis plots. Sequence data are available from the European Nucleotide Archive (accession code PRJEB24573).

Statistical analysis

Permutational Multivariate Analysis of Variance (PERMANOVA) (999 permutations) was performed in QIIME using UniFrac distance matrices and OTU tables as inputs. All other statistical analysis was carried out using SPSS® (IBM®, USA).

Results

Variation in seawater D-DNA at Station L4

The concentration of D-DNA, including dissolved 'free' DNA, viruses and uncharacterised bound DNA, at Station L4 showed significant changes over the 16 week sampling period ($F_{16, 50}$ = 28.40, p<0.001), increasing from 0.09 µg L^{-1} in early spring to a maximum of 1.5 µg L^{-1} in the summer (Tukey, p<0.001) (Figure 1A). The mean concentration over the sampling period was 0.55 µg L^{-1}. A comparison between this study and other studies is shown in Table 1.

Patterns of nutrients and seawater temperature were typical for Station L4 (Smyth *et al.*, 2010), with nutrients declining steeply during the spring diatom bloom. Throughout the sampling period, D-DNA concentration and surface seawater temperature showed a positive correlation (Spearman; *r* 0.705; *p* <0.005) (Figure 1A, Supplementary Table 1) and a concurrent negative correlation with nutrients (Spearman; Nitrate *r* -0.64, *p*<0.01; Ammonia *r* -0.62, *p*<0.05; Silicate *r* -0.72, *p*<0.005; Phosphate *r* -0.67, *p*<0.005) (Figure 1B).

Assessment of the changes in D-DNA concentration and the abundance of specific phytoplankton groups showed strong positive correlations with pigmented dinoflagellates (phototrophs or mixotrophs) (Spearman, *r* 0.69, p<0.005), non-pigmented dinoflagellates (heterotrophs) (Spearman r 0.82, p<0.000) and diatoms (Spearman *r* 0.57, *p*<0.02). There was no significant correlation with chlorophyll-a over the study, however there was a cross correlation between D-DNA and chlorophyll, with chlorophyll leading by 3 weeks followed by peaks in D-DNA (Spearman *r* 0.84, p<0.001) (Figure 1C). A strong positive correlation was also seen between D-DNA concentration and high nucleic acid containing bacterioplankton (Spearman *r* 0.59, p<0.02) (Figure 1D).

Impact of dDNA on bacterioplankton active diversity and dDNA assimilation

Surface seawater samples were collected for the microcosm experiments from Station L4 approximately 3 weeks after the spring diatom bloom when the water column was thermally stratified, with physicochemical parameters at the time of sampling typical for the time of

278 year (Smyth *et al.*, 2010). In the surface water above the thermocline, nutrients were
279 depleted relative to values reported in the winter (Smyth *et al.*, 2010) and the D-DNA
280 concentration was 0.42 μg L^{-1}.

281 Weighted unifrac distance matrices showed that the active bacterioplankton
282 communities after 24 hours were significantly different in the dDNA amended microcosms
283 compared to the communities at T0 (i.e. un-amended seawater) and to the no addition
284 control (i.e. no dDNA addition) after 24 hours (PERMANOVA, $P<0.05$) (Figure 2A). No
285 significant differences were seen between the treatments and time points based on the un-
286 weighted (i.e. presence/absence) communities distance matrices (Figure 2B).

287 At the time of sampling (T0) the active bacterioplankton communities were typical for
288 the time of year at Station L4 (Taylor *et al.*, 2014), dominated by the orders Flavobacteriales,
289 Rhodobacterales, Oceanospirillales, Alteromonadales and the candidate taxa Marine Group
290 A-SAR406 (Arctic96B-7), representing 89 % of the total sequences (Figure 3). In the dDNA-
291 amended treatments, the relative abundance of the order Rhodobacterales was significantly
292 increased compared to the no addition controls ($p<0.005$) (Figure 3). Two OTUs made up
293 the majority of the Rhodobacterales 16S rRNA reads (OTU_1, OTU_3) (~15%) (Figure 4Bi).
294 Both were affiliated to unclassified members of the Marine Roseobacter Clade (MRC).
295 OTU_1 was most closely related to *Amylibacter* and OTU_3 was closer to *Halocynthiibacter*
296 based on partial 16S rRNA encoding genes (Figure 4A). There was no significant
297 enrichment of any other groups (Figure 3).

298 At the OTU level there was significant enrichment in the dDNA amended treatments
299 compared to the no addition control in four OTUs, OTU_3, OTU_37, OTU_410 and
300 OTU_414 from the order Rhodobacterales (Figure Bi). All four OTUs were from the MRC,
301 with the highest difference in relative abundance in OTU_3 (ANOVA, Tukey $p<0.004$)
302 (Figure 4Bi). Of the OTUs significantly enriched in the dDNA amended treatments, OTU_3
303 and OTU_37 also showed enrichment in the ^{13}C-dDNA RNA SIP heavy fractions compared
304 to the ^{12}C control RNA SIP fractions (Figure 4 Bii) indicating that the taxa had assimilated the
305 dDNA carbon.

306 **Discussion**

307 The majority of studies on seawater DNA have focused on bacterial e.g. (Paul & Carlson,
308 1984, DeFlaun *et al.*, 1987) and viral sources e.g. (Jiang & Paul, 1995, Brum, 2005), with
309 links to specific phytoplankton taxa generally overlooked. Although seawater D-DNA
310 concentrations at Station L4 did not directly correlate with chlorophyll-a, D-DNA did show a
311 cross correlation with chlorophyll-a, with concentrations of D-DNA increasing after the
312 collapse of the spring diatom bloom. Dinoflagellates also showed a strong positive
313 correlation with D-DNA, in particular during the period when D-DNA concentration was
314 greatest at Station L4, suggesting that they may be an important source of DNA in seawater.
315 Dinoflagellates did not contribute greatly to total phytoplankton abundance in terms of cell
316 numbers at Station L4 compared to other phytoplankton groups, however dinoflagellates can
317 contain large amounts of genomic DNA (up to 85 pg DNA.cell^{-1}) compared to other
318 phytoplankton groups, such as diatoms (2 pg DNA.cell^{-1}) (Veldhuis *et al.*, 1997). Many
319 dinoflagellates can also be predatory, particularly non-pigmented cells (Sherr & Sherr, 2007),
320 and predation could also be a source of seawater DNA (Strom *et al.*, 1997). The individual
321 D-DNA pools at Station L4 (i.e. dissolved 'free' DNA, viruses and uncharacterised bound
322 DNA <0.2 μm) was not assessed in this study. Other studies have shown that there can be
323 substantial variation within the D-DNA pool. For example, at Station ALOHA dDNA can vary
324 between 27 to 51 % of the D-DNA pool and viruses between 49 to 63 %, with
325 uncharacterised bound DNA undetectable (Brum, 2005). Therefore in this study, the D-DNA
326 associated with specific phytoplankton taxa discussed above could be produced directly or
327 as released viruses.

328 The positive correlation between HNA-containing bacterioplankton and D-DNA
329 concentration at Station L4 suggests they could also contribute to the seawater DNA pool.
330 However, as we show here, some bacterioplankton are able to assimilate dDNA and
331 increase in abundance could also be caused in part by utilisation of dDNA. The dominant
332 bacterioplankton groups at Station L4 during the summer included Rhodobacterales,

333 Flavobacteriales and Alteromonadales, which are categorised as HNA-containing
334 bacterioplankton (Schattenhofer *et al.*, 2011, Vila-Costa *et al.*, 2012).
335 Rhodobacterales have been well established as ecologically linked to marine
336 phytoplankton, and often increase in abundance during and following phytoplankton blooms
337 in response to increased substrate availability (Gilbert *et al.*, 2012, Teeling *et al.*, 2012,
338 Taylor *et al.*, 2014). In this study, Rhodobacterales showed a significant increase in
339 abundance due to dDNA additions with OTUs specifically from the MRC being enriched.
340 Furthermore, two specific MRC OTUs were also shown to assimilate ^{13}C-dDNA in the RNA-
341 SIP experiments, indicating that they utilise dDNA as a carbon source. These results
342 corroborate previous culture-based studies that isolated dDNA-utilising Rhodobacterales
343 (Lennon, 2007). Laboratory-based experiments with the model MRC *Ruegeria pomeroyi*
344 DSS-3 have shown that bacterioplankton can utilise purines (a major component of nucleic
345 acids) as a carbon and nitrogen source (Cunliffe, 2015).
346 It could have been possible that those taxa enriched in dDNA-amended treatments
347 but not enriched in the SIP experiment were utilising other elements in the dDNA such as
348 nitrogen or phosphorus rather than carbon. Several studies have suggested that the MRC
349 rely on organic and reduced nitrogen compounds (e.g. ammonium) as nitrogen sources
350 (Moran & Miller, 2007, Newton *et al.*, 2010, Chen, 2012, Gifford *et al.*, 2013) and are also
351 able to utilise organic phosphorus sources (Moran & Miller, 2007). Other studies have also
352 linked members of the Rhodobacterales to degradation of DON in the form of dissolved
353 protein using SIP (Orsi *et al.*, 2016), suggesting they may be able to utilise a diverse range
354 of organic compounds for growth. Whether various taxa within the MRC exhibit resource
355 partitioning between them for different organic compounds remains to be determined.
356 In conclusion, in coastal marine waters MRC bacterioplankton in particular appear
357 important in the degradation of phytoplankton-derived dDNA and assimilation of dDNA
358 carbon. This observation further reinforces the ‘master recycler’ ecological role that the MRC
359 hold proposed by Buchan et al 2014. Bacterioplankton dDNA processing is ecologically and
360 biogeochemically important because this is a potential mechanism through which

phytoplankton-derived nitrogen and phosphorous-containing DOM compounds could be regenerated to sustain wider productivity throughout the summer once inorganic nutrients have been depleted by the spring bloom. Future work should consider using molecular tools (metagenomics or metabarcoding) to sequence seawater dDNA to identify organisms contributing to the D-DNA pool, and also determine the underpinning biological mechanisms used by bacterioplankton to process seawater dDNA.

Funding

This work was supported by a Marine Biological Association (MBA) Research Fellowship awarded to MC.

Acknowledgements

We thank the crews of the RV *Plymouth Quest* and RV *Sepia* for facilitating seawater sample collection, the MBA Phytoplankton Culture Collection for providing the diatom culture and Malcolm Woodward and Glen Tarran for generating nutrient and flow cytometry data, respectively. We also acknowledge the Western Channel Observatory, which is funded as part of the UK Natural Environmental Research Council's National Capability programme.

References

Agustí S & Duarte CM (2013) Phytoplankton lysis predicts dissolved organic carbon release in marine plankton communities. *Biogeosciences* **10**: 1259-1264.

Azam F, Fenchel T, Field JG, Gray J, Meyer-Reil L & Thingstad F (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 257-263.

Benner R (2002) Chemical composition and reactivity. *Biogeochemistry of marine dissolved organic matter* **3**: 56-90.

Berg GM & Jorgensen NOG (2006) Purine and pyrimidine metabolism by estuarine bacteria. *Aquatic Microbial Ecology* **42**: 215-226.

Berges JA, Franklin DJ & Harrison PJ (2001) Evolution of an artificial seawater medium: improvements in enriched seawater, artificial water over the last two decades. *Journal of Phycology* **37**: 1138-1145.

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

Boehme J, Frischer M, Jiang S, Kellogg C, Pichard S, Rose J, Steinway C & Paul JH (1993) Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. *Mar Ecol Prog Ser* 1-10.

Breter H-J, Kurelec B, Müller W & Zahn R (1977) Thymine content of sea water as a measure of biosynthetic potential. *Marine Biology* **40**: 1-8.

Brum JR (2005) Concentration, production and turnover of viruses and dissolved DNA pools at Stn ALOHA, North Pacific Subtropical Gyre. *Aquatic Microbial Ecology* **41**: 103-113.

Brum JR, Steward GF & Karl DM (2004) A novel method for the measurement of dissolved deoxyribonucleic acid in seawater. *Limnology and Oceanography: Methods* **2**: 248-255.

Buchan A, LeCleir GR, Gulvik CA & González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nature Reviews Microbiology* **12**: 686-698.

406 Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer
407 N & Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of
408 sequences per sample. *Proc Natl Acad Sci U S A* **108 Suppl 1**: 4516-4522.
409 Caporaso JG, Kuczynski J, Stombaugh J*, et al.* (2010) QIIME allows analysis of high-
410 throughput community sequencing data. *Nature methods* **7**: 335-336.
411 Chen Y (2012) Comparative genomics of methylated amine utilization by marine
412 Roseobacter clade bacteria and development of functional gene markers (tmm, gmaS).
413 *Environmental microbiology* **14**: 2308-2322.
414 Cunliffe M (2015) Purine catabolic pathway revealed by transcriptomics in the model marine
415 bacterium Ruegeria pomeroyi DSS-3. *FEMS microbiology ecology* **92**: fiv150.
416 DeFlaun MF, Paul JH & Davis D (1986) Simplified method for dissolved DNA determination
417 in aquatic environments. *Applied and environmental microbiology* **52**: 654-659.
418 DeFlaun MF, Paul JH & Jeffrey WH (1987) Distribution and molecular weight of dissolved
419 DNA in subtropical estuarine and oceanic environments. *Mar Ecol Prog Ser* 65-73.
420 DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu
421 P & Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database
422 and workbench compatible with ARB. *Applied and environmental microbiology* **72**: 5069-
423 5072.
424 Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST.
425 *Bioinformatics (Oxford, England)* **26**: 2460-2461.
426 Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads.
427 *Nature methods* **10**: 996-998.
428 Edgar RC, Haas BJ, Clemente JC, Quince C & Knight R (2011) UCHIME improves
429 sensitivity and speed of chimera detection. *Bioinformatics (Oxford, England)* **27**: 2194-
430 2200.
431 Gifford SM, Sharma S, Booth M & Moran MA (2013) Expression patterns reveal niche
432 diversification in a marine microbial assemblage. *The ISME journal* **7**: 281-298.

433 Gilbert JA, Steele JA, Caporaso JG*, et al.* (2012) Defining seasonal marine microbial
434 community dynamics. *ISME J* **6**: 298-308.
435 Guillard RR & Ryther JH (1962) Studies of marine planktonic diatoms: I. Cyclotella Nana
436 Hustedt, and Detonula Confervacea (CLEVE) Gran. *Canadian journal of microbiology* **8**:
437 229-239.
438 Jiang SC & Paul JH (1995) Viral contribution to dissolved DNA in the marine environment as
439 determined by differential centrifugation and kingdom probing. *Applied and*
440 *environmental microbiology* **61**: 317-325.
441 Karl DM & Bailiff MD (1989) The measurement and distribution of dissolved nucleic acids in
442 aquatic environments. *Limnology and Oceanography* **34**: 543-558.
443 Karl DM & Björkman KM (2015) Dynamics of Dissolved Organic Phosphorus.
444 *Biogeochemistry of Marine Dissolved Organic Matter,*(Hansell DA & Carlson CA, eds.),
445 p.^pp. 233-334. Elsevier.
446 Lennon JT (2007) Diversity and metabolism of marine bacteria cultivated on dissolved DNA.
447 *Applied and environmental microbiology* **73**: 2799-2805.
448 Lozupone C, Lladser ME, Knights D, Stombaugh J & Knight R (2011) UniFrac: an effective
449 distance metric for microbial community comparison. *The ISME journal* **5**: 169.
450 Maeda M & Taga N (1974) Occurrence and distribution of deoxyribonucleic acid-hydrolyzing
451 bacteria in sea water. *Journal of Experimental Marine Biology and Ecology* **14**: 157-169.
452 Maruyama A, Oda M & Higashihara T (1993) Abundance of virus-sized non-DNase-
453 digestible DNA (coated DNA) in eutrophic seawater. *Applied and environmental*
454 *microbiology* **59**: 712-717.
455 McCarthy M, Hedges J & Benner R (1993) The chemical composition of dissolved organic
456 matter in seawater. *Chemical Geology* **107**: 503-507.
457 McCarthy M, Pratum T, Hedges J & Benner R (1997) Chemical composition of dissolved
458 organic nitrogen in the ocean. *Nature* **390**: 150-154.
459 Moran MA & Miller WL (2007) Resourceful heterotrophs make the most of light in the coastal
460 ocean. *Nature reviews Microbiology* **5**: 792-800.

461 Newton RJ, Griffin LE, Bowles KM*, et al.* (2010) Genome characteristics of a generalist
462 marine bacterial lineage. *Isme j* **4**: 784-798.
463 Orsi WD, Smith JM, Liu S, Liu Z, Sakamoto CM, Wilken S, Poirier C, Richards TA, Keeling
464 PJ & Worden AZ (2016) Diverse, uncultivated bacteria and archaea underlying the
465 cycling of dissolved protein in the ocean. *The ISME journal* **10**: 2158-2173.
466 Paul JH & Carlson DJ (1984) Genetic material in the marine environment: implication for
467 bacterial DNA. *Limnology and oceanography* **29**: 1091-1096.
468 Paul JH, Jeffrey WH & DeFlaun MF (1987) Dynamics of extracellular DNA in the marine
469 environment. *Applied and Environmental Microbiology* **53**: 170-179.
470 Paul JH, DeFlaun MF & Jeffrey WH (1988) Mechanisms of DNA utilization by estuarine
471 microbial populations. *Applied and environmental microbiology* **54**: 1682-1688.
472 Pillai T & Ganguly A (1970) Nucleic acids in the dissolved constituents of sea-water. *Current*
473 *Science* 501-504.
474 Pinto FL, Thapper A, Sontheim W & Lindblad P (2009) Analysis of current and alternative
475 phenol based RNA extraction methodologies for cyanobacteria. *BMC molecular biology*
476 **10**: 79.
477 Schattenhofer M, Wulf J, Kostadinov I, Glöckner FO, Zubkov MV & Fuchs BM (2011)
478 Phylogenetic characterisation of picoplanktonic populations with high and low nucleic
479 acid content in the North Atlantic Ocean. *Systematic and applied microbiology* **34**: 470-
480 475.
481 Sherr EB & Sherr BF (2007) Heterotrophic dinoflagellates: a significant component of
482 microzooplankton biomass and major grazers of diatoms in the sea. *Mar Ecol Prog Ser*
483 **352**: 187-197.
484 Siuda W, Chróst RJ & Güde H (1998) Distribution and origin of dissolved DNA in lakes of
485 different trophic states. *Aquatic microbial ecology* **15**: 89-96.
486 Smyth TJ, Fishwick JR, Al-Moosawi L, Cummings DG, Harris C, Kitidis V, Rees A, Martinez-
487 Vicente V & Woodward EMS (2010) A broad spatio-temporal view of the Western
488 English Channel observatory. *Journal of Plankton Research* **32**: 585-601.

Strom SL, Benner R, Ziegler S & Dagg MJ (1997) Planktonic grazers are a potentially important source of marine dissolved organic carbon. *Limnology and Oceanography* **42**: 1364-1374.

Suzuki MT, Taylor LT & DeLong EF (2000) Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 5′-Nuclease Assays. *Applied and Environmental Microbiology* **66**: 4605-4614.

Tarran GA & Bruun JT (2015) Nanoplankton and picoplankton in the Western English Channel: abundance and seasonality from 2007–2013. *Progress in Oceanography* **137**: 446-455.

Taylor JD & Cunliffe M (2017) Coastal bacterioplankton community response to diatom-derived polysaccharide microgels. *Environmental Microbiology Reports* **9**: 151-157.

Taylor JD, Cottingham SD, Billinge J & Cunliffe M (2014) Seasonal microbial community dynamics correlate with phytoplankton-derived polysaccharides in surface coastal waters. *Isme j* **8**: 245-248.

Taylor JD, McKew BA, Kuhl A, McGenity TJ & Underwood GJ (2013) Microphytobenthic extracellular polymeric substances (EPS) in intertidal sediments fuel both generalist and specialist EPS-degrading bacteria. *Limnology and Oceanography* **58**: 1463-1480.

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM, Kassabgy M, Huang S, Mann AJ & Waldmann J (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. *Science* **336**: 608-611.

Throndsen J (1978) *Preservation and storage.* In: Sournia A. (ed.) *Phytoplankton Manual*. UNESCO, Paris 69–74.

Utermöhl vH (1931) Neue Wege in der quantitativen Erfassung des Plankton.(Mit besonderer Berücksichtigung des Ultraplanktons.) Mit 4 Abbildungen im Text. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen* **5**: 567-596.

Veldhuis MJ, Cucci TL & Sieracki ME (1997) Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. *Journal of Phycology* **33**: 527-541.

Vila-Costa M, Gasol JM, Sharma S & Moran MA (2012) Community analysis of high-and low-nucleic acid-containing bacteria in NW Mediterranean coastal waters using 16S rDNA pyrosequencing. *Environmental microbiology* **14**: 1390-1402.

Weinbauer MG, Fuks D & Peduzzi P (1993) Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. *Applied and Environmental Microbiology* **59**: 4074-4082.

Weinbauer MG, Fuks D, Puskaric S & Peduzzi P (1995) Diel, seasonal, and depth-related variability of viruses and dissolved DNA in the Northern Adriatic Sea. *Microbial Ecology* **30**: 25-41.

Whiteley AS, Thomson B, Lueders T & Manefield M (2007) RNA stable-isotope probing. *Nature Protocols* **2**: 838-844.

Widdicombe C, Eloire D, Harbour D, Harris R & Somerfield P (2010) Long-term phytoplankton community dynamics in the Western English Channel. *Journal of Plankton Research* **32**: 643-655.

Table 1 Dissolved DNA (dDNA) concentrations in seawater showing the range of values obtained in this study and values from other studies.

Location	dDNA range ($\mu g\ L^{-1}$)	Reference
L4, Plymouth, (U.K)	0.09-1.50	This study
N. Adriatic Sea (Europe)	2.9-25.8	Weinbauer *et al.* (1993)
N. Adriatic Sea (Europe)	3.1-26.5	Weinbauer *et al.* (1995)
N. Adriatic Sea (Europe)	0.05-0.8	Breter *et al.* (1977)
Tampa Bay & Charlotte Harbour (Florida)	10-19	DeFlaun *et al.* (1987)
Tampa Bay (Florida)	11.9-16.8	Boehme *et al.* (1993)
Gulf of Mexico - Oceanic	3.5-6.7	Boehme *et al.* (1993)
Gulf of Mexico - Coastal	5-15	DeFlaun *et al.* (1986)
Kaneohe Bay (Hawaii)	3.41	Brum *et al.* (2004)
Kaneohe Bay (Hawaii)	2.66-3.15	Karl & Bailiff (1989)
Ala Moana Beach (Hawaii)	19-21	Karl & Bailiff (1989)
Magic Island (Hawaii)	14-19	Karl & Bailiff (1989)
Mamala Bay (Hawaii)	4.7	Karl & Bailiff (1989)
Tokyo Bay (Japan)	9-19	Maruyama *et al.* (1993)
Bombay Harbour (India)	13.4-80.6	Pillai & Ganguly (1970)

Figure Legends

Figure 1. dDNA and other parameters measured at Station L4 during the study period (x axes are Julian days). A) dDNA concentration (µg µl $^{-1}$) ± SE (n 3) and seawater temperature (^{o}C). B) Nitrate, nitrite, silicate, phosphate (µM) and chlorophyll a (mg m^{-3}), C) diatoms, dinoflagellates, colourless dinoflagellates (cells mL^{-1}) D) High-nucleic acid bacterioplankton and low-nucleic acid bacterioplankton (cells mL^{-1}).

Figure 2. Principal coordinates analyses (PCoA) plot describing diversity of active bacterioplankton communities determined from 16S rRNA libraries using A) weighted and B) un-weighted UniFrac distance matrices generated from OTU (97% similarity) data. The blue circles represent the T0 samples, green circles represent the no addition control treatments after 24 hrs and the orange circles represent the dDNA amended treatments after 24 hrs.

Figure 3. Normalized abundance of major bacterioplankton orders showing T0 blue bars, no addition control after 24 hrs green bars and dDNA amended treatments after 24 hrs orange. Values shown are means ± standard error (n 3).

Figure 4. A) Phylogenetic analysis of OTUs within the Rhodobacterales that showed increases in relative abundance in dDNA amended treatments compared to the no addition controls. The tree is based on a maximum likelihood method using a Tamura-Nei model. The numbers on the nodes indicate bootstrap percentages and the scale bar represents sequence divergence. Bi) Normalized abundance of OTUs within the order Rhodobacterales showing enrichment, showing T0 blue bars, no addition control after 24 hrs green bars and dDNA amended treatments after 24 hrs orange. Values are means ± standard error (n 3). (Bii) Comparison of the normalized relative abundance of 16S rRNA gene sequences from ^{13}C-labelled fraction and ^{12}C control fraction at the OTU level. Taxa >0.5 are those taxa enriched in the ^{13}C libraries relative to the ^{12}C control libraries, indicating that they had assimilated ^{13}C-labelled dDNA.

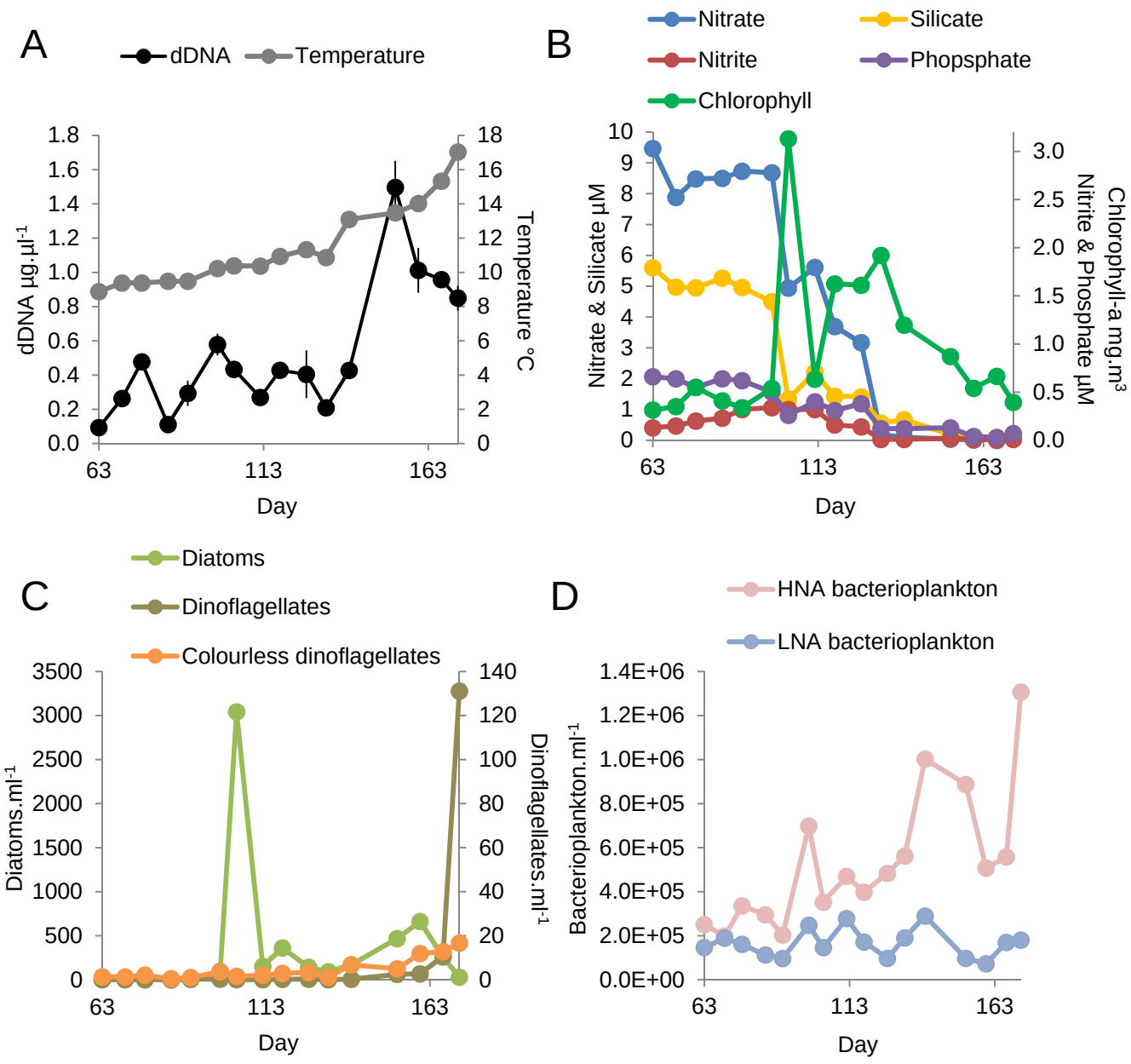

A
dDNA
Temperature
dDNA µg.µl-1
Temperature °C
Day
B
Nitrate
Silicate
Nitrite
Phopsphate
Chlorophyll
Nitrate & Silicate µM
Nitrite & Phosphate µM
Chlorophyll-a mg.m3
Day
C
Diatoms
Dinoflagellates
Colourless dinoflagellates
Diatoms.ml-1
Dinoflagellates.ml-1
Day
D
HNA bacterioplankton
LNA bacterioplankton
Bacterioplankton.ml-1
Day

Figure 1

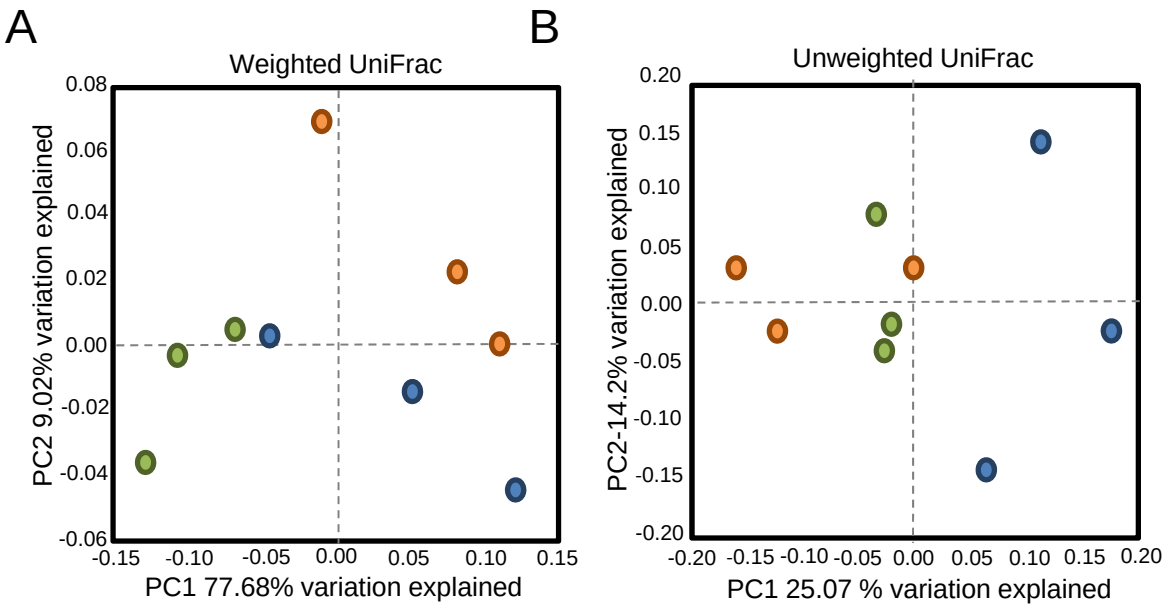

A
Weighted UniFrac
PC2 9.02% variation explained
PC1 77.68% variation explained
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
B
Unweighted UniFrac
PC2-14.2% variation explained
PC1 25.07 % variation explained
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20

Figure 2

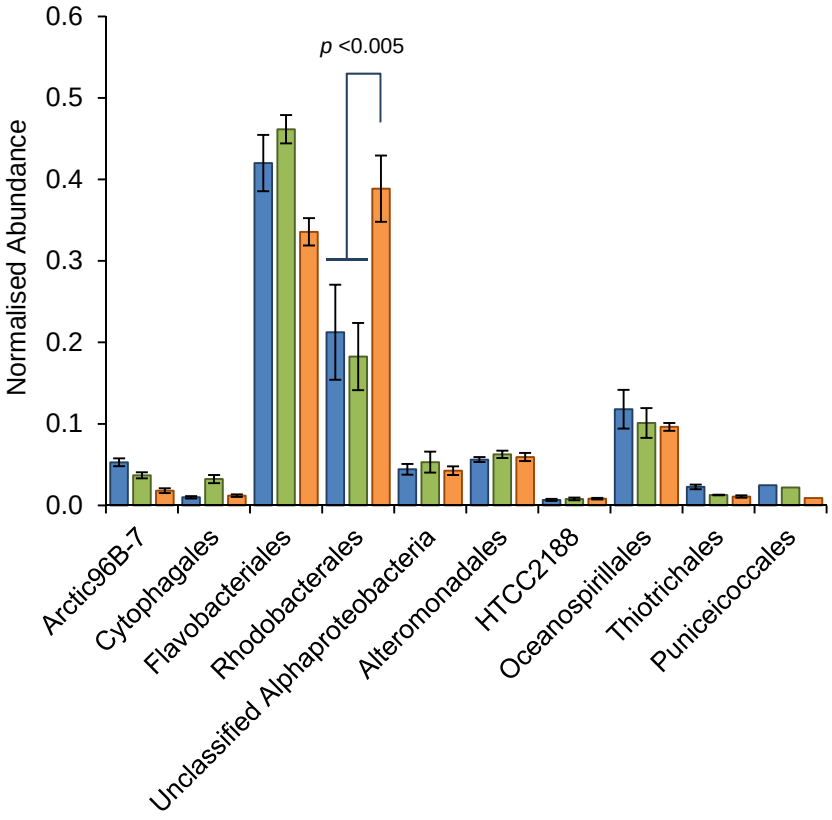

0.6
0.5
0.4
0.3
0.2
0.1
0.0
Normalised Abundance
p <0.005
Arctic96B-7
Cytophagales
Flavobacteriales
Rhodobacterales
Unclassified Alphaproteobacteria
Alteromonadales
HTCC2188
Oceanospirillales
Thiotrichales
Puniceicoccales

Figure 3

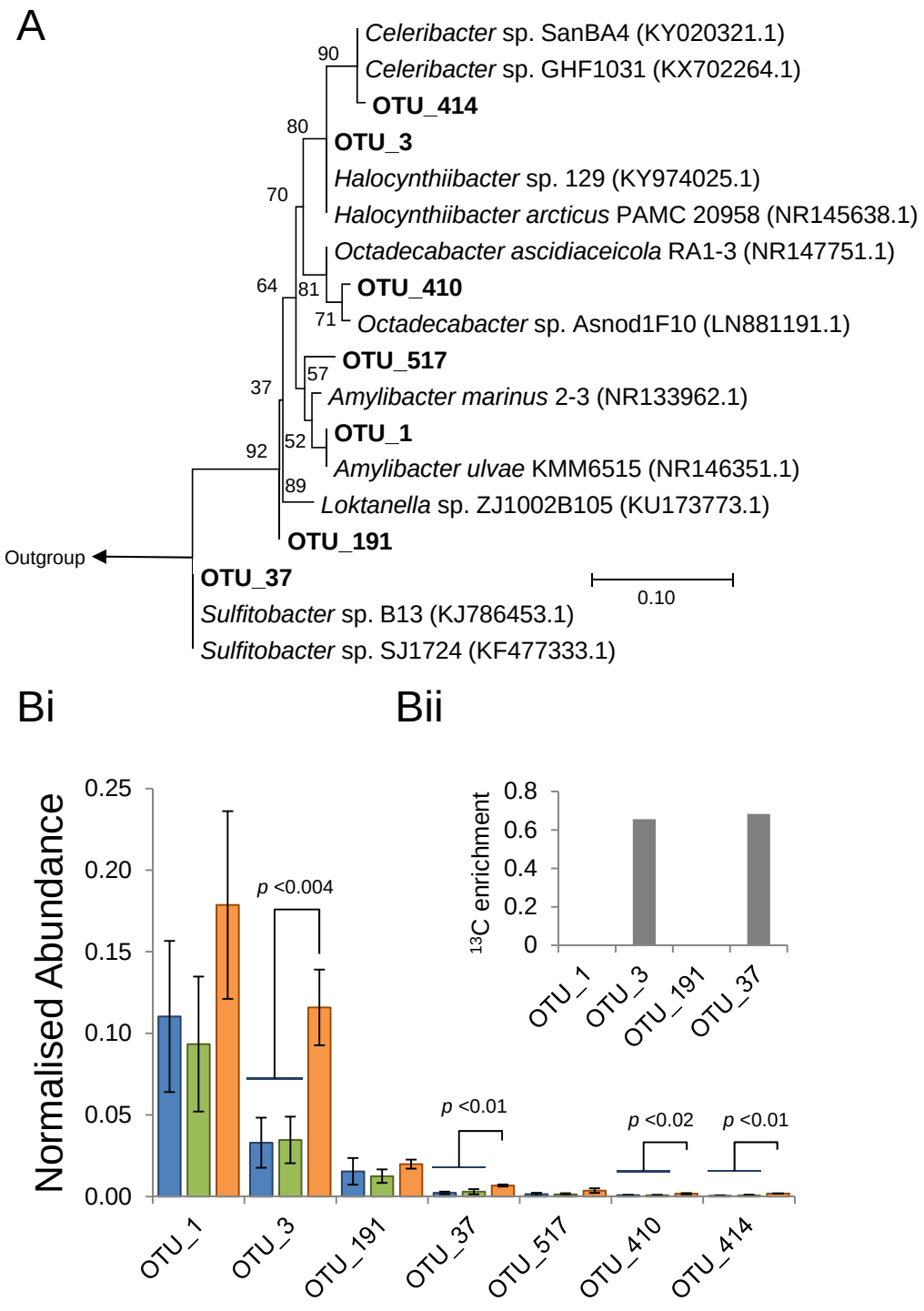
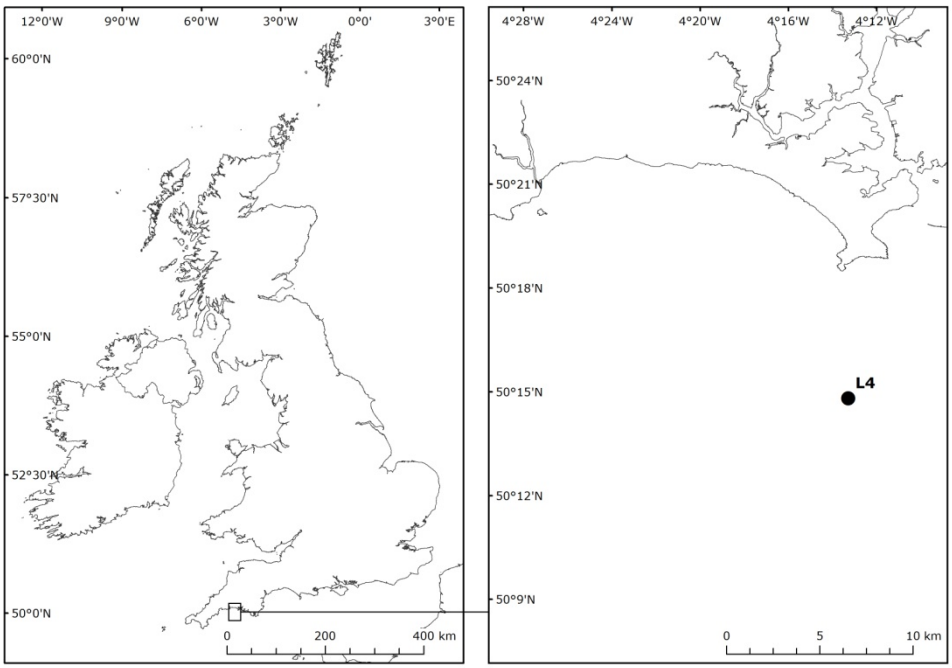

A
Celeribacter sp. SanBA4 (KY020321.1)
Celeribacter sp. GHF1031 (KX702264.1)
OTU_414
OTU_3
Halocynthiibacter sp. 129 (KY974025.1)
Halocynthiibacter arcticus PAMC 20958 (NR145638.1)
Octadecabacter ascidiaceicola RA1-3 (NR147751.1)
OTU_410
Octadecabacter sp. Asnod1F10 (LN881191.1)
OTU_517
Amylibacter marinus 2-3 (NR133962.1)
OTU_1
Amylibacter ulvae KMM6515 (NR146351.1)
Loktanella sp. ZJ1002B105 (KU173773.1)
OTU_191
Outgroup
OTU_37
0.10
Sulfitobacter sp. B13 (KJ786453.1)
Sulfitobacter sp. SJ1724 (KF477333.1)
90
80
70
64
81
71
57
37
52
92
89
Bi
Bii
Normalised Abundance
0.25
0.20
0.15
0.10
0.05
0.00
p <0.004
p <0.01
p <0.02
p <0.01
OTU_1
OTU_3
OTU_191
OTU_37
OTU_517
OTU_410
OTU_414
13C enrichment
0.8
0.6
0.4
0.2
0

Figure 4

Supplementary Figure 1. Location of the Western Channel Observatory coastal sampling station L4.



Supplementary Figure 1

Supplementary Table 1 Spearman's Correlation of measured dDNA concentration with cognate physicochemical and biological factors, bold text indicates significant (p< .5) correlations.

Variables	*p* value	Spearman's
Temperature	**0.003**	**0.705**
Salinity	0.729	0.093
Nitrite	0.318	-0.266
Nitrate	**0.009**	**-0.641**
Ammonia	**0.012**	**-0.619**
Silicate	**0.002**	**-0.724**
Phosphate	**0.006**	**-0.670**
Chlorophyll	0.416	0.216
Diatoms	**0.023**	**0.571**
Dinoflagellates	**0.004**	**0.694**
Colourless Dinoflagellates	**<0.001**	**0.818**
Coccolithophores	0.098	-0.430
Phyto-flagellates	0.571	0.157
Zoo-flagellates	0.460	0.197
Ciliates	0.376	0.235
HNA bacterioplankton	**0.018**	**0.588**
LNA bacterioplankton	0.498	-0.182